New Glycine derived peptides bearing benzenesulphonamide as antiplasmodial agent

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Abstract

Background : In the tropics, malaria is among the most serious infections disease in developing countries. The discovery of artemisinin antimalarial drug not too long ago was a major breakthrough in effort to combat malaria disease. However, recent reports of resistance even to combination therapy involving artemisinin are very worrisome and have led to search for new chemical agents to sustain the fight against malaria. Carboxamide functionality has been shown as an important pharmacophore in over 25 % of commercial chemotherapeutic agents. **Method :** Three benzensulphonamides (3a-c) were prepared from the reaction of 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), 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. The compounds were characterized and evaluated for their antiplasmodial activity **Results :** Computed molecular descriptors and assessed biochemical parameters showed that the compounds were drug-like and safe. All the compounds had favourable binding interaction with residues at PABA binding site of homology modeled *P. falciparum* dihydropteroate synthase and henceforward in vitro and in vivo antiplasmodial activities were evaluated. Compounds 7a-7x showed activity against the *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 post-infection day. The percentages of parasites cleared by 20 mg/kg of the three most effective compounds (7g , 7n and 7r) were 74.98, 74.98 and 74.07 respectively **Conclusion :** In coclusion, this class of glycine derived sulfonamides, especially 7r (MIC 0.71 μM), ability to clear 74.07 % of *P. berghei* from blood of infected mice at 20 mg/kg and interesting pharmacokinetic profile (MW = 430.31 Da, HBA = 7, HBD = 3, log P = 2.56, NRB = 9 and TPSA = 104.37 $\text{\AA}^2$), could serve as leads in developing new antiplasmodial agent.

Introduction

Malaria is an infectious disease caused by protozoa 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 insecticides⁵⁻⁶ 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 parasites¹¹⁻¹⁴ evidencing the urgent need for new drugs

The prevalence of carboxamide in biological systems, pharmacologically active molecules and favourable properties of amides makes it one of the most popular and reliable functional group in organic chemistry.¹⁵ Carboxamide functionality has been shown to appear in over 25% of commercial chemotherapeutic agents.¹⁶ This finding makes the amide bond a sort 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 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 agent. 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).¹⁸⁻¹⁹

Antifolate action of sulpha drugs

Several literatures have described other sulphonamides with antimalarial activity.²⁰⁻²⁵ 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 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 liver²⁶. The human body needs folate to synthesize DNA, repair DNA, methylate DNA as well as act as 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 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 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 replication²⁷⁻³¹

Some studies have linked point mutations in *Plasmodium falciparum* to sulfa drug resistance.^{32,33}

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, increasing similarity in results of computational and experimental methods, further presents the former as a good alternative to the later method. Although no drug molecule has moved from computer to market, the method has been employed at one point in the development of much food and drug agency (FDA) approved drugs. Therefore, both methods work in tandem during the development of new drugs.³⁴⁻³⁵

We report herein the successful synthesis of twenty four new glycine derived sulfonamides that possessed fascinating antiplasmodial property that can become drug-lead in sustaining the fight against malaria. This

work was designed based on the reported antimalarial properties of short peptide and benzenesulphonamides and the need to develop newer chemotherapeutic agents that will overcome the reported emerging resistance against artemisinin based therapy. 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 Plasmodium falciparum dihydroptarate synthase para aminobenzoic acid (PABA) binding site was carried out and finally, in-vitro and in-vivo antiplasmodial activity of the compounds were 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 Perkin-Elmer RX 1 spectrometer and the wave numbers are reported in cm^{-1} . ^1H NMR and ^{13}C NMR spectral data were recorded on Advance Bruker 400 spectrometer (^1H 400 MHz/ ^{13}C 100 MHz) with DMSO- d_6 as solvent and tetramethylsilane (TMS) as internal standard and reported in δ (ppm). J values are in hertz (Hz). The molecular mass of the compounds were obtained using high resolution positive ion electrospray ionization on Brucker 10152 mass spectrometer pos_tune_low_msq.m.

General procedure for the synthesis of substituted benzene sulphonamoyl alkanamides (3a-c) ³⁶

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 crystal was filtered via suction and washed with 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 ³⁷

A mixture of Boc-glycine (4, 0.45 g, 1.84 mmol), 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide hydrochloride (EDC, 0.53 g, 2.76 mmol), 1-hydroxybenzotriazole (HOBt, 0.248, 1.84 mmol), triethylamine (TEA), 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), 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) was used directly for the next stage of reaction.

