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New glycine derived peptides bearing benzenesulphonamide as an antiplasmodial agent†

Daniel Izuchukwu Ugwuja,[a] Uchechukwu Okoro,[b] Shubhanji Soman,[c] Akachukwu Ibezim,[d] David Ugwu,[b] Rina Soni,[c] Bonaventure Obi,[e] James Ezugwu[b] and Ogechi Ekoh[f]

In the tropics, malaria is among the most serious infectious diseases in developing countries. The discovery of the artemesinin antimalarial drug not too long ago was a major breakthrough in the effort to combat the malaria disease. However, recent reports of resistance even to combination therapy involving artemisinin are very worrisome and have led to the search for new chemical agents to sustain the fight against malaria. The carboxamide functionality has been shown to be an important pharmacophore in over 25% of commercial chemotherapeutic agents. Three benzensulphonamides (**3a–c**) were prepared from the reaction of the appropriate benzensulphonyl chloride (**1a–c**) and alanine (**2**) in aqueous basic medium. Eight *tert*-butylamino-oxo-ethylcarbamates (**5a–h**) were also prepared from reacting commercially available boc-glycine (**4**) and different amines using peptide coupling reagents such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBt), with triethyl amine and dichloromethane (DCM) as solvents. The target compounds were prepared by reacting compounds **3a–c** with compounds **5a–h** in the presence of coupling reagents to get twenty four (24) different compounds. The compounds were characterized and evaluated for their antiplasmodial activity. Computed molecular descriptors and assessed biochemical parameters showed that the compounds were drug-like and safe. All the compounds had favourable binding interactions with residues at the PABA binding site of homologically modeled *P. falciparum* dihydropteroate synthase and henceforward the *in vitro* and *in vivo* antiplasmodial activities were evaluated. Compounds **7a–7x** showed activity against *P. falciparum* (W2 strain) at MIC values ranging from 3.52 to 0.09 μM. Moreover, seven of the compounds (**7c**, **7d**, **7i**, **7j**, **7p**, **7r** and **7s**) showed better activity than quinine (MIC = 0.72 μM). In addition, 16 of the 24 compounds were found to clear more than 50 percent of *P. berghei* (NK-65 strain) from the blood of infected mice at 12 days post-infection. The percentages of parasites cleared by 20 mg kg^{-1} of the three most effective compounds (**7g**, **7n** and **7r**) were 74.98, 74.98 and 74.07, respectively. In conclusion, **7r** (MIC 0.71 μM) from this class of glycine derived sulfonamides has the ability to clear 74.07% of *P. berghei* from blood of infected mice at 20 mg kg^{-1} and an interesting pharmacokinetic profile (M_W = 430.31 Da, HBA = 7, HBD = 3, log *P* = 2.56, NRB = 9 and TPSA = 104.37 $Å^2$), which is in agreement with the Lipinski rule of 5 for a compound to be qualified as a drug candidate. **7r** could serve as a lead in developing new antiplasmodial agents.

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Introduction

Malaria is an infectious disease caused by a protozoan of the genus *Plasmodium* and is transmitted to humans by the *Anopheles* mosquito. Since this malady is mainly present in tropical regions and primarily affects poor people in developing countries, its occurrence is often associated with socioeconomic problems.[1] In spite of the recent progress, there are not yet vaccines available for clinical treatment against malaria and the problem is even greater due to increasing parasite resistance.[2–4] In order to contain the epidemic, different technologies, methods and drugs are currently being used. Such procedures range from using nets treated with

[a] *Department of Chemical Sciences, Federal University, Wukari, Nigeria. E-mail: danielizuchukwu77@gmail.com*
[b] *Department of Pure and Industrial Chemistry, University of Nigeria, Nsukka, Nigeria*
[c] *Department of Chemistry, The Maharaja Sayajirao University of Baroda, Vadodara, India*
[d] *Department of Pharmaceutical and Medicinal Chemistry, University of Nigeria, Nsukka, Nigeria*
[e] *Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, Nigeria*
[f] *Evangel University Akaeze, Ebonyi State, Nigeria*

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insecticides[5,6] to employing a combination of drugs with distinct effects on the parasites.[7–10] However, even with these actions, tests indicate a continuous increase in the resistance of the parasites,[11–14] evidencing the urgent need for new drugs.

The prevalence of carboxamide in biological systems, pharmacologically active molecules and amides with favourable properties makes it one of the most popular and reliable functional groups in organic chemistry.[15] The carboxamide functionality has been shown to appear in over 25% of commercial chemotherapeutic agents.[16] This finding makes the amide bond a sought after bond in the design of new chemotherapeutic agents. Similarly, peptides are among the most versatile bioactive molecules and play crucial roles in the human body and other organisms.[17] Peptides have been used as a base for many drugs as they possess good cell penetrating properties.[18] Sulphonamides are widely used in medicinal chemistry because of their low cost, low toxicity and excellent biological activity. Several analogues of sulphonamide have been reported as antimalarial agents. For example, sulfadoxine, sulfadiazine, and sulfalene are effective malaria drugs that possess sulphonamide groups attached to a heterocyclic ring. Krungkrai and coworkers reported a library of aromatic/heteroaromatic sulfonamides with diverse scaffolds and assayed these compounds for the inhibition of carbonic anhydrase from *Plasmodium falciparum* (pfCA).[19,20]

Antifolate action of sulfa drugs

Several studies have described other sulphonamides with antimalarial activity.[21–26] Some of these studies involved the inhibition of folate metabolic enzymes that are crucial for the growth of the malaria parasite. Dihydropteroate synthase (dhps) is one of the essential enzymes in the folate metabolic pathway and has been well characterized to be the target of the sulphonamide class of antimalarial drugs. Folic acid is itself not biologically active but its biological importance is due to tetrahydrofolate and other derivatives after its conversion to dihydrofolic acid in the liver.[27] The human body needs folate to synthesize DNA, repair DNA, and methylate DNA as well as act as a cofactor in certain biological reactions. Antifolate sulfa antimalarial drugs interfere with folate metabolism, a pathway essential to malaria parasite survival. Dihydrofolate reductase (DHFR) is an enzyme that reduces dihydrofolic acid to tetrahydrofolic acid, using NADPH as an electron donor, which can be converted to the kinds of tetrahydrofolate cofactors used in 1-carbon transfer chemistry. This enzyme is encoded by DHFR in humans. It is found in the q11–q22 region of chromosome-5. Disruption of folate synthesis by DHFR and DHPS inhibitors leads to decreased levels of fully reduced tetrahydrofolate, a necessary cofactor in important one-carbon transfer reactions in the purine, pyrimidine, and amino acid biosynthetic pathways (Ferone, 1977). The lower levels of tetrahydrofolate result in decreased conversion of glycine to serine, reduced methionine synthesis, and lower thymidylate levels with a subsequent arrest of DNA replication.[28–32] Some studies have linked point mutations in *Plasmodium falciparum* to sulfa drug resistance.[33,34] The use of computational techniques has become rampant in drug design and discovery to bridge the high cost and time involved in experimental procedures. Moreover, the increasing similarity in the results of computational and experimental methods further presents the former as a good alternative to the latter method. Although no drug molecule has moved from computer to market, the method has been employed at one point in the development of many food and drug agency (FDA) approved drugs. Therefore, both methods work in tandem during the development of new drugs.[35,36]

We report herein the successful synthesis of twenty four new glycine derived sulfonamides that possessed a fascinating antiplasmodial property that can become drug leads in sustaining the fight against malaria. This work was designed based on the reported antimalarial properties of short peptides and benzenesulphonamides and the need to develop newer chemotherapeutic agents that will overcome the reported emerging resistance against artemisinin based therapy. The oral bioavailability and safety profile of the compounds were evaluated by analyzing computed molecular descriptors commonly used to compare "drug-likeness" properties and testing biochemical parameters, respectively. Molecular docking toward the *Plasmodium falciparum* dihydropterate synthase *para*-aminobenzoic acid (PABA) binding site was carried out and finally the *in vitro* and *in vivo* antiplasmodial activity of the compounds was exploited.

Materials and methods

Chemistry

Reagent-grade chemicals and solvents were purchased from a commercial supplier and used after purification. Thin-layer chromatography (TLC) was performed on silica-gel F254 plates (Merck). Merck silica gel (60–120 mesh) was used for column chromatographic purification. All reactions were carried out in a nitrogen atmosphere. Melting points are uncorrected and were measured in open capillary tubes, using a Rolex melting-point apparatus. IR spectra were recorded as KBr pellets on a PerkinElmer RX 1 spectrometer and the wave numbers are reported in cm^{-1}. ^{1}H NMR and ^{13}C NMR spectral data were recorded on a Bruker Advance 400 spectrometer (^{1}H 400 MHz/^{13}C 100 MHz) with DMSO-d_6 as a solvent and tetramethylsilane (TMS) as an internal standard and reported in δ (ppm). J values are in hertz (Hz). The molecular mass of the compounds was obtained using high resolution positive ion electrospray ionization on a Bruker 10152 mass spectrometer.

General procedure for the synthesis of substituted benzene sulphonamoyl alkanamides (3a–c)[37]

Sodium carbonate (Na_2CO_3, 1.590 g, 15 mmol) was added to a solution of amino acids (**2**, 12.5 mmol) in water (15 mL) with continuous stirring until all the solutes had dissolved. The solution was cooled to −5 °C and the appropriate benzenesulphonyl chloride **1a–c** (15 mmol) was added in four portions over a period of 1 h. The slurry was further stirred at room temperature for about 4 h. The progress of the reaction was monitored using TLC (MeOH/DCM, 1 : 9). Upon completion, the mixture was acidified using 20% aqueous hydrochloric acid to pH 2. The crystals were filtered *via* suction and washed with

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pH 2.2 buffer. The pure products (**3a–c**) were dried over self-indicating fused silica gel in a desiccator.

Synthesis of compounds 6a–6h[38]

A mixture of Boc-glycine (**4**, 0.45 g, 1.84 mmol), 1-ethyl-3-(3-dimethyl aminopropylcarbodiimide hydrochloride (EDC, 0.53 g, 2.76 mmol), 1-hydroxybenzotriazole (HOBT, 0.248, 1.84 mmol), triethylamine (TEA), and amines (1.84 mmol) in dichloromethane (DCM, 50 mL) was stirred at room temperature for sixteen (16) hours. The reaction was monitored using TLC. On completion of the reaction, it was washed with water (2 × 20 mL), brine (1 × 10 mL), and dried over anhydrous sodium sulphate and the solvent was evaporated under reduced pressure to give the crude product (**5a–h**), which was then purified by flash column chromatography on silica gel. The products (**5a–h**) were then deprotected by addition of 10% TFA in DCM and allowed to stir for more than 1 h while been monitored with TLC. When the reaction was completed, the solvent was evaporated and the products (**6a–h**) were used directly for the next stage of the reaction.

