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Quest for a potent antimalarial drug lead: Synthesis and evaluation of 6,7-dimethoxyquinazoline-2,4-diamines

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ABSTRACT

Quinazolines have long been known to exert varied pharmacologic activities that make them suitable for use in treating hypertension, viral infections, tumors, and malaria. Since 2014, we have synthesized approximately 150 different 6,7-dimethoxyquinazoline-2,4-diamines and evaluated their antimalarial activity via structure-activity relationship studies. Here, we summarize the results and report the discovery of 6,7-dimethoxy-N^4-(1-phenylethyl)-2-(pyrrolidin-1-yl)quinazolin-4-amine (**20**, SSJ-717), which exhibits high antimalarial activity as a promising antimalarial drug lead.

1. Introduction

Alongside acquired immunodeficiency syndrome (i.e., AIDS) and tuberculosis, malaria is one of the three major worldwide epidemic diseases. Various species of malaria parasites have been identified, including *Plasmodium falciparum, P. vivax*, *P. malariae*, and *P. ovale*. These species are widely distributed in the tropical and subtropical regions near the equator, including areas of sub-Saharan Africa, Asia, and Latin America. A recent report by the World Health Organization indicated that 228 million cases of malaria infection occurred worldwide in 2018, causing an estimated 400,000 deaths, 67% of which were children under 5 years of age.[1]

Chloroquine has been used for many years as an antimalarial drug, but it is no longer effective due to the emergence of resistant *P. falciparum* strains in many regions of the world. Current malaria treatment relies largely on artemisinin and artemisinin-based combination drug therapy (ACT), although mutants resistant to artemisinin and ACT have emerged in southeast Asia between 2001 and 2018. To combat this drug resistance, there is an acute need for new, highly effective drugs that are preferably both orally available and inexpensive.[1]

Quinazolines have long been known to exert physiologic activities. A several number of patent applications have been filed for the use of quinazolines as antihypertensive[2–7] and antiviral[8,9] agents. In addition, a report[10] and patent[11] have been published regarding their use as antitumor agents. It was also reported that 6,7-dimethoxyquinazoline-2,4-diamines (DMQDAs) exhibit highly selective antilysine methyltransferase activity.[12–14] Another report indicated that quinazoline-2,4-amines (QDAs) are effective in the treatment of leishmaniasis.[15] Several studies have examined the antimalarial activity of QDAs. For example, Werbel and co-workers tested approximately 100 QDAs for activity against *P. falciparum*.[16] Nzila and co-workers reported that QDAs with dihydrofolate reductase inhibitory activity also exhibited antimalarial activity.[17] Garcia-Bustos and colleagues assayed 2 million compounds

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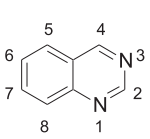
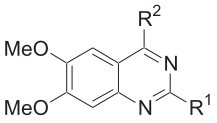
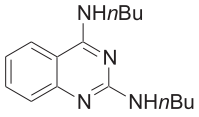
Table 1
Effect of 6,7-dimethoxy groups and 2-position functional group on antimalarial activity and cytotoxicity of QDAs



quinazoline



1: $R^1 = R^2 = NHnBu$
3: $R^1 = NHnBu$, $R^2 = NHEt$
4: $R^1 = Cl$, $R^2 = NHEt$
5: $R^1 = OMe$, $R^2 = NHEt$
6: $R^1 = H$, $R^2 = NHnBu$



2

Compound	IC_{50} (nM)[a,c] *P. falciparum* NF54	IC_{50} (μM)[b,c] L6 rat myoblasts	Selectivity index (SI)[d]
1	2.10 ± 0.5	3.67 ± 0.12	1,750
2	121 ± 16	3.01 ± 0.57	25.6
3	23.0 ± 6.6	5.29 ± 0.16	230
4	15,800 ± 2,600	52.6 ± 4.8	3.33
5	34,800 ± 6,000	67.3 ± 1.4	1.90
6	4,710 ± 230	73.6 ± 5.5	15.6
Chloroquine (control)	6.25		
podophyllotoxin(control)		0.0145	

[a] IC_{50} values for inhibition of *P. falciparum* NF54 growth. [b] IC_{50} values for inhibition of rat L6 cell growth. [c] Data are expressed as the average of two independent determinations of IC_{50} ± deviation from the mean. [d] SI is the ratio of IC_{50} for cytotoxicity versus antiplasmodial activity (L6/*P. falciparum*).

selected from a GSK library for *in vitro* activity against *P. falciparum* and identified 8,000 compounds that exhibited 80% inhibition of *P. falciparum* at a concentration of 2 μM, ultimately obtaining 44 antimalarial drug leads that included two DMQDAs.[18] Fuchter and coworkers reported that DMQDA with a six-membered ring amino group (piperidine, piperazine) or a 7-membered ring amino group at the 2-position of the quinazoline ring exhibits selective antilysine methyltransferase activity and potent antimalarial activity *in vitro*.[19,20] Finally, Sleebs et al. reported the results of a detailed analysis of the antimalarial activity of 56 types of QDAs containing various aniline derivatives at the 2-position of the quinazoline ring.[21]

Since 2014, we have been engaged in the development of orally available antimalarial drugs, with particular attention centered on DMQDAs. To date, we have synthesized approximately 150 compounds and screened their *in vitro* antimalarial activity. Some promising compounds demonstrating high potency have advanced to *in vivo* evaluations using malaria-infected mice. Here, we report the chemical synthesis and antimalarial activity of these DMQDAs in detail. Our efforts have culminated in the discovery of a promising compound exhibiting high antimalarial activity, 6,7-dimethoxy-N^4-(1-phenylethyl)-2-(pyrrolidin-1-yl)quinazolin-4-amine (**20**), which is expected to become a promising antimalarial drug lead.

2. Results and discussion

2.1. Synthesis of DMQDAs

In developing QDA-based antimalarial drugs, we first addressed the necessity of the two methoxy groups at the 6- and 7-positions of the QDA skeleton by investigating their effects on antimalarial activity and cytotoxicity. *In vitro* antimalarial assays were conducted using *P. falciparum* NF54, whereas rat-derived L6 cells were used for cytotoxicity assays. The selectivity index (SI) was determined by dividing the IC_{50} value for cytotoxicity by the IC_{50} value for antimalarial activity.

Compound **1**, which has methoxy groups at the 6- and 7-positions of the N^2,N^4-di-*n*-butyl-quinazoline-2,4-diamine, exhibited 60-fold higher antimalarial activity (against *P. falciparum* NF54) than compound **2**, which has no 6,7-dimethoxy groups (Table 1), whereas the cytotoxicity (against L6 rat myoblasts) of the compounds was similar. The antimalarial activity of **1** was also higher than that of chloroquine. These results indicate that the dimethoxy groups play an important role in the antimalarial activity of QDAs. Thus, we decided to prepare various QDAs with 6,7-dimethoxy groups.

We next focused on the effect on antimalarial activity of an amino group at the 2-position. Three compounds (**3**, **4**, and **5**) with an ethylamino substituent at the 4-position of the 6,7-dimethoxyquinazoline were synthesized. Although compound **3** with *n*-butylamino group at the 2-position exhibited good activity, no activity was observed with a chlorine atom (**4**), or a methoxy group (**5**) at the 2-position (Table 1). Compound **6**, which has no substitution at the 2-position, exhibited only low activity. These results indicate that the presence of an amino group at the 2-position of the 6,7-dimethoxy-quinazoline skeleton has a significant effect on antimalarial activity.

Based on these results, we synthesized ca. 150 DMQDAs with the goal of identifying promising antimalarial drug candidates. Each synthesis was carried out in two steps using commercially available 2,4-dichloro-6,7-dimethoxyquinazoline as the common starting material (Scheme 1). Because the chlorine atom at the 4-position is more reactive than the chlorine atom at the 2-position, first substitution with the chosen amine (R^2) at 30–50 °C gave a 2-chloro-N^4-(R^2)-quinazolin-4-amine intermediate. The solution containing the intermediate was added to a solution containing the second amine (R^1), and the reaction temperature was increased to 130–150 °C to allow substitution of the R^1-amino group into the 2-position of the 6,7-dimethoxyquinazoline skeleton.

2.2. In vitro antiplasmodial activity and cytotoxicity

The antimalarial activity (against *P. falciparum* NF54) and cytotoxicity (against L6 rat myoblasts) were also tested *in vitro*. The selectivity index (SI) was calculated from the values thus obtained. Table 2 shows the data for 40 compounds as representatives of the 150 synthesized products. Twenty-nine compounds exhibited high antimalarial activity, with IC_{50} values < 30 nM, which was stronger than values reported for QDA.[7–12] Compound **18**, having 2- and 4-amino groups without an active hydrogen, exhibited extremely low antimalarial activity (IC_{50} > 1,000 nM), which was also the case for compound **14**, which has an NH_2-group at the 4-position. These results suggest that an amino group containing one active hydrogen at the 4-position of the 6,7-dimethoxyqunazoline is essential for antimalarial activity. Sixteen compounds

exhibited potent activity (IC_{50} values < 10 nM),[7–12] all of which were substituted at the 2-position with an alkylamino group (**1** and **7**), (pyridin-3-ylmethyl)amino group (**12**), (pyridin-4-ylmethyl)amino group (**13**), pyrrolidin-1-yl group (**15**–**19**, **20**, and **21**), (pyridin-4-yl)amino group (**24**–**27**, **29**, and **30**), or (pyridin-3-yl)amino group (**35**). In contrast, compound **8**, which has an alkoxyamino group at the 2-position, exhibited less-potent activity. Compounds **9** and **10**, which have benzylamino and methylbenzylamino groups at the 2-position, exhibited IC_{50} values > 10 nM. In addition, compounds **22**, **23**, **31**–**34**, and **36**–**39** exhibited antimalarial IC_{50} values > 10 nM. Thus, with respect to inhibitory activity against *P. falciparum* NF54, there was no significant difference between compounds with amino substituents at the 2-position. However, eight compounds with a pyrrolidin-1-yl (**16**), (pyridin-3-ylmethyl)amino (**12**), pyridin-4-yl (**24**–**27**, and **30**), or (pyridin-3-yl) amino (**35**) group exhibited better inhibitory profiles, with SI values > 3,000. With the exception of **16**, these compounds all have a pyridine moiety at the 2-position. Thus, installation of a pyridine ring at the 2-position is an effective means of significantly reducing cytotoxicity.

Although compounds having a pyrrolidin-1-yl group at the 2-position demonstrated high antimalarial activity, most compounds did not exhibit a particularly good SI value (i.e., <1000) because of their relatively high cytotoxicity against L6 cells. Compounds having a different substituent, such as an alkyl group (**1**, **3**, and **7**) or benzyl group (**9**), at the 2-position exhibited potent to moderate antimalarial activity but relatively high cytotoxicity against L6 cells. In contrast, compound **40**, which was reported by Garcia-Bustos and co-workers,[18] exhibited lower activity.