Synthesis of glycine derived peptides 7a-7x

A mixture of 2-[phenylsulfonyl]amido]propanoic acid (3a-c, 1.84 mmol), EDC (0.53 g, 2.76 mmol), HOBT (0.248, 1.84 mmol), TEA, 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, 14267, (C = C), 1349, 1309 (2SO₂), 1169 (SO₂NH), 781 (C-Cl). ¹H NMR (DMSO-d₆, 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). ¹³C NMR (DMSO-d₆, 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 C₁₇H₁₈ClN₄O₆S, found value is (m/z): 441.0637 (M + H), 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 (2SO₂), 1170 (SO₂NH), 741 (C-Cl). ¹H NMR(DMSO-d₆, 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). ¹³C NMR (DMSO-d₆, 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 C₁₇H₁₈ClN₄O₆S, 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 (SO₂), 1169 (SO₂NH), 782 (C-H). ¹H NMR(DMSO-d₆, 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). ¹³C NMR (DMSO-d₆, 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 C₁₇H₁₈FN₄O₆S, 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-FI). ^1H NMR (DMSO- d_6 , 400 MHz) δ : 1.10 (s, 1H), 3.70 (s, 2H), 3.96 (s, 3H), 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 $\text{C}_{17}\text{H}_{17}\text{FN}_4\text{O}_6$, 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). ^1H 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 $\text{C}_{18}\text{H}_{20}\text{N}_4\text{O}_6\text{S}$ 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). ^1H NMR (DMSO- d_6 , 400 MHz) δ : 1.13–1.16 (m, 2H), 1.61–1.63 (d, J = 8 Hz, 3H), 1.98 (s, 3H), 2.49–2.50 (m, 2H), 3.74–3.75 (d, J = 4 Hz, 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 $\text{C}_{17}\text{H}_{18}\text{N}_4\text{O}_6\text{S}$ found value is (m/z): 406.0949, M^+ , calculated value is 406.0947.

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 $\text{C}_{21}\text{H}_{20}\text{N}_4\text{O}_6\text{S}$ found value is (m/z): 456.1108, M^+ , calculated value is 456.1104.

N-(2-morpholino-2-oxoethyl)-2-(4-nitrophenylsulfonamido)propanamide (7 h)

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 $\text{C}_{15}\text{H}_{20}\text{N}_4\text{O}_7\text{S}$ found value is (m/z): 400.1057, M^+ , calculated value is 400.1053.

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 $\text{C}_{19}\text{H}_{21}\text{ClN}_4\text{O}_5\text{S}$ 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 $\text{C}_{19}\text{H}_{21}\text{ClN}_4\text{O}_5\text{S}$ 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 (7 k)

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 $\text{C}_{19}\text{H}_{21}\text{FN}_4\text{O}_5\text{S}$ 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 (7 l)

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-d₆ 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). ¹³C NMR (DMSO-d₆, 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 C₁₉H₂₁ClN₄O₅S 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 (7 m)

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 (SO₂), 1165 (SO₂NH). ¹H NMR (DMSO-d₆, 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). ¹³C NMR (DMSO-d₆ 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 C₂₀H₂₄N₄O₅S 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 (SO₂), 1166 (SO₂NH). ¹H NMR (DMSO-d₆, 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).¹³C NMR (DMSO-d₆, 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 C₁₉H₂₂N₄O₅S found value is (m/z): 419.1391, M + H, calculated value is 419.1389.

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 (SO₂), 1162 (SO₂NH). ¹H NMR (DMSO-d₆, 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 (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).¹³CNMR (DMSO-d₆ 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 C₂₃H₂₄N₄O₅S 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 $\text{C}_{17}\text{H}_{24}\text{N}_4\text{O}_6\text{S}$ 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) ^1H 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 $\text{C}_{17}\text{H}_{17}\text{Cl}_2\text{N}_4\text{O}_6\text{S}$ found value is (m/z): 430.0398, $\text{M} + \text{H}$, calculated value is 430.0395.

2-(4-chlorophenylsulfonamido)-N-(2-((4-chlorophenyl)amino)-2-oxoethyl)propanamide (7r)

Yield 84% mp 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-Cl). ^1H 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 $\text{C}_{17}\text{H}_{17}\text{Cl}_2\text{N}_4\text{O}_6\text{S}$ found value is (m/z): 430.0396, $\text{M} + \text{H}$, calculated value is 429.0395.

2-(4-chlorophenylsulfonamido)-N-(2-((3-fluorophenyl)amino)-2-oxoethyl)propanamide (7 s)

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). ^1H 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 $\text{C}_{17}\text{H}_{17}\text{FN}_4\text{O}_6\text{S}$ 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). ^1H 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). ¹³C NMR (DMSO-d₆, 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 C₁₇H₁₇FN₄O₆S 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 (SO₂), 1168 (SO₂NH), 757 (C = Cl). ¹H NMR (DMSO-d₆, 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) ¹³C NMR (DMSO-d₆, 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 C₁₈H₂₀ClN₃O₄S found value is (m/z): 427.1075, M + NH₄, calculated value is 427.1078.