Synthesis of glycine derived peptides 7a–7x

A mixture of 2-[(phenylsulfonyl)amido]propanioc acid (**3a–c**, 1.84 mmol), EDC (0.53 g, 2.76 mmol), HOBT (0.248, 1.84 mmol), TEA, and deprotected boc-glycine amine (**6a–h**, 1.84 mmol) in dichloromethane (50 mL) was stirred at room temperature for sixteen (16) hours. The reaction was monitored using TLC (MeOH/DCM, 1 : 9). On completion of the reaction, the mixture was acidified using 20% aqueous hydrochloric acid to pH 2. The crystals were filtered *via* suction and washed with pH 2.2 buffer. The pure products (**7a–x**) were dried over self-indicating fused silica gel in a desiccator.

***N*-(2-(3-Chlorophenylamino)-2-oxoethyl)-2-(4-nitrophenylsulfonamido)propanamide (7a).** Yield 78%, melting point 120–124 °C. FTIR (KBr, cm^{-1}) 3352 (NH), 3106, 2984 (C–H Ar), 2872 (C–H), 1667, 1595 (2C═O), 1523, 1481, 1426 (C═C), 1349, 1309 ($2SO_2$), 1169 (SO_2NH), 781 (C–Cl). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.12–1.16 (t, J = 6.72 Hz, 5H), 3.75–3.76 (d, J = 5.6 Hz 3H), 3.98–4.05 (m, 1H), 7.09–7.35 (m, 7H), 7.40–7.42 (d, J = 8.4 Hz, 1H), 7.76 (s, 1H), 8.02–8.04 (d, J = 8.8 Hz 2H), 8.31–8.41 (m, 3H), 10.09 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 19.48, 46.14, 51.32, 51.32 (aliphatic carbon), 117.80, 118.90, 123.46, 124.69, 128.16, 128.63, 130.93, 133.57, 140.64, 147.07, 149.88 (aromatic carbon), 168.07, 171.75 (carbonyl carbon). HRMS-ESI $C_{17}H_{18}ClN_4O_6S$, the found value is (m/z): 441.0637 (M + H), the calculated value is 441.0635.

N-(2-(4-Chlorophenylamino)-2-oxoethyl)-2-(4-nitrophenylsulfonamido)propanamide (7b). Yield 72%, melting point 180–182 °C; FTIR (KBr, cm^{-1}): 3386, 3344 (2NH), 3109, 3067 (C–H Ar), 2973, 2934 (C–H), 1890, 1652 (2C═O), 1597, 1525, 1492, 1460, 1433 (C═C), 1383, 1349 ($2SO_2$), 1170 (SO_2NH), 741 (C–Cl). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.13–1.15 (d, J = 5.73 Hz, 3H), 3.74–3.75 (d, J = 6.88 Hz, 2H), 3.97–4.02 (m, 1H), 7.35–7.38 (m, 2H), 7.56–7.59 (m, 2H), 8.01–8.04 (m, 2H), 8.29–8.34 (m, 1H), 8.34–8.36 (m, 2H), 8.49 (s, 1H), 10.02 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 19.50, 42.81, 52.31 (aliphatic carbon), 120.95, 124.70, 127.30, 128.63, 129.13, 138.17, 147.06, 149.88 (aromatic carbon), 167.82, 171.71 (carbonyl carbon). HRMS-ESI $C_{17}H_{18}ClN_4O_6S$, found value is (m/z): 441.0634, M + H, calculated value is 441.0635.

***N*-(2-(3-Fluorophenyl)amino)-2-oxoethyl)-2-(4-nitrophenylsulfonamido)propanamide (7c).** Yield 83%, melting point 124–126 °C. FTIR (KBr, cm^{-1}): 3354, 3309 (N–H), 3104 (C–H ArH), 2984, 2935 (C–H aliph), 1671, 1611 (C═O), 1529, 1492, 1446 (C═C), 1351 (SO_2), 1169 (SO_2NH), 782 (C–H). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.14–1.15 (d, J = 4 Hz, 3H), 2.45 (s, 3H), 3.05–3.04 (d, J = 4 Hz 2H), 3.37 (s, 2H), 3.75–3.76 (d, J = 4 Hz 1H), 3.99–4.02 (m, 2H), 6.85–6.88 (m, 2H), 7.24–7.36 (m, 3H), 7.53–7.56 (d, J = 12 Hz, 2H), 8.02–8.04 (d, J = 8 Hz, 1H), 8.32–8.35 (m, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 19.50, 40.11, 52.29 (aliphatic carbon), 106.05, 110.09, 115.10, 124.70, 128.63, 130.84, 130.93, 147.01, 149.83, 157.98 (aromatic carbon), 168.06, 171.13 (carbonyl carbon). HRMS-ESI $C_{17}H_{18}FN_4O_6S$, found value is (m/z): 425.0925, M + H, calculated value is 425.0922.

***N*-(2-(4-Fluorophenyl)amino)-2-oxoethyl)-2-(4-nitrophenylsulfonamido)propanamide (7d).** Yield 72%, melting point 180–182 °C. FTIR (KBr, cm^{-1}): 3388, 3343 (NH), 3111, 3067 (C–H ArH), 2971, 2870 (C–H), 1649, 1613 (2C═O), 1525, 1510, 1461, 1448, 1432 (C═C), 1386, 1350 (SO_2), 1171 (SO_2NH), 686 (C–Fl). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.10 (s, 1H), 3.70 (s, 2H), 3.96 (s, 31), 7.12 (s, 1H aro), 8.31 (s, 2H), 8.46 (m, 2H aro), 9.91 (s, 1H NH). ^{13}C NMR (DMSO-d_6 100 MHz) δ: 19.41, 42.73, 52.32 (aliphatic carbon), 115.67, 115.89, 121.11, 121.19, 124.70, 128.63, 135.53, 147.03, 149.88, 137.24 (aromatic carbon), 167.55, 171.69 (carbonyl carbon). HRMS-ESI $C_{17}H_{17}FN_4O_6$, found value is (m/z): 424.0854, M^+, calculated value is 424.0852.

2-(4-Nitrophenylsulfonamido)-*N*-(2-oxo-2-(*p*-tolylamino)ethyl)propanamide (7e). Yield 76%, melting point, 140–142 °C. FTIR (KBr, cm^{-1}): 3323, 3270 (N–H), 3102, 3070 (C–H ArH), 2977, 2936 (C–H), 1707, 1669 (C═O), 1351 (SO_2), 1170 (SO_2NH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.13–1.15 (d, J = 8 Hz, 2H), 2.24 (s, 3H), 3.71–3.73 (d, J = 8 Hz, 2H), 3.99–4.01 (d, J = 8 Hz, 2H), 4.87–4.90 (d, J = 12 Hz, 1H), 7.09–7.11 (d, J = 8 Hz, 3H), 7.43–7.46 (m, 2H), 8.02–8.04 (d, J = 8 Hz, 1H), 8.30–8.44 (m, 3H), 8.53 (s, broad 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 19.49, 42.73, 46.15, 52.32 (aliphatic carbon), 119.40, 124.72, 128.63, 129.60, 131.12, 132.63, 136.65, 143.51, 147.02, 149.84, 151.16, 157.60, 165.21 (aromatic carbon) 167.37, 171.66 (carbonyl carbon). HRMS-ESI $C_{18}H_{20}N_4O_6S$ found value is (m/z): 420.1105, M^+, calculated value is 420.1104.

2-(4-Nitrophenylsulfonamido)-*N*-(2-oxo-2-(phenylamino)ethyl)-propanamide (7f). Yield 81%, melting point 126–128 °C. FTIR (KBr, cm^{-1}): 3273 (N–H), 3105 (C–H, ArH), 1703, 1669 (C═O), 1601, 1526, 1499, 1446 (C═C), 1350 (SO_2), 1169 (SO_2NH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.13–1.16 (m, 2H), 1.61–1.63 (d, J = 8Hz 3H), 1.98 (s, 3H), 2.49–2.50 (m, 2H), 3.74–3.75 (d, J = 4Hz, 1H), 3.99–4.03 (m, 1H), 7.02–7.07 (m, 2H), 7.28–7.33 (m, 3H), 7.56–7.59 (m, 3H), 8.02–8.04 (d, J = 8 Hz, 1H), 8.35–8.43 (m 2H), 8.54 (s, 1H), 9.91 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 14.53, 19.50, 22.53, 31.42, 42.20, 46.03, 52.33 (aliphatic carbon), 119.39, 123.75, 124.74, 128.53, 131.12, 139.21, 143.51, 149.85, 151.16 (aromatic carbon), 166.22, 171.70 (carbonyl carbon). HRMS-ESI $C_{17}H_{18}N_4O_6S$ found value is (m/z): 406.0949, M^+, calculated value is 406.0947.

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***N*-(2-(Naphthalene-2-ylamino)-2-oxoethyl)-2-(4-nitrophenylsulfonamido)propanamide (7g).** Yield 76%, melting point 198–200 °C. FTIR (KBr, cm^{-1}): 3319, 3253 (2NH), 3109, 3016 (C–H Ar), 2967, 2928 (C–H), 1665, 1637 (2C═O), 1601, 1552, 1460, 1431 (C═C), 1395, 1352 (SO_2), 1172 (SO_2NH). ^1H NMR (DMSO-d_6 400 MHz) δ: 1.15–1.16 (d, J = 4 Hz, 3H), 3.93–3.94 (d, J = 4.4 Hz, 2H), 4.00–4.04 (m, 1H), 7.47–7.55 (m, 3H), 7.76–7.78 (d, J = 8.4 Hz, 1H), 7.93–7.95 (m, 1H), 8.03–8.05 (d, J = 8.0 Hz, 3H), 8.35–8.37 (d, J = 8.4 Hz, 3H), 8.50–8.52 (d, J = 7.6 Hz, 1H), 9.86 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 19.45, 52.39 (aliphatic carbon), 123.11, 124.75, 126.00, 126.29, 126.53, 128.58, 128.63, 133.58, 134.16, 147.11, 149.88, 171.83 aromatic carbon). HRMS-ESI $C_{21}H_{20}N_4O_6S$ found value is (m/z): 456.1108, M^+, calculated value is 456.1104.