2.3. In vivo efficacy studies

For the *in vivo* assay, we used a rodent malaria parasite (*P. berghei*) as an animal model that has a very similar lifecycle to that of *P. falciparum*. Blood of mice infected with *P. berghei* NK65 was collected and used to infect other mice (SLc: ICR females 25–27 g, n = 3 or 5) via tail vein injection. The test compounds were suspended in 0.5% methylcellulose aqueous solution and administered orally once per day for 4 days. In order to enhance the bioavailability of the compounds, corresponding hydrochloride salts of each test compound were prepared to increase the water solubility. The number of HCl and H_2O molecules in each hydrochloride salt was confirmed by elemental analysis, and the empirical formula was determined. The oral dose was adjusted to a hydrochloride salt equivalent of 50 mg of the free form per kg of mouse body weight. Four days after infection, blood was collected from the tail of each mouse and stained with Diff-Quik (Sysmex). Thin-layer smears were prepared and observed under a microscope to calculate the suppression of infection ratio (%) relative to that of the control group. The mean survival days (MSD) of infected mice was compared to that of the control group.

Based on the results of the *in vitro* studies described above, an *in vivo* assay was conducted using 6,7-dimethoxy-2-(pyrrolidin-1-yl)quinazolin-4-amines **15**, **17**, **20**–**23**, and N^2–(pyridin-4-yl)-2,4-diamino compounds **24**, **25**, **29**–**31**, **33**, and **34** based on their high antimalarial activity and very low cytotoxicity, which resulted in good SI values. Table 3 shows the assay results obtained after dosing with hydrochloride salts of the compounds equivalent to 50 mg of the free form per kg of mouse body weight (n = 3; 3 mice were used in each case). The empirical formula of each salt, including the number of attached HCl and H_2O molecules determined by elemental analysis, is also shown in the table.

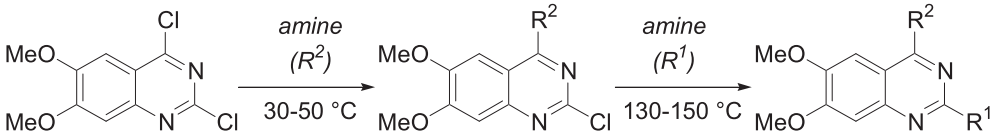
Six 2-(pyrrolidin-1-yl) compounds, **15**, **17**, and **20**–**23**, which exhibited fairly high antimalarial activity and some degree of cytotoxicity in the *in vitro* assay, were selected. Four of the six compounds (**15**, **20**, **22**, and **23**) exhibited inhibition rates > 80% (Table 3). In particular, compounds **15** and **20** were highly active, with **15** exhibiting an inhibition rate of 96.5% and **20** (SSJ-717) an inhibition rate of 99.6%. In contrast, among the seven N^2–(pyridin-4-yl)amino compounds (**24**, **25**, **29**–**31**, **33**, and **34**), compounds **29** and **31** exhibited an inhibition rate of > 50% after 4 days. Contrary to our expectations based on the *in vitro* assay results, however, even the best compound, **31**, exhibited only a 60.8% inhibition rate. These results suggest that the presence of a pyridine ring at the 2-position of the 6,7-dimethoxyquinazoline increases the overall water solubility of the molecule, thus making it too hydrophilic and consequently either impeding adequate translocation of the molecule through the small intestine into the bloodstream or possibly facilitating overly rapid metabolism in the blood stream. The former assumption would also explain why most of the N^2–(pyridin-4-yl)amino compounds exhibited such low cytotoxicity in the *in vitro* assay (i.e., difficulty in entering L6 cells due to high hydrophilicity).

Figure 1 shows the correlation between the C log P of the six compounds (**15**, **17**, **20**–**23**) having a pyrrolidin-1-yl group at the 2-position and the mean malarial activity suppression rate of the corresponding hydrochloride salts. An optimum C log P value of 4.5 was observed, indicating that the *in vivo* activity of these compounds is affected by their hydrophobicity/hydrophilicity balance. However, no correlation was observed between inhibition rate and C log P for the group of pyridine-containing compounds, as listed in Table 3.

As the preliminary results shown in Table 3 indicated that the 2-(pyrrolidin-1-yl) compounds are highly active antimalarial agents, *in vivo* studies were conducted using compounds **15**, **20**, and **23**. Animals were dosed with 50 mg/kg of the free form, 50 mg of free form per kg equivalent of the hydrochloride salt, or 100 mg of free form per kg equivalent of the hydrochloride salt, using five mice in each assay, including the control. The parasitemia (%) and MSD values were compared against the control group using the Student's *t*-test unless otherwise indicated. The results are summarized in Table 4. Compound **15** exhibited a 60% suppression rate at a dose of 50 mg/kg of free form (vs control, $p < 0.05$), whereas its corresponding hydrochloride exhibited a much better suppression rate of up to 96% (free form vs control, $p < 0.05$) and a dramatic increase in MSD from 6.0 days (control group) to 17.5 days. In addition, dosing of the hydrochloride salt at 100 mg/kg free-form equivalent resulted in potent antimalarial activity, such that no infected blood cells were found on day 4 (vs control, $p < 0.01$); however, the MSD was shortened to 11.6 days, contrary to our expectations. Although there was no significant difference between the antimalarial activity of the free form and corresponding hydrochloride salt at a dose of 50 mg/kg (free-form equivalent), compound **20** (SSJ-717) exhibited potent antimalarial activity (90.4% and 92.1% suppression rate, respectively) and an MSD of approximately 20 days (vs control, $p < 0.01$). Mice dosed with 100 mg/kg free-form equivalent of **20** (SSJ-717) and the corresponding hydrochloride salt exhibited a remarkable suppression rate as high as > 99% (no infected blood cells were observed) and an MSD of > 28 days (vs control, $p < 0.01$). It should be noted that one of the five mice was completely cured, and this is the first reported case of a malaria-infected mouse being cured by a quinazoline derivative. The effect of a salt dose equivalent to 50 mg/kg free form on MSD was not examined for **23**; however, the MSD was prolonged to 14.5 days with a salt dose equivalent of 100 mg/kg free form (vs control, $p < 0.05$).

In addition, Kaplan-Meier survival analyses were carried out using the MSD values of **15** and **20**, and the results are shown in Figure 2. Although **15** suppressed parasitemia by > 60% at a dose of 50 mg/kg, no significant difference in MSD was observed versus the control. However, the MSD value was markedly prolonged for the corresponding hydrochloride salt, as shown in Figure 2a (vs control, $p < 0.05$). No significant differences in suppression rate or MSD were observed between free compound **20** and the corresponding hydrochloride salt, but the MSD value was prolonged in a dose-dependent manner to > 28 days (vs control, $p < 0.01$, Figure 2b).

Our data suggest that these compounds **15** and **20** could be strong inhibitors of histone lysine methyltransferase,[6–7] resulting in potent antimalarial activity.[12] However, it has been reported that these compounds might also inhibit human G9a.[14] As such, the detailed



Scheme 1. Synthesis of DMQDAs.

mechanism underlying the activity of these compounds should be investigated in the future. Development of a method to realize strong *in vivo* activity for 2-(pyridin-4-yl) derivatives is another future subject for research due to the potent *in vitro* activity of these compounds.

In summary, we synthesized approximately 150 DMQDAs and evaluated their antimalarial activity. A total of 40 DMQDAs were subsequently selected and examined in the present SAR study. Hydrochloride salts of DMQDAs having a pyrrolidin-1-yl group at the 2-position (**15**, **20**, and **23**) exhibited improved transfer from the small intestine to the blood when administered orally, resulting in strong *in vivo* antimalarial activity. Compound **20** (SSJ-717) in particular was identified as a potent lead compound capable of curing malaria in mice. Further SAR studies and investigations of the mode of action of **20** are currently underway in our laboratories.

3. Experimental section

3.1. Chemistry

All reagents and solvents were obtained from FUJIFILM Wako Pure Chemical or TCI and used without further purification. 2,4-Dichloro-6,7-dimethoxyquinazoline and 2,4-dichloroquinazoline with 98% and 97% purity, respectively, were purchased from FUJIFILM Wako Pure Chemical.

3.2. General procedure a (for 2-chloro-6,7-dimethoxyquinazolin-4-amines [intermediates]):

To a solution of commercially obtained 2,4-dichloro-6,7-dimethoxyquinazoline (1.04 g, 4 mmol), 2–2.5 equivalents of the selected amine in acetonitrile (10 mL) was added, and the solution was heated and stirred at 35–50 °C for 3–5 h. After insoluble solids were filtered off, the filtrate was poured into water to afford the intermediates at 70–95% yield. The purity of the obtained intermediates was > 95% (high-performance liquid chromatography [HPLC] analysis), and they were used in the final step without further purification.

3.3. General procedure B (for 6,7-dimethoxyquinazoline-2,4-diamines):

A mixture of the intermediates (2 mmol) and selected amine (5 mmol) in dimethylacetamide (DMA, 5 mL) was heated at 120–150 °C for 3–10 h, and the reaction mixture was then poured into water to afford a crude solid. The solid was washed with water and dried at room temperature and then purified using the methods described below to obtain pure solids (purity > 96%). The yields after purification were 50–72%.

3.4. General procedure C (for hydrochloride salt preparation):

To a solution of 6,7-dimethoxyquinazoline-2,4-diamines (2.5 mmol) in MeOH (20 mL), 37% hydrochloride (HCl, 0.23 mL) was added dropwise at 5–10 °C, and the mixture was stirred for 1 h at room temperature. The reaction mixture was then concentrated under reduced pressure. Ethylacetate (EtOAc, 150 mL) was added to the residue, and the resulting mixture was stirred for 1 h at room temperature. The solids were then filtered and washed with EtOAc to afford the HCl salts at yields of 70–90%. The empirical formula of the salts, including attached H_2O molecules, was determined by elemental analysis (JM10, Yanaco Technical Science).

3.5. Purification method A:

The solid obtained according to general procedure B was heated and stirred in EtOAc for 1–3 h at 90 °C and then filtered off after cooling to room temperature to obtain the pure solid.

3.6. Purification method B:

The solid obtained according to general procedure B was recrystallized in acetonitrile to afford the pure solid.

3.7. Purification method C:

In cases in which the purity of the 6,7-dimethoxyquinazoline-2,4-diamine was < 96% after purification method A or B, the product was purified again by column chromatography using silica gel (35–60 mesh) and elution with a mixture of EtOAc and MeOH (90:10 to 60:40).

