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Novel stereoselective 2,3-disubstituted quinazoline-4(3*H*)-one derivatives derived from glycine as a potent antimalarial lead†

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An optimization and modification of Grimmel's method leading to cyclization and incorporation of glycine linked sulphonamide at position-2 in 4-quinazolin-(3*H*)-ones was accomplished. Generation of a lipophilic site at position-3 of 4-quinazolinones was explored by synthesis of imines, unfortunately leading to an isomeric mixture of stereoisomers. These stereoisomeric mixtures were further converted to a single isomer utilizing the novel methodology developed by the use of an aprotic solvent system. Moreover, a mixture of (*Z*)/(*E*)-isomers and single configuration was identified and ascertained using NMR, HMQC, HMBC and NOESY spectroscopic techniques. The synthesized entities were further screened for their antimalarial efficacy pertaining two active scaffolds **8m** and **8s**. The active molecules were sent forth for enzyme inhibitory study against presumed receptors h-DHFR and Pf-DHFR computationally as well as *in vitro*, proving their potency as dihydrofolate reductase inhibitors. The oral bioavailability of these active molecules was also predicted by the study of ADME properties, indicating good bioavailability of the active entities.

1. Introduction

Malaria, a life threatening disease, is probably one of the oldest diseases known to mankind. Mentions of this disease can be found in ancient Chinese, Indian and Egyptian manuscripts. In the 7th century AD, the Italians named the disease malaria meaning bad air, due to its association with ill-smelling vapours from the swamp near Rome. The first recorded treatment of malaria dates back to 1600, when the bark of the Cinchona tree was first used by the native Peruvian Indians to treat the intermittent fevers associated with this illness.[1] The greatest challenge lies in the parasite's ability to quickly adapt and overcome eradication efforts when these are fragmented and uncoordinated. Malaria quickly rebounded from the mass insecticide spraying campaigns in the 1950s and 1960s. Since the 1980s parasite resistance to chloroquine, the most commonly available antimalarial drug has emerged as a major challenge. Malaria continues to be a major infectious disease, with a global estimate of 200–500 million cases per year, and annual mortality of some 1.2 million.[2] In humans, four species are responsible for malaria: *P. falciparum; P. vivax; P. ovale* and *P. malariae.* The first one is the most dangerous. Two aspects have currently stimulated new efforts regarding medical and molecular studies about malaria: the rapid emergence of *P. falciparum* strains resistant to currently available antimalarial drugs;[3–6] and the inefficacy of malarial vaccines.[7] To overcome the resistance of the strain against the available antimalarial drugs, an urge for development of newer class of antimalarials has diverted the medicinal chemists towards design of inhibitors of various key enzymes. Dihydrofolate reductase (DHFR) is one of the key enzymes in the process of DNA replication, it catalyzes the transformation of 7,8-dihydrofolate into tetrahydrofolate.[8] 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.[9]

The exploration of heterocycles as privileged structures in drug discovery is an important major area in medicinal chemistry.[10] Among them, the quinazoline ring system is a ubiquitous structural

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† Electronic supplementary information (ESI) available: Results and discussion as well as experimental details of intermediates, spectral data including spectral copies (HPLC, ^{1}H-NMR, ^{13}C-NMR, 2D-NMR and Mass) and crystallographic data of some compounds. CCDC 932142. For ESI and crystallographic in CIF or other electronic format see DOI: 10.1039/c5nj01408e

unit and important pharmacophore found in a number of alkaloids and many biologically active compounds.[11] Synthesis of quinazolin-4(3*H*)-ones has been extensively investigated,[12] quinazolinone derivatives have become especially noteworthy in recent years due to their wide spectrum of biological activity.[13–19] On the basis of biological and medicinal importance, the synthesis and bioactivity of the quinazolinone nucleus have got impetus along with the chemists and biologists in recent years. Owing to the excellent biological properties of 4-quinazolin-(3*H*)-one derivatives, particularly, 2,3-disubstituted-4(3*H*)-quinazolinone, numerous synthetic methods have been found in the book chapters and in recent literature reviews.[20,21]

The discovery and development of sulphonamides as antibacterial agents was one of the most fascinating and informative field in medicinal chemistry, highlighting the roles of skillful planning and serendipity in drug research.[22] Sulfonamides were the first effective agents against most Gram-positive and many Gram-negative organisms, and were employed systematically for the prevention and cure of bacterial infections.[22,23] Many sulphonamide derivatives, analogues as well as related compounds have been synthesized,[24,25] and this has lead to the discovery of many useful drugs, which are effective as antimicrobial agents as well as diuretics, antidiabetic, antimalarial, antileprotic, antithyroid agents, and several other agents.[22–25] The α-amino acids have great potential as chiral building blocks for synthesis of optically active materials; polyfunctional unusual amino acids, amino polyols, and peptide mimics like enzyme inhibitors, amino sugar antibiotics, and sympatho mimetic amines.[26] Numerous efforts were made towards syntheses of optically pure amino acids: asymmetric derivatization of glycine, electrophilic amination of enolates, nucleophilic amination of α-substituted acids, asymmetric Strecker synthesis, asymmetric hydrogenation of dehydro amino acids, and enzymatic syntheses of α-amino acids.[27] The synthesis of an efficient assembly of complex molecules from readily available building blocks is an important task for organic chemists.[28] On the other hand, Schiff bases have gained importance because of the physiological and pharmacological activities related with them.[29] Compounds containing azomethine/imine/aldimine groups (–C═N–) in the structure are known as Schiff bases, which are usually synthesized by the condensation of primary amines and active carbonyl groups. The anticonvulsant action of Schiff base is attributed to its unique property of being an electron donor moiety and its ability to act as a controlled pharmacophore at the receptor site.

In continuation of our previous work, with interest in the synthesis of sulfonamide linked quinazolinone with a lipophilic side arm at position-3 as an antimalarial scaffold with medicinal significance and a potent lead molecule, we attempted to synthesize different substituted benzylideneamino derivatives containing a quinazolin-4(3*H*)-one moiety. To get an insight of the potency of the synthesized compounds, the *in vitro* efficacy was investigated and further for comprehensive know how of the mechanism of action, the active entities were scrutinized through a molecular modeling approach and were finally examined for their oral bioavailability as well as for their enzyme inhibitory activity.

2. Results and discussion

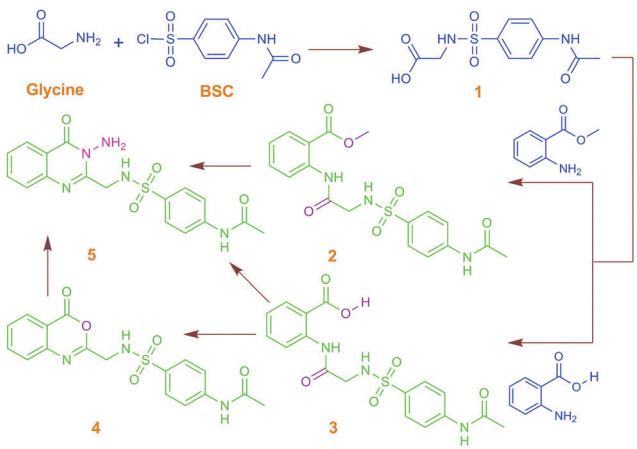
2.1. Synthetic strategies

Since the last few years, we have been engaged in the synthesis of various 2,3-disubstituted-4(3*H*)-quinazolinone derivatives of biological interest.[30] With the relative experience in hand and keeping in mind the literature methods, synthesis of desired quinazolinone–sulfonamide hybrid molecules from amino acids as initial precursors 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–sulfonamide hybrid molecules from glycine in our laboratory, and is outlined in Scheme 1. The general methods and reaction conditions employed by the early investigators were examined to establish suitable experimental procedures and all the needful modifications were carried out to obtain the desired entitled heterocycles, which is further described along with the appropriate explanations. Various methodologies, modifications and optimizations employed for procurement of the chief heterocyclic motif (compound **5**) are discussed in the ESI† and further the best pertained methodology leading to quinazolinone–sulfonamide hybrid molecule **5** is delineated below.

