"CONVERGENCE OF COMPUTATIONAL DOCKING, MICROWAVE-ASSISTED SYNTHESIS, CHARACTERIZATION, AND ANTIMALARIAL ASSESSMENT OF QUINAZOLIN-(3H)-ONE-SULPHONAMIDE HYBRIDS"

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Abstract

This study presents a comprehensive investigation into the synthesis, characterization, and antimalarial assessment of Quinazolin-(3H)-one-sulphonamide hybrid compounds. The research encompasses the use of computational docking techniques to predict the binding interactions between the hybrid compounds and target proteins, offering insights into potential bioactivity. Leveraging the efficiency of microwave-assisted synthesis, 24 compounds **(S2M1-S2M24**) were synthesized with improved yields and reduced reaction times. Thorough characterization methods, including TLC, IR spectroscopy, NMR and Mass spectrometry, confirm the structures and purities of the synthesized compounds. Subsequently, the synthesized hybrids are subjected to antimalarial evaluation to assess their efficacy against malaria parasites. Two compounds **S2M4 (0.23 µg/mL)** and **S2M15 (0.36 µg/mL)** showed promising activity as compared to Quinine as reference drug. This multidisciplinary approach contributes to a deeper understanding of the structure-activity relationships and potential therapeutic applications of Quinazolin-(3H)-one-sulphonamide hybrids in combating malaria.

KeyWords: Quinazolinone-sulphonamidehybrids,1-n-butyl-3-methylimidazolium tetrafluoroborate, Docking, antimalarial activity.

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Introduction

A parasitic infectious disease called malaria poses a hazard to around half of the world's population. A female Anopheles mosquito feeding on blood can spread this parasite disease to people. [1]In the subtropical areas, young children and pregnant women are more susceptible to contracting malaria.[2] There are five parasites in the genus Plasmodium that cause malaria, but Plasmodium falciparum and Plasmodium vivax are the most deadly.[3] To stop or delay the onset of drug resistance, combination therapy entails the concurrent administration of two or

more medications with various mechanisms of action. Additionally, it is frequently more effective if, throughout the course of the infection, a mutant parasite resistant to one of the treatments appears, allowing the other drug to kill the parasite.[4] Designing hybrid substances with antimalarial activity has special benefits, including lower cost and drug-drug interaction risk. Hybrid medications have a uniform distribution, metabolization, and excretion rate and don't compete with one another for plasma protein binding, which can lead to interactions with other medications. The medications' pharmacokinetic behaviour is governed by the linker. [5] Potential antimalarial hybrid compounds with quinoline and sulphonamide moieties have been reported. In order to attach the arylsulfonamide moieties to the aminoquinolin, Pinheiro et al. created quinoline-sulfonamide hybrids 40 with a linker group.[6] As medicinally significant nitrogen heterocycles, quinazoline and quinazolinone exhibit a wide range of biological activities, including the inhibition of dihydrofolate reductase, cellular phosphorylation, kinase, anti-cholinesterase, anti-microbial, anti-convulsant, anti-cancer, anti-malarial, anti-hypertensive, anti-inflammatory, and anti-tumor processes. Numerous medications made from synthetic and natural products that include the quinazoline and quinazolinone moiety are utilised in clinical settings to treat a wide range of medical conditions.[7]

Experimental

Computational work

Docking studies were performed for hybrid molecules with the target protein by Glide v5.6 module of Schrodinger suite, Molecular Modeling Interface Inc., LLC, New York, USA. Glide uses a hierarchical series of filter to search for possible location of the ligand in the active-site region of the receptor. The shape and properties of the receptor are represented on a grid by several different sets of field that provide progressively more accurate scoring of the ligand poses. The X-ray crystal structures of the proteins human protein Plasmodium falciparum Dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) PDB id (4DPD) was obtained from the RCSB protein data bank (http://www.rcsb.org/pdb). Protein preparation wizard of Schrodinger suite has been used to prepare the proteins. The A chain was selected of the protein of PDB id 4DPD and treated to add missing hydrogen, assign proper bond orders (i.e., breaking bonds to metal and correct the formal charge on it and neighboring atoms), and to delete water molecules that were more than 5Å from the heterogeneous groups. The H bonds were optimized

using sample orientations. On the basis of docking results we have synthesized compounds. The docking results showed in table 1.