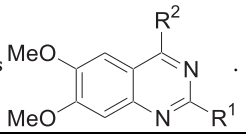
Nuclear magnetic resonance (NMR) spectra were recorded at room temperature on a JEOL JNM-ECA600 II (600 MHz for ^{1}H, 150 MHz for ^{13}C) spectrometer or JEOL JNM-ECA 500 (500 MHz for ^{1}H, 125 Hz for ^{13}C) spectrometer in the solvent indicated. All ^{1}H NMR chemical shifts are reported in ppm (δ) downfield of TMS. All ^{13}C NMR chemical shifts are reported in ppm (δ) relative to the signals for chloroform (77 ppm), DMSO (39.5 ppm), or MeOH (49.3 ppm) with ^{1}H decoupled observation. Data for ^{1}H NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, brs = broad singlet, d = doublet, dd = double doublet, t = triplet, q = quartet, m = multiplet), integration, and J coupling constant (Hz). Data for ^{13}C NMR spectra are reported in chemical shift (δ ppm). Mass spectrometry (MS) was performed on a Xevo G2 TOF (Waters) in positive ion mode using an atmospheric solids analysis probe. High-resolution MS (HRMS) spectra with electrospray ionization (ESI) or fast atom bombardment (FAB) were obtained using a JEOL JMS-T100LC or JEOL JMS-700 instrument and are reported in terms of mass-to-charge ratio (m/z). HPLC analyses were performed on a LC-10ADvp (Shimadzu) instrument with ultraviolet (UV) detector set to 254 nm. Samples were dissolved in MeOH or mixed solvent of MeOH and ethylacetate (50:50) and injected onto a TSK-gel 100 V 5.0 μm, 4.6 mm × 150 mm column maintained at 40 °C. The flow rate was 1.0 mL/min. A binary gradient of 0.1% acetic acid and 0.1% triethylamine in MeOH (A) and H_2O (B) at 0.01–3 min (hold: A/B = 50/50), 3–20 min (linear gradient: A/B = 50/50 to A:100%), 20–25 min (A: 100%). All final compounds were obtained at > 95% purity using the HPLC method described above.

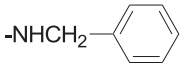
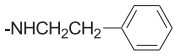
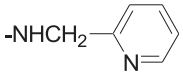
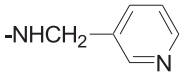
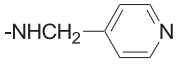
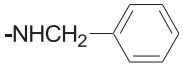
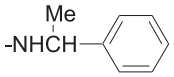
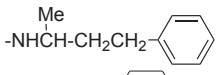
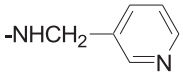
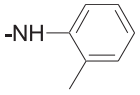
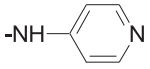
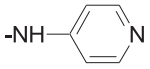
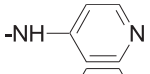
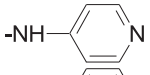
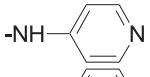
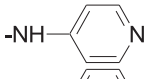
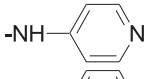
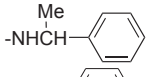
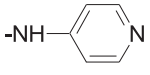
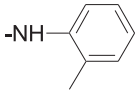
3.8. N^2,N^4-Di-n-butyl-6,7-dimethxyquinazoline-2,4-diamine (**1**):

To a solution of 2,4-dichloro-6,7-dimethoxyquinazoline (1.04 g, 4 mmol: Wako Chemicals) in acetonitrile (10 mL), *n*-butylamine (0.584 g, 8 mmol) was added at room temperature, and the mixture was stirred for 3 h at 35–40 °C. The reaction mixture was then poured into water (100 mL), and the precipitated solid was dried at room temperature to afford 0.931 g (79.0% yield) of N^4-*n*-butyl-2-chloro-6,7-dimethoxyquinazolin-4-amine.

A solution of N^4-*n*-butyl-2-chloro-6,7-dimethoxyquinazolin-4-amine (0.593 g, 2 mmol) and *n*-butylamine (0.365 g, 5 mmol) in DMA (5 mL) was heated at 140–145 °C and stirred for 12 h. The reaction mixture was then poured into water (100 mL), and the precipitated solid was collected. After drying at room temperature, the solid was purified

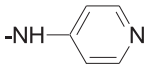
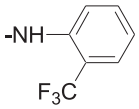
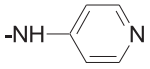
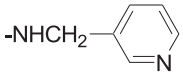
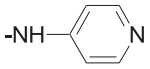
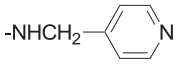
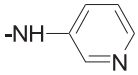
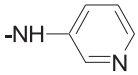
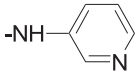
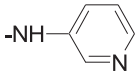
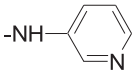
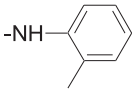
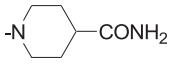
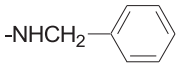
Table 2

In vitro antimalarial activity and cytotoxicity (IC_{50} values) of newly synthesized DMQDAs  .

Compound	R^1	R^2	IC_{50}[a,c] (nM)*P. falciparum*	IC_{50}[b,c] (μM) L6 cells	Selectivity index (SI)[d]
1	-NH*n*Bu	-NH*n*Bu	2.10 ± 0.5	3.67 ± 0.12	1,750
7	-NH*n*Bu	-NH*i*Pr	3.14 ± 0.8	5.31 ± 0.33	1,690
8	–NH(CH_2)$_3$OEt	-NH*n*Bu	27.6 ± 5.2	4.46 ± 0.20	161
9		-NHEt	14.8 ± 2.9	5.44 ± 0.18	367
10		-NHEt	17.0 ± 0	1.68 ± 0.22	99
11		-NH*n*Bu	10.9 ± 1.3	10.6 ± 0.77	1,000
12		-NH*n*Bu	2.17 ± 0	11.1 ± 0.63	5,120
13		-NH*n*Bu	8.16 ± 4.0	11.0 ± 5.3	1,360
14		–NH_2	1,660 ± 84	28.2 ± 1.5	17.0
15		-NH*n*Bu	1.80 ± 0	61.8 ± 3.4	2,650
16		-NH*i*Pr	47.1 ± 11	12.5 ± 0.63	3,430
17		-NH*n*C_8H_{17}	1.40 ± 0.1	1.48 ± 0.13	1,060
18			1,040 ± 15	3.96 ± 0.93	3.80
19			5.49 ± 1.4	9.49 ± 4.5	1,900
20 (SSJ-717)			10.0 ± 1.3	4.81 ± 0.53	481
21			7.30 ± 3.7	4.48 ± 0.39	613
22			13.6 ± 1.4	27.3 ± 6.8	2,000
23			32.9 ± 4.1	8.46 ± 2.3	257
24		-NHEt	3.07 ± 0	55.1 ± 5.5	17,900
25		-NH*i*Pr	2.90 ± 0.2	81.4 ± 13	28,000
26		-NH*n*Bu	5.67 ± 2.8	105 ± 5.1	5,300
27		-NHCHMeEt	2.83 ± 0	47.7 ± 5.8	16,800
28		-NHCHEt_2	51.77 ± 2.7	110 ± 6.8	2,130
29		-NH*n*C_8H_{17}	7.30 ± 3.6	1.76 ± 0.63	241
30			0.40 ± 0.10	62.7 ± 4.1	156,000
31			49.0 ± 3.9	213 ± 45	4,350

(*continued on next page*)

Table 2 (*continued*)

Compound	R^1	R^2	IC_{50}[a,c] (nM)*P. falciparum*	IC_{50}[b,c] (μM) L6 cells	Selectivity index (SI)[d]
32			22.6 ± 10	138 ± 11	6,100
33			12.8 ± 1.3	138 ± 18.4	10,700
34			30.9 ± 1.3	138 ± 33	4,470
35		-NHEt	6.14 ± 1.5	112 ± 0.77	18,200
36		-NH*i*Pr	14.7 ± 1.5	249 ± 35	16,900
37		-NH*n*Bu	25.4 ± 0	150 ± 1.8	5,900
38		$-NHCHEt_2$	19.0 ± 1.4	155 ± 27	8,190
39			20.6 ± 2.6	170 ± 16	8,250
40			180 ± 60.6	4,980 ± 273	27.7
chloroquine (control for *P. falciparum* NF54)			6.25		
podophyllotoxin (control for L6 cells)				0.0145	

[a] IC_{50} values for inhibition of *P. falciparum* NF54 growth.
[b] IC_{50} values for inhibition of rat L6 cell growth.
[c] Data are expressed as the average of two independent determinations of IC_{50} ± deviation from the mean.
[d] SI is the ratio of IC_{50} for cytotoxicity versus antiplasmodial activity (L6/*P. falciparum*).

according to method A to obtain 0.545 g of **1** (82% yield); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.88 (s, 1H), 6.75 (s, 1H), 5.26 (s, 1H), 4.73 (s, 1H), 3.94 (s, 3H), 3.91 (s, 3H), 3.59 (t, J = 7.1 Hz, 2H), 3.47 (t, J = 7.1 Hz, 2H), 1.75–1.53 (m, 4H), 1.51–1.37 (m, 4H), 0.99–0.95 (m, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.5, 159.3, 154.3, 148.9, 145.2, 105.7, 103.6, 100.8, 56.2, 55.9, 41.3, 40.9, 40.7, 32.2, 31.7, 20.3, 20.2, 13.91, 13.88; ESI-MS (*m/z*) calcd for $C_{18}H_{29}N_4O_2$ $[M + H]^+$ 333.23, found 333.29.

3.9. N^2,N^4-Di-n-butylquinazoline-2,4-diamine (2):

Starting with 2,4-dichloroquinazoline, the title compound was prepared according to general procedures A and B and purified according to method A (65% overall yield); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.65 (brs, 1H), 7.48 (q, J = 6.6, 1.2 Hz, 1H), 7.42 (brs, 1H), 7.06 (t, J = 6.6 Hz, 1H), 6.28–5.52 (brs, 2H), 3.59 (t, J = 7.2 Hz, 2H), 3.49 (t, J = 7.2 Hz, 2H), 1.67 (m, 2H), 1.59 (m, 2H), 1.42 (m, 4H), 0.96 (m, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.1, 158.9, 150.1, 132.7, 124.0, 121.2, 121.1, 110.8, 41.1, 40.9, 32.0, 31.3, 20.2, 20.1, 13.8, 13.8; ESI-MS (*m/z*) calcd for $C_{16}H_{25}N_4$ $[M + H]^+$ 273.21, found 273.27.

3.10. N^2-n-Butyl-6,7-dimethoxy-N^4-ethylquinazoline-2,4-diamine (3):

The title compound was prepared according to general procedures A and B and purified according to method A (62% overall yield); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.92 (s, 1H), 6.85 (s, 1H), 5.82 (brs, 1H), 5.20 (brs,1H), 3.93 (s, 3H), 3.90 (s, 3H), 3.63 (m, 2H), 3.47 (m, 2H), 1.60 (m, 2H), 1.43 (m, 2H), 1.32 (t, J = 7.2 Hz, 3H), 0.94 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.2, 159.1, 158.5, 154.5, 145.4, 104.6, 103.4, 101.3, 56.3, 56.0, 41.2, 41.1, 36.1, 35.9, 32.1, 20.2, 14.8, 13.9; ESI-MS (*m/z*) calcd for $C_{16}H_{25}N_4O_2$ $[M + H]^+$ 305.20, found 305.26.

