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Synthesis and biological activity of 2-[2-(7-chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-*N*-phenylacetamide derivatives as antimalarial and cytotoxic agents

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Abstract

A novel series of 2-[2-(7-chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-*N*-phenylacetamide derivatives is synthesized via substitution with 2-mercapto-4-methyl-5-thiazoleacetic acid at position 4 of 4,7-dichloroquinoline to obtain an intermediate acetic acid derivative. The chemical behavior of these reactants was investigated using different reaction conditions to optimize the nucleophilic substitution at position 4. The final compounds are prepared using a modified version of the Steglich esterification reaction between the acetic acid intermediate **3** and different anilines. The structures are confirmed by infrared, 1H, 13C, distortionless enhancement by polarization transfer 135°, Correlated Spectroscopy, heteronuclear correlation spectroscopy and (Long range HETCOR using three BIRD pulses) FLOCK-NMR spectral studies, and by elemental analysis. The synthesized compounds are tested in vitro and in vivo for their potential antimalarial and anticancer activities, with derivative **11** being the most promising candidate.

Keywords

anticancer, antimalarial, *Plasmodium*, quinoline, thiazoleacetic acid

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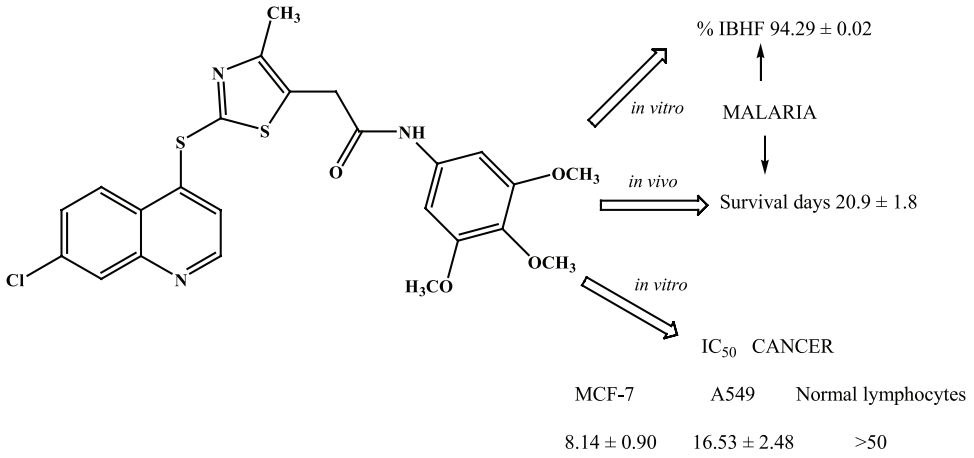

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Introduction

Malaria is a mosquito-transmitted (female *Anopheles*) infectious disease caused by *Plasmodium* parasites. Five parasite species can cause malaria in humans. In particular, *Plasmodium falciparum* and *Plasmodium vivax* are the most dangerous. Around 219 million cases of malaria were accounted for in 2017, in 87 countries, along with 435,000 deaths, mostly in sub-Saharan Africa.[1–3]

Vector control has been used as a preventive measure in malaria. However, mosquitos have become resistant to pesticides as well as there being an increase in parasite resistance, and consequently, the number of cases has not dropped.[4]

Cancer has been defined as a severe disease caused by the uncontrolled growth of cells, tissue destruction, and death. It is the second principal cause of death worldwide. In 2018 alone, 17 million new cases were reported along with 9.6 million deaths.[5] Since cancer is a complex disease, treatment strategies that involve surgery, radiotherapy, and chemotherapy result in limited efficacy depending on the stage and type of cancer. Aggressive therapy is also associated with a significant number of side effects, and consequently, life quality is reduced.[6]

Chloroquine (CQ), available since 1947, and its equivalents, have been successfully used for treatment of malaria infection.[7–9] Despite widespread resistance to CQ in most of the malaria-endemic areas, compounds with the quinoline scaffold remain an important class of potential antimalarial and anticancer drugs that require more considerable research.[10,11]

A valuable approach to obtain novel antimalarial and anticancer drugs is represented by the assembly of fused molecules with a dual mechanism of action.[12–17] A further approach refers to a superimposition in a single molecular scaffold of the structural features responsible for the activity of different antimalarial and anticancer agents.[18–23]

In our previous reports,[24–27] different quinoline compounds were designed and synthesized. The in vitro and in vivo biological activities of these compounds showed promising potential as antimalarial and anticancer compounds.

We report herein the synthesis of a hybrid scaffold by incorporating 2-mercapto-4-methyl-5-thiazoleacetic acid (**2**) on position 4 of 4,7-dichloroquinoline (**1**) and the chemical behavior of these reactants using different reaction conditions to optimize the nucleophilic substitution at position 4 of compound **1**. In vitro and in vivo analyses were used to assay the antimalarial activity of the products. Anticancer activity was assessed in vitro using the A549 and MCF-7 cell lines.

Results and discussion

Chemistry

The synthetic route to compounds **3-32** is depicted in Scheme 1. The synthesis begins with nucleophilic substitution at position 4 of 4,7-dichloroquinoline (**1**) with 2-mercapto-4-methyl-5-thiazoleacetic acid (**2**). Variables such as the solvent, temperature, and reaction time were studied. When dry acetonitrile and triethylamine (catalyst) were used at reflux temperature for 12 h, product **3** was not obtained. Changing the solvent to dry MeOH and using triethylamine (catalytic) and a temperature from room temperature to reflux for 24 h produced a mixture consisting of 6% of the desired product **3** and byproduct **1a** in 75% yield. When dry EtOH was used along with triethylamine (catalytic) and a temperature from room temperature to reflux for 24 h, a mixture of **3** (14%) and **1b** (63%) was obtained. The best result was obtained when dry EtOH was used as the solvent, a temperature of 50 °C, and a reaction time of 4 h, which led to formation of **3** as a solid white powder in a yield of 89%. The infrared (IR) spectrum of **3** revealed the presence of one broad band at 3088 cm^{-1} (OH) along with an intense stretching band (C=O) at 1705 cm^{-1} for the acid moiety confirming the structure assigned to **3**.

Additional support for the assignment of the structure was based on 1H NMR data, which showed the presence of a singlet for the methyl protons at 2.32 ppm, a singlet at 3.87 ppm due to the methylene protons (2H, $–CH_2–COOH$), several signals at 7.36–8.81 ppm due to the quinoline protons, and a broad singlet at 12.70 ppm due to the –COOH proton. Using the techniques of 13C NMR, distortionless enhancement by polarization transfer 135° (DEPT), heteronuclear correlation spectroscopy (HETCOR), and FLOCK (Long range HETCOR using three BIRD pulses), the chemical shifts at 15.5, 32.2, and 171.5 ppm confirmed the existence of CH_3, CH_2, and C=O carbons, respectively, along with signals for the carbons of the quinoline nucleus.

