European Journal of Medicinal Chemistry 127 (2017) 434–441

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

N-(2-(arylmethylimino)ethyl)-7-chloroquinolin-4-amine derivatives, synthesized by thermal and ultrasonic means, are endowed with anti-Zika virus activity

Giselle Barbosa-Lima [a, b, c, 1], Ligia S. da Silveira Pinto [d, 1], Carlos R. Kaiser [d], James L. Wardell [e, f], Caroline S. De Freitas [a, b], Yasmine R. Vieira [a, b, c], Andressa Marttorelli [a, b], José Cerbino Neto [c], Patrícia T. Bozza [a], Solange M.S.V. Wardell [g], Marcus V.N. de Souza [d, e, *, 2], Thiago M.L. Souza [a, b, c, 2]

[a] *Instituto Oswaldo Cruz, Fiocruz - Fundação Oswaldo Cruz, Rio de Janeiro, Brazil*
[b] *Centro de Desenvolvimento Tecnológico em Saúde, Fiocruz - Fundação Oswaldo Cruz, Rio de Janeiro, Brazil*
[c] *Instituto Nacional de Infectologia Evandro Chagas, Fiocruz - Fundação Oswaldo Cruz, Rio de Janeiro, Brazil*
[d] *Departamento de Química Orgânica, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil*
[e] *Instituto de Tecnologia em Fármacos - Far-Manguinhos, Fiocruz - Fundação Oswaldo Cruz, Rio de Janeiro, Brazil*
[f] *Department of Chemistry, University of Aberdeen, Old Aberdeen, Scotland, UK*
[g] *CHEMSOL, 1 Harcourt Road, Aberdeen AB15 5 NY, Scotland, UK*

ARTICLE INFO

Article history:
Received 24 October 2016
Received in revised form
4 January 2017
Accepted 5 January 2017
Available online 6 January 2017

Keywords:
Zika virus
Quinoline derivatives
Sonochemical method

ABSTRACT

Zika virus (ZIKV), an emerging *Flavivirus*, was recently associated with severe neurological complications and congenital diseases. Therefore, development of antiviral agents capable of inhibiting ZIKV replication is urgent. Chloroquine is a molecule with a confirmed safety history for use with pregnant women, and has been found to exhibit anti-ZIKV activity at concentrations around 10 μM. This suggests that modifications to the chloroquine structure could be promising for obtaining more effective anti-ZIKV agents. Here, we report the ability of a series of *N*-(2-(arylmethylimino)ethyl)-7-chloroquinolin-4-amine derivatives to inhibit ZIKV replication *in vitro*. We have found that the quinoline derivative, *N*-(2-((5-nitrofuran-2-yl)methylimino)ethyl)-7-chloroquinolin-4-amine, **40,** was the most potent compound within this series, reducing ZIKV replication by 72% at 10 μM. Compound **40** exhibits an EC_{50} value of 0.8 ± 0.07 μM, compared to that of chloroquine of 12 ± 3.2 μM. Good activities were also obtained for other compounds, including those with aryl groups = phenyl, 4-fluorophenyl, 4-nitrophenyl, 2,6-dimethoxyphenyl, 3-pyridinyl and 5-nitrothien-2-yl. Syntheses of these quinoline derivatives have been obtained both by thermal and ultrasonic means. The ultrasonic method produced comparable yields to the thermal (reflux) method in very much shorter times 30–180 s compared to 30–180 min reactions times. These results indicate that this group of compounds is a good follow-up point for the potential discovery of new drugs against the Zika disease.

1. Introduction

Quinolinyl derivatives are found in many synthetic and natural products possessing a wide range of biological and pharmacological activities. In particular, quinolinyl derivatives have found importance over many years in antimalarial drug research [1–3]. Many of these quinolinyl compounds have subsequently been used as starting points for research in other medical areas, including as antituberculosis [4,5], anticancer [6–11] and, more recently, as anti-Zika virus (ZIKV) agents [12].

ZIKV is a *Flavivirus*, which belongs to the *Flaviviridae* family. This family includes other agents of clinical significance, such as dengue (DENV), West Nile (WNV) and Japanese encephalitis (JEV) viruses.

* Corresponding author. Instituto de Tecnologia em Fármacos-Far-Manguinhos, FioCruz-Fundação Oswaldo Cruz, P.O. Box: 21041-250, Rio de Janeiro, Brazil. Tel.: +55 2139772404; fax: +55 2125602518.
E-mail address: marcos_souza@far.fiocruz.br (M.V.N. de Souza).
[1] These authors contributed equally as first author.
[2] These authors contributed equally as last author.

http://dx.doi.org/10.1016/j.ejmech.2017.01.007
0223-5234/

Besides being an arthropod-born virus, transmitted by mosquitoes of the genus *Aedes* [13], ZIKV is also transmitted by sexual contact [14–16]. Prior to 2007 only a few cases of ZIKV infection in humans has been confirmed [13,17]. Nevertheless, by the year of 2016, 52 countries and territories of the Americas, Africa, Asia and Pacific had reported autochthonous ZIKV transmission [18]. A dramatic increase in neurological disorders, during fetal development and adulthood, associated with ZIKV infection led the World Health Organization (WHO) to declare Zika as a Public Health Emergency of International Concern [19]. Effective antiviral drugs able to inhibit ZIKV replication are necessary. In this regard, various molecules are under investigation, showing promising results [12,20–23], such the anthelmintic drug niclosamide [22,23]; nucleoside/nucleotides analogs [20–22], as the anti-hepatitis C (HCV) drug sofosbuvir, and the antimalarial substance, chloroquine [12,22]. Among these, chloroquine is the only molecule with confirmed safety history for use with pregnant women and fetus. However, chloroquine's anti-ZIKV potency is around 10 μM, at least 10-times higher than observed for compounds with antiviral activity [24,25]. While this information indicates that the chloroquine chemical structure is promising against ZIKV, medicinal chemistry-driven approaches could lead to derivatives with improved potencies in anti-ZIKV therapies.

In this study, we have evaluated the anti-ZIKV activity of *N*-(2-(arylmethylimino)ethyl)-7-chloroquinolin-4-amine derivatives – which contain the 7-chloroquinoline unit found in both amodiaquine and chloroquine. We have measured the ability of this family of compounds to inhibit ZIKV replication, by reducing viral RNA levels. Indeed, we have found that the *N*-(2-(arylmethylimino) ethyl)-7-chloroquinolin-4-amine derivatives could highlight potential lead molecules for the development of novel anti-ZIKV drugs.

2. Results and discussion

2.1. Synthesis and characterization

Reaction of 4,7-dichloroquinoline with ethylenediamine on heating formed the *N*-(7-chloroquinolin-4-yl)ethane-1,2-diamine **1**. Subsequently, reactions of substituted benzaldehydes, **2**–**21**, with **1** generated the *N*-(2-(arylmethylimino)ethyl)-7-chloroquinolin-4-amine derivatives, **22**–**41**, using both thermal and ultrasonic methods in ethanol solutions (Scheme 1). Comparisons of the yields and reaction times in the two processes are listed in Table 1. The optimum results using ultrasonics were obtained with a frequency of 20 kHz, 50% of the maximum power output, without pulsing and with 30–180 s reaction times. Comparable yields using the reflux method were only obtained after 30–180 min reactions times because of the effects of cavitation.