3.11. 2-Chloro-N^4-ethyl-6,7-dimethoxyquinazolin-4-amine (4):

To a solution of 2,4-dichloro-6,7-dimethoxyquinazoline (1.04 g, 4 mmol) in acetonitrile (10 mL), ethylamine (0.43 g, 9.6 mmol) was added at room temperature. The solution was heated and stirred at 35–40 °C for 2 h. The reaction mixture was then poured into water (100 mL) to afford a crude solid. The solid was purified according to method A to obtain 0.88 g of **4** (82.2% yield); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.34 (t, J = 4.8 Hz, 1H), 7.61 (s, 1H), 7.06 (s, 1H), 3.91 (s, 6H), 3.51 (m, 2H), 1.24 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.8, 155.2, 154.3, 148.4, 147.1, 106.9, 106.5, 102.2, 56.1, 55.8, 35.6, 14.3; FAB-HRMS (*m/z*) calcd for $C_{12}H_{15}N_3O_2Cl$ $[M + H]^+$ 268.0853, found 268.0854.

3.12. N^4-Ethyl-2,6,7-trimethoxyquinazolin-4-amine (5):

To a solution of **4** (0.534 g, 2 mmol) in DMA (10 mL), sodium methoxide in MeOH (4 mmol) was added dropwise at 5–10 °C and stirred for 4 h at room temperature. The reaction solution was then poured into water to afford a crude solid. The solid was purified according to method A to obtain 0.421 g of **5** (80% yield); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.09 (s, 1H), 7.04 (s, 1H) 4.03 (s, 3H), 3.99 (s, 6H), 3.71 (q, J = 7.2 Hz, 2H), 1.36 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.4, 160.6, 154.4, 148.0, 146.8, 106.6, 105.2, 100.5, 56.1, 56.1, 54.0, 36.2, 14.7; ESI-MS (*m/z*) calcd for $C_{13}H_{18}N_3O_3$ $[M + H]^+$ 264.13, found 264.19.

3.13. N^4-n-Butyl-6,7-dimethoxyquinazolin-4-amine (6):

To a solution of commercially available 4-chloro-6,7-dimethoxyquinazoline (674 mg, 3 mmol: TCI) in acetonitrile (5 mL), *n*-butylamine

Table 3
SAR of 6,7-dimethoxy-2-(pyrrolidin-1-yl)quinazolin-4-amines and 6,7-dimethoxy-N^2-(pyridin-4-yl)quinazolin-2,4-diamines in *in vivo* oral assays (n = 3).

Compound	C log P[a]	Empirical formula of the salt	Dose[b] (mg/kg × 4)	Suppression[c] (%)	MSD[d] (days)
Control	–	–	–	0.0	6.2
2-(pyrrolidin-1-yl) compounds					
15	3.83	$C_{18}H_{26}N_4O_2$ $HCl{\cdot}H_2O$	58.2	96.5	17.5
17	5.50	$C_{22}H_{34}N_4O_2$ $HCl{\cdot}H_2O$	57.0	48.8	6.7
20 (SSJ-717)	4.64	$C_{22}H_{26}N_4O_2$ $HCl{\cdot}H_2O$	57.2	99.6	12.0
21	5.34	$C_{24}H_{30}N_4O_2$ 1/ 2HCl	63.7	74.4	11.7
22	2.99	$C_{20}H_{23}N_5O_2$ $HCl{\cdot}3/2H_2O$	59.0	80.3	8.0
23	5.05	$C_{21}H_{24}N_4O_2$ $HCl{\cdot}H_2O$	57.5	83.0	14.7
2-(pyridin-4-yl)amino compounds					
24	2.46	$C_{17}H_{19}N_5O_2$ $HCl{\cdot}H_2O$	58.4	1.0	7.0
25	2.77	$C_{18}H_{21}N_5O_2$ $2HCl{\cdot}5/2H_2O$	58.2	39.5	8.3
29	5.03	$C_{23}H_{31}N_5O_2$ $HCl{\cdot}2H_2O$	58.4	54.8	7.3
30	4.17	$C_{23}H_{23}N_5O_2$ $2HCl{\cdot}2H_2O$	63.7	6.6	8.3
31	4.57	$C_{22}H_{21}N_5O_2$ HCl	54.7	60.8	7.3
33	2.51	$C_{21}H_{20}N_5O_2$ $2HCl{\cdot}3H_2O$	66.3	27.1	8.7
34	2.51	$C_{21}H_{20}N_5O_2$ $2HCl{\cdot}3H_2O$	66.3	29.4	7.7

[a] Calculated using ChemDraw.
[b] Vehicle, 0.5% MC.
[c] Mean suppression of parasitemia on day 4 (%) versus that of the control (n = 5).
[d] Mean survival days (n = 3).

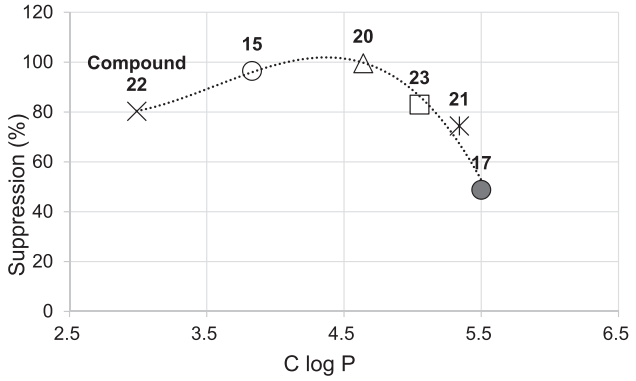


Figure 1. Correlation between C log P of 6,7-dimethoxy-2-(pyrrolidin-1-yl)-quinazolin-4-amines and antimalarial activity of the corresponding hydrochloride salts.

(0.99 mL, 10 mmol) was added at room temperature, and the mixture was heated for 5 h at 70–75 °C. Then, the reaction mixture was poured into water (50 mL) and the precipitated solid was dried at room temperature to afford 0.650 g of **6** (83% yield); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.44 (s, 1H), 7.06 (s, 1H), 6.82 (s, 1H), 5.59 (brs, 1H), 3.86–3.83 (m, 6H), 3.55–3.51 (m, 2H), 1.95 (s, 2H), 1.63–1.57 (m, 2H), 1.37–1.33 (m, 2H), 0.87–0.84 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.5, 154.3, 154.2, 148.99, 146.5, 108.7, 107.8, 99.6, 77.4, 77.2, 76.9, 56.3, 56.3, 41.4, 31.8, 20.4, 14.0; ESI-HRMS (*m/z*) calcd for $C_{14}H_{20}N_3O_2$ $[M + H]^+$ 262.1556, found 262.1559.

Table 4
In vivo antimalarial activity of quinazoline derivatives and hydrochloride salts administered via the oral route.

No.	Form	Dose (mg/kg × 4)	Parasitemia (%)	Suppression (%)[c]	MSD (days)[a]
Control		0.5% MC	28.4 ± 10.2[b]	0.0	6.2 ± 0.5[b]
15	Free	50	10.8 ± 4.9	60.0	6.6 ± 1.1
	H_2O, HCl	58.2 (50)	1.5 ± 1.3	95.5	17.5 ± 2.2
	H_2O, HCl	116.4 (100)	ND[d]	>99	11.6 ± 1.4
20	Free	50	2.5 ± 1.3	90.4	19.8 ± 1.0
	H_2O, HCl	57.2 (50)	2.0 ± 1.1	92.1	18.6 ± 2.2
	H_2O, HCl	114.4 (100)	ND[d]	>99	28.2 ± 1.7[e]
23	Free	50	23.4 ± 9.4	26.8	6.8 ± 1.2
	H_2O, HCl	57.5 (50)	14.7 ± 11.1	53.9	7.0 ± 0.9
	H_2O, HCl	115.0 (100)	11.6 ± 2.1	63.8	14.5 ± 4.5

[a] Mean survival days.
[b] Average value of four experiments (19 mice).
[c] Suppression on day 4 calculated from the reduction in parasitemia compared with each control group (n = 5 or 4).
[d] No detection of infected blood cells.
[e] Results included one censored case.

3.14. N^2-n-Butyl-6,7-dimethoxy-N^4-isopropylquinazoline-2,4-diamine (7):

The title compound was prepared according to general procedures A and B, and purified according to method A (65% overall yield); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.87 (s, 1H), 6.76 (s, 1H), 5.08 (brs, 1H), 4.73 (brs, 1H), 4.48 (m, 1H), 3.93 (s, 6H), 3.47 (m, 2H), 1.61 (m, 2H), 1.44 (m, 2H), 1.32 (s, 6H), 0.95 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.5, 158.5, 154.3, 149.0, 145.1, 105.8, 103.6, 100.9, 56.3, 55.9, 42.5, 41.2, 41.1, 32.2, 22.9, 22.8, 20.2, 13.9; ESI-MS (*m/z*) calcd for $C_{17}H_{27}N_4O_2$ $[M + H]^+$ 319.21, found 319.28.

3.15. N^4-n-Butyl-6,7-dimethoxy-N^2-(2-ethoxypropyl)quinazoline-2,4-diamine (8):

The title compound was prepared according to general procedures A and B, and purified according to method A (61% overall yield); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.86 (s, 1H), 6.76 (s, 1H), 5.36 (brs, 1H), 5.01 (brs, 1H), 3.92–3.88 (m, 6H), 3.60–3.53 (m, 6H), 3.45–3.60 (m, 2H), 3.21 (s, 1H), 1.93–1.87 (m, 2H), 1.69–1.63 (m, 2H), 1.46–1.39 (m, 2H), 1.22–1.19 (m, 3H), 0.98–0.95 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.5, 159.4, 154.4, 149.0, 145.3, 105.8, 103.8, 101.0, 77.4, 77.2, 76.9, 69.0, 66.4, 56.3, 56.1, 41.0, 39.4, 37.2, 31.8, 30.2, 20.4, 15.4, 14.0; ESI-HRMS (*m/z*) calcd for $C_{19}H_{31}N_4O_3$ $[M + H]^+$ 363.2396, found 363.2396.

3.16. N^2-Benzyl-6,7-dimethoxy-N^4-ethylquinazoline-2,4-diamine (9):

The title compound was prepared according to general procedures A and B, and purified according to method A (58% overall yield); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 (d, J = 6.6 Hz, 2H), 7.31 (m, 2H), 7.26 (m, 1H), 6.90 (s, 1H), 6.78 (s, 1H), 5.27 (s, 1H), 5.10 (s, 1H), 4.70 (d, J = 5.4 Hz, 2H), 3.94 (s, 3H), 3.91 (s, 3H), 3.59 (m, 2H), 1.27 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.2, 159.1, 154.3, 148.6, 145.4, 140.2, 128.4, 127.5, 126.9, 56.2, 56.0, 45.6, 36.0, 14.8; ESI-MS (*m/z*) calcd for $C_{19}H_{23}N_4O_2$ $[M + H]^+$ 339.18, found 339.24.