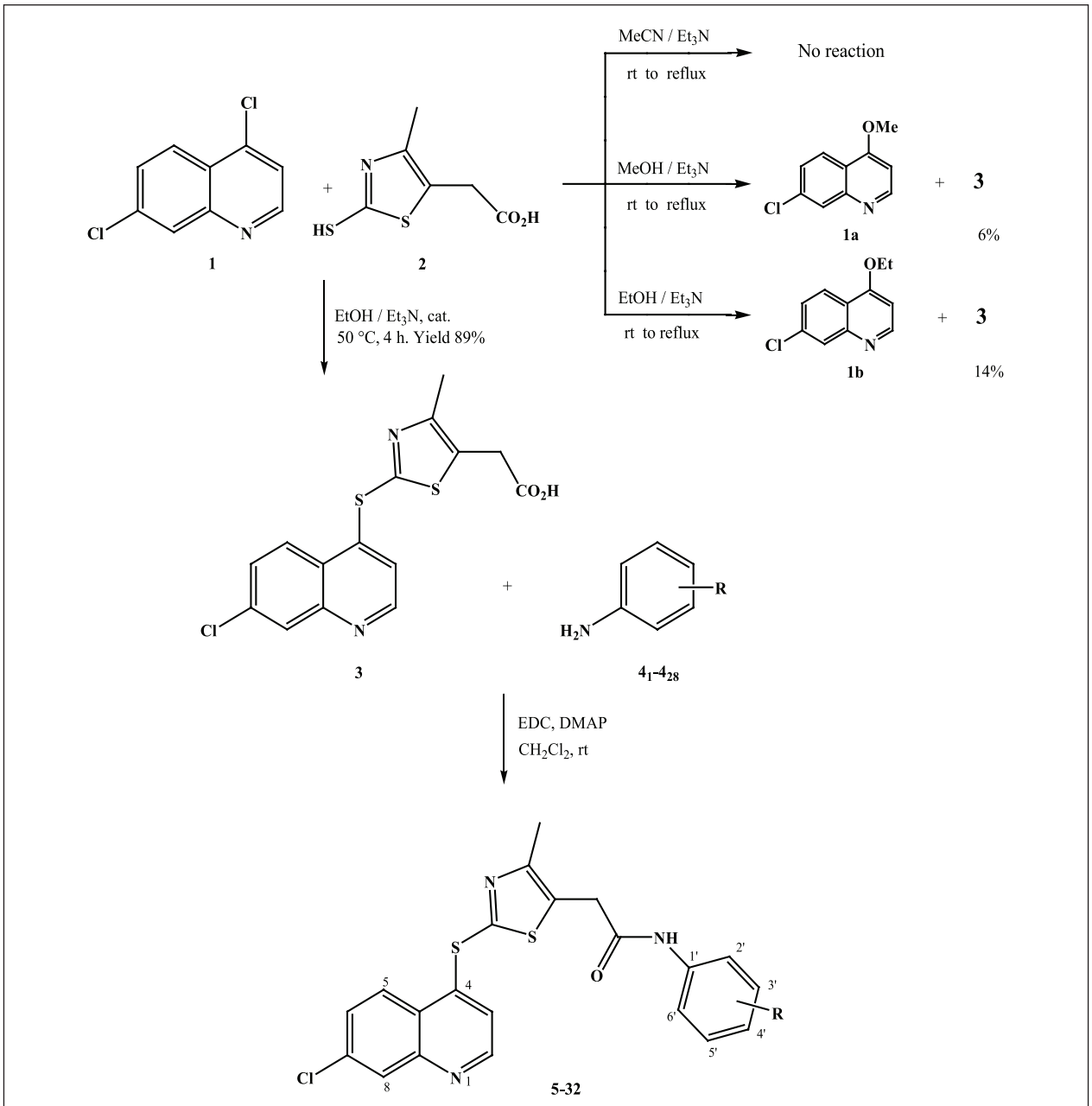
The corresponding *N*-phenylacetamide derivatives **5-32** were acquired by a modified form of the Steglich esterification reaction, at room temperature, between acid **3** with different anilines using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) and 4-(dimethylamino)pyridine (DMAP) in dichloromethane.[28]

Data from 1H NMR and 13C NMR spectroscopy were used to identify the products. The 1H NMR spectra showed the presence of singlets for methyl protons (4-methylthiazol-5-yl) at 2.42–2.48 ppm, singlets at δ 3.78–3.92 ppm due to the methylene protons, the methoxy protons at 3.72–3.84 ppm, and methyl protons at 2.06–2.30 ppm indicating that the *N*-phenylacetamide had formed. All the structures reported were confirmed by 13C NMR spectroscopy, with the chemical shifts at 166.0–168.5 ppm confirming the existence of the –NH–C=O moiety (Supplemental material).

The IR spectra of compounds **5-32** revealed intense bands at 3225–3456 cm^{-1} for NH, along with C=O stretches at 1648–1699 cm^{-1}.

Antimalarial activity

The novel synthesized compounds **5-32** were tested in vitro as inhibitors of β-hematin formation, and in vivo in a murine model (see Table 1).[27,29,30] Compounds **7** and **11** significantly reduced heme crystallization, half maximal inhibitory concentration (IC_{50}) < 10 μM. Most of the tested compounds (**5-6**, **8-10**, and **12-32**) exhibited inhibition percentages in the range of <50%. Compounds **7** and **11** were able to inhibit heme crystallization 87.75 ± 0.01 and 94.29 ± 0.02 with IC_{50} values of 9.10 ± 0.41 μM and 7.06 ± 0.36 μM,



	R		R		R		R		R		R		R
5	H	**9**	2',4'-OMe	**13**	3'-Me	**17**	3',4'-Me	**21**	2'-Cl	**25**	2',4'-Cl	**29**	2',4'-F
6	2'-OMe	**10**	2',5'-OMe	**14**	4'-Me	**18**	3',5'-Me	**22**	3'-Cl	**26**	3',4'-Cl	**30**	3',4'-F
7	3'-OMe	**11**	3',4',5'-OMe	**15**	2',4'-Me	**19**	3'-Cl-4'-OMe	**23**	4'-Cl	**27**	3'-F	**31**	3'-CF_3
8	4'-OMe	**12**	2'-Me	**16**	2',5'-Me	**20**	5'-Cl-2'-OMe	**24**	2',3'-Cl	**28**	4'-F	**32**	4'-Br

Scheme 1. Synthesis of 2-[2-(7-chloroquinolin-4-ylthio)-4-methylthiazol-5-yl)-*N*-phenylacetamide derivatives **5-32**.

respectively. The values are comparable to those of CQ 98.52 ± 0.01% with an IC_{50} value of 0.18 ± 0.03 μM.

Compounds **7** and **11**, as inhibitors of β-hematin formation in vitro, were tested in vivo in mice infected with *Plasmodium berghei* ANKA, a CQ-susceptible strain of murine malaria. The antimalarial potential of these compounds was assessed by their ability to reduce parasitemia and increase survival at the fourth-day post-infection as compared to the untreated control group. Mice were treated, intraperitoneal once daily, with the test compounds (20 mg kg^{-1}) or CQ (20 mg kg^{-1}) for consecutive days (days 1–4 post-infection). The survival times and percentage of parasitemia on day 4 were compared with those of control mice receiving only saline.[27,29,30] The Institute of Immunology Bioethical Committee approved the study according to universal guidelines of the National Research Council's Institute for Laboratory Animal Research (ILAR) and the ethical principles for medical research by the World Medical Association Declaration of Helsinki.

Table 1. The half maximal inhibitory concentrations (IC_{50}) of quinoline derivatives for the formation of β-hematin (βHF) and the effect on *Plasmodium berghei*–infected mice (20 mg kg^{-1}).

Com.	R	IC_{50} (μM)	%IβHF (±SEM)	Sd (±SEM)	%P (±SEM)
7	3′-OMe	9.10 ± 0.41	87.75 ± 0.01	21.4 ± 2.1*	3.9 ± 0.8*
11	3′,4′,5′-OMe	7.06 ± 0.36	94.29 ± 0.02	20.9 ± 1.8*	3.7 ± 0.5*
CQ		0.18 ± 0.03	98.52 ± 0.01	30	1.40 ± 0.2
CiSS		–	–	6.31 ± 0.8	22.1 ± 1.4

Com.: compounds **5-6**, **8-10**, and **12-32**; %IβHF < 50; IC_{50}: inhibitory concentration 50 (βHF) ($n = 3$); SEM: standard error of the mean; Sd: survival days; %P: percentage of parasitemias; CQ: chloroquine; CiSS: control infected and treated with saline solution.
*$p < 0.001$ compared to CiSS. $n = 6$.

Table 2. The activity of quinoline derivatives on cell viability.