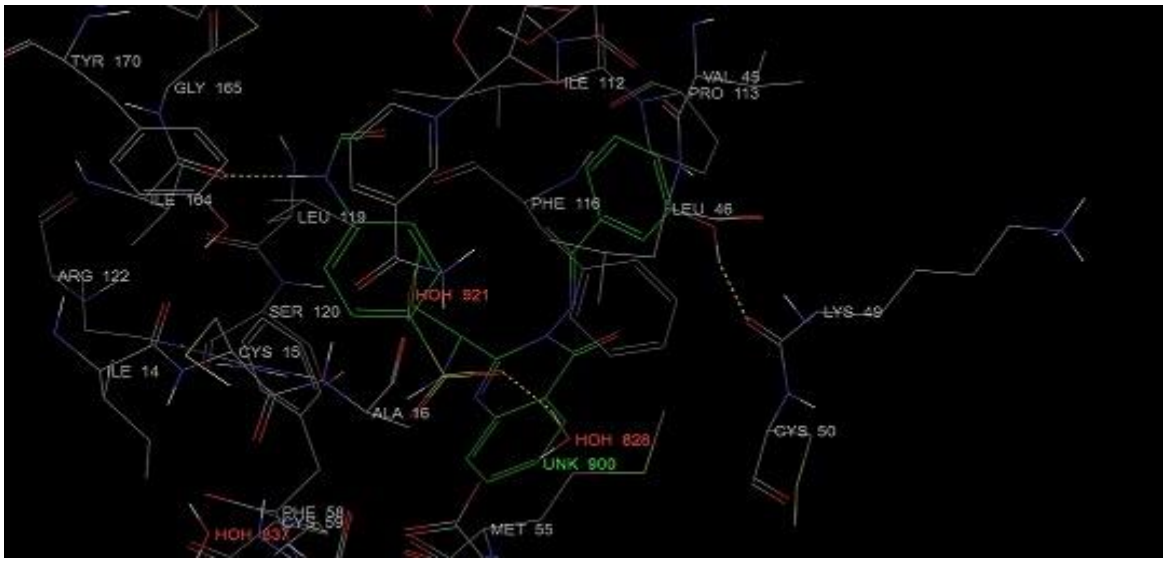


Figure 1: The Plasmodium Falciparum Dihydrofolate reductase thymidylate synthase Protein (PDB ID: 4DPD) with docked conformation of compound S2M4 at the ligand binding domain under XP- flexible docking indicating hydrogen bonding interaction with amino acid residue LYS-49 and ILE-164 (using GLIDE XP visualizer)

Table: 1 Docking results

S.No	Ligand code	Glide Score	Glide Eng.	H Bond	Rot Penal	Lipophilic EvdW	Electro	Site map	Phob En
1	S2M4	-10.64	-45.07	-0.64	0.14	-5.05	-0.36	-0.88	-0.95
2	S2M9	-10.22	-39.25	-071	0.24	-4.45	-0.34	-0.97	-0.74
3	S2M10	-9.94	-57.62	0	0.11	-6.43	-0.14	-0.66	-0.95
4	S2M15	-9.27	-44.84	-0.7	0.22	-5.16	-0.19	-0.93	-0.77
5	S2M22	-8.28	-44.82	0	0.13	-5.88	-0.12	-0.41	-0.32

6	S2M2	-8.25	-47.02	-0.93	0.22	-5.12	-0.34	-1.03	-1.05
7	S2M6	-7.76	-59.41	-0.93	0.15	-6.02	-0.29	-0.93	-0.84
8	S2M12	-7.65	-56.00	-1.20	0.23	-5.20	-0.48	-1.13	-1.02
9	S2M1	-7.57	-31.64	0	0.24	-5.39	-0.22	-0.41	-0.92
10	S2M11	-7.55	-47.15	-0.27	0.31	-4.82	-0.21	-0.40	-0.63
11	S2M5	-7.53	-39.64	0	0.22	-4.92	-0.27	-0.16	-0.75
12	S2M14	-7.28	-51.29	-1.33	0.19	-5.32	-0.22	-0.51	-0.65
13	S2M3	-7.10	-36.23	-0.7	0.32	-4.64	-0.12	-0.74	-0.78
14	S2M7	-7.00	-32.13	0	0.21	-4.50	-0.21	-0.62	-0.80
15	S2M27	-6.91	-51.04	0	0.19	-4.76	-0.01	-0.85	-0.65
16	S2M19	-6.60	-26.54	0	0.21	-4.37	-0.09	-0.86	-0.72
17	S2M13	-6.45	-47.13	0	0.18	-4.63	-0.25	-0.63	-0.72
18	S2M16	-6.32	-24.13	-0.7	0.23	-4.99	-0.32	-0.61	-0.85
19	S2M17	-6.24	-38.59	-0.60	0.19	-5.72	-0.29	-0.82	-0.75
20	S2M26	-5.46	-36.45	0	0.19	-4.78	-0.29	-0.52	-0.94
21	S2M18	-5.21	-34.83	0	0.19	-4.61	-0.23	-0.56	-0.91
22	S2M20	-4.98	-33.88	0	0.19	-4.25	-0.02	-0.51	-0.78
23	S2M23	-4.76	-37.54	0	0.18	-4.98	-0.04	-0.73	-0.98

24	S2M25	-4.69	-31.23	0	0.19	-3.11	-0.03	-0.94	-0.95
25	S2M21	-3.92	-22.34	0	0.18	-3.72	-0.03	-0.45	-0.58
26	S2M24	-3.81	-50.16	-1.14	0.22	-2.95	-0.23	-0.51	-1.00

Material & Methods

All the chemicals and reagents were purchased from Avra synthesis, Hyderabad, India. and BLD pharm Hyderabad, India. The Infrared spectra of the synthesized intermediate and compounds were determined by FT-IR Brucker alpha spectrophotometer. C^{13} NMR spectra were recorded on a Bruker's AVANCE-III 500MHz FT NMR spectrometers using DMSO/CDC_{l3} as solvent and TMS as an internal standard at Indian Institute of Science & Research, Bhopal. The LCMS/mass recorded on Bruker microTOF QII mass spectrometer at Indian Institute of Science & Research, Bhopal. The antimalarial activity of the synthesized compounds was evaluated by the SYBER Green I-based assay method on Plasmodium falciparum at Microcare Laboratories, Surat (Gujarat).

