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Aminoquinoline-Pyrimidine-Based Alkyl-Piperazine Tethered Hybrids: Synthesis, Antiplasmodial Activity, and Mechanistic Studies

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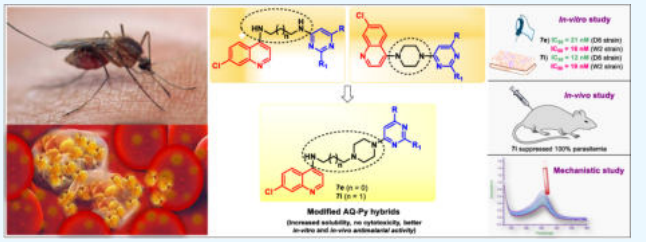
ABSTRACT: Though great progress has been made to eliminate malaria globally, effective and inexpensive strategies to design new antimalarials are still required due to the problem of drug resistance to the currently used antimalarials. Herein, in continuation of our efforts to improve the therapeutic efficacy of 4-aminoquinoline-pyrimidine (4-AQ-Py) based molecular hybrids, a series of 4-AQ-Py hybrids linked through diamine-piperazine (flexible and rigid) linkers was synthesized and assessed for *in vitro* antiplasmodial activity. In the *in vitro* assay, these hybrids exhibited excellent potency and selectivity index against both the chloroquine (**CQ**)-sensitive (D6) and **CQ**-resistant (W2) strains of *Plasmodium falciparum*. Compound **7i** was found to be the most potent (5-fold more active than **CQ**) against the D6 strain, while compound **7e** displayed the most potency (53-fold more potent than **CQ**) against the W2 strain. Furthermore, nine compounds (**7d, 7f−i**, **7l**, and **7o−q**) showed better antiplasmodial activity than the reference drug artemisinin (**ART**) against the D6 strain, and compared to **ART**, seven compounds (**7d−e, 7i−k**, and **7p−q**) demonstrated better activity against the W2 strain. All the synthesized hybrids were found noncytotoxic against the mammalian VERO cell lines. Two potent compounds, **7e** and **7i,** were evaluated for their *in vivo* antiplasmodial activity against *P. berghei*-infected mouse models. Additionally, one of the best active compounds, **7i**, was tested for heme binding, and docking studies were conducted with *Pf*-DHFR to determine the primary mechanism of action of these hybrids.



1. INTRODUCTION

Despite modern medicine and various control measures, malaria caused by *Plasmodium falciparum* (*P. falciparum*) still remains the most common parasitic disease and haunts the majority of human society across the globe. From the beginning of the human race, this vector-borne disease has been transmitted to humans by the female *Anopheles* mosquito[1] and *P. falciparum* is the most virulent parasite, responsible for the vast majority of malarial cases and more than 90% of deaths, especially in tropical and subtropical regions of the African continent. As per the WHO World Malaria Report 2023, approximately 249 million people got infected in 2022, with an estimated 608,000 deaths worldwide.[2] Most of the malarial cases in 2022 were reported from the African Region (94%) followed by the Southeast Asia Region (2%).[2]

Compounds bearing an aminoquinoline (AQ) core have played a pivotal role in combating malaria. They remained the drug of choice due to their outstanding ability to bind and modulate numerous biological targets that are critical for treatment. Chloroquine (**CQ**), a 4-aminoquinoline (4-AQ), was discovered during World War II and was considered a miracle medication to treat all kinds of malaria with minimal side effects.[3,4] However, the parasite has developed resistance against **CQ,** while other alternative AQ agents such as amodiaquine and mefloquine (more effective than **CQ** against **CQ**-resistant strains) were abandoned due to reported cases of toxicity (agranulocytosis, neutropenia, and hepatotoxicity).[4−7] Currently, five artemisinin-based combination therapies (ACTs) are recommended by WHO for the treatment of malarial infection.[8] In ACTs, artemisinin-based compounds are combined with different classes of drugs. Artemisinin derivatives include artesunate, dihydroartemisinin, and artemether, while companion drugs include mefloquine, lumefantrine, piperaquine, dapsone/chlorproguanil, and pyrimethamine/sulfadoxine.[9,10] However, recent reports of artemisinin resistance in the Greater-Mekong Region, i.e., five Southeast Asian countries (the Lao People's Democratic Republic, Cambodia, Vietnam, Thailand, and Myanmar) over the past decade highlight the need for novel drugs to treat severe malaria.[11−13] As the malaria parasite has developed resistance to nearly all classes of the existing antimalarials, there is a dire

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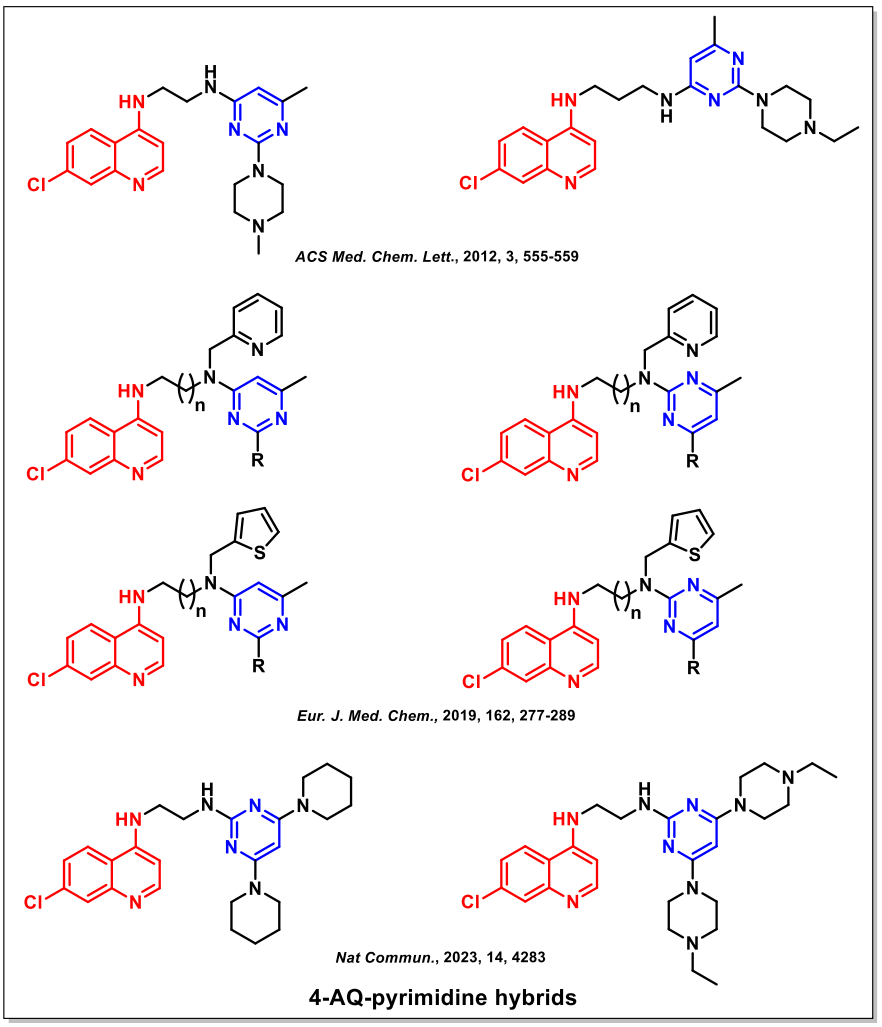

Figure 1. Structures of previously synthesized 4-AQ-pyrimidine hybrids.

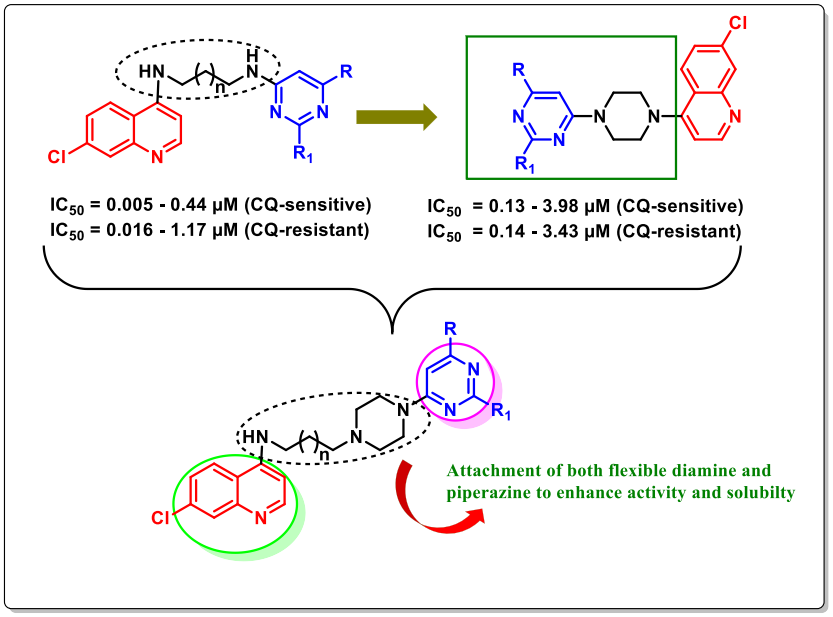

Figure 2. Design of 4-AQ-pyrimidine hybrids containing diamine and piperazine linker.

need to develop new chemical entities that can solve the problem of drug resistance and eradicate this deadly disease.

In an effort to overcome the issue of drug resistance and in the development of new antimalarial drugs, various drug

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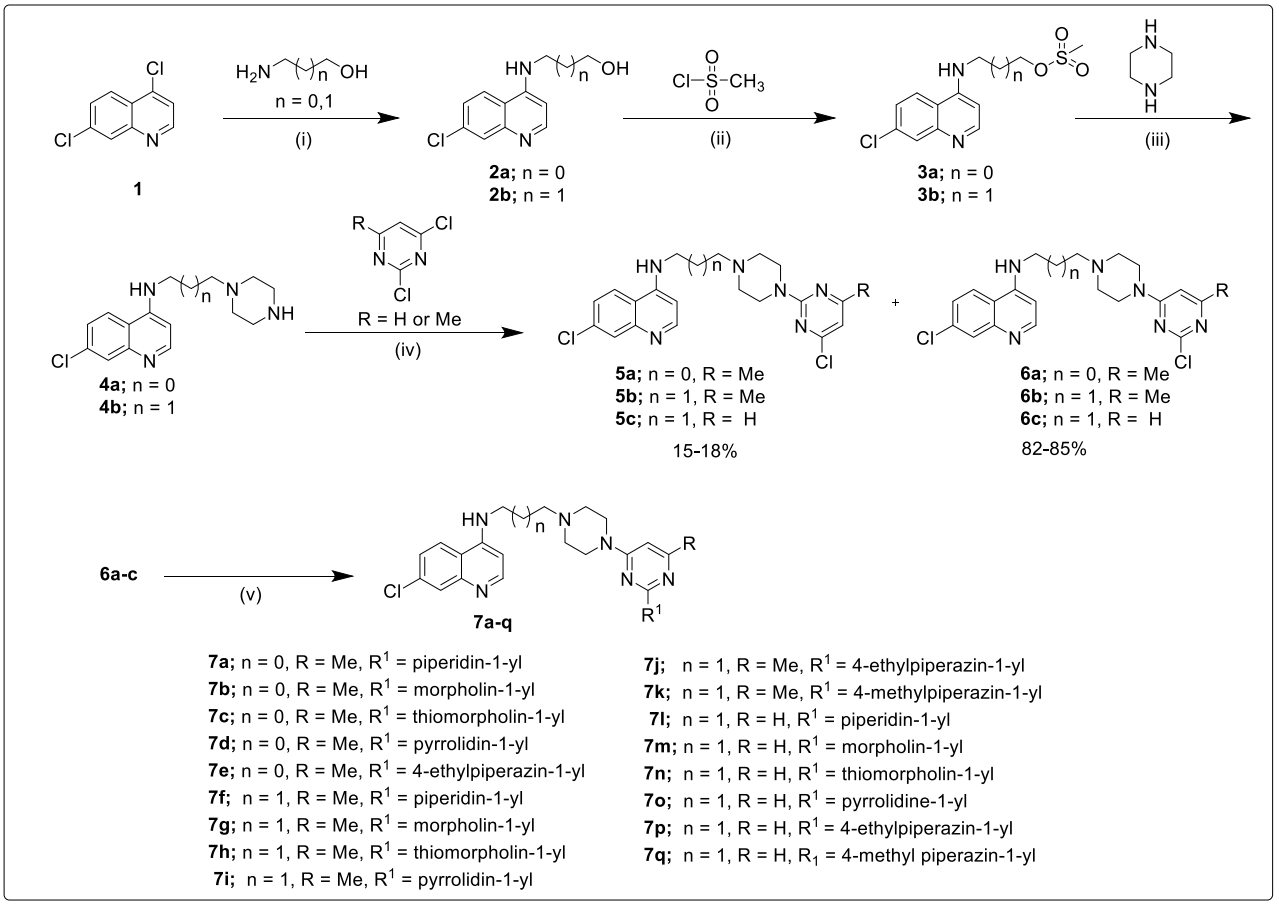
Scheme 1. (i) Neat, 130−150 °C, 10−12 h, 80−90%; (ii) Triethylamine, THF, 0 °C-RT, under N_2, 1 h, 75−80%; (iii) DMF, 80-90 °C, 3−4 h, 90−95%; (iv) DIPEA, THF, 0−15 °C, 12−14 h; (v) Secondary Amines, K_2CO_3, DMF, 100−120 °C, 10−12 h, 75−85%