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 (SO₂), 1165 (SO₂NH), 754 (C = Cl). ¹H NMR (DMSO-d₆, 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). ¹³C NMR (DMSO-d₆, 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 C₁₇H₁₈ClN₃O₄S 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. ¹H NMR (DMSO-d₆, 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). ¹³C NMR (DMSO-d₆, 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 C₂₁H₂₀ClN₃O₄S 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 (SO₂), 1159 (SO₂NH). ¹H NMR (DMSO-d₆, 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, 3H), 7.99 (s, 1H), 8.11 (s, 2H), 8.22–8.24 (d, J = 8 Hz, 2H). ¹³C NMR (DMSO-d₆, 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 C₁₅H₂₀ClN₃O₅S found value is (m/z): 388.0736, M-H, calculated value is 388.0734.

Molecular Modeling

Homology modeling of target protein and Docking

Since there is no three-dimensional (3-D) structure of Plasmodium falciparum dihydropteroate synthase (Pfdhps) in Protein Databank, the crystal structure of Pfdhps was homology modeled using the Pfdhps sequence fragment retrieved from UniprotKB database as the target sequence (A0A3G2LE00_PLAFA).³⁸ SWISS-MODEL³⁹ use their own set of modeling algorithm to automatically build model using P. vivax homodimer (PDB code: 5Z79; resolution 1.7 Å)⁴⁰ as template. The constructed model was subjected to molecular dynamics simulation using GROMOS96 force field.⁴¹ The system was placed in a cubic box simple point charge (SPC) and solvated using an explicit 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 Psi/Phi Ramachandran plot package in Discovery Studio,⁴² stability of the protein structure of MD simulation and alignment to 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.⁴³

The chemical structures of the compounds were prepared using the builder protocol in Molecular Operating Environment (MOE) software⁴⁴ and energy minimized to 0.001 kcal/mol gradient. Molecular descriptors employed in calculating the following basic physicochemical features: molecular weight (MW), hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), lipophilicity (logP), number of rotatable bond (NRB) and total polar surface area (TPSA) were computed using the QuSAR module of MOE. Docking of the compounds toward Pfdhps_model binding site was performed using "triangle" matcher as 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 P. falciparum (W2 strain). Briefly, sorbitol synchronized, 0.1% parasitemia, ring stage P. falciparum strain W2 parasites were cultured under the atmosphere of 3% O₂, 6% CO₂ and 91% N₂ 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 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 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)⁴⁵ [33].

In vivo antiplasmodial activity

In vivo antimalarial activity of the synthetic compounds (7a-7x) was carried out against *P. berghei* (NK-65 strain) infected mice as described by Peter et al⁴⁶ and Kalra et al⁴⁷ 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%. By 7 days post-infection, animals were administered with the synthesized compounds (7a-7x) for 12 consecutive days and were monitored with constant check of the percentage of parasitemia after one-day interval. 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 microscope with an oil immersion objective of 100x magnification power and percentage inhibition of parasitemia was calculated comparing the treated group with untreated group by means of the following formula⁴⁸ $[(A-B)/A] \times 100$; 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 reduce parasitaemia by 30–40% or less than 30% were deemed partially active and inactive respectively.⁴⁹ 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; NHREC/05/01/2008B. The study protocol was approved by our institution's Ethics Committee.⁵⁰

Biochemical Assessment

By twelfth day post-infection, 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 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 aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). Standard laboratory procedure according to was used for the determination of parameters⁵¹

Renal or Kidney Function Test

Kidney function tests carried out with the blood of the rats fed with the sulphonamide derivatives were, creatinine and albumin. The method reported by⁵² were 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 contaminations and intestinal sickness. Instances of medication obstruction against current antimalarial sulfonamides are on the expansion. Thus, there is 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 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-hydroxybenzotriazole (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. Compound 7 was converted into their salt by addition of 10% trifluoroacetic acid (TFA) in DCM. The salt on further reaction with acid in the presence of peptide coupling agents EDCI, HOBt, TEA or DMAP, gave the desired diamide derivative of the glycine. The synthesized compounds were crystallized in their analytical grade using n-hexane while FTIR, ^1H NMR, ^{13}C NMR and high resolution mass spectroscopy (HRMS) were used to characterize their chemical structures. The IR showed 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 group which indicate the successful formation of the compounds. ^1H NMR of compounds showed the methylene group of glycine exhibiting multiplet at δ 3.5-4.00 due to the interaction CH_2 and NH

of the glycine. There is a doublet at 1.0-1.2 due to the CH and CH₃ interaction of the alanine amide. The compound also exhibited multiplet from 7.1–7.9 representing aromatic protons. ¹³CNMR showed strong peak between 168.16 and 172 which indicate 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 carbon.

Scheme 1.