Chemistry and synthesis

Synthetic scheme for *2-(4-acetamidophenylsulfonamido)propanoic acid* (1) (Scheme II) The *p*-acetamidobenzenesulfonyl chloride (11.683 g, 0.05 mol) was added portion wise with stirring to a clear solution of Alanine (0.05 mol) in aqueous potassium carbonate (K_2CO_3, 6.91 g, 0.11 mol) solution (15 % or 2 $molL^{-1}$). The mixture was then stirred vigorously and heated at 70-80 °C for 30-35 min. After completion of reaction (TLC), the resulting clear solution was cooled to room temperature and then acidified in an ice bath to pH ~2.5 with drop wise addition of dil. HCl solution (2 $molL^{-1}$). The product thus obtained was filtered off, washed with water and recrystallized from *R*-spirit to afford appropriate of 2-(4-acetamidophenylsulfonamido)propanoic acid (**1**) in good yields.

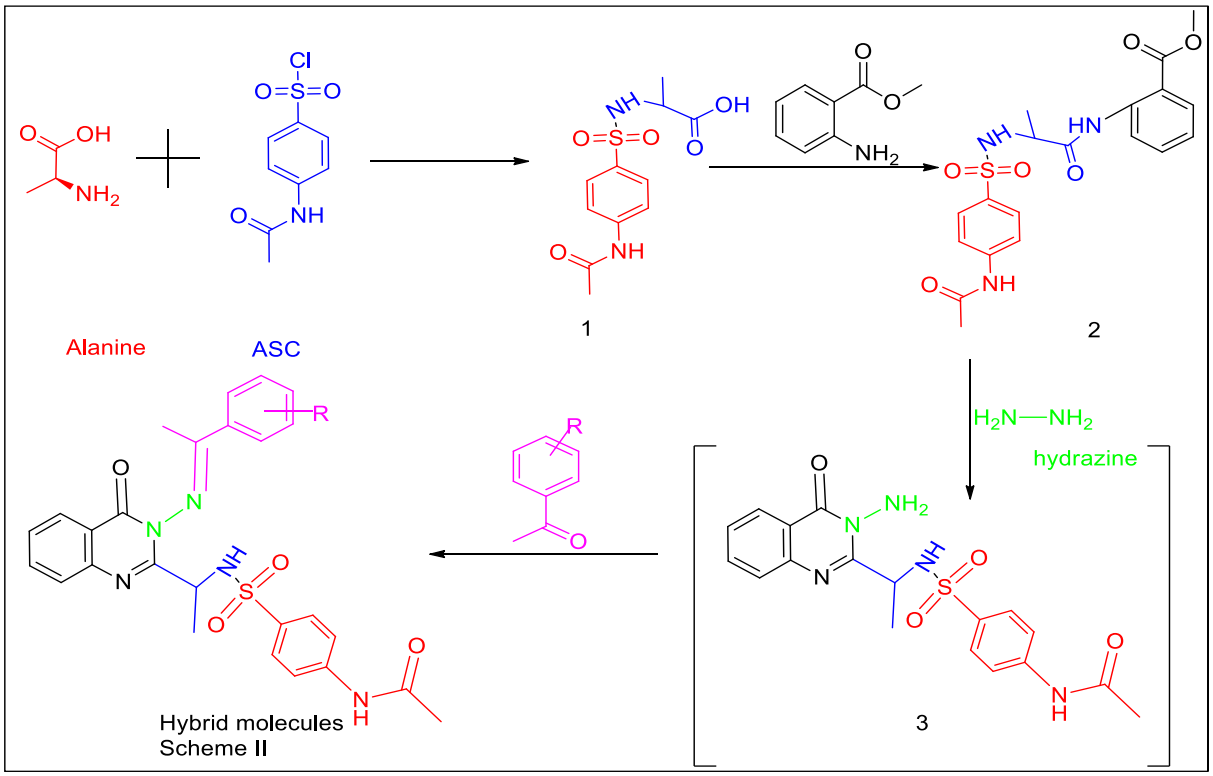
Synthetic scheme for *2-(2-(4-acetamidophenylsulfonamido) propanamido)benzoate* (2) (Scheme II)

To a mixture of 2-(4-acetamidophenylsulfonamido)propanamido) benzoic acid (0.05 mol) and methyl anthranilate (8.314 g, 0.055 mol) in appropriate solvent (THF-165 mL), phosphorous

trichloride (PCl_3, 6.87 g, 0.05 mol) in same solvent (10-15 mL) was added with continuous stirring over a period of 5 min. The resulting mixture (suspension) was heated for an appropriate time till completion of reaction (TLC) and the content in the flask was diluted with water and treated with 10 % $NaHCO_3$ solution. The product thus obtained was filtered off, washed with water and recrystallized from methanol to afford appropriate methyl *N*-acylanthranilate derivatives (**2**) in quantitative yields.