Characterization of compounds **22**–**41** was achieved generally from IR and NMR spectral and HRMS (TOFF) data, and specifically for compound, **24**, as its trihydrate, by X-ray crystallography. Two sets of signals for the N=CH hydrogens in the ^{1}H NMR spectra indicated that (*E*): (Z) isomers had been formed: ratios of the isomers are given in Table 1. Some general spectral findings are (i) the chemical shifts of the N=CH, C$\underline{H}_2$N=CH and NHC$\underline{H}_2$ protons in the ^{1}H NMR spectra are found in the ranges 8.24–8.62, 3.83–3.97 and 3.56–3.76 ppm, respectively and (ii) the N–H and N=C stretching vibrations in the IR spectra occur in the ranges 3019–3426 and 1526–1582 cm^{-1}, respectively.

2.2. Anti-zika virus activity

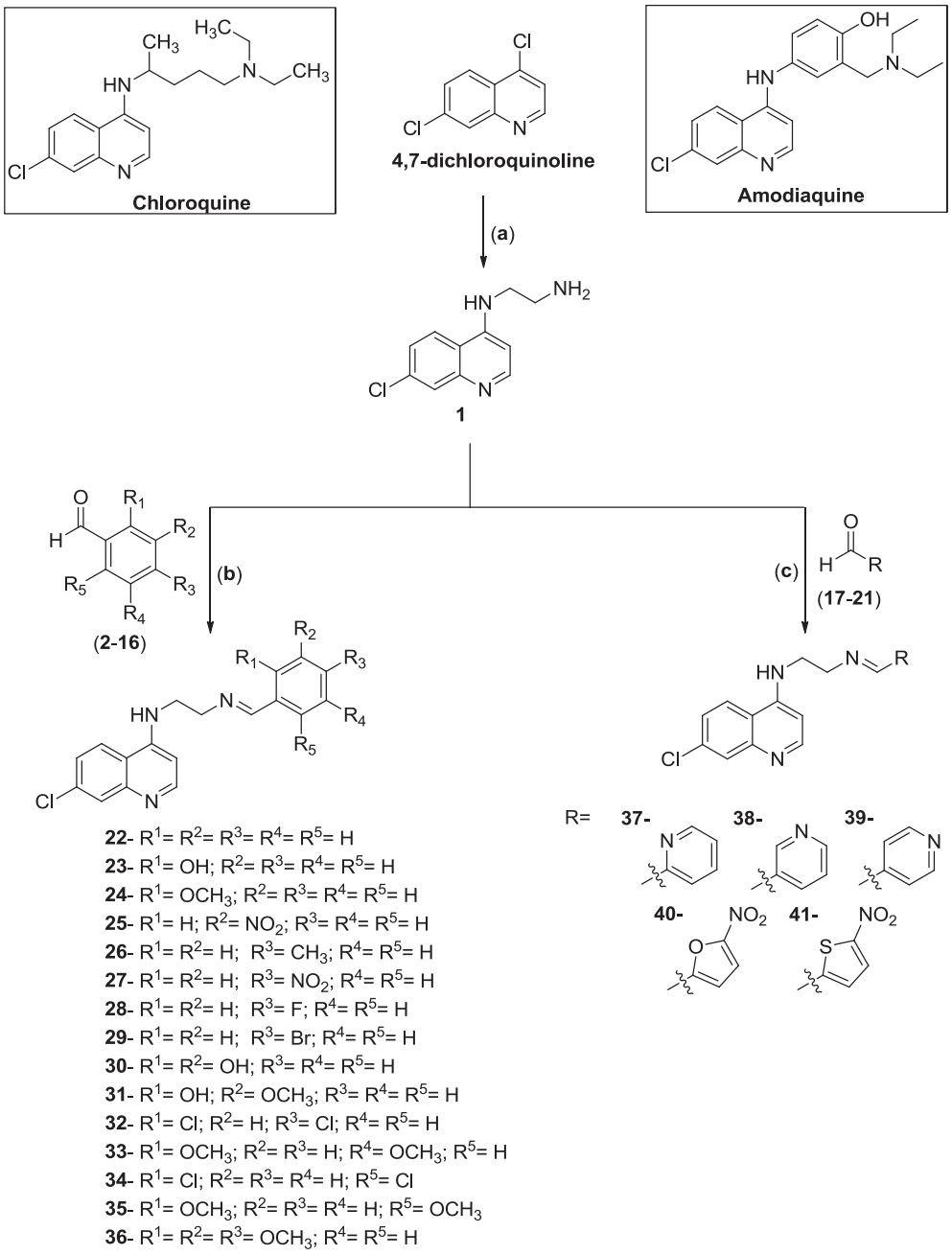
Considering that chloroquine has shown anti-ZIKV activity, with an EC_{50} of approximately 10 μM [12,22], we screened the chloroquine analogs, **22**–**41**, for inhibition of ZIKV replication at this concentration. Positively, almost the entire series showed improved antiviral activities when compared to chloroquine (Table 1). Subsequently, we further examined the pharmacological activity of the best hits against ZIKV, that is, those molecules inhibiting approximately or greater than 75% of virus replication. Derivatives, **22**, **27**, **28**, **31**, **35**, **38**, **39**, **40** and **41,** were selected (Table 1). All the tested compounds had potencies around 10-times greater than did chloroquine to inhibit ZIKV replication, with the exception of **38**, which was around 5-times more potent (Table 2). Among these molecules, compound **40** was the most potent, with an EC_{50} value of 0.8 ± 0.07 μM, whereas this pharmacological parameter for chloroquine was 12 ± 3.2 μM (Table 2). The medicinal chemistry approach improved the antiviral activity of the chloroquine analogs without affecting their cytotoxicity, because all tested molecules had similar CC_{50} values to that of chloroquine (Table 2). Of note, compound **38** was the least cytotoxic molecule (Table 2). The selective index (SI) – which represents the ratio between the CC_{50} and EC_{50} values, and thus the *in vitro* safety – for **40** was 515 (Table 2). This means that SI value for compound **40** is 15-times higher than this parameter for chloroquine (Table 2). When compared to other compounds with antiviral activity against ZIKV and potential clinical use, such as sofosbuvir, **40** presented a comparable SI value [20]. Altogether, these results indicate that *N*-(2-(arylmethylimino)ethyl)-7-chloroquinolin-4-amine derivatives, especially, compound **40**, are endowed with an higher anti-ZIKV activity than chloroquine.

The structure-activity relationship (SAR) analysis indicated that the imino group is critical for the activity since the ethylenediamine group does not present any anti-ZIKV activity. The number, the positions, and the types of substituents attached to the aromatic ring are also critical factors for biological activity (Scheme 2). For example, compound, **35**, with methoxy groups at C-2 and 6 was more active compound **34,** with chloro groups at C-2 and 6. Compound **35** was also more active than compounds **24**, **31**, **33** and **36,** indicating that the number and positions of methoxy groups are critical for anti-ZIKV activity. The importance of the substituents and position into the aromatic ring were demonstrated by the compounds **27**, **28** and **29** since fluorine **28** and nitro **27** at *para*-position was more active than bromine **29** at the same position. It is necessary to mention that compound **22** without substituent also displayed good anti-ZIKV activity. In the case of heterocyclic rings the position of the nitrogen of pyridine is critical for biological activity. The most active compound **40** of this series indicated that the furan nucleus is more active than thiophene nucleus **41**.