Com.	R	MCF-7	A549	Nor. Lymps	SI	SI
			IC_{50} (±SD)	IC_{50} (±SD)	MCF-7	A549
11	3′,4′,5′-OMe	8.14 ± 0.90	16.53 ± 2.48	>50	>6.14	>3.02
13	3′-Me	14.63 ± 1.41	21.56 ± 1.60	>50	>3.42	>2.32
19	2′-Cl-4′-OMe	27.62 ± 2.14	>50	>50	>1.81	–
21	2′-Cl	21.67 ± 1.50	24.06 ± 1.52	>50	>2.31	>2.08
22	3′-Cl	32.00 ± 3.78	20.55 ± 1.84	>50	>1.56	>2.43
26	3′,4′-Cl	45.98 ± 4.13	22.02 ± 1.78	>50	>1.09	>2.27
29	2′,4′-F	27.23 ± 3.38	31.21 ± 3.22	>50	>1.84	>1.60
30	3′,4′-F	>50	24.78 ± 2.17	>50	–	>2.02
CQ	–	>100	>100	74.08 ± 1.51	<1.0	<1.0
Dox	–	0.42 ± 0.07	1.21 ± 0.14	3.49 ± 0.26	8.31	2.88
As_2O_3	–	5.64 ± 1.48	12.35 ± 0.61	33.66 ± 1.33	5.97	2.73

Com.: compounds; MCF-7: human breast cancer cell line; A549: human lung tumor cell line; Nor. Lymps: normal lymphocytes; SI: selectivity index tumor cells versus/normal lymphocytes; SD: standard deviation; CQ: chloroquine; Dox: doxorubicin.
Data are expressed as the mean ± standard deviation of five independent assays.

Structures **7** and **11**, used as a single therapy, extended the average survival time of infected mice to 21.4 ± 2.1 days and 20.9 ± 1.8 days, respectively; however, they were not able to decrease or delay the evolution of malaria (3.9 ± 0.8% and 3.7 ± 0.5%). CQ prolonged the mouse survival time to 30 days and decreased the development of malaria to 1.4 ± 0.2%.

Cytotoxicity evaluation

All the synthesized compounds were assessed for cell death induction in vitro. The cell lines A549 (human lung tumor), MCF-7 (breast tumor), and normal human peripheral blood mononuclear cells (PBMCs), isolated from healthy donors, were used. The Bioethical Committee of the Institute of Immunology approved the study. Written consent was obtained from each donor.

Doxorubicin, CQ, and As_2O_3 were used as the reference drugs, and the results are summarized in terms of IC_{50} values (Table 2). Compounds **5-9**, **12-15**, **17**, **20-22**, **24**, **25**, and **28-32** showed weak or no activity as cytotoxic agents against the A549 and MCF-7 cancer cell lines. Moderate results were observed for compounds **10**, **16**, **18**, **19**, **23**, **26**, and **27** against the A549 and MCF-7 cancer cell lines. Only compound **11** exhibited excellent cytotoxic activity against the A549 and MCF-7 cancer cell lines, with a better selectivity index (killing more tumor cells than healthy cells) than those observed for CQ, doxorubicin, and As_2O_3. The specificity of the compounds was assessed by dividing the IC_{50} values recorded for lymphocytes from healthy donors against those recorded from tumor cells.

We can see that the type of substituent on the phenyl ring had a significant effect on the potential antimalarial and antiproliferative activities of the target compounds. When the phenyl group had three methoxy substituents at positions 3, 4, and 5, the corresponding compound exhibited excellent activity as an inhibitor of β-hematin formation, and as an antimalarial in vivo, and an antiproliferative in vitro. When the phenyl group was mono-substituted at position 3 with a methoxy group, the activity as a potential antimalarial compound was comparable to the trimethoxy-substituted compound. However, it did not exhibit antiproliferative activity against the two cancer cell lines. Compounds di- or mono-substituted with methoxy groups at other positions were not effective. It was found that when the phenyl group was substituted with Me, Cl, F, CF_3, or Br, the corresponding compounds were not able to inhibit β-hematin formation in vitro, but exhibited moderate antiproliferative responses against the two tumor cell lines in vitro.

Our studies have demonstrated that compound **11** can inhibit malaria progression and the induction of cell death. In both models (parasite and cell lines), the mechanism of action of **11** can be associated with the blockage of heme

synthesis and the inhibition of autophagy induced by lysosomes. Three types of interactions for these compounds are possible: coordination, hydrogen bonding, and π–π stacking. These interactions have been proposed for quinidine, quinine, and CQ.[31–34]

Conclusion

In summary, 29 novel compounds were obtained after substitution at position 4 of the 4,7-dichloroqunoline (**1**) with 2-mercapto-4-methyl-5-thiazoleacetic acid (**2**) and acyl substitution with different anilines. All the structures were characterized by IR, 1H NMR, 13C NMR, and elemental analyses. The antimalarial and cytotoxic activities of the target compounds were tested. The biological results revealed that compound **11** exhibited high activity against malaria progression and induction of cell death. Our results are still in the introductory phase and need further experiments with CQ-susceptible and CQ-resistant *P. falciparum* strains.

Experimental

NMR spectra were recorded on a JEOL Eclipse™ 270 MHz for 1H NMR and at 67.9 MHz for 13C NMR using $CDCl_3$ or dimethyl sulfoxide (DMSO)-d_6, and are reported in ppm downfield from the residual $CHCl_3$ or DMSO. Elemental analyses were obtained using a PerkinElmer 2400 CHN elemental analyzer; the results were within ±0.4% of the calculated values. A Nicolet™ IS5 FTIR (ID3 Zn-Zr) spectrophotometer was used to determine the IR spectra. The melting points (m.p.) were determined by using a Thomas micro hot-stage device. The 4,7-dichloroquinoline and 2-mercapto-4-methyl-5-thiazoleacetic acid were purchased from Sigma-Aldrich Group, USA. Solvents were used directly or distilled and dried in the usual manner. Thin-layer chromatography was performed on Merck silica gel F254 (0.255-mm plates), and spots were analyzed by UV fluorescence at 254 nm.

Synthesis of 2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]acetic acid (**3**): To a solution of 4,7-dichloroquinoline (**1**) (1 mmol) in (10 mL) of dry ethanol was heated to a constant temperature of 50 °C until no insoluble material existed. 2-Mercapto-4-methyl-5-thiazoleacetic acid (**2**) (1 mmol) and (10 drops) Et_3N were added. The reaction mixture was stirred for 4 h at a constant temperature of 50 °C, affording a white precipitate. The product was filtered, washed with cold ethanol and diethyl ether, recrystallized from ethanol and then dried, to afford **3** as a white solid. Yield 89%. m.p.: 202–204 °C. IR (Zn-Zr) cm^{-1}; 3088 (OH), 1705 (C=O). 1H NMR (270 MHz, DMSO-d_6): δ = 2.32 (s, 3H, CH_3), 3.87 (s, 2H, CH_2), 7.36 (d, 1H, H3, J = 4.61 Hz), 7.75 (dd, 1H, H6, J_1 = 8.10 Hz, J_2 = 1.97 Hz), 8.14 (d, 1H, H8, J = 1.97 Hz), 8.23 (d, 1H, H5, J = 8.10 Hz), 8.82 (d, 1H, H2, J = 4.61 Hz), 12.70 (br s, 1H, OH). 13C NMR (67.9 MHz, DMSO-d_6): δ = 15.5, 32.2, 122.5, 125.1, 126.4, 128.8, 128.9, 130.5, 135.6, 143.9, 148.7, 152.1, 152.2, 152.9, 171.5. Anal. calcd for (%) $C_{15}H_{11}ClN_2O_2S_2$: C, 51.35; H, 3.16; N, 7.98; found: C, 51.37; H, 3.16; N, 8.14.

General technique for the synthesis of 2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-phenylacetamide derivatives **5-32**

To a solution of 2-*[*2-(7-chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]acetic acid (**3**) (0.5 mmol) in dry CH_2Cl_2 (20 mL) was added in one portion EDC (0.65 mmol) and DMAP (0.04 mmol) and the mixture was stirred at 0 °C for 1 h. Subsequently, the corresponding aniline **4**1-**4**$_{28}$ was added, and the mixture was stirred for 24 h at room temperature. Water (10 mL) was added to the mixture, and the organic phase was washed with 1% HCl (3 × 2 mL), saturated $NaHCO_3$ solution (3 × 2 mL), and saturated NaCl solution (3 × 2 mL). The organic layer was dried with Na_2SO_4, filtered, and evaporated under reduced pressure to give the crude product. The residue was purified by recrystallization from ethyl acetate/CH_2Cl_2 (1:1).