***N*-(2-Morpholino-2-oxoethyl)-2-(4-nitrophenylsulfonamido)-propanamide (7h).** Yield 68%, melting point 120–122 °C. FTIR (KBr, cm^{-1}): 3338, 3263 (N–H), 3108 (C–H Ar), 2971, 2929 (C–H aliph), 1679, 1644 (C═O), 1518, 1451 (C═C), 1352 (SO_2), 1163 (SO_2NH). ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.09–1.10 (d, J = 4 Hz, 3H), 3.23–3.42 (m, 2H), 3.53–3.54 (d, J = 4 Hz, 1H), 3.78–3.79 (d, J = 4 Hz, 2H), 3.99–4.03 (m, 1H), 8.00–8.07 (m 2H Ar), 8.34–8.43 (m, 1H Ar), 8.50–8.52 (d, J = 8 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 19.46, 42.09, 44.84, 52.29, 56.47, 66.35, 66.41 (aliphatic carbon), 124.76, 128.59, 147.06, 149.86 (aromatic carbon), 167.08, 171.33 (carbonyl carbon). HRMS-ESI $C_{15}H_{20}N_4O_7S$ found value is (m/z): 400.1057, M^+, calculated value is 400.1053.

***N*-2-((4-Acetamidophenyl)sulfonamido)-*N*-(2-((3-chlorophenyl)-amino)-2-oxoethyl)propanamide (7i).** Yield 78%, melting point 118–120 °C. FTIR (KBr, cm^{-1}): 3329 (NH), 3102 (C–H Ar), 2939, 2857 (C–H aliph), 1669 (CO), 1593, 1534, 1483, 1449 (C═C), 1374 (SO_2), 1162 (SO_2NH), 781 (C–Cl). ^1H NMR (DMSO-d_6, 400 MHz) δ: 0.82 (s, 3H), 1.10–1.12 (d, J = 8 Hz, 3H), 2.05 (s, 1H), 3.76 (s, 3H), 7.08 (s, 1H), 7.39–7.95 (m, 2H), 8.20 (s, 1H), 10.07–10.31 (m, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 18.99, 24.59, 43.06, 52.26 (aliphatic carbon), 110.13, 118.87, 119.53, 127.87, 127.59, 128.21 (aromatic carbon), 130.59, 143.26 (carbonyl carbon). HRMS-ESI $C_{19}H_{21}ClN_4O_5S$ found value is (m/z): 452.0919, M^+, calculated value is 452.0921.

2-((4-Acetamidophenyl)sulfonamido)-*N*-(2-((4-chlorophenyl)-amino)-2-oxoethyl)propanamide (7j). Yield 78%, melting point 182–184 °C. ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.04–1.06 (d, J = 8 Hz, 2H), 2.06 (s, 2H), 3.75–3.78 (d, J = 12 Hz, 2H), 3.82–3.86 (m, 1H), 7.34–7.36 (d, J = 8 Hz, 1H ArH), 7.59–7.61 (d, J = 8 Hz, 1H), 7.73 (s, 1H), 7.92–7.94 (d, J = 8 Hz, 2H aro), 8.19 (s, 1H), 10.29 (S, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 19.03, 24.58, 43.08, 52.31 (aliphatic carbon), 118.90, 121.05, 127.31, 128.19, 134.87, 138.16, 143.27 (aromatic carbon), 167.92, 169.42, 172.28 (carbonyl carbon). HRMS-ESI $C_{19}H_{21}ClN_4O_5S$ found value is (m/z): 452.0925, M^+, calculated value is 452.0921.

2-((4-Acetamidophenyl)sulfonamido)-*N*-(2-((3-fluorophenyl)-amino)-2-oxoethyl)propanamide (7k). Yield 82%, melting point 128–130 °C. FTIR (KBr, cm^{-1}): 3330 (NH), 3111, 2988 (C–H Ar), 2988, 2930 (C–H), 1674, 1647 (C═O), 1593, 1534, 1494, 1446 (C═C), 1376, 1321 (SO_2), 1161 (SO_2NH), 637 (C–F). ^1H NMR (DMSO-d_6 400 MHz) δ: 1.04–1.06 (d, J = 6.8 Hz, 3H), 2.07 (s, 3H), 3.76–3.84 (m, 1H), 4.02–4.03 (d, J = 6.8 Hz, 2H), 6.86–6.90 (m, 2H), 7.22–7.37 (m, 2H), 7.55–7.57 (d, J = 8 Hz, 2H), 7.69–7.94 (m, 4H), 8.18–8.21 (m, 1H), 10.06 (s, 1H), 10.29 (s, 1H). ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 19.00, 24.58, 43.10, 52.30 (aliphatic carbon), 106.17, 106.43, 110.32, 115.25, 118.91, 128.20, 130.85, 130.94, 140.86, 140.97, 143.27, 163.79 (aromatic carbon), 168.15, 169.40, 172.30 (carbonyl carbons). HRMS-ESI $C_{19}H_{21}ClN_4O_5S$ found value is (m/z): 436.1218, M^+, calculated value is 436.1217.

2-((4-Acetamidophenyl)sulfonamido)-*N*-(2-((4-fluorophenyl)-amino)-2-oxoethyl)propanamide (7l). Yield 79%, melting point 180–182 °C. FTIR (KBr, cm^{-1}): 3415, 3161 (N–H), 3108 (CH aro), 2938 (C–H aliph), 1679, 1649 (C═O), 1592, 1573, 1529, 1506, 1452 (C═C), 1374 (SO_2), 1168 (SO_2NH), 799 (C–F). ^1H NMR (DMSO-d_6 400 MHz) δ: 1.98 (s, 3H), 2.07 (s, 1H), 3.01–3.07 (m, 1H), 3.74–3.87 (m, 3H), 4.02–4.03 (d, J = 7.2 Hz, 1H), 7.12–7.22 (m, 2H), 7.56–7.61 (m, 2H), 7.71–7.76 (m, 5H), 7.93–7.79 (d, J = 6.8 Hz, 2H), 8.12–8.21 (m, 1H), 9.90 (s, 1H), 10.51 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 14.53, 19.00, 21.21, 24.58, 43.01, 52.34 (aliphatic carbon). 115.68, 115.90, 118.91, 121.23, 121.30, 127.12, 128.20, 134.84, 135.58, 143.28, 157.26 (aromatic carbon), 167.67, 169.43, 172.27 (carbonyl carbon). HRMS-ESI $C_{19}H_{21}ClN_4O_5S$ found value is (m/z): 436.1217, M^+, calculated value is 436.1217.

2-((4-Acetamidophenyl)sulfonamido)-*N*-(2-oxo-2-(*p*-tolylamino)-ethyl)propanamide (7m). Yield 68%, melting point 178–180 °C. FTIR (KBr, cm^{-1}): 3296, 3161 (N–H), 3044 (C–H aro), 2992, 2931 (C–H aliph), 1669, 1650 (C═O), 1534, 1429, 1592 (C═C), 1370 (SO_2), 1165 (SO_2NH). ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.04–1.05 (d, J = 6.4 Hz, 3H), 2.07 (s, 3H), 2.24 (s, 3H), 3.74–3.84 (m, 3H), 3.96–4.03 (m, 2H), 7.09–7.11 (d, J = 7.2 Hz, 2H), 7.44–7.46 (d, J = 7.6 Hz, 4H), 7.73 (s, 2H), 7.92–7.95 (d, J = 7.2 Hz, 1H), 8.17 (s, 1H), 9.72 (s, 1H), 10.26 (s, 1H). ^{13}C NMR (DMSO-d_6 400 MHz) δ: 18.97, 20.88, 24.59, 43.04, 52.33 (aliphatic carbon), 118.92, 119.54, 128.20, 129.60, 132.67, 134.85, 136.68, 143.28 (aromatic carbon), 167.46, 169.47, 172.23 (carbonyl carbon). HRMS-ESI $C_{20}H_{24}N_4O_5S$ found value is (m/z): 433.1538, M + H, calculated value is 433.1535.

2-((4-Acetamidophenyl)sulfonamido)-*N*-(2-oxo-2-(phenylamino)ethyl)propanamide (7n). Yield 83%, melting point 180–182 °C. FTIR (KBr, cm^{-1}) δ: 3299, 3259 (N–H), 1702, 1669, 1648 (C═O), 1591, 1537, 1499, 1446 (C═C), 1371 (SO_2), 1166 (SO_2NH). ^1H NMR (DMSO-d_6, 400 MHz): 1.03–1.05 (d, J = 8 Hz, 3H), 2.07 (s, 3H), 2.45 (s, 2H), 3.37 (s, 3H), 3.75–3.90 (m, 1H), 7.03–7.06 (m, 2H), 7.28–7.23 (m, 3H), 7.56–7.58 (d, J = 8 Hz, 1H), 7.74 (s, 1H), 7.97–7.99 (d, J = 8 Hz, 2H), 8.21–8.24 (m, 2H), 9.86 (s, 1H), 10.32 (s 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 19.00, 24.60, 43.03, 50.36 (aliphatic carbon), 118.89, 119.48, 123.76, 123.91, 128.22, 129.24, 134.82, 139.19, 143.27 (aromatic carbon), 116.23, 169.44, 172.26 (carbonyl carbon). HRMS-ESI $C_{19}H_{22}N_4O_5S$ found value is (m/z): 419.1391, M + H, calculated value is 419.1389.

2-((4-Acetamidophenyl)sulfonamido)-*N*-(2-(naphthalen-1-ylamino)-2-oxoethyl)propanamide (7o). Yield 75%, melting point 178–180 °C. FTIR (KBr, cm^{-1}): 3318, 3257 (NH), 3065 (C–H Aro), 2968, 2931 (C–H aliphatic), 1679, 1640 (C═O), 1590, 1523, 1439 (C═C), 1369 (SO_2), 1162 (SO_2NH). ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.07–1.08 (d, J = 6.8 Hz, 3H), 2.08 (s, 3H), 3.81–3.99 (d, J = 7.2 Hz, 3H), 7.47–7.51 (m, 3H), 7.52–7.54 (m, 1H), 7.65–7.67 (d, J = 6.0 Hz, 2H), 7.27–7.80 (m, 5H), 7.92–7.94 (m, 2H), 7.98–7.99

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(d, J = 5.2 Hz, 2H), 8.06–8.08 (d, J = 5.2 Hz, 1H), 8.34 δ (s, 1H), 9.92 (s, 1H), 10.56 (s, 1H). ^{13}C NMR (DMSO-d_6 100 MHz) δ: 18.98, 24.56, 43.16, 52.45 (aliphatic carbon), 118.947, 122.093, 123.267, 125.847, 126.004, 126.293, 126.511, 128.146, 128.514, 133.603, 134.142, 135.806, 143.386 (aromatic carbon), 168.66, 169.56, 172.42 (carbonyl carbon). HRMS-ESI $C_{23}H_{24}N_4O_5S$ found value is (m/z): 467.1388, M–H, calculated value is 468.1389.

