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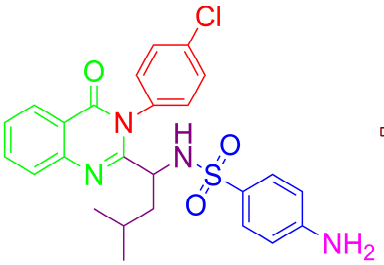

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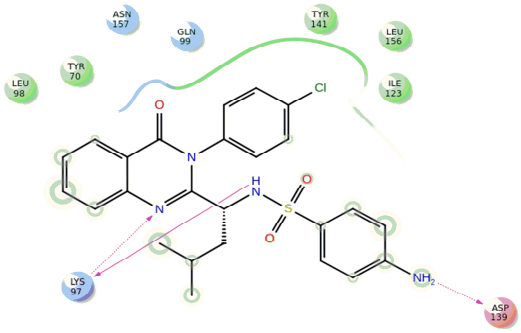

Enzyme Inhibition

IC_{50} = 0.028±0.002 µg/mL

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Novel 2,3-disubstituted quinazoline-4(3*H*)-one molecules derived from amino acid linked sulphonamide as a potent malarial antifolates for DHFR inhibition

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Abstract

An optimization of a modified Grimmel's method for *N*-heterocyclization of Leucine linked sulphonamide leading to 2,3-disustituted-4-quinazolin-(3*H*)-ones was accomplished. Further, nineteen hybrid quinaloinone motifs (**5a**-**5s**) were synthesized by *N*-heterocyclization reaction under microwave irradiation using TEAA (IL) as green solvent as well as catalyst. The *in vitro* screening of the hybrid entities against the plasmodium species *P*. *falciparum* yielded five antimalarial potent molecules **5g**, **5l**, **5m**, **5n** & **5p** owing comparable activity to the reference drugs. The active scaffolds were further evaluated for enzyme inhibition efficacy against alleged receptor *Pf*-DHFR computationally as well as *in vitro*, proving their candidature as lead dihydrofolate reductase inhibitors. The prediction of the ADMET properties of the potent molecules also indicated their good oral bioavailability.

Keywords: Triethylammonium Acetate; *p*-LDH assay; MTT assay; Maestro; Glide; Cytotoxicity

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1. Introduction

Malaria is a life threatening disease caused by parasites that are transmitted to people through the bites of infected female mosquitoes. In 2015 the World Health Organization has estimated approximately 3.2 billion people; nearly half of the world's population was at risk of malaria. Most malaria cases and deaths occur in sub-Saharan Africa. However, Asia, Latin America, and, to a lesser extent, the Middle East, are also at risk. In 2015, 97 countries and territories had ongoing malaria transmission [1]. Malaria is caused by different parasites of Plasmodium, of which Plasmodium falciparum is the most vicious one [2]. The rapid emergence of *P. falciparum* strains resistant to currently available antimalarial drugs [3-6]; and the inefficacy of malarial vaccines [7] have alarmed the emergence to stimulate new efforts regarding medical and molecular studies about malaria. The resistance of the strain against the available antimalarial drugs and an urge for development of newer class of antimalarials has motivated the medicinal chemists towards design of inhibitors of various key enzymes involved in the lifecycle of the parasite [8]. Among the various target enzymes, Dihydrofolate reductase is the key enzyme that catalyses the NADPH-dependent reduction of 7,8-dihydrofolate to 5,6,7,8-tetrahydrofolate, which is the precursor of the co-factors required for the synthesis of purine nucleotides, thymidylate and several amino acids [9]. Thus, inhibition of DHFR can lead to the disruption of DNA synthesis and the death of the rapidly proliferating cells [9, 10]. Early studies have shown that the parasite DHFR and TS, as with other protozoa, reside on the same polypeptide as a DHFR-TS bifunctional protein [11].

More than past decade, synthesis of the heterocyclic compounds has become an important landmark of synthetic organic chemistry as a result of broad diversity of their application in medicinal and pharmaceutical chemistry [12]. The important major area in medicinal chemistry comprises of investigation of heterocycles as privileged structures in drug discovery [13]. Among them, quinazoline ring systems from natural or synthetic origin are known to give a wide variety of biological responses [14-20], which has stimulated the synthesis and pharmacological evaluation of a great number of quinazolinone derivatives. Accounting the excellent biological properties of 4-quinazolin-(3*H*)-one derivatives [21], particularly 2,3-disubstituted-4(3*H*)-quinazolinone, numerous synthetic methods have been investigated and studied by the researchers, which are found in the book chapters and in recent literature reviews [22, 23].

Sulphonamides were the first effective agents against most Gram-positive and many Gram-negative organisms, as well as employed systematically for the prevention and cure of bacterial infections [24, 25]. The discovery and development of sulphonamides as an antibacterial agent, was one of the most intriguing and enlightening breakthrough in the field of medicinal chemistry demonstrating skillful planning and fate in drug research [24]. Since discovery, the sulphonamide group has been found as a key structural motif shared by a large number of bioactive compounds, spanning a wide variety of biological effects, such as, specific enzyme inhibition, hormone regulation, and several others [26]. Apart from the commercialized application as antibacterial/antibiotic agents, various sulphonamides such as Celecoxib and Valdecoxib (COX-2 inhibitor), Darunavir, Tipranavir and Fosamprenavir (Protease Inhibitors), Probenecid (PBN), Sulfasalazine (SSZ), Sumatriptan (SMT) among

others are also known to inhibit several enzymes [27]. The proteinogenic α-amino acids serve as precursors to a wide range of naturally occurring heteroaromatic substances including alkaloids, antibiotics and peptides [28]. Further, special attention has been devoted to the development of methodologies for the synthesis of optically active heterocyclic compounds, due to their biological importance. In this context, proteinogenic α-amino acids are the most extensively used precursors for the synthesis of enantiomerically pure heterocycles, for the reason that they constitute a natural source of optical activity [29]. Foreseeing, the mutations and resistance developing in the parasite against currently prescribed drug regime, the best way to combat the diseased condition is by developing hybrid molecules owing pharmacological activities [30]. Some of the potent hybrid molecules rendering antimalarial potency are depicted in Figure 1, demonstrating analogical relationship with the targeted 2,3-disubstituted quinazolinone-sulphonamide hybrids derived from amino acid.

Heating chemical reactions by microwave energy continues to be a popular theme in the organic and medicinal chemistry community. It has been known since the first published reports in 1986 by groups of Gedye and Giguere, on the use of microwave irradiation to carry out organic chemical transformations, numerous articles have been published in this fast moving and exciting field, generally referred to as microwave assisted organic synthesis [31]. This “non conventional” synthetic method has shown broad applications as a very efficient way to accelerate the course of many organic reactions, producing high yields and higher selectivity, lower quantities of side products and, consequently, easier work up and purification of the products [8, 31, 32].

In continuation of our previous work [8, 33], with an aim to synthesize potent antimalarial leads derived from 2,3-disubstituted quinazolinone motifs withholding leucine linked sulphonamide side arm at position-2. We hereby report the synthesis of 2,3-disubstituted quinazolinone molecules utilizing conventional as well as microwave assisted irradiation. Further, to get an insight of the potency of the synthesized molecules, *in vitro* efficacy was evaluated against *P. falciparum* employing MTT assay and LDH assay. Progressively the active entities were scrutinized via docking experimentation against *Pf*-DHFR enzyme for the comprehensive know how of the mechanism of action as well as their bioavailability parameters were predicted. Finally, the potent molecules screened for their enzyme inhibitory activity as well as toxicity against vero cells to establish their selectivity.

2. Chemistry

A series of novel quinazolinone-sulphonamide hybrid derivatives derived from Leucine were designed and synthesized as depicted in Scheme 1. The desired entities were synthesized by *N*-hetero cyclization of previously synthesized *N*-acylanthranilic acid (**3**) with various aromatic amines. The synthetic protocol was optimized utilizing the classical catalysts as well as TEAA as green catalyst and reaction medium under conventional and microwave assisted heating conditions. Finally, the targeted sulphonamide incorporated 2,3-disubstituted quinazolinone hybrids **4a-s** were synthesized using TEAA as green solvent as well as catalyst under microwave irradiation as a source of energy at 350 watts power level. Further, the final

entitled de-acetylated derivatives **5a-s** were procured by acidic hydrolysis of **4a-s** under conventional as well as microwave assisted heating conditions.

3. Pharmacology

3.1 In vitro antimalarial activity

The newly synthesized Quinazolinone-Sulphonamide hybrids were evaluated against *P. falciparum* for their antimalarial potency considering Chloroquine, Pyrimethamine and Trimethoprime as standard reference drugs. The outcomes of the antimalarial screening are expressed as the concentration of the molecules resulting in 50% inhibition (IC_{50}) of parasite growth and are as outlined in Table 5.

3.2 The antiplasmodial assay (p-LDH and MTT)

The antiplasmodial efficacy of the synthesized Quinazolinone-Sulphonamide hybrid entities was evaluated by *in vitro* quantitative enzyme-based method (Malstat® assay) involving spectrophotometric measurement of the parasite lactate dehydrogenase activity (*p*-LDH). The inhibition rendered by each drug concentration was calculated as compared to the untreated control to obtain the IC_{50} values.

The *in vitro* antiplasmodial and cytotoxic effect of Quinazolinone-Sulphonamide hybrids on parasite growth and normal uninfected RBCs was tested by the quantitative enzyme based method (MTT assay) involving spectrophotometric measurement of the host RBC's *Mitochondrial Succinate Dehydrogenase* catalysed MTT reduction. The effective concentration (EC_{50}) and cytotoxic concentration (CC_{50}) were ascertained as well as selectivity was determined from the values obtained.

3.3 The enzyme inhibition assay

The inhibitory efficacy of the synthesized Quinazolinone-Sulphonamide hybrids on *Pf*-DHFR was determined against bovine DHFR owing resemblance to *Pf*-DHFR. Results are reported as % inhibition of enzymatic activity.

3.4 The cytotoxicity against Vero cells

The cytotoxic behaviour of the synthesized hybrid motifs was evaluated against Vero cells using MTT assay. The toxicity of the compounds was evaluated and enumerated as IC_{50} values indicating 50% inhibitory concentration of the molecules.

4. Computational and Docking study

To study and understand the functionality and mode of action of the *in vitro* active Quinazolinone-Sulphonamide hybrids, *in silico* studies including prediction of physicochemical properties, docking as well as prediction of the pharmacokinetic properties was accomplished against mutant *Pf*-DHFR as well as human DHFR (1MVT) obtained from RSC protein databank.

5. Result and discussion

5.1 Synthetic aspect

To establish a more general, efficient and convenient synthetic method, our efforts begins with the hetero cyclization approach to derive desired quinazolinone-sulphonamide hybrids **(4a-s)** under milder reaction conditions and to obtain high isolated yield. We recently have been engaged for the reinvestigation of this heterocyclization approach, and the successful modifications is reported in our article describing the synthesis of various *N*-(4-(*N*-((3-substituted-4-oxo-3,4-dihydroquinazolin-2-yl)methyl)sulfamoyl) phenyl) acetamide derivatives [33]. With the relative experience in hand and keeping in mind the literature methods, synthesis of desired quinazolinone-sulphonamide hybrid molecules from amino acids as initial precursor would not be a difficult task. Hence, it was decided to explicit various approaches which are feasible in common laboratories. Therefore, we chose to explore some plausible synthetic approaches based on the intermediates to be opted in the crucial reaction step, i.e. ring formation or ring closure step for the synthesis of diverse quinazolinone-sulphonamide hybrid molecules from Leucine in our laboratory, and is outlined in Scheme 1. Consequently, to probe the scope and limitations of newly developed protocol for the synthesis of desired quinazolinone-sulphonamide hybrid **4a** under classical heating conditions, it was planned to employ optimized conditions for the hetero cyclization of
N-acylanthranilic acid (**3**) with a aniline as an amine (Scheme 2). Therefore, it was decided to reinvestigate again the solvent and catalyst effects on the efficiency of cyclization reactions with the hope to identify an ideal combination that could improve reaction duration and contribute eventually the higher conversion to quinazolinone-sulphonamide hybrids in the ring formation step as well as proved itself to be environmentally benign. Hence, the effect of various solvents and catalyst on the reaction time and yield were examined as shown in Table 1. An excellent conversion of **3** to **4a** was achieved (entry 7) within short reaction span (14 min.) as indicated by isolated yield (90 %) utilizing an ionic liquid triethylammonium acetate (TEAA) as reaction medium as well as catalyst.

In addition to this, to study the effect of microwave irradiation on the reaction time and yield of the product it was decided further to employ microwave heating conditions for the synthesis of the quinazolinone-sulphonamide hybrid (**4a**). The optimization of reaction between **3** with appropriate aryl amine (Scheme 2) was examined under microwave

irradiation conditions for the formation of **4a** was achieved by considering the effect of change of power levels, solvents and catalysts (Table 2). Amongst the solvents examined, good results were observed again when the TEAA was employed as a solvent as well as catalyst (Entry 9-10). Under the optimized condition (Entry 9), reaction under microwave irradiation (350 W) has afforded desired **4a** in excellent yield (96 %). Thus, the optimized condition utilizing TEAA as reaction medium and catalyst as well under microwave irradiation at 350 Watts power level for the conversion of *N*-acylanthranilic acid (**3**) to quinazolinone-sulphonamide derivative (**4a**) was further employed for the synthesis of quinazolinone-sulphonamide hybrids (**4a-s**) and the substitution pattern is as depicted in Table 3.