discovery efforts have been explored. These include the development of analogs of existing drugs, combining drugs with different modes of action (using combination therapy), and the incorporation of drug resistance reversers.[14−17] In recent years, the molecular hybrid approach, where two or more pharmacophores are linked through a linker, has attracted the attention of medicinal chemists worldwide.[16−18] The molecular hybrid approach has distinct advantages such as a lower risk of drug−drug interactions, a more predictable pharmacokinetic profile, increased druggable characteristics, prolonged duration of effectiveness, reduced dosage and, more importantly, patient compliance.[17] Our research group has strategically applied this approach and synthesized various series of 4-AQ-pyrimidine hybrids[19−21] for the development of new lead compounds with antiplasmodial and anti-Parkinson properties (Figure 1). AQ-Pyrimidine (AQ-Py) hybrids have been recently reported for their anti-Parkinson activity by our research group[21] and one of the compounds, ATH-399A (code given by the company HL192), has entered phase-I clinical trials.[22] In previous works, while understanding the effect of linkers in the structure modification of AQ-Py hybrids, we observed a sharp decrease in the activity of compounds when AQ and Py were linked through a rigid linker, piperazine.[23] However, these hybrids showed better solubility and were noncytotoxic toward mammalian cells in comparison to their flexible diamine linker counterpart (Figure 2). In order to study the combined effect of the flexible diamine and rigid piperazine linkers on the pharmacokinetic behavior and antiplasmodial activity of 4-aminoquinoline-pyrimidine hybrids, we decided to introduce both diamine and piperazine linkers to our previously reported 4-AQ-pyrimidine hybrids (Figure 2).

In the present work, taking advantage of the SAR studies of our previous approach to further improve the antiplasmodial activity, we report the synthesis, antiplasmodial activity (*in vitro* and *in vivo*), and cytotoxicity of a series of 4-AQ-pyrimidine hybrids in conjunction with diamine and piperazine linkers. Furthermore, we performed heme binding and *in silico* docking studies with the reported crystal structure of *P. falciparum* dihydrofolate reductase-thymidylate synthase (*Pf*-DHFR-TS) to validate the probable mode of action, with the inclusion of ADME properties to assess the pharmacokinetic behavior of the active compounds.

2. RESULTS AND DISCUSSION

2.1. Synthesis. The synthetic pathway to synthesize the desired hybrids is depicted in Scheme 1. In brief, commercially available 4,7-dichloroquinoline (**1**) was first treated with excess alkanolamines (2-aminoethan-1-ol and 3-aminopropan-1-ol) via S_NAr reaction under neat conditions at 130−150 °C, which resulted in the formation of intermediates **2a**−**b**.[24] These intermediates (**2a** and **2b**) containing a free hydroxyl group, on further reaction with mesyl chloride in the presence of triethylamine, afforded mesylated 4-AQ **3a** and **3b**, respec-

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Table 1. *In Vitro* Antiplasmodial Activity and Cytotoxicity of Diamine and Piperazine-Linked 4-AQ-Pyrimidine Hybrids

Compd No.	n	R	R[1]	*P. falciparum* (D6 strain) IC_{50} (μM)[a]	SI[b]	*P. falciparum* (W2 strain) IC_{50} (μM)[a]	SI[b]	Cytotoxicity (VERO cells) IC_{50} (μM)[c]	Resistance Index (RI)[d]
5a	0	Me	Cl	0.070	>160.6	0.21	>52.7	NC	3.0
5b	1	Me	Cl	0.10	>101	0.35	>310	NC	3.50
5c	1	H	Cl	0.062	>183	0.23	>480	NC	3.70
6a	0	Me	Cl	0.079	>143.6	0.50	>22.7	NC	6.33
6b	1	Me	Cl	0.066	>167.2	0.20	>52.8	NC	3.03
6c	1	H	Cl	0.073	>155	0.42	>26.9	NC	5.75
7a	0	Me	Piperidin-1-yl	0.032	>313	0.040	>251	NC	1.25
7b	0	Me	Morpholin-1-yl	0.095	>106.6	0.049	>206.6	NC	0.52
7c	0	Me	Thiomorpholin-1-yl	0.038	>256	0.064	>154	NC	1.68
7d	0	Me	Pyrrolidin-1-yl	0.013	>758	0.020	>506	NC	1.54
7e	0	Me	4-Ethylpiperazin-1-yl	0.021	>446	0.016	>570	NC	0.76
7f	1	Me	Piperidin-1-yl	0.015	>654	0.034	>285	NC	2.27
7g	1	Me	Morpholin-1-yl	0.015	>644	0.043	>227	NC	2.87
7h	1	Me	Thiomorpholin-1-yl	0.018	>523	0.050	>188	NC	2.78
7i	1	Me	Pyrrolidin-1-yl	0.012	>812	0.019	>516	NC	1.58
7j	1	Me	4-Ethylpiperazin-1-yl	0.019	>487	0.020	>452	NC	1.05
7k	1	Me	4-Methylpiperazin-1-yl	0.019	>482	0.022	>426	NC	1.16
7l	1	H	Piperidin-1-yl	0.014	>722	0.031	>327	NC	2.21
7m	1	H	Morpholin-1-yl	0.020	>488	0.034	>294	NC	1.70
7n	1	H	Thiomorpholin-1-yl	4.08	>2.4	>9.83	1	NC	2.40
7o	1	H	Pyrrolidin-1-yl	0.015	>668	0.030	>343	NC	2.0
7p	1	H	4-Ethylpiperazin-1-yl	0.013	>710	0.020	>467	NC	1.54
7q	1	H	4-Methylpiperazin-1-yl	0.014	>65.8	0.017	>568	NC	1.21
CQ	-	-	-	0.06	-	0.853	-	NC	14.21
ART	-	-	-	0.019	-	0.0281	-	NC	1.48
doxorubicin	-	-	-	-	-	-	-	8.6 ± 0.4	-

[a]IC_{50}: Drug concentration that causes 50% growth inhibition. [b]S.I.: Selectivity index (IC50 value for cytotoxicity/IC_{50} value for antiplasmodial activity). [c]Cytotoxicity against VERO cells; NC: no cytotoxicity; compounds were not cytotoxic up to the highest concentration of 4760 ng/mL. [d]RI = IC50 (W2 strain)/IC50 (D6 strain). % viability: % decrease in cell viability was <10%.

tively. On nucleophilic substitution of these intermediates in DMF with an excess of piperazine, intermediates **4a** and **4b** were afforded. Subsequent reaction of intermediates **4a−b** with commercially available 2,4-dichloropyrimidine or 2,4-dichloro-6-methyl-pyrimidine yielded three sets of regioisomers namely, **5a−c** as minor products and **6a−c** as major products. On nucleophilic substitution of these major regioisomers (**6a−c**) with different secondary cyclic amines at elevated temperatures (100−120 °C) in the presence of K_2CO_3 and DMF, the desired final compounds (**7a−q**) were afforded in excellent yields.

2.2. *In Vitro* Antiplasmodial Activity, Structure−Activity Relationship, and Cytotoxicity. As mentioned in Table 1, *the in vitro* antiplasmodial activity of all the synthesized diamine and piperazine-linked 4-aminoquinoline-pyrimidine hybrids (**7a−q**) along with their intermediates (**5a−c** and **6a−c**) were evaluated against *P. falciparum* D6 and W2 strains. The *in vitro* activity results indicate that almost all of the tested compounds were potent, with IC_{50} values in the range of 0.012−0.10 μM against the D6 strain and IC_{50} values in the range of 0.016−9.83 μM against the W2 strain of *P. falciparum*. Compounds **7a, 7c−m,** and **7o−q** showed better activity (IC_{50} 0.012−0.038 μM), and compounds **5a, 5c,** and **6a−c** (IC_{50} 0.062−0.079) showed comparable activity to the reference compound **CQ** (IC_{50} 0.06 μM) against the D6 strain. Compound **7i** was found to be the most potent against the D6 strain, with an IC_{50} value of 0.012 μM (5-fold better activity than **CQ**) and a selectivity index >812.

Almost all the compounds (**5a−c, 6a−c, 7a−m,** and **7o−q**) showed better activity (IC_{50} 0.016−0.50 μM) than the reference compound **CQ** (IC_{50} 0.853 μM) against the W2 strain. Compound **7e** was found to be the most potent against the W2 strain with an IC_{50} value of 0.016 μM (53-fold better activity than **CQ**) and a selectivity index >570. Comparison of the antiplasmodial activity of these compounds with another reference compound, artemisinin (**ART**), against the D6 strain revealed that nine compounds (**7d, 7f−i, 7l,** and **7o−q**) exhibited superior activity, two compounds (**7j−k**) showed comparable activity, and two compounds (**7e** and **7m**) demonstrated activity equivalent to **ART**. Against the W2 strain, four compounds (**7f, 7l−m,** and **7o**) demonstrated activity comparable to **ART**, while seven compounds (**7d−e, 7i−k,** and **7p−q**) demonstrated better activity. All of the regioisomeric intermediates (**5a−c** and **6a−c**) exhibited superior activity against the W2 strain (IC_{50} 0.20−50 μM) compared to the D6 strain (IC_{50} 0.062−0.10 μM). Remarkably, substituting the chloro group in the regioisomers (**5a−c** and **6a−c**) with various cyclic secondary amines significantly enhanced the activity of nearly all resulting hybrids (**7a−q**) against both D6 and W2 strains. Notable exceptions were compound **7b**, which showed a slight reduction in activity against the D6 strain, and compound **7n**, which exhibited decreased activity against both