2-((4-Acetamidophenyl)sulfonamido)-*N*-(2-morpholino-2-oxoethyl)-propanamide (7p). Yield 80%, melting point 110–114 °C. FTIR (KBr, cm^{-1}), 3269 (NH), 3187 (C–H aro), 2983, 2930 (C–H aliphatic), 1682 (C═O), 1591, 1533, 1496 (C═C), 1367 (SO_2), 1161 (SO_2NH). ^{13}C NMR (DMSO-d_6 100 MHz) δ: 19.04, 21.35, 24.65, 52.31 (aliphatic carbon), 110.04, 118.30, 120.23, 125.83, 128.17, 129.59, 131.05, 135.00, 143.21, 145.34 (aromatic carbon), 167.09, 169.44, 171.21 (carbonyl carbon). HRMS-ESI $C_{17}H_{24}N_4O_6S$ found value is (m/z): 412.1419, M$^+$, calculated value is 412.1417.

2-(4-Chlorophenylsulfonamido)-*N*-(2-((3-chlorophenyl)amino)-2-oxoethyl)propanamide (7q). Yield 78%, melting point 140–142 °C. FTIR (KBr, cm^{-1}): 3341 (NH), 3082 (C–H Ar), 2979 (C–H), 1693, 1665 (C–H ArH), 1598, 1531, 1499, 1445 (C═C), 1324 (SO_2), 1167 (SO_2NH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.10–1.10 (d, J = 8 Hz 3H), 1.16–1.18 (m, 1H), 2.45 (s, 2H), 3.05–3.07 (d, J = 8 Hz, 1H), 3.37 (s, 1H), 3.78–3.80 (m, 2H), 3.85–3.91 (m, 1H), 7.09–7.11 (d, J = 8 Hz, 1H), 7.31–7.35 (m, 2H), 7.42–7.44 (d, J = 8 Hz, 3H), 7.59–7.73 (m, 1H), 7.79–7.81 (d, J = 8 Hz, 1H), 8.12–8.29 (m, 2H). HRMS-ESI $C_{17}H_{17}Cl_2N_4O_6S$ found value is (m/z): 430.0398, M + H, calculated value is 430.0395.

2-(4-Chlorophenylsulfonamido)-*N*-(2-((4-chlorophenyl)amino)-2-oxoethyl)propanamide (7r). Yield 84%, m.p. 184–186 °C. FTIR (KBr, cm^{-1}): 3325, 3259 (2NH), 3159, 3088 (C–H Ar), 2988, 2968 (C–H), 1672, 1646 (C═O), 1553, 1534, 1513, 1477 (C═C), 1389, 1333 (SO_2), 1116 (SONH), 682 (C–Fl). ^{1}H NMR (DMSO-d_6 400 MHz) δ: 1.08–1.09 (d, J = 5.2 Hz, 3H), 3.76–83 (m, 1H), 7.14 (s, 2H), 7.59 (s, 3H), 7.78–7.79 (d, J = 6.8 Hz, 3H), 8.18–8.25 (m, 3H), 9.93 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 19.28, 42.83, 52.28 (aliphatic carbon), 115.68, 115.91, 121.18, 121.26, 128.98, 129.53, 135.61, 137.64, 148.32 (aromatic carbon), 167.62, 171.94 (carbonyl carbon). HRMS-ESI $C_{17}H_{17}Cl_2N_4O_6S$ found value is (m/z): 430.0396, M + H, calculated value is 429.0395.

2-(4-Chlorophenylsulfonamido)-*N*-(2-((3-fluorophenyl)amino)-2-oxoethyl)propanamide (7s). Yield 81%, melting point 158–160 °C. FTIR (KBr, cm^{-1}): 3325, 3269 (N–H), 3091 (C–H aro), 2987, 2967 (C–H aliph), 1646, 1612 (C═O), 1546, 1491, 1478, 1446 (C═C), 1332 (SO_2), 1166 (SO_2NH), 776 (C–F). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.09–1.11 (d, J = 8 Hz, 3H), 3.79–3.80 (d, J = 4 Hz, 1H), 3.89–3.93 (m, 1H), 6.85–6.89 (m, 2H), 7.27–7.37 (m, 3H), 7.58–7.61 (m, 3H), 7.79–7.81 (d, J = 8 Hz, 1H), 8.24–8.26 (d, J = 8 Hz, 2H), 8.32–8.35 (m, 3H), 10.16 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 24.05, 47.66, 57.00 (aliphatic carbon), 110.87, 111.13, 114.84, 115.05, 119.91, 133.74, 134.29, 135.59, 135.59, 135.66, 142.40, 145.04, 145.16 (aromatic carbon), 168.55, 176.76 (carbonyl carbon). HRMS-ESI $C_{17}H_{17}FN_4O_6S$ found value is (m/z): 413.0615, M$^+$, calculated value is 413.0612.

2-(4-Chlorophenylsulfonamido)-*N*-(2-((4-fluorophenyl)amino)-2-oxoethyl)propanamide (7t). Yield 79%, melting point 160–162 °C. FTIR (KBr, cm^{-1}): 3341, 3262 (2NH), 3088, 3114 (C–H Ar), 2974, 2935 (C–H), 1675, 1647 (2C═O), 1595, 1526, 1491, 1451 (C═C), 1401, 1352 (SO_2), 1168 (SO_2NH), 755 (C–Cl). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.083 (s, 1H), 2.49 (s, 2H), 3.36 (s, 3H), 3.76–3.88 (d, J = 4.8 Hz, 2H), 7.37 (s, 1H), 7.59–7.60 (d, J = 4 Hz, 2H), 7.77 (s, 1H), 8.22–8.30 (m, 2H), 10.06 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 19.30, 42.85, 52.23 (aliphatic carbon), 120.97, 127.27, 128.98, 129.17, 137.63, 138.19, 140.29 (aromatic carbon), 167.90, 171.96 (carbonyl carbon). HRMS-ESI $C_{17}H_{17}FN_4O_6S$ found value is (m/z): 413.0618, M$^+$, calculated value is 413.0612.

2-(4-Chlorophenylsulfonamido)-*N*-(2-oxo-2-(*p*-tolylamino)ethyl)-propanamide (7u). Yield 82%, melting point 180–182 °C. FTIR (KBr, cm^{-1}): 3328, 3271 (N–H), 1671, 1646 (C═O), 1600, 1533, 1477 (C═C), 1345 (SO_2), 1168 (SO_2NH), 757 (C═Cl). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.08–1.10 (d, 6.8 Hz, 3H), 3.06 (s, 3H), 3.75–3.77 (m, 2H), 3.87–3.90 (m, 1H), 7.09–7.12 (d, J = 8 Hz, 2H), 7.45–7.47 (d, J = 8 Hz, 2H), 7.59–7.62 (d, J = 8 Hz, 2H), 7.78–7.81 (d, J = 8.8 Hz, 2H), 8.18–8.24 (m, 2H), 9.77 (s, 1H) ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 19.27, 20.88, 42.87, 52.30 (aliphatic carbon), 119.51, 128.99, 129.55, 129.61, 132.66, 136.66, 136.66, 137.66, 140.32 (aromatic carbon), 167.42, 171.91 (carbonyl carbon). HRMS-ESI $C_{18}H_{20}ClN_3O_4S$ found value is (m/z): 427.1075, M + NH_4, calculated value is 427.1078.

2-((4-Chlorophenyl)sulfonamido)-*N*-(2-oxo-2-(phenylamino)-ethyl)propanamide (7v). Yield 89%, melting point 160–162 °C. FTIR (KBr, cm^{-1}): 326, 3262 (N–H), 3086, 3063 (C–H aro), 2988, 2967 (C–H aliph), 1670, 1644 (C═O), 1602, 1549, 1529, 1498, 1476 (C═C), 1332 (SO_2), 1165 (SO_2NH), 754 (C═Cl). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.09–1.11 (d, J = 8 Hz, 3H), 3.77–3.79 (m, 1H), 3.88–3.92 (m, 1H), 7.02–7.06 (m, 2H), 7.28–7.33 (m, 3H), 7.57–7.62 (m, 1H), 7.79–7.81 (d, J = 8 Hz, 1H), 8.23–8.25 (d, J = 8 Hz, 1H), 8.29–8.32 (m, 2H), 9.91 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 19.31, 42.89, 52.28 (aliphatic carbon), 119.45, 123.76, 129.00, 129.26, 129.55, 137.66, 138.23, 140.29 (aromatic carbon), 161.71, 171.71 (carbonyl carbon). HRMS-ESI $C_{17}H_{18}ClN_3O_4S$ found value is (m/z): 395.0704, M$^+$, calculated value is 395.0706.

2-((4-Chlorophenyl)sulfonamido)-*N*-(2-(naphthalene-2-ylamino)-2-oxoethyl)propanamide (7w). Yield 89%, melting point 190–192 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.08–1.09 (s, J = 7.2 Hz, 3H), 3.85 (m, 1H), 4.02–4.04 (d, J = 7.2 Hz, 1H), 7.48–7.55 (m, 8H), 7.56–7.62 (m, 4H), 7.64 (d, J = 1.2 Hz, 2H), 7.66–7.69 (m, 3H), 7.77–7.79 (m, 1H), 7.81–7.81 (m, 2H), 7.93–7.96 (m, 5H), 8.06–8.13 (m, 4H), 8.29–8.31 (d, J = 5.2 Hz, 2H), 9.85 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 19.05, 43.10, 52.39 (aliphatic carbon), 122.00, 123.16, 125.85, 126.03, 126.31, 126.54, 127.00, 127.18, 128.17, 128.57, 129.49, 129.67, 132.88, 133.59, 134.16, 141.37 (aromatic carbon), 168.59, 172.24 (carbonyl carbon). HRMS-ESI $C_{21}H_{20}ClN_3O_4S$ found value is (m/z): 468.0864, M + Na, calculated value is 468.0863.