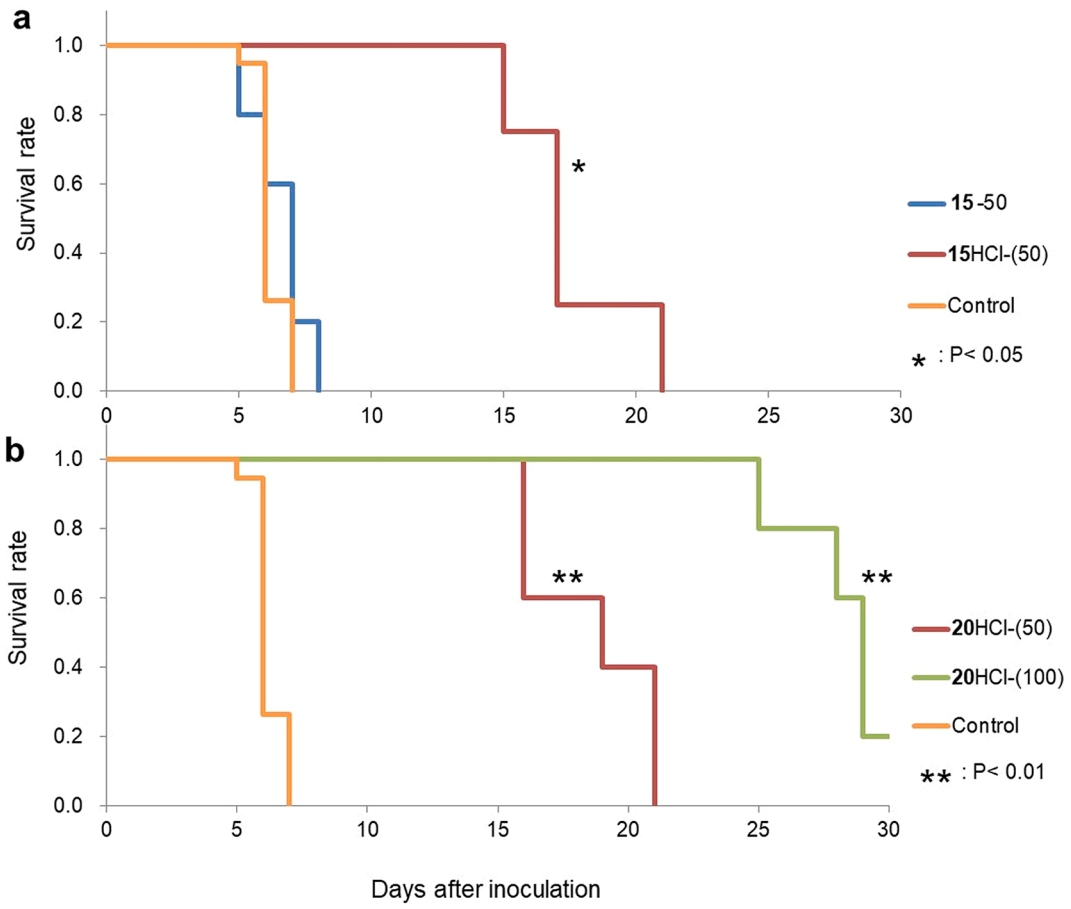


Figure 2. Kaplan-Meier survival estimates for the antimalarial activity of compounds **15** and **20**; (a) effect of the hydrochloride salt; (b) dose dependence.

*3.17. 6,7-Dimethoxy-N^4-ethyl-N^2-(2-phenylethyl)quinazoline-2,4-diamine (**10**):*

The title compound was prepared according to general procedures A and B, and purified according to method A (60% overall yield); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22–7.19 (m, 3H), 7.14–7.10 (m, 3H), 6.69 (s, 1H), 3.84–3.83 (m, 6H), 3.58–3.53 (m, 4H), 2.86–2.83 (t, J = 7.2 Hz, 2H), 2.05 (s, 3H) 1.29–1.26 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.7, 159.5, 155.3, 146.5, 139.2, 128.8, 128.6, 126.4, 103.1, 102.2, 77.4, 77.2, 76.9, 56.5, 56.4, 42.8, 36.8, 36.1, 24.7, 14.5; ESI-HRMS (m/z) calcd for $C_{20}H_{25}N_4O_2$ $[M + H]^+$ 353.1998, found 353.1990.

*3.18. N^4-n-Butyl-6,7-dimethoxy-N^2-(pyridin-2-ylmethyl)quinazoline-2,4-diamine (**11**):*

The title compound was prepared according to general procedures A and B, and purified according to method A (56% overall yield); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.54 (s, 1H), 7.61–7.57 (m, 1H), 7.37–7.35 (m, 1H), 7.14–7.12 (m, 1H), 6.88 (s, 1H), 6.79 (s, 1H), 5.72 (brs, 1H), 5.47 (brs, 1H), 4.83–4.82 (s, 2H), 3.92 (s, 3H), 3.87 (s, 3H), 3.54–3.47 (m, 2H), 1.61–1.55 (m, 2H), 1.40–1.34 (m, 2H), 0.92–0.89 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.9, 159.7, 159.5, 154.7, 149.3, 149.1, 145.7, 136.8, 122.1, 121.8, 106.1, 104.2, 101.2, 77.7, 77.4, 77.2, 56.6, 56.4, 47.4, 41.3, 32.0, 20.6, 14.3; ESI-HRMS (m/z) calcd for $C_{20}H_{26}N_5O_2$ $[M + H]^+$ 368.2087, found 368.2111.

*3.19. N^4-n-Butyl-6,7-dimethoxy-N^2-(pyridin-3-ylmethyl)quinazoline-2,4-diamine (**12**):*

The title compound was prepared according to general procedures A and B, and purified according to method C (58% overall yield); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.63 (s, 1H), 8.47–8.46 (m, 1H), 7.71–7.69 (m, 1H), 7.22–7.21 (m, 1H), 6.85 (s, 1H), 6.80 (s, 1H), 5.54 (brs, 1H), 5.24 (brs, 1H), 4.70–4.68 (brs, 2H), 3.91 (s, 3H), 3.86 (s, 3H), 3.52–3.48 (m, 2H), 1.62–1.56 (m, 2H), 1.40–1.33 (m, 2H), 0.92–0.89 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.8, 159.3, 154.8, 149.6, 149.0, 148.6, 145.9, 136.4, 135.5, 123.7, 106.1, 104.3, 101.2, 77.7, 77.4, 77.2, 56.6, 56.4, 43.5, 41.3, 32.0, 20.6, 14.3; ESI-HRMS (m/z) calcd for $C_{20}H_{26}N_5O_2$ $[M + H]^+$ 368.2087, found 368.2078.

*3.20. N^4-n-Butyl-6,7-dimethoxy-N^2-(pyridin-4-ylmethyl)quinazoline-2,4-diamine (**13**):*

The title compound was prepared according to general procedures A and B, and purified according to method C (56% overall yield); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.43–8.42 (br s, 2H), 7.57 (br s, 1H), 7.38 (br s, 1H), 7.29–7.28 (d, J = 5.6 Hz, 2H), 6.88 (br s, 1H), 6.64 (s, 1H), 4.51–4.50 (d, J = 6.3 Hz, 2H), 3.78–3.77 (d, J = 5.7 Hz, 6H), 1.50 (br s, 2H), 1.29 (br s, 2H), 0.87 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 159.2, 158.7, 153.7, 150.9, 149.2, 148.2, 144.6, 122.1, 103.2, 55.9, 55.3, 43.3, 40.0, 39.9, 39.7, 39.5, 39.4, 39.2, 39.0, 31.1, 19.9, 13.9; ESI-HRMS (m/z) calcd for $C_{20}H_{26}N_5O_2$ $[M + H]^+$ 368.2087, found 368.2093.

*3.21. 6,7-Dimethoxy-2-(pyrrolidin-1-yl)quinazolin-4-amine (**14**):*

The title compound was prepared according to general procedures A and B, and purified according to method B (62% overall yield); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.95 (s, 1H), 6.83 (s, 1H), 3.93 (s, 3H), 3.89 (s, 3H), 3.62 (m, 4H), 2.09 (s, 2H), 1.95 (m, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ

160.5, 157.8, 154.9, 150.2, 145.0, 105.4, 102.4, 101.4, 56.0, 46.6, 25.5; ESI-MS (*m/z*) calcd for $C_{14}H_{19}N_4O_2$ $[M + H]^+$ 275.15, found 275.21.

3.22. N^4-n-Butyl-6,7-dimethoxy-2-(pyrrolidin-1-yl)quinazolin-4-amine (15):

The title compound was prepared according to general procedures A and B, and purified according to method B (57% overall yield); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.93 (s, 1H), 6.75 (s, 1H), 5.23 (brs, 1H), 3.93 (s, 3H), 3.90 (s, 3H), 3.66–3.61 (m, 6H), 1.97–1.95 (m, 4H), 1.69 (m, 2H), 1.45 (m, 2H), 0.99 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.8, 157.9, 154.2 149.4, 144.7, 105.8, 102.8, 100.9, 56.2, 56.0, 46.5, 40.8, 40.7, 31.7, 25.6, 20.3, 13.9; ESI-MS (*m/z*) calcd for $C_{18}H_{27}N_4O_2$ $[M + H]^+$ 331.21, found 331.28.

The hydrochloride salt was prepared according to general procedure C. Elemental analysis, found: C, 56.12; H, 7.50; N, 14.56; Empirical formula $C_{18}H_{26}N_4O_2 \cdot HCl \cdot H_2O$: C, 56.17; H, 7.59; N, 14.56.