2.3. Crystal structure determination of the trihydrate of compound 24

Attempts to grow suitable crystals of compound **24** by slow evaporation from a moist ethanol solution at room temperature led to the isolation of the trihydrate, [(24).3(H_2O)]. Data for [(24).3(H_2O)] were collected at 120(2) K [26–28]. The trihydrate crystallises in the *triclinic* space group, P-1, with Z = 2. The atom arrangement and numbering scheme of the trihydrate are illustrated in Fig. 1a. Features of the molecular configuration of **24** are (*a*) a quasi T″ shape, see Fig. 1b, with the dihedral angle between the quinoline and phenyl rings of 62.18(5)°, (*b*) the methoxy substituent in the phenyl ring is sited away from the N2-C9-C10-N3 link between the two aryl units and (*c*) the N2-C9-C10-N3 fragment has a syn arrangement as shown by the dihedral angle of 72.72(14)°, which is ideal for coordinating to metals.

The intermolecular interactions in [(24).3(H_2O)] are set of π—π stacking interactions and classical O-H—O, N-H—O and O-H—N hydrogen bonds, which link the molecules of water and **24** into a



Scheme 1. Synthesis of *N*-(2-(arylmethylimino)ethyl)-7-chloroquinolin-4-amine derivatives.
Conditions. (a) Ethylenediamine, 80 °C → 1 h, without stirring, 135 °C → 3 h, with stirring, 94% (b) EtOH, ultrasound, 30–180 s, 52–88%, or reflux, 30–180 min; 58–81% yields (c) R = heteroaryl, EtOH, U.S. 30–120 s, 40–77%, or reflux, 30–60 min, 47–70% yields.

three dimensional array.

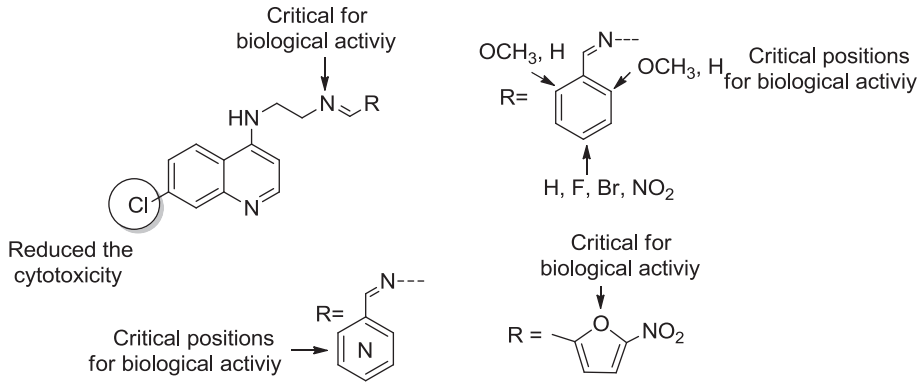
3. Experimental

3.1. Chemistry

Chemical reagents and solvents were used as obtained from Merck and Aldrich. A Multiwave Eco-sonics QR750 ultrasonic generator (20 kHz, 750 W) equipped with a converter/transducer, and titanium oscillator (horn diameter = 4 mm) was used for the ultrasonic irradiation. NMR spectra were recorded at room temperature in deuterated dimethyl sulfoxide on a Bruker Avance 500 spectrometer operating at 400.00 and 500.00 MHz (^{1}H) and 100 and 125.0 MHz (^{13}C). Melting points were determined on a Büchi apparatus. Infrared spectra were recorded in a Thermo Nicolet Nexus 670 spectrometer, as potassium bromide pellets and frequencies are expressed in cm^{-1}. HRMS were performed on Bruker Compact QTOF mass spectrometer system. TLC plates, coated with silica gel, were run in an ethyl acetate: methanol: triethylamine mixture (8:1:1). The spots were developed and viewed under ultraviolet light.

3.2. General ultrasonic irradiation protocol for the synthesis of the imino-quinoline derivatives (22–41)

In a round bottom flask 25.0 mL equipped with magnetic stirrer



Scheme 2. Critical factors for the biological activity: number, the positions and the types of substituents.

Table 1
Comparison of classical and ultrasonic methods for the preparation of the *N*-(2-(arylmethylimino)ethyl)-7-chloroquinolin-4-amine derivatives, the geometric isomeric product ratios as determined by ^{1}H NMR spectra and the inhibition of ZIKV replication.

Entry	ZIKV RNA inhibition at 10 μM (%)	Ultrasound Yied %/time (s)	Thermal Yied %/time (min)	Relative proportion between the isomers
1	43 ± 8.0	–	–	–
22	76 ± 1.8	52/120	58/60	–
23	57 ± 5.1	74/30	69/30	82:18
24	70 ± 2.7	69/180	76/60	–
25	59 ± 5.6	80/60	80/60	93:7
26	66 ± 8.1	76/60	74/30	85:15
27	82 ± 3.8	80/60	81/60	86:14
28	75 ± 3.2	64/120	59/180	79:21
29	52 ± 5.2	83/60	80/30	84:16
30	NE	88/30	65/30	82:18
31	74 ± 5.1	74/60	78/30	72:28
32	NE	74/45	71/30	–
33	51 ± 1.3	59/30	68/30	–
34	NE	77/30	73/30	69:31
35	75 ± 3.2	70/30	73/30	79:21
36	41 ± 3.1	60/30	74/30	85:15
37	70 ± 2.3	64/120	53/60	76:24
38	80 ± 6.7	40/120	47/30	86:14
39	74 ± 4.2	50/120	52/30	74:26
40	72 ± 5.3	49/30	60/30	78:22
41	78 ± 3.5	77/30	70/60	81:19
Chloroquine	47 ± 5.3	–	–	–

NE – Non evaluated. These substances did not have their antiviral activity evaluated because they were cytotoxic, reducing the cell viability in more than 80% at 10 μM.

Table 2
Potency, cytotoxicity and lipophilicity of the most active quinoline derivatives against ZIKV replication.

Compound	EC_{50}	CC_{50}	SI[a]	*c*Log*P*[b]
22	1.2 ± 0.05	489 ± 16	407	4.14
27	1.5 ± 0.03	512 ± 27	341	4.10
28	1.5 ± 0.08	534 ± 42	356	4.31
31	1.6 ± 0.02	487 ± 41	304	3.38
35	1.5 ± 0.08	458 ± 36	305	4.16
38	3.4 ± 0.08	578 ± 26	170	2.91
39	1.5 ± 0.09	571 ± 52	380	2.91
40	0.8 ± 0.07	412 ± 26	515	3.48
41	1.5 ± 0.03	478 ± 34	318	4.13
Chloroquine	12 ± 3.2	412 ± 24	34	5.00

[a] SI, selective index is determined by the ratio between CC_{50} and EC_{50} values.
[b] Calculated using www.molinspiration.com.

and reflux condenser was added 4,7-dichloroquinoline (1 g; 5 mmol) and ethylenediamine (1.17 g; 25.3 mmol). The reaction mixture was heated at 80 °C without stirring for 1 h. After this period, heating was increased to 135 °C and was stirred for 3 h. The flask containing the reaction mixture was allowed to stand at room temperature for 12 h. After this time, finely divided ice was added to the precipitation of the product. The solid **1** formed was filtered and washed with distilled water (20 mL) and ethyl ether (20 mL).