Proceedingly to obtain our final entitled de-acetylated derivatives (Scheme 3) it was decided to employ acidic hydrolysis conditions which utilized dilute HCl (40 % solution) in *R*-spirit and H_2O mixture under conventional and microwave-assisted heating conditions to afford entitled amino derivatives (**5a-s**) in quantitative yields as compared in Table 4.

5.2 Analytical aspect

The structures of all the synthesized compounds were confirmed using various physico-chemical methods and spectroscopic investigations, and the relevant analytical data have been given in experimental section.

Mass spectra and elemental analysis of compounds reveals that they are consistent with the proposed structures. Further, the results of elemental analysis of all compounds were in good agreement (± 0.4 %) with their predicted molecular formula. All the ESI-MS (positive mode) spectra of synthesized compounds showed molecular ion peak $[M+H]^+$ corresponding to either exact mass or molecular weight of respective compound [34, 35]. The mass spectrum of compound **5a** exhibited a parent peak at *m/z* 463.0 $[M+H]^+$, which confirms the proposed formula. Compounds, **5k** (chloro-derivative) and **5n** (bromo-derivative) exhibited ionization peaks ($[M+H]^+$and $[M+H]^{+2}$) at *m/z* (497.0, 499) and at *m/z* (540.8, 542.8) respectively. Further, ESI-MS spectra of some compounds appeared with $[M+H+Na]^+$ peak along with molecular ion peak $[M+H]^+$.

The FT-IR spectra represent an obvious tool for initial identification of the presence of various functional groups in the moiety [34, 35]. In IR spectra of all the compounds, presence of $–SO_2NH–$ group was evidenced by the two strong bands appeared at 1318-1340 and 1144-1165 cm^{-1} due to asymmetric and symmetric S═O stretching vibrations respectively, and further absorption band at ~3250 cm^{-1}assigned to $–SO_2N–H$ stretching. For the quinazolinone derivatives, strong intensity bands appeared in the IR spectra at 1690-1670, 1610-1650 and at ~1495 cm^{-1} attributed to C=O, C=N and C=C stretching vibrations respectively, provides a strong evidence for the parent heterocyclic ring skeleton vibrations. In IR spectra of **5a-s** bands observed at ~2930 and ~2850 (methylene) due to the aliphatic C–H strechtching vibrations, while C–H bending vibrations due to isobutyl group was observed at 1395-1385 cm^{-1}.

In ^{1}H-NMR spectra of compounds **4a-s** with acetamido group (Ar–N**H**COC**H**$_3$), protons of –COC**H**$_3$ and –N**H**CO were appeared as a singlet at δ >10.0 ppm due to –N**H**CO and between δ 2.0-2.3 ppm due to –COC**H**$_3$ respectively. After the hydrolysis, disappearance of these two signals (siglets) in **5a-s** and appearance of signal (singlet at δ 5.6-6.3 ppm) due to Ar–N**H**$_2$ supports the formation of compounds, **5a-s**. In addition to this, the aromatic protons of benzenesulfonyl ring (**Ar**-NH$_2$ in **5a-s**) appeared as two separate doublets (J=8.8/8.4) and with two integral proton to each peak (at δ 7.4-7.2 and 6.2-6.8 ppm), however, these four protons **(Ar**–NHAc group in **4a-s)** appeared as broad multiplet (at δ 7.5-7.8 ppm) due to the symmetry might have been influenced by acetamido group. The proton at C$_5$ of quinazolinone ring appeared as a doublet around δ >8 ppm. All the other protons of aromatic and heteroaromatic ring resonated in aromatic region (δ 6.2-8.6 ppm). All ^{13}C-NMR spectra of **5a-s** were characterized by two signals (quarternary carbons) at low frequencies (δ 150-157 and 160-162 ppm) associated with a cyclic amide or lactam ring of quinazolinone (with a cyclic –N–C═N carbon conjugated with C═O group) further, –**C**H$_2$CH(CH$_3$)$_2$ (secondary carbon) appeared at δ ~42 ppm. All the other carbons (CH, C) of aryl and quinazolinone ring were resonated in expected region (δ 112-150 ppm).

5.3 In vitro antimalarial screening and assay

The antimalarial screening of the synthesized compounds was carried out against *P. falciparum* strain which resulted in varied results as given in Table 5. The spectrum of activity of entitled compounds showed improved potency IC$_{50}$<0.20 µg/mL, even better than reference compound Pyrimethamine. The results of *in vitro* assay suggest that moderate to good activity was achieved from the synthesized 2,3-disubstituted quinazolinones. Among the screened scaffolds, compounds **5g, 5l, 5m, 5n and 5p** were found to be most potent entities having IC$_{50}$ value of 0.068 µg/mL.

The core Quinazolinone motif consisting of sulphonamide linked amino acid at position-2 was found to be most promising structure owing comparatively better potency against plasmodium species. Considering substitutional pattern at position-3, varied results were noted which indicated the presence of electron withdrawing group greatly enhanced the efficacy of the molecules. The compounds with 4-OMe (**5g**), 4-Cl (**5m**), 3-Cl (**5l**), 4-Br (**5p**) and 2-Br (**5n**) substituted phenyl ring were found to have enhanced potency against *P. falciparum*.

Progressively to cross validate the results of the above *in vitro* study and to determine the selectivity of the potent molecules as true antimalarial drugs we executed *p*-LDH assay and MTT assay as a pure indication of the efficacy as well as cytotoxicity of the synthesized entities. The results of the *p*-LDH assay as enumerated in Table 6 indicated that the compounds demonstrated lower to moderate potency as compared to standard drug Chloroquine. Considering the results of lactate dehydrogenase assay, molecules **5l** and **5m** noted their candidature to be most potent among the tested molecules.

Further, the results of MTT assay against *P. falciparum* indicated the potency of the molecules as well as toxicity against RBC. The results in form of effective concentration and

cytotoxic concentration revealed that the molecules were effective against the parasite but had lower toxicity against blood cells. Based on the calculation of EC_{50} and CC_{50} values, selectivity indices (SI) were calculated for *P. falciparum*. All the compounds tested have exhibited the SI values of >1. Therefore, these compounds are specific against the malarial parasites and not against the RBCs.

5.4 Docking study

To enhance the knowhow of mechanism of the antimalarial potency of the *in vitro* active scaffolds (**5g**, **5l**, **5m**, **5n** and **5p**), the molecules were further evaluated by docking them against wild type mutant *Pf*-DHFR (4DPD) protein structure obtained from protein data bank (RSC). For completion of docking study, 3D structures of the molecules were prepared and were docked against the active site of the enzyme using glide 6.6.

A potential interaction was observed between the active molecules and the *Pf*-DHFR enzyme. The molecules interacted with the protein active pocket by forming H-bonds as well as *π-π* stacking interactions. As enumerated in Table 7, docking score as well as binding energies of the molecules against the active site indicated higher affinity of the molecules to bind with the protein leading to its inhibition, further more the RMSD values were found to be lower than 2 which can be considered to be in acceptable range. The docking score of the molecules (**5g**, **5l**, **5m**, **5n** and **5p**) was found to be ranging from -3.07 to -4.21 and binding energies ranging from -34.56 to -43.08 which were found to be better as compared to standard drugs Trimethoprime and Pyrimethamine. The compound **5g** interacted with the active pocket of the enzyme by forming hydrogen bonds with Phe295 at a distance of 2.732 Å and Asn294 with bond length 1.993 Å (Figure 2). The molecule **5l** occupied the active pocket with great ease as depicted in Figure 3. A hydrogen bond was observed between the nitrogen of backbone of Quinazolinone hybrid and Lys97 with bond length of 1.938 Å, further side chain amide group interacted with same amino acid Lys97 at distance of 1.99 Å and terminal amine group if the hybrid interacted with Asp139 at a bond length of 1.791 Å. For molecule **5m** (Figure 4) similar mode of interaction was observed where a hydrogen bonding between nitrogen and Lys97 was observed at a distance of 2.26 Å. The same amino acid Lys97 also established hydrogen bond with amide group of side chain with bond length of 2.214 Å, finally a hydrogen bond was observed between amine group and Asp139 at a distance of 1.75 Å. The compound **5n** interacted in the similar fashion forming hydrogen bonding as well as *π-π* stacking interactions with amino acids Leu98 and Lys97 with the bond length of 2.23 Å and 3.927 Å respectively as elucidated in Figure 5. The active site invited **5p** molecule to occupy the pocket by forming hydrogen bond between nitrogen and Lys297 as well as Asn294 at a distance of 2.43 Å and 2.071 Å respectively as visualized in Figure 6. The 2D and 3D binding model of the standard inhibitors showing the interaction with the protein can be observed in Figure S1 and Figure S2 in supplementary material.

Further, to understand the selectivity of the quinazolinone-sulphonamide hybrids they were again docked against human DHFR (1MVT) procured from protein data bank (RSC). The glide score and energies displayed in Table 7 as well as interactions of the ligand molecules

against the active site indicated that the potent molecules poorly interacted with *h*-DHFR as compared to *Pf*-DHFR. Moreover, the active site of the *Pf*-DHFR was found to be flexible accommodating the ligand molecules. Thus the results of docking study also indicated the selectivity of the potent ligand molecules.

5.5 Pharmacokinetic properties prediction

The drug likeness properties of the synthesized molecules demonstrating superior antimalarial potency was evaluated by predicting the pharmacokinetic properties *in silico*. The potent molecules (**5g**, **5l**, **5m**, **5n** and **5p**) were preliminarily screened considering the basic parameters of Lipinski's rule of 5. The results of the Quickprop module with their permissible rang are delineated in Table 8. For an orally active compound, 2 violations of the Lipinski's rule are acceptable. The molecules of the present study were found to follow the rule with maximum violation of one rule and thus proving their drug likeness properties.

The oral bioavailability of the drug molecules are greatly influenced by the optimum values of the descriptors, polar surface area and rotatable bonds. The important pharmacokinetic parameters with their permissible ranges are delineated in Table 9. The active test quinazolinone derivatives show results of the optimum value of rotatable bonds (<15) and polar surface area (7-200 Å) to be in the prescribed range [36] thus owing good bioavailability. The Caco-2 cell permeability (QPPCaco), used as model for gut-blood barrier [37] acts as the important factor to be studied in concern with the absorption of the drug molecule across the intestine. Caco-2 cell permeability prediction of the test compounds indicates excellent results predicting good intestinal absorption. Further, the compounds were tested for human serum albumin binding indicated by the predicted values of QPlogkhsa descriptor of Quickprop and all the test molecules were found to fall in the permissible range (-1.5 to 1.5). Also, the Quickprop descriptor for blood/brain partition coefficient QPlogBB showed reliable prediction for all the test compounds and reference drugs. The test entities were assessed for aqueous solubility parameter (QPlogS) and the compounds were found to be falling in the permissible range (-6.5-0.5). Finally, the active molecules were accessed for IC_{50} value of HERG K+ channel blockage prediction, which indicated that the predicted values fall in the acceptable range (<-5) as compared to the standard reference entities.

5.6 DHFR inhibition assay and Cytotoxicity assay

The potent entities traced out of *in vitro* antimalarial screening were further evaluated for inhibitory efficacy against bovine liver DHFR enzyme and cytotoxicity against vero cells. As enumerated in Table 10, the test molecules rendered a good inhibitory potency against the enzymes as compared to the standard inhibitors. The inhibitory concentrations of the compounds were obtained as IC_{50} values which indicated that among the tested molecules, compounds **5g**, **5l** and **5m** were found to be most potent inhibitors. The cytotoxic concentration of the test compounds against vero cells was found to be exceeding 15 μg/mL, and thus showing up a non toxic behavior. The selective index calculated was found to be

more than 100 which support the harmony towards utilization of the scaffolds as active antimalarial compounds inhibiting dihydrofolate reductase enzyme.

6. Conclusion

Withholding the aim of synthesizing Leucine linked sulphonamide incorporated hybrid quinazolinone molecules via environmental friendly methodology; we have modified and developed an efficient, convergent and facile methodology of *N*-heterocyclization leading towards synthesis of 2,3-disustituted quinazolinone motifs in satisfactory yields. We hereby report synthesis of nineteen qinazolinone-sulphonamide hybrids (**5a**-**5s**) under microwave irradiation technique utilizing an ionic liquid TEAA as inexpensive, environmentally benign and recyclable reaction medium as well as catalyst. Among the synthesized molecules, **5g**, **5l** and **5m** were found to be the most potent entities against *Plasmodium falciparum* species. The *in silico* docking study directed towards their potentiality of interacting and inhibiting the enzyme *Plasmodium falciparum* dihydrofolate reductase occupying the active binding pocket with great ease. The antimalarial efficacy was further proved by the molecules exhibiting an active inhibition of enzyme proving their potency as dihydrofolate reductase inhibitors and thus interrupting malarial folate pathway. The calculated pharmacokinetic properties for the test compounds predicted good oral bioavailability. Thus promising *in vitro* antimalarial activity, inhibition as well as interactions with the enzyme *Plasmodium falciparum* dihydrofolate reductase and the pharmacokinetic properties revealed in the present study, indicates their potential for further development as antimalarial lead entities.