3.23. 6,7-Dimethoxy-N^4-isopropyl-2-(pyrrolidin-1-yl)quinazolin-4-amine (16):

The title compound was prepared according to general procedures A and B, and purified according to method B (68% overall yield); ^{1}H NMR (500 MHz, CD_3OD) δ 7.28 (s, 1H), 6.90 (s, 1H), 4.91 (s, 4H), 3.89–3.86 (m, 6H), 3.60–3.51 (m, 6H), 1.98–1.95 (m, 4H), 1.74–1.72 (m, 2H), 1.00–0.97 (t, J = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 160.7, 159.2, 155.5, 149.5, 146.3, 105.2, 104.7, 104.0, 56.8, 56.1, 49.5, 49.3, 49.2, 48.8, 48.7, 48.5, 47.6, 43.8, 26.5, 23.6, 12.0; FAB-HRMS (*m/z*) calcd for $C_{17}H_{25}N_4O_2$ $[M + H]^+$ 317.1978, found 317.1959

3.24. 6,7-Dimethoxy-N^4-octyl-2-(pyrrolidin-1-yl)quinazolin-4-amine (17):

The title compound was prepared according to general procedures A and B, and purified according to method B (70% overall yield); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.96 (s, 1H), 6.81 (s, 1H), 5.41 (brs, 1H), 3.93 (s, 6H), 3.66 (t, J = 6.6 Hz, 4H), 3.59 (m, 2H), 1.96 (m, 4H), 1.70 (m, 2H), 1.44–1.23 (m, 10*H*), 0.88 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.8, 157.7, 154.1, 149.2, 144.8, 105.6, 102.8, 100.9, 56.2, 56.0, 46.5, 41.2, 31.8, 29.4, 27.2, 25.6, 22.6, 14.1; ESI-MS (*m/z*) calcd for $C_{22}H_{35}N_4O_2$ $[M + H]^+$ 387.28, found 387.35.

The hydrochloride salt was prepared according to general procedure C. Elemental analysis, found: C, 60.21; H, 8.40; N, 12.75; Empirical formula $C_{22}H_{34}N_4O_2 \cdot HCl \cdot H_2O$: C, 59.90; H, 8.46; N, 12.70.

3.25. 2,4-Bis(pyrrolidin-1-yl)-6,7-dimethoxyquinazoline (18):

The title compound was prepared according to general procedures A and B, and purified according to method B (77% overall yield); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.44 (s, 1H), 7.06 (s, 1H), 3.82–3.77 (m, 10*H*), 3.49–3.46 (br t, 4H), 3.86–3.83 (m, 6H), 1.93–1.86 (m, 8H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 159.3, 156.7, 153.4, 151.0, 143.0, 106.1, 105.2, 104.1, 55.6, 55.3, 49.9, 46.1, 25.3, 25.2; ESI-HRMS (*m/z*) calcd for $C_{18}H_{25}N_4O_2$ $[M + H]^+$ 329.1978, found 329.1969.

3.26. N^4-Benzyl-6,7-dimethoxy-2-(pyrrolidin-1-yl)quinazolin-4-amine (19):

The title compound was prepared according to general procedures A and B, and purified according to method B (60% overall yield); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (d, J = 6.9 Hz, 2H), 7.36 (t, J = 7.4 Hz, 2H), 7.30 (s, 1H), 6.95 (s, 1H), 6.71 (s, 1H), 4.84 (d, J = 5.7 Hz, 2H), 3.96 (s, 3H), 3.89 (s, 3H), 3.65 (d, J = 6.3 Hz, 4H), 1.95 (d, J = 6.9 Hz, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.8, 158.0, 154.4, 149.8, 145.0, 139.7, 128.7, 128.2, 127.4, 105.9, 102.9, 101.0, 77.4, 77.2, 76.9, 56.3, 56.1, 46.7, 45.1, 25.7; ESI-HRMS (*m/z*) calcd for $C_{21}H_{25}N_4O_2$ $[M + H]^+$ 365.1978, found 365.1994.

3.27. 6,7-Dimethoxy-N^4-(1-phenylethyl)-2-(pyrrolidin-1-yl)quinazolin-4-amine (20):

The title compound was prepared according to general procedures A and B, and purified according to method B (68% overall yield); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43 (t, J = 8.4 Hz, 2H), 7.33 (t, J = 7.8 Hz, 2H), 7.26 (brs, 1H), 6.96 (s, 1H), 6.81 (s, 1H), 5.53 (m, 1H), 3.93 (s, 6H), 3.65–3.50 (m, 4H), 1.92 (m, 4H), 1.65 (d, J = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.8, 154.3, 149.6, 144.8, 144.6, 128.4, 127.0, 126.3, 105.7, 102.7, 100.8, 56.3, 56.0, 50.2, 46.5, 25.5, 22.1; ESI-MS (*m/z*) calcd for $C_{22}H_{27}N_4O_2$ $[M + H]^+$ 379.21, found 379.29.

The hydrochloride salt was prepared according to general procedure C. Elemental analysis, found: C, 61.08; H, 6.69; N, 12.88; Empirical formula $C_{22}H_{26}N_4O_2 \cdot HCl \cdot H_2O$: C, 61.03; H, 6.75; N, 12.94.

3.28. 6,7-Dimethoxy-N^4-(1-methyl-3-phenylpropyl)-2-(pyrrolidin-1-yl) quinazolin-4-amine (21):

The title compound was prepared according to general procedures A and B, and purified according to method B (68% overall yield); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.25 (d, J = 5.4 Hz, 3H), 7.18 (d, J = 7.8 Hz, 3H), 6.98 (s, 1H), 6.67 (s, 1H), 4.52 (t, J = 6.6 Hz, 1H), 3.96 (s, 3H), 3.92 (s, 3H), 3.61 (brs, 4H), 2.74 (m, 2H), 2.03 (m, 2H), 1.95 (m, 4H), 1.34 (d, J = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.1, 154.2, 147.8, 144.8, 142.0, 128.4, 128.4, 125.8, 105.7, 102.7, 100.9, 56.3, 56.0, 46.6, 46.3, 38.5, 32.7, 25.6, 20.8; ESI-MS (*m/z*) calcd for $C_{24}H_{31}N_4O_2$ $[M + H]^+$ 407.24, found 407.32.

The hydrochloride salt was prepared according to general procedure C. Elemental analysis, found: C, 68.17; H, 7.12; N, 13.20; Empirical formula $C_{24}H_{30}N_4O_2 \cdot 1/2HCl$: C,67.86; H,7.25; N,13.19.

3.29. 6,7-Dimethoxy-N^4-(pyridin-3-ylmethyl)-2-(pyrrolidin-1-yl) quinazolin-4-amine (22):

The title compound was prepared according to general procedures A and B, and purified according to method B (69% overall yield); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.63 (s, 1H), 8.44 (d, J = 3.6 Hz, 1H), 8.32 (m, 1H), 7.79 (s, 1H), 7.45 (s, 1H), 7.35 (d, J = 4.8 Hz, 1H), 6.75 (s, 1H), 4.70 (d, J = 5.4 Hz, 2H), 3.85 (s, 3H), 3.80 (s, 3H), 3.45 (d, J = 6.6 Hz, 4H), 1.86 (t, J = 6.6 Hz, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.5, 157.1, 153.9, 149.2, 148.9, 147.9, 144.4, 136.0, 135.3, 123.4, 105.1, 102.9, 102.8, 55.8, 55.4, 46.1, 41.4, 25.1; ESI-MS (*m/z*) calcd for $C_{24}H_{31}N_4O_2$ $[M + H]^+$ 407.24, found 407.32. ESI-MS (*m/z*) calcd for $C_{20}H_{24}N_5O_2$ $[M + H]^+$ 366.19, found 366.27.

The hydrochloride salt was prepared according to general procedure C. Elemental analysis, found: C, 56.00; H, 6.34; N, 16.33; Empirical formula $C_{20}H_{23}N_5O_2 \cdot HCl \cdot 3/2 \cdot H_2O$: C, 55.68; H, 5.96; N, 16.23.

3.30. 6,7-Dimethoxy-N^4-(2-methylphenyl)-2-(pyrrolidin-1-yl) quinazolin-4-amine (23):

The title compound was prepared according to general procedures A and B, and purified according to method B (70% overall yield); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.25 (t, J = 7.2 Hz, 3H), 7.09 (d, J = 6.6 Hz, 1H), 7.00 (br, 1H), 6.89 (s, 1H), 3.98 (s, 3H), 3.93 (s, 3H), 3.60 (s, 4H), 2.38 (s, 3H), 1.95 (t, J = 7.7 Hz, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.2, 156.8, 154.7, 150.5, 145.2, 137.5, 130.4, 129.5, 126.4, 124.1, 123.3, 105.9, 103.0, 100.7, 56.2, 56.1, 46.6, 25.5, 18.2; ESI-MS (*m/z*) calcd for $C_{21}H_{25}N_4O_2$ $[M + H]^+$ 365.20, found 365.27.

The hydrochloride salt was prepared according to general procedure C. Elemental analysis, found: C, 60.00; H, 6.42; N, 13.38; Empirical formula $C_{21}H_{24}N_4O_2 \cdot HCl \cdot H_2O$: C, 60.21; H, 6.50; N, 13.37.

3.31. 6,7-Dimethoxy-N^4-ethyl-N^2-(pyridin-4-yl)quinazoline-2,4-diamine (24):

The title compound was prepared according to general procedures A and B, and purified according to method C (64% overall yield); ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.03 (s, 2H), 7.79 (s, 1H), 7.08 (s, 1H), 6.84 (s, 2H), 3.91 (s, 6H), 3.66 (q, J = 7.2 Hz, 2H), 1.30 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 161.2, 159.5, 154.5, 150.4, 148.3, 145.6, 135.7, 110.2, 107.0, 106.7, 103.0, 56.2, 55.8, 35.7, 14.2; ESI-MS (m/z) calcd for $C_{17}H_{20}N_5O_2$ $[M + H]^+$ 326.16, found 326.22.

The hydrochloride salt was prepared according to general procedure C. Elemental analysis, found: C, 53.75; H, 5.84; N, 18.44; Empirical formula $C_{17}H_{19}N_5O_2 \cdot HCl \cdot H_2O$: C, 53.75; H, 5.90; N, 18.22.

3.32. 6,7-Dimethoxy-N^4-isopropyl-N^2-(pyridin-4-yl)quinazoline-2,4-diamine (25):

The title compound was prepared according to general procedures A and B, and purified according to method C (60% overall yield); ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.28 (t, J = 7.4 Hz, 2H), 8.93 (s, 2H), 8.50 (d, J = 7.8 Hz, 1H), 7.88 (s, 1H), 7.04 (d, J = 7.2 Hz, 2H), 4.66 (m, 1H), 3.94 (s, 6H), 1.36 (t, J = 7.2 Hz, 6H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.7, 158.9, 154.7, 150.2, 148.7, 145.6, 137.8, 109.1, 107.3, 106.9, 103,1, 56.5, 55.8, 42.8, 22.1; ESI-MS (m/z) calcd for $C_{18}H_{22}N_5O_2$ $[M + H]^+$ 340.18, found 340.24.

The hydrochloride salt was prepared according to general procedure C. Elemental analysis, found: C, 47.32; H, 5.95; N, 15.34; Empirical formula $C_{18}H_{21}N_5O_2 \cdot 2HCl \cdot 5/2H_2O$: C, 47.27; H, 6.17; N, 15.32.