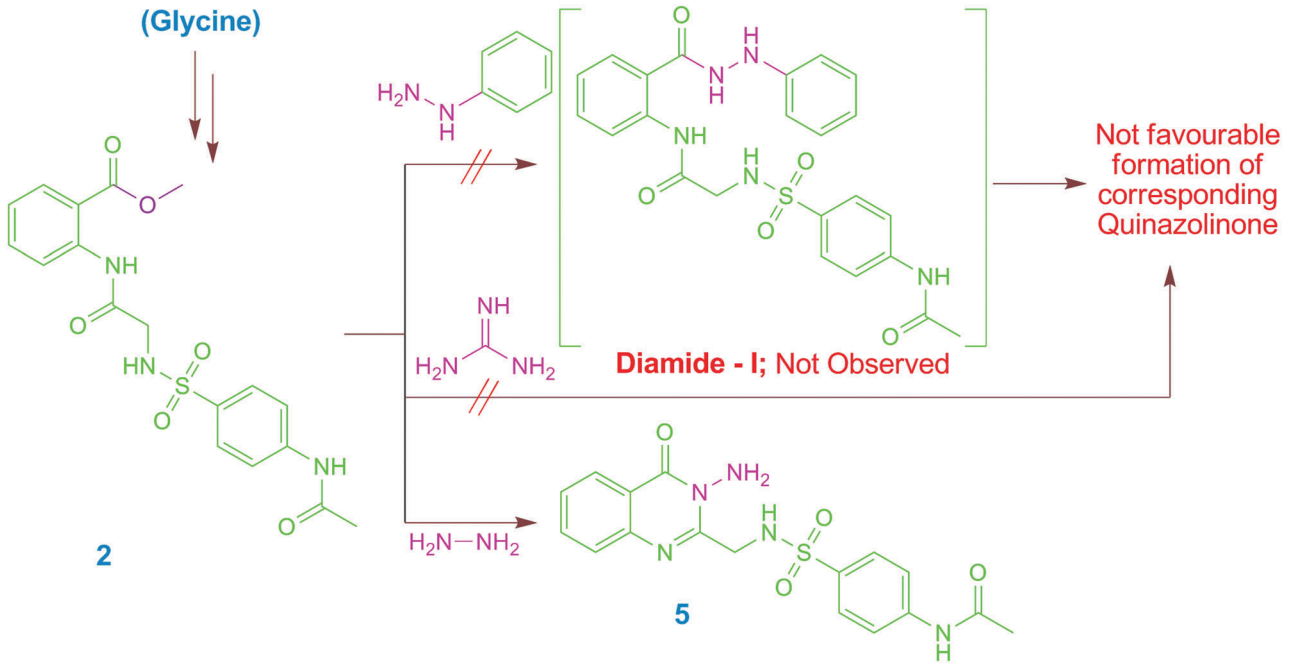
2.2. Multi step condensation approach *via* methyl *N*-acylanthranilates

Another strategy for the synthesis of desired quinazolinone–sulfonamide derivatives involves the use of methyl anthranilate as an initial precursor which upon treatment with appropriate acids could yield the methyl *N*-acylanthranilate esters, the latter upon interaction with appropriate amines would generate either a cyclized product, quinazolinone, or an open chain product, *N*-acylanthranilamide respectively.[31,32] Therefore, it was decided to examine the reaction of methyl *N*-acylanthranilate esters (**2**) with various amines under classical as well as microwave assisted heating conditions (Scheme 2).

Initially, the reaction of **2** with fewer guanidine as well as phenyl hydrazine was examined. The reaction of **2** and guanidine



Scheme 1 Synthetic pathway leading to major intermediate **5**.



Scheme 2 Multi step condensation approach for the synthesis of 4-quinazolinones from methyl *N*-acylanthranilates *Reagents and conditions*: phenyl hydrazine, Py, Δ/MWI OR guanidine, Py or EtOH, Δ/MWI OR hydrazine hydrate, EtOH, Δ/MWI.

Table 1 Synthesis of quinazolinone–sulfonamide hybrids **5** & **7**

				Method-I CH[a]		Method-II MW[a]	
Entry	Comp.	Group (R = R′$_2$)	Group (R′$_3$)	Time	Yield (%)	Time (min)	Yield (%)
1	**5**	–H	$-NH_2$	45 min	91	5	97
2	7	–H	$-NHC_6H_5$	48 h	00	20	00

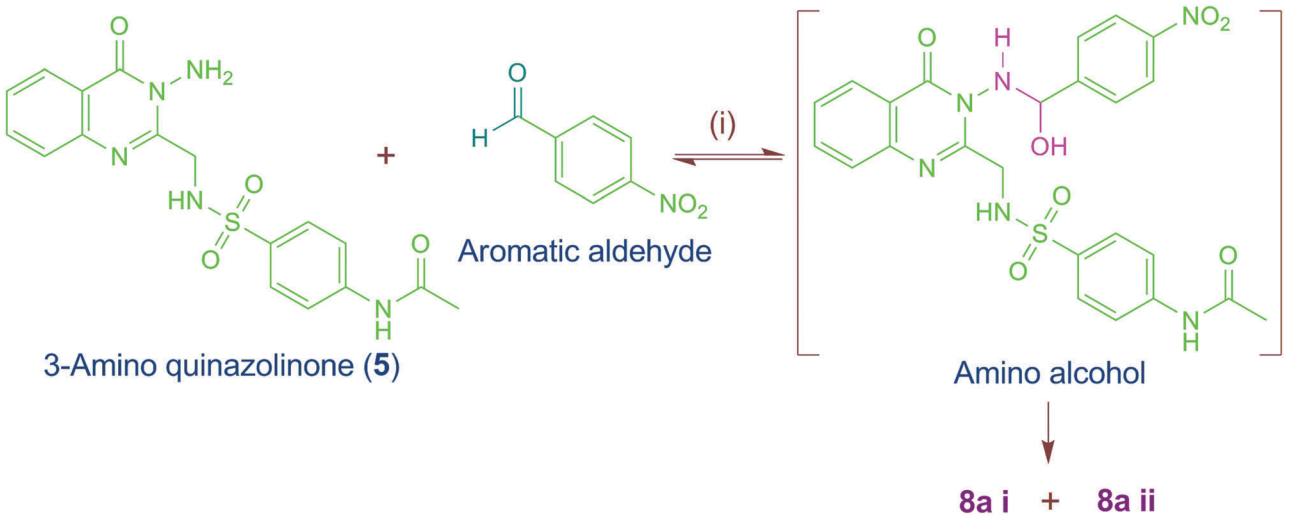
[a] CH = conventional heating, MW = microwave irradiation.

or phenyl hydrazine (1.25 equiv.) in pyridine or ethanol under classical heating conditions did not show the formation of open chain or cyclized product **7** even after 2–3 days. Furthermore, when the reaction was performed under microwave irradiation, TLC monitoring showed unreacted starting materials and previously identified byproducts. In addition, when various hydrazines were examined under microwave irradiation as well as classical heating conditions, it was observed that the ratio of byproduct formation was found to be higher with the latter amines. However, we managed the synthesis of the corresponding 2,3-disubstituted-4-quinazolinone in good yield by the reaction of **2** and reactive hydrazine hydrate under microwave mediated heating conditions (Table 1).