7. Experimental section

7.1 Experimental protocols and analytical data

*7.1.1 Synthesis of N-{4-[({1-[3-(aryl substituted)-4-oxo-3,4-dihydroquinazolin-2-yl]-2-phenylethyl}amino)sulfonyl]phenyl}acetamide (**4a**-**4s**)*

To a mixture of *N*-acylanthranilic acid (**3**) (0.783 g, 0.002 mol) and appropriate mono-substituted aromatic amines (0.022 mol) {for instance, aniline and its *o*-, *m*-, *p*- substituted derivatives of toludine, anisidine, nitroaniline, chloroaniline, bromoaniline, fluoroaniline and phenylhydrazine respectively} in were taken in 5.0 mL of TEAA in round bottom flask and heated at 100 °C for specific time interval as visualized by TLC. To carry out the reaction under microwave irradiation, the reaction mass was irradiated under microwave for required time period as indicated in Table 3 at 350 W power level. After completion of the reaction, the system was cooled to room temperature and finally poured onto crushed ice. The solid product thus separated was collected, washed with acetone and finally recrystallized from ethanol to obtain pure product. TEAA was recovered from the aqueous medium by heating it

under reduced pressure at 80 °C. The ionic liquid recovered can be reused again for the same experimental protocol.

N-[4-({[1-(3-phenyl-4-oxo-3,4-dihydroquinazolin-2-yl)-3-methylbutyl]amino}sulfonyl)-phenyl]acetamide **(4a)**: White solid; **mp**: 164-169 °C; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3358, 3253 (N–H str.), 3065, 2956 (C–H str.), 1679 (C═O str.), 1602, 1592, 1496 (C═N, C═C str.), 1326, 1152 (S═O str.); **^{1}H-NMR** (400 MHz, DMSO-d_6, δ_H, ppm): 10.01 (s, 1H, Ar–N*H*Ac), 8.20 (d, *J* = 8.4 Hz, 1H, SO_2N*H*), 8.04 (dd, *J* = 8, 1.2 Hz, 1H, Ar*H*), 7.82 (dt, *J* = 8, 1.2 Hz, 1H, Ar*H*), 7.67-7.52 (m, 6H, Ar*H*), 7.47-7.21 (m, 5H, Ar*H*), 3.79 (m, 1H, NH–C*H*), 2.01 (s, 3H, Ar–NHCOCH_3), 1.55-1.30 (m, 3H, C*H*–CH_2), 0.77, 0.55 (d, *J* = 6.4 Hz, 6H, CH–(CH_3)$_2$); **^{13}C-NMR** (100 MHz, DMSO-d_6, δ_C, ppm): 168.7, 161.2, 156.7, 146.7, 142.5, 135.6, 134.5, 133.7, 129.7, 129.4, 128.5, 127.6, 127.1, 126.9, 126.2, 120.6, 118.3, 52.1, 41.8, 24.1, 23.5, 23.1, 19.2; **MS (ESI)** *m/z*: 504.5 $[M+H]^+$; Anal. Calcd. for $C_{27}H_{28}N_4O_4S$: C, 64.27; H, 5.59; N, 11.10. Found: C, 64.49; H, 5.67; N, 11.22 %.

*7.1.2 Synthesis of 4-amino-N-[(4-oxo-3-substituted aryl-3,4-dihydroquinazolin-2-yl)alkyl]benzenesulfonamide derivatives (**5a-5s**)*

The appropriate product obtained in ring-closure step (**4a-4s**) (0.001 mol) was taken in a two-necked round bottomed flask containing dilute HCl solution (40 %) in *R*-spirit and H_2O (1:1) mixture (15-20 mL). The reaction mixture was heated to reflux for 45-75 min till the clear solution obtained. Under microwave irradiation method, the reaction mixture was heated under microwave irradiation at 210 W for an appropriate time (Table 4). After cooling to room temperature, the reaction mixture was filtered, diluted with distilled water (100 mL) and neutralized with saturated $NaHCO_3$ solution. The precipitated product was filtered and recrystallized from *R*-spirit.

4-amino-N-[1-(4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)-3-methylbutyl]benzene-sulfonamide **(5a)**: White solid; **mp**: 183-191 °C; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3378, 3253 (N–H str.), 3060, 2954 (C–H str.), 1656 (C═O str.), 1623, 1593, 1502 (C═N, C═C str.), 1314, 1152 (S═O str.) ; **^{1}H-NMR** (400 MHz, DMSO-d_6, δ_H, ppm): 8.05 (d, *J* = 7.6 Hz, 1H, Ar*H*), 7.85-7.59 (m, 4H, SO_2N*H* & Ar*H*), 7.49-7.32 (m, 5H, Ar*H*), 7.29 (d, *J* = 8.8 Hz, 2H, Ar*H*), 6.34 (d, *J* = 8.4 Hz, 2H, Ar*H*), 5.91 (s, 2H, Ar–NH_2), 3.72 (m, 1H, NH–C*H*), 1.54-1.31 (m, 3H, C*H*–CH_2), 0.75, 0.51 (d, *J* = 6.4 Hz, 6H, CH–(CH_3)$_2$); **^{13}C-NMR** (100 MHz, DMSO-d_6, δ_C, ppm): 161.1, 156.7, 152.5, 146.6, 135.6, 134.6, 130.0, 129.2, 128.6, 128.5, 127.2, 127.0, 126.2, 124.5, 120.5, 112.4, 51.9, 42.0, 23.5, 23.1, 19.1; **MS (ESI)** *m/z*: 463.0 $[M+H]^+$, 484.9 $[M+H+Na]^+$; Anal. Calcd. for $C_{25}H_{26}N_4O_3S$: C, 64.91; H, 5.67; N, 12.11. Found: C, 65.16; H, 5.76; N, 12.23 %.

4-amino-N-{1-[3-(2-methylphenyl)-4-oxo-3,4-dihydroquinazolin-2-yl]-3-methylbutyl}-benzenesulfonamide **(5b)**: White solid; **mp**: 204-213 °C; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3361, 3240 (N–H str.), 3055, 2955 (C–H str.), 1683 (C═O str.), 1613, 1592, 1503 (C═N, C═C str.), 1312, 1151 (S═O str.), 1370 (C–H def.); **^{1}H-NMR** (400 MHz, DMSO-d_6, δ_H, ppm): 8.08 (dd,

J = 8, 1.2 Hz, 1H, Ar*H*), 7.88 (t, *J* = 8 Hz, 1H, Ar*H*), 7.71-7.55 (m, 3H, SO_2N*H* & Ar*H*), 7.45-7.39 (m, 3H, Ar*H*), 7.30-7.14 (m, 3H, Ar*H*), 6.78 (d, *J* = 8.8 Hz, 2H, Ar*H*), 5.86 (s, 2H, Ar–NH_2), 3.67 (m, 1H, NH–C*H*), 2.41 (s, 3H, Ar–CH_3), 1.55-1.34 (m, 3H, C*H*–CH_2), 0.75, 0.53 (d, *J* = 6.4 Hz, 6H, CH–(CH_3)$_2$); **^{13}C-NMR** (100 MHz, DMSO-d_6, δ_C, ppm): 161.3, 155.6, 152.6, 146.4, 141.4, 134.6, 134.5, 131.7, 129.8, 128.9, 128.4, 127.2, 126.8, 126.4, 125.5, 125.1, 120.6, 112.4, 51.6, 42.3, 23.5, 23.1, 21.5, 19.1; **MS (ESI)** *m/z*: 477.0 $[M+H]^+$; Anal. Calcd. for $C_{26}H_{28}N_4O_3S$: C, 65.52; H, 5.92; N, 11.76. Found: C, 65.74; H, 5.99; N, 11.88 %.

4-amino-N-{1-[3-(3-methylphenyl)-4-oxo-3,4-dihydroquinazolin-2-yl]-3-methylbutyl}-benzenesulfonamide **(5c)**: White solid; **mp**: 188-195 °C; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3375, 3251 (N–H str.), 3059, 2957 (C–H str.), 1689 (C═O str.), 1616, 1590, 1508 (C═N, C═C str.), 1313, 1154 (S═O str.), 1371 (C–H def.); **^{1}H-NMR** (400 MHz, DMSO-d_6, δ_H, ppm): 8.08 (d, *J* = 8 Hz, 1H, Ar*H*), 7.79-7.64 (m, 3H, SO_2N*H* & Ar*H*), 7.59 (d, *J* = 8 Hz, 1H, Ar*H*), 7.40-7.33 (m, 4H, Ar*H*), 7.31 (d, *J* = 8.8 Hz, 2H, Ar*H*), 6.74 (d, *J* = 8.8 Hz, 2H, Ar*H*), 5.87 (s, 2H, Ar–NH_2), 3.63 (m, 1H, NH–C*H*), 2.41 (s, 3H, Ar–CH_3), 1.57-1.33 (m, 3H, C*H*–CH_2), 0.74, 0.52 (d, *J* = 6.4 Hz, 6H, CH–(CH_3)$_2$); **^{13}C-NMR** (100 MHz, DMSO-d_6, δ_C, ppm): 161.2, 155.8, 152.5, 146.5, 140.6, 135.7, 134.5, 130.6, 129.5, 128.8, 128.4, 127.1, 126.8, 126.3, 125.2, 123.5, 120.5, 112.6, 51.7, 42.3, 23.5, 23.1, 21.3, 19.1; **MS (ESI)** *m/z*: 476.9 $[M+H]^+$; Anal. Calcd. for $C_{26}H_{28}N_4O_3S$: C, 65.52; H, 5.92; N, 11.76. Found: C, 65.71; H, 5.97; N, 11.85 %.

4-amino-N-{1-[3-(4-methylphenyl)-4-oxo-3,4-dihydroquinazolin-2-yl]-3-methylbutyl}-benzenesulfonamide **(5d)**: White solid; **mp**: 169-178 °C; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3367, 3255 (N–H str.), 3056, 2953 (C–H str.), 1685 (C═O str.), 1615, 1596, 1505 (C═N, C═C str.), 1314, 1153 (S═O str.), 1370 (C–H def.); **^{1}H-NMR** (400 MHz, DMSO-d_6, δ_H, ppm): 8.04 (d, *J* = 7.6, Hz, 1H, Ar*H*), 7.82-7.78 (m, 2H, SO_2N*H* & Ar*H*), 7.56-7.49 (m, 2H, Ar*H*), 7.43 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.34-7.27 (m, 4H, Ar*H*), 6.71 (d, *J* = 8.8 Hz, 2H, Ar*H*), 5.87 (s, 2H, Ar–NH_2), 3.69 (m, 1H, NH–C*H*), 2.41 (s, 3H, Ar–CH_3), 1.58-1.34 (m, 3H, C*H*–CH_2), 0.73, 0.51 (d, *J* = 6.4 Hz, 6H, CH–(CH_3)$_2$); **^{13}C-NMR** (100 MHz, DMSO-d_6, δ_C, ppm): 161.2, 155.9, 152.5, 146.4, 143.1, 137.8, 134.4, 129.9, 128.8, 128.3, 127.0, 126.9, 126.4, 125.2, 120.6, 112.6, 51.7, 41.3, 23.5, 23.1, 21.4, 19.1; **MS (ESI)** *m/z*: 477.1 $[M+H]^+$; Anal. Calcd. for $C_{26}H_{28}N_4O_3S$: C, 65.52; H, 5.92; N, 11.76. Found: C, 65.73; H, 5.98; N, 11.89 %.

4-amino-N-{1-[3-(2-methoxyphenyl)-4-oxo-3,4-dihydroquinazolin-2-yl]-3-methylbutyl}-benzenesulfonamide **(5e)**: White solid; **mp**: 173-178 °C; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3377, 3250 (N–H str.), 3054, 2959 (C–H str.), 1688 (C═O str.), 1619, 1591, 1507 (C═N, C═C str.), 1317, 1150 (S═O str.), 1263 (C–O–C str.); **^{1}H-NMR** (400 MHz, DMSO-d_6, δ_H, ppm): 8.21 (d, *J* = 8 Hz, 1H, Ar*H*), 8.08 (d, *J* = 8 Hz, 1H, Ar*H*), 7.81-7.56 (m, 3H, SO_2N*H* & Ar*H*), 7.44-7.34 (m, 3H, Ar*H*), 7.19-6.87 (m, 3H, Ar*H*), 6.72 (d, *J* = 8.4 Hz, 2H, Ar*H*), 5.89 (s, 2H, Ar–NH_2), 3.93 (s, 3H, Ar–OCH_3), 3.81 (m, 1H, NH–C*H*), 1.53-1.29 (m, 3H, C*H*–CH_2), 0.75, 0.54 (d, *J* = 6.4 Hz, 6H, CH–(CH_3)$_2$); **^{13}C-NMR** (100 MHz, DMSO-d_6, δ_C, ppm): 161.2, 157.6, 156.7, 152.6, 146.3, 134.7, 134.4, 132.9, 128.4, 127.3, 127.2, 126.9, 126.3, 124.8, 124.0, 120.5, 117.3, 112.5, 56.8, 51.8, 42.2, 23.5, 23.1, 19.1; **MS (ESI)** *m/z*: 493.1 $[M+H]^+$; Anal. Calcd. for $C_{26}H_{28}N_4O_4S$: C, 63.40; H, 5.73; N, 11.37. Found: C, 63.64; H, 5.81; N, 11.52 %.