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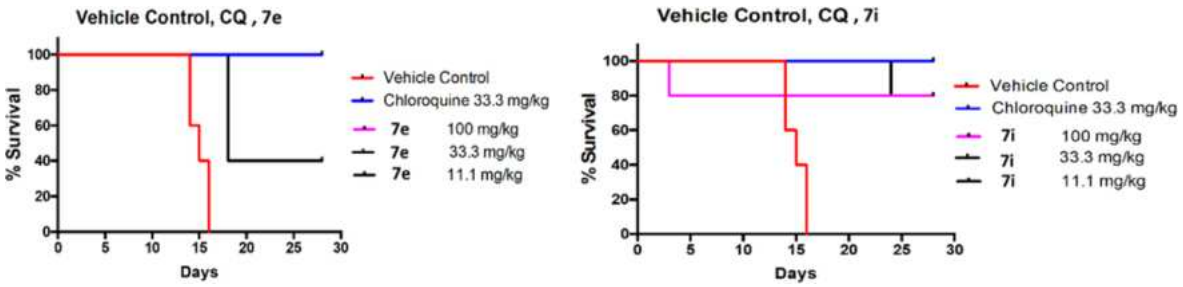

Figure 3. Dose regime (compounds **7e** and **7i**) and survival curve.

strains. On comparing the activity of compounds **7a−e** (having a two-carbon chain linker and methyl-substituted pyrimidine ring) with compounds **7f−k** (having a three-carbon chain linker and methyl-substituted pyrimidine ring), it was observed that compounds with a three-carbon chain linker (**7f−k**) showed better activity than compounds with a two-carbon chain linker (**7a−e**) against the D6 strain, while the activity of both sets (**7a−e** and **7f−k**) was comparable against the W2 strain. On comparing the activity of compounds **7a−e** (having a two-carbon chain linker and methyl-substituted pyrimidine ring), it was observed that piperidin-1-yl-, pyrrolidin-1-yl-, and 4-ethylpiperazin-1-yl-substituted compounds (**7a, 7d,** and **7e**, respectively) showed better activity against both D6 and W2 strains than the compounds having morpholin-1-yl and thiomorpholine-1-yl (**7b** and **7c**, respectively) substitution.

Among compounds **7a−e,** compound **7d** with pyrrolidin-1-yl was found to be the most active against the D6 strain, and compound **7e** with 4-ethylpiperazin-1-yl substitution was the most potent against the W2 strain. On comparing the activity of compounds **7f−k** (having a three-carbon chain linker and methyl-substituted pyrimidine ring), it was observed that compounds with piperidin-1-yl, morpholin-1-yl, and pyrrolidine-1-yl (**7f, 7g,** and **7i,** respectively) substitution were more potent than compounds having thiomorpholine-1-yl, 4-ethylpiperazin-1-yl, and 4-methylpiperazin-1-yl (**7h**, **7j**, and **7k**, respectively) substitution against the D6 strain, while compounds **7i−k** (pyrrolidine-1-yl, 4-ethylpiperazin-1-yl, and 4-methylpiperazin-1-yl, respectively) showed better activity against the W2 strain. Compound **7i** with pyrrolidine-1-yl substitution was found to be the most active against both strains. On comparing the activity of compounds **7l−q** (having a three-carbon chain linker and pyrimidine ring), all the compounds showed better activity than the regio-isomeric intermediates (**5c** and **6c**) except in the case of compound **7n** the activity was decreased by morpholin-1-yl substitution. Even the selectivity index was superior to the standard drug chloroquine for many compounds against both strains (D6 and W2), while its value ranged from 2.4 to 812 for all compounds. All synthesized compounds were tested for cytotoxicity against mammalian VERO cells, and even at the highest tested concentration of 4760 ng/mL, none showed any cytotoxic effects. According to the resistance index (RI), which is calculated as the ratio of the IC50 value for the **CQ**-resistant strain to that for the **CQ**-sensitive strain, the synthesized hybrids demonstrated potent efficacy against both **CQ**-sensitive and **CQ**-resistant strains of *P. falciparum*. Compared to those of **CQ** (RI = 14.21), the RI values of these compounds were much lower (up to 0.52). Additionally, a few compounds had RI values that were lower or similar to those of **ART** (RI = 1.48). On comparing the RI values of the most active compounds, **7e** and **7i**, it was found that compound **7e** showed a lower RI value (0.76) than **ART**, while **7i** showed a comparable RI value (1.58). A lower RI of 20 indicates a potentially effective lead antiplasmodial since it demonstrates its equipotent nature regardless of the strain's susceptibility, further indicating its usefulness in addressing the problem of drug resistance.

2.3. *In Vivo* Antiplasmodial Efficacy. On the basis of *in vitro* antiplasmodial activity and cytotoxicity profile, two potent molecules (**7e** and **7i**) were evaluated for their *in vivo* antiplasmodial potential in *P. berghei*-infected mice models.- Post-infection, the *P. berghei*-infected mice were administered with compounds **7e** and **7i** *via* oral gavage at doses of 11.1, 33.3, and 100 mg/kg once daily on days 0, 1, and 2 (Figure 3). The mice were then monitored for apparent signs of toxicity, parasitemia, and survival until day 28. It was observed that compounds **7e** and **7i** both displayed promising results and were effective as they were able to suppress 100% parasitemia and cured all 5/5 mice ata 33.33 mg/kg dose with a mean survival time of 28 days in comparison to **CQ** which cured only 2/5 mice (Table 2). However, compound **7i** displayed a better antiplasmodial activity profile than **7e** as it suppressed 100% parasitemia at all doses (*i.e.*, 11.1, 33.3, and 100.0 mg/kg).

2.4. Heme Binding Studies. The malarial parasite (*P. falciparum*) breaks down host red blood cells (RBCs) within its acidic food vacuole (pH = 5.6) to utilize globin, the protein component, as a source of essential amino acids required for its

Table 2. *In Vivo* Antiplasmodial Evaluation of Selected Diamine and Piperazine-Linked 4-AQ-Pyrimidine Hybrids in the *P. berghei*−Mouse Malaria Model

	% Parasitemia suppression[a]					
Treatment	dose (mg/kg × no. of days post infection)	day 5	day 7	survival[b]	MST[c]	cure[d,e]
Vehicle	NA × 3	-	-	0/5	14	0/5
CQ	33.3 × 3	100.00	100.00	5/5	28	2/5
7e	100 × 3	100.00	100.00	4/5	28	5/5
7e	33.3 × 3	100.00	100.00	5/5	28	5/5
7e	11.1 × 3	94.31	41.53	2/5	21.4	0/5
7i	100 × 3	100.00	100.00	5/5	22.8	4/4
7i	33.3 × 3	100.00	100.00	5/5	28	5/5
7i	11.1 × 3	100.00	100.00	4/5	27	3/5

[a]% parasitemia suppression is calculated by considering the mean parasitemia in the vehicle control as 100%. Parasitemia suppression of less than 80% is considered nonsignificant. [b]Number of animals that survived on day 28/total animals in the group (the day of the death postinfection). [c]% MST—mean survival time (days). [d]Number of mice without parasitemia (cured) until day 28 postinfection. [e]**Host**: Swiss Webster Hsd:ND4 mice were used for the study.

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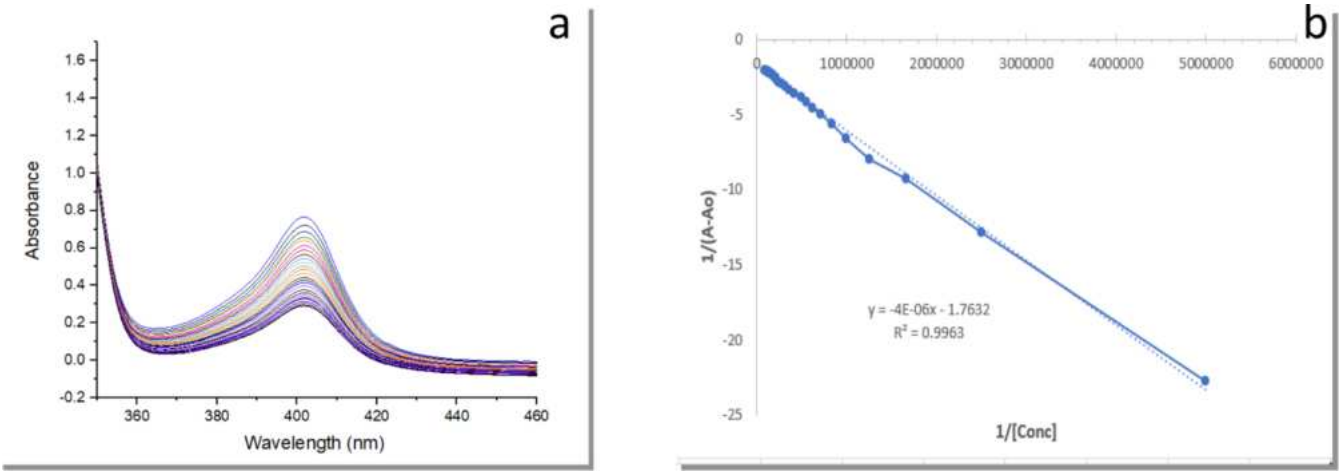

Figure 4. (a) Monomeric heme titration with increasing concentration of compound **7i** at pH 7.4 (using HEPES buffer). (b) Spectro-photometric titrations of compound **7i**.

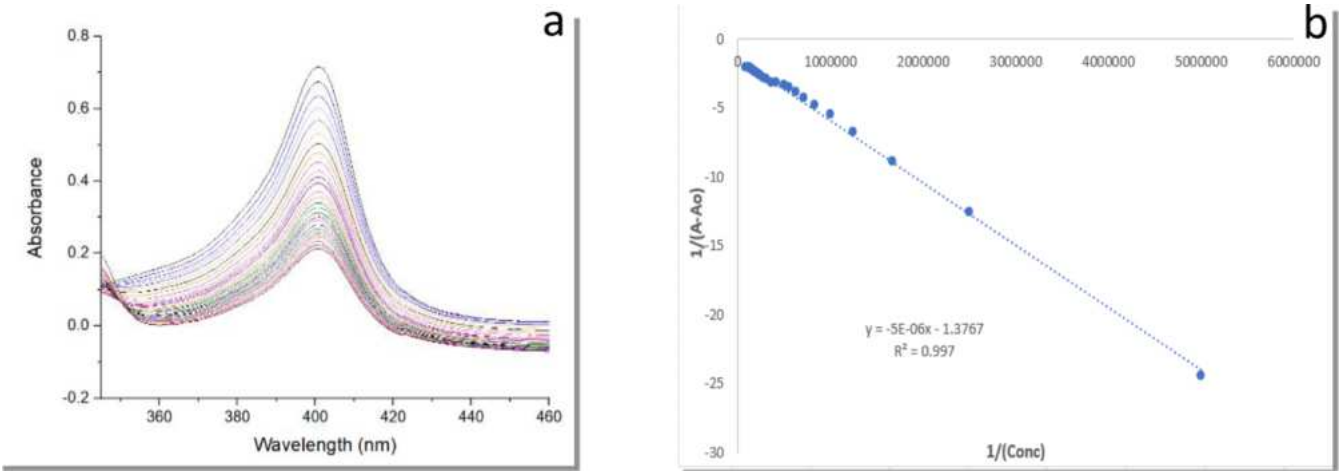

Figure 5. (a) Monomeric heme titration with increasing concentration of compound **7i** at pH 5.6 (using MES buffer). (b) Spectro-photometric titrations of compound **7i**.