2.3. Synthesis of imine derivatives

To satisfy our interest to build up heterocyclic entities at position-3 of **5**, we tried to introduce aldehydes leading to imine substituted heterocycles. With the study and experience of several reactions leading to synthesis of imines especially aldimines utilizing various aromatic aldehyde derivatives with the synthesized substituted sulphonamide 3-aminoquinazolinones (**5**) gave two different components as indicated by TLC. It was further decided to investigate various methodologies and to optimize the reactions leading to aldimines (**8a–v**), for the purpose 3-amino quinazolinones (**5**) were further reacted with 4-nitrobenzaldehyde considering **8a** as a representative molecule of the study for a further series of imines. Initially the synthesized sulphonamide substituted 3-amino quinazolinones (**5**) were reacted with 4-nitrobenzaldehyde using acetic acid as the catalyst in ethanol using conventional as well as microwave irradiation for synthesis of aldimines, resulting in two different compounds as indicated by TLC (Scheme 3).

Considering two spots on TLC we employed an alternate pathway utilizing mineral acid as the catalyst and ethanol by conventional and microwave irradiation, but there was no change in the product formed. In another path, DMF was used as the catalyst as well as solvent by conventional and microwave irradiation for synthesis of imines giving two different products as other methods. Synthesis of a single compound was our prime motto, therefore we further selected an alternative of solid phase reaction by microwave irradiation but unfortunately, we again obtained two products as visualized by TLC having two spots of same intensities. Since we could not obtain our desired product we finally tried to synthesize aldimines by reacting the amine and aldehyde in acetic acid upon conventional and microwave irradiation as well, which gave two spots on TLC. Further observation indicated that the quantity of the compounds varied distinguishably which was confirmed by the intensities of compounds on TLC. For instance, considering the structure and reactivity of **5**, our thoughts rendered two different products that is, fused heterocyclic entity and imines at position-3. To satisfy our curiosity we employed column



Scheme 3 Synthesis of **8a** quinazolinone–sulfonamide hybrid molecules under acidic conditions *Reagents and conditions*: (i) Method-I: conventional OR microwave irradiation method for synthesis of **8a**, AcOH/EtOH, Δ. (i) Method-II: conventional OR microwave irradiation method for synthesis of **8a**, H_2SO_4/EtOH, Δ. (i) Method-III: conventional OR microwave irradiation method for synthesis of **8a**, DMF, Δ. (i) Method-IV: conventional OR microwave irradiation method for synthesis of **8a**, AcOH, Δ.

chromatography to separate the obtained mixture of two compounds **8a**(**i** and **ii**) using chloroform : methanol as an eluent in a ratio of 95 : 5 respectively. Further analysis of the two separated compounds by ^{1}H-NMR and APT shows negligible difference in the spectra indicating the presence of isomerism among the compounds. To study the presence of isomerism, 2D-NMR analysis was employed to prove that the two compounds showed isomerism and could be stereoisomers. Heteronuclear multiple bond coherence (HMBC) is one of the 2D-NMR spectrometry techniques which can determine long range ^{1}H-^{13}C connectivities *via* two or three bonds with high sensitivity as well as the heteronuclear multiple quantum coherence (HMQC) spectrum which can determine short-range ^{1}H-^{13}C connectivities were utilized for understanding the configurations of the compound. The HMBC and HMQC spectrum of **8a**(**i** and **ii**) was determined, and correlations were observed between a proton signal of H_{18} 9.28 singlet (–N=C*H*–) which correlates with a carbon signal of $C_{2''}$ 129.89 and $C_{3''}$ 124.06, H_{11} 4.32 doublet (–CH_2–NHSO$_2$) shows correlation with carbon C_2 150.79 ppm, while carbon C_{18} 165.59 ppm correlates with proton $H_{2''}$ 8.39 doublet (Ar–*H*) and $H_{3''}$ 8.19 doublet (Ar–*H*) respectively. The correlation between H_{11} of –CH_2–NHSO$_2$ and C_{18} of –CH–NH– as well as H_{18} of –NH–C*H*– and C_{11} was not observed, also correlation between C_{18} and H_{17} of –N–N*H*–CH– was not detected. These results are illustrated in Fig. 1. From the above data it can be concluded that the two isolated entities were isomers, specifically stereoisomers of aldimines and not a fused heterocyclic stereoisomer. These findings created a tornado of questions regarding the conformation of the stereoisomers in our mind. Moreover, literature survey reveals that resonance structures may exist in the compounds containing azomethine groups and these compounds may form *E* and *Z* isomers together in diverse percentage.[33] Finally, to stabilize the mental restlessness related to conformation, we intended to utilize NOESY.

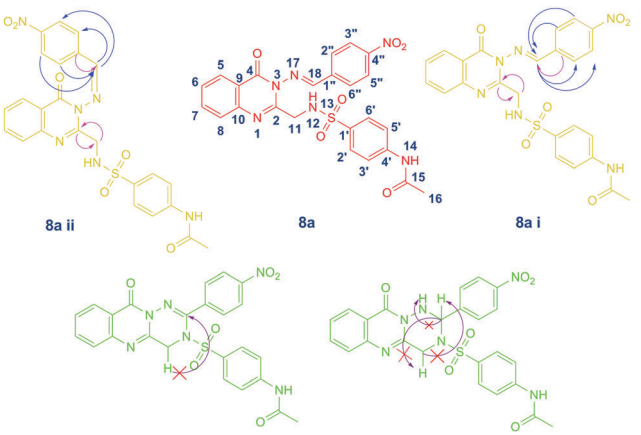


Fig. 1 Significant short-range and long-range correlation observed in HMQC & HMBC spectrum of **8a** molecule (blue line arrows indicate HMBC connectivities and Pink line arrows indicate HMQC connectivities).

In continuation to the above study, more spectroscopic evidence for the regiochemical assignment of the aldimine derivatives was obtained using nuclear Overhauser effect spectroscopy (NOESY). The experiment was run to establish the stereo-orientation for aldimine derivatives **8a** as either *E* or *Z* isomers. NOESY experiments for two separated compounds **8a**(**i** and **ii**) showed an enhanced interaction between (H_{18}) and (H_{11}) at δ_H 9.28 ppm and 4.322 ppm respectively as well as between (H_{18}) and (H_{12}) at δ_H 9.28 ppm and 8.050 ppm respectively in compound **8a**(**i**), which shows that the two groups are on the opposite side elucidating *E*-configuration, in contrast no interaction between (H_{18}) and (H_{11}) was observed in compound **8a**(**ii**) which proves that two bulky groups are on the same sides of the double bond as required by the *Z*-configuration (Fig. 2).