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-phenylacetamide (**5**): Mustard-colored powder. Yield: 74%. m.p.: 176–177 °C. IR (Zn-Zr) cm^{-1}; 3225 (NH), 1662 (C=O). 1H NMR (270 MHz, DMSO-d_6): δ = 2.43 (s, 3H, CH_3), 3.89 (s, 2H, CH_2), 7.05 (t, 1H, H4′, J = 7.18 Hz), 7.29 (t, 2H, H3′, 5′, J = 7.42 Hz), 7.35 (d, 1H, H3, J = 5.18 Hz), 7.60 (d, 2H, H6′, 2′, J = 7.66 Hz), 7.86 (dd, 1H, H6, J_1 = 1.97 Hz, J_2 = 8.91 Hz), 8.26 (d, 1H, H8, J = 1.97 Hz), 8.32 (d, 1H, H5, J = 8.91 Hz), 8.89 (d, 1H, H2, J = 5.18 Hz), 10.56 (br s, 1H, NH). 13C NMR (67.9 MHz, DMSO-d_6): δ = 15.8, 39.6, 119.9, 121.2, 124.1, 124.8, 126.4, 126.7, 129.3, 129.5, 132.1, 136.9, 139.4, 145.4, 149.9, 151.1, 152.2, 167.7. Anal. calcd for $C_{21}H_{16}ClN_3OS_2$: C, 59.21; H, 3.79; N, 9.86; found: C, 59.17; H, 3.86; N, 10.07.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(2-methoxyphenyl)acetamide (**6**): Yellow powder. Yield: 62%. m.p.: 144–146 °C. IR (Zn-Zr) cm^{-1}; 3312 (NH), 1680 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.49 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 3.89 (s, 2H, CH_2), 6.86 (dd, 1H, H3′, J_1 = 1.24 Hz, J_2 = 8.15 Hz), 6.94 (t, 1H, H4′, J = 8.15 Hz), 7.06 (td, 1H, H5′, J_1 = 1.24 Hz, J_2 = 8.15 Hz), 7.29 (d, 1H, H3, J = 4.69 Hz), 7.58 (dd, 1H, H6, J_1 = 1.72 Hz, J_2 = 8.88 Hz), 7.91 (br s, 1H, NH), 8.18 (m, 2H, H8, 5), 8.28 (dd, 1H, H6′, J_1 = 0.99 Hz, J_2 = 8.15 Hz), 8.70 (d, 1H, H2, J = 4.69 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.5, 35.5, 55.9, 110.2, 119.9, 120.2, 120.5, 121.3, 124.5, 124.8, 125.1, 125.3, 126.9, 127.3, 129.1, 141.9, 142.4, 147.9, 152.6, 165.7, 168.4. Anal. calcd for $C_{22}H_{18}ClN_3O_2S_2$: C, 57.95; H, 3.98; N, 9.22; found: C, 58.01; H, 4.03; N, 9.38.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(3-methoxyphenyl)acetamide (**7**): Mustard-colored powder. Yield: 65%. m.p.: 162–164 °C. IR (Zn-Zr) cm^{-1}; 3456 (NH), 1680 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.44 (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 3.89 (s, 2H, CH_2), 6.62 (dd, 1H, H4′, J_1 = 2.24 Hz, J_2 = 8.18 Hz), 6.95 (d, 1H, H6′, J = 8.18 Hz), 7.14 (t, 1H, H5′, J = 8.18 Hz), 7.25 (s, 1H, H2′), 7.29 (d, 1H, H3, J = 4.94 Hz), 7.58 (dd, 1H, H6, J_1 = 2.21 Hz, J_2 = 9.15 Hz), 8.16 (d, 1H, H5, J = 9.15 Hz), 8.18 (d, 1H, H8, J = 2.24 Hz), 8.64 (d, 1H, H2, J = 4.94 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.5, 35.1, 55.4, 105.9,