rapid growth and development inside the host.[25] This process is accompanied by the release of the byproduct heme (Fe(III)-protoporphyrin-IX), which is toxic to the parasite and leads to the death of the parasite. To avoid this toxic heme, the parasite converts it to a nontoxic substance known as hemozoin (a polymer of heme).[25] 4-AQ-based antimalarials are believed to inhibit the hemozoin formation within the *P. falciparum* food vacuole through $\pi-\pi$ stacking interactions between the aminoquinoline moiety and the porphyrin ring of heme. The classical antimalarial drug **CQ** (4-aminoquinoline), a weak diprotic base, diffuses through the membranes of the erythrocyte and accumulates in the parasite's acidic food vacuole (pH 5.0). Herein, the **CQ** protonates and thus accumulates inside the food vacuole, where it binds with the heme and forms a complex and inhibits the hemozoin formation. Thus, **CQ**–heme complex retains the toxicity of heme and kills the parasite.[26] To validate that the synthesized compounds have heme binding as the possible mode of action, we studied the binding interactions of the compound **7i** with monomeric heme, and its binding constant was calculated and compared with **CQ** as per the standard methods reported in the literature.[27−29] As the concentration of the compound increases, the intensity of the Soret band near 402 nm in a 40% hemin-DMSO solution decreases, indicating that the compound binds to the monomeric heme. Compound **7i** was subjected to spectrophotometric titrations using monomeric heme solutions at two distinct pH values: physiological pH (7.4) and the acidic pH (5.6) of the parasite's digestive vacuole. The intensity of the monomeric heme's Soret band significantly decreased at both pH values, suggesting that **7i** interacts with heme (shown in Figures 4 and 5). Further, binding constants of compound **7i** with heme were calculated and obtained more in comparison to standard **CQ**–heme (Table 3) at both pH values .[30]

2.5. Molecular Modeling Studies. *2.5.1. Binding Mode Analysis.* The major antifolate drugs used for the treatment of malaria are pyrimethamine (PYR), proguanil (converted to the active form cycloguanil), and sulfa drugs.[31] Both pyrimethamine and cycloguanil target the dihydrofolate reductase-

Table 3. Binding Constant of Log K for Compound 7i and Chloroquine

	Monomeric Heme log K_b	
Compound	pH 5.6 (MES buffer)	pH 7.4 (HEPES buffer)
7i	5.4	5.6
CQ	5.3	5.2

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thymidylate synthase (DHFR-TS), a bifunctional enzyme of the *P. falciparum* involved in the folate metabolism pathway.[32] However, the emergence of S108N, C59R, and N51I mutations in *P. falciparum* DHFR-TS has conferred resistance to pyrimethamine. The quadruple mutant (N51I, C59R, S108N, I164L) shows resistance to pyrimethamine and cycloguanil but is still sensitive to WR99210.[33]

To analyze the interactions of novel 4-aminoquinoline-pyrimidine hybrid compounds with *Pf*-DHFR-TS, we tried to dock the hybrid compounds into the X-ray structures of wild-type *Pf*-DHFR-TS and quadruple mutant *Pf*-DHFR-TS. Molecular docking studies were conducted for the most active compounds (**7d**, **7e**, **7f**, **7i**, **7l**, and **7p**) identified through *in vitro* studies. These studies were performed with the crystal structures of quadruple mutant *Pf*-DHFR-TS (PDB ID:1J3K) and wild-type *Pf*-DHFR-TS (PDB ID:3QGT). The results summarized in Table 4 demonstrate that the highly active compounds exhibited notable binding affinities.

Table 4. Glide Docking Energies and Docking Scores, Along with the Reference Compounds, in Wild and Mutant *Pf*-DHFR-TS

	Docking results with wild *Pf*-DHFR		Docking results with mutant *Pf*-DHFR	
Compound	Glide GScore	Glide Energy	Glide GScore	Glide Energy
7d	−7.964	−35.33	−5.319	−32.628
7e	−6.542	−38.441	−6.574	−40.657
7f	−6.931	−48.716	−5.596	−42.325
7i	−7.515	−49.514	−5.84	−32.099
7l	−7.33	−45.813	−5.777	−31.392
7p	−6.536	−47.616	−7.318	−48.956
PYR	−8.903	−46.976	−7.337	−40.38
cycloguanil	−8.396	−31.309	−6.612	−40.24
dihydrofolate	−8.406	−61.021	−9.764	−66.293

This is evident from their Glide energies, which ranged from −48.7 kcal mol^{-1} to −35.33 kcal mol^{-1} for the wild type and from −48.95 kcal mol^{-1} to −31.39 kcal mol^{-1} for the quadruple mutant type.

Figures 6 and 7 illustrate the binding configuration of the most active compound (**7i**) with both the wild-type and quadruple mutant *Pf*-DHFR-TS. **Compound 7i** showed hydrogen bonding interactions with the binding residue Asp54 and cation-pi bonding with Lys115 of the wild-type *Pf*-DHFR-TS. The same Asp54 residue also showed hydrogen bonding interactions with the quadruple mutant *Pf*-DHFR-TS. Additionally, the Phe116 residue of the mutant exhibited pi−pi interactions.

2.5.2. Prediction of Pharmacokinetic Properties. Pharmacokinetic and ADME properties of the studied compounds were evaluated *in silico* using QikProp v3.5.[34] The key properties and their permissible ranges are detailed in Tables 5 and 6. The active compounds were assessed against Lipinski's Rule of Five (Ro5), a set of computational criteria used to predict the likelihood of high oral absorption.[35] According to these guidelines, an orally active compound should not exceed four violations of the rule.[36] All the compounds successfully met the criteria, indicating their potential as orally active drugs.[35]

The *in silico* predictions for oral drug absorption (PercentHumanOralAbsorption) indicated values within an acceptable range. Factors such as size, polarity, lipophilicity, and conformational dynamics influence oral bioavailability, which can be further evaluated using properties like the number of rotatable bonds (<15) and polar surface area (70 $Å^2$−200 $Å^2$).[35,37] The compounds analyzed in this study exhibited fewer than 15 rotatable bonds and polar surface area values within the permissible range (Table 6). Additional properties, such as permeability in Caco-2 cells (QPPCaco), which reflects intestinal drug absorption, showed favorable outcomes with some exceptions. Transcellular absorption, involving passive diffusion across cell membranes, is typically studied using the human colorectal carcinoma (Caco-2) cell model.[35] Furthermore, parameters such as human serum albumin binding (QPlogKhsa), brain-to-blood partition coefficient (QPlogBB), and blood−brain barrier permeability using the MDCK cell model (QPPMDCK) were within the acceptable range for most active compounds.

3. CONCLUSIONS

To summarize, we reported the synthesis of 4-AQ-pyrimidine hybrids linked through the *N*-aminoalkyl piperazine linker. Most of the compounds in the series displayed better potency against **CQ**-sensitive and **CQ**-resistant *P. falciparum* strains without causing cytotoxicity to the mammalian VERO cell lines up to a concentration of 4760 ng/mL. Compound **7i** was found to be the most potent (5-fold more active than **CQ**) against the D6 strain, while compound **7e** was found to be the most potent (53-fold more active than **CQ**) against the W2 strain of *P. falciparum*. Further, two potent compounds (**7e** and **7i**) were evaluated for *in vivo* studies against *P. berghei*-infected mouse models, and compound **7e** was found to be the most active. Mechanistic heme binding studies of compound **7i** showed strong binding interaction with monomeric heme compared to chloroquine, thus providing evidence that heme may be a potential target for these derivatives. Moreover, molecular docking studies of one of the best active compounds, **7i**, were conducted with both wild and mutant *Pf*-DHFR-TS. The results showed good binding interactions in the active site, and ADMET prediction was used to analyze the pharmacokinetic properties of some active compounds.

4. EXPERIMENTAL SECTION

An EZ automated melting point device was used to record the uncorrected melting points of the synthesized compounds. To monitor the progress and completion of the reaction, TLC sheets (E. Merck Kieselgel 60 F254) were used, and visualization of spots was accomplished using UV light, iodine stain, or ninhydrin stain. As mentioned in the procedure, silica gel column chromatography was utilized to purify the intermediates and final compounds. A Jeol Spectrospin spectrometer was used to record the ^1H NMR and ^{13}C NMR at 400 and 100 MHz, respectively, while the values for chemical shifts are shown in parts per million (ppm) on the delta scale (δ). These shifts are referenced to the internal standard tetramethylsilane (TMS). IR spectra were recorded using a PerkinElmer FT-IR spectrophotometer, and the results are shown as λ_{max} per centimeter. Mass spectra were recorded on Agilent 6550 iFunnel Q-TOFs. HPLC data were recorded on a Shimadzu LC2010CHT with a UV detector.

4.1. General Procedure for the Synthesis of 2-((7-Chloro-quinolin-4-yl)amino)-ethanol (2a) and Related Compound (2b). In the initial step, aromatic nucleophilic

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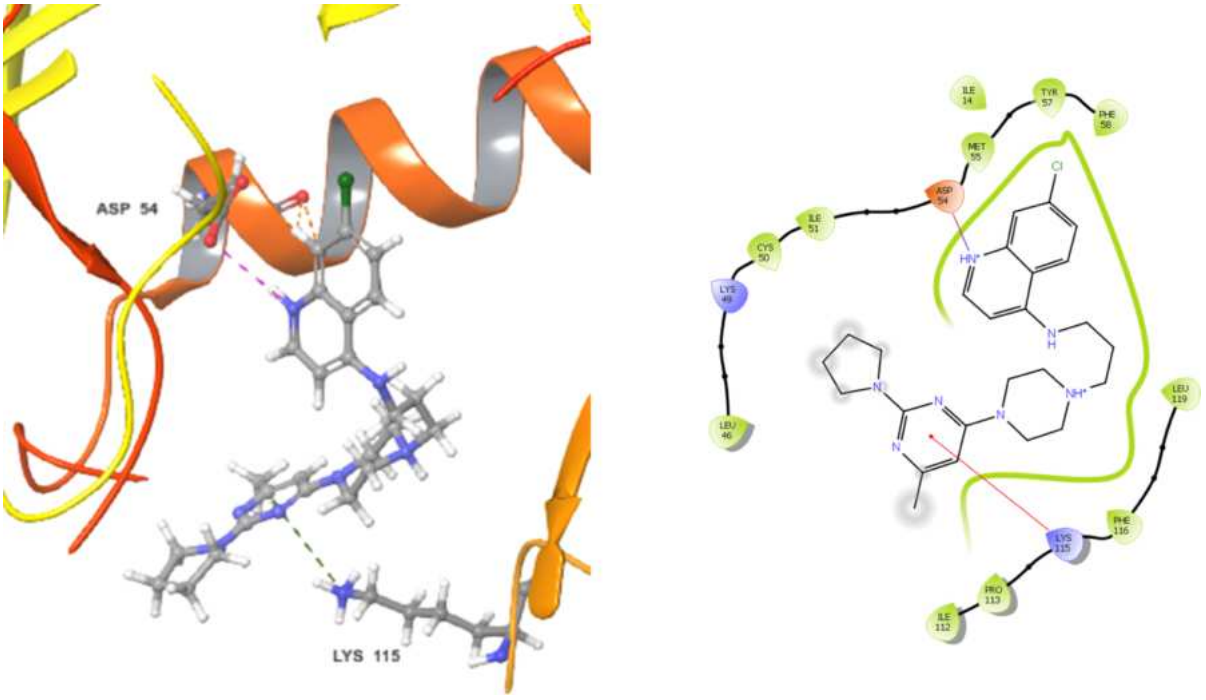

Figure 6. Interactions in 2D and 3D for compound **7i** in the binding site of the wild-type *Pf*-DHFR-TS (PDB ID: 3QGT). Interactions include hydrogen bonds, $\pi-\pi$ interactions, and salt-bridge formation.