(i) Na₂CO₃, DCM/H₂O, HCl, 0OC, 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

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

compd	R	R ¹	R ²	MW	HBA	HBD	logP	NRB	TPSA
7a	NO ₂	H	3-ClC ₆ H ₄	440.86	9	4	1.29	11	133.47
7b	NO ₂	H	4-ClC ₆ H ₄	440.86	9	4	1.25	11	133.47
7c	NO ₂	H	3-FC ₆ H ₄	420.40	9	4	0.85	11	133.47
7d	NO ₂	H	4-FC ₆ H ₄	420.40	9	4	0.82	11	133.47
7e	NO ₂	H	4-CH ₃ C ₆ H ₄	420.44	9	4	0.96	11	133.47
7f	NO ₂	H	C ₆ H 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 ₄ H ₉ O	400.10	10	3	-2.02	10	133.91
7i	NHCOCH ₃	H	3-ClC ₆ H ₄	452.91	10	3	1.94	10	150.19
7j	NHCOCH ₃	H	4-ClC ₆ H ₄	452.91	10	3	1.10	10	150.19
7k	NHCOCH ₃	H	3-FC ₆ H ₄	436.46	10	3	1.50	10	150.19
7l	NHCOCH ₃	H	4-FC ₆ H ₄	436.46	10	3	1.46	10	150.19
7m	NHCOCH ₃	H	4-CH ₃ C ₆ H ₄	418.10	10	3	1.60	10	150.19
7n	NHCOCH ₃	H	C ₆ H 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 ₄ H ₉ O	412.10	11	2	-1.38	9	150.63
7q	Cl	H	3-ClC ₆ H ₄	429.10	7	3	2.59	9	104.37
7r	Cl	H	4-ClC ₆ H ₄	430.31	7	3	2.56	9	104.37
7s	Cl	H	3-FC ₆ H ₄	431.10	7	3	2.15	9	104.37
7t	Cl	H	4-FC ₆ H ₄	413.86	7	3	2.12	9	104.37
7u	Cl	H	3-CH ₃ C ₆ H ₄	409.89	7	3	2.26	9	104.37
7v	Cl	H	C ₆ H 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 ₄ H ₉ O	380.10	8	2	-0.72	8	104.81

The parameters are in their standard units: MW in Dalton, and TPSA in Å²

Oral bioavailability and safety

Failures of many drug candidates with excellent pharmacological features due to poor ADME/Tox properties have necessitate evaluation of physicochemical features of potential drug molecules at early stage of drug discovery.⁵³ As a rule of thumb, it has been found that molecules with the following criteria MW < 500 Da, HBA < 10, HBD < 5, logP < 10, NRB < 5 and TPSA < 140 Å², are orally bioavailable.⁵⁴ The MW, HBD and logP of the compounds are respectively in ranges of 380.85 – 468.53 Da, 2 – 4 and -2.02 to 3.22 values. Only **7p** had number of HBA above ten (HBA = 11). Seven different compounds possess NRB and TPSA values above the recommended ranges (Table 1). In spite of some outliers in NRB and TPSA values, analysis of the computed molecular descriptors suggests **7a-7x** are drug-like, hence, good candidate for biological screening. Furthermore, to ascertain the safety profile of some of the compounds, their effect on blood glucose, kidney function, haematology, 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 sulphonamide pharmacophore are known to exhibit antimalarial activity through inhibition of PfCA and/or dhps enzymes.⁵⁵⁻⁵⁷ Since we could not obtain PfCa template with acceptable Qmean score, only the homology model of *Pfdhps* was used. 222 amino acids sequence of *Pfdhps* was used to build 3-dimensional structure of *Pfdhps* homology model (*Pfdhps_model*) in order to investigate the affinity of the newly synthesized compounds for *Pfdhps*. The *Pfdhps_model* (Figure 1a) has sequence similarity identity 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 Qmean score, Ramachandran plot and alignment of C α . It was respectively observed that the modeled protein lies in the Z-score of other protein crystal structures that exist in PDB (Figure 1b), contains above 95.0 % of *Pfdhps_model* residues in the favored region (Figure 1c) and closely aligned with *P. vivax*_dhps C α 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 RMSD which has maximum value of 0.426 Å (Figure 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 -15125 kcal/mol to -18154 kcal/mol indicating stabilization as the simulation progressed (Figure 1e). Having ascertained *Pfdhps_model* is of good quality, the COACH online program, which uses their in-house algorithm to predict protein binding sites, was employed. *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 occupy 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 COACH derived *Pfdhps*-pABA complex. The docking protocol (A grid box size of 4, 3.5, 2 Å³ points and centered on the mass center on 63.3, 47.5, 42.3) which identified rmsd between COACH-based and docked pose of pABA lower than 2.0 Å (Figure 1f) was retained and used for docking studies. The best docked poses characterized by the lowest theoretical binding energy revealed that **7a-7x** take place preferentially within the *Pfdhps_model* pABA binding site. Narrow range of binding affinity was observed (-2.80 to -4.68 kcal/mol) (Table 2) which eluded any observable significant structure-activity relationship within the studied series. However, the negative free binding energies of each of the **7a** to **7x** suggested they favourably interacted with the protein and could serve as inhibitor and subsequently antimalarial agent. Therefore, their ability to kill *P. falciparum* in culture and clear *P. berghei* in infected mice were investigated.