Solutions of *N*-(7-chloroquinolin-4-yl)ethane-1,2-diamine (100 mg) in ethanol (3 mL), and the benzaldehyde (0.45 mmol) in ethanol (2 mL) were mixed and ultrasonically irradiated (frequency = 50 KHz, amplitude = 50% of the maximum power output) without a pulse for 30–180 s. After the ultrasonic irradiation, the resulting mixture was concentrated under reduced pressure, the residue was washed with cold water and ether, to leave derivatives **22**–**41**.

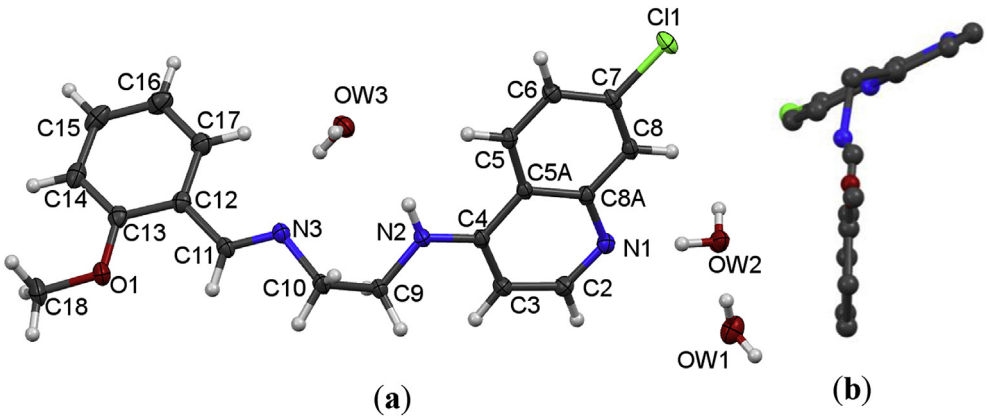


Fig. 1. (***a***) Atom arrangements and numbering scheme for [(24).3H_2O], (***b***) view of the conformation of molecule **24**.

3.2.1. N-(2-(Benzylideneamino)ethyl)-7-chloroquinolin-4-amine (22)

The product was obtained as a white solid; m.p.: 170–174 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.41 (d, 1H, *J* = 5.4 Hz, H2); 8.35 (s, 1H, N=C<u>H</u>); 8.25 (d, 1H, *J* = 9.0 Hz, H5); 7.78 (d, 1H, *J* = 2.0 Hz, H8); 7.73–7.71 (m, 1H, H6); 7.45–7.42 (m, 5H, H2'/H3'/H4'/H5'/H6′); 6.60 (d, 1H, *J* = 5.4 Hz, H3); 3.88 (t, 2H, *J* = 6.0 Hz, C<u>H</u>$_2$N=CH); 3.62 (q, 2H, *J* = 6.0, NHC<u>H</u>$_2$); ^{13}C NMR (100 MHz, DMSO) δ162.2; 151.9; 150.0; 149.1; 136.0; 133.4; 130.7; 128.6; 127.9; 127.8; 127.5; 124.1; 124.0; 117.4; 99.0; 58.8; 43.1. IR (KBr): *ν* = 3211 (N–H), 1574 (C=N) cm^{-1}. HRMS *m*/*z* [M+H]$^+$ = 310.1054, calc. 310.1033.

3.2.2. N-(2-((2-Hydroxyphenyl)methylimino)ethyl)-7-chloroquinolin-4-amine (23)

yellow solid, m.p.: 161–164 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.54 (s, 1H, N=C<u>H</u>); 8.41 (d, 1H, *J* = 5.4 Hz, H2); 8.24 (d, 1H, *J* = 9.1 Hz, H5); 7.79 (d, 1H, *J* = 2.2 Hz, H8); 7.48–7.43 (m, 2H, H6 and H6′); 7.39 (td, 1H, *J* = 9.1 Hz and *J* = 2.2 Hz, H5′); 7.33–7.29 (m, 1H, H4′); 6.88–6.85 (m, 1H, H3′); 6.62 (d, 1H, *J* = 5.4 Hz, H3); 3.89 (t, 2H, *J* = 6.0 Hz, C<u>H</u>$_2$N=CH); 3.64 (m, 2H, NHC<u>H</u>$_2$); ^{13}C NMR (100 MHz, DMSO) δ167.0; 160.6; 151.9; 149.9; 149.1; 133.4; 132.3; 131.6; 127.5; 124.2; 124.0; 118.6; 117.5; 116.4; 99.0; 58.7; 56.6; 43.0. IR (KBr): *ν* = 3232 (N–H), 1578 (C=N) cm^{-1}. HRMS *m*/*z* [M+H]$^+$ = 326.1074, calc. 326.0982.

3.2.3. N-(2-((2-methoxyphenyl)methylimino)ethyl)-7-chloroquinolin-4-amine (24)

The product was obtained as a white solid; m.p.: 108–110 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.62 (s, 1H, N=C<u>H</u>); 8.40 (d, 1H, *J* = 5.3 Hz, H2); 8.24 (d, 1H, *J* = 9.0 Hz, H5); 7.84–7.78 (m, 2H, H8 and H3′); 7.44–7.40 (m, 2H, H4′ and H6′); 7.06 (d, 1H, *J* = 9.0 Hz, H6); 6.97 (t, 1H, *J* = 7.4 Hz, H5′); 6.59 (d, 1H, *J* = 5.3 Hz, H3); 3.88–3.85 (m, 2H, C<u>H</u>$_2$N=CH); 3.75 (s, 3H, OC<u>H</u>$_3$); 3.61–3.59 (m, 2H, NHC<u>H</u>$_2$); ^{13}C NMR (100 MHz, DMSO) δ 158.3; 157.3; 151.8; 150.1; 149.1; 133.4; 132.2; 127.5; 126.6; 124.0; 123.8; 120.4; 117.4; 111.7; 99.1; 59.2; 55.5; 43.1. IR (KBr): *ν* = 3338 (N–H), 1581 (C=N) cm^{-1}. HRMS *m*/*z* [M+H]$^+$ = 340.1112, calc. 340.1138.

3.2.4. N-(2-((3-nitrophenyl)methylimino)ethyl)-7-chloroquinolin-4-amine (25)

The product was obtained as a yellow solid; m.p.: 170–172 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.52 (s, 1H, N=C<u>H</u>); 8.50 (s, 1H, H2′); 8.42 (d, 1H, *J* = 5.6 Hz, H2); 8.29 (m, 2H, H5 and H4′); 8.12 (d, 1H, *J* = 7.6 Hz, H6′); 7.78 (d, 1H, *J* = 2.1 Hz, H8); 7.73 (t, 1H, *J* = 7.6 Hz, H5′); 7.47 (d, 1H, *J* = 9.0 Hz, H6); 6.67 (d, 1H, *J* = 5.6 Hz, H3); 3.93 (t, 2H, *J* = 6.0 Hz, C<u>H</u>$_2$N=CH); 3.69 (m, 2H, NHC<u>H</u>$_2$); ^{13}C NMR (100 MHz, DMSO) δ 160.5; 160.4; 150.9; 150.5; 148.1; 137.5; 134.2; 134,0; 130.3; 125.0; 124.5; 124.2; 121.9; 117.1; 99.1; 60.5; 58.7; 43.0. IR (KBr): *ν* = 3213 (N–H), 1526 (C=N) cm^{-1}. HRMS *m*/*z* [M+H]$^+$ = 355.0956, calc. 355.0884.