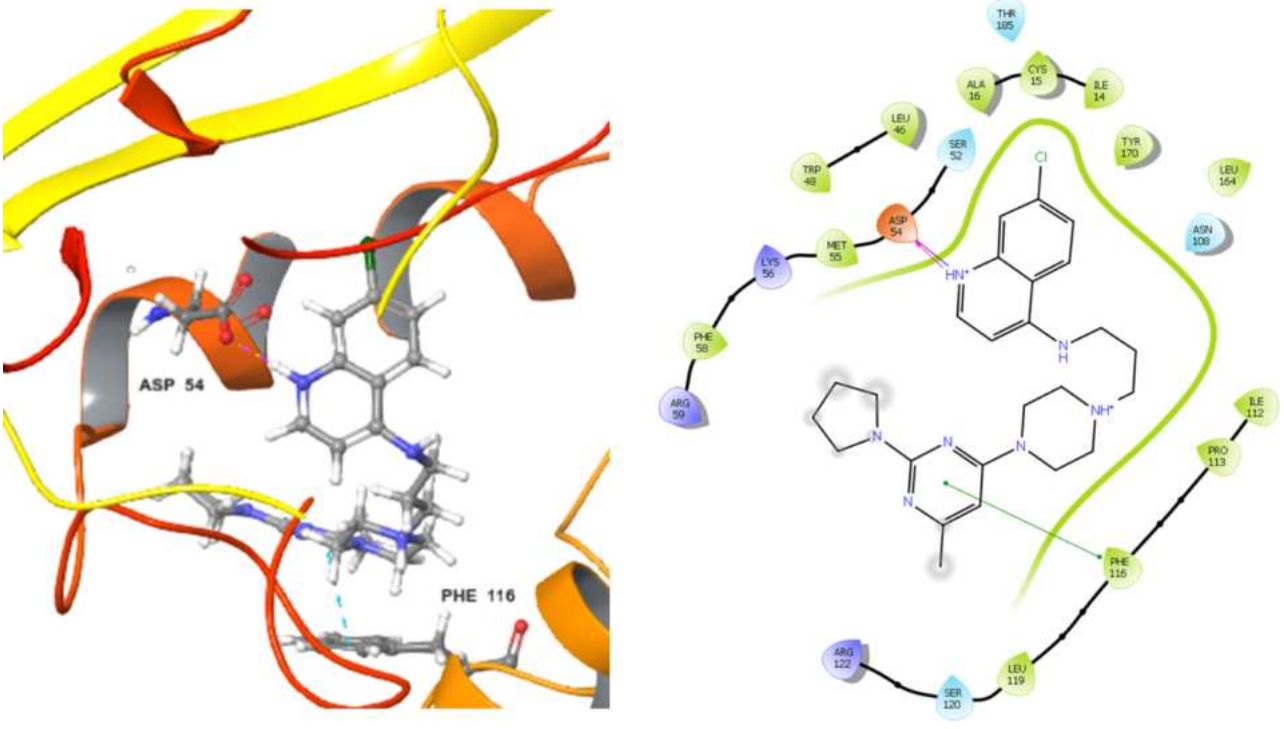

Figure 7. Interactions in 2D and 3D for compound **7i** in the binding site of the mutant type *Pf*-DHFR-TS (PDB ID: 1J3K). Interactions include hydrogen bonds, $\pi-\pi$ interactions, and salt-bridge formation.

substitution of 4,7-dichloroquinoline (**1**, 10.0 g, 50.49 mmol) with an excess of 2-aminoethan-1-ol (9.15 mL, 151.47 mmol) at 130−150 °C in neat condition for 10 h was performed (Scheme 1). TLC was employed to track the progress of the reaction. After the reaction was finished, it was cooled to room temperature, and ice-cold water was added to it. The resulting product **2a** was filtered and thoroughly washed with excess ice-cold water. Likewise, intermediate **2b** was obtained from 3-aminopropan-1-ol. Ethanol was used to recrystallize the crude products. These intermediates were characterized and compared to data that had already been published.[38]

4.2. General Procedure for Synthesizing 2-((7-Chloroquinolin-4-yl)amino)ethylmethanesulfonate (3a) and Related Compound (3b). Methanesulfonyl chloride (6.97

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Table 5. Prediction of Lipinski's 'Rule of 5'

Compound	mol_MW (<500)	donorHB (<5)	acceptHB (<10)	QPlogPo/w (<5)	RuleOfFive (<4)
7d	452.001	1	7	5.104	1
7e	495.069	1	9	4.791	0
7f	480.054	1	7	5.567	1
7i	466.028	1	7	5.512	1
7l	466.028	1	7	5.197	1
7p	495.069	1	9	4.739	0
Pyrimethamine	248.71	4	3	1.82	0
Cycloguanil	251.72	4	3.5	1.63	0

Table 6. Calculated ADMET Properties

Compound	[a]PercentHumanOralAbsorption (>80% high,<25% poor)	[a]QPPCaco nms^{-1} (<25 poor, >500 great)	[a]QPlogBB (−3.0−1.2)	[a]QPPMDCK (<25 poor >500 great)	[a]QPlogKhsa (−1.5to1.5)	[a]PSA (7.0−200.0)	[a]#rotor (0−15)
7d	96.161	834.402	0.335	1108.897	0.978	54.549	4
7e	100	281.515	0.812	379.08	0.922	56.851	5
7f	100	1001.742	0.404	1305.672	1.139	51.653	5
7i	100	890.97	0.296	1190.345	1.1	53.386	5
7l	96.931	859.17	0.349	1113.105	0.978	52.275	5
7p	95.686	195.065	0.55	254.985	0.877	59.458	6
wr99210	91.09	396.41	−0.98	2171.83	−0.07	89.06	8
Pyrimethamine	84.39	412.17	−0.79	468.71	−0.24	73.73	4
Cycloguanil	84.92	507.32	−0.52	586.49	−0.22	73.49	2

[a]Calculations were performed using QikProp v 3.5. The ranges/recommended values (shown in parentheses) were derived from 95% of known drugs.

mL, 90.0 mmol) in THF (22 mL) was gradually added to a solution of **2a** (8.0 g, 36 mmol) dissolved in THF (24 mL) at 0 °C. The resulting mixture was then stirred for 1 h at room temperature under a nitrogen atmosphere. Following reaction completion, a 17% aqueous ammonia solution (25 mL) was added to dilute the reaction mixture, extracted with CH_2Cl_2 (3 × 50 mL), dried over anhydrous $MgSO_4$, concentrated, and recrystallized from methanol/water to obtain the desired compound **3a** as white crystals. The obtained compound was characterized and compared to previously reported data.[38]

4.3. General Procedure for Synthesizing of 7-Chloro-N-(2-(piperazin-1-yl)ethyl)quinolin-4-amine (4a) and Related Compound (4b). Compound **3a** (5 g, 16.62 mmol) was dissolved in DMF (15 mL), and piperazine (7.15 g, 83 mmol) was added to it and stirred for 4 h at 80−90 °C (Scheme1). As evident from TLC, on completion of the reaction, crushed ice (50 mL) was added to the reaction mixture. The obtained precipitate was separated by filtration, washed with 100 mL of water to remove excess piperazine, and finally dried, followed by crystallization with ethanol to obtain the pure product (**4a**). Similarly, intermediate **4b** was prepared from **3b**. Ethanol was used to crystallize the resulting crude product, and all intermediates were characterized and compared to previously published data.[39]

4.4. General Procedure for the Synthesis of Compounds 5a−c and 6a−c. THF (50 mL) was used to dissolve compound **4a** (5 g, 17.2 mmol) and N,N-diisopropylethylamine (3.9 mL, 22.3 mmol), which were then stirred for 20 min at 0 °C. After adding (2.80 g, 17.2 mmol) of 2,4-dichloro-6-methyl-pyrimidine, the reaction mixture was left to stir for 12−14 h at 10−15 °C. Two regioisomers, **5a** and **6a,** were formed as a result of the reaction, which was observed by TLC (**5a** being minor and **6a** being major). The excess THF was removed *in vacuo*. The reaction mixture was treated with water, and EtOAc (3 × 50 mL) was used to extract the regioisomers. Na_2SO_4 was added to the combined organic layers, while the excess solvent was evaporated *in vacuo*. Pure compounds **5a** and **6a** were obtained from column chromatography in MeOH−$CHCl_3$ as the eluent. Similarly, compounds **5b−c** and **6b−c** were obtained.

*4.4.1. 7-Chloro-N-(2-(4-(4-chloro-6-methylpyrimidin-2-yl)-piperazin-1-yl)ethyl)quinolin-4-amine (**5a**).* Yield: 15%; Purity: 99.54%, RT = 10.11 min with method A; mp 208−210 °C; IR (cm^{-1}, Film): 3359, 2948, 2854, 1573, 1533, 1446, 1371, 1292, 1139, 996, 755; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.54 (d, J = 5.5 Hz, 1H), 7.97 (d, J = 2.3 Hz, 1H), 7.70 (d, J = 8.7 Hz, 1H), 7.37 (dd, J = 8.7, 2.3 Hz, 1H), 6.41 (brs, 1H), 6.40 (d, J = 5.5 Hz, 1H), 5.94−5.99 (m, 1H), 3.89 (t, J = 5.0 Hz, 4H), 3.31−3.42 (m, 2H), 2.83 (t, J = 6.4 Hz, 2H), 2.59 (t, J = 5.0 Hz, 4H), 2.31 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 169.30, 161.23, 160.98, 152.03, 149.68, 149.01, 134.88, 128.74, 125.40, 121.02, 117.28, 108.61, 99.30, 55.66, 52.52, 43.86, 38.90, 24.00. HRMS (ESI-MS, m/z): calculated: 417.1356 $[C_{20}H_{22}Cl_2N_6+H]^+$, found: 417.1344 $[C_{20}H_{22}Cl_2N_6+H]^+$.

*4.4.2. 7-Chloro-N-(3-(4-(4-chloro-6-methylpyrimidin-2-yl)-piperazin-1-yl)propyl)quinolin-4-amine (**5b**).* Yield: 18%; Purity: 99.66%, RT = 10.00 min with method A; mp 188−190 °C; IR (cm^{-1}, Film): 3261, 2926, 2851, 1572, 1521, 1445, 1333, 1267, 1145, 1079, 982, 752; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.52 (d, J = 5.1 Hz, 1H), 7.95 (d, J = 2.2 Hz, 1H), 7.78 (d, J = 8.8 Hz, 1H), 7.31 (dd, J = 8.8, 2.2 Hz, 1H), 7.18 (brs, 1H), 6.45 (s, 1H), 6.36 (d, J = 5.1 Hz, 1H), 3.95−3.97 (m, 4H), 3.39−3.45 (m, 2H), 2.60−2.66 (m, 6H), 2.36 (s, 3H), 1.96−2.02 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 169.35, 161.09, 161.02, 152.05, 150.36, 149.00, 134.67, 128.60, 124.87, 121.67, 117.39, 108.70, 98.57, 58.43, 53.38, 43.96, 43.68, 23.99, 23.72. HRMS (ESI-MS, m/z): calculated: 431.1512 $[C_{21}H_{24}Cl_2N_6+H]^+$, found: 431.1500 $[C_{21}H_{24}Cl_2N_6+H]^+$.