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

Compds	7a	7b	7c	7d	7e	7f	7g	7h	7i	7j	7k	7l
ΔG (kcal/mol)	-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)	-3.61	-3.94	-4.47	-4.31	-4.01	-3.16	-3.58	-4.22	-4.14	-3.26	-3.43	-3.09

Antiplasmodial screening

The antiplasmodial activity of the 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 action of compounds as possible drug leads.⁵⁸ 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 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 more 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 effect of malaria and the rise in **CLQ** and **Qn** resistance strains, any molecule with antimalarial activity deserves attention. Therefore, the antiplasmodial effect of the compounds was tested at 100 and 200 mg/kg in *P. berghei* (NK-65 strain) infected mice. It was observed that all the compounds exhibited marginal to appreciable activity at the two dose level 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 post-infection days (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 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** were lesser 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 promising hit due to its low MIC values at *in vitro* test (0.71 μM), reasonable *in vivo* potency at 200 mg/kg (74.07 %) and interesting pharmacokinetic profile (MW = 430.31 Da, HBA = 7, HBD = 3, log P = 2.56, NRB = 9 and TPSA = 104.37 $\text{\AA}^2$). Predicted binding mode for **7r** toward the *Pfdhps_model* PABA binding site described in Figure 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 kidney in all groups of the mice means that the administered compounds have no membrane labializing effect on these organs. Enzyme activities in the tissues are often used as 'marker' to ascertain early toxic effects of administered foreign compounds

to experimental animals⁵⁹⁻⁶⁰ ALP is a membrane bound enzyme while ALT and AST are cytosolic enzymes. These enzymes are highly concentrated in the liver and kidney and are only found in the serum in significant quantities when the cell membrane becomes leaky and even completely ruptured⁶¹. A rise in serum level or decrease in tissue level of these intracellular enzymes is an index of damage to liver and kidney cells⁶².

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 parasite in mice

Compound	Dosage (mg/kg)	% Inhibition of parasitaemia		% survival	% survival	
		Day 7	Day 12	Day 7	Day 12	
	100	33.33	29.61	60	40	
	200	30.36	44.44	60	40	
	100	47.61	57.14	60	60	
	200	57.45	63.33	60	60	
	100	30.00	35.01	40	40	
	200	50.00	43.75	40	40	
	100	50.00	56.67	60	60	
	200	46.37	57.67	60	60	
	100	54.00	54.67	60	40	
	200	43.90	60.97	60	60	
	100	44.00	36.78	40	20	
	200	50.00	43.35	40	20	
	100	53.49	58.14	60	40	
	200	57.14	74.98	80	60	
	100	42.05	52.05	60	40	
	200	58.56	65.85	60	60	
	40	100	37.50	34.56	20	20
		200	31.25	37.50	40	
		100	51.16	58.14	60	40
		200	57.89	65.79	60	60
100		51.17	60.86	40	40	
200		53.33	63.33	60	40	
100		50.00	56.26	60	40	
200		46.32	57.69	40	40	

100	45.83	58.33	60	40
200	54.84	64.52	60	40
100	57.14	69.05	60	60
200	57.14	74.98	80	60
100	21.95	39.00	40	20
200	48.71	56.41	40	40
100	47.86	57.40	60	40
200	63.63	60.61	80	60
100	50.00	56.25	60	
200	47.50	55.00	40	40
100	47.36	57.89	60	60
200	51.85	74.07	80	80
100	34.46	65.38	60	60
200	53.57	64.29	60	60
100	30.00	42.67	40	40
200	52.06	60.34	60	40
100	41.38	50.00	60	40
200	37.00	55.00	40	40
100	51.61	64.37	60	40
200	61.53	69.23	80	60
100	36.67	46.66	40	40
200	50.00	60.00	60	60
100	36.58	58.54	40	40
200	48.72	56.41	60	40
100	75.60	78.00	100	100
200	86.22	82.34	100	100
Q	-	-	0	0
;	-	-	100	100

Qn = Quninin; NIT= Non treated control; NIC= Non infected control

Table 5. Biochemical parameters

Parameters	Compound	Day 0 (pre-infection)	Day 7	Day 12
Blood glucose conc. (mg/dl)	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)	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)	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)	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)	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)	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)	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)X10 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
	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)X10 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)	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			

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 the compounds are both safe and drug-like. Molecular docking suggested the compounds had favourable interaction with *P. falciparum* dihydropteroate synthase homology model and therefore could be a potential inhibitor of the protein. Finally, in vitro screening against the *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 sulfonamides antiplasmodial agent.