4-amino-N-{1-[3-(3-methoxyphenyl)-4-oxo-3,4-dihydroquinazolin-2-yl]-3-methylbutyl}-benzenesulfonamide **(5f)**: White solid; **mp**: 159-163 °C; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3373, 3249 (N–H str.), 3059, 2952 (C–H str.), 1686 (C═O str.), 1618, 1593, 1509 (C═N, C═C str.), 1318, 1152 (S═O str.), 1260 (C–O–C str.); **^{1}H-NMR** (400 MHz, DMSO-d_6, δ_H, ppm): 8.08 (d, J = 8 Hz, 1H, Ar*H*), 7.88 (d, J = 8 Hz, 1H, Ar*H*), 7.79-7.64 (m, 3H, SO_2NH & Ar*H*), 7.39-7.36 (m, 2H, Ar*H*), 7.31 (d, J = 8.8 Hz, 2H, Ar*H*), 7.18-7.08 (m, 2H, Ar*H*), 6.75 (d, J = 8.8 Hz, 2H, Ar*H*), 5.79 (s, 2H, Ar–NH_2), 3.86 (s, 3H, Ar–OCH_3), 3.73 (m, 1H, NH–C*H*), 1.55-1.30 (m, 3H, C*H*–CH_2), 0.76, 0.53 (d, J = 6.4 Hz, 6H, CH–$(CH_3)_2$); **^{13}C-NMR** (100 MHz, DMSO-d_6, δ_C, ppm): 161.3, 157.5, 156.7, 152.5, 146.3, 134.5, 133.8, 132.6, 128.4, 127.2, 127.1, 126.2, 126.2, 124.7, 120.5, 116.5, 112.6, 110.2, 56.1, 51.8, 42.2, 23.5, 23.1, 19.1; **MS (ESI)** *m/z*: 493.0 $[M+H]^+$; Anal. Calcd. for $C_{26}H_{28}N_4O_4S$: C, 63.40; H, 5.73; N, 11.37. Found: C, 63.62; H, 5.79; N, 11.49 %.

4-amino-N-{1-[3-(4-methoxyphenyl)-4-oxo-3,4-dihydroquinazolin-2-yl]-3-methylbutyl}-benzenesulfonamide **(5g)**: White solid; **mp**: 179-184 °C; α_{obs} = + 11.8 (AcOH); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3368, 3251 (N–H str.), 3056, 2960 (C–H str.), 1689 (C═O str.), 1614, 1596, 1506 (C═N, C═C str.), 1313, 1153 (S═O str.), 1262 (C–O–C str.); **^{1}H-NMR** (400 MHz, DMSO-d_6, δ_H, ppm): 8.08 (d, J = 7.6 Hz, 1H, Ar*H*), 7.83 (t, J = 7.6 Hz, 1H, Ar*H*), 7.77-7.48 (m, 5H, SO_2NH & Ar*H*), 7.34 (d, J = 8.8 Hz, 2H, Ar*H*), 7.15 (d, J = 8.8 Hz, 2H, Ar*H*), 6.73 (d, J = 8.4 Hz, 2H, Ar*H*), 5.86 (s, 2H, Ar–NH_2), 3.80 (m, 1H, NH–C*H*), 3.88 (s, 3H, Ar–OCH_3), 1.58-1.33 (m, 3H, C*H*–CH_2), 0.75, 0.52 (d, J = 6 Hz, 6H, CH–$(CH_3)_2$); **^{13}C-NMR** (100 MHz, DMSO-d_6, δ_C, ppm): 161.3, 159.0, 156.6, 152.6, 146.3, 134.4, 129.7, 128.3, 128.2, 127.1, 127.0, 126.2, 124.8, 120.4, 114.7, 112.6, 55.5, 51.8, 42.2, 23.5, 23.1, 19.1; **MS (ESI)** *m/z*: 492.9 $[M+H]^+$; Anal. Calcd. for $C_{26}H_{28}N_4O_4S$: C, 63.40; H, 5.73; N, 11.37. Found: C, 63.65; H, 5.81; N, 11.50 %.

4-amino-N-{1-[3-(2-nitrophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl]-3-methylbutyl}-benzenesulfonamide **(5h)**: White solid; **mp**: 208-212 °C; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3379, 3249 (N–H str.), 3063, 2953 (C–H str.), 1680 (C═O str.), 1616, 1589, 1495 (C═N, C═C str.), 1315, 1148 (S═O str.), 1530, 1350 (N═O str.); **^{1}H-NMR** (400 MHz, DMSO-d_6, δ_H, ppm): 8.64 (d, J = 8.4 Hz, 1H, Ar*H*), 8.31 (d, J = 8.4 Hz, 1H, Ar*H*), 8.14 (d, J = 8 Hz, 1H, Ar*H*), 7.94 (d, J = 6 Hz, 1H, SO_2NH), 7.86-7.63 (m, 5H, Ar*H*), 7.36 (d, J = 8.8 Hz, 2H, Ar*H*), 6.71 (d, J = 8.8 Hz, 2H, Ar*H*), 5.84 (s, 2H, Ar–NH_2), 4.10 (m, 1H, NH–C*H*), 1.50-1.36 (m, 3H, C*H*–CH_2), 0.77, 0.49 (d, J = 5.6 Hz, 6H, CH–$(CH_3)_2$); **^{13}C-NMR** (100 MHz, DMSO-d_6, δ_C, ppm): 160.8, 155.3, 152.6, 146.6, 146.5, 139.0, 135.3, 129.9, 128.4, 127.5, 127.2, 126.4, 125.8, 125.3, 125.1, 122.0, 120.2, 112.6, 52.1, 41.7, 23.5, 23.1, 19.1; **MS (ESI)** *m/z*: 507.9 $[M+H]^+$; Anal. Calcd. for $C_{25}H_{25}N_5O_5S$: C, 59.16; H, 4.96; N, 13.80. Found: C, 59.39; H, 5.05; N, 13.93 %.

4-amino-N-{1-[3-(3-nitrophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl]-3-methylbutyl}-benzenesulfonamide **(5i)**: White solid; **mp**: 176-185 °C; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3376, 3258 (N–H str.), 3061, 2950 (C–H str.), 1685 (C═O str.), 1617, 1591, 1498 (C═N, C═C str.), 1311, 1146 (S═O str.), 1532, 1353 (N═O str.); **^{1}H-NMR** (400 MHz, DMSO-d_6, δ_H, ppm): 8.54 (s, 1H, Ar*H*), 8.16 (d, J = 8 Hz, 1H, Ar*H*), 7.93-7.78 (m, 4H, SO_2NH & Ar*H*), 7.71-7.48 (m, 3H, Ar*H*), 7.36 (d, J = 8.4 Hz, 2H, Ar*H*), 6.69 (d, J = 8.4 Hz, 2H, Ar*H*), 5.88 (s, 2H, Ar–NH_2), 3.97 (m, 1H, NH–C*H*), 1.51-1.37 (m, 3H, C*H*–CH_2), 0.78, 0.50 (d, J = 6 Hz, 6H, CH–

(C<u>*H*</u>$_3$)$_2$); **^{13}C-NMR** (100 MHz, DMSO-d_6, δ_C, ppm): 160.7, 155.5, 152.4, 148.5, 146.5, 137.9, 135.3, 132.2, 131.9, 128.3, 127.6, 127.2, 126.4, 124.9, 124.3, 122.0, 120.2, 112.6, 52.3, 41.8, 23.5, 23.1, 19.1; **MS (ESI)** *m/z*: 508.0 $[M+H]^+$; Anal. Calcd. for $C_{25}H_{25}N_5O_5S$: C, 59.16; H, 4.96; N, 13.80. Found: C, 59.34; H, 5.02; N, 13.91 %.

4-amino-N-{1-[3-(4-nitrophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl]-3-methylbutyl}-benzenesulfonamide **(5j):** White solid; **mp**: 204-209 °C; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3374, 3248 (N–H str.), 3060, 2858 (C–H str.), 1689 (C═O str.), 1613, 1595, 1497 (C═N, C═C str.), 1310, 1149 (S═O str.), 1535, 1354 (N═O str.); **^{1}H-NMR** (400 MHz, DMSO-d_6, δ_H, ppm): 8.51 (d, *J* = 8.8 Hz, 2H, Ar<u>*H*</u>), 8.36 (d, *J* = 8.8 Hz, 2H, Ar<u>*H*</u>), 8.14 (d, *J* = 7.6 Hz, 1H, Ar<u>*H*</u>), 7.89-7.84 (m, 2H, SO_2N<u>*H*</u> & Ar<u>*H*</u>), 7.74 (t, *J* = 7.6 Hz, 1H, Ar<u>*H*</u>), 7.51 (d, *J* = 7.6 Hz, 1H, Ar<u>*H*</u>), 7.37 (d, *J* = 8.8 Hz, 2H, Ar<u>*H*</u>), 6.73 (d, *J* = 8.8 Hz, 2H, Ar<u>*H*</u>), 5.79 (s, 2H, Ar–N<u>*H*</u>$_2$), 4.08 (m, 1H, NH–C<u>*H*</u>), 1.52-1.38 (m, 3H, C<u>*H*</u>–C<u>*H*</u>$_2$), 0.79, 0.51 (d, *J* = 6 Hz, 6H, CH–(C<u>*H*</u>$_3$)$_2$); **^{13}C-NMR** (100 MHz, DMSO-d_6, δ_C, ppm): 160.8, 155.5, 152.6, 147.7, 146.5, 141.2, 135.2, 130.9, 128.4, 127.6, 127.1, 126.5, 125.1, 124.8, 120.1, 112.8, 52.3, 41.8, 23.5, 23.1, 19.1; **MS (ESI)** *m/z*: 508.1 $[M+H]^+$; Anal. Calcd. for $C_{25}H_{25}N_5O_5S$: C, 59.16; H, 4.96; N, 13.80. Found: C, 59.38; H, 5.04; N, 13.92 %.

4-amino-N-{1-[3-(2-chlorophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl]-3-methylbutyl}-benzenesulfonamide **(5k)**: White solid; **mp**: 196-201 °C; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3367, 3255 (N–H str.), 3051, 2855 (C–H str.), 1682 (C═O str.), 1620, 1596, 1496 (C═N, C═C str.), 1319, 1147 (S═O str.), 750 (C–Cl str.); **^{1}H-NMR** (400 MHz, DMSO-d_6, δ_H, ppm): 8.08 (d, *J* = 8 Hz, 1H, Ar<u>*H*</u>), 7.91-7.81 (m, 3H, SO_2N<u>*H*</u> & Ar<u>*H*</u>), 7.74-7.59 (m, 3H, Ar<u>*H*</u>), 7.51-7.47 (m, 2H, Ar<u>*H*</u>), 7.34 (d, *J* = 8.8 Hz, 2H, Ar<u>*H*</u>), 6.72 (d, *J* = 8.8 Hz, 2H, Ar<u>*H*</u>), 5.87 (s, 2H, Ar–N<u>*H*</u>$_2$), 3.78 (m, 1H, NH–C<u>*H*</u>), 1.56-1.30 (m, 3H, C<u>*H*</u>–C<u>*H*</u>$_2$), 0.73, 0.52 (d, *J* = 6.4 Hz, 6H, CH–(C<u>*H*</u>$_3$)$_2$); **^{13}C-NMR** (100 MHz, DMSO-d_6, δ_C, ppm): 161.1, 157.0, 152.6, 146.6, 135.0, 134.9, 132.4, 131.4, 130.2, 129.8, 128.8, 128.6, 127.3, 127.0, 126.3, 125.1, 120.4, 112.6, 51.8, 42.2, 23.5, 23.1, 19.1; **MS (ESI)** *m/z*: 497.0 $[M+H]^+$, 499.0$[(M+H)+2]^+$; Anal. Calcd. for $C_{25}H_{25}ClN_4O_3S$: C, 60.41; H, 5.07; N, 11.27. Found: C, 60.71; H, 5.15; N, 11.41 %.