3.2.5. N-(2-((4-Methylphenyl)methylimino)ethyl)-7-chloroquinolin-4-amine (26)

The product was obtained as a white solid; m.p.: 180–182 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.40 (d, 1H, *J* = 5.4 Hz, H2); 8.30 (s, 1H, N=C<u>H</u>); 8.28–8.23 (m, 1H, H5); 7.78 (d, 1H, *J* = 2.2 Hz, H8); 7.62–7.58 (m, 2H, H2′ and H6′); 7.43 (dd, 1H, *J* = 8.9 Hz and *J* = 2.2 Hz, H6); 7.24 (d, 2H, *J* = 7.9 Hz, H3′ and H5′); 6.60 (d, 1H, *J* = 5.4 Hz, H5); 3.87–3.84 (m, 2H, C<u>H</u>$_2$N=CH); 3.62–3.58 (m, 2H, NHC<u>H</u>$_2$); 2.33–2.32 (m, 6H, OC<u>H</u>$_3$) ^{13}C NMR (100 MHz, DMSO) δ 161.9; 151.8; 149.9; 149.0; 140.4; 133.3; 129.1; 127.8; 127.7; 123.9; 117.3; 98.9; 60.8; 58.7; 43.1; 20.9. IR (KBr): *ν* = 3219 (N–H), 1574 (C=N) cm^{-1}. HRMS *m*/*z* [M+H]$^+$ = 324.1276, calc. 324.1189.

3.2.6. N-(2-((4-nitrophenyl)methylimino)ethyl)-7-chloroquinolin-4-amine (27)

The product was obtained as a yellow solid; m.p.: 120–123 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.50 (s, 1H, N=C<u>H</u>); 8.41 (d, 1H, *J* = 5.5 Hz, H2); 8.27 (m, 3H, H5, H3′ and H5′); 7.98 (d, 1H, *J* = 8.6 Hz, H2′ and H6′); 7.78 (d, 1H, *J* = 1.9 Hz, H8); 7.44 (dd, 1H, *J* = 8.6 Hz and *J* = 1,9 Hz, H6); 6.62 (d, 1H, *J* = 5.5 Hz, H3); 3.95 (t, 2H, *J* = 5.8 Hz, C<u>H</u>$_2$N=CH); 3.67 (m, 2H, NHC<u>H</u>$_2$); ^{13}C NMR (100 MHz, DMSO) δ 160.7; 151.5; 150.1; 148.6; 148.4; 141.4; 133.4; 128.9; 128.7; 127.2; 124.1; 123.9; 123.8; 117.3; 99.0; 60.6; 58.9; 42.8. IR (KBr): *ν* = 3212 (N–H), 1579 (C=N) cm^{-1}. HRMS *m*/*z* [M+H]$^+$ = 355.0980, calc. 355.0884.

3.2.7. N-(2-((4-fluorophenyl)methylimino)ethyl)-7-chloroquinolin-4-amine (28)

The product was obtained as a white solid; m.p.: 179–181 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.40 (d, 1H, *J* = 5.4 Hz, H2); 8.34 (s, 1H, N=C<u>H</u>); 8.25–8.21 (m, 1H, H5); 7.79–7.76 (m, 3H, H8, H3′ and H5′); 7.44–7.41 (m, 1H, H6); 7.26 (t, 2H, *J* = 8.8 Hz, H2′ and H6′); 6.59 (d, 1H, *J* = 5.4 Hz, H3); 3.86 (t, 2H, *J* = 6.2 Hz; C<u>H</u>$_2$N=CH); 3.60 (q, 2H, *J* = 6.2 Hz; NHC<u>H</u>$_2$); ^{13}C NMR (100 MHz, DMSO) δ 160.9; 151.8; 150.1; 149.0; 133.4; 130.2; 130.1; 127.5; 124.1; 124.0; 117.4; 115.7; 115.5; 99.0; 58.7; 43.1. IR (KBr): *ν* = 3215 (N–H), 1578 (C=N) cm^{-1}. HRMS *m*/*z* [M+H]$^+$ = 328.1026, calc. 328.0939.

3.2.8. N-(2-((4-bromophenyl)methylimino)ethyl)-7-chloroquinolin-4-amine (29)

The product was obtained as a white solid; m.p.: 211–213 °C; ^{1}H

NMR (400 MHz, DMSO) δ 8.40 (d, 1H, J = 4.3 Hz, H2); 8.33 (s, 1H, N=CH); 8.24 (d, 1H, J = 8.8 Hz, H5); 7.79 (dd, 1H, J = 8.8 Hz and J = 1.8 Hz, H6); 7.68–7.63 (m, 3H, H8, H2′ and H6′); 7.47–7.43 (m, 2H, H3′ and H5′); 6.60 (d, 1H, J = 4.5 Hz, H3); 3.87 (t, 2H, J = 4.6 Hz; CH_2N=CH); 3.63–3.60 (m, 2H, NHCH_2); ^{13}C NMR (100 MHz, DMSO) δ 161.1; 151.8; 151.0; 149.9; 149.0; 135.0; 133.3; 131.6; 129.7; 127.4; 124.0; 123.9; 117.3; 98.9; 58.7; 42.9; 40.6. IR (KBr): ν = 3204 (N–H), 1578 (C=N) cm^{-1}.

3.2.9. N-(2-((2,3-dihydroxyphenyl)methylimino)ethyl)-7-chloroquinolin-4-amine (30)

yellow solid. m.p.: 139–143 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.49 (s, 1H, N=CH); 8.41 (d, 1H, J = 5.4 Hz, H2); 8.26 (d, 1H, J = 9.1 Hz, H5); 7.79 (d, 1H, J = 2.2 Hz, H8); 7.51 (s, 1H, OH); 7.45 (dd, 1H, J = 9.1 Hz and J = 2.2 Hz, H6′); 6.86–6.80 (m, 2H, H4′ and H6); 6.67–6.56 (m, 2H, H5′ and H3); 3.90 (t, 2H, J = 5.8 Hz, CH_2N=CH); 3.64 (d, 2H, J = 5.8 Hz, NHCH_2); ^{13}C NMR (100 MHz, DMSO) δ 167.0; 151.8; 151.6; 149.9; 148.9; 145.9; 133.3; 127.4; 124.1; 123.9; 121.7; 117.9; 117.4; 117.4; 117.3; 98.9; 55.4; 42.9. IR (KBr): ν = 3379 (N–H), 1575 (C=N) cm^{-1}. HRMS m/z [M+H]$^+$ = 342.1029, calc. 342.0931.