Declarations

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References

1. World Health Organization (2011) World malaria report 2011. World Health Organization, Genova
2. Aguiar ACC, Rocha EMM, Souza NBD, Franc, a TCC, Krettli AU. New approaches in antimalarial drug discovery and development: a review. *Mem Inst Oswaldo Cruz*. 2012; 107:831–845.

3. Flannery EL, Chatterjee AK, Winzeler EA. Antimalarial drug discovery [mdash] approaches and progress towards new medicines. *Nat Rev Microbiol.* 2013; 11:849–862.
4. Jensen K, Plichta D, Panagiotou G, Kouskoumvekaki I. Mapping the genome of *Plasmodium falciparum* on the drug-like chemical space reveals novel anti-malarial targets and potential drug leads. *Mol BioSyst.* 2012; 8:1678–1685.
5. Eisele TP, Miller JM, Moonga HB, Hamainza B, Hutchinson P, Keating J. Malaria infection and anemia prevalence in Zambia's Luangwa District: an area of near-universal insecticide-treated mosquito net coverage. *Am J Trop Med Hyg.* 2011; 84:152–157.
6. Metropolis H, State R, Harcourt P. Effectiveness of insecticide-treated mosquito nets (Itns) in the control of malaria disease among slum dwellers in port. *Res Hum Soc Sci.* 2014; 4:79–8.
7. Desgrouas C, Dormoi J, Chapus C, Ollivier E, Parzy D, Taudon N. In vitro and in vivo combination of cepharanthine with anti-malarial drugs. *Malar J.* 2014; 13:90–95.
8. Guiguemde WA, Hunt NH, Guo J, Marciano A, Haynes RK, Clark J, Guy RK, Golenser J. Treatment of murine cerebral malaria by artemisone in combination with conventional antimalarial drugs: antiplasmodial effects and immune responses. *Antimicrob Agents Chemother.* 2014; 58:4745–4754.
9. Mutabingwa TK. Artemisinin-based combination therapies (ACTs): best hope for malaria treatment but inaccessible to the needy! *Acta Trop.* 2005; 95:305–315.
10. Santelli AC, Ribeiro I, Daher A, Boulos M, Marchesini PB, dos Santos RLC, Lucena MBF, Magalhães I, Leon AP, Junger W, Ladislau JLB. Effect of artesunate-mefloquine fixed-dose combination in malaria transmission in Amazon basin communities. *Malar J.* 2012; 11:286–297.
11. Ku'mpornsin K, Modchang C, Heinberg A, Ekland EH, Jirawatcharadech P, Chobson P, Suwanakitti N, Chaotheing S, Wilairat P, Deitsch KW, Kamchonwongpaisan S, Fidock DA, Kirkman LA, Yuthavong Y, Chookajorn T. Origin of robustness in generating drug-resistant malaria parasites. *Mol Biol Evol.* 2014; 31:1649–1660.
12. Mita T, Ohashi J, Venkatesan M, Marma ASP, Nakamura M, Plowe CV, Tanabe K. Ordered accumulation of mutations conferring resistance to sulfadoxine-pyrimethamine in the *Plasmodium falciparum* parasite. *J Infect Dis.* 2014; 209:130–139.
13. Perakslis ED. Cybersecurity in health care. *N Engl J Med.* 2014; 371:395–397.
14. Winzeler EA, Manary MJ. Drug resistance genomics of the antimalarial drug artemisinin. *Genome Biol.* 2014; 15:544–555.
15. Colombo M, Bossolo S, Aramini A. Phosphorus trichloride-mediated and microwave-associated synthesis of small collection of amides bearing strong electron-withdrawing group substituted anilines. *J Comb Chem.* 2009; 11(3):335e337.
16. Montalbetti CAGN, Falque V. Amide bond formation and peptide coupling. *Tetrahedron.* 2005; 61:10827e.
17. Day T, Greenfield SA. Bioactivity of a peptide derived from acetylcholinesterase in hippocampal organotypic culture. *Exp Brain Res.* 2004; 155:500-508.