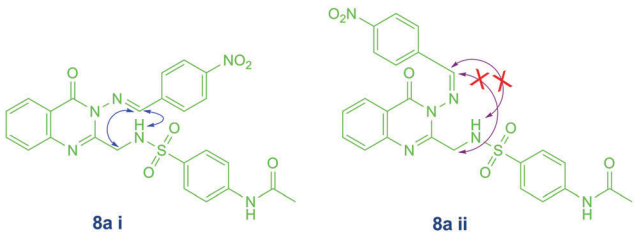
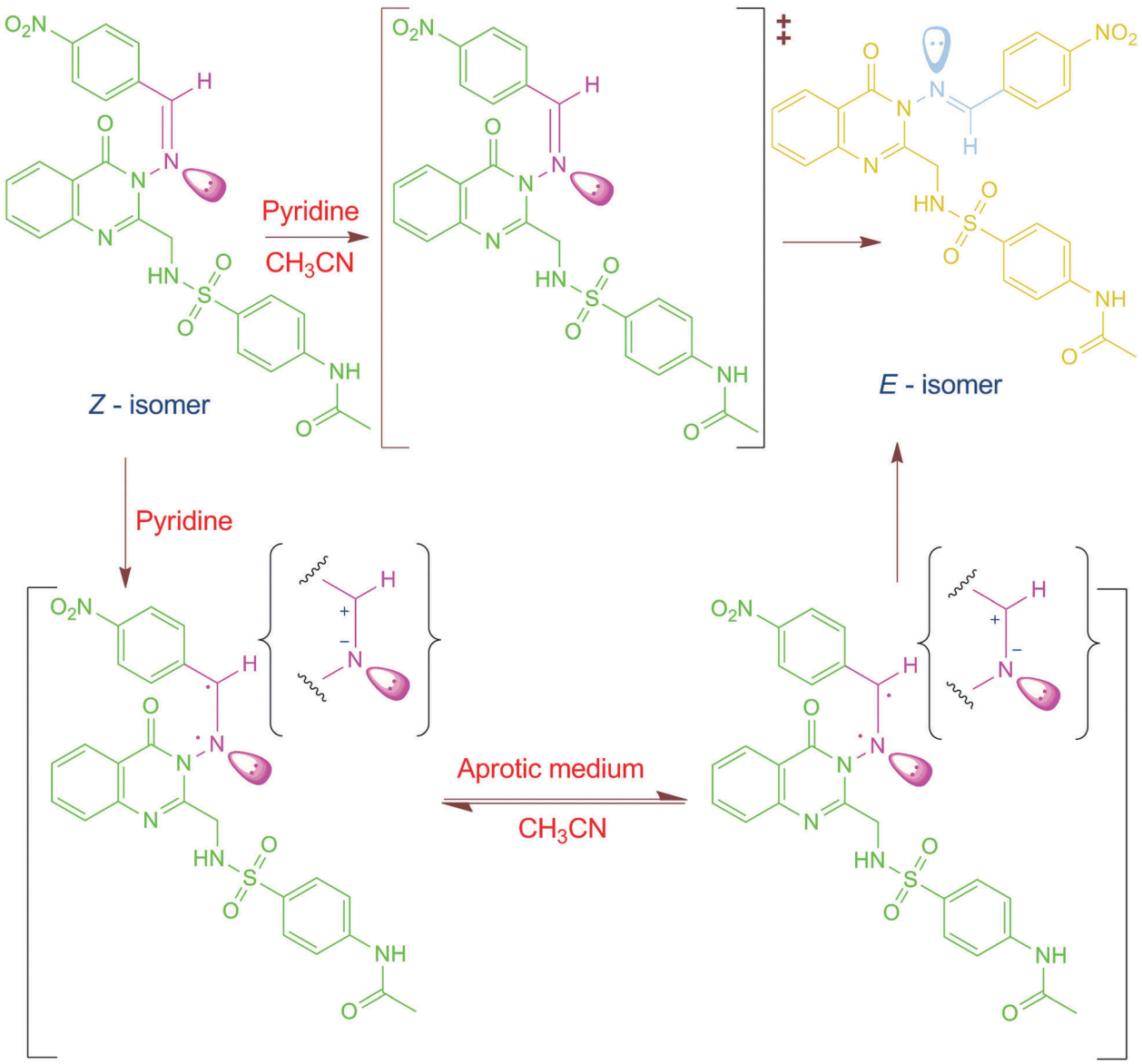


Fig. 2 Significant correlation observed in the NOESY spectrum of **8a** molecule.

E and *Z* configurational isomers were obtained during the reaction whose ratio was 71 : 29 respectively, which was derived from HPLC as shown in Fig. S3, ESI.† Now, our track of research turned toward obtaining individual configurations separately or to obtain a single configuration only, which could be achieved by physical or chromatographic separation, which was found to be a tedious and time-consuming procedure. We were more concerned with obtaining single configuration that is *E* configuration which was a major product as per HPLC results. Therefore, we had designed a methodology utilizing pyridine and aprotic medium acetonitrile for continuous conversion of *Z* configuration to *E* configuration *in vitro*. These studies pointed out that in an aprotic medium, isomerization occurs by inversion of the nitrogen atom, while the plausible mechanism of inter conversion of the isomers is shown in Scheme 4 which indicates that the interconversion follows an ionic or radical pathway for conversion into a single configurational isomer.

A sample was withdrawn during the reaction and was analyzed by HPLC indicating incomplete conversion as shown in Fig. S4 (ESI†). Considering the obtained results, the reaction



Scheme 4 Plausible mechanism of inter conversion of the isomers.

Table 2 Synthesis of quinazolinone–sulfonamide hybrids **8a**

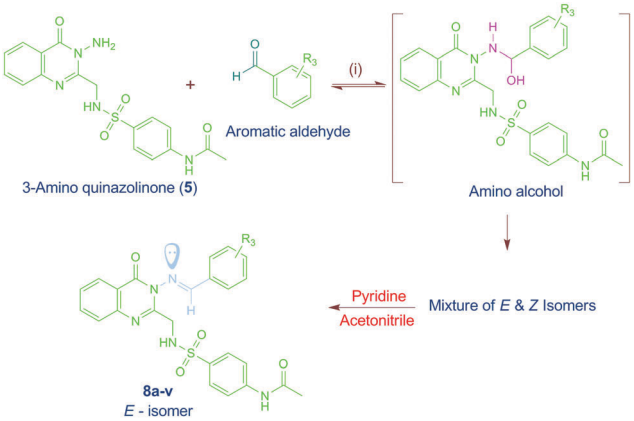
Method	Group (R = R′$_2$)	Group (R$_3$ = R′$_3$)	CH[a] Time (h)	CH[a] Yield (%)	MW[a] Time (min)	MW[a] Yield (%)
I	–H	–C_6H_4–(4-NO_2)	10	82	21	88
II	–H	–C_6H_4–(4-NO_2)	12	77	37	85
III	–H	–C_6H_4–(4-NO_2)	14	80	64	84
IV	–H	–C_6H_4–(4-NO_2)	4	91	12	97

[a] CH = conventional heating, MW = microwave irradiation.

was allowed to proceed further until the final product as *E* configuration was obtained, which was further ascertained by HPLC as illustrated in Fig. S5 (ESI†). From the above optimization steps, we found that the alternative using acetic acid directly by conventional as well as microwave irradiation turned out to be superior (Table 2) and was further employed for synthesis of all the entitled compounds (**8a–v**). The comparative yields of isomeric mixture obtained by the two techniques are displayed in Table 3. Furthermore, the isomeric mixtures were subjected to the method developed for inter-conversion of *Z* to *E* configuration *in situ*, to obtain the *E*-isomer predominantly as a single component (Scheme 5).

2.4. *In vitro* antimalarial screening

Antimalarial screening data for entitled compounds **8a–8v** are given in Table 4. The spectrum of activity of entitled compounds showed improved potency $IC_{50} < 0.20$ μg mL^{-1}, 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 **8m** and **8s**



Scheme 5 Synthesis of **8a–v** quinazolinone–sulfonamide hybrid molecules under acidic conditions *Reagents and conditions*: (i) conventional OR microwave irradiation method for synthesis of **8a–v**, AcOH, Δ.