5.3.1.3 Synthetic scheme for *N-(4-(N-(1-(3-amino-4-oxo-3,4-dihydroquinazolin-2-yl)ethyl)sulfamoyl)phenyl)acetamide* (3) (Scheme II)

In a two necked round bottomed flask fitted with a device condenser, appropriate methyl *N*-acylanthranilate esters (**2**) (0.002 mol) and appropriate hydrazins (0.0022 mol) were taken in 1-*n*-butyl-3-methylimidazolium tetrafluoroborate ([Bmim][BF_4]-H_2O) and was stirred at 80 °C temperature. The progress of the reaction was monitored by TLC and further used in synthesis of final product *in situ.*



Scheme 1: **Reagents and conditions:** (i) Potassium carbonate solution (15% or 2 $molL^{-1}$), stirring 70-80^{0}C for 30-35min; (ii) methyl anthranilate (0.055 mol), THF, PCl_3 (0.05 mol),

stirring 5 min and Heat; (iii) Hydrazine (0.0022 mol), ([Bmim][BF4]-H2O), stirring 80^0C; (iv) respective acetophenones (0.022 mol), MW 70-90^0C, 350W, 10 min.

General procedure for synthesis of S2M1-S2M9 derivatives

To a washed, cleaned, and dried 30 ml Pyrex microwave glass vial**,** 3-Aminoquinazolinone (**3**) and different acetophenone (0.022mol) was added. The reaction mass was irradiated under microwave at 350W power level. After completion of the reaction, as indicated by TLC, the reaction mixture was washed with a mixture of chloroform and methanol.

Table: 2 Reaction parameter for synthesis of compounds S2M1-S2M9

Compound code	Substitution	Reaction time (Min.)	Reaction temperature(°C)	Solvent system Chloroform: Methanol
S2M1	2-OH acetophenone	10	80	9:1
S2M2	3-OH acetophenone	10.5	75	8:2
S2M3	4-OH acetophenone	10	75	9:1
S2M4	2-Nitro acetophenone	9.5	80	7:3
S2M5	3-Nitro acetophenone	8	90	9:1
S2M6	4-Nitro acetophenone	10	80	9:1
S2M7	2-Methyl acetophenone	9.5	90	9:1
S2M8	3-Methyl acetophenone	10.5	75	7:3
S2M9	4-Methyl acetophenone	10.5	75	8:2

General procedure for synthesis of S2M10-S2M18 derivatives

To a washed, cleaned, and dried 30 ml Pyrex microwave glass vial**,** 3-Aminoquinazolinone (**3**) and different acetophenone (0.022 mol) was added. The reaction mass was irradiated under microwave at 350W power level. After completion of the reaction, as indicated by TLC, the reaction mixture was washed with a mixture of chloroform and methanol.

Table: 3 Reaction parameter for synthesis of compounds S2M10-S2M18

Compoun	Substitution	Reaction	Reaction	Solvent system

d code		time (Min.)	temperature (°C)	Chloroform: Methanol
S2M10	2-Methoxy acetophenone	8.5	90	8:2
S2M11	3-Methoxy acetophenone	10.5	75	9:1
S2M12	4-Methoxy acetophenone	10	80	7:3
S2M13	2-Chloro acetophenone	10	80	9:1
S2M14	3-Chloro acetophenone	10.5	75	7:3
S2M15	4-Chloro acetophenone	9.5	90	9:1
S2M16	2-Bromo acetophenone	10	80	8:2
S2M17	3-Bromo acetophenone	10	80	9:1
S2M18	4-Bromo acetophenone	10	80	9:1

General procedure for synthesis of S2M19-S2M24 derivatives

To a washed, cleaned, and dried 30 ml Pyrex microwave glass vial**,** 3-Aminoquinazolinone (**3**) and different acetophenone (0.022 mol) was added. The reaction mass was irradiated under microwave at 350W power level. After completion of the reaction, as indicated by TLC, the reaction mixture was washed with a mixture of chloroform and methanol.