18. Krungkrai J, Krungkrai SR, Supuran CT. Carbonic anhydrase inhibitors: Inhibition of *Plasmodium falciparum* carbonic anhydrase with aromatic/heterocyclic sulfonamides—*In vitro* and *in vivo* *Bioorg Med Chem Lett*. 2008; 18:5466-5471.
19. Krungkrai J, Scozzafava A, Reungprapavut S, Krungkrai SR, Rattanajak, R, Kamchonwongpaisan S, Supuran CT. Carbonic anhydrase inhibitors. Inhibition of *Plasmodium falciparum* carbonic anhydrase with aromatic sulfonamides: Towards antimalarials with a novel mechanism of action? *Bioorg Med Chem*. 2005; 13: 483-489.
20. Johann L, Pegraro S, Dormeyer M, Michael L, Aschenbrenner A, Karmer B. Sulfonylphenyl-ureido benzamidines: A novel structural class of potent antimalarial agents. *Bioorg Med Chem Lett*. 2004; 14:1979-1982.
21. Ugwu DI, Okoro UC, Ukoha PO, Okafor S, Ibezim A, Kumar NM. Synthesis, characterization, molecular docking and in vitro antimalarial properties of new carboxamides bearing sulphonamide *Eur J Med Chem*. 2017; 135:349-369.
22. Ryckebusch A, Deprez-Poulain R, Debreu-Fontaine MA, Vandaele R, Mouray E, Grellier P, Sergheraert C. Parallel synthesis and anti-malarial activity of a sulfonamide library. *Bioorg Med Chem Lett*. 2002; 12:2595-2598.
23. Posner GH, Maxwell JP, O'Dowd H, Krasavin M, Xie S, Shapiro TA. Antimalarialsulfide, sulfone, and sulfonamide trioxanes. *Bioorg Med Chem*. 2000; 8:1361-1370.
24. Parai MK, Panda G, Srivastava K, Puri SK. Design, synthesis and antimalarial activity of benzene and isoquinoline sulfonamide derivatives. *Bioorg Med Chem Lett*. 2008; 18:776-781.
25. Martyn DC, Cortese JF, Tyndall E, Dick J, Mazitschek R, Munoz B, Clardy J. Antiplasmodial activity of piperazine sulfonamides. *Bioorg Med Chem Lett*. 2010; 20:218-221.
26. Supuran, C.T., Scozzafava, H. and Casini, A. (2003). Carbonic anhydrase inhibitors, *Medicinal Research Review*, 23 (2), 146-189.
27. Schellenberg KA and Coatney GR (1961) The influence of antimalarial drugs on nucleic acid synthesis in *Plasmodium gallinaceum* and *Plasmodium berghei*. *Biochem*
28. *Pharmacol* 6:143–152.
29. Gutteridge WE and Trigg PI (1971) Action of pyrimethamine and related drugs against *Plasmodium knowlesi* in vitro. *Parasitology* 62:431–444.
30. Newbold CI, Boyle DB, Smith CC, and Brown KN (1982) Stage specific protein and nucleic acid synthesis during the asexual cycle of the rodent malaria *Plasmodium chabaudi*. *Mol Biochem Parasitol* 5:33–44.
31. Gritzmacher CA and Reese RT (1984) Protein and nucleic acid synthesis during synchronized growth of *Plasmodium falciparum*. *J Bacteriol* 160:1165–1167.
32. Triglia T and Cowman AF (1999) The mechanism of resistance to sulfa drugs in *Plasmodium falciparum*. *Drug Resist Updat* 2:15–19
33. Ekoue-Kovi K, Yearick K, Iwaniuk DP, Natarajan JK, Alumasa J, Dios AC, Roepe PD, Wolf C. Synthesis and antimalarial activity of new 4-amino-7- chloroquinolylamides, sulfonamides, ureas and thioureas. *Bioorg Med Chem*. 2009; 17:270-283.

34. Wang P, Read M, Sims PF, Hyde JE. Sulfadoxine resistance in the human malaria parasite *Plasmodium falciparum* is determined by mutations in dihydropteroate synthase and an additional factor associated with folate utilization. *Mol Microbiol.* 1997; 23, 979-986
35. Pornthanakasem W, Riengrunroj P, Chitnumsub P, Ittarat W, Kongkasuriyachai D, Uthapibull C, Yuthavong Y, Leartsakulpanich U. Role of *Plasmodium vivax* dihydropteroate synthase polymorphisms in sulfa drug resistance. *Agent Chemother.* 2016;60: 4453-4463.
36. Ibezim A, Olubiyi OO, Ata K, Mbah CJ, Nwodo NJ. Structure-based study of natural products with anti-Schistosoma activity. *Curr Comp Aided Drug Des.* 2017; 13:91-100.
37. Ugwu DI, Okoro UC, Mishra NK, Synthesis, characterization and anthelmintic activity evaluation of pyrimidine derivatives bearing carboxamide and sulphonamide moieties. *J Serb Chem Soc.* 2018, 83, 166-179.
38. Sharma R, Soman SS. Design and synthesis of novel diamide derivatives of glycine as antihyperglycemic agents. *Synthetic Communic.* 2016; 46:1307-1317.
39. The Uniprot Consortium. Uniprot: a worldwide hub of protein knowledge. *Nucleic Acids Res.* 2019; 47:D506-515.
40. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-Pdbviewer: an environment for comparative protein modeling, *Electrophoresis.* 1997; 18:2714–2723.
41. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. Protein databank. *Nucleic Acids Res.* 2000; 28:235–342.
42. Discovery Studio Modeling Environment, Release 3.5; Accelrys Software Inc.: San Diego, CA, USA, 2012.
43. Oostenbrink C. Soares TA, van der Vegt NFA, van Gunsteren WF. Validation of the 53A6 GROMOS force field. *Biophys. J.* 2005; 34:273-284.
44. Yang J, Roy A, Zhang Y. HTQC: a fast quality control toolkit for illumine sequencing data. *BMC Bioinformatics.* 2013; 29:2588-2595.
45. Chemical Computing Group, Molecular Operating Environment (MOE) software, 2010.
46. Peters W, Portus JH, Robinson BL. The chemotherapy of rodent malaria, XXII The value of drug resistant strains of berghei in screening for blood schizontocidal activity. *Ann Trop Med Parasitol.* 1975; 69:155–171.
47. Kalra BS, Chawla S, Gupta P, Valecha N. Screening of antimalarial drugs: an *Indian J Pharmacol.* 2006; 38:5-12.
48. Dolabela MF, Oliveira SG, Nescimento MJ, Peres JM, Wagner H, Pova MM, de-Oliveira AB. *In-vitro* antiplasmodial activity of extract and constituents from *Esenbeckia febrifuga*, a plant traditionally used to treat malaria in the Brazilian Amazon. *Phytomedicine.* 2008; 15(5):367-372.
49. Souza MC, Goncalves-Silva T, Moreth M, Gomes CR, Kaiser CR, Henriques MO, Souza MV. Synthesis and *in vivo* antimalarial evaluation of novel hydroxyethylamine derivatives. *Med Chem.* 2012; 8:266-272.