2-(4-Chlorophenylsulfonamido)-*N*-(2-morpholino-2-oxoethyl)-propanamide (7x). Yield 67%, melting point 110–112 °C. FTIR (KBr, cm^{-1}): 3296 (N–H), 3091 (C–H aro), 2983, 2928 (C–H aliph), 1660, 1629 (C═O), 1585, 1549, 1475 (C═C), 1361 (SO_2), 1159 (SO_2NH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.04–1.06 (d, J = 8 Hz, 3H), 3.37–3.55 (m, 1H), 3.82 (s, 2H), 3.87–3.92 (m, 2H), 7.60–7.62 (d, J = 8 Hz, 3H), 7.71–7.73 (d, J = 8 Hz, 1H), 7.77–7.79 (d, J = 8 Hz,

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3H), 7.99 (s, 1H), 8.11 (s, 2H), 8.22–8.24 (d, J = 8 Hz, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 19.27, 42.13, 44.87, 52.24, 66.36, 66.44 (aliphatic carbon), 128.97, 129.55, 129.69, 129.90, 130.48, 131.45, 137.60, 140.38 (aromatic carbon), 167.07, 171.52 (carbonyl carbon). HRMS-ESI $C_{15}H_{20}ClN_3O_5S$ found value is (m/z): 388.0736, M−H, calculated value is 388.0734.

Molecular modeling

Homology modeling of the target protein and docking. Since there is no three-dimensional (3-D) structure of *Plasmodium falciparum* dihydropteroate synthase (*Pfdhps*) in the Protein Databank, the crystal structure of *Pfdhps* was homologically modeled using the *Pfdhps* sequence fragment retrieved from the UniprotKB database as the target sequence (A0A3G2LE00_PLAFA).[39] SWISS-MODEL[40] uses its own set of modeling algorithms to automatically build a model using the *P. vivax* homodimer (PDB code: 5Z79; resolution 1.7 Å)[41] as a template. The constructed model was subjected to molecular dynamics simulation using the GROMOS96 force field.[42] The system was placed in a cubic box and solvated using an explicit simple point charge (SPC) water model for all atom simulation. The protein had at least 10 Å buffer in every direction of the box. The system was maintained at a neutral charge while adjusting the NaCl concentration to 150 mM and equilibrated at 300 K using Berendsen's algorithm. The entire system was subjected to energy minimization using both the steepest descent and the conjugate gradient algorithms. All non-hydrogen atoms were subsequently restrained in position while solvent molecules and ions were allowed to relax around the solute molecules for a simulation time of 1000 ps. The evolutions of all quantities considered in this study were recorded per 1 ns. The accuracy of the built *Pfdhps*_model was evaluated by backbone conformation analysis using the Psi/Phi Ramachandran plot package in Discovery Studio,[43] the stability of the protein structure of the MD simulation and alignment to the *P. vivax* homodimer template. In order to dock the newly synthesized sulfa compounds, the binding site of *Pfdhps*_model was identified using the online program COACH.[44]

The chemical structures of the compounds were prepared using the builder protocol in Molecular Operating Environment (MOE) software[45] and energy minimized to a 0.001 kcal mol^{-1} gradient. The molecular descriptors employed in calculating the following basic physicochemical features: molecular weight (M_W), hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), lipophilicity ($\log P$), number of rotatable bonds (NRB) and total polar surface area (TPSA), were computed using the QuSAR module of MOE. Docking of the compounds toward the *Pfdhps_model* binding site was performed using "*triangle*" matcher for placement and "*affinity dG*" for scoring and force field refinement was also applied.

Biological activities

***In vitro* antiplasmodial activity.** The effect of the synthesized compounds (**7a–7x**) *in vitro* was evaluated against chloroquine resistant *P. falciparum* (W2 strain). Briefly, sorbitol synchronized, 0.1% parasitemia, ring stage *P. falciparum* strain W2 parasites were cultured under an atmosphere of 3% O_2, 6% CO_2 and 91% N_2 in RPMI-1640 medium supplemented with 10% human serum in the presence of inhibitors for 48 h without media change. Inhibitors were added from 1000 DMSO stocks. After 48 h, the culture medium was removed and replaced with 1% formaldehyde in PBS pH 7.4 for an additional 48 h at room temperature to fix the cells. Fixed parasites were transferred into 0.1% Triton-X-100 in PBS containing 1 nM YOYO-1 dye (Molecular Probes). Parasitemia was determined from dot plots (forward scatter *vs.* fluorescence) acquired on a FACS sort flow cytometer using Cell Quest software (Beckton Dickinson). The MICs of the compounds were the minimum concentrations at which more than 99% of the parasites, relative to the control, were inhibited from developing to schizonts (parasites with six or more chromatin dots).[46]

***In vivo* antiplasmodial activity.** The *in vivo* antimalarial activity of the synthetic compounds (**7a–7x**) was tested against *P. berghei* (NK-65 strain) infected mice as described by Peter *et al.*[47] and Kalra *et al.*[48] with minor modifications. The animals were obtained from Nigerian Institute for Trypanosoma and Onchocercarioses Research (NTIR), Vom, Plateau State, Nigeria and were kept under standard conditions for 7 days to adapt to the laboratory animal housing facilities. The permission and approval for the use of animals were granted by the Animal Ethics Committee, Federal College of Veterinary Medical Laboratory, Vom, Plateau State. Briefly, eighty infected mice were randomly divided into 27 groups of five mice in each group. The inoculum was prepared from a donor mouse with rising parasitemia of 60.42%. At 7 days post-infection, the animals were administered with the synthesized compounds (**7a–7x**) for 12 consecutive days and were monitored with constant checking of the percentage of parasitemia after one-day intervals. Artemisinin was used as the positive control of the experiment. Group four was not treated and group five was not infected. All the compounds and the drugs were given orally by using a standard intragastric tube. For all parasitemia determination, blood samples were collected from a tail snip of each mouse on days 7 and 8 and thin smears prepared and stained with 10% Giemsa solution. The uniform fields of each stained slide (for each mouse) were examined under a microscope with an oil immersion objective of 100× magnification power and the percentage inhibition of parasitemia was calculated comparing the treated group with the untreated group by means of the following formula[49] $[(A - B)/A] \times 100$, where A = parasitaemia in the untreated group and B = parasitemia in the test group. Compounds that reduced parasitemia by 40% were considered active, whereas those that reduced parasitaemia by 30–40% or less than 30% were deemed partially active and inactive, respectively.[50] Furthermore, the percentage survival rate of each group was determined.

All animal experiments were conducted in compliance with the National Institute of Health guide for care and use of laboratory animals (Pub No. 85-23, revised 1985) and in accordance with the University of Nigeria ethics committee on the use of laboratory animals, registered by the National Health Research Ethics Committee (NHREC) of Nigeria, with the number

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NHREC/05/01/2008B. The study protocol was approved by our institution's ethics committee.[51]

Biochemical assessment

On the twelfth day post-infection, the animals were sacrificed by cervical dislocation and blood samples obtained for further biochemical analysis. Blood samples were collected firstly at the beginning of the experiment (day 0) to assay for basal biochemical levels. Subsequently, blood samples were recollected from the animals on days 7 and 12 post-treatment to re-assay for serum biochemical levels. The blood glucose concentration (BGC) was measured using a One Touch Ultra® glucometer (Lifescan; Johnson & Johnson, Milpitas, CA, USA). The serum creatinine and albumin, total protein and bilirubin levels were analyzed according to standard methods. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were assayed using standard procedures. The haematological parameters red blood cell (RBC) count, packed cell volume (PCV), white blood cell (WBC) count and haemoglobin concentration (HB) were estimated according to a standard procedure using an automated machine (automated CBC analyser: Sysmex KX-21).

Liver function tests (LFTs)

The liver function tests carried out with the blood of the rats fed with the sulphonamide derivatives were aspartate aminotransperase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). The standard laboratory procedure according to ref. 52 was used for the determination of the parameters.

Renal or kidney function test

The kidney function tests carried out with the blood of the rats fed with the sulphonamide derivatives were creatinine and albumin. The method reported in ref. 53 was used in the determination of creatinine.

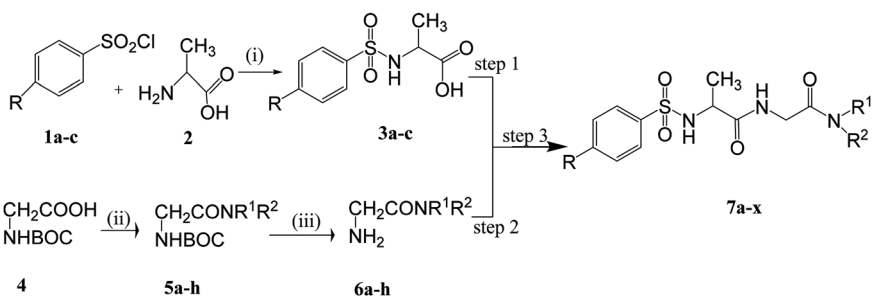
Results and discussion

Synthesis of the glycine derived sulfonamides

Sulfonamides are broadly utilized as pharmaceuticals to treat numerous sicknesses, for example, bacterial contamination and intestinal sickness. Instances of medication obstruction against current antimalarial sulfonamides are on the rise. Thus, there is a consistent quest for new sulfonamides with astounding antimalaria power. We along these lines have arranged boc-glycine-amine-derived sulfonamides **7a–x** as shown in Scheme 1. The first phase of the reaction was the combination of phenylsulphonamido acids by reacting substituted benzenesulphonyl chloride and an amino acid (alanine) in the presence of Na_2CO_3 as a base; the acid was formed after 4 h reaction and on acidification of the reacting mixture. The diamide derivatives of glycine were synthesized by reacting commercially available boc-protected glycine with amines in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI), 1-hydroybenzotriazole (HOBT), and trimethylamine (TEA) to yield various C-substituted amide derivatives of glycine. EDCI alone could not sufficiently activate the carboxylic acid group of glycine and thus addition of HOBt was necessary for the coupling reaction. Compounds 7 were converted into their salts by addition of 10% trifluoroacetic acid (TFA) in DCM. The salt on further reaction with acid in the presence of peptide coupling agent EDCI, HOBt, TEA or DMAP gave the desired diamide derivative of glycine. The synthesized compounds were crystallized in analytical grade using *n*-hexane, while FTIR, ^{1}H NMR, ^{13}C NMR and high resolution mass spectroscopy (HRMS) were used to characterize their chemical structures. The IR showed a strong band between 3300 and 3400 showing the presence of two NH (amide) bonds of the derivatives. Strong bonds were also observed between 1650 and 1800 for amide carbonyls and a band at 1355 cm^{-1} for sulphonamide groups, which indicate the successful formation of the compounds. ^{1}H NMR of the compounds showed the methylene group of glycine exhibiting a multiplet at δ 3.5–4.00 due to the interaction of CH_2 and NH of glycine. There is a doublet at 1.0–1.2 due to the CH and CH_3 interaction of the alanine amide. The compound also exhibited a multiplet from 7.1–7.9 representing aromatic protons. ^{13}C NMR showed a strong peak between 168.16 and 172 which indicates the presence of two carbonyl carbons. The appearance of peaks at 19.02, 42.99, and 53.30 showed the presence of three aliphatic carbons. The peaks at 117.82, 118.88, 123.48, 127.00, 129.49, 131.02, 132.89, 133.56, 140.67, and 141.30 indicated the presence of aromatic carbons.