110.7, 112.2, 120.9, 124.9, 125.3, 127.6, 128.9, 129.7, 129.8, 137.4, 138.6, 146.5, 147.4, 148.9, 152.7, 153.3, 160.3, 166.5. Anal. calcd for $C_{22}H_{18}ClN_3O_2S_2$: C, 57.95; H, 3.98; N, 9.22; found: C, 57.93; H, 3.99; N, 9.35.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(4-methoxyphenyl)acetamide (**8**): Yellow powder. Yield: 66%. m.p.: 158–159 °C. IR (Zn-Zr) cm^{-1}; 3440 (NH), 1680 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.48 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 3.89 (s, 2H, CH_2), 6.81 (d, 2H, H5′, 3′, J = 8.91 Hz), 7.31 (d, 1H, H3, J = 4.94 Hz), 7.39 (d, 2H, H2′, 6′, J = 8.91 Hz), 7.63 (dd, 1H, H6, J_1 = 8,99 Hz, J_2 = 1.99 Hz), 7.82 (br s, 1H, NH), 8.19 (d, 1H, H5, J = 8.99 Hz), 8.29 (d, 1H, H8, J = 1.99 Hz), 8.65 (d, 1H, H2, J = 4.94 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.6, 34.9, 55.5, 114.3, 120.2, 122.1, 124.7, 125.3, 126.7, 129.3, 130.5, 130.7, 138.1, 147.9, 152.1, 152.7, 156.8, 166.4. Anal. calcd for $C_{22}H_{18}ClN_3O_2S_2$: C, 57.95; H, 3.98; N, 9.22; found: C, 57.98; H, 4.01; N, 9.40.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(2,4-dimethoxyphenyl)acetamide (**9**): Mustard-colored powder. Yield: 72%. m.p.: 155–157 °C. IR (Zn-Zr) cm^{-1}; 3440–3328 (NH), 1680 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.48 (s, 3H, CH_3), 3.77 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.86 (s, 2H, CH_2), 6.42–6.45 (m, 2H, H5′, 3′), 7.28 (d, 1H, H_3, J = 2.97 Hz), 7.57 (d, 1H, H6, J = 9.15 Hz), 7.69 (br s, 1H, NH), 8.11–8.19 (m, 3H, H6′, 5, 8), 8.69 (s, 1H, H2). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.4, 34.9, 56.2, 56.4, 110.4, 111.7, 121.7, 123.0, 125.2, 126.4, 128.8, 129.8, 135.0, 143.9, 148.7, 148.9, 151.8, 152.1, 166.3. Anal. calcd for $C_{23}H_{20}ClN_3O_3S_2$: C, 56.83; H, 4.15; N, 8.65; found: C, 56.87; H, 4.19; N, 8.83.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(2,5-dimethoxyphenyl)acetamide (**10**): Dark gray powder. Yield: 68%. m.p.: 154–155 °C. IR (Zn-Zr) cm^{-1}; 3440–3328 (NH), 1680 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.46 (s, 3H, CH_3), 3.72 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.87 (s, 2H, CH_2), 6.54 (dd, 1H, H6, J_1 = 8.91 Hz, J_2 = 2.72 Hz), 6.75 (d, 1H, H5, J = 8.91 Hz), 7.26 (d, 1H, H3, J = 4.45 Hz), 7.53 (d, 1H, H3′, J = 8.88 Hz), 7.98 (d, 1H, H8, J = 2.72 Hz), 8.04 (br s, 1H, NH), 8.11–8.15 (m, 2H, H4′, 6′), 8.68 (s, 1H, H2). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.4, 35.4, 55.9, 56.4, 106.3, 109.3, 111.0, 121.2, 125.1, 125.3, 127.7, 128.4, 128.6, 129.3, 136.5, 142.2, 145.2, 148.0, 150.2, 152.6, 154.1, 166.0. Anal. calcd for $C_{23}H_{20}ClN_3O_3S_2$: C, 56.83; H, 4.15; N, 8.65; found: C, 56.81; H, 4.21; N, 8.77.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(3,4,5-trimethoxyphenyl)-acetamide (**11**): Beige powder. Yield: 67%. m.p.: 138–140 °C. IR (Zn-Zr) cm^{-1}; 3328 (NH), 1677 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.46 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 3.79 (s, 6H, OCH_3), 3.89 (s, 2H, CH_2), 6.84 (s, 2H, H2′, 6′), 7.31 (d, 1H, H3, J = 4.90 Hz), 7.59 (dd, 1H, H6, J_1 = 10.88 Hz, J_2 = 1.99 Hz), 8.04 (br s, 1H, NH), 8.19 (d, 1H, H5, J = 10.88 Hz), 8.22 (d, 1H, H8, J = 1.99 Hz), 8.67 (d, 1H, H2, J = 4.90 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.4, 31.2, 56.2, 59.8, 104.9, 121.7, 125.2, 126.7, 127.8, 128.6, 129.1, 131.9, 134.8, 140.9, 144.9, 148.6, 151.7, 152.0, 153.3, 166.8. Anal. calcd for $C_{24}H_{22}ClN_3O_4S_2$: C, 55.86; H, 4.30; N, 8.14; found: C, 55.87; H, 4.34; N, 8.35.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-o-tolylacetamide (**12**): Beige powder. Yield: 65%. m.p.: 150–152 °C. IR (Zn-Zr) cm^{-1}; 3250 (NH), 1660 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.09 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 3.84 (s, 2H, CH_2), 7.07–7.17 (m, 3H, H3′, 4′, 5′), 7.32 (d, 1H, H3, J = 4.70 Hz), 7.53 (dd, 1H, H6, J_1 = 1.97 Hz, J_2 = 8.91 Hz), 7.69 (d, 1H, H6′, J = 7.91 Hz), 8.09 (d, 1H, H8, J = 1.97 Hz), 8.16 (d, 1H, H5, J = 8.91 Hz), 8.72 (d, 1H, H2, J = 4.70 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.4, 17.6, 34.9, 122.2, 123.2, 125.3, 125.5, 125.9, 126.9, 128.4, 128.7, 129.2, 130.7, 134.9, 136.3, 140.4, 148.9, 150.8, 152.6, 155.6, 166.3. Anal. calcd for $C_{22}H_{18}ClN_3OS_2$: C, 60.06; H, 4.12; N, 9.55; found: C, 60.04; H, 4.11; N, 9.67.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-m-tolylacetamide (**13**): Beige powder. Yield: 75%. m.p.: 152–154 °C. IR (Zn-Zr) cm^{-1}; 3264 (NH), 1660(C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.30 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 3.81 (s, 2H, CH_2), 6.93 (d, 1H, H2′, J = 2.45 Hz), 7.17–7.19 (m, 1H, H6′), 7.29–7.31 (m, 1H, H5′), 7.36–7.41 (m, 1H, H4′), 7.54 (dd, 1H, H6, J_1 = 1.91 Hz, J_2 = 8.88 Hz), 8.10 (s, 1H, H8), 8.17 (d, 1H, H5, J = 8.88 Hz), 8.72 (d, 1H, H2, J = 4.69 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.4, 21.4, 35.1, 117.2, 120.8, 122.0, 125.3, 125.5, 125.9, 128.3, 128.9, 129.2, 136.4, 137.1, 139.2, 142.4, 143.8, 148.9, 150.8, 152.4, 155.3, 166.2. Anal. calcd for $C_{22}H_{18}ClN_3OS_2$: C, 60.06; H, 4.12; N, 9.55; found: C, 60.10; H, 4.15; N, 9.80.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-p-tolylacetamide (**14**): Light gray powder. Yield: 67%. m.p.: 152–153 °C. IR (Zn-Zr) cm^{-1}; 3245 (NH), 1661 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.27 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 3.78 (s, 2H, CH_2), 7.07 (d, 2H, H3′, 5′, J = 8.18 Hz), 7.28–7.31 (m, 3H, H3, 2′, 6′), 7.53 (dd, 1H, H6, J_1 = 1.73 Hz, J_2 = 8.91 Hz), 7.66 (br s, 1H, NH), 8.08 (d, 1H, H8, J = 1.73 Hz), 8.14 (d, 1H, H5, J = 8.91 Hz), 8.65 (d, 1H, H2, J = 4.69 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.4, 20.9, 35.0, 120.3, 121.9, 125.3, 125.4, 128.3, 128.8, 129.1, 129.6, 134.7, 134.8, 136.2, 143.9, 148.8, 150.8, 152.3, 155.1, 166.3. Anal. calcd for $C_{22}H_{18}ClN_3OS_2$: C, 60.06; H, 4.12; N, 9.55; found: C, 60.13; H, 4.10; N, 9.73.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(2,4-dimethylphenyl)acetamide (**15**): Beige powder. Yield: 74%. m.p.: 164–165 °C. IR (Zn-Zr) cm^{-1}; 3296 (NH), 1651 (C=O). 1H NMR (270 MHz $CDCl_3$): δ = 2.09 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 3.89 (s, 2H, CH_2), 6.93–6.95 (m, 2H, H5′, 3′), 7.30 (d, 1H, H3, J = 4.94 Hz), 7.45 (d, 1H, H6′, J = 8.91 Hz), 7.58 (dd, 1H, H6, J_1 = 1.99 Hz, J_2 = 8.91 Hz), 8.16 (d, 1H, H5, J = 8.91 Hz), 8.21 (d, 1H, H8, J = 1.99 Hz), 8.65 (d, 1H, H2, J = 4.94 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.3, 17.7, 20.6, 34.9, 121.1, 123.8, 124.3, 125.3, 127.4, 127.7, 128.9, 129.9, 131.3, 135.8, 142.9, 146.7, 148.9, 149.6, 152.2, 152.7, 161.0, 166.3. Anal. calcd for $C_{23}H_{20}ClN_3OS_2$: C, 60.85; H, 4.44; N, 9.26; found: C, 60.81; H, 4.43; N, 9.41.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(2,5-dimethylphenyl)acetamide (**16**): Beige powder. Yield: 71%. m.p.: 196–198 °C. IR (Zn-Zr) cm^{-1}; 3296 (NH), 1651 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.06 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 3.85 (s, 2H,