50. Ibezim A, Obi CB, Oforkansi NM, Mbah CJ, Nwodo NJ. Discovery of Trypanocidal bioactive leads by docking study, molecular dynamic simulation and in vivo screening. *ChemistrySelect*. 2018; 3:2386-2389.
51. Ibezim A, Debnath B, Ntie-Kang F, Mbah CJ. Binding of antitrypanosoma natural products from Africa flora against selected drug targets: a docking study. *Med Chem Res*. 2017; 26:562–579.
52. Reitman S and Frankel S (1957). A Colorimetric Method for the Determination of Serum Glutamic Oxaloacetic and Glutamic Pyruvic Transaminase, *J. Clin. Pathol.* , **28**: 56-63.
53. Kaplan A and Tengt LL (1982). Selected Methods of Clinical Chemistry, W.R. Faulkner and S. Meits, *AACC, Washington*. **9**: 357-363
54. Ibezim A, Onyia K, Ntie-Kang F, Nwodo NJ. Drug-like properties of potential anti-cancer compounds from Cameroonian flora: a virtual study. *J Appl Pharm Sci*. 2015; 5(06):133–137.
55. Supuran CT, Briganti F, Tilli S, Chegwidde WR, Scozzafava A. "Carbonic anhydrase inhibitors: sulfonamides as antitumor agents?" *Bioorg Med Chem*. 2001; 9(3):703–714.
56. al-Rashida M, Hussain S, Hamayoun M, Altaf A, Iqbal Sulfa Drugs as Inhibitors of Carbonic Anhydrase: New Targets for the Old Drugs. *BioMed Res Int*. 2014; doi.org/10.1155/2014/162928
57. Zhang Y, Meshnick SR. Inhibition of Plasmodium falciparum dihydropteroate synthase and growth in vitro by sulfa drugs. *Antimicrob Agents Chemother*. 1991; 35(2): 267-71.
58. Wang P, Read M, Sims PF, Hyde JE. Sulfadoxine resistance in the human malaria parasite Plasmodium falciparum is determined by mutations in dihydropteroate synthase and an additional factor associated with folate utilization. *Mol Microbiol*, 1997; 23:979-986.
59. Onoabedje EA, Ibezim A, Okafor SN, Onoabedje US, Okoro UC. Oxazin-5-ones as a novel class of penicillin binding protein inhibitors: design, synthesis and structure activity relationship. *PLoS ONE*. 2016; 11(10):e0163467.
60. Akanji MA and Ngaha EO (1989). Effect of repeated administration of Berenil on urinary enzyme excretion with corresponding tissue pattern in rats. *Pharmacol Toxicol*. 64:272-279.
61. Adesokan AA and Akanji MA (2004). Effect of administration of aqueous extract of Enantia chlorantha on the activities of some enzymes in the small intestine of rats. *Nig J Biochem Mol Biol* 18:103-105.
62. Cotran R., Kumar V and Robins S (1989). Robin's pathological basis of disease. 4th edn. W.B Saunders Co. Harcourt. Pp: 212-217.
63. Moss DW and Rosalki SB (1986). Enzyme tests in diagnosis. Edward Arnold. London. Pp: 88-89

Supplemental Files

Please see the supplemental files section for NMR data.

Figures

Figure 1

Pfdhps_model and its properties. (a) 3-D model of dihydropteroate synthase (b) Qmean score of the 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 COACH program (in sticks) and dock program (in lines).

Figure 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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