3.2.10. N-(2-((2-Hydroxy-3-methoxyphenyl)methylimino)ethyl)-7-chloroquinolin-4-amine (31)

The product was obtained as a yellow solid; m.p.: 122–123 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.57 (s, 1H, N=CH); 8.52 (s, 1H, H8); 8.42 (d, 1H, J = 5.6 Hz, H2); 8.28 (d, 1H, J = 8.9 Hz, H5); 7.81–7.80 (m, 1H, H8′); 7.51–7.47 (m, 1H, H4′); 7.03–6.95 (m, 1H, H9′); 6.78 (d, 1H, J = 8.9 Hz, H6); 6.65 (d, 1H, J = 5.6 Hz, H3); 3.92 (s, 2H, CH_2N=CH); 3.76 (d, 5H, J = 1.8 Hz; OCH_3 and NHCH_2); ^{13}C NMR (100 MHz, DMSO) δ 167.0; 151.5; 151.0; 150.4; 147.9; 133.8; 126.6; 124.3; 124.0; 123.0; 118.2; 117.6; 117.2; 114.6; 98.9; 58.2; 56.0; 55.6; 42.9. IR (KBr): ν = 3426 (N–H), 1575 (C=N) cm^{-1}. HRMS m/z [M+H]$^+$ = 356.1184, calc. 356.1088.

3.2.11. N-(2-((2,4-dichlorophenyl)methylimino)ethyl)-7-chloroquinolin-4-amine (32)

white solid; m.p.: 134–137 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.58 (s, 1H, N=CH); 8.42–8.40 (m, 1H, H2); 8.25 (t, 1H, J = 7.1 Hz, H5); 7.96–7.92 (m, 1H, H6′); 7.79 (dd, 1H, J = 7.9 Hz and J = 2.2 Hz, H8); 7.69–7.67 (m, 1H, H3′); 7.56–7.45 (m, 2H, H6 and H5′); 6.61 (t, 1H, J = 6.4 Hz, H3); 3.97–3.93 (m, 2H, CH_2N=CH); 3.66–3.63 (m, 2H, NHCH_2); ^{13}C NMR (100 MHz, DMSO) δ157.5; 135.8; 134.6; 133.8; 131.5; 131.4; 129.3; 129.2; 129.2; 127.8; 124.2; 124.0; 117.3; 99.0; 98.6; 60.3; 58.8; 42.7; 40.7. IR (KBr): ν = 3208 (N–H), 1581 (C=N) cm^{-1}. HRMS m/z [M+H]$^+$ = 378.0353, calc. 378.0253.

3.2.12. N-(2-((2,5-dimethoxyphenyl)methylimino)ethyl)-7-chloroquinolin-4-amine (33)

The product was obtained as a yellow solid; m.p.: 103–106 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.57 (s, 1H, N=CH); 8.40 (d, 1H, J = 5.3 Hz, H2); 8.24 (d, 1H, J = 8.9 Hz, H5); 7.77 (s, 1H, H8); 7.46 (m, 1H, H6); 7.34 (s, 1H, H6′); 6.99 (s, 2H, H3′ and H4′); 6.61 (d, 1H, J = 5.3 Hz, H3); 3.87–3.85 (m, 2H, CH_2N=CH); 3.75–3.69 (m, 6H, OCH_3); 3.62 (m, 2H, NHCH_2); ^{13}C NMR (100 MHz, DMSO) δ 157.1; 153.0; 152.7; 151.4; 150.2; 148.6; 133.4; 127.1; 124.3; 124.0; 124.0; 118.1; 117.3; 113.2; 110.2; 99.0; 59.1; 55.9; 55.3. IR (KBr): ν = 3377 (N–H), 1582 (C=N) cm^{-1}. HRMS m/z [M+H]$^+$ = 370.1229, calc. 370.1244.

3.2.13. N-(2-((2,6-dichlorophenyl)methylimino)ethyl)-7-chloroquinolin-4-amine (34)

The product was obtained as a white solid; m.p.: 179–185 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.49 (s, 1H, N=CH); 8.40 (d, 1H, J = 5.4 Hz, H2); 8.24 (d, 1H, J = 9.0 Hz, H5); 7.78 (d, 1H, J = 2.1 Hz, H8); 7.52–7.37 (m, 4H, H6/H5′/H3′/H4′); 6.58 (d, 1H, J = 5.4 Hz, H3); 3.97 (t, 2H, J = 5.7 Hz, CH_2N=CH); 3.65 (q, 2H, J = 5.7, NHCH_2); ^{13}C NMR (100 MHz, DMSO) δ158.2; 157.6; 151.7; 149.8; 148.9; 133.5; 133.3; 131.1; 128.9; 128.7; 127.3; 124.0; 123.9; 117.4; 98.8; 61.2; 58.9; 42.4. IR (KBr): ν = 3211 (N–H), 1575 (C=N) cm^{-1}. HRMS m/z [M+H]$^+$ = 378.0333, calc. 378.0253.

3.2.14. N-(2-((2,6-dimethoxyphenyl)methylimino)ethyl)-7-chloroquinolin-4-amine (35)

The product was obtained as a yellow solid; m.p.: 71–73 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.40 (s, 1H, N=CH); 8.22 (d, 1H, J = 9.0 Hz, H5); 7.78 (d, 1H, J = 2.2 Hz, H8); 7.44 (dd, 1H, J = 9.0 Hz and J = 2.2 Hz, H6); 7.34–7.28 (m, 2H, H2 and H4′); 6.64 (d, 2H, J = 8.4 Hz, H3′ and H5′); 3.66–3.64 (m, 6H, OCH_3); 3.83 (t, 2H, J = 5.7 Hz, CH_2N=CH); 3.56 (q, 2H, J = 5.7, NHCH_2); ^{13}C NMR (100 MHz, DMSO) δ158.9; 157.2; 151.8; 150.0; 149.0; 133.4; 131.4; 127.4; 124.0; 117.5; 113.2; 104.3; 99.0; 60.1; 55.7; 42.9. IR (KBr): ν = 3339 (N–H), 1574 (C=N) cm^{-1}.

3.2.15. N-(2-((3,4,5-trimethoxyphenyl)methylimino)ethyl)-7-chloroquinolin-4-amine (36)

The product was obtained as a white solid; m.p.: 92–93 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.43 (d, 1H, J = 5.6 Hz, H2); 8.30 (d, 1H, J = 9.0 Hz, H5); 8.24 (s, 1H, N=CH); 7.80 (d, 1H, J = 2.2 Hz, H8); 7.48 (dd, 1H, J = 2.2 Hz and J = 9.0 Hz, H6); 7.03–7.02 (m, 2H, H2′ and H6′); 6.67 (d, 1H, J = 5.6 Hz, H3); 3.87–3.84 (m, 2H, J = 6.1 Hz, CH_2N=CH); 3.79–3.76 (m, 9H, OCH_3); 3.68–3.65 (m, 2H, NHCH_2); ^{13}C NMR (100 MHz, DMSO) δ 191.9; 161.9; 153.3; 153.0; 139.6; 131.5; 124.6; 124.3; 117.1; 106.7; 105.0; 99.2; 60.1; 58.8; 55.8; 43.2. IR (KBr): ν = 3019 (N–H), 1582 (C=N) cm^{-1}. HRMS m/z [M+H]$^+$ = 400.1423, calc. 400.1350.