4-amino-N-{1-[3-(3-chlorophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl]-3-methylbutyl}-benzenesulfonamide **(5l)**: White solid; **mp**: 157-164 °C; α_{obs} = -27.6 (AcOH); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3365, 3252 (N–H str.), 3050, 2861 (C–H str.), 1680 (C═O str.), 1625, 1594, 1499 (C═N, C═C str.), 1312, 1144 (S═O str.), 746 (C–Cl str.); **^{1}H-NMR** (400 MHz, DMSO-d_6, δ_H, ppm): 8.09 (d, *J* = 8.8 Hz, 1H, Ar<u>*H*</u>), 7.84-7.75 (m, 3H, SO_2N<u>*H*</u> & ArH), 7.62-7.48 (m, 3H, Ar<u>*H*</u>), 7.44-7.39 (m, 2H, Ar<u>*H*</u>), 7.29 (d, *J* = 8.8 Hz, 2H, Ar<u>*H*</u>), 6.71 (d, *J* = 8.8 Hz, 2H, Ar<u>*H*</u>), 5.68 (s, 2H, Ar–N<u>*H*</u>$_2$), 3.74 (m, 1H, NH–C<u>*H*</u>), 1.57-1.32 (m, 3H, C<u>*H*</u>–C<u>*H*</u>$_2$), 0.75, 0.51 (d, *J* = 6.4 Hz, 6H, CH–(C<u>*H*</u>$_3$)$_2$); **^{13}C-NMR** (100 MHz, DMSO-d_6, δ_C, ppm): 161.1, 157.2, 152.5, 146.6, 135.7, 134.9, 132.8, 132.3, 131.3, 130.8, 128.5, 128.3, 127.2, 127.1, 126.2, 125.0, 120.3, 112.6, 51.8, 42.2, 23.5, 23.1, 19.1; **MS (ESI)** *m/z*: 496.9 $[M+H]^+$, 498.9 $[(M+H)+2]^+$; Anal. Calcd. for $C_{25}H_{25}ClN_4O_3S$: C, 60.41; H, 5.07; N, 11.27. Found: C, 60.67; H, 5.13; N, 11.38 %.

4-amino-N-{1-[3-(4-chlorophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl]-3-methylbutyl}-benzenesulfonamide **(5m):** White solid; **mp**: 183-188 °C; α_{obs} = -41.9 ($CHCl_3$); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3375, 3250 (N–H str.), 3055, 2958 (C–H str.), 1681 (C═O str.), 1628, 1597, 1504 (C═N, C═C str.), 1310, 1148 (S═O str.), 744 (C–Cl str.); **^{1}H-NMR** (400 MHz, DMSO-d_6,

δ_H, ppm): 8.09 (d, *J* = 7.6 Hz, 1H, Ar*H*), 7.91-7.84 (m, 2H, SO_2NH & Ar*H*), 7.70 (d, *J* = 8.4 Hz, 1H, Ar*H*), 7.63-7.52 (m, 3H, Ar*H*), 7.29 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.21 (dd, *J* = 8.4, 2.4 Hz, 2H, Ar*H*), 6.41 (d, *J* = 8.4 Hz, 2H, Ar*H*), 5.84 (s, 2H, Ar–NH_2), 3.70 (m, 1H, NH–C*H*), 1.57-1.23 (m, 3H, C*H*–CH_2), 0.69, 0.51 (d, *J* = 6.4 Hz, 6H, CH–(CH_3)$_2$); **^{13}C-NMR** (100 MHz, DMSO-d_6, δ_C, ppm): 161.0, 157.4, 152.4, 146.9, 134.8, 134.8, 134.0, 131.3, 130.7, 128.6, 127.0, 126.9, 126.3, 125.0, 120.2, 112.4, 51.8, 42.2, 23.5, 23.1, 19.1; **MS (ESI)** *m/z*: 496.8 $[M+H]^+$, 498.8 $[(M+H)+2]^+$; Anal. Calcd. for $C_{25}H_{25}ClN_4O_3S$: C, 60.41; H, 5.07; N, 11.27. Found: C, 60.69; H, 5.16; N, 11.40 %.

4-amino-N-{1-[3-(2-bromophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl]-3-methylbutyl}-benzenesulfonamide **(5n)**: White solid; **mp**: 167-171 °C; α_{obs} = -18.3 (AcOH); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3368, 3254 (N–H str.), 3059, 2955 (C–H str.), 1684 (C═O str.), 1624, 1590, 1503 (C═N, C═C str.), 1313, 1153 (S═O str.), 610 (C–Br str.); **^{1}H-NMR** (400 MHz, DMSO-d_6, δ_H, ppm): 8.12 (dd, *J* = 8, 1.2, 1H, Ar*H*), 7.89-7.78 (m, 3H, SO_2NH & Ar*H*), 7.71 (d, *J* = 8, 1H, Ar*H*), 7.64-7.58 (m, 2H, Ar*H*), 7.51-7.48 (m, 2H, Ar*H*), 7.33 (d, *J* = 8.4 Hz, 2H, Ar*H*), 6.51 (d, *J* = 8.4 Hz, 2H, Ar*H*), 5.88 (s, 2H, Ar–NH_2), 3.66 (m, 1H, NH–C*H*), 1.55-1.33 (m, 3H, C*H*–CH_2), 0.75, 0.53 (d, *J* = 6.4 Hz, 6H, CH–(CH_3)$_2$); **^{13}C-NMR** (100 MHz, DMSO-d_6, δ_C, ppm): 161.1, 157.0, 152.4, 146.4, 135.0, 134.6, 133.6, 131.3, 130.8, 129.5, 128.6, 127.3, 127.2, 126.4, 124.7, 122.2, 120.4, 112.4, 52.2, 42.0, 23.5, 23.1, 19.1; **MS (ESI)** *m/z*: 540.8 $[M+H]^+$, 542.8 $[(M+H)+2]^+$; Anal. Calcd. for $C_{25}H_{25}BrN_4O_3S$: C, 55.46; H, 4.65; N, 10.35. Found: C, 55.71; H, 4.76; N, 10.48 %.

4-amino-N-{1-[3-(3-bromophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl]-3-methylbutyl}-benzenesulfonamide **(5o)**: White solid; **mp**: 184-189 °C; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3357, 3250 (N–H str.), 3057, 2926 (C–H str.), 1685 (C═O str.), 1621, 1594, 1500 (C═N, C═C str.), 1312, 1154 (S═O str.), 597 (C–Br str.); **^{1}H-NMR** (400 MHz, DMSO-d_6, δ_H, ppm): 8.11 (d, *J* = 8 Hz, 1H, Ar*H*), 7.88-7.79 (m, 2H, SO_2NH & Ar*H*), 7.74-7.69 (m, 3H, Ar*H*), 7.59-7.50 (m, 2H, Ar*H*), 7.45 (s, 1H, Ar*H*), 7.32 (d, *J* = 8.8 Hz, 2H, Ar*H*), 6.65 (d, *J* = 8.8 Hz, 2H, Ar*H*), 5.89 (s, 2H, Ar–NH_2), 3.62 (m, 1H, NH–C*H*), 1.56-1.35 (m, 3H, C*H*–CH_2), 0.77, 0.54 (d, *J* = 6.4 Hz, 6H, CH–(CH_3)$_2$); **^{13}C-NMR** (100 MHz, DMSO-d_6, δ_C, ppm): 159.9, 157.2, 152.6, 146.3, 137.2, 134.8, 132.2, 131.5, 131.2, 128.5, 127.9, 127.2, 127.2, 126.3, 124.8, 122.0, 120.3, 112.5, 51.8, 42.1, 23.5, 23.1, 19.1; **MS (ESI)** *m/z*: 541.0 $[M+H]^+$, 543.0 $[(M+H)+2]^+$; Anal. Calcd. for $C_{25}H_{25}BrN_4O_3S$: C, 55.46; H, 4.65; N, 10.35. Found: C, 55.69; H, 4.71; N, 10.47 %.

4-amino-N-{1-[3-(4-bromophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl]-3-methylbutyl}-benzenesulfonamide **(5p)**: White solid; **mp**: 193-199 °C; α_{obs} = -36.7 (AcOH); **FT-IR** (KBr, ν_{max}, cm^{-1}): 3367, 3258 (N–H str.), 3053, 2924 (C–H str.), 1682 (C═O str.), 1617, 1598, 1501 (C═N, C═C str.), 1317, 1151 (S═O str.), 619 (C–Br str.); **^{1}H-NMR** (400 MHz, DMSO-d_6, δ_H, ppm): 8.10 (d, *J* = 7.6 Hz, 1H, Ar*H*), 7.82-7.78 (m, 2H, SO_2NH & Ar*H*), 7.74 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.68 (d, *J* = 7.6 Hz, 1H, Ar*H*), 7.56 (t, *J* = 7.6 Hz, 1H, Ar*H*), 7.38 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.32 (d, *J* = 8.4 Hz, 2H, Ar*H*), 6.26 (d, *J* = 8.4 Hz, 2H, Ar*H*), 5.89 (s, 2H, Ar–NH_2), 3.65 (m, 1H, NH–C*H*), 1.58-1.36 (m, 3H, C*H*–CH_2), 0.78, 0.57 (d, *J* = 6.4 Hz, 6H, CH–(CH_3)$_2$); **^{13}C-NMR** (100 MHz, DMSO-d_6, δ_C, ppm): 160.0, 157.3, 152.4, 146.3, 137.7, 135.4, 134.8, 131.0, 127.2, 127.1, 126.5, 126.3, 124.7, 122.9, 120.4, 112.4, 51.8, 42.1, 23.5,

23.1, 19.1; **MS (ESI)** *m/z*: 540.9 $[M+H]^+$, 542.9 $[(M+H)+2]^+$; Anal. Calcd. for $C_{25}H_{25}BrN_4O_3S$: C, 55.46; H, 4.65; N, 10.35. Found: C, 55.72; H, 4.74; N, 10.48 %.

4-amino-N-{1-[3-(2-fluorophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl]-3-methylbutyl}-benzenesulfonamide **(5q)**: White solid; **mp**: 166-170 °C; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3364, 3259 (N–H str.), 3059, 2859 (C–H str.), 1683 (C═O str.), 1622, 1593, 1504 (C═N, C═C str.), 1319, 1152 (S═O str.), 1213 (C–F str.); **^{1}H-NMR** (400 MHz, DMSO-d_6, δ_H, ppm): 8.09 (d, *J* = 8 Hz, 1H, Ar*H*), 7.83-7.72 (m, 3H, SO_2NH & Ar*H*), 7.68-7.56 (m, 2H, Ar*H*), 7.34 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.32-7.18 (m, 3H, Ar*H*), 6.60 (d, *J* = 8.4 Hz, 2H, Ar*H*), 5.84 (s, 2H, Ar–NH_2), 3.77 (m, 1H, NH–C*H*), 1.52-1.34 (m, 3H, C*H*–CH_2), 0.77, 0.54 (d, *J* = 6 Hz, 6H, CH–$(CH_3)_2$); **^{13}C-NMR** (100 MHz, DMSO-d_6, δ_C, ppm): 161.4, 158.2, 156.5, 152.6, 146.6, 135.7, 134.9, 130.6, 129.3, 128.6, 128.3, 128.2, 127.1, 126.3, 125.2, 120.3, 114.7, 112.2, 52.0, 41.8, 23.5, 23.1, 19.1; **MS (ESI)** *m/z*: 480.9 $[M+H]^+$; Anal. Calcd. for $C_{25}H_{25}FN_4O_3S$: C, 62.48; H, 5.24; N, 11.66. Found: C, 62.76; H, 5.32; N, 11.79 %.

4-amino-N-{1-[3-(4-fluorophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl]-3-methylbutyl}-benzenesulfonamide **(5r)**: White solid; **mp**: 201-208 °C; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3375, 3256 (N–H str.), 3059, 2862 (C–H str.), 1686 (C═O str.), 1625, 1596, 1502 (C═N, C═C str.), 1316, 1150 (S═O str.), 1206 (C–F str.); **^{1}H-NMR** (400 MHz, DMSO-d_6, δ_H, ppm): 8.10 (d, *J* = 7.6 Hz, 1H, Ar*H*), 7.84-7.64 (m, 4H, SO_2NH & Ar*H*), 7.42 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.36 (d, *J* = 8.4 Hz, 2H, Ar*H*), 7.28 (d, *J* = 8.8 Hz, 2H, Ar*H*), 6.73 (d, *J* = 8.4 Hz, 2H, Ar*H*), 5.91 (s, 2H, Ar–NH_2), 3.76 (m, 1H, NH–C*H*), 1.55-1.34 (m, 3H, C*H*–CH_2), 0.79, 0.57 (d, *J* = 6.4 Hz, 6H, CH–$(CH_3)_2$); **^{13}C-NMR** (100 MHz, DMSO-d_6, δ_C, ppm): 161.1, 160.9, 156.8, 152.5, 146.6, 134.7, 132.3, 128.6, 128.4, 127.0, 126.9, 126.2, 125.2, 120.3, 116.6, 112.2, 52.0, 41.9, 23.5, 23.1, 19.1; **MS (ESI)** *m/z*: 481.0 $[M+H]^+$, 502.9 $[M+Na]^+$; Anal. Calcd. for $C_{25}H_{25}FN_4O_3S$: C, 62.48; H, 5.24; N, 11.66. Found: C, 62.72; H, 5.31; N, 11.77 %.