CH_2), 6.88 (d, 1H, H4′, J = 7.40 Hz), 7.02 (d, 1H, H3′, J = 7.67 Hz), 7.09 (br s, 1H, NH), 7.32 (d, 1H, H3, J = 4.69 Hz), 7.54 (m, 2H, H6, 6′), 8.10 (d, 1H, H8, J = 1.97 Hz), 8.17 (d, 1H, H5, J = 8.88 Hz), 8.73 (d, 1H, H2, J = 4.69 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.4, 17.1, 21.1, 34.9, 122.2, 123.7, 125.3, 125.5, 125.8, 126.6, 128.4, 128.8, 129.1, 130.4, 134.8, 136.3, 136.8, 144.6, 148.8, 150.8, 152.7, 154.7, 166.2. Anal. calcd for $C_{23}H_{20}ClN_3OS_2$: C, 60.85; H, 4.44; N, 9.26; found: C, 60.89; H, 4.53; N, 9.51.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(3,4-dimethylphenyl)acetamide (**17**): Light yellow powder. Yield: 82 %. m.p.: 174–176 °C. IR (Zn-Zr) cm^{-1}; 3312 (NH), 1680 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.18 (s, 6H, CH_3), 2.42 (s, 3H, CH_3), 3.79 (s, 2H, CH_2), 7.02 (d, 1H, H5′, J = 8.15 Hz), 7.12 (dd, 1H, H6′, J_1 = 1.73 Hz, J_2 = 8.15 Hz), 7.23 (s, 1H, H2′), 7.28 (d, 1H, H3, J = 4.69 Hz), 7.53 (dd, 1H, H6, J_1 = 1.99 Hz, J_2 = 8.88 Hz), 8.08 (d, 1H, H8, J = 1.99 Hz), 8.15 (d, 1H, H5, J = 8.88 Hz), 8.70 (d, 1H, H2, J = 4.46 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.4, 19.2, 19.8, 35.0, 117.7, 121.6, 121.9, 125.3, 125.4, 128.3, 128.9, 129.1, 130.1, 133.5, 134.9, 136.3, 137.5, 143.9, 148.8, 150.8, 152.3, 155.1, 166.2. Anal. calcd for $C_{23}H_{20}ClN_3OS_2$: C, 60.85; H, 4.44; N, 9.26; found: C, 60.86; H, 4.47; N, 9.38.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(3,5-dimethylphenyl)acetamide (**18**): Beige powder. Yield: 68%. m.p.: 176–177 °C. IR (Zn-Zr) cm^{-1}; 3408 (NH), 1680 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.24 (s, 6H, CH_3), 2.46 (s, 3H, CH_3), 3.86 (s, 2H, CH_2), 6.74 (s, 1H, H4′), 7.09 (s, 2H, H6′, 2′), 7.29 (d, 1H, H3, J = 4.46 Hz), 7.58 (dd, 1H, H6, J_1 = 1.97 Hz, J_2 = 9.13 Hz), 7.76 (br s, 1H, NH), 8.17 (d, 1H, H5, J = 9.13 Hz), 8.18 (d, 1H, H8, J = 1.97 Hz), 8.66 (d, 1H, H2, J = 4.46 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.5, 21.3, 35.1, 117.9, 121.2, 125.1, 125.4, 126.8, 128.2, 128.7, 129.4, 137.0, 137.1, 138.9, 147.3, 149.6, 152.4, 154.0, 166.3. Anal. calcd for $C_{23}H_{20}ClN_3OS_2$: C, 60.85; H, 4.44; N, 9.26; found: C, 60.92; H, 4.51; N, 9.43.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(3-chloro-4-methoxyphenyl) acetamide (**19**): Light pink powder. Yield: 70%. m.p.: 170–172 °C. IR (Zn-Zr) cm^{-1}; 3248 (NH), 1664 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.42 (s, 3H, CH_3), 3.78 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 6.82 (d, 1H, H5′, J = 8.91 Hz), 7.28 (dd, 1H, H6′, J_1 = 2.70 Hz, J_2 = 8.91 Hz), 7.31 (d, 1H, H3, J = 4.70 Hz), 7.48 (d, 1H, H2′, J = 2.70 Hz), 7.54 (dd, 1H, H6, J_1 = 1.97 Hz, J_2 = 8.91 Hz), 7.58 (br s, 1H, NH), 8.08 (d, 1H, H8, J = 1.97 Hz), 8.15 (d, 1H, H5, J = 8.91 Hz), 8.71 (d, 1H, H2, J = 4.70 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.4, 34.9, 56.5, 112.4, 120.0, 122.1, 122.9, 125.3, 125.5, 128.4, 129.1, 130.7, 136.3, 143.7, 148.8, 150.8, 152.4, 152.6, 155.4, 166.4. Anal. calcd for $C_{22}H_{17}Cl_2N_3O_2S_2$: C, 53.88; H, 3.49; N, 8.57; found: C, 53.95; H, 3.57; N, 8.71.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(5-chloro-2-methoxyphenyl) acetamide (**20**): Violet solid. Yield: 66%. m.p.: 164–166 °C. IR (Zn-Zr) cm^{-1}; 3280 (NH), 1693 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.39 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 4.10 (s, 2H, CH_2), 7.05–7.15 (m, 2H, H3′, 4′), 7.38 (d, 1H, H3, J = 4.69 Hz), 7.77 (d, 1H, H6, J = 8.88 Hz), 8.06 (s, 1H, H6′), 8.16 (s, 1H, H8), 8.25 (d, 1H, H5, J = 8.88 Hz), 8.84 (d, 1H, H2, J = 4.69 Hz), 9.72 (br s, 1H, NH). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.7, 34.4, 56.8, 113.5, 121.4, 122.5, 124.4, 124.5, 126.4, 128.8, 129.0, 130.9, 135.5, 135.6, 143.9, 144.0, 148.0, 148.7, 148.9, 151.9, 152.1, 168.5. Anal. calcd for $C_{22}H_{17}Cl_2N_3O_2S_2$: C, 53.88; H, 3.49; N, 8.57; found: C, 53.87; H, 3.50; N, 8.63.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(2-chlorophenyl)acetamide (**21**): Brown powder. Yield: 68%. m.p.: 146–148 °C. IR (Zn-Zr) cm^{-1}; 3280 (NH), 1648 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.49 (s, 3H, CH_3), 3.89 (s, 2H, CH_2), 7.05 (t, 1H, H5′, J = 7.91 Hz), 7.23–7.35 (m, 3H, H3′, 4′, 3), 7.55 (dd, 1H, H6, J_1 = 1.97 Hz, J_2 = 9.15 Hz), 7.76 (br s, 1H, NH), 8.09 (s, 1H, H8), 8.17 (d, 1H, H5, J = 9.15 Hz), 8.30 (d, 1H, H6′, J = 7.91 Hz), 8.74 (d, 1H, H2, J = 4.70 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.4, 35.3, 121.6, 122.1, 123.0, 125.3, 125.4, 127.9, 128.1, 128.4, 129.1, 129.2, 133.9, 136.3, 143.4, 148.9, 150.9, 153.0, 155.8, 166.2. Anal. calcd for $C_{21}H_{15}Cl_2N_3OS_2$: C, 54.78; H, 3.28; N, 9.13; found: C, 54.71; H, 3.25; N, 9.25.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(3-chlorophenyl)acetamide (**22**): Light yellow powder. Yield: 61%. m.p.: 180–182 °C. IR (Zn-Zr) cm^{-1}; 3248 (NH), 1696 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.42 (s, 3H, CH_3), 3.81 (s, 2H, CH_2), 7.08 (d, 1H, H4′, J = 7.39 Hz), 7.18–7.28 (m, 2H, H5′, 6′), 7.33 (d, 1H, H3, J = 4.69 Hz), 7.53–7.58 (m, 2H, H6, 2′), 8.09 (d, 1H, H8, J = 1.97 Hz), 8.88 (d, 1H, H5, J = 8.91 Hz), 8.73 (d, 1H, H2, J = 4.69 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.4, 35.3, 117.9, 120.2, 122.1, 125.2, 125.4, 125.5, 128.0, 128.4, 129.1, 130.2, 134.9, 136.3, 138.3, 143.5, 148.9, 150.9, 152.5, 155.6, 166.4. Anal. calcd for $C_{21}H_{15}Cl_2N_3OS_2$: C, 54.78; H, 3.28; N, 9.13; found: C, 54.77; H, 3.23; N, 9.32.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(4-chlorophenyl)acetamide (**23**): Light yellow powder. Yield: 66%. m.p.: 192 °C. IR (Zn-Zr) cm^{-1}; 3248 (NH), 1690 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.43 (s, 3H, CH_3), 3.80 (s, 2H, CH_2), 7.26 (d, 2H, H5′, 3′ J = 8.67 Hz), 7.34 (d, 1H, H3, J = 4.94 Hz), 7.38 (d, 2H, H2′, 6′,J = 8.67 Hz), 7.54 (dd, 1H, H6, J_1 = 1.97 Hz, J_2 = 9.15 Hz), 8.10 (d, 1H, H8, J = 1.97 Hz), 8.17 (d, 1H, H5, J = 9.15 Hz), 8.73 (d, 1H, H2, J = 4.69 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.5, 35.1, 121.3, 122.3, 125.2, 125.4, 125.5, 127.9, 128.4, 129.2, 130.4, 133.5, 135.2, 142.1, 145.2, 148.7, 150.8, 152.5, 166.2. Anal. calcd for $C_{21}H_{15}Cl_2N_3OS_2$: C, 54.78; H, 3.28; N, 9.13; found: C, 54.85; H, 3.30; N, 9.27.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(2,3-dichlorophenyl)acetamide (**24**): Beige powder. Yield: 68%. m.p.: 174–176 °C. IR (Zn-Zr) cm^{-1}; 3225 (NH), 1660 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.48 (s, 3H, CH_3), 3.90 (s, 2H, CH_2), 7.19–7.25 (m, 2H, H5′, 4′), 7.35 (d, 1H, H3, J = 4.70 Hz), 7.55 (dd, 1H, H6, J_1 = 1.97 Hz, J_2 = 8.91 Hz), 7.81 (br s, 1H, NH), 8.11 (d, 1H, H8, J = 1.97 Hz), 8.18 (d, 1H, H5, J = 8.91 Hz), 8.25 (m, 1H, H6′), 8.76 (d, 1H, H2, J = 4.70 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.4, 35.3, 119.4, 122.4, 125.5, 125.9, 127.6, 128.0, 128.5, 129.2, 133.0, 135.4, 136.2, 148.8, 150.8, 153.1, 166.3. Anal. calcd for $C_{21}H_{14}Cl_3N_3OS_2$: C, 50.97; H, 2.85; N, 8.49; found: C, 51.08; H, 2.93; N, 8.73.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(2,4-dichlorophenyl)acetamide (**25**): Brown powder. Yield: 59%. m.p.: 104–106 °C. IR (Zn-Zr) cm^{-1}; 3248 (NH), 1661 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.47 (s, 3H, CH_3), 3.88 (s, 2H, CH_2), 7.23 (dd, 1H, H5′, J_1 = 2.24 Hz, J_2 = 8.88 Hz), 7.33 (d, 1H, H3, *J* = 4.70 Hz), 7.36 (d, 1H, H3′, *J* = 2.24 Hz), 7.55 (dd, 1H, H6, J_1 = 1.97 Hz, J_2 = 8.91 Hz), 7.70 (br s, 1H, NH), 8.10 (d, 1H, H8, *J* = 1.97 Hz), 8.17 (d, 1H, H5, *J* = 8.91 Hz), 8.27 (d, 1H, H6′, *J* = 8.88 Hz), 8.74 (d, 1H, H2, *J* = 4.70 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.4, 35.2, 122.4, 125.4, 125.5, 127.6, 128.1, 128.4, 128.9, 129.2, 132.6, 136.3, 143.2, 148.9, 150.8, 153.1, 156.2, 166.2. Anal. calcd for $C_{21}H_{14}Cl_3N_3OS_2$: C, 50.97; H, 2.85; N, 8.49; found: C, 51.03; H, 2.89; N, 8.65.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(3,4-dichlorophenyl)acetamide (**26**): Brown powder. Yield: 62%. m.p.: 190–192 °C. IR (Zn-Zr) cm^{-1}; 3248 (NH), 1661(C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.43 (s, 3H, CH_3), 3.81 (s, 2H, CH_2), 7.25 (dd, 1H, H6′, J_1 = 2.45 Hz, J_2 = 7.66 Hz), 7.34–7.37 (m, 2H, H3, 5′), 7.42 (br s, 1H, NH), 7.55 (dd, 1H, H6, J_1 = 2.21 Hz, J_2 = 8.91 Hz), 7.68 (d, 1H, H2′, *J* = 1.97 Hz), 8.10 (d, 1H, H8, *J* = 2.24 Hz), 8.18 (d, 1H, H5, *J* = 8.91 Hz), 8.74 (d, 1H, H2, *J* = 4.69 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.4, 34.9, 119.1, 121.8, 122.3, 125.4, 125.5, 127.7, 128.4, 129.2, 130.7, 133.1, 136.3, 136.6, 143.4, 148.8, 150.8, 152.6, 155.8, 166.4. Anal. calcd for $C_{21}H_{14}Cl_3N_3OS_2$: C, 50.97; H, 2.85; N, 8.49; found: C, 50.92; H, 2.87; N, 8.47.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(3-fluorophenyl)acetamide (**27**): Light yellow powder. Yield: 69%. m.p.: 180–182 °C. IR (Zn-Zr) cm^{-1}; 3280 (NH), 1696 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.43 (s, 3H, CH_3), 3.82 (s, 2H, CH_2), 6.82 (t, 1H, H4′, *J* = 6.91 Hz), 7.06 (d, 1H, H6′, *J* = 7.67 Hz), 7.19–7.24 (m, 1H, H5′), 7.32 (d, 1H, H3, *J* = 4.69 Hz), 7.43 (dd, 1H, H2′, J_1 = 10.33 Hz, J_2 = 1.97 Hz), 7.55 (dd, 1H, H6, J_1 = 1.73 Hz, J_2 = 8.88 Hz), 7.59 (br s, 1H, NH), 8.09 (d, 1H, H8, *J* = 1.73 Hz), 8.17 (d, 1H, H5, *J* = 8.88 Hz), 8.72 (d, 1H, H2, *J* = 4.69 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.6, 35.1, 109.7 (d, *J* = 245 Hz), 115.1, 122.2, 122.9, 125.2, 125.4, 128.1, 128.4, 129.1, 129.2, 130.4, 136.3, 143.6, 148.8, 150.7, 152.4, 155.6, 166.3. Anal. calcd for $C_{21}H_{15}ClFN_3OS_2$: C, 56.81; H, 3.41; N, 9.47; found: C, 56.87; H, 3.45; N, 9.71.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(4-fluorophenyl)acetamide (**28**): Beige powder. Yield 71%. m.p.: 194–195 °C. IR (Zn-Zr) cm^{-1}; 3264 (NH), 1689 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.43 (s, 3H, CH_3), 3.81 (s, 2H, CH_2), 6.98 (t, 2H, H5′, 3′, *J* = 8.64 Hz), 7.32 (d, 1H, H3, *J* = 4.46 Hz), 7.37–7.42 (m, 2H, H2′, 6′), 7.54 (dd, 1H, H6, J_1 = 1.97 Hz, J_2 = 8.91 Hz), 8.10 (d, 1H, H8, *J* = 1.97 Hz), 8.17 (d, 1H, H5, *J* = 8.91 Hz), 8.72 (d, 1H, H2, *J* = 4.46 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.4, 34.9, 115.5 (d, *J* = 21.3 Hz), 121.9, 122.0, 122.1, 125.5, 128.4, 129.0, 133.0, 136.4, 137.4, 144.5, 148.7, 150.7, 152.6, 155.3, 166.4. Anal. calcd for $C_{21}H_{15}ClFN_3OS_2$: C, 56.81; H, 3.41; N, 9.47; found: C, 56.75; H, 3.39; N, 9.63.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(2,4-difluorophenyl)acetamide (**29**): Light brown powder. Yield 60%. m.p.: 182–184 °C. IR (Zn-Zr) cm^{-1}; 3280 (NH), 1689 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.46 (s, 3H, CH_3), 3.86 (s, 2H, CH_2), 6.82- 6.89 (m, 2H, H6′, 5′), 7.31 (d, 1H, H3, *J* = 4.69 Hz), 7.39 (br s, 1H, NH), 7.54 (dd, 1H, H6, J_1 = 2.24 Hz, J_2 = 8.88 Hz), 8.10 (d, 1H, H8, *J* = 2.24 Hz), 8.14 (s, 1H, H3′),8.17 (d, 1H, H5, *J* = 8.88 Hz), 8.73 (d, 1H, H2, *J* = 4.69 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.5, 34.9, 110.2, 119.9, 120.2, 121.3, 124.5, 124.9, 125.2, 126.9, 127.3, 128.4, 129.2, 141.9, 142.4, 147.9 150.8, 152.8, 155.6, 166.3. Anal. calcd for $C_{21}H_{14}ClF_2N_3OS_2$: C, 54.60; H, 3.05; N, 9.10; found: C, 54.69; H, 3.12; N, 9.31.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(3,4-difluorophenyl)acetamide (**30**): Brown powder. Yield: 65%. m.p.: 167–169 °C. IR (Zn-Zr) cm^{-1}; 3280 (NH), 1696 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.43 (s, 3H, CH_3), 3.81 (s, 2H, CH_2), 7.02–7.09 (m, 2H, H6′, 5′), 7.34 (d, 1H, H3, *J* = 4.46 Hz), 7.53–7.57 (m, 2H, H6, 2′), 8.09 (d, 1H, H8, *J* = 1.74 Hz), 8.17 (d, 1H, H5, *J* = 8.91 Hz), 8.73 (d, 1H, H2, *J* = 4.69 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.4, 34.8, 110.20 (d, *J* = 22.3 Hz), 115.6 (d, *J* = 6.22 Hz), 117.4 (d, *J* = 17.6 Hz), 122.2, 125.4, 125.5, 127.8, 128.4, 129.2, 136.2, 143.4, 148.9, 150.9, 152.5, 166.4. Anal. calcd for $C_{21}H_{14}ClF_2N_3OS_2$: C, 54.60; H, 3.05; N, 9.10; found: C, 54.65; H, 3.09; N, 9.27.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-[3-(trifluoromethyl)phenyl] acetamide (**31**): Light pink powder. Yield 58%. m.p.: > 300 °C. IR (Zn-Zr) cm^{-1}; 3258 (NH), 1699 (C=O). 1H NMR (270 MHz, $CDCl_3$): δ = 2.47 (s, 3H, CH_3), 3.85 (s, 2H, CH_2), 7.36 (d, 1H, H3, *J* = 4.69 Hz), 7.39–7.44 (m, 2H, H4′, 5′), 7.55 (dd, 1H, H6, J_1 = 2.24 Hz, J_2 = 8.91 Hz), 7.63 (dd, 1H, H6′, J_1 = 2.30 Hz, J_2 = 8.81 Hz), 7.77 (s, 1H, H2′), 8.10 (d, 1H, H8, *J* = 2.24 Hz), 8.18 (d, 1H, H5, *J* = 8.91 Hz), 8.76 (d, 1H, H2, *J* = 4.69 Hz). 13C NMR (67.9 MHz, $CDCl_3$): δ = 15.4, 34.8, 110.2 (d, *J* = 242.3 Hz), 115.6 (d, *J* = 4.1 Hz), 117.4 (d, *J* = 27.6 Hz), 122.2, 125.4, 125.5, 127.8, 128.4, 129.2, 131.2, 133.0, 136.2, 143.4, 148.9, 150.9, 152.5, 166.4. Anal. calcd for $C_{22}H_{15}ClF_3N_3OS_2$: C, 53.49; H, 3.06; N, 8.51; found: C, 53.51; H, 3.10; N, 8.75.