3.33. N^4-n-Butyl-6,7-dimethoxy-N^2-(pyridin-4-yl)quinazoline-2,4-diamine (26):

The title compound was prepared according to general procedures A and B, and purified according to method C (58% overall yield); ^{1}H NMR (500 MHz, CD_3OD) δ 9.10 (dd, J = 18.6, 7.2 Hz, 2H), 7.33 (d, J = 13.2 Hz, 1H), 6.91 (d, J = 18.3 Hz, 1H), 6.84–6.81 (m, 2H), 3.84 (t, J = 5.2 Hz, 6H), 3.55–3.50 (m, 2H), 3.25–3.21 (m, 2H), 1.68–1.62 (m, 2H), 1.42–1.38 (m, 2H), 0.93 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 138.9, 110.0, 56.9, 56.6, 49.5, 49.3, 49.2, 49.0, 48.8, 48.7, 48.5, 42.4, 32.2, 21.4, 14.3; ESI-HRMS (m/z) calcd for $C_{19}H_{24}N_5O_2$ $[M + H]^+$ 354.1930, found 354.1930.

3.34. 6,7-Dimethoxy-N^4-(1-methylpropyl)–N^2-(pyridin-4-yl) quinazoline-2,4-diamine (27):

The title compound was prepared according to general procedures A and B, and purified according to method C (60% overall yield); ^{1}H NMR (500 MHz, CD_3OD) δ 9.29 (d, J = 7.4 Hz, 2H), 7.63 (s, 1H), 7.12 (s, 1H), 6.97 (d, J = 8.0 Hz, 2H), 4.50 (q, J = 6.7 Hz, 1H), 3.97 (s, 6H), 1.84–1.78 (m, 1H), 1.77–1.71 (m, 1H), 1.38 (t, J = 6.3 Hz, 3H), 1.03 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 162.8, 161.4, 156.7, 151.7, 151.0, 147.7, 139.2, 110.2, 109.2, 108.5, 103.4, 56.9, 50.3, 49.7–48.7, 30.4, 20.5, 11.5; ESI-HRMS (m/z) calcd for $C_{19}H_{24}N_5O_2$ $[M + H]^+$ 354.1930, found 354.1944.

3.35. 6,7-Dimethoxy-N^4-(diethylmethyl)–N^2-(pyridin-4-yl)quinazoline-2,4-diamine (28):

The title compound was prepared according to general procedures A and B, and purified according to method B (72% overall yield); ^{1}H NMR (500 MHz, CD_3OD) δ 7.65 (s, 1H), 7.12 (s, 1H), 6.94–6.93 (d, J = 8.0 Hz, 2H), 4.50 (q, J = 6.7 Hz, 1H), 3.94 (s, 6H), 3.27–3.26 (m, 2H), 1.77–1.67 (m, 4H), 0.98–0.95 (m, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 162.7, 162.1, 156.6, 151.6, 150.9, 147.7, 139.1, 110.1, 109.0, 108.4, 103.1, 56.9, 56.6, 55.8, 28.4, 11.2; ESI-HRMS (m/z) calcd for $C_{20}H_{26}N_5O_2$ $[M + H]^+$ 368.2087, found 368.2116.

3.36. 6,7-Dimethoxy-N^4-octylamino-N^2–(pyridin-4-yl)quinazoline-2,4-diamine (29):

The title compound was prepared according to general procedures A and B, and purified according to method C (58% overall yield); ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.24 (d, J = 7.2 Hz, 2H), 8.99 (brs, 1H), 8.91 (d, J = 4.8 Hz, 2H), 7.88 (s, 1H), 7.05 (d, J = 4.2 Hz, 2H), 3.92 (s, 6H), 3.62 (s, 2H), 1.70 (m, 2H), 1.40 (m, 2H), 1.35 (m, 2H), 1.24 (m, 6H), 0.83 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.7, 159.8, 154.6, 150.1, 148.6, 145.4, 137.6, 109.0, 107.4, 106.8, 103.1, 56.4, 55.8, 40.8, 31.2, 28.8, 28.7, 26.6, 22.1, 13.9; ESI-MS (m/z) calcd for $C_{23}H_{32}N_5O_2$ $[M + H]^+$ 410.26, found 410.33.

The hydrochloride salt was prepared according to general procedure C. Elemental analysis, found: C, 57.50; H, 7.24; N, 14.51; Empirical formula $C_{23}H_{31}N_5O_2 \cdot HCl \cdot 2H_2O$: C, 57.31; H, 7.53; N, 14.53.

3.37. 6,7-Dimethoxy-N^4-(1-phenylethyl)–N^2-(pyridin-4-yl)quinazoline-2,4-diamine (30):

The title compound was prepared according to general procedures A and B, and purified according to method C (56% overall yield); ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.19 (d, J = 7.8 Hz, 2H), 9.13 (d, J = 7.8 Hz, 1H), 8.95 (s, 2H), 8.06 (s, 1H), 7.59 (d, J = 7.2 Hz, 2H), 7.35 (t, J = 7.8 Hz, 2H), 7.23 (t, J = 7.2 Hz, 1H), 7.01 (d, J = 4.8 Hz, 2H), 5.68 (m, 1H), 3.98 (s, 3H), 3.93 (s, 3H), 1.69 (d, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.6, 159.0, 154.8, 149.8, 148.8, 145.8, 144.7, 137.6, 128.4, 126.8, 126.3, 109.0, 107.3, 106.9, 103.2, 56.6, 55.9, 50.3, 22.3; ESI-MS (m/z) calcd for $C_{23}H_{24}N_5O_2$ $[M + H]^+$ 402.19, found 402.27.

The hydrochloride salt was prepared according to general procedure C. Elemental analysis, found: C, 53.58; H, 5.88; N, 13.80; Empirical formula $C_{23}H_{23}N_5O_2 \cdot 2HCl \cdot 2H_2O$: C, 54.01; H, 5.91; N, 13.69.

3.38. 6,7-Dimethoxy-N^4-(2-methylphenyl)–N^2-(pyridin-4-yl) quinazoline-2,4-diamine (31):

The title compound was prepared according to general procedures A and B, and purified according to method C (54% overall yield); ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.12 (s, 1H), 8.91 (d, J = 7.2 Hz, 2H), 8.76 (s, 2H), 8.02 (s, 1H), 7.44 (dd, J = 18.0, 7.2 Hz, 2H), 7.33 (m, 2H), 6.92 (s, 2H), 3.97 (s, 6H), 2.26 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.5, 159.1, 155.2, 150.0, 149.2, 146.3, 137.3, 136.2, 134.5, 130.6, 127.3, 126.8, 126.3, 109.1, 107.4, 106.9, 103.0, 56.3, 56.0, 17.9; ESI-MS (m/z) calcd for $C_{22}H_{22}N_5O_2$ $[M + H]^+$ 388.18, found 388.25.

The hydrochloride salt was prepared according to general procedure C. Elemental analysis, found: C, 62.09; H, 5.38; N, 16.46; Empirical formula $C_{22}H_{21}N_5O_2 \cdot HCl$: C, 62.34; H, 5.23; N, 16.52.

3.39. 6,7-Dimethoxy-N^4-(o-trifluoromethylphenyl)–N^2-(pyridin-4-yl) quinazoline-2,4-diamine (32):

The title compound was prepared according to general procedures A and B, and purified according to method B (52% overall yield); ^{1}H NMR (500 MHz, CD_3OD) δ 9.00 (d, J = *8.0* Hz, 2H), 7.90–7.62 (m, 6H), 7.30 (s, 1H), 6.86 (d, J = *8.0* Hz, 2H), 4.02–4.00 (m, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 162.7, 162.0, 157.5, 151.6, 151.2, 148.7, 138.8, 137.6, 134.3, 132.6, 129.1, 129.0, 128.1, 110.0, 109.0, 108.3, 102.8, 56.9, 49.4–48.5; ESI-HRMS (m/z) calcd for $C_{21}H_{21}N_6O_2$ $[M + H]^+$ 389.1726, found 389.1750.

3.40. 6,7-Dimethoxy-N^4-(pyridin-3-ylmethyl)–N^2-(pyridin-4-yl) quinazoline-2,4-diamine (33):

The title compound was prepared according to general procedures A and B, and purified according to method C (50% overall yield); ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.35 (s, 1H), 9.32 (d, J = 7.8 Hz, 2H), 8.79 (s, 2H), 8.74 (d, J = 2.4 Hz,1H), 8.48 (s, 1H), 7.87 (s, 1H), 7.83 (s, 1H), 7.38

(dd, $J = 8.4$, 4.8 Hz, 1H), 6.99 (d, $J = 7.8$ Hz, 2H), 4.94 (d, $J = 5.4$ Hz, 2H), 3.94 (s, 3H), 3.92 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.7, 159.8, 155.0, 150.0, 149.2, 148.9, 148.4, 145.8, 137.9, 135.5, 134.7, 123.7, 109.1, 107.3, 106.9, 102.7, 56.2, 56.0, 41.8; ESI-MS (m/z) calcd for $C_{21}H_{21}N_6O_2$ $[M + H]^+$ 389.17, found 389.24.

The hydrochloride salt was prepared according to general procedure C. Elemental analysis, found: C, 48.92; H, 5.22; N, 16.23; Empirical formula $C_{21}H_{20}N_5O_2 \cdot 2HCl \cdot 3H_2O$: C, 48.94; H, 5.47; N, 16.30.

3.41. 6,7-Dimethoxy-N^2-(pyridin-4-yl)-N^4-(pyridin-4-ylmethyl) quinazoline-2,4-diamine (**34**):

The title compound was prepared according to general procedures A and B, and purified according to method C (54% overall yield); ^{1}H NMR (600 MHz, DMSO-d_6) 9.65 (t, $J = 5.4$ Hz, 1H), 9.19 (d, $J = 7.8$ Hz, 2H), 8.93 (s, 2H), 8.52 (d, $J = 5.4$ Hz, 2H), 7,98 (s, 1H), 7.49 (d, $J = 6.0$, 2H), 6.99 (d, $J = 7.8$ Hz, 2H), 4.91 (d, $J = 5.4$ Hz, 2H), 3.94 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.7, 159.9, 155.0, 149.9, 149.6, 148.9, 148.3, 147.7, 137.7, 122.6, 109.1, 107.4, 106.9, 103.0, 56.3, 55.9, 43.1; ESI-MS (m/z) calcd for $C_{21}H_{21}N_6O_2$ $[M + H]^+$ 389.17, found 389.24.

The hydrochloride salt was prepared according to general procedure C. Elemental analysis, found: C, 48.37; H, 5.29; N, 16.11; Empirical formula $C_{21}H_{20}N_5O_2 \cdot 2HCl \cdot 3H_2O$: C, 48.94; H, 5.47; N, 16.30.