Oral bioavailability and safety

Failures of many drug candidates with excellent pharmacological features due to poor ADME/Tox properties have necessitated



Scheme 1 (i) Na_2CO_3, DCM/H_2O, HCl, 0 °C, r.t, pH 2, 4 h. (ii) EDCI, HOBt, TEA, DCM, amines, 16 h. (iii) 10% TFA in DCM. (V) EDCI, HOBt, TEA or DMAP, 16 h.

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evaluation of the physicochemical features of potential drug molecules at the early stage of drug discovery.[54] As a rule of thumb, it has been found that molecules with the following criteria $M_W < 500$ Da, HBA < 10, HBD < 5, $\log P < 10$, NRB < 5 and TPSA < 140 Å^2 are orally bioavailable.[55] The M_W, HBD and $\log P$ of the compounds are respectively in the ranges of 380.85–468.53 Da, 2–4 and −2.02 to 3.22. Only **7p** had HBA above ten (HBA = 11). Seven different compounds possess NRB and TPSA values above the recommended ranges (Table 1). It has been reported that the number of rotatable bonds (NoRB) influences bioavailability in rats and a cut off of NoRB ≤ 10 is recommended for good oral bioavailability.[56] All the compounds reported here have NoRB ≤ 10. The total polar surface area (TPSA) has also been used as a surrogate property for cell permeability. A molecule with TPSA ≤ 140 Å^2 would be able to permeate the cell. Again, most of the compounds had TPSA less than 140 and as such can permeate the cell membrane. In spite of some outliers in NRB and TPSA values, analysis of the computed molecular descriptors suggests that **7a–7x** are drug-like, and hence good candidates for biological screening. Furthermore, to ascertain the safety profile of some of the compounds, their effects on blood glucose, kidney function, haematology, and liver marker enzymes were assessed. The obtained result of the biochemical analysis (Table 4) indicates that the blood glucose, kidney function, haemopoietic system and liver enzymes of the infected animals did not differ significantly ($p > 0.05$) as compared with the control and baseline values, at day 12. Hence, the compounds are safe at the studied concentrations.

Homology modeling and docking calculations

Compounds bearing the sulphonamide pharmacophore are known to exhibit antimalarial activity through inhibition of the PfCA and/or dhps enzymes.[57–59] Since we could not obtain a PfCa template with an acceptable Qmean score, only the homology model of *Pfdhps* was used. The 222 amino acid sequence of *Pfdhps* was used to build the 3-dimensional structure of the *Pfdhps* homology model (*Pfdhps*_model) in order to investigate the affinity of the newly synthesized compounds for *Pfdhps*. *Pfdhps*_model (Fig. 1a) has a sequence similarity of 75.23% with *P. vivax* and therefore was used as the target protein template (PDB code 5Z79). The structural quality of *Pfdhps*_model was assessed by calculating the Qmean score, Ramachandran plot and alignment of Cα. It was respectively observed that the modeled protein lies in the *Z*-score range of other protein crystal structures that exist in the PDB (Fig. 1b), contains above 95.0% of *Pfdhps*_model residues in the favored region (Fig. 1c) and closely aligned with *P. vivax*_dhps Cα to an RMS value of 0.188. These showed that the modeled protein is reliable and has good quality. MD simulation was carried out to probe *Pfdhps*_model. Analysis of the RSMD, which has a maximum value of 0.426 Å (Fig. 1d), showed that the model undergoes very minimal positional and conformational fluctuation. The potential energy of the protein starting from the end of the equilibrium phase dropped from −15 125 kcal mol^{-1} to −18 154 kcal mol^{-1} indicating stabilization as the simulation progressed (Fig. 1e). Having ascertained that *Pfdhps*_model is of good quality, the COACH online program, which uses an in-house algorithm to predict protein binding sites, was employed. The *Pfdhps*_model region occupied by pABA as returned by the COACH program was considered as the binding site in this study because sulphonamides are known to bind the same binding site that pABA occupies in *Pfdhps*.

Meanwhile, the reliability of the MOE docking software was evaluated by checking for its ability to retrieve a binding pose of pABA comparable to that found in the COACH derived

Table 1 Basic physicochemical data of the boc-glycine–amine derived sulphonamides

Compd	R	R^1	R^2	M_W	HBA	HBD	$\log P$	NRB	TPSA
7a	NO_2	H	3-ClC_6H_4	440.86	9	4	1.29	11	133.47
7b	NO_2	H	4-ClC_6H_4	440.86	9	4	1.25	11	133.47
7c	NO_2	H	3-FC_6H_4	420.40	9	4	0.85	11	133.47
7d	NO_2	H	4-FC_6H_4	420.40	9	4	0.82	11	133.47
7e	NO_2	H	4-$CH_3C_6H_4$	420.44	9	4	0.96	11	133.47
7f	NO_2	H	C_6H_5	406.41	9	4	0.66	11	133.47
7g	NO_2	H	$C_{10}H_9$	456.47	9	4	1.92	11	133.47
7h	NO_2	H	C_4H_9O	400.10	10	3	−2.02	10	133.91
7i	$NHCOCH_3$	H	3-ClC_6H_4	452.91	10	3	1.94	10	150.19
7j	$NHCOCH_3$	H	4-ClC_6H_4	452.91	10	3	1.10	10	150.19
7k	$NHCOCH_3$	H	3-FC_6H_4	436.46	10	3	1.50	10	150.19
7l	$NHCOCH_3$	H	4-FC_6H_4	436.46	10	3	1.46	10	150.19
7m	$NHCOCH_3$	H	4-$CH_3C_6H_4$	418.10	10	3	1.60	10	150.19
7n	$NHCOCH_3$	H	C_6H_5	391.40	9	2	1.87	9	138.16
7o	$NHCOCH_3$	H	$C_{10}H_9$	468.53	10	3	2.57	10	150.19
7p	$NHCOCH_3$	H	C_4H_9O	412.10	11	2	−1.38	9	150.63
7q	Cl	H	3-ClC_6H_4	429.10	7	3	2.59	9	104.37
7r	Cl	H	4-ClC_6H_4	430.31	7	3	2.56	9	104.37
7s	Cl	H	3-FC_6H_4	431.10	7	3	2.15	9	104.37
7t	Cl	H	4-FC_6H_4	413.86	7	3	2.12	9	104.37
7u	Cl	H	3-$CH_3C_6H_4$	409.89	7	3	2.26	9	104.37
7v	Cl	H	C_6H_5	381.10	6	2	2.53	8	92.34
7w	Cl	H	$C_{10}H_9$	445.93	7	3	3.22	9	104.37
7x	Cl	H	C_4H_9O	380.10	8	2	−0.72	8	104.81

The parameters are in their standard units: M_W in Dalton, and TPSA in Å^2

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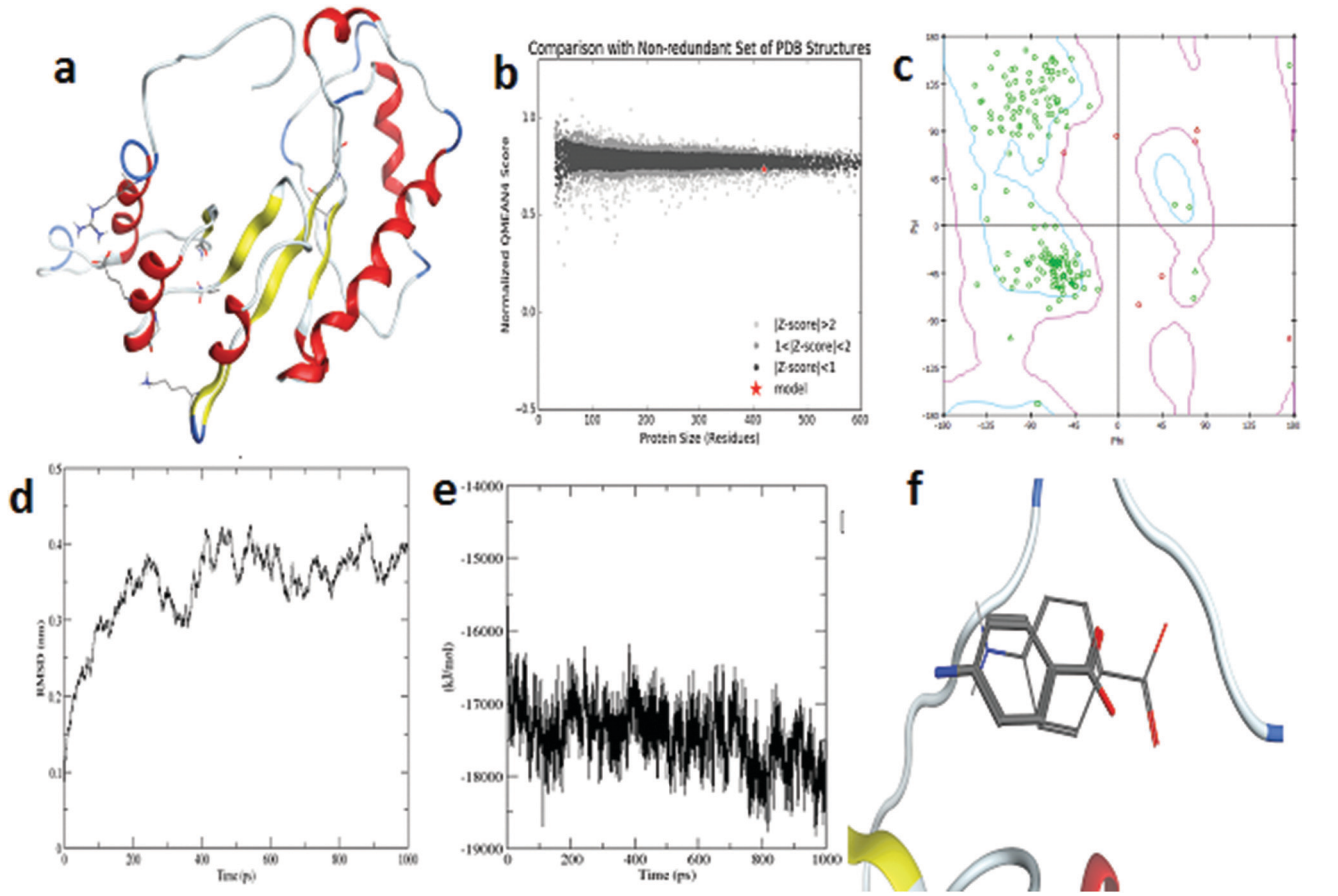