2-[2-(7-Chloroquinolin-4-ylthio)-4-methylthiazol-5-yl]-N-(4-bromophenyl)acetamide (**32**): Yellow powder. Yield 62%. m.p.: 206–208 °C. IR (Zn-Zr) cm^{-1}; 3754–3228 (NH), 1689 (C=O). 1H NMR (270 MHz, DMSO-d_6): δ = 2.36 (s, 3H, CH_3), 3.92 (s, 2H, CH_2), 7.35 (d, 1H, H3, *J* = 4.70 Hz), 7.45 (d, 2H, H3′, 5′, *J* = 8.91 Hz), 7.52 (d, 2H, H2′, 6′, *J* = 8.91 Hz), 7.73 (dd, 1H, H6, J_1 = 2.21 Hz, J_2 = 8.91 Hz), 8.12 (d, 1H, H8, *J* = 2.21 Hz), 8.21 (d, 1H, H5, *J* = 8.91 Hz), 8.80 (d, 1H, H2, *J* = 4.70 Hz), 10.45 (br s, 1H, NH). 13C NMR (67.9 MHz, DMSO-d_6): δ = 15.6, 39.4, 115.8, 121.9, 122.5, 125.1, 126.4, 128.8, 128.9, 130.6, 132.1, 135.7, 138.5, 143.9, 148.6, 151.9, 152.0, 153.2, 167.9. Anal. calcd for $C_{22}H_{15}BrClN_3OS_2$: C, 49.96; H, 2.99; N, 8.32; found: C, 50.08; H, 3.06; N, 8.63.