Table: 4 reaction parameters for synthesis of compounds S2M19-S2M24

Compound code	Substitution	Reaction time (Min.)	Reaction temperature(°C)	Solvent system Chloroform: Methanol
S2M19	2-Fluoro acetophenone	10	85	9:1
S2M20	3-Fluoro acetophenone	10	90	7:3
S2M21	4-Fluoro acetophenone	10	80	9:1
S2M22	4 Bromo 2Hydroxy acetophenone	10	85	8:2
S2M23	2,4-Dichloro acetophenone	10	85	9:1
S2M24	2,4-Dihydroxy acetophenone	10	90	9:1

Result and Discussion

On completion of the flexible docking process on Phenyl alanine linked Quinazoline-(3H)-one sulphonamide derivatives, the resulting conformation/poses of the ligands in the binding site of *Plasmodium Falciparum* Dihydro folate reductase thymidylate synthase were studied for the interaction pattern within 5A^{o} from the centre of the grid. The designed compounds were showing the same binding pattern as shown by the reported ligands. The docked molecule residues in the active site make hydrogen bond with LEU-49 and ILE-164 molecules. In Scheme-2, S2M4, S2M9 and S2M15 ligands showed good interactions with amino acids, and the Glide scores were found to be -10.64, -10.22 and -9.27, respectively.

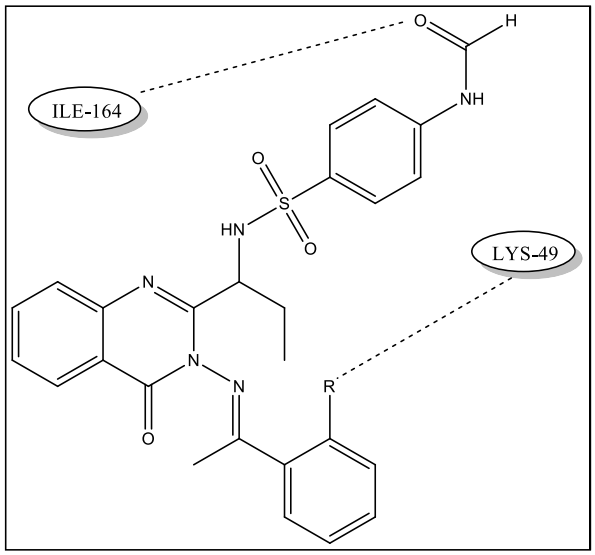


Figure 2: Interaction diagram of the ligand with LYS-49 and ILE-164 of Plasmodium Falciparum Dihydrofolate reductase thymidylate synthase Protein (PDB ID: 4DPD)

The purity of all the synthesized compounds was determined by TLC. Silica gel G-254 was used as adsorbent on prepared aluminium foils and Pet ether: methanol as mobile phase, the compounds were observed under UV cabinet and iodine vapors. For each compound single spot was observed. Melting points were determined by open capillary method and are uncorrected. Finally, the structure of the compounds was confirmed by IR, NMR and Mass spectrometry. Seventeen compounds were confirmed by IR data, which showed appearance of stretching frequencies of C-H at 3195-3026cm^{-1}. Appearance of C-N frequencies around 1310-1395

cm^{-1}, C=O around 1720-1785 cm^{-1}, S=O around 1294-1385 cm^{-1} and C=C frequencies around 1602-1695 cm^{-1}. 13C NMR spectra, this showed appearance of –C=O at 193.5 ppm which indicates the reaction of Quinazolinone with acetophenones.

The mass spectra of all the compounds clearly indicate the presence of M+ or M+1 or M-1 peak.

All the seventeen compounds of scheme-2 were screened for in vitro antimalarial activity at Microcare laboratories, Surat.

Most of the compounds were found to be potent against the *Plasmodium Falciparum*. Two compounds S2M4 and S2M15 are showing most promising results with maximum potency (MIC value-0.23 µg/mL and 0.36 µg/mL) against *Plasmodium Falciparum.* Most of the compounds from Scheme-2 have shown results between the MIC values of 0.23-1.95 g/mL against *Plasmodium Falciparum*.

In scheme two good antimalarial activity is shown by S2M4 and S2M15 which have 2-nitro acetophenone and 4 chloro acetophenone substitutions at 2^{nd} and 4^{th} position of phenyl ring on quinazoline-4(3*H*)-one. The nitro group is an electron withdrawing group whose positive charge becomes concentrated at ortho-para positions and they show electrophilic aromatic substitution. The chloro group is a halogen which increases drug solubility. S2M9 also has moderate activity which is substituted with 4 methyl acetophenone, methyl group at 4^{th} position of phenyl ring on quinazoline-4(3*H*)-one. Methyl group is also an electron withdrawing group which increases it activity. After the successful synthesis and characterization on Quinazolinone-sulphonamide hybrids, they were analyzed for in vitro antimalarial activity against Plasmodium Falciparum. The antimalarial activity of Quinazolinone-sulphonamide hybrids was done at the Microcare Laboratories, Surat, Gujarat, India by the SYBER Green assay technique, Quinine and Chloroquine were used as reference drug. The results of antimicrobial activity are summarized in table

Table 5 Antimalarial activity of the hybrid compounds (scheme 1)