4-amino-N-[1-(3-anilino-4-oxo-3,4-dihydroquinazolin-2-yl)-3-methylbutyl]benzene-sulfonamide **(5s)**: White solid; **mp**: 198-203 °C; **FT-IR** (KBr, ν_{max}, cm^{-1}): 3350, 3252 (N–H str.), 3051, 2920 (C–H str.), 1692 (C═O str.), 1612, 1596, 1496 (C═N, C═C str.), 1314, 1152 (S═O str.); **^{1}H-NMR** (400 MHz, DMSO-d_6, δ_H, ppm): 8.93 (s, 1H, N*H*–Ar), 8.09 (d, *J* = 8 Hz, 1H, Ar*H*), 7.83-7.73 (m, 3H, SO_2NH & Ar*H*), 7.55 (t, *J* = 8 Hz, 1H, Ar*H*), 7.35 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.19 (d, *J* = 8 Hz, 2H, Ar*H*), 6.84 (t, *J* = 8 Hz, 1H, Ar*H*), 6.56 (d, *J* = 8 Hz, 2H, Ar*H*), 6.43 (d, *J* = 8.8 Hz, 2H, Ar*H*), 5.92 (s, 2H, Ar–NH_2), 4.17 (m, 1H, NH–C*H*), 1.57-1.39 (m, 3H, C*H*–CH_2), 0.79, 0.56 (d, *J* = 6 Hz, 6H, CH–$(CH_3)_2$); **^{13}C-NMR** (100 MHz, DMSO-d_6, δ_C, ppm): 161.1, 156.9, 152.5, 146.8, 146.5, 134.8, 129.4, 128.6, 127.9, 127.2, 126.3, 124.9, 120.3, 120.2, 112.6, 112.5, 52.1, 42.0, 23.5, 23.1, 19.1; **MS (ESI)** *m/z*: 478.0 $[M+H]^+$; Anal. Calcd. for $C_{25}H_{27}N_5O_3S$: C, 62.87; H, 5.70; N, 14.66. Found: C, 63.06; H, 5.78; N, 14.79 %.

7.2 In vitro antimalarial assay

A positive control with reference antimalarial drugs (Chloroquine, Pyrimethamine and Trimethoprime) in standard concentrations was used in each experiment. The stock solutions were further diluted in complete medium (RPMI 1640 plus 10% human serum) to each of the

used concentrations (0.0001 up to 100 mg/mL in seven dilutions). The halfmaximal inhibitory (IC_{50}) responses as compared to the drug-free controls were estimated by interpolation using Microcal Origin software. Each duplicate experiment was repeated three times and blood smears were read blind [38]. Results from above study are summarized in Table 5 as IC_{50} values.

7.3 The Parasite Lactate Dehydrogenase assay (p-LDH)

The *in vitro* antiplasmodial effect of synthetic Quinazolinone-Sulphonamide hybrids on parasite growth is tested by the quantitative enzyme-based method (Malstat® assay) involving spectrophotometric measurement of the parasite lactate dehydrogenase activity (*p*-LDH) as described by Makler and Hinrichs [39], at 650 nm using a micro plate reader (EL 800, Biotech Instruments Inc.). The simultaneous control parasite cultures devoid of drug were referred to as having 100 % *p*-LDH activity. The inhibition of each drug concentration was calculated as compared to the untreated control obtain the IC_{50} values.

7.4 The Tetrazolium based colorimetric assay (MTT-assay)

The *in vitro* antiplasmodial and cytotoxic effect of Quinazolinone-Sulphonamide hybrids on parasite growth and normal uninfected RBCs was tested by the quantitative enzyme based method (MTT assay) involving spectrophotometric measurement of the host RBC's *Mitochondrial Succinate Dehydrogenase* catalysed MTT reduction as described by Ayisi, N.K. *et al*. [40], at 565 & 690 nm using a micro plate reader (EL 800, Biotech Instruments Inc.). The simultaneous control RBC cultures devoid of drug and parasite were referred to as having maximum enzyme activity. The inhibition and cytotoxicity of each drug concentration was calculated as compared to the untreated, uninfected and cell free controls to obtain the EC_{50} and CC_{50} values. Finally, the ratio, CC_{50}/EC_{50} was calculated to obtain selective indices (SI), showing that the drug is only selective against parasite.

7.5 DHFR inhibitory activity

The inhibitory activity was carried out using bovine liver DHFR as model enzymes resembling to *Pf*-DHFR [41]. The utilized assay mixture contained 50 mM Tris-HCl buffer (pH 7.4), 50 μM NADPH, 20 μL DMSO or the same volume of DMSO solution containing the test compounds to a final concentration of 10^{-11}-10^{-5} M, and 0.02 units of bovine liver DHFR, in a final volume of 2.0 ml. After addition of the enzyme, the mixture was incubated at room temperature for 2.0 min, and the reaction was initiated by adding 25 μM FH2, the change in absorbance (ΔA/min) was measured at 340 nm. The activity under these conditions was linear for 10 min [41]. Results are reported as % inhibition of enzymatic activity calculated using the following formula:

$$\% \text{ Inhibition} = \left(1 - \frac{\Delta A/min_{test}}{\Delta A/min_{DMSO}}\right) \times 100$$

The % inhibition values were plotted versus drug concentration (log scale). The 50% inhibitory concentration (IC_{50}) of each compound was obtained using the Graph Pad Prism program, version 3 (San Diego, CA).

7.6 Cytotoxicity study by MTT assay

MTT assay was used to determine the inhibition of vero cell proliferation by the synthesized entities using Promega CellTiter 96 Non-radioactive Cell Proliferation Assay (Promega, Madison, WI, USA). The viability was assessed on the basis of cellular conversion of MTT into a formazan by the vero cells after 72 hours of incubation at 37 °C and the activity was assessed by measuring the absorbance at 540 nm followed by the calculation of percentage cell viability.

Percentage cell viability = $\left[100 - \left(\frac{A_o - A_t}{A_o}\right) \times 100\right]$

Where, A_o = Absorbance of cells treated with 0.1% DMSO medium, A_t = Absorbance of cells treated with various concentration of the samples.

Each treatment was performed in triplicate and the 50% inhibitory concentration (IC_{50}) of each compound was obtained using the Graph Pad Prism program, version 3 (San Diego, CA).

7.7 Computational studies

The molecular structures of all the compounds were drawn using ChemBioDraw Ultra 14.0 (www.cambridgesoft.com). These structures were then imported into Maestro implemented in Schrödinger, further energy of the 3D structures was minimized using Ligprep 3.3 module. The possible Lewis structure, tautomers and ionization states (pH 7.0 ± 2.0) for each of these compounds were generated and optimized with default settings (Ligprep 3.3, Schrödinger, LLC, New York, NY, 2015). The crystal structure of mutant *Pf*-DHFR (PDB ID: 4DPD) and *h*-DHFR (PDB ID: 1MVT) was extracted from protein data bank (www.rcsb.org). The protein structure was further refined and hydrogen atoms were added to the structure utilizing Protein Preparation Wizard (Maestro 10.1 Schrödinger, LLC, New York, NY, 2015). The binding site in the protein molecules was predicted using Sitemap 3.4 wizard and further the centre of the grid was defined, which was generated using Glide 6.6 (Schrödinger, LLC, New York, NY, 2015) with default settings for all parameters. The grid size was kept sufficiently high so as to include the atoms participating in the interaction and then all the compounds were docked against the grid of prepared receptor (mutant *Pf*-DHFR & *h*-DHFR) using Glide in XP (Extra Precession) mode [42] with other default setting for scoring function.

7.8 ADMET prediction method

The pharmacokinetic profile of the test compounds showing good antimalarial activity were predicted by using programs Qikprop v4.3 (Schrödinger, Inc., New York, NY, 2015). The compounds prepared by LigPrep 3.3 were utilized for the calculation of pharmacokinetic parameters by QikProp v4.3. The program QikProp v4.3, utilizes the method of Jorgensen [43] to compute pharmacokinetic properties and descriptors such as octanol/water partitioning coefficient, aqueous solubility, brain/blood partition coefficient, intestinal wall permeability, plasma protein binding and others.

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Fig. 3 The binding model of docked compound **5l** with *Pf*-DHFR enzyme. **(a)** 2D model of binding pose with bond length and interacting amino acid **(b)** 3D model of ligand bonded to active site, the protein molecules are displayed as cartoon and ligand as ball and stick

Fig. 4 The binding model of docked compound **5m** with *Pf*-DHFR enzyme. **(a)** 2D model of binding pose with bond length and interacting amino acid **(b)** 3D model of ligand bonded to active site, the protein molecules are displayed as cartoon and ligand as ball and stick

Fig. 5 The binding model of docked compound **5n** with *Pf*-DHFR enzyme. **(a)** 2D model of binding pose with bond length and interacting amino acid **(b)** 3D model of ligand bonded to active site, the protein molecules are displayed as cartoon and ligand as ball and stick

Fig. 6 The binding model of docked compound **5p** with *Pf*-DHFR enzyme. **(a)** 2D model of binding pose with bond length and interacting amino acid **(b)** 3D model of ligand bonded to active site, the protein molecules are displayed as cartoon and ligand as ball and stick

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Table 1 Optimization of reaction condition under conventional heating for synthesis of compound **4a**

Entry	**Solvent**	**Catalyst**	**Conventional Method**		
			Temp. [°C]	**Time [min.]**	**Yield [%]**
1	Toluene	PCl_3 [1 equiv.]	110	120	52
2	Xylene	PCl_3 [1 equiv.]	130	120	56
3	CH_2Cl_2	PCl_3 [1 equiv.]	40	180	42
4	CH_3CN	PCl_3 [1 equiv.]	50	120	66
5	THF	PCl_3 [1 equiv.]	60	30	81
6	Pyridine	PCl_3 [1 equiv.]	80	30	65
7	TEAA	—	100	14	90

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Table 2 Optimization of microwave promoted reaction condition for synthesis of compound **4a**

Entry	**Solvent**	**Catalyst**	**Microwave Irradiation Method**		
			Power [Watt]	**Time [min.]**	**Yield [%]**
1	CH_2Cl_2	PCl_3 [1 equiv.]	210	10	42
2	CH_3CN	PCl_3 [1 equiv.]	245	8	55
3	THF	PCl_3 [1 equiv.]	245	5	82
4	THF	PCl_3 [1 equiv.]	280	3	86
5	THF	PCl_3 [1 equiv.]	350	3	87
6	THF	PCl_3 [1 equiv.]	420	2	81
7	Pyridine	PCl_3 [1 equiv.]	350	4	51
8	Pyridine	PCl_3 [1 equiv.]	420	4	47
9	TEAA	—	350	2.5	96
10	TEAA	—	420	2.5	96

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Table 3 Synthesis of quinazolinone-sulfonamide hybrids using TEAA **4a-4s**

Compound	**(R_3)**	**Microwave Conditions (350 Watts)**	
		Reaction Time	**Yield [%]**
4a	$–C_6H_5$	3 min.	96
4b	$–C_6H_4$–(2–Me)	3 min.	91
4c	$–C_6H_4$–(3–Me)	3 min.	93
4d	$–C_6H_4$–(4–Me)	3 min.	92
4e	$–C_6H_4$–(2–OMe)	3 min.	87
4f	$–C_6H_4$–(3–OMe)	3 min.	85
4g	$–C_6H_4$–(4–OMe)	3 min.	90
4h	$–C_6H_4$–(2–NO_2)	4 min.	80
4i	$–C_6H_4$–(3–NO_2)	3 min.	82
4j	$–C_6H_4$–(4–NO_2)	3 min.	81
4k	$–C_6H_4$–(2–Cl)	3 min.	87
4l	$–C_6H_4$–(3–Cl)	3 min.	91
4m	$–C_6H_4$–(4–Cl)	3 min.	89
4n	$–C_6H_4$–(2–Br)	3 min.	85
4o	$–C_6H_4$–(3–Br)	3 min.	90
4p	$–C_6H_4$–(4–Br)	3 min.	87
4q	$–C_6H_4$–(2–F)	3 min.	85
4r	$–C_6H_4$–(4–F)	3 min.	88
4s	–NH–C_6H_5	3 min.	74

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Table 4 Synthesis of quinazolinone-sulfonamide hybrids **5a-s**

Compound	**(R_3)**	**Method-I: CH**		**Method-II: MW (210 Watts)**	
		Reaction Time	**Yield [%]**	**Reaction Time**	**Yield [%]**
5a	$–C_6H_5$	45 min.	73	4 min.	81
5b	$–C_6H_4–$(2–Me)	45 min.	66	4 min.	70
5c	$–C_6H_4–$(3–Me)	45 min.	70	4 min.	72
5d	$–C_6H_4–$(4–Me)	45 min.	73	4 min.	78
5e	$–C_6H_4–$(2–OMe)	75 min.	71	5 min.	73
5f	$–C_6H_4–$(3–OMe)	75 min.	69	7 min.	74
5g	$–C_6H_4–$(4–OMe)	75 min.	74	5 min.	82
5h	$–C_6H_4–$(2–NO_2)	60 min.	76	5 min.	77
5i	$–C_6H_4–$(3–NO_2)	60 min.	85	5 min.	79
5j	$–C_6H_4–$(4–NO_2)	60 min.	80	5 min.	83
5k	$–C_6H_4–$(2–Cl)	45 min.	75	4 min.	78
5l	$–C_6H_4–$(3–Cl)	45 min.	79	4 min.	81
5m	$–C_6H_4–$(4–Cl)	45 min.	82	4 min.	85
5n	$–C_6H_4–$(2–Br)	45 min.	73	4 min.	77
5o	$–C_6H_4–$(3–Br)	45 min.	77	4 min.	84
5p	$–C_6H_4–$(4–Br)	45 min.	81	4 min.	86
5q	$–C_6H_4–$(2–F)	45 min.	70	4 min.	73
5r	$–C_6H_4–$(4–F)	45 min.	78	4 min.	76
5s	$–NH–C_6H_5$	45 min.	65	5 min.	71