3.42. 6,7-Dimethoxy-N^4-ethyl-N^2-(pyridin-3-yl)quinazoline-2,4-diamine (**35**):

The title compound was prepared according to general procedures A and B, and purified according to method C (59% overall yield); ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.36 (s, 1H), 9.20 (d, $J = 5.7$ Hz, 1H), 9.01 (t, $J = 4.9$ Hz, 1H), 7.88 (dd, $J = 7.7$, 5.4 Hz, 2H), 7.83 (d, $J = 8.0$ Hz, 1H), 6.92 (s, 2H), 3.95 (s, 6H), 3.73 (q, $J = 6.5$ Hz, 2H), 1.33 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 159.9, 155.1, 150.9, 149.8, 148.7, 145.3, 130.4, 127.7, 126.9, 124.1, 108.6, 107.4, 103.2, 56.7, 56.2, 40.2–39.2, 36.1, 14.5; ESI-HRMS (m/z) calcd for $C_{17}H_{20}N_5O_2$ $[M + H]^+$ 326.1617, found 326.1645.

3.43. 6,7-Dimethoxy-N^4-isopropyl-N^2-(pyridin-3-yl)quinazoline-2,4-diamine (**36**):

The title compound was prepared according to general procedures A and B, and purified according to method C (59% overall yield); ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.34 (d, $J = 2.4$ Hz, 1H), 9.21 (d, $J = 7.0$ Hz, 1H), 8.55 (d, $J = 7.8$ Hz, 1H), 7.88 (m, 2H), 7.78 (t, $J = 7.2$ Hz, 1H), 6.89 (s, 2H), 4.70 (m, 1H), 3.97 (s, 3H), 3.93 (s, 3H), 1.37 (d, $J = 7.7$ Hz, 6H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.1, 154.9, 150.8, 149.6, 148.5, 145.3, 130.3, 127.5, 126.8, 123.9, 108.2, 107.3, 102.9, 56.5, 56.0, 43.0, 22.1; ESI-MS (m/z) calcd for $C_{18}H_{22}N_5O_2$ $[M + H]^+$ 340.18, found 340.25.

3.44. N^4-n-Butyl-6,7-dimethoxy-N^2-(pyridin-3-yl)quinazoline-2,4-diamine (**37**):

The title compound was prepared according to general procedures A and B, and purified according to method C (57% overall yield); ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.35 (s, 1H), 9.18 (d, $J = 7.0$ Hz, 1H), 9.09 (t, $J = 5.4$ Hz, 2H), 7.97 (s, 1H), 7.88 (m, 2H), 6.97 (s, 2H), 3.98 (s, 3H), 3.95 (s, 3H), 3.68 (q, $J = 6.6$ Hz, 2H), 1.72 (m, 2H), 1,45 (m, 2H), 0.96 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.9, 154.9, 150.7, 149.4, 148.5, 145.1, 130.2, 127.5, 126.6, 123.9, 108.4, 107.2, 103.2, 56.5, 56.0, 40.7, 30.7, 19.8, 13.9; ESI-MS (m/z) calcd for $C_{19}H_{24}N_5O_2$ $[M + H]^+$ 354.19, found 354.27.

3.45. 6,7-Dimethoxy-N^4-diethylmethyl-N^2-(pyridin-3-yl)quinazoline-2,4-diamine (**38**):

The title compound was prepared according to general procedures A and B, and purified according to method C (61% overall yield); ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.34 (s, 1H), 9.19 (d, $J = 7.7$ Hz, 1H), 8.56 (d, $J = 8.4$ Hz, 1H), 8.04 (s, 1H), 7.89–7.85 (m, 2H), 6.96 (s, 2H), 4.45 (m, 1H), 3.98 (s, 3H), 3.96 (s, 3H), 1.74–1.72 (m, 4H), 0.96 (q, $J = 7.2$ Hz, 6H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.3, 154.9, 150.7, 149.6, 148.6, 145.3, 130.3, 127.5, 126.7, 123.8, 108.2, 107.3, 103.2, 56.7, 56.0, 54.1, 26.8, 10.7; ESI-MS (m/z) calcd for $C_{20}H_{26}N_5O_2$ $[M + H]^+$ 368.21, found 368.28.

3.46. 6,7-Dimethoxy-N^4-(2-methylphenyl)–N^2-(pyridin-3-yl) quinazoline-2,4-diamine (**39**):

The title compound was prepared according to general procedures A and B, and purified according to method C (53% overall yield); ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.31 (s, 1H), 9.10 (t, $J = 2.4$ Hz, 1H), 8.68 (d, $J = 6.0$ Hz, 1H), 8.11 (s, 1H), 7.86–7.75 (m, 2H), 7.46–7.31 (m, 4H), 6.87 (s, 2H), 4.00 (s, 6H), 2.27 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.3, 155.4, 150.7, 150.0, 148.4, 146.0, 136.0, 134.5, 130.7, 130.3, 127.4, 127.3, 126.9, 126.4, 123.7, 108.4, 107.2, 103.3, 56.6, 56.1, 18.0; ESI-MS (m/z) calcd for $C_{22}H_{22}N_5O_2$ $[M + H]^+$ 388.18, found 388.25.

3.47. N^4-Benzyl-6,7-dimethoxy-N^2-(4-carbamoylpiperidyl)quinazolin-4-amine (**40**):

The title compound was prepared according to general procedures A and B, and purified according to method C (63% overall yield). HPLC purity was 99.8%; ^{1}H NMR (500 MHz, CD_3OD) δ 7.38–7.36 (t, $J = 5.7$ Hz, 3H), 7.29–7.26 (t, $J = 7.5$ Hz, 2H), 7.21–7.19 (d, $J = 7.4$ Hz, 1H), 6.89 (s, 1H), 4.00 (s, 11*H*), 6.80 (s, 1H), 4.75–4.70 (m, 4H), 3.90 (s, 3H), 3.87 (s, 3H), 2.91–2.87 (m, 2H), 3.86 (s, 3H), 2.48 (br, 1H), 1.80–1.78 (d, $J = 10.4$ Hz, 2H), 1.61–1.57 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ180.7, 160.7, 158.8, 156.02, 147.3, 141.1, 129.4, 128.4, 127.9, 104.7, 104.6, 103.8, 56.7, 56.3, 49.5, 49.3, 49.2, 48.8, 48.65, 48.5, 45.7, 45.2, 44.1, 29.7; ESI-HRMS (m/z) calcd for $C_{23}H_{28}N_5O_3$ $[M + H]^+$ 422.2192, found 422.2186.

4. Biology

4.1. Activity against P. Falciparum

In vitro activity against the erythrocytic stages of *P. falciparum* was determined using a ^{3}H-hypoxanthine incorporation assay[22] with the drug-sensitive NF54 strain.[23] Compounds were dissolved in DMSO at 10 mg/mL and further diluted in medium before addition to parasite cultures incubated in Roswell Park Memorial Institute (RPMI) 1640 medium without hypoxanthine, supplemented with 4-(2-hydroxyethy)-1-piperazineethanesulfonic acid (HEPES, 5.94 g/L), $NaHCO_3$ (2.1 g/L), neomycin (100 U/mL), AlbumaxR (5 g/L), and washed human red cells A + at 2.5% hematocrit (0.3% parasitemia). Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 μg/mL were prepared. The 96-well plates were incubated in a humidified atmosphere (4% CO_2, 3% O_2, 93% N_2) at 37 °C. After 48 h, 50 μL of ^{3}H-hypoxanthine (=0.5 μCi) was added to each well of the plate. The plates were incubated for a further 24 h under the same conditions. The cells were then harvested from the plate using a Betaplate™ cell harvester (Wallac, Zurich, Switzerland), and the red blood cells were transferred onto a glass fiber filter and lysed with distilled water. The dried filters were inserted into plastic foil with 10 mL of scintillation fluid and counted in a Betaplate™ liquid scintillation counter (Wallac, Zurich, Switzerland). IC_{50} values were calculated from sigmoidal inhibition curves by linear regression and 4-parameter logistic regression using GraphPad Prism S. Chloroquine (Sigma C6628) was used as a control.

Assays were performed in at least two independent replicates.

4.2. In vitro cytotoxicity with L6 cells

Assays were performed in 96-well microtiter plates, each well containing 100 μL of RPMI 1640 medium supplemented with 1% L-glutamine (200 mmol) and 10% fetal bovine serum with 4000 L6 cells (a primary cell line derived from rat skeletal myoblasts).[24,25] Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 μg/mL were prepared 24 h post L6 cell seeding. The plates were incubated for 70 h and inspected under an inverted microscope to confirm sterile conditions and growth of the controls. Next, 10 μL of resazurin solution (12.5 mg resazurin dissolved in 100 mL water) was added to each well, and the plates were incubated for another 2 h. The plates were then read using a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) at an excitation wavelength of 536 nm and emission wavelength of 588 nm. The IC_{50} values were calculated by linear regression and 4-parameter logistic regression from the sigmoidal dose-inhibition curves using SoftmaxPro software (Molecular Devices Corp., Sunnyvale, CA, USA).

4.3. In vivo antimalarial assay

In vivo evaluation of the antimalarial efficacy of the compounds was carried out using a rodent malaria model as described by Fidock and coworkers,[26] with some modifications. Briefly, female ICR mice (25–27 g, n = 3–5; Sankyo Lab Service, Tokyo, Japan) were infected with *P. berghei*, strain NK 65 (2×10^7 parasitized red blood cells in saline), via tail vein injection on day 0. For oral administration, a solution of aqueous vehicle, 0.5% (w/v) methyl cellulose 400 (0.5% MC, FUJIFILM Wako Pure Chemical), was used. Compounds **15**, **17**, **20–25**, **29–31**, **33**, and **34** and the corresponding hydrochloride salts were dissolved in 0.5% MC. Four hours after inoculation, the treatment groups were administered the formulated compounds at 50 or 100 mg/kg body weight. The control group was administered approximately 100 μL of vehicle. On days 1 to 3, the experimental groups were treated with the same dose. On day 4, peripheral blood samples were obtained from the tail veins of the experimental mice. Red blood cells were observed in thin smears stained using a Diff-Quik stain kit (Sysmex, Kobe, Japan) with a Leica DM6000B microscope (Leica Microsystems, Wetzlar, Germany). Parasitemia was determined by counting over 10 fields of view. The percent reduction (% suppression = activity) in parasitemia was calculated from the difference between the parasitemia mean value of the control group (taken as 100%) and that of the experimental group. Additionally, the survival day of the treated mice was recorded, and the MSD values were calculated in comparison to the MSD value of the control group. Mice exhibiting no signs of parasitemia on day 30 post-inoculation were considered cured. Kaplan-Meier curves were generated to graphically display the efficacy using BellCurve for Excel (Social Survey Research Information Co., Ltd, Tokyo, Japan). All experiments were performed in accordance with the Guiding Principles for Care and Use of Laboratory Animals of Hoshi University.

Notes

The authors report that no financial support was obtained for this study and declare no competing financial interests.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data (NMR spectra are available free of charge. Almost all compounds in Table 2 are available on demand.) to this article can be found online at https://doi.org/10.1016/j.bmc.2021.116018.

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