3.2.16. N-(2-((pyridin-2-yl)methylimino)ethyl)-7-chloroquinolin-4-amine (37)

The product was obtained as a white solid; m.p.: 130–133 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.61 (d, 1H, J = 4.8 Hz, H3′); 8.40 (d, 1H, J = 5.4 Hz, H2); 8.35 (s, 1H, N=CH); 8.24 (d, 1H, J = 9.0 Hz, H5); 7.96 (d, 1H, J = 7.5 Hz, H6′); 7.87 (td, 1H, J = 7.5 Hz and J = 1.3 Hz, H5′); 7.77 (d, 1H, J = 2.2 Hz, H8); 7.46–7.42 (m, 2H, H6 and H4′); 7.61 (d, 1H, J = 5.4 Hz, H3); 3.95 (t, 2H, J = 6.1 Hz, CH_2N=CH); 3.67–3.62 (m, 2H, NHCH_2); ^{13}C NMR (100 MHz, DMSO) δ 163.1; 153.9; 151.8; 149.9; 149.2; 149.0; 136.7; 133.3; 127.4; 125.1; 124.0; 123.9; 120.4; 117.3; 99.0; 58.5; 42.8. IR (KBr): ν = 3237 (N–H), 1580 (C=N) cm^{-1}. HRMS m/z [M+H]$^+$ = 311.1081, calc. 311.0985.

3.2.17. N-(2-((pyridin-3-yl)methylimino)ethyl)-7-chloroquinolin-4-amine (38)

The product was obtained as a white solid; m.p.: 160–162 °C; ^{1}H NMR (500 MHz, DMSO) δ 8.86 (d, 1H, J = 1.5 Hz, H5′); 8.63 (dd, 1H, J = 4.8 Hz and J = 1.5 Hz, H4′); 8.41–8.40 (m, 2H, N=CH and H2); 8.24 (d, 1H, J = 9.0 Hz, H5); 8.10 (d, 1H, J = 9.0 Hz, H6); 7.78 (d, 1H, J = 2.2 Hz, H8); 7.48–7.42 (m, 3H, H2′, H3′ and H3); 3.90 (t, 2H, J = 6.0 Hz, CH_2N=CH); 3.64 (q, 2H, J = 6.0 Hz; NHCH_2); ^{13}C NMR (125 MHz, DMSO) δ 160.1; 151.9; 151.4; 150.0; 149.5; 149.1; 134.5; 133.4; 131.4; 127.5; 124.1; 123.9; 123.9; 117.4; 99.1; 59.0; 42.9. IR (KBr): ν = 3216 (N–H), 1579 (C=N) cm^{-1}. HRMS m/z [M+H]$^+$ = 311.1068, calc. calc. 311.0985.

3.2.18. N-(2-((pyridin-4-yl)methylimino)ethyl)-7-chloroquinolin-4-amine (39)

The product was obtained as a white solid; m.p.: 186–188 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.66 (d, 2H, J = 5.9 Hz, H3′ and H5′); 8.41 (d, 1H, J = 5.4 Hz, H2); 8.38 (s, 1H, N=CH); 8.23 (d, 1H, J = 9.0 Hz, H5); 7.78 (d, 1H, J = 2.2 Hz, H8); 7.65 (d, 2H, J = 5.9 Hz, H2′ and H6′); 7.45–7.42 (m, 1H, H6); 6.61 (d, 1H, J = 5.4 Hz, H3); 3.93 (t, 2H, J = 6.2 Hz, CH_2N=CH); 3.64 (q, 2H, J = 6.2 Hz; NHCH_2); ^{13}C NMR

(100 MHz, DMSO) δ 160.5; 151.3; 149.7; 149.4; 148.5; 141.9; 132.8; 126.9; 123.5; 123.4; 121.2; 116.8; 98.5; 58.3; 42.2. IR (KBr): $\nu = 3212$ (N–H), 1577 (C=N) cm^{-1}. HRMS m/z $[M+H]^+ = 311.0972$, calc. 311.0985.

3.2.19. N-(2-((5-nitrofuran-2-yl)methylimino)ethyl)-7-chloroquinolin-4-amine (40)

The product was obtained as a black solid; m.p.: 236–237 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.43 (d, 1H, $J = 5.6$ Hz, H2); 8.31–8.26 (m, 2H, H5 and N=CH); 7.81 (d, 1H, $J = 2.2$ Hz, H8); 7.75 (d, 1H, $J = 3.9$ Hz, H4′); 7.49 (dd, 1H, $J = 8.9$ Hz and $J = 2.2$ Hz, H6); 7.23 (d, 1H, $J = 3.9$ Hz, H5′); 6.66 (d, 1H, $J = 5.6$ Hz, H3); 3.94 (t, 2H, $J = 5.8$, CH_2N=CH); 3.70–3.65 (m, 2H, NHCH_2); ^{13}C NMR (100 MHz, DMSO) δ 152.1; 151.1; 150.6; 150.5; 147.5; 133.9; 126.3; 124.4; 124.1; 117.2; 117.1; 116.3; 113.8; 99.0; 58.8; 42.8. IR (KBr): $\nu = 3238$ (N–H), 1574 (C=N) cm^{-1}. HRMS m/z $[M+H]^+ = 345.0751$, calc. 345.0676.

3.2.20. N-(2-((5-nitrothiophen-2-yl)methylimino)ethyl)-7-chloroquinolin-4-amine (41)

The product was obtained as a brown solid; m.p.: 116–118 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.50 (s, 1H, N=CH); 8.40 (d, 1H, $J = 5.4$ Hz, H2); 8.23 (d, 1H, $J = 9.0$ Hz, H5); 8.10 (d, 1H, $J = 4.2$ Hz, H4′); 7.78 (d, 1H, $J = 2.1$ Hz, H8); 7.51 (d, 1H, $J = 4.2$ Hz, H5′); 7.46–7.43 (m, 1H, H6); 6.60 (d, 1H, $J = 5.4$ Hz, H3); 3.91–3.88 (m, 2H, CH_2N=CH); 3.65–3.61 (m, 2H, NHCH_2); ^{13}C NMR (100 MHz, DMSO) δ 155.9; 151.8; 151.7; 149.9; 148.9; 148.3; 133.3; 130.2; 130.1; 127.3; 124.1; 123.9; 117.3; 99.0; 58.4; 42.5. IR (KBr): $\nu = 3218$ (N–H), 1581 (C=N) cm^{-1}. HRMS m/z ($[M+H]^+ = 361.0545$, calc. 361.0448.

3.3. Biological assays

3.3.1. Cells

African green monkey kidney cells (Vero) were cultured in DMEM (Life Technologies, Grand Island, NY). *Aedes albopictus* cells (C6/36) were grown in L-15 medium supplemented with 0.3% tryptose phosphate broth, 0.75 g/L sodium bicarbonate, 1.4 mM glutamine, and nonessential amino acids. The culture medium of the cell types was supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, Utah), 100 U/mL penicillin and 100 μg/mL streptomycin. Mammals cells were kept at 37 °C in 5% CO_2, whereas mosquito cells were maintained at 26 °C.

3.3.2. Virus

ZIKV strain used is representative of the virus circulating in Brazil [20]. ZIKV was grown in C6/36 cells, tittered by 50% endpoint of ZIKV cytopathic effect in tissue cultures ($TCID_{50}$) and further passaged at the multiplicity of infection (MOI) of 0.01 [29]. After 9 days post-infection, cells were lysed by freezing and thawing and centrifuged at 1500×*g* at 4 °C for 20 min to remove cellular debris. Infectious virus titers were determined by $TCID_{50}$/mL in Vero cells and stored at −70 °C for further studies.