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Table 5 *In vitro* antimalarial assay (IC_{50}) values

Comp.	IC_{50} $\mu g/mL$	Comp.	IC_{50} $\mu g/mL$
5a	0.68	**5l**	**0.068**
5b	0.72	**5m**	**0.068**
5c	1.28	**5n**	**0.068**
5d	0.67	**5o**	0.78
5e	0.72	**5p**	**0.068**
5f	0.78	**5q**	1.012
5g	**0.068**	**5r**	1.12
5h	0.92	**5s**	1.052
5i	0.78	**Chloroquine**	0.020 µg/mL
5j	0.84	**Pyrimethamine**	0.25 µg/mL
5k	0.136	**Trimethoprime**	0.38 µg/mL

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Table 6 Antiplasmodial activity and cytotoxicity of the tested hybrids

Compd.	***p*-LDH Assay**	**MTT Assay (*P. falsiparum*)**		**SI**
	IC_{50} (ppm)	**EC_{50} (ppm)**	**CC_{50} (ppm)**	
5g	8317	1009	19630000	19454.9
5l	524.8	368.97	18660	50.57
5m	96.38	115.61	10140	87.71
5n	3380	2831	58240	20.57
5p	37240	1909	29310	15.35
ClQ	17.45	28.97	466.66	16.11

Selectivity Index = SI = CC50/EC50

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Table 7 Glide docking score and docking energies of the active entries and reference compound

Comp.	Docking result of *Pf*-DHFR			Docking result of *h*-DHFR		
	Glide score	Glide energy	RMSD* (Å)	Glide score	Glide energy	RMSD* (Å)
5g	-4.21	-43.08	0.9644	-3.22	-39.05	1.2254
5l	-3.41	-36.05	0.9433	-2.73	-31.03	0.9873
5m	-3.68	-34.56	0.9784	-3.05	-42.96	0.9914
5n	-3.65	-40.74	0.9294	-2.90	-40.29	1.5621
5p	-3.07	-39.99	0.9655	-2.50	-35.58	0.9733
TMP	-2.51	-28.25	—	-2.44	-26.54	—
PYM	-2.07	-28.36	—	-1.97	-26.21	—

* The standard deviation was calculated considering Trimethoprime as reference molecule; TMP: Trimethoprime; PYM: Pyrimethamine.

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Table 8 Prediction of Lipinski RO5 for active test entities

Comp.	mol_MW (<500amu)	donorHB (<5)	accptHB (<10)	QPlogPo/w (<5)	N of violation (<2)	#rotor (0–15)
5g	492.592	2.5	10.25	3.315	0	8
5l	497.010	2.5	09.5	3.389	0	7
5m	497.010	2.5	09.5	3.37	0	7
5n	541.461	2.5	09.5	3.252	1	7
5p	541.461	2.5	09.5	3.441	1	7
TMP	290.321	4	3	0.918	0	7
PYM	248.714	4	3	1.798	0	4

Mol_MW: Molecular Weight; donorHB: Hydrogen Bond Donor; accptHB: Hydrogen Bond Acceptor; QPlogPo/w: Partition Coefficient; #rotor: Rotatable Bonds; 'N' of violations: Number of Lipinski Rule Violations; TMP: Trimethoprime; PYM: Pyrimethamine.

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Table 9 Prediction of ADME parameters* of the active test compounds

Comp.	Percent Human Oral Absorption (>80% - high & <25% - poor)	QPPCaco (<25 poor, >500 great)	QPlogBB (-3.0 – 1.5)	QPlogKhsa (-1.5 – 1.5)	QPlogHERG (below -5)	QPlogS (-6.5 – 5)	PSA (70–200Å)
5g	91.451	330.915	-1.511	0.200	-6.443	-5.200	118.28
5l	94.528	464.792	-0.938	0.201	-5.228	-4.538	106.096
5m	94.058	443.958	-0.951	0.193	-5.235	-4.571	106.084
5n	81.306	498.179	-0.958	0.212	-5.272	-4.675	106.084
5p	81.520	443.953	-0.945	0.212	-5.272	-4.675	106.084
TMP	78.695	389.915	-1.126	-0.286	-3.882	-2.804	93.874
PYM	84.285	412.669	-0.770	-0.247	-4.263	-2.930	73.768

*Calculated using QikProp v 3.5. Range/recommended values calculated for 95% known drugs.

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Table 10 Enzyme inhibition assay and Cytotoxicity of 2,3-disubstituted quinazolinone*

Compounds	**DHFR inhibition (IC_{50} µg/mL)**	**Cytotoxicity Vero Cells (IC_{50} µg/mL)**	**S.I.**
5g	0.048±0.003	N.C.	312.50
5l	0.036±0.002	N.C.	416.66
5m	0.028±0.002	N.C.	535.71
5n	0.091±0.006	N.C.	164.83
5p	0.087±0.005	N.C.	172.14
TMP	0.042±0.003	N.T.	
PYM	0.035±0.002	N.T.	
CQ	0.010±0.0005	N.T.	

* The results indicated are average of triplicate, N.C.: No cytotoxicity observed up to a concentration of 15 µg/mL, N.T.: Not tested, S.I.: Selective Index (IC_{50} value of cytotoxicity assay/ IC_{50} value of enzyme inhibition assay)

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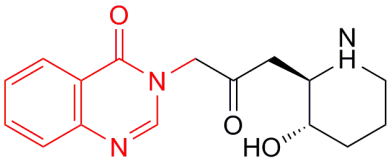

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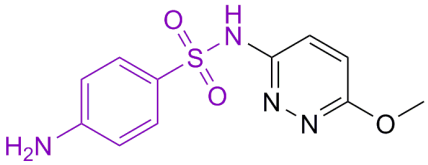

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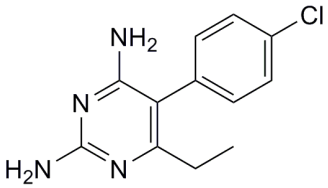

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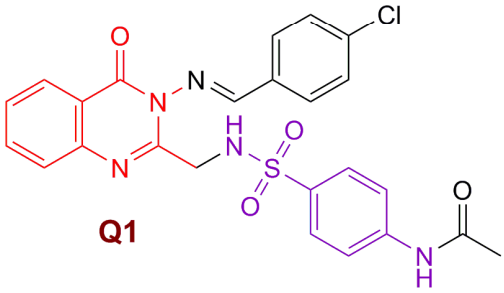
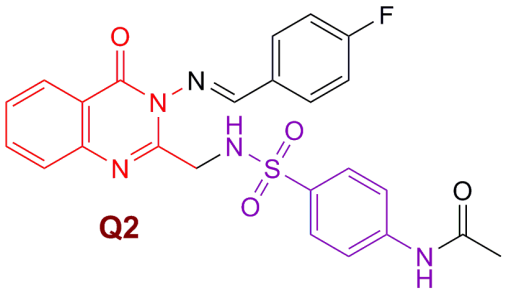
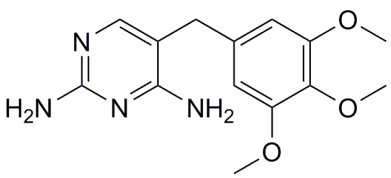


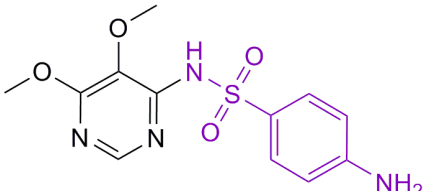
Q1



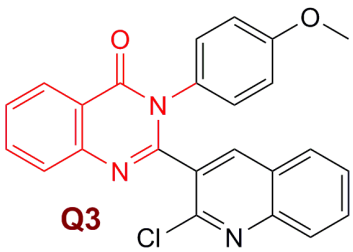
Q2



Trimethoprim



Sulfadoxine



Q3

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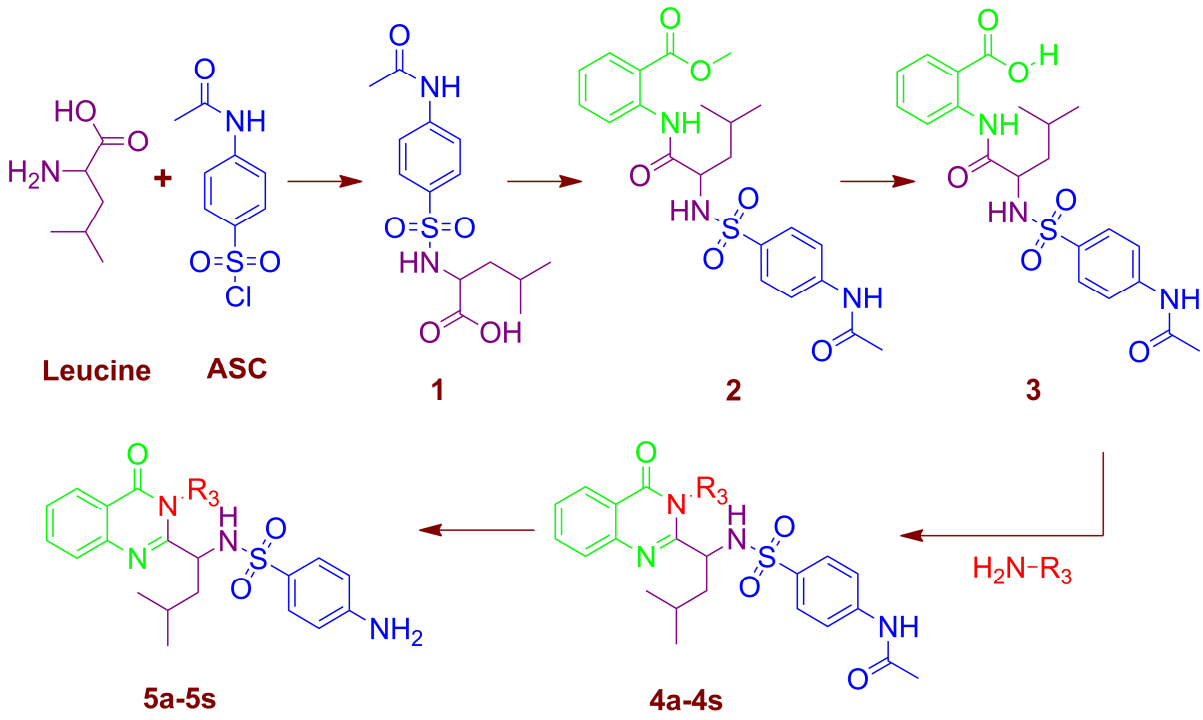

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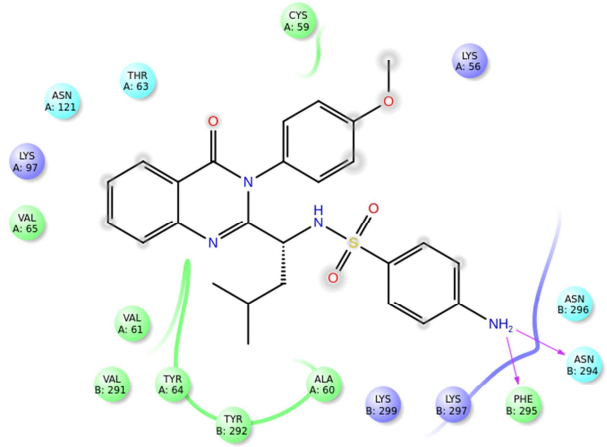
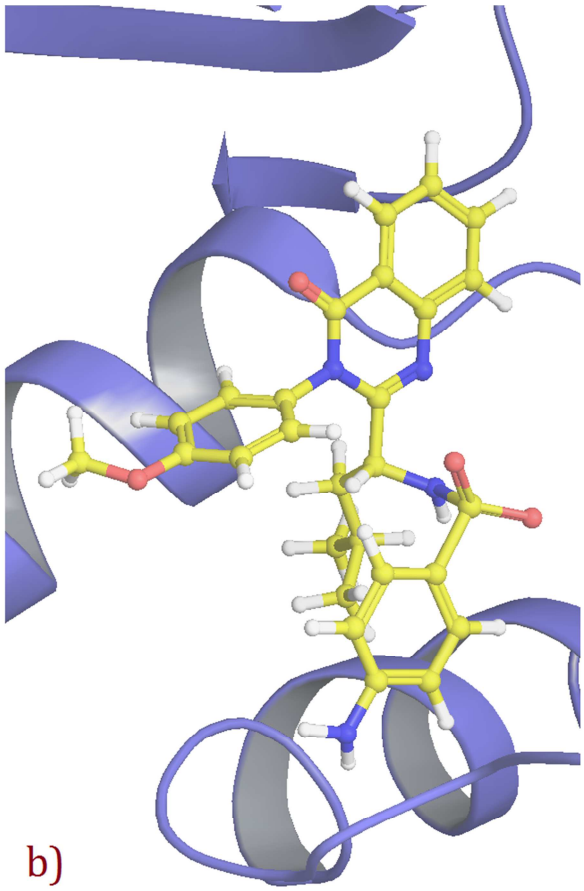


a)