Table 4 *In vitro* antimalarial assay (IC_{50}) values

Comp.	IC_{50} (μg mL^{-1})	Comp.	IC_{50} (μg mL^{-1})
8a	0.68 ± 0.04	**8m**	0.068 ± 0.003
8b	0.72 ± 0.04	**8n**	1.24 ± 0.08
8c	1.28 ± 0.07	**8o**	0.78 ± 0.05
8d	0.67 ± 0.04	**8p**	0.72 ± 0.04
8e	1.024 ± 0.06	**8q**	1.012 ± 0.06
8f	0.78 ± 0.05	**8r**	1.052 ± 0.06
8g	0.72 ± 0.04	**8s**	0.068 ± 0.003
8h	1.012 ± 0.06	**8t**	0.72 ± 0.04
8i	1.052 ± 0.06	**8u**	1.28 ± 0.07
8j	0.84 ± 0.05	**8v**	1.84 ± 0.11
8k	0.92 ± 0.05	**CQ**	0.020 ± 0.002
8l	1.70 ± 0.09	**PYM**	0.25 ± 0.02

Table 3 Synthesis of quinazolinone–sulfonamide hybrids **8a–8v**

	(*E*)-*N*-(4-(*N*-((3-(Substituted benzylideneamino)-4-oxo-3,4-dihydroquinazolin-2-yl)methyl)sulfamoyl)phenyl) acetamide (**8a–v**)					
	Groups		Method-I: CH		Method-II: MW	
Comp.	(R′$_2$)	(R′$_3$)	Reaction time (h)	Yield [%]	Reaction time (min)	Yield [%]
8a	–H	–C_6H_4–(4-NO_2)	4.0	91	12	97
8b	–H	–C_6H_4–(3-NO_2)	4.5	86	14	89
8c	–H	–C_6H_4–(2-NO_2)	4.5	78	14	84
8d	–H	–C_6H_4–(4-OH)	7.5	76	18	82
8e	–H	–C_6H_4–(3-OH)	6.0	78	16	85
8f	–H	–C_6H_4–(2-OH)	4.5	80	14	89
8g	–H	–C_6H_4–(4-OMe)	6.0	78	17	83
8h	–H	–C_6H_4–(3-OMe)	6.5	81	18	87
8i	–H	–C_6H_4–(2-OMe)	6.0	79	17	88
8j	–H	–C_6H_4–(4-Me)	4.5	84	14	90
8k	–H	–C_6H_4–(3-Me)	4.5	86	14	95
8l	–H	–C_6H_4–(2-Me)	5.0	89	16	94
8m	–H	–C_6H_4–(4-Cl)	5.0	80	15	88
8n	–H	–C_6H_4–(3-Cl)	4.5	83	14	92
8o	–H	–C_6H_4–(2-Cl)	4.5	88	14	97
8p	–H	–C_6H_4–(4-Br)	5.0	90	16	95
8q	–H	–C_6H_4–(3-Br)	4.5	79	14	94
8r	–H	–C_6H_4–(2-Br)	4.5	85	14	91
8s	–H	–C_6H_4–(4-F)	4.5	90	14	95
8t	–H	–C_6H_4–(3-F)	4.5	79	14	89
8u	–H	–C_6H_4–(2-F)	4.5	75	15	86
8v	–H	–C_6H_5	4.0	85	13	96

were found to be the most potent entities having an IC_{50} value of 0.068 μg mL^{-1}. The results indicate that the electron withdrawing property and lipophilicity of the substituent greatly enhanced the activity of the halogenated active scaffolds.

2.5. Docking study

To rationalize the potency of the fabricated scaffolds, novel quinazolinone derivatives **8m** and **8s** which were found to be active *in vitro* were docked against human DHFR (1MVT) and wild type mutant Pf-DHFR (4DPD) protein structures obtained from protein data bank (RSC). For docking purpose the prepared 3D structures of the ligands were docked in the active site of the proteins using Glide 6.6.

The active quinazolinone–sulfonamide hybrids interacted with the human and mutant DHFR forming H-bonds as well as π–π stacking interactions. The docking results are delineated in Table 5 which enumerates the glide score and binding energy of ligand binding. The values of the glide score and binding energies obtained for the *in vitro* active entities proved that the compounds **8m** and **8s** have significant binding affinity with energies ranging between −40.21 kcal mol^{-1} and −37.24 kcal mol^{-1} towards Pf-DHFR while −70.58 kcal mol^{-1} and −68.94 kcal mol^{-1} against h-DHFR, and energy ranges are comparable with the standard reference drugs chloroquine and pyrimethamine.

As depicted in Fig. 3 the amino acids of the hydrophobic pocket of the protein h-DHFR invited **8s** motif by formation of a hydrogen bond between the acetyl oxygen of the ligand with hydrogen of Gly20 as well as hydrogen of the sulphonamide group with oxygen of Val115 at bond lengths of 2.23 Å and 2.01 Å respectively. For compound **8m** (Fig. 4), amine hydrogen of Gly20 interacted with oxygen of acetyl amide at a distance of 2.23 Å and carbonyl oxygen of Val115 bonded with hydrogen of amide linkage of the sulphonamide side chain having a bond length of 1.95 Å, both the interactions leading to hydrogen bonding. Furthermore, π–π stacking interaction was observed between the fused benzene ring of quinazolinone of **8s** and the aromatic region of Phe34, while compound **8m** did not exhibit such interaction.

The binding study of the active ligands towards Pf-DHFR enzyme revealed that the compound **8s** exhibited hydrogen bonding between nitrogen of the imine linkage and hydrogen of the amine group of Asn521 at a distance of 2.09 Å (Fig. 5). The active site of the receptor molecule also invited the compound **8m** forming a hydrogen bond between the nitrogen of the imine linkage and the hydrogen of Asn521 amino acid in the binding pocket with a bond length of 2.18 Å, as well as another hydrogen bond was observed between the hydrogen of the amide side chain of the sulphonamide linkage and the oxygen of Arg402 with a bond length of 1.99 Å in the active site as visualized in Fig. 6.

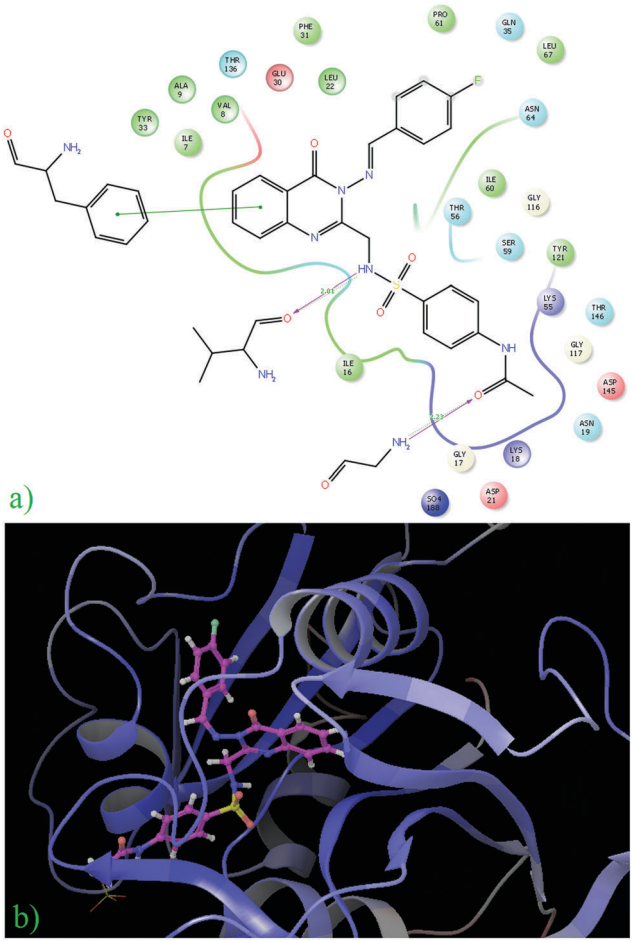


Fig. 3 The binding model of docked compound **8s** with h-DHFR enzyme. (a) 2D model of binding pose with bond length and interacting amino acid; (b) 3D model of ligand bonded to active site, the protein molecules are displayed as cartoon and ligand as ball and stick.

Table 5 Glide docking score and docking energies of the active entries and reference compounds

	Docking result of hDHFR		Docking result of PfDHFR	
Comp.	Glide score	Glide energy	Glide score	Glide energy
8m	−10.27	−70.58	−05.61	−43.08
8s	−10.55	−68.94	−05.99	−43.28
CQ	−06.99	−41.04	−05.18	−30.44
PYM	−06.20	−37.71	−04.12	−28.76

For the above study two reference molecules chloroquine and pyrimethamine were also docked with the active receptor pockets of h-DHFR as well as Pf-DHFR. Fig. 7 displays the accommodation of the standard reference molecules as well as active synthesized ligands revealing the fact that the ligands **8m** and **8s** occupy the receptor pocket with great ease and exhibit enhanced binding affinity as compared to reference molecules. The interactions of the active ligand molecules with binding pockets of the two protein molecules (h-DHFR and Pf-DHFR) exemplify the potency and active participation of lipophilic residues of the active entities binding with the active site and further inhibition of the enzymes.