*4.4.3. 7-Chloro-N-(3-(4-(4-chloropyrimidin-2-yl)piperazin-1-yl)propyl)quinolin-4-amine (**5c**).* Yield: 17%; mp 144−146

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°C; IR (cm^{-1}, Film): 3261, 2926, 2851, 1572, 1521, 1445, 1333, 1267, 1145, 982, 752; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.51 (d, J = 5.9 Hz, 1H), 8.19 (d, J = 5.1 Hz, 1H), 7.95 (d, J = 2.2 Hz, 1H), 7.78 (d, J = 8.8 Hz, 1H), 7.30 (dd, J = 8.8, 2.2 Hz, 1H), 7.17 (brs, 1H), 6.56 (d, J = 5.1 Hz, 1H), 6.37 (d, J = 5.9 Hz, 1H), 3.97 (t, J = 4.4 Hz, 4H), 3.41−3.45 (m, 2H), 2.66 (t, J = 5.1 Hz, 2H), 2.62 (t, J = 5.1 Hz, 4H), 1.97−2.03 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 161.28, 161.23, 158.86, 151.43, 150.67, 148.31, 135.06, 128.16, 125.12, 121.71, 117.23, 109.53, 98.53, 58.41, 53.33, 44.03, 43.69, 23.65. HRMS (ESI-MS, m/z): calculated: 417.1356 $[C_{20}H_{22}Cl_2N_6+H]^+$, found: 417.1344 $[C_{20}H_{22}Cl_2N_6+H]^+$.

*4.4.4. 7-Chloro-N-(2-(4-(2-chloro-6-methylpyrimidin-4-yl)-piperazin-1-yl)ethyl)quinolin-4-amine (**6a**).* Yield: 85%; mp 176−178 °C; IR (cm^{-1}, Film): 3367, 2953, 2854, 1575, 1525, 1491, 1440, 1370, 1309, 1221, 1076, 990, 845, 753; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.54 (d, J = 5.1 Hz, 1H), 7.97 (d, J = 2.2 Hz, 1H), 7.67 (d, J = 8.8 Hz, 1H), 7.37 (dd, J = 8.8, 2.2 Hz, 1H), 6.40 (d, J = 5.9 Hz, 1H), 6.26 (s, 1H), 5.81−5.91 (m, 1H), 3.67−3.78 (m, 4H), 3.36−3.40 (m, 2H), 2.84 (t, J = 5.31 Hz, 2H), 2.61 (t, J = 5.3 Hz, 4H), 2.35 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 167.99, 163.14, 160.14, 151.99, 149.59, 148.95, 134.94, 128.73, 125.46, 120.92, 117.21, 99.70, 99.32, 55.58, 52.17, 44.06, 38.89, 24.02. HRMS (ESI-MS, m/z): calculated: 417.1356 $[C_{20}H_{22}Cl_2N_6+H]^+$, found: 417.1343 $[C_{20}H_{22}Cl_2N_6+H]^+$.

*4.4.5. 7-Chloro-N-(3-(4-(2-chloro-6-methylpyrimidin-4-yl)-piperazin-1-yl)propyl)quinolin-4-amine (**6b**).* Yield: 82%; Purity: 98.03%, RT = 9.94 min with method A; mp 208−210 °C; IR (cm^{-1}, Film): 3270, 2943, 2816, 1581, 1492, 1437, 1365, 1277, 1136, 1075, 981, 807, 753; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.52 (d, J = 5.1 Hz, 1H), 7.95 (d, J = 2.2 Hz, 1H), 7.71 (d, J = 9.5 Hz, 1H), 7.32 (dd, J = 8.8, 2.2 Hz, 1H), 6.77 (brs, 1H), 6.38 (d, J = 5.1 Hz, 1H), 6.28 (brs, 1H), 3.74−3.77 (m, 4H), 3.41−3.45 (m, 2H), 2.61−2.63 (m, 6H), 2.36 (s, 3H), 1.96−2.01 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 168.08, 163.07, 161.01, 152.04, 150.20, 148.98, 134.77, 128.73, 124.98, 121.34, 117.31, 99.71, 98.70, 58.02, 52.98, 43.94, 43.62, 24.03, 23.99. HRMS (ESI-MS, m/z): calculated: 431.1512 $[C_{21}H_{24}Cl_2N_6+H]^+$, found: 431.1504 $[C_{21}H_{24}Cl_2N_6+H]^+$.

*4.4.6. 7-Chloro-N-(3-(4-(2-chloropyrimidin-4-yl)piperazin-1-yl)propyl)quinolin-4-amine (**6c**).* Yield: 83%; Purity: 99.65%, RT = 9.97 min with method A; mp 215−217 °C; IR (cm^{-1}, Film): 3273, 2942, 2815, 1580, 1534, 1490, 1355, 1246, 1164, 1144, 980, 803, 758; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.52 (d, J = 6.1 Hz, 1H), 8.08 (d, J = 6.1 Hz, 1H), 7.95 (d, J = 1.5 Hz, 1H), 7.71 (d, J = 8.4 Hz, 1H), 7.32 (dd, J = 8.4, 1.5 Hz, 1H), 6.73 (brs, 1H), 6.43 (d, J = 6.1 Hz, 1H), 6.38 (d, J = 5.3 Hz, 1H), 3.76 (m, 4H), 3.40−3.44 (m, 2H), 2.60−2.66 (m, 6H), 1.96−2.02 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 162.60, 160.93, 157.64, 150.69, 135.28, 128.21, 125.32, 121.69, 101.33, 98.85, 57.96, 53.04, 44.02, 43.67, 24.14. HRMS (ESI-MS, m/z): calculated: 417.1356 $[C_{20}H_{22}Cl_2N_6+H]^+$, found: 417.1368 $[C_{20}H_{22}Cl_2N_6+H]^+$.

4.5. General Protocol for Synthesizing Compounds 7a−7q. To a dissolved solution of intermediate **6a** or **6b** or **6c** (1 equiv) in K_2CO_3 and DMF, the respective amine (3 equiv) was added dropwise. The reaction mixture was stirred at 100−120 °C for 10−12 h (Scheme 1). After the reaction was completed (as monitored by TLC), ice-cold water was added, and EtOAc was used to extract the compound. The obtained organic layer was dried with Na_2SO_4 and later concentrated *in vacuo* using a rotary evaporator. The obtained residue was purified using silica gel column chromatography in MeOH/$CHCl_3$ as an eluent to afford the desired hybrids **7a−7q** in excellent yield.

*4.5.1. 7-Chloro-N-(2-(4-(6-methyl-2-(piperidin-1-yl)-pyrimidin-4-yl)piperazin-1-yl)ethyl) quinolin-4-amine (**7a**).* Yield: 79%; Purity: 98.25%, RT = 10.15 min with method A; mp 148−150 °C; IR (cm^{-1}, Film): 3374, 2928, 2846, 1566, 1442, 1368, 1237, 1199, 992, 747; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.55 (d, J = 5.1 Hz, 1H), 7.96 (d, J = 2.2 Hz, 1H), 7.67 (d, J = 8.8 Hz, 1H), 7.38 (dd, J = 8.8, 2.2 Hz, 1H), 6.39 (d, J = 5.9 Hz, 1H), 5.90−6.03 (m, 1H), 5.72 (s, 1H), 3.73 (t, J = 5.9 Hz, 4H), 3.61−3.63 (t, J = 4.4 Hz, 4H), 3.34−3.38 (m, 2H), 2.83 (t, J = 5.9 Hz, 2H), 2.59 (t, 4H), 2.23 (s, 3H), 1.57−1.61 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): 166.46, 163.26, 161.74, 152.11, 149.65, 149.08, 134.86, 128.83, 125.42, 120.96, 117.30, 99.31, 90.96, 55.55, 52.36, 44.74, 44.01, 38.87, 25.86, 24.99, 24.63. HRMS (ESI-MS, m/z): calculated: 466.2480 $[C_{25}H_{32}ClN_7+H]^+$, found: 466.2496 $[C_{25}H_{32}ClN_7+H]^+$.

*4.5.2. 7-Chloro-N-(2-(4-(6-methyl-2-morpholinopyrimidin-4-yl)piperazin-1-yl)ethyl) quinolin-4-amine (**7b**).* Yield: 81%; mp 114−116 °C; IR (cm^{-1}, Film): 3372, 2954, 2848, 1574, 1442, 1366, 1272, 1115, 995, 881, 751; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.54 (d, J = 5.9 Hz, 1H), 7.97 (d, J = 2.2 Hz, 1H), 7.68 (d, J = 9.5 Hz, 1H), 7.37 (dd, J = 8.8, 2.2 Hz, 1H), 6.40 (d, J = 5.1 Hz, 1H), 5.92−6.00 (m, 1H), 5.80 (s, 1H), 3.73−3.74 (m, 8H), 3.63 (t, J = 4.2 Hz, 4H), 3.32−3.41 (m, 2H), 2.83 (t, J = 5.9 Hz, 2H), 2.59 (t, J = 5.5 Hz, 4H), 2.24 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 166.53, 163.12, 161.72, 152.09, 149.64, 149.05, 134.88, 128.81, 125.43, 120.94, 117.28, 99.31, 91.99, 66.98, 55.57, 52.33, 44.37, 43.97, 38.87, 24.51. HRMS (ESI-MS, m/z): calculated: 468.2273 $[C_{24}H_{30}ClN_7O+H]^+$, found: 468.2274 $[C_{24}H_{30}ClN_7O+H]^+$.

*4.5.3. 7-Chloro-N-(2-(4-(6-methyl-2-thiomorpholinopyrimidin-4-yl)piperazin-1-yl)ethyl) quinolin-4-amine (**7c**).* Yield: 78%; Purity: 99.06%, RT = 10.14 min with method A; mp 190−192 °C; IR (cm^{-1}, Film): 3368, 2949, 2839, 1570, 1413, 1368, 1319, 1234, 1142, 993, 745; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.54 (d, J = 5.9 Hz, 1H), 7.96 (d, J = 2.2 Hz, 1H), 7.67 (d, J = 9.5 Hz, 1H), 7.37 (dd, J = 9.5 Hz, J = 2.2 Hz, 1H), 6.39 (d, J = 5.1 Hz, 1H), 5.91−6.00 (m, 1H), 5.76 (brs, 1H), 4.09−4.11 (m, 4H), 3.62 (t, J = 4.4 Hz, 4H), 3.34−3.38 (m, 2H), 2.82 (t, J = 5.9 Hz, 2H), 2.57−2.64 (m, 8H), 2.22 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 166.53, 163.18, 161.07, 152.04, 149.62, 149.00, 134.85, 128.73, 125.38, 120.95, 117.24, 99.27, 91.51, 55.52, 52.29, 46.24, 43.97, 38.85, 26.93, 24.53. HRMS (ESI-MS, m/z): calculated: 484.2045 $[C_{24}H_{30}ClN_7S+H]^+$, found: 484.2042 $[C_{24}H_{30}ClN_7S+H]^+$.

*4.5.4. 7-Chloro-N-(2-(4-(6-methyl-2-(pyrrolidin-1-yl)-pyrimidin-4-yl)piperazin-1-yl)ethyl) quinolin-4-amine (**7d**).* Yield: 83%; mp 189−191 °C; IR (cm^{-1}, Film): 3350, 2948, 2861, 1565, 1447, 1412, 1332, 1243, 1077, 996, 746; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.53 (d, J = 5.1 Hz, 1H), 7.96 (d, J = 2.2 Hz, 1H), 7.68 (d, J = 8.8 Hz, 1H), 7.37 (dd, J = 8.8, 2.2 Hz, 1H), 6.39 (d, J = 5.1 Hz, 1H), 5.96−6.05 (m, 1H), 5.74 (s, 1H), 3.63−3.65 (m, 4H), 3.52−3.55 (m, 4H), 3.34−3.38 (m, 2H), 2.82 (t, J = 5.9 Hz, 2H), 2.58 (t, J = 5.1 Hz, 4H), 2.25 (s, 3H), 1.93−2.00 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 166.48, 163.16, 160.58, 152.19, 149.78, 149.15, 134.98, 128.86, 125.52, 121.12, 117.39, 99.39, 90.84, 55.68, 52.49, 46.54, 44.03, 38.96, 25.61, 24.69. HRMS (ESI-MS, m/z):

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calculated: 452.2324 $[C_{24}H_{30}ClN_7+H]^+$, found: 452.2324 $[C_{24}H_{30}ClN_7+H]^+$.