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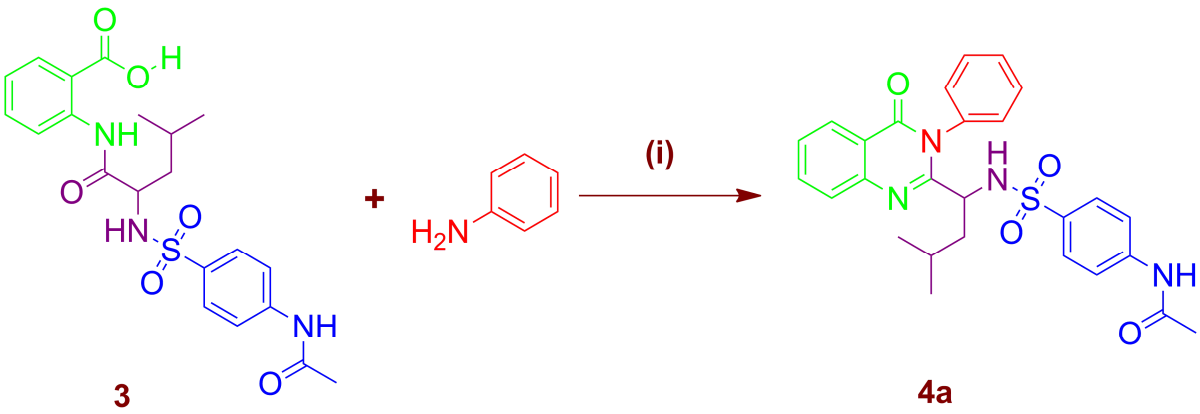

3 4a

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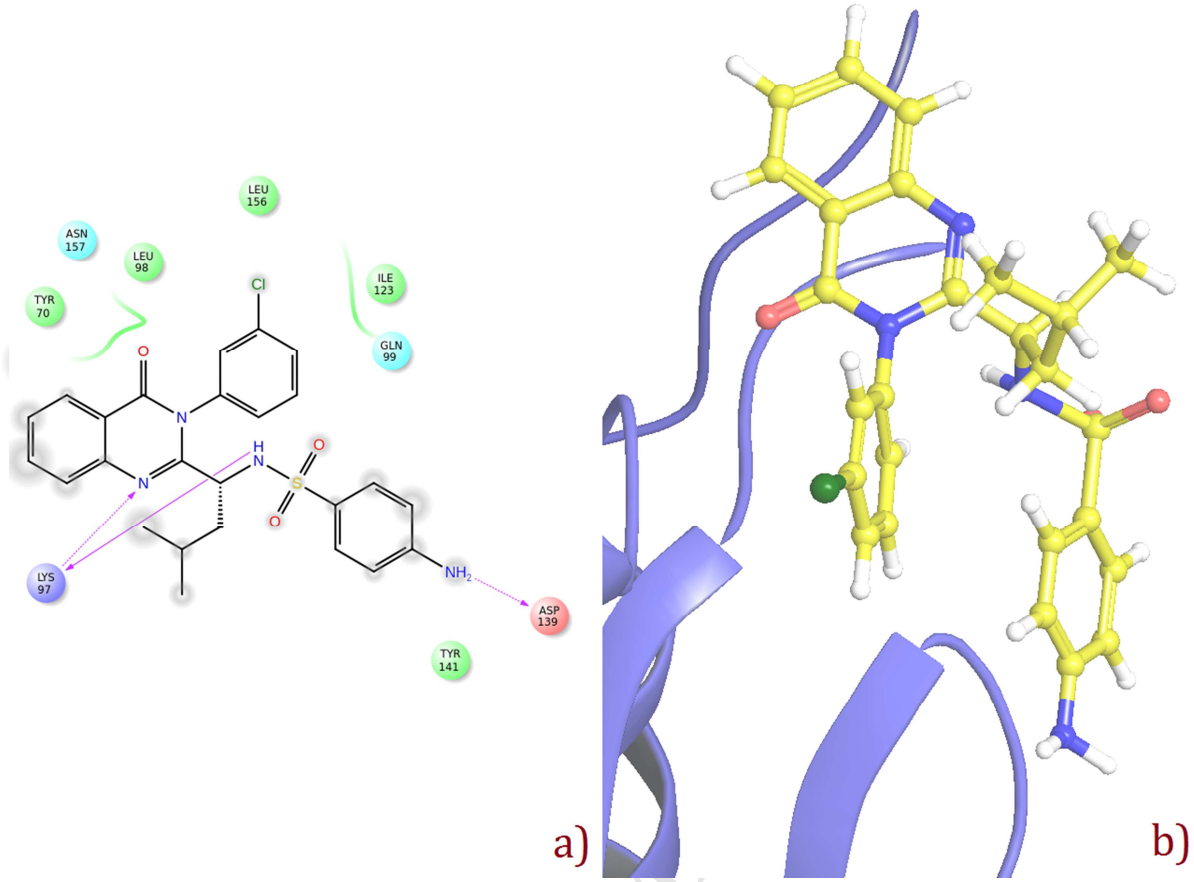

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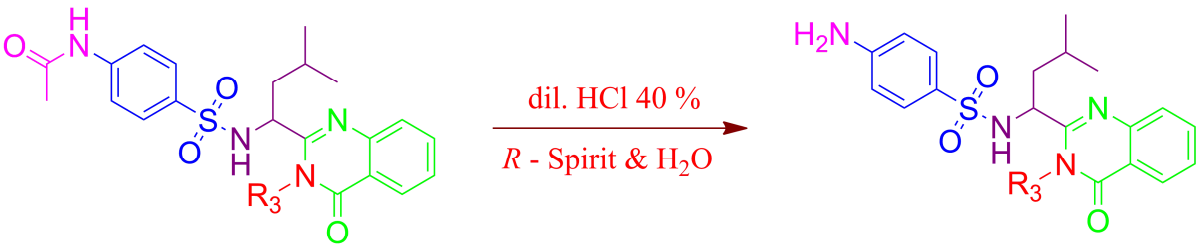

4a - 4s **5a - 5s**

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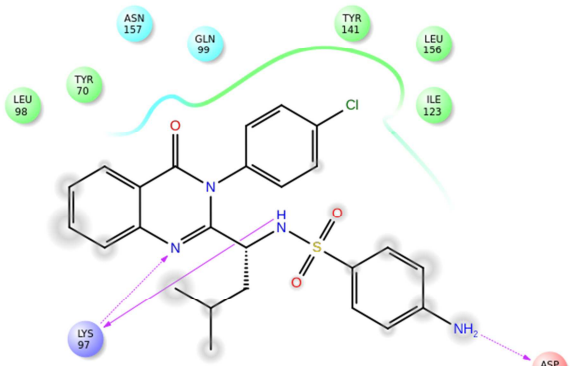
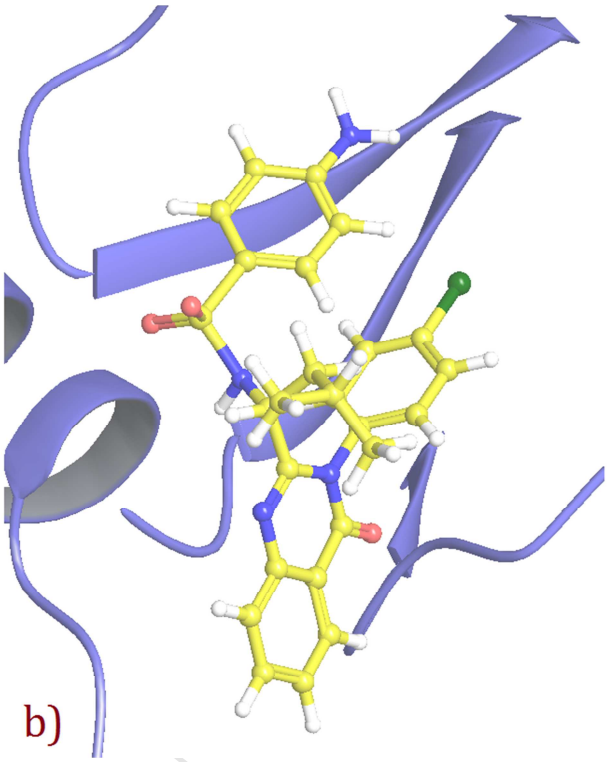


a)



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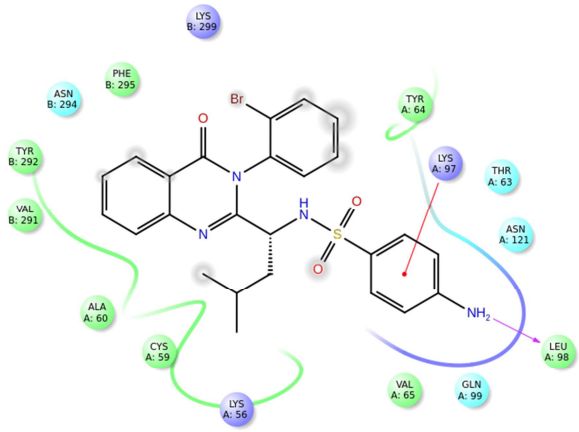

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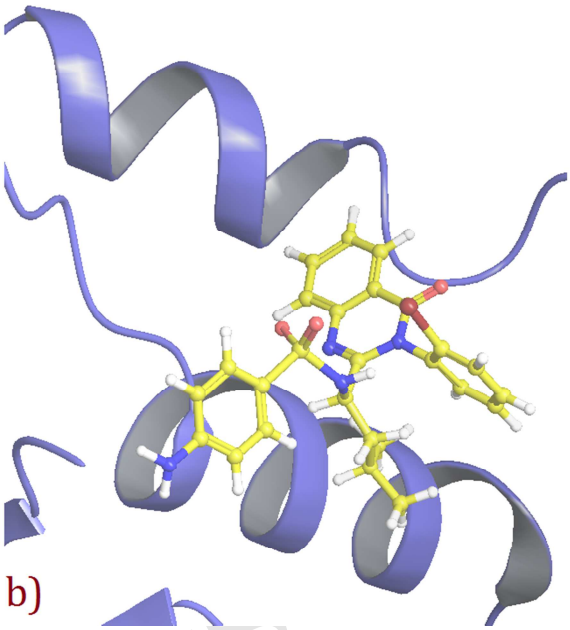

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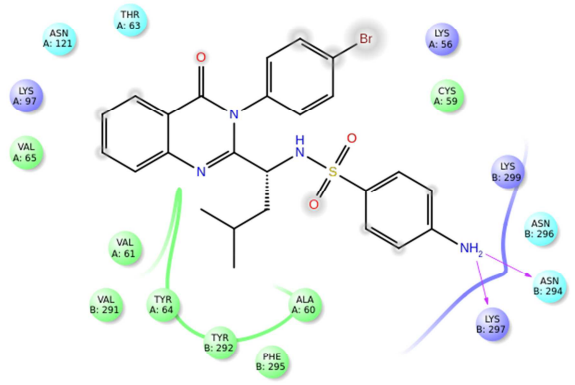

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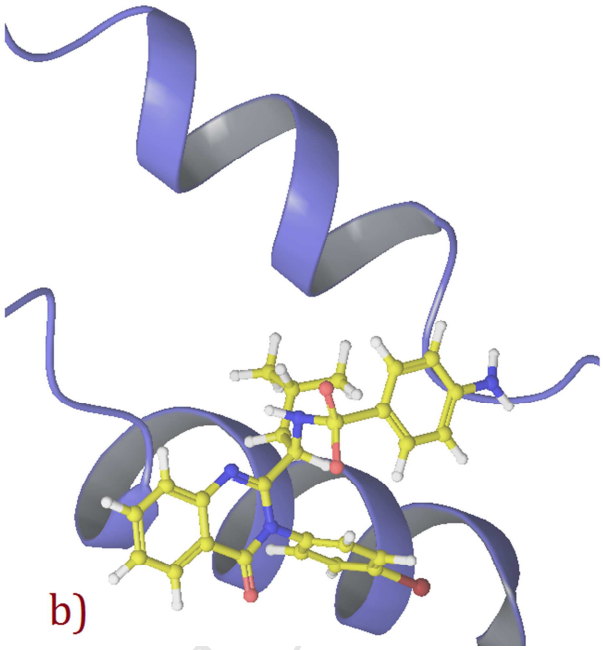

b)

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Highlights

- A modified methodology leading to synthesis of 2,3-disubstituted quinazolinones derived from of sulfonamide linked Leucine.
- Novel hybrid Leucine linked sulfonamide clubbed quinazoline-4(3*H*)-one molecules were synthesized under microwave irradiation using TEEA as reaction media.
- Antimalarial screening of quinazolinones and their DHFR inhibitory potency.
- Molecular docking of active quinazolinones at the active pocket of DHFR enzyme.
- Prediction of ADME parameters leading to oral bioavailability evaluation.

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