S.no	**Compound codes**	**MIC value (µg/mL)**

1	S2M2	1.15
2	S2M3	0.76
3	**S2M4**	**0.23**
4	S2M5	1.42
5	S2M6	1.02
6	S2M7	0.87
7	S2M8	0.94
8	S2M9	0.55
9	S2M10	0.96
10	S2M11	0.92
11	S2M12	1.25
12	S2M14	0.87
13	**S2M15**	**0.36**
14	S2M17	1.95
15	S2M18	0.90
16	S2M21	0.97
17	S2M23	1.05
18	Quinine	0.268
19	Chloroquine	0.020
20	TMP	0.042

Table 6 IR Spectral data of Quinazolinone-sulphonamide hybrids (Scheme-1)

S.no	Compounds Codes	IR Spectra
1	S2M2	IR:3124.58cm^{-1}(Ar C-H) (str.), 1742.47cm^{-1} (C=O) 1630.45cm^{-1} (C=C), 1345.98cm^{-1} (C-N) (str.) 1298.88cm^{-1} (S=O), 1612.23cm^{-1} (C=N) (str.)
2	S2M3	IR:3112.62cm^{-1}(Ar C-H) (str.), 1741.73cm^{-1} (C=O) 1645.31cm^{-1} (C=C), 1369.82cm^{-1} (C-N) (str.) 1269.89cm^{-1} (S=O), 1606.81cm^{-1} (C=N) (str.)
3	S2M4	IR:3090.27cm^{-1}(Ar C-H) (str.), 1742.88cm^{-1} (C=O) 1690.81cm^{-1} (C=C), 1334.63cm^{-1} (C-N) (str.) 1262.88cm^{-1} (S=O), 1644.81cm^{-1} (C=N) (str.)
4	S2M5	IR:3097.27cm^{-1}(Ar C-H) (str.), 1741.66cm^{-1} (C=O) 1641.34cm^{-1} (C=C), 1338.80cm^{-1} (C-N) (str.) 1307.23cm^{-1} (S=O), 1513.96cm^{-1} (C=N) (str.)
5	S2M6	IR:3160.14cm^{-1}(Ar C-H) (str.), 1742.40cm^{-1} (C=O) 1616.42cm^{-1} (C=C), 1394.34cm^{-1} (C-N) (str.) 1310.09cm^{-1} (S=O), 1595.56cm^{-1} (C=N) (str.)
6	S2M7	IR:3095.12cm^{-1}(Ar C-H) (str.), 1742.45cm^{-1} (C=O) 1635.24cm^{-1} (C=C), 1369.25cm^{-1} (C-N) (str.) 1335.54cm^{-1} (S=O), 1612.55cm^{-1} (C=N) (str.)
7	S2M8	IR:3119.54cm^{-1}(Ar C-H) (str.), 1741.84cm^{-1} (C=O) 1619.34cm^{-1} (C=C), 1308.36cm^{-1} (C-N) (str.) 1295.02cm^{-1} (S=O), 1518.57cm^{-1} (C=N) (str.)
8	S2M9	IR:3156.41cm^{-1}(Ar C-H) (str.), 1740.23cm^{-1} (C=O) 1692.28cm^{-1} (C=C), 1310.36cm^{-1} (C-N) (str.) 1288.04cm^{-1} (S=O), 1632.65cm^{-1} (C=N) (str.)
9	S2M10	IR:3111.09cm^{-1}(Ar C-H) (str.), 1743.08cm^{-1} (C=O) 1684.47cm^{-1} (C=C), 1392.10cm^{-1} (C-N) (str.) 1345.59cm^{-1} (S=O), 1648.12cm^{-1} (C=N) (str.)

10	S2M11	IR:3026.30cm^{-1}(Ar C-H) (str.), 1738.45cm^{-1} (C=O) 1673.14cm^{-1} (C=C), 1333.57cm^{-1} (C-N) (str.) 1350.73cm^{-1} (S=O), 1646.89cm^{-1} (C=N) (str.)
11	S2M12	IR:3115.62cm^{-1}(Ar C-H) (str.), 1735.62cm^{-1} (C=O) 1651.01cm^{-1} (C=C), 1340.90cm^{-1} (C-N) (str.) 1325.41cm^{-1} (S=O), 1620.10cm^{-1} (C=N) (str.)
12	S2M14	IR:3046.53cm^{-1}(Ar C-H) (str.), 1741.54cm^{-1} (C=O) 1584.40cm^{-1} (C=C), 1396.55cm^{-1} (C-N) (str.) 1262.40cm^{-1} (S=O), 1522.09cm^{-1} (C=N) (str.)
13	S2M15	IR:3084.55cm^{-1}(Ar C-H) (str.), 1742.45cm^{-1} (C=O) 1650.25cm^{-1} (C=C), 1330.79cm^{-1} (C-N) (str.) 1325.02cm^{-1} (S=O), 1640.10cm^{-1} (C=N) (str.)
14	S2M17	IR:3111.04cm^{-1}(Ar C-H) (str.), 1742.47cm^{-1} (C=O) 1693.66cm^{-1} (C=C), 1322.99cm^{-1} (C-N) (str.) 1263.91cm^{-1} (S=O), 1518.32cm^{-1} (C=N) (str.)
15	S2M18	IR:3111.68cm^{-1}(Ar C-H) (str.), 1741.99cm^{-1} (C=O) 1693.87cm^{-1} (C=C), 1393.90cm^{-1} (C-N) (str.) 1325.10cm^{-1} (S=O), 1647.99cm^{-1} (C=N) (str.)
16	S2M21	IR:3114.51cm^{-1}(Ar C-H) (str.), 1740.60cm^{-1} (C=O) 1592.38cm^{-1} (C=C), 1384.21cm^{-1} (C-N) (str.) 1313.50cm^{-1} (S=O), 1513.48cm^{-1} (C=N) (str.)
17	S2M23	IR:3116.74cm^{-1}(Ar C-H) (str.), 1741.00cm^{-1} (C=O) 1694.57cm^{-1} (C=C), 1380.11cm^{-1} (C-N) (str.) 1286.81cm^{-1} (S=O), 1647.10cm^{-1} (C=N) (str.)