Fig. 1 *Pfdhps*_model and its properties. (a) 3-D model of dihydropteroate synthase. (b) Qmean score of *Pfdhps*_model. (c) Ramachandran plot of the model. (d) RMSD across time along the production phase for the protein model. (e) Potential energy of the protein model across the production phase trajectory. (f) Predicted pABA binding poses by the COACH program (as sticks) and dock program (as lines).

Pfdhps–pABA complex. The docking protocol (a grid box size of 4, 3.5, 2 Å^3 points and centered on the mass center at 63.3, 47.5, 42.3) which identified an RMSD between the COACH-based and docked pose of pABA lower than 2.0 Å (Fig. 1f) was retained and used for docking studies. The best docked poses characterized by the lowest theoretical binding energy revealed that **7a–7x** dock preferentially within the *Pfdhps_model* pABA binding site. A narrow range of binding affinity was observed (−2.80 to −4.68 kcal mol^{-1}) (Table 2) which eluded any observable significant structure–activity relationship within the studied series. However, the negative free binding energies of each of **7a** to **7x** suggested that they favourably interacted with the protein and could serve as an inhibitor and subsequently an antimalarial agent. Therefore, their ability to kill *P. falciparum* in culture and clear *P. berghei* in infected mice was investigated.

Antiplasmodial screening

The anitplasmodial activity of compounds **7a–x** was measured against *P. falciparum* (W2 strain). The MIC values were calculated from experiments carried out in triplicate and compared to chloroquine (**CLQ**) and quinine (**Qn**) (Table 3). Studies have shown that the *in vitro* system, as an experimental model, aids in understanding the pathophysiology of a disease state as well as screening and identifying the action of compounds as possible drug leads.[60] All the compounds showed activity against the tested *P. falciparum* strain at MIC values ranging from 3.52 to 0.09 μM. Apart from **7p** (MIC = 0.09 μM) with activity comparable

Table 2 Binding free energy of the newly synthesized compounds towards the homology model of *Plasmodium falciparum* dihydropteroate synthase

Compds	**7a**	**7b**	**7c**	**7d**	**7e**	**7f**	**7g**	**7h**	**7i**	**7j**	**7k**	**7l**
ΔG (kcal mol^{-1})	−3.17	−4.68	−3.08	−3.66	−3.48	−2.80	−3.41	−3.22	−3.77	−3.85	−3.68	−4.64
Compds	**7m**	**7n**	**7o**	**7p**	**7q**	**7r**	**7s**	**7t**	**7u**	**7v**	**7w**	**7x**
ΔG (kcal mol^{-1})	−3.61	−3.94	−4.47	−4.31	−4.01	−3.16	−3.58	−4.22	−4.14	−3.26	−3.43	−3.09

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Table 3 Minimum inhibitory concentration of the compounds against chloroquine sensitive *P. falciparum*

Compounds	7a	7b	7c	7d	7e	7f	7g	7h	7i	7j	7k	7l	7m
MIC (μM)	0.92	0.87	0.33	0.56	2.56	0.77	3.52	1.78	0.33	0.56	0.82	0.77	0.92
Compounds	7n	7o	7p	7q	7r	7s	7t	7u	7v	7w	7x	CLQ	Qn
MIC (μM)	1.08	1.09	0.09	0.97	0.71	0.67	0.87	1.37	2.38	2.67	2.43	0.08	0.72

Table 4 Percentage survival and inhibition of the parasite in mice

Compound	Dosage (mg kg^{-1})	% Inhibition of parasitaemia Day 7	Day 12	% Survival Day 7	% Survival Day 12
7a	100	33.33	29.61	60	40
	200	30.36	44.44	60	40
7b	100	47.61	57.14	60	60
	200	57.45	63.33	60	60
7c	100	30.00	35.01	40	40
	200	50.00	43.75	40	40
7d	100	50.00	56.67	60	60
	200	46.37	57.67	60	60
7e	100	54.00	54.67	60	40
	200	43.90	60.97	60	60
7f	100	44.00	36.78	40	20
	200	50.00	43.35	40	20
7g	100	53.49	58.14	60	40
	200	57.14	74.98	80	60
7h	100	42.05	52.05	60	40
	200	58.56	65.85	60	60
7i	100	37.50	34.56	20	20
	200	31.25	37.50	40	40
7j	100	51.16	58.14	60	40
	200	57.89	65.79	60	60
7k	100	51.17	60.86	40	40
	200	53.33	63.33	60	40
7l	100	50.00	56.26	60	40
	200	46.32	57.69	40	40
7m	100	45.83	58.33	60	40
	200	54.84	64.52	60	40
7n	100	57.14	69.05	60	60
	200	57.14	74.98	80	60
7o	100	21.95	39.00	40	20
	200	48.71	56.41	40	40
7p	100	47.86	57.40	60	40
	200	63.63	60.61	80	60
7q	100	50.00	56.25	60	40
	200	47.50	55.00	40	40
7r	100	47.36	57.89	60	60
	200	51.85	74.07	80	80
7s	100	34.46	65.38	60	60
	200	53.57	64.29	60	60
7t	100	30.00	42.67	40	40
	200	52.06	60.34	60	40
7u	100	41.38	50.00	60	40
	200	37.00	55.00	40	40
7v	100	51.61	64.37	60	40
	200	61.53	69.23	80	60
7w	100	36.67	46.66	40	40
	200	50.00	60.00	60	60
7x	100	36.58	58.54	40	40
	200	48.72	56.41	60	40
Qn	100	75.60	78.00	100	100
	200	86.22	82.34	100	100
NTC	—	—	—	0	0
NIC	—	—	—	100	100

Qn = quinine; NIT = non treated control; NIC = non infected control.

to **CLQ** (MIC = 0.08 μM), the W2 strain was more susceptible to **CLQ** than all the compounds. However, seven of the compounds, **7c** (MIC = 0.33 μM), **7d** (MIC = 0.56 μM), **7i** (MIC = 0.33 μM), **7j** (0.56 μM), **7p** (0.09 μM), **7r** (0.71 μM) and **7s** (0.67 μM), showed a greater antiplasmodial effect against the W2 strain than the standard drug widely used in malaria treatment, quinine (MIC = 0.72 μM).

Given the health and economic effects of malaria and the rise in **CLQ** and **Qn** resistant strains, any molecule with antimalarial activity deserves attention. Therefore, the antiplasmodial effect of the compounds was tested at 100 and 200 mg kg^{-1} in *P. berghei* (NK-65 strain) infected mice. It was observed that all the compounds exhibited marginal to appreciable activity at the two dose levels studied. 16 out of the 24 compounds were found to clear more than 50 percent of *P. berghei* from the blood of *P. berghei* (NK-65 strain) infected mice at 12 days post-infection (Table 4). The fact that these compounds recorded activity *in vivo* confirmed our *in silico* prediction of their drug-like properties. Also, the relatively high survival rate of the experimental animals speaks about the safety profile of the new sulphonamides as earlier suggested by the biochemical data. The percentages of parasites cleared by 200 mg kg^{-1} of the three most effective compounds (**7g**, **7n** and **7r**) were 74.98, 74.98 and 74.07, respectively. Although more potent, the percentage survival of infected mice treated with **7g** and **7n** was less than that of **7r** (60 and 60 *vs.* 80 percentage survival of *P. berghei* infected mice on 12 day, respectively). Hence, 2-(4-chlorophenylsulfonamido)-*N*-(2-((4-chlorophenyl)amino)-2-oxoethyl)propanamide (**7r**) is considered a promising hit due to its low MIC values in the *in vitro* test

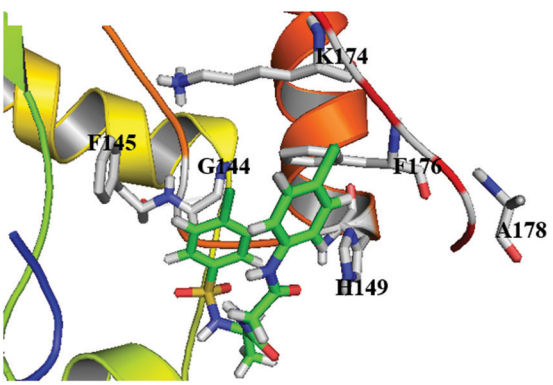

Fig. 2 Theoretical binding pose of **7r** in the *Pfdhps_model* pABA binding site. Carbons of **7r** and protein residues are respectively coloured green and grey, while oxygen, nitrogen and sulphur are coloured red, blue and yellow in both molecules.