2.6. Pharmacokinetic properties prediction

The synthesized compounds showing good antimalarial activity were further evaluated for their pharmacokinetic properties leading to drug likeness. The compounds were preliminarily screened considering the basic parameters of Lipinski's rule of 5.

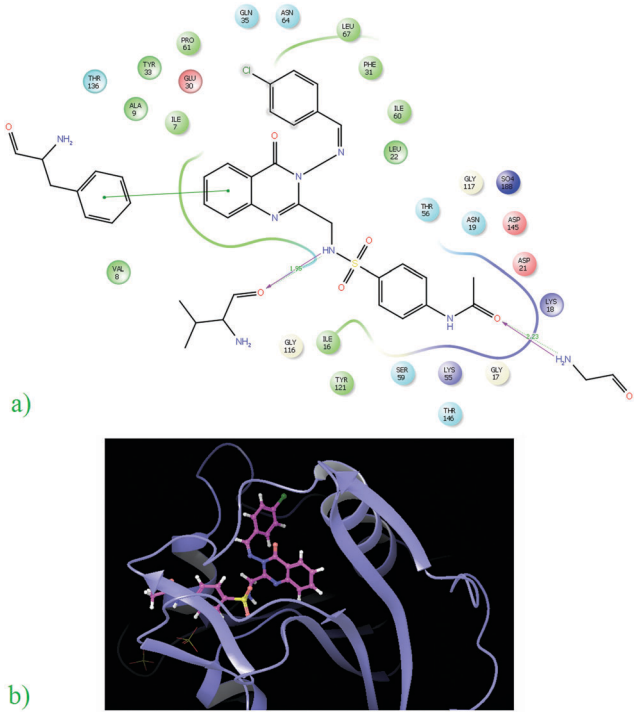


Fig. 4 The binding model of docked compound **8m** with h-DHFR enzyme. (a) 2D model of binding pose with bond length and interacting amino acid; (b) 3D model of ligand bonded to active site, the protein molecules are displayed as cartoon and ligand as ball and stick.

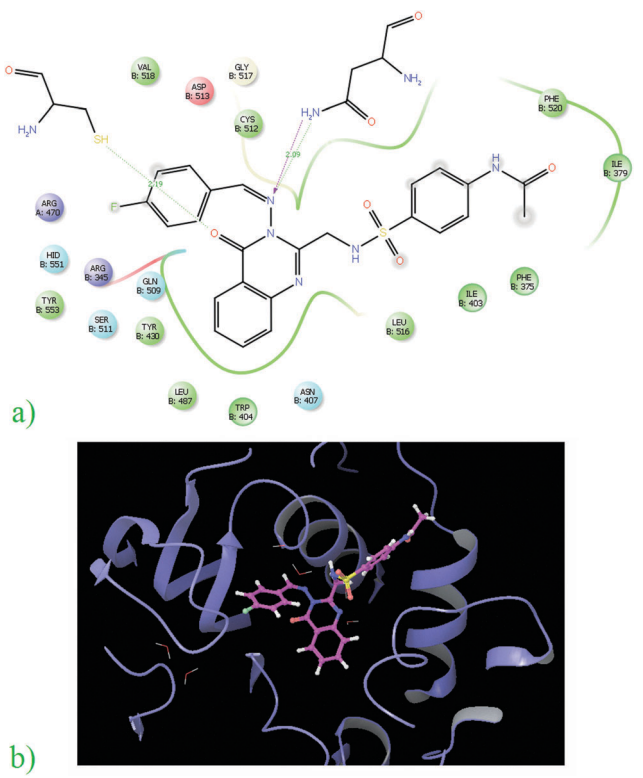


Fig. 5 The binding model of docked compound **8s** with Pf-DHFR enzyme. (a) 2D model of binding pose with bond length and interacting amino acid (b) 3D model of ligand bonded to active site, the protein molecules are displayed as cartoon and ligand as ball and stick.

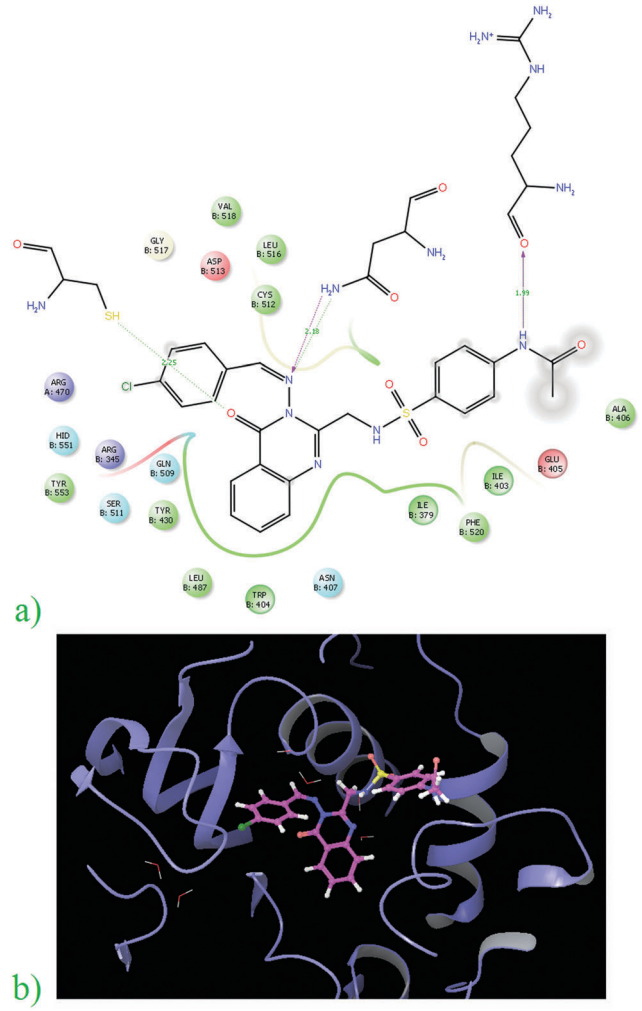


Fig. 6 The binding model of docked compound **8m** with Pf-DHFR enzyme. (a) 2D model of binding pose with bond length and interacting amino acid (b) 3D model of ligand bonded to active site, the protein molecules are displayed as cartoon and ligand as ball and stick.

Table 6 shows the results obtained from Quickprop with their permissible range. In general an orally active compound should not have more than 2 violations of the Lipinski rule. The active test compounds in the present study were not found to be violating the rule more than the maximum permissible limits and thus proving their drug likeness properties.