Table 7: NMR Spectral data of Quinazolinone-sulphonamide hybrids (Scheme-1)

S.no	Compounds	NMR Spectra

	Codes	
1	S2M2	^{13}C NMR: (500MHz, CDCl3) δ 22.5, 33, 61.3 ,105.5, 111.4, 115.9, 125.4 , 127.2 , 129.4, 130.2 , 138.5-138.7, 142.9 , 144.8 , 152.5 , 153.4, 165.3, 168.5, 186.7 ,191.5.
2	S2M3	^{13}C NMR: (500MHz, CDCl3) δ 20.1 , 32.9 , 45.7 ,102.5 , 110.7 , 113.7 , 119.5 , 121.3 , 124.5, 126.1 , 130.4-130.8, 139.3 , 145.9 , 150.7 , 153.4 , 165.4 , 185.9 , 186.9 , 197.4 .
3	S2M4	^{13}C NMR: (500MHz, CDCl3) δ 22.8 , 30.5 , 53.7 ,103.7 , 112.4 , 114.7 , 120.4 , 125.1 , 127.7-127.8 , 128.0 , 128.1-128.3 , 132.5 , 136.1 , 139.8 , 155.5 , 156.75, 168.3 ,182.5 , 188.3 .
4	S2M5	^{13}C NMR: (500MHz, CDCl3) δ 21.6 , 50.5 , 109.8 ,113.5 , 115.7 , 118.8 , 122.6 , 125.6 , 127.7-127.8 , 128.5 , 128.1-128.3 , 128.3-128.5 , 130.7, 133.5 , 134.7 , 135.6 , 136.8 , 138.6 ,157.4 , 189.8 .
5	S2M6	^{13}C NMR: (500MHz, CDCl3) δ 20.2 , 35.9 , 51.1 ,55.7 , 110.1 , 113.7 , 117.2 , 121.1 , 124.3, 125.1 , 127.7-127.8 , 128.0 , 128.1-128.3 , 128.3-128.5 , 133.1 , 134.8 , 136.7 , 138.3 ,160.1 , 164.8 , 191.9 .
6	S2M7	^{13}C NMR: (500MHz, CDCl3) δ 21.3 , 50.0 , 109.7 ,112.0 , 115.4 , 118.7 , 121.4 , 125.1 , 127.7-127.8 , 128.0 , 128.1-128.3 , 128.3-128.5 , 132.5, 133.1 , 134.8 , 135.5 , 136.7 , 138.3 ,162.5 , 188.9 .
7	S2M8	^{13}C NMR: (500MHz, CDCl3) δ 21.6 , 50.5 , 109.8 ,113.5 , 115.7 , 118.8 , 122.6 , 125.6 , 127.7-127.8 , 128.5 , 128.1-128.3 , 128.3-128.5 , 130.7, 133.5 , 134.7 , 135.6 , 136.8 , 138.6 ,157.4 , 189.8 .
8	S2M9	^{13}C NMR: (500MHz, CDCl3) δ 21.3 , 50.0 , 109.7 , 112.0 , 115.4 , 118.7 , 121.4 , 125.1 , 127.7-127.8 , 128.0 , 128.1-128.3 , 128.3-128.5 , 132.5, 133.1 , 134.8 , 135.5 , 136.7 , 138.3 , 162.5 , 188.9 .
9	S2M10	^{13}C NMR: (500MHz, CDCl3) δ 21.3 , 50.0 , 109.7 ,112.0 , 115.4 , 118.7 , 121.4 , 125.1 , 127.7-127.8 , 128.0 , 128.1-128.3 , 128.3-128.5 , 132.5, 133.1 , 134.8 , 135.5 , 136.7 , 138.3 ,162.5 , 188.9 .
10	S2M11	^{13}C NMR: (500MHz, CDCl3) δ 21.6 , 50.5 , 109.8 ,113.5 , 115.7 , 118.8 , 122.6 , 125.6 , 127.7-127.8 , 128.5 , 128.1-128.3 , 128.3-128.5 , 130.7, 133.5 , 134.7 , 135.6 , 136.8 , 138.6 ,157.4 , 189.8 .