Antimalarial activity

Inhibition of β-hematin formation. The assay was performed according to previously reported protocols.[26,29,35] Hemin chloride (50 μL, 4 mM) in DMSO (5.2 mg mL^{-1}) was

pipetted into 96-well microplates. Different concentrations (5–100 mM) of the compounds in DMSO were added in triplicate (50 µL). Water (50 µL) or DMSO (50 µL) was used as control. Acetate buffer (100 µL, 0.2 M, pH 4.4) initiated the formation of β-hematin. Next, the plates were incubated at 37 °C for 48 h and subsequently centrifuged (4000 r min^{-1} × 15 min). The supernatent was washed twice with DMSO (200 µL) and resuspended in NaOH (200 µL, 0.2 N). The solubilized products were diluted (1:2) with NaOH (0.1 N), and the plates were read in an enzyme-linked immunosorbent assay (ELISA) reader at 405 nm (Microplate Reader, BIORAD-550). The results are expressed as %IβHF.

Parasite, the experimental host and strain maintenance. Male Balb-C mice, weight 18–22 g, were fed with a commercial diet at libitum under standard procedures of animal care following the aforementioned method approved by the Ethics Committee of the Institute of Immunology. The animals were infected with a rodent malaria strain of *P. berghei* ANKA. A million infected erythrocytes, in phosphate-buffered saline solution (PBS, 10 mM, pH 7.4, 0.1 mL), were inoculated intraperitoneally to infect the animal. The parasitemia was inspected by continuous microscopic examination of Giemsa-stained smears.[26,29,36]

Four-day suppressive test. One million *P. berghei*, injected in the caudal vein intravenously, were used to infect the mice (18–23 g, $n = 6$). After 2 h of inoculation, the compounds that were active in vitro were dissolved in DMSO (0.1 M) and subsequently diluted with Saline-Tween 20 solution (2%) and administered intraperitoneally for 4 days (10 mg kg^{-1}). The parasite load was evaluated on day 4 by examining Giemsa-stained smears. The positive control was CQ (10 mg kg^{-1}), and the negative control was saline solution. Non-treated mice were used as a baseline control for survival times.[26,29,30] The percentage of parasitemia was used to express the results.

Antitumor activity

Cell lines. The human cell lines, obtained from the American Tissue Culture Collection (ATCC), MCF-7 (human breast cancer cell line) and A549 (human lung tumor cell line), were maintained in Roswell Park Memorial Institute (RPMI) 1640 media supplemented as recommended by the ATCC. The experimental analyses were performed by culturing 3×10^5 cells/well with the different compounds in 96 well microtiter plates. Five different assays in triplicate were performed per compound.[26,29]

Isolation of human total lymphocytes. Normal volunteers' blood was used with written consent, as specified by the Ethical Committee at the Institute of Immunology, in order to obtain PBMCs by the standard Ficoll-Paque gradient (Histopaque 1077, Sigma, Poole, UK) as described previously.[26,29] The cells, 85% T lymphocytes, 8% B lymphocytes, and 7% natural killer (NK) cells, were used as the primary culture for assessment of compound specificity. The selectivity index refers to the ratio of IC_{50} required to induce cell death in healthy cells divided by the IC_{50} required to induce cell death in tumor cell lines. Ideal compounds have high selectivity indices.

The 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide viability assay. The protocol used is a slight modification of Mossmann's protocol[37] and was described previously.[38] Specifically, different concentrations of compounds **3** and **5-32** (0, 1, 5, 10, 25, 50, and 100 µM) were incubated with 5×10^4 cells/well in 96-well microtiter plates for 24 h, and then, the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was performed.[38] Cells incubated with CQ, doxorubicin (Dox), or As_2O_3 were used as positive controls.

Data analysis. One-way analysis of variance (ANOVA) and *t* tests for specific group comparisons were used for data analysis. The program used was Graph Pad Prism 4.02.[39]

Declaration of conflicting interests

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Supplemental material

Supplemental material for this article is available online.

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