The optimum values of the descriptors, polar surface area and rotatable bonds also have a great influence on the oral bioavailability of the drug molecules. The important parameters with their permissible ranges are delineated in Table 7. The optimum value of rotatable bonds (<15) and polar surface area (7–200 Å) holds great importance in the oral bioavailability of the drug molecules.[34] The active test quinazolinone derivatives show results of the descriptors to be in the prescribed range thus showing good bioavailability. Intestinal absorption or permeation is also one of the important factors to be studied in relation with the absorption of the drug molecule, which was further confirmed by predicted Caco-2 cell permeability

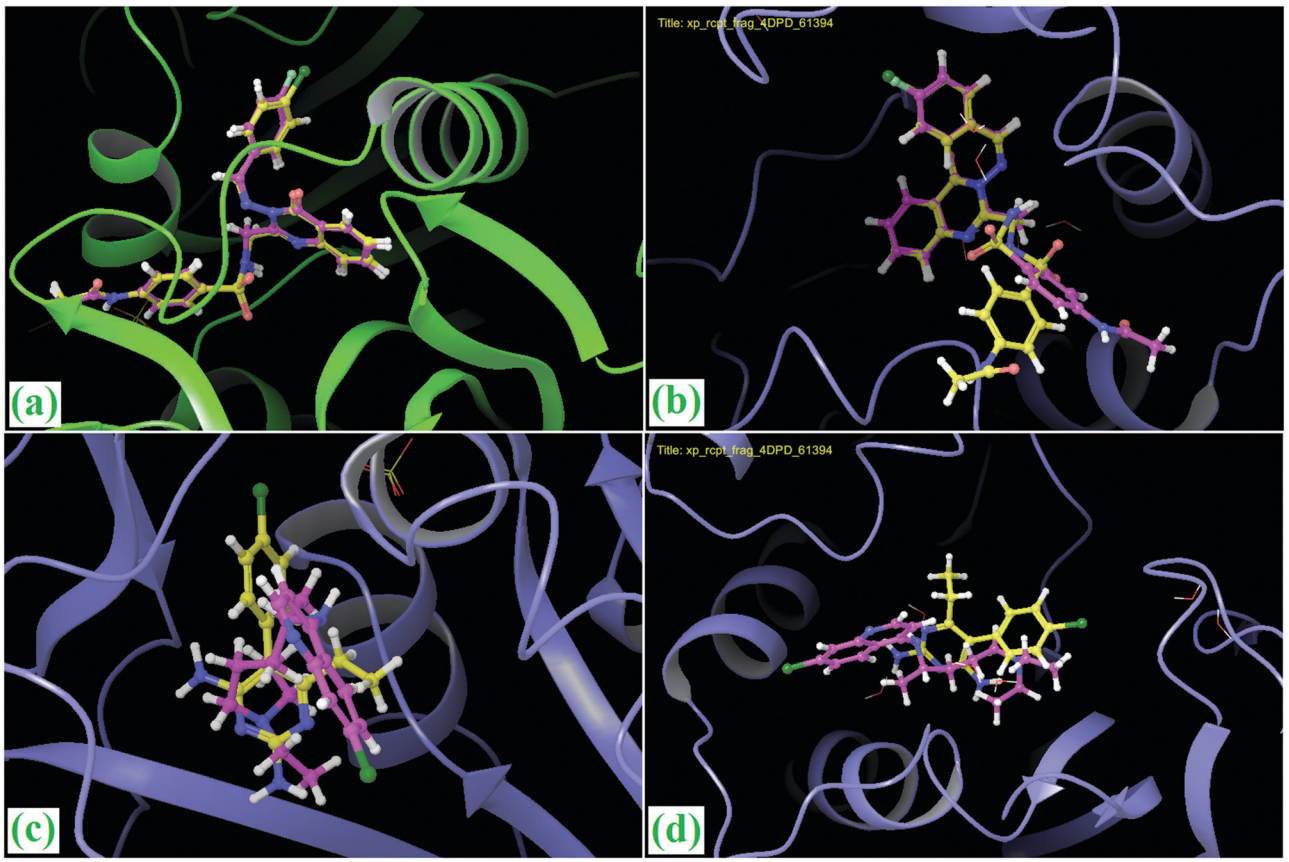


Fig. 7 Correlative representations of the standard drugs [chloroquine (purple) and pyrimethamine (yellow)] and the active entities **8s** (purple) and **8m** (yellow) circumscribed to active receptor site. (a) Active ligands bonded to h-DHFR active site (b) active ligands bonded to Pf-DHFR active site (c) standard drug molecules bonded to h-DHFR active site (d) standard drug molecules bonded to Pf-DHFR active site.

Table 6 Prediction of Lipinski RO5 for active test entities

Comp.	MW (<500 amu)	DonorHB (<5)	AccptHB (<10)	QP log Po/*w* (<5)	*N* of violation (<2)	#Rotor (0–15)
8m	509.966	2	10.5	3.589	1	8
8s	493.511	2	10.5	3.243	0	8
CQ	319.876	1	4	3.827	0	8
PYM	248.714	4	3	1.861	0	4

(QPPCaco), used as a model for the gut–blood barrier.[35] Caco-2 cell permeability prediction of the test compounds indicates excellent results predicting good intestinal absorption. Furthermore, the compounds were tested for QP log k_{hsa} descriptor of Quickprop indicating the predicted values of human serum albumin binding 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 QP log BB showed reliable prediction for all the test compounds and reference drugs. The aqueous solubility parameter (QP log *S*) of the test entities was assessed 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} values 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.

2.7. DHFR inhibition assay and cytotoxicity assay

The active molecules obtained from antimalarial screening were further tested for their inhibitory efficacy against bovine

Table 7 Prediction of ADME parameters of the active test compounds

Comp.	Percent human oral absorption (>80% – high & <25% – poor)	QPPCaco (<25 poor, >500 great)	QP log BB (−3.0–1.5)	QP log K_{hsa} (−1.5–1.5)	QP log HERG (below −5)	QP log *S* (−6.5–5)	PSA (70–200 Å)
8m	79.313	299.187	−1.552	0.135	−7.32	−6.113	130.479
8s	88.886	251.088	−1.669	0.057	−7.218	−5.679	131.32
CQ	100.00	1127.096	0.343	0.342	−5.08	−2.952	26.129
PYM	85.33	450.568	−0.769	−0.24	−4.486	−3.075	71.933

Table 8 Enzyme inhibition assay and cytotoxicity of 2,3-disubstituted quinazolinone[a]

Compounds	DHFR inhibition (IC_{50} μg mL^{-1})	Cytotoxicity vero cells (IC_{50} μg mL^{-1})	S.I.
8m	0.047 ± 0.003	N.C.	319.18
8s	0.039 ± 0.002	N.C.	384.615
CQ	0.010 ± 0.0005	N.T.	—
PYM	0.035 ± 0.002	N.T.	—

[a] The results indicated are average of triplicate, N.C.: no cytotoxicity observed up to a concentration of 15 μg mL^{-1}, N.T.: not tested, S.I.: selective index (IC_{50} value of cytotoxicity assay/IC_{50} value of enzyme inhibition assay).

liver DHFR enzyme and cytotoxicity against vero cells. The results obtained are delineated as IC_{50} values of enzyme inhibition and cell viability (Table 8). The entities **8m** and **8s** demonstrated good inhibitory activity as compared to reference drugs chloroquine and pyrimethamine. The results of cytotoxicity assay disclosed the non-toxic behavior of the screened molecules. The compounds rendered no toxicity up to a concentration of 15 μg mL^{-1} thus showing their non-toxic nature. The selective index of the entities was found to be more than 100 which shows concurrence for utilization of the scaffolds as active antimalarial compounds inhibiting dihydrofolate reductase enzyme.

3. Conclusions

Progressing towards our goal of synthesizing amino acid and sulfonamide incorporated quinazolinone derivatives as antimalarial active scaffolds; we have modified and developed efficient, convergent and facile methodologies leading towards synthesis of **5** in satisfactory yields. The intermediate was further mechanized and aldimines were generated in a stereoisomeric mixture that can be further utilized for the development of heterocycles. The hurdles of diastereoisomers encountered on the path were eminently rectified by development of a new rapid and reproducible methodology for conversion of *Z*-configuration to *E*-configuration *in situ*. In a nutshell, sulfonamide–quinazolinone hybrids were successfully developed and synthesized from glycine, among them compounds **8m** and **8s** were found to be active antimalarial scaffolds (IC_{50} = 0.068 μg mL^{-1}) as compared to standard drugs chloroquine and pyrimethamine. The active molecules established a potential interaction *in silico* with the enzymes human dihydrofolate reductase and *Plasmodium falciparum* dihydrofolate reductase occupying the active binding pocket with great ease. The molecules exhibited active inhibition of enzymes proving their potency as dihydrofolate reductase inhibitors and thus interrupting the malarial folate pathway leading to antimalarial efficacy. The calculated oral bioavailability parameters for the test compounds predicted good pharmacokinetic properties. Thus promising *in vitro* antimalarial activity, docking pattern in the enzymes human dihydrofolate reductase and *Plasmodium falciparum* dihydrofolate reductase, also pharmacokinetic properties portrayed in the present study, indicating their potential for further development as antimalarial lead compounds.