11	S2M12	^{13}C NMR: (500MHz, CDCl3) δ 21.3 , 50.0 , 109.7 ,112.0 , 115.4 , 118.7 , 121.4 , 125.1 , 127.7-127.8 , 128.0 , 128.1-128.3 , 128.3-128.5 , 132.5, 133.1 , 134.8 , 135.5 , 136.7 , 138.3 ,162.5 , 188.9 .
12	S2M14	^{13}C NMR: (500MHz, CDCl3) δ 20.0 , 53.5 , 108.3 , 112.5 , 115.4 , 118.9 , 121.4 , 125.1 , 126.4, 128.2 , 128.3-128.5 , 128.9 , 129.8 , 132.5, 133.4 , 135.3 , 135.5 , 136.7 , 162.5 , 191.5 .
13	S2M15	^{13}C NMR: (500MHz, CDCl3) δ 21.6 , 50.5 , 109.8 , 113.5 , 115.7 , 118.8 , 122.6 , 125.6 , 127.7-127.8 , 128.5 , 128.1-128.3 , 128.3-128.5 , 130.7, 133.5 , 134.7 , 135.6 , 136.8 , 138.6 , 157.4 , 189.8 .
14	S2M17	^{13}C NMR: (500MHz, CDCl3) δ 22.3 , 56.5 , 62.7 ,105.8 , 111.3 , 113.7 , 118.1 , 121.3 , 125.6-125.9 , 129.4 , 132.2-132.5 , 135.3-135.5 , 138.5, 140.1 , 143.2 , 146.5 , 151.3 , 158.4 ,165.5 , 187.3 .
15	S2M18	^{13}C NMR: (500MHz, CDCl3) δ 24.3 , 33.5 , 52.3 ,112.5 , 115.4 , 118.9 , 121.4 , 125.1 , 126.4, 128.2 , 128.3-128.5 , 128.9 , 129.8 , 132.5 , 133.4 , 135.3 , 135.5 , 136.7 , 162.5 ,191.5 .
16	S2M21	^{13}C NMR: (500MHz, CDCl3) δ 21.3 , 50.0 , 109.7 , 112.0 , 115.4 , 118.7 , 121.4 , 125.1 , 127.7-127.8 , 128.0 , 128.1-128.3 , 128.3-128.5 , 132.5, 133.1 , 134.8 , 135.5 , 136.7 , 138.3 ,162.5 , 188.9 .
17	S2M23	^{13}C NMR: (500MHz, CDCl3) δ 21.6 , 50.5 , 109.8 , 113.5 , 115.7 , 118.8 , 122.6 , 125.6 , 127.7-127.8 , 128.5 , 128.1-128.3 , 128.3-128.5 , 130.7, 133.5 , 134.7 , 135.6 , 136.8 , 138.6 , 157.4 , 189.8 .

Conclusion:

In conclusion, the battle against malaria continues to be a formidable global health challenge, especially devastating for children, as it takes a toll of 2-3 million lives annually. This grim reality underscores the urgent need for new therapeutic avenues to combat this pervasive and deadly parasitic disease. The emergence of chloroquine resistance, attributed to mutations in the Plasmodium transmembrane protein PfCRT, has underscored the necessity for innovative drugs capable of overcoming these resistance mechanisms. In this context, the investigation into

Quinazolinone-sulphonamide hybrids, which exhibit antimalarial activity by inhibiting the PfDHFR enzyme, offers a ray of hope.

This study serves as a crucial stride towards meeting the demand for fresh antimalarial agents. Through the application of molecular docking techniques, a thorough exploration of more than 50 compounds against the PfDHFR enzyme (Pdb Id-4DPD) was conducted, culminating in the identification of a selection of 24 Quinazolinone-sulphonamide hybrids with promising binding scores. Employing microwave-assisted methods expedited the synthesis of these hybrids, with the progress of reactions being tracked meticulously via thin-layer chromatography.

The synthesized compounds' structural elucidation was achieved through a combination of analytical techniques, including IR spectroscopy, 1H NMR, and Mass spectrometry, solidifying their chemical identities. Furthermore, to assess their viability as potential drug candidates, the compounds underwent rigorous ADME screening, meticulously evaluating their pharmacokinetic and pharmacodynamic profiles.

The pinnacle of this study was the in vitro evaluation of the synthesized compounds for their antimalarial activity, a pivotal step in gauging their potential effectiveness against Plasmodium falciparum. Through the amalgamation of computational docking, synthetic chemistry, molecular characterization, ADME profiling, and biological assessment, this research substantially contributes to the pursuit of groundbreaking antimalarial agents. The identified Quinazolinone-sulphonamide hybrids emerge as promising contenders, potentially offering a solution to the urgent need for effective treatments in the face of drug-resistant malaria parasites.

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