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Table 5 Biochemical parameters

Parameters	Compound	Day 0 (pre-infection)	Day 7	Day 12
Blood glucose conc. (mg dl^{-1})	**7a**	5.60	4.90	5.30
	7b	5.50	5.00	5.70
	7c	6.00	5.30	5.60
	7d	5.60	5.40	5.50
	7e	5.90	5.00	5.70
	7f	5.80	5.30	5.20
	7g	5.50	5.00	5.50
	7h	5.20	5.40	5.00
	7k	6.10	5.70	5.90
	7l	5.60	5.40	5.40
	7o	5.90	5.30	5.80
	7p	6.00	5.80	5.40
	7q	6.00	5.50	5.60
	7s	5.90	5.90	5.60
	7t	5.80	5.40	5.70
	7w	5.90	5.30	5.60
	Control	6.30		
ALT (μ L^{-1})	**7a**	25.00	23.00	24.00
	7b	23.20	22.00	22.50
	7c	24.00	23.20	25.50
	7d	26.40	23.20	23.50
	7e	23.40	24.30	26.50
	7f	25.40	22.30	24.50
	7g	23.50	24.30	23.20
	7h	25.60	24.20	24.80
	7k	24.60	23.70	24.00
	7l	23.80	24.70	23.00
	7o	25.00	24.00	25.60
	7p	24.30	24.60	23.70
	7q	24.00	25.60	23.00
	7s	24.30	24.00	23.70
	7t	23.50	24.50	24.30
	7w	25.80	25.60	24.90
	Control	24.20		
AST (μ L^{-1})	**7a**	65.00	62.00	64.50
	7b	67.00	66.00	68.00
	7c	64.00	63.00	62.00
	7d	66.00	65.00	67.00
	7e	62.00	63.00	64.10
	7f	64.30	62.80	64.00
	7g	66.20	62.10	65.30
	7h	65.20	64.00	66.10
	7k	64.50	64.20	64.50
	7l	66.30	67.30	65.00
	7o	64.30	65.30	65.00
	7p	65.50	64.00	65.70
	7q	65.40	66.70	65.80
	7s	64.40	63.60	66.50
	7t	64.50	64.30	65.70
	7w	67.30	65.30	66.00
	Control	65.50		
TP (g dl^{-1})	**7a**	5.00	4.30	4.90
	7b	5.40	4.80	4.80
	7c	6.00	4.80	5.20
	7d	5.60	5.10	5.30
	7e	5.70	4.90	5.40
	7f	5.30	5.00	5.40
	7g	5.80	4.90	5.10
	7h	6.00	5.80	5.50
	7k	5.00	4.80	4.90
	7l	5.50	5.50	5.40
	7o	6.00	5.50	5.70
	7p	5.70	5.40	5.40
	7q	6.20	5.30	5.10
	7s	5.80	5.40	5.60
	7t	6.00	5.40	5.80
	7w	5.80	5.30	5.50
	Control	5.90		
Bilirubin (mg dl^{-1})	**7a**	0.30	0.20	0.40
	7b	0.40	0.30	0.20
	7c	0.30	0.20	0.50
	7d	0.40	0.20	0.30
	7e	0.30	0.30	0.40
	7f	0.40	0.30	0.30
	7g	0.30	0.40	0.30
	7h	0.40	0.20	0.30
	7k	0.30	0.40	0.20
	7l	0.30	0.30	0.40
	7o	0.40	0.30	0.40
	7p	0.30	0.20	0.40
	7q	0.30	0.40	0.40
	7s	0.40	0.30	0.40
	7t	0.30	0.30	0.20
	7w	0.40	0.40	0.30
	Control	0.30		
Creatinine (mg dl^{-1})	**7a**	0.40	0.40	0.30
	7b	0.30	0.40	0.30
	7c	0.30	0.50	0.30
	7d	0.30	0.40	0.40
	7e	0.30	0.40	0.30
	7f	0.30	0.40	0.30
	7g	0.40	0.40	0.30
	7h	0.30	0.40	0.30
	7k	0.30	0.30	0.30
	7l	0.40	0.50	0.30
	7o	0.30	0.50	0.40
	7p	0.40	0.50	0.40
	7q	0.40	0.50	0.50
	7s	0.30	0.50	0.40
	7t	0.30	0.30	0.40
	7w	0.40	0.50	0.50
	Control	0.30		
Albumin (g dl^{-1})	**7a**	3.70	4.10	3.50
	7b	3.80	4.00	3.50
	7c	3.90	3.90	4.00
	7d	4.00	3.90	3.90
	7e	3.50	3.50	3.00
	7f	4.00	4.00	3.60
	7g	3.90	3.80	3.50
	7h	3.80	3.90	3.60
	7k	3.70	3.60	3.80
	7l	4.00	4.10	3.60
	7o	3.90	4.00	3.90
	7p	3.80	3.90	3.70
	7q	3.80	3.90	3.60
	7s	4.00	4.30	4.10
	7t	4.00	4.00	3.90
	7w	3.90	4.10	4.00
	Control	3.90		
RBC (mm^3) × 10^6	**7a**	6.90	5.50	6.20
	7b	7.00	6.00	6.80
	7c	7.30	6.10	6.50
	7d	6.80	5.70	6.00
	7e	7.10	5.90	6.70
	7f	7.00	6.00	6.80
	7g	6.80	5.80	6.50
	7h	7.00	5.70	6.00
	7k	7.50	6.10	6.80
	7l	7.00	5.80	6.30
	7o	6.90	5.50	6.00
	7p	7.40	6.40	6.00
	7q	7.20	6.60	6.00
	7s	7.00	6.10	6.70
	7t	6.90	5.50	6.00
	7w	7.10	6.80	6.80
	Control	7.00		
PCV (%)	**7a**	37	33	35
	7b	38	35	35
	7c	36	32	34
	7d	38	34	36

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Table 5 (*continued*)

Parameters	Compound	Day 0 (pre-infection)	Day 7	Day 12
	7e	36	30	34
	7f	37	33	35
	7g	38	34	36
	7h	36	33	34
	7k	36	35	35
	7l	37	34	34
	7o	39	34	36
	7p	38	35	37
	7q	39	33	37
	7s	36	34	34
	7t	37	35	36
	7w	38	35	34
	Control	38		
WBC (mm^3) $\times$ 10^3	**7a**	12.00	9.00	10.00
	7b	11.50	9.53	10.20
	7c	9.78	9.00	9.05
	7d	12.45	10.2	11.03
	7e	11.00	9.74	10.02
	7f	10.66	9.23	9.93
	7g	12.10	9.84	10.52
	7h	11.55	9.67	10.44
	7k	12.23	11.00	11.77
	7l	11.00	9.80	10.24
	7o	11.45	9.55	10.32
	7p	12.23	11.00	11.77
	7q	13.00	11.24	11.67
	7s	12.01	9.56	10.78
	7t	11.46	10.89	11.10
	7w	12.33	10.22	11.00
	Control	12.22		
HB (g dl^{-1})	**7a**	15.20	10.20	11.00
	7b	12.50	9.70	10.50
	7c	13.50	11.30	11.80
	7d	12.80	10.56	11.90
	7e	13.50	11.10	12.70
	7f	12.40	10.60	11.30
	7g	11.90	9.00	11.00
	7h	12.30	11.10	11.90
	7k	11.56	10.68	11.00
	7l	12.76	11.21	10.60
	7o	13.56	10.88	11.09
	7p	11.44	10.01	10.67
	7q	13.70	12.02	12.90
	7s	12.34	11.85	11.80
	7t	11.57	11.20	12.00
	7w	12.89	10.56	10.45
	Control	12.5		

(0.71 μM), reasonable *in vivo* potency at 200 mg kg^{-1} (74.07%) and interesting pharmacokinetic profile (M_W = 430.31 Da, HBA = 7, HBD = 3, log*P* = 2.56, NRB = 9 and TPSA = 104.37 $Å^2$). The predicted binding mode for **7r** toward the *Pfdhps_model* PABA binding site described in Fig. 2 revealed interesting interactions which could be targeted in chemical modifications during structure–activity optimization. The fact that the levels of the enzymes were maintained in the liver and kidneys in all groups of mice means that the administered compounds have no membrane labializing effect on these organs. Enzyme activities in tissues are often used as a 'marker' to ascertain early toxic effects of administered foreign compounds on experimental animals.[61,62] ALP is a membrane bound enzyme while ALT and AST are cytosolic enzymes. These enzymes are highly concentrated in the liver and kidneys and are only found in the serum in significant quantities when the cell membrane becomes leaky and even completely ruptured.[63] A rise in the serum level or decrease in the tissue level of these intracellular enzymes is an index of damage to liver and kidney cells (Tables 5 and 6).[64]

Table 6 Rat biochemical reference ranges

Glucose 50–135 mg dl^{-1}	Calcium 5.3–13 mg dl^{-1}
Albumin 3.8–4.8 g dl^{-1}	Alt 17.5–30.2 μl^{-1}
Bun 15–21 mg dl^{-1}	Ast 45.7–80.8 μl^{-1}
Total protein 5.6–7.6 g dl^{-1}	Alp 56.8–128 μl^{-1}
Creatinine 0.2–0.8 mg dl^{-1}	Cholesterol 40–130 mg dl^{-1}
Sodium 143–156 mg l^{-1}	Total bilirubin 0.2–0.55 mg dl^{-1}
Chloride mg l^{-1}	Amylase 128–313 mg dl^{-1}
Phosphorous 3.11–11 mg dl^{-1}	

Conclusion

In conclusion, twenty-four new boc-glycine-amine-derived sulfonamides (**7a–x**) were synthesized and characterized in this study. Analysis of biochemical parameters and computed molecular descriptors used to evaluate drug-likeness revealed that the compounds are both safe and drug-like. Molecular docking suggested that the compounds had a favourable interaction with a *P. falciparum* dihydropteroate synthase homology model and therefore could be a potential inhibitor of the protein. Finally, *in vitro* screening against *P. falciparum* (W2 strain) and *in vivo* testing against *P. berghei* (NK-65 strain) infected mice revealed their antiplasmodial activity. The promising candidate identified in this study could be a template for the development of boc-glycine-amine-derived sulfonamide antiplasmodial agents.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All dataset are contained in the manuscript.

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Authors contributions

Conceptualization – Prof. U. C. Okoro, Ugwuja D. I. Data curation – Ugwuja D. I., Rina Soni. Formal analysis – Akachukwu Ibezim. Funding acquisition – Prof. Shubhangi S. Soman. Investigation – Ugwuja D. I., Roni Soni, Ugwu D. I. Methodology – Prof. U. C. Okoro, Prof. Shubhanji Soman, Ugwuja D. I. Project

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administration – Ugwuja Daniel. Resources – Ezugwu James, Ogechi Ekoh, Bonaventure Obi. Software – Akachukwu Ibezim. Supervision – Prof. U. C. Okoro, Prof. Shubhanji Soman. Validation – Ugwuja Daniel Izuchukwu. Visualization – Ugwuja Daniel Izuchukwu. Writing – original draft – Ugwuja Daniel Izuchukwu. Writing – review & editing – Ugwuja Daniel Izuchukwu.

Conflicts of interest

The authors declare that they have no competing interests.

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