4. Experimental section

4.1. Experimental protocols and analytical data

4.1.1. Synthesis of *N*-(4-(*N*-((3-substituted-4-oxo-3,4-dihydroquinazolin-2-yl)methyl)sulfamoyl)phenyl) acetamide derivatives. A mixture of methyl *N*-acylanthranilate esters (**2**) (0.002 mol) and appropriate hydrazines (0.0022 mol) in ethanol (25 mL) was allowed to warm at appropriate temperature (40–70 °C) for 25–35 min, after completion of the reaction as indicated by TLC. To accomplish microwave assisted synthesis, the resulting suspension was irradiated in a microwave oven for an appropriate time (shown in Table 1) with a power of 350 W. The mixture was allowed to cool at room temperature, poured into 100 mL icecold water. The solid product thus separated was filtered off, washed with distilled water and recrystallized from R-spirit to give appropriate 4-quinazolone derivatives in excellent yields (as shown in Table 1).

N-(4-(*N*-((3-Amino-4-oxo-3,4-dihydroquinazolin-2-yl)methyl)-sulfamoyl)phenyl)acetamide (**5**). White solid; mp: 226–230 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3223, 3115 (N–H str.), 3062, 2925 (C–H str.), 1762, 1676 (C═O str.), 1612, 1590, 1493 (C═N, C═C str.), 1318, 1155 (S═O str.); ^{1}H-NMR (400 MHz, DMSO-d_6, δ_H, ppm): 10.17 (s, 1H, Ar–N*H*–CO), 8.07 (dd, *J* = 8, 1.2 Hz, 1H, Ar*H*), 7.80–7.74 (m, 4H, Ar*H*), 7.63 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.54 (d, *J* = 8 Hz, 1H, Ar*H*), 7.49 (dt, *J* = 8, 1.2 Hz, 1H, Ar*H*), 5.60 (s, 2H, –NH_2), 4.33 (s, 2H, NH–CH_2), 2.04 (s, 3H, Ar–NHCOCH_3); ^{13}C-NMR (100 MHz, DMSO-d_6, δ_C, ppm): 167.8, 160.4, 153.1, 146.0, 142.7, 134.1, 134.0, 127.8, 126.9, 126.5, 125.8, 120.0, 118.2, 44.3, 24.0; MS (ESI) *m*/*z*: 388.0 $[M + H]^+$; anal. calcd for $C_{17}H_{17}N_5O_4S$: C, 52.70; H, 4.42; N, 18.08. Found: C, 52.77; H, 4.39; N, 18.07%.

4.1.2. Synthesis of *N*-(4-(*N*-((3-(substituted benzylideneamino)-4-oxo-3,4-dihydroquinazolin-2-yl)alkyl)sulfamoyl)phenyl) acetamide derivatives 8a–v. A mixture of 3-amino quinazolinone (0.002 mol) and appropriate aromatic aldehydes (0.0022 mol) in acetic acid (30 mL) was heated under classical as well as microwave irradiation to reflux for appropriate time (Table 2), after completion of the reaction as indicated by TLC, the mixture was allowed to cool at room temperature, poured into 100 mL icecold water and neutralized with 10% $NaHCO_3$ solution. The solid product thus separated was filtered off, washed with distilled water and dried *in vacuo* to afford the desired products.

4.1.3. Method for interconversion of *Z* into *E* isomer. In a two necked round bottomed flask fitted with a device condenser, a mixture of isomers (1 mol) and pyridine (0.6 mol) in acetonitrile (50 mL) was stirred and refluxed for 4–6 h. A sample was withdrawn *in situ* and further analyzed by HPLC, the reaction was carried out further until total conversion was accomplished as indicated by HPLC. The solvent was distilled out under reduced pressure, to give the residual compound *E* isomer. The final product was dried in a vacuum oven. To obtain the desired products in extra pure form, it was recrystallized from R-spirit to obtain appropriate 4-quinazolone derivatives in above 99% purity.

(*E*)-*N*-(4-(*N*-((3-(4-Nitrobenzylideneamino)-4-oxo-3,4-dihydroquinazolin-2-yl)methyl)sulfamoyl)phenyl)acetamide (**8a**). White solid; mp: 176–178 °C; $[\alpha]_D^{25}$ −97.8 (*c* 0.75 in $CHCl_3$); FT-IR (KBr, ν_{max}, cm^{-1}): 3268, 3189 (N–H str.), 3061, 2929 (C–H str.), 1685 (C═O str.), 1611, 1592, 1493 (C═N, C═C str.), 1329, 1156 (S═O str.), 1530, 1349 (N═O str.); ^{1}H-NMR (400 MHz, DMSO-d_6, δ_H, ppm): 10.18 (s, 1H, Ar–N*H*Ac), 9.28 (s, 1H, –N═C*H*–), 8.39 (d, *J* = 8.8 Hz, 2H, Ar*H*), 8.19 (d, *J* = 8.8 Hz, 2H, Ar*H*), 8.14 (dd, *J* = 8.0, 1.2 Hz, 2H, Ar*H*), 8.09 (t, *J* = 6 Hz, 1H, SO_2NH–CH_2), 7.84 (dt, *J* = 7.6, 1.2 Hz, 1H, Ar*H*), 7.71 (d, *J* = 8.8 Hz, 2H, Ar*H*), 7.60 (d, *J* = 7.6 Hz, 1H, Ar*H*), 7.58–7.54 (m, 2H, Ar*H*), 4.32 (d, *J* = 6 Hz, 2H, NH–CH_2), 2.04 (s, 3H, Ar–NHCOCH_3); ^{13}C-NMR (100 MHz, DMSO-d_6, δ_C, ppm): 168.8, 165.6, 157.4, 150.8, 149.5, 145.3, 142.7, 138.6, 134.7, 134.0, 129.9, 127.7, 127.2, 126.7, 124.1, 121.0, 118.2, 44.9, 24.0; MS (ESI) *m*/*z*: 520.8 $[M + H]^+$; anal. calcd for $C_{24}H_{20}N_6O_6S$: C, 55.38; H, 3.87; N, 16.15. Found: C, 55.25; H, 3.90; N, 16.25%.

4.2. *In vitro* antimalarial assay

A positive control with reference antimalarial drugs (chloroquine and quinine) 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^{-1} in seven dilutions). The half-maximal inhibitory (IC_{50}) responses as compared to the drug-free controls were estimated by interpolation using the Microcal Origin software. Each duplicate experiment was repeated three times and blood smears were read blind.[36] Results from the above study are summarized in Table 4 as IC_{50} values.

4.3. DHFR inhibitory activity

The inhibitory activity was determined using bovine liver DHFR as model enzymes resembling human DHFR and Pf-DHFR.[37] 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 (Δ*A*/min) was measured at 340 nm. The activity under these conditions was linear for 10 min.[37] Results are reported as % inhibition of enzymatic activity calculated using the following formula:

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

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).

4.4. Cell viability 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.

$$\text{Percentage cell viability} = \left[100 - \left(\frac{A_0 - A_t}{A_0}\right) \times 100\right]$$

where, A_0 = absorbance of cells treated with 0.1% DMSO medium, A_t = absorbance of cells treated with various concentrations 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).

4.5. 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 the 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 human DHFR (PDB ID: 1MVT) were 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 center 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 receptors (mutant Pf-DHFR and human DHFR) using Glide in XP (Extra Precession) mode[38] with other default setting for scoring function.

4.6. ADMET prediction method

The pharmacokinetic profiles of the test compounds showing good antimalarial activity were predicted 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[39] 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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