*4.5.5. 7-Chloro-N-(2-(4-(2-(4-ethylpiperazin-1-yl)-6-methylpyrimidin-4-yl)piperazin-1-yl) ethyl)quinolin-4-amine (**7e**).* Yield: 75%; Purity: 99.97%, RT = 9.94 min with method A; mp 138−140 °C; IR (cm^{-1}, Film): 3342, 2940, 2819, 1571, 1443, 1369, 1235, 1198, 1079, 995, 746; ^1H NMR (400 MHz, $CDCl_3$): δ ppm 8.53 (d, J = 5.1 Hz, 1H), 7.96 (d, J = 2.2 Hz, 1H), 7.67 (d, J = 8.8 Hz, 1H), 7.37 (dd, J = 8.8, 2.2 Hz, 1H), 6.39 (d, J = 5.1 Hz, 1H), 5.91−6.02 (m, 1H), 5.76 (brs, 1H), 3.77−3.85 (m, 4H), 3.59−3.66 (m, 4H), 3.34−3.37 (m, 2H), 2.82 (t, J = 5.9 Hz, 2H), 2.58 (t, J = 4.4 Hz, 4H), 2.41−2.49 (m, 6H), 2.23 (s, 3H), 1.12 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 166.48, 163.16, 161.54, 152.07, 149.65, 149.03, 134.89, 128.80, 125.43, 120.95, 117.27, 99.31, 91.60, 55.55, 52.86, 52.49, 52.34, 43.98, 43.69, 38.86, 24.56, 11.90. HRMS (ESI-MS, m/z): calculated: 495.2746 $[C_{26}H_{35}ClN_8+H]^+$, found: 495.2732 $[C_{26}H_{35}ClN_8+H]^+$.

*4.5.6. 7-Chloro-N-(3-(4-(6-methyl-2-(piperidin-1-yl)-pyrimidin-4-yl)piperazin-1-yl)propyl) quinolin-4-amine (**7f**).* Yield: 83%; mp 160−162 °C; IR (cm^{-1}, Film): 3262, 2927, 2846, 1569, 1441, 1414, 1368, 1305, 1238, 1139, 988, 745; ^1H NMR (400 MHz, $CDCl_3$): δ ppm 8.51 (d, J = 5.1 Hz, 1H), 7.93 (d, J = 2.2 Hz, 1H), 7.77 (d, J = 8.8 Hz, 1H), 7.24−7.31 (m, 2H), 6.35 (d, J = 5.1 Hz, 1H), 5.74 (s, 1H), 3.73−3.76 (m, 4H), 3.68−3.70 (m, 4H), 3.39−3.43 (m, 2H), 2.59−2.66 (m, 6H), 2.25 (s, 3H), 1.97−1.99 (m, 2H), 1.58−1.61 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 166.53, 163.06, 161.70, 152.07, 150.36, 148.99, 134.67, 128.59, 124.86, 121.74, 117.37, 98.53, 90.87, 58.50, 53.29, 44.71, 44.02, 43.79, 25.83, 24.96, 24.62, 23.61. HRMS (ESI-MS, m/z): calculated: 480.2637 $[C_{26}H_{34}ClN_7+H]^+$, found: 480.2653 $[C_{26}H_{34}ClN_7+H]^+$.

*4.5.7. 7-Chloro-N-(3-(4-(6-methyl-2-morpholinopyrimidin-4-yl)piperazin-1-yl)propyl) quinolin-4-amine (**7g**).* Yield: 84%; mp 170−172 °C; IR (cm^{-1}, Film): 3267, 2923, 2847, 1572, 1439, 1421, 1365, 1239, 1116, 993, 753; ^1H NMR (400 MHz, $CDCl_3$): δ ppm 8.52 (d, J = 5.0 Hz, 1H), 7.94 (s, 1H), 7.77 (d, J = 8.7 Hz, 1H), 7.29 (dd, J = 8.7, 2.2 Hz, 1H), 7.19 (brs, 1H), 6.36 (d, J = 5.5 Hz, 1H), 5.82 (s, 1H), 3.75−3.77 (m, 8H), 3.69−3.71 (m, 4H), 3.39−3.44 (m, 2H), 2.60−2.67 (m, 6H), 2.26 (s, 3H), 1.96−2.02 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 166.63, 162.97, 157.10, 152.10, 150.35, 134.72, 128.66, 124.90, 121.65, 98.59, 91.92, 66.98, 58.49, 53.26, 44.35, 44.02, 43.79, 24.52, 23.66. HRMS (ESI-MS, m/z): calculated: 482.2430 $[C_{25}H_{32}ClN_7O+H]^+$, found: 482.2428 $[C_{25}H_{32}ClN_7O+H]^+$.

*4.5.8. 7-Chloro-N-(3-(4-(6-methyl-2-thiomorpholinopyrimidin-4-yl)piperazin-1-yl)propyl) quinolin-4-amine (**7h**).* Yield: 81%; mp 192−194 °C; IR (cm^{-1}, Film): 3261, 2949, 2838, 1568, 1414, 1336, 1282, 1196, 1078, 988, 950, 745; ^1H NMR (400 MHz, $CDCl_3$): δ ppm 8.51 (d, J = 5.9 Hz, 1H), 7.94 (d, J = 1.8 Hz, 1H), 7.76 (d, J = 8.8 Hz, 1H), 7.28−7.30 (m, 1H), 7.15 (brs, 1H), 6.35 (d, J = 5.1 Hz, 1H), 5.79 (s, 1H), 4.10−4.13 (m, 4H), 3.67−3.71 (m, 4H), 3.41−3.43 (m, 2H), 2.60−2.65 (m, 10H), 2.25 (s, 3H), 1.97−2.00 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 166.66, 163.04, 161.08, 152.11, 150.33, 149.02, 134.68, 128.67, 124.86, 121.67, 117.37, 98.58, 91.46, 58.49, 53.25, 46.23, 44.02, 43.81, 26.98, 24.58, 23.64. HRMS (ESI-MS, m/z): calculated: 498.2201 $[C_{25}H_{32}ClN_7S+H]^+$, found: 498.2207 $[C_{25}H_{32}ClN_7S+H]^+$.

*4.5.9. 7-Chloro-N-(3-(4-(6-methyl-2-(pyrrolidin-1-yl)-pyrimidin-4-yl)piperazin-1-yl)propyl) quinolin-4-amine (**7i**).* Yield: 77%; Purity: 99.59%, RT = 10.11 min with method A; mp 184−186 °C; IR (cm^{-1}, Film): 3263, 2948, 2853, 1566, 1447, 1413, 1368, 1244, 1216, 1137, 991, 744; ^1H NMR (400 MHz, $CDCl_3$): δ ppm: 8.51 (d, J = 5.1 Hz, 1H), 7.93 (d, J = 2.2 Hz, 1H), 7.78 (d, J = 8.8 Hz, 1H), 7.25−7.30 (m, 2H), 6.35 (d, J = 5.9 Hz, 1H), 5.76 (s, 1H), 3.70−3.72 (m, 4H), 3.54−3.58 (m, 4H), 3.38−3.42 (m, 2H), 2.59−2.65 (m, 6H), 2.27 (s, 3H), 1.91−1.98 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 166.47, 162.94, 160.47, 152.10, 150.38, 149.03, 134.69, 128.64, 124.88, 121.74, 117.40, 98.55, 90.67, 58.56, 53.32, 46.44, 44.08, 43.76, 25.49, 24.60, 23.62. HRMS (ESI-MS, m/z): calculated: 466.2480 $[C_{25}H_{32}ClN_7+H]^+$, found: 466.2466 $[C_{25}H_{32}ClN_7+H]^+$.

*4.5.10. 7-Chloro-N-(3-(4-(2-(4-ethylpiperazin-1-yl)-6-methylpyrimidin-4-yl)piperazin-1-yl) propyl)quinolin-4-amine (**7j**).* Yield: 85%; mp 150−152 °C; IR (cm^{-1}, Film): 2922, 2850, 1572, 1442, 1417, 1368, 1273, 1136, 992, 747; ^1H NMR (400 MHz, $CDCl_3$): δ ppm 8.51 (d, J = 5.13 Hz, 1H), 7.92−7.96 (m, 1H), 7.77 (d, J = 9.5 Hz, 1H), 7.28−7.31 (m, 1H), 7.22 (brs, 1H), 6.36 (d, J = 5.1 Hz, 1H,), 5.79 (s, 1H), 3.79−3.85 (m, 4H), 3.67−3.73 (m, 4H), 3.38−3.45 (m, 2H), 2.61−2.65 (m, 6H), 2.43−2.50 (m, 6H), 2.25 (s, 3H), 1.86−1.92 (m, 2H), 1.13 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 166.58, 162.98, 161.59, 152.11, 150.34, 149.02, 134.67, 128.66, 124.87, 121.69, 117.37, 98.56, 91.48, 58.52, 53.28, 52.88, 52.48, 44.05, 43.78, 43.70, 24.57, 23.61, 11.94. HRMS (ESI-MS, m/z): calculated: 509.2902 $[C_{27}H_{37}ClN_8+H]^+$, found: 509.2906 $[C_{27}H_{37}ClN_8+H]^+$.

*4.5.11. 7-Chloro-N-(3-(4-(6-methyl-2-(4-methylpiperazin-1-yl)pyrimidin-4-yl)piperazin-1-yl) propyl)quinolin-4-amine (**7k**).* Yield: 84%; mp 170−172 °C; IR (cm^{-1}, Film): 3265, 2936, 2842, 1569, 1440, 1418, 1365, 1238, 1139,994, 747; ^1H NMR (400 MHz, $CDCl_3$): δ ppm 8.50 (d, J = 5.3 Hz, 1H), 7.93−7.94 (m, 1H), 7.77 (d, J = 8.4 Hz, 1H), 7.28−7.31 (m, 1H), 7.18 (brs, 1H), 6.36 (d, J = 6.1 Hz, 1H), 5.79 (s, 1H), 3.79−3.82 (m, 4H), 3.68−3.71 (m, 4H), 3.39−3.44 (m, 2H), 2.59−2.66 (m, 6H), 2.44−2.47 (m, 4H), 2.33 (s, 3H), 2.25 (s, 3H), 1.97−2.00 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 166.57, 162.96, 161.62, 152.05, 150.35, 148.95, 134.68, 128.57, 124.87, 121.69, 117.34, 98.54, 91.56, 58.44, 55.06, 53.25, 46.24, 43.97, 43.76, 43.68, 24.54, 23.61. HRMS (ESI-MS, m/z): calculated: 495.2746 $[C_{26}H_{35}ClN_8+H]^+$, found: 495.2745 $[C_{26}H_{35}ClN_8+H]^+$.

*4.5.12. 7-Chloro-N-(3-(4-(2-(piperidin-1-yl)pyrimidin-4-yl)-piperazin-1-yl)propyl)quinolin-4-amine (**7l**).* Yield: 75%; mp 174−176 °C; IR (cm^{-1}, Film): 3351, 2930, 2847, 1574, 1545, 1436, 1335, 1229, 1140, 1077, 1024, 879, 793; ^1H NMR (400 MHz, $CDCl_3$): δ ppm 8.45 (d, J = 5.0 Hz, 1H), 7.94−7.96 (m, 2H), 7.78 (d, J = 9.2 Hz, 1H), 7.38 (brs, 1H), 7.27 (dd, J = 9.2, 1.4 Hz, 1H), 6.34 (d, J = 5.5 Hz, 1H), 5.82 (d, J = 5.9 Hz, 1H), 3.66−3.72 (m, 8H), 3.40−3.47 (m, 2H), 2.58−2.64 (m, 6H), 1.96−2.00 (m, 2H), 1.56−1.62 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 162.30, 161.38, 156.77, 151.44, 150.65, 148.34, 135.02, 128.12, 125.06, 121.79, 117.22, 98.49, 92.07, 58.38, 53.23, 44.75, 43.98, 43.66, 25.76, 24.91, 23.63. HRMS (ESI-MS, m/z): calculated: 466.2480 $[C_{25}H_{32}ClN_7+H]^+$, found: 466.2468 $[C_{25}H_{32}ClN_7+H]^+$.

*4.5.13. 7-Chloro-N-(3-(4-(2-morpholinopyrimidin-4-yl)-piperazin-1-yl)propyl)quinolin-4-amine (**7m**).* Yield: 76%; mp 160−162 °C; IR (cm^{-1}, Film): 3264, 2952, 2847, 1575, 1546, 1436, 1338, 1231, 1116, 997, 850, 753; ^1H NMR (400 MHz, $CDCl_3$): δ ppm 8.42−8.44 (m, 1H), 7.96−7.97 (m, 2H), 7.81 (d, J = 9.2 Hz, 1H), 7.56 (brs, 1H), 7.27 (dd, J = 8.7, 2.3 Hz, 1H), 6.35 (d, J = 5.5 Hz, 1H), 5.90 (d, J = 5.9 Hz, 1H),

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3.65−3.72 (m, 12H), 3.41−3.45 (m, 2H), 2.58−2.65 (m, 6H), 1.95−2.01 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 162.19, 161.54, 156.77, 151.22, 150.71, 148.11, 135.08, 127.93, 125.06, 121.80, 117.15, 98.48, 93.15, 66.87, 58.24, 53.15, 44.24, 43.87, 43.64, 23.68. HRMS (ESI-MS, *m*/*z*): calculated: 468.2273 $[C_{24}H_{30}ClN_7O+H]^+$, found: 468.2275 $[C_{24}H_{30}ClN_7O+H]^+$.

*4.5.14. 7-Chloro-N-(3-(4-(2-thiomorpholinopyrimidin-4-yl)piperazin-1-yl)propyl)quinolin-4-amine (**7n**).* Yield: 82%; mp 202−204 °C; IR (cm^{-1}, Film): 3263, 2946, 2839, 1575, 1468, 1345, 1224, 1140, 957, 750; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.52 (d, *J* = 5.5 Hz, 1H), 7.98 (d, *J* = 5.9 Hz, 1H), 7.94 (d, *J* = 2.3 Hz, 1H), 7.76 (d, *J* = 8.7 Hz, 1H), 7.30 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.12 (brs, 1H), 6.37 (d, *J* = 5.0 Hz, 1H), 5.90 (d, *J* = 5.9 Hz, 1H), 4.10−4.12 (m, 4H), 3.69−3.71 (m, 4H), 3.40−3.45 (m, 2H), 2.60−2.67 (m, 10H), 1.96−2.02 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 162.29, 160.91, 156.87, 152.13, 150.27, 149.03, 134.67, 128.71, 124.86, 121.58, 117.35, 98.59, 92.70, 58.41, 53.18, 46.32, 43.94, 43.68, 26.85, 23.69. HRMS (ESI-MS, *m*/*z*): calculated: 484.2045 $[C_{24}H_{30}ClN_7S+H]^+$, found: 484.2047 $[C_{24}H_{30}ClN_7S+H]^+$.

*4.5.15. 7-Chloro-N-(3-(4-(2-(pyrroldin-1-yl)pyrimidin-4-yl)-piperazin-1-yl)propyl)quinoline-4-amine (**7o**).* Yield: 81%; mp 153−155 °C; IR (cm^{-1}, Film): 3262, 2944, 2859, 1574, 1548, 1445, 1323, 1237, 1138, 1017, 973, 750; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.45 (d, *J* = 5.5 Hz, 1H), 7.94−7.96 (m, 2H), 7.78−7.81 (m, 1H), 7.40−7.44 (m, 1H), 7.26−7.28 (m, 1H), 6.34 (d, *J* = 5.5 Hz, 1H), 5.84 (d, *J* = 5.9 Hz, 1H), 3.68−3.71 (m, 4H), 3.51−3.54 (m, 4H), 3.40−3.43 (m, 2H), 2.63 (t, *J* = 5.5 Hz, 2H), 2.58 (t, *J* = 5.0 Hz, 4H), 1.91−1.98 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 162.11, 159.91, 156.52, 151.59, 150.56, 148.50, 134.87, 128.19, 124.94, 121.82, 117.24, 98.48, 91.76, 58.34, 53.20, 46.35, 43.91, 43.61, 25.46, 23.62. HRMS (ESI-MS, *m*/*z*): calculated: 452.2324 $[C_{24}H_{30}ClN_7+H]^+$, found: 452.2283 $[C_{24}H_{30}ClN_7+H]^+$.

*4.5.16. 7-Chloro-N-(3-(4-(2-(4-ethylpiperazin-1-yl)-pyrimidin-4-yl)piperazin-1-yl)propyl) quinolin-4-amine (**7p**).* Yield: 81%; mp 165−167 °C; IR (cm^{-1}, Film): 3269, 2923, 2851, 1578, 1549, 1440, 1341, 1226, 1139, 1000, 751; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.52 (d, *J* = 5.0 Hz, 1H), 7.98 (d, *J* = 5.9 Hz, 1H), 7.94 (d, *J* = 2.3 Hz, 1H), 7.76 (d, *J* = 8.7 Hz, 1H), 7.30 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.13 (brs, 1H), 6.36 (d, *J* = 5.5 Hz, 1H), 5.89 (d, *J* = 5.5 Hz, 1H), 3.80−3.82 (m, 4H), 3.70−3.72 (m, 4H), 3.39−3.43 (m, 2H), 2.66 (t, *J* = 5.5 Hz, 2H), 2.62 (t, *J* = 5.0 Hz, 4H), 2.43−2.51 (m, 6H), 1.97−2.01 (m, 2H), 1.15 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 162.23, 161.45, 156.82, 152.12, 150.30, 149.02, 134.68, 128.69, 124.89, 121.61, 117.36, 98.59, 92.72, 58.44, 53.22, 52.79, 52.45, 43.97, 43.67, 23.67, 11.94. HRMS (ESI-MS, *m*/*z*): calculated: 495.2746 $[C_{26}H_{35}ClN_8+H]^+$, found: 495.2747 $[C_{26}H_{35}ClN_8+H]^+$.

*4.5.17. 7-Chloro-N-(3-(4-(2-(4-methylpiperazin-1-yl)-pyrimidin-4-yl)piperazin-1-yl)propyl) quinolin-4-amine (**7q**).* Yield: 76%; mp 196−198 °C; IR (cm^{-1}, Film): 3261, 2938, 2843, 1575, 1546, 1436, 1338, 1227, 1139, 998, 746; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.52 (d, *J* = 5.5 Hz, 1H), 7.99 (d, *J* = 5.9 Hz, 1H), 7.94 (d, *J* = 1.8 Hz, 1H), 7.76 (d, *J* = 8.7 Hz, 1H), 7.30 (dd, *J* = 8.7, 1.8 Hz, 1H), 7.14 (brs, 1H), 6.36 (d, *J* = 5.5 Hz, 1H), 5.90 (d, *J* = 5.9 Hz, 1H), 3.78−3.82 (m, 4H), 3.69−3.76 (m, 4H), 3.39−3.43 (m, 2H), 2.61−2.67 (m, 6H), 2.46 (t, *J* = 4.6 Hz, 4H), 2.34 (s, 3H), 1.98−2.00 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 162.24, 161.48, 156.83, 152.11, 150.30, 149.02, 134.69, 128.70, 124.89, 121.60, 117.35, 98.59, 92.78, 58.44, 55.01, 53.22, 46.26, 43.98, 43.67, 23.68. HRMS (ESI-MS, *m*/*z*): calculated: 481.2589 $[C_{25}H_{33}ClN_8+H]^+$, found: 481.2589 $[C_{25}H_{33}ClN_8+H]^+$.

5. ASSAY FOR *IN VITRO* ANTIPLASMODIAL ACTIVITY AND CYTOTOXICITY

The antiplasmodial activity of the synthesized series was assessed against the **CQ**-resistant (W2) and **CQ**-sensitive (D6) strains of *P. falciparum*. This was determined by measuring plasmodial lactate dehydrogenase (LDH) activity as mentioned earlier.[19,40] A 96-well plate containing 10 μL of serially diluted test samples was used. The wells of the plate were filled with red blood cell suspension infected with the aforementioned strain of *P. falciparum* (200 μL, with hematocrit (2%) and parasitemia (2%) in RPMI 1640 medium supplemented with 60 μg/mL amikacin and 10% human serum). After the plates were flushed with a gas mixture of 90% N_2, 5% CO_2, and 5% O_2, they were incubated in an incubation chamber at 37 °C for 72 h. The parasitic LDH activity was assessed using the methodology outlined by Makler et al.[41] The Malstat reagent (100 μL) was combined with 20 μL of the incubation mixture. It was further incubated for 30 min at room temperature. Twenty μL of a 1:1 mixture of NBT/PES was then added, and the plate was again incubated for 1 h in the dark. Then,100 μL of a 5% acetic acid solution was added to stop the reaction. At 650 nm, the plate was read. Chloroquine (**CQ**) and artemisinin (**ART**) were included as antimalarial drug controls in each assay with DMSO as the vehicle control. All screenings were performed in triplicate with each no-drug negative control and chloroquine positive control, while IC_{50} values were obtained from dose−response studies.

For selectivity index (SI) of compounds, *in vitro* cytotoxicity to mammalian cells was also determined. As described in the literature, an assay was conducted in 96-well tissue culture-treated plates to ascertain cytotoxicity.[42] A 96-well plate was seeded with Vero cells at a density of 25,000 cells per well, followed by incubation for 24 h. After the addition of samples at varying concentrations, the plates were once more incubated for 48 h. The Neutral Red assay was employed to determine the number of viable cells. From the dose−response curves, IC50 values were derived. Doxorubicin was used as a positive control for the cytotoxicity.

6. *IN VIVO* BIOLOGICAL STUDIES

The *in vivo* antiplasmodial testing was carried out by following the ethical and scientific guidelines of the University of Mississippi. The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the animal protocol. The University of Mississippi's animal care and use program is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). All animal experiments were conducted in strict accordance with the University of Mississippi's Animal Welfare Assurance (Assurance Number: D16−00232). This institution is guided by the "U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training.

7. PROCEDURE FOR MONOMERIC HEME BINDING STUDIES

6.5 mg of hemin chloride was dissolved in 10 mL of DMSO to create a 1.0 mM heme stock solution. Next, 100 μL of the

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hemin stock solution was dissolved in 4 mL of DMSO and 1 mL of 20 mM HEPES buffer (pH 7.4) to create a 10.0 μM working solution of monomeric heme. The final volume was adjusted to 10 mL using distilled water. The 1 mM stock solution of the compound was prepared in DMSO. A working solution of heme (10.0 μM) was titrated with increasing concentrations of the compound (0.5−30.0 μM). After each addition of the compound, the sample was mixed well, and the absorbance was measured around 402 nm. The preparation of a second working solution of hemin was identical, with the exception that MES buffer (pH 5.6) was utilized in place of HEPES buffer and titrated similarly.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c08363.

Experimental details for heme binding studies with CQ and characterization of compounds, including ^{1}H NMR spectra, ^{13}C NMR spectra, and HPLC methods and chromatograms of the synthesized compounds, are provided as electronic (PDF)

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Notes

The authors declare no competing financial interest.

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