3.3.3. Cytotoxity assay

Monolayers of 2×10^4 Vero cells in 96-well culture plates were incubated with the compounds at different concentrations for 72 h. Then, 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2*H*-Tetrazolium-5-Carboxanilide (XTT) at 5 mg/ml was added to DMEM in the presence of 0.01% N-methyl-dibenzopirazina methyl sulfate (PMS). After incubation for 4 h at 37 °C, the plates were read in a spectrophotometer at 492 nm and 620 nm [30]. The 50% cytotoxic concentration (CC_{50}) was calculated by linear regression analysis of the dose–response curves generated from the data.

3.3.4. Antiviral assay

Monolayers of Vero cells (2×10^4 cell/well) in 96-well plates were infected with ZIKV at MOI of 0.1 for 1 h at 37 °C. Cells were washed to remove residual viruses and various concentrations of the compounds were added. After 24 h, viruses in the supernatant were harvested, RNA was extracted and ZIKV RNA was quantified by RT-PCR (see molecular biology analysis below). For comparison, the reference compound chloroquine was used as a positive control. Linear regression of the dose-response curve was performed to determine the 50% inhibitory effect on viral replication (EC_{50}) for the tested and reference compounds.

3.3.5. Molecular biology analysis

Total RNA from the culture supernatant was extracted using QIAamp Viral RNA Mini Kit (Qiagen®), according to manufacturer's instructions. Quantitative RT-PCR was performed using QuantiTect Probe RT-PCR Kit (Quiagen®) in an ABI PRISM 7300 Sequence Detection System (Applied Biosystems). Amplifications were carried out in 25 μL reaction mixtures containing 2 × reaction mix buffer, 50 μM of each primer, 10 μM of probe and 5 μL of RNA template. Primers, probes and cycling conditions recommended by the Centers for Disease Control and Prevention (CDC) were used to detect the ZIKV [17]. The standard curve method was employed for virus quantification.

3.3.6. Statistical analyses

The dose-response curves used to calculate the EC_{50} and CC_{50} values and the % of ZIKV replication inhibition were generated by Excel for Windows. All of the experiments were performed at least three times, and the results are displayed as mean ± standard error of the mean (SEM).

4. Conclusion

In this study we report the synthesis of a series of twenty *N*-(2-(arylmethylimino)ethyl)-7-chloroquinolin-4-amine derivatives obtained by thermal and ultrasonic means. The ultrasonic procedures are simple, safe and with short reaction times. Furthermore, we report the anti-ZIKV activity of these derivatives, which represent an improvement of chloroquine chemical structure towards the development of future anti-ZIKV therapies. Compound **40** was the most potent in inhibiting ZIKV replication, without affecting the cytotoxicity when compared to chloroquine. This study also reveals the importance of the number, the positions, and the types of substituents, as: phenyl, 4-fluorophenyl, 4-nitrophenyl and 2,6-dimethoxyphenyl, attached to the aromatic ring as being critical factors for the biological activity. The 3-pyridinyl, indicating that the positions of N atom attached to pyridinyl ring haven't significant change at the activity and 5-nitro -thiophen and -furan substituents indicated that the furan nucleus are more active than thiophene nucleus. These factors can be critical for the biological activity and a good starting point to the discovery of new prototypes against Zika.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. The authors (Giselle B. Lima and Ligia S. da S. Pinto; Thiago M. L. Souza and Marcus V. N. de Souza) contributed equally.

Funding

This work was supported by grants from PAPES/Fiocruz (www.ioc.fiocruz.br), CNPq (www.cnpq.br), CAPES (www.capes.gov.br) and Faperj (www.faperj.br). The funders had no role in study design, data collection and analysis, decision to publish, or

preparation of the manuscript.

Acknowledgment

We thank the Dr Ana Bispo de Fillips for critical discussion. Thanks are due to the the Oswaldo Cruz Institute and Evandro Chagas Clinical Research Institute.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2017.01.007.

References

[1] W. Cunico, C.A. Cechinel, H.G. Bonacorso, M.A.P. Martins, N. Zanatta, M.V.N. de Souza, I.O. Freitas, R.P.P. Soares, A.U. Krettli, Antimalarial activity of 4-(5-trifluoromethyl-1h-pyrazol-1-yl)-chloroquine analogues, Bioorg. Med. Chem. Lett. 16 (2006) 649–653.
[2] A.A. Andrade, F. de P. Varotti, I.O. de Freitas, M.V.N. de Souza, T.R.A. Vasconcelos, N. Boechat, A.U. Krettli, Enhanced activity of mefloquine and artesunic acid against plasmodium falciparum in vitro and p. berghei in mice by combination with ciprofloxacin, Eur. J. Pharmacol. 558 (2007) 194–198.
[3] F. de P. Varotti, A.C.C. Botelho, A.A. Andrade, R.C. de Paula, E.M.S. Fagundes, A. Valverde, L.M.U. Mayer, J.S. Mendonça, M.V.N. de Souza, N. Boechat, A.U. Krettli, Synthesis, antimalarial activity, and intracellular targets of mefas, a new hybrid compound derived from mefloquine and artesunate, Antimicrob. Agents Chemother. 52 (2008) 3868–3874.
[4] M.V.N. de Souza, K.C. Pais, C.R. Kaiser, M.A. Peralta, M. de L Ferreira, M.C.S. Lourenço, Synthesis and in vitro antitubercular activity of a series of quinoline derivatives, Bioorg. Med. Chem. 17 (2009) 1474–1480.
[5] R.S.B. Gonçalves, C.R. Kaiser, M.C.S. Lourenço, M.V.N. de Souza, J.L. Wardell, S.M.S.V. Wardell, A.D. da Silva, Synthesis and antitubercular activity of new mefloquine-oxazolidine derivatives, Eur. J. Med. Chem. 45 (2010) 6095–6100.
[6] M. de L.F. Bispo, C.C. de Alcantara, M.O. de Moraes, C. do Ó Pessoa, F.A.R. Rodrigues, C.R. Kaiser, S.M.S.V. Wardell, J.L. Wardell, M.V.N. de Souza, A new and potent class of quinoline derivatives against cancer, Monatsh. Chem. 146 (2015) 2041–2052.
[7] V. Facchinetti, F.A. Guimarães, M.V.N. de Souza, C.R.B. Gomes, M.C.B.V. de Souza, J.L. Wardell, S.M.S.V. Wardell, T.R.A. Vasconcelos, Synthesis of novel ethyl (substituted)phenyl-4-oxothiazolidin-3-yl)-1-ethyl-4-oxo-1,4-dihydroquinoline-3-carboxylates as potential anticancer agents, J. Heterocycl. Chem. 52 (2015) 1245–1252.
[8] R.C. Montenegro, L.V. Lotufo, M.O. de Moraes, C. do Ó. Pessoa, F.A.R. Rodrigues, M. de L.F. Bispo, L.N. de F. Cardoso, C.R. Kaiser, M.V.N. de Souza, Synthesis and antitumoral evaluation of 7-chloro-4-quinolinylhydrazones derivatives, Med. Chem. 7 (2011) 599–604.
[9] R.C. Montenegro, L.V. Lotufo, M.O. de Moraes, C. do O Pessoa, F.A.R. Rodrigues, M. de L.F. Bispo, B.A. Freire, C.R. Kaiser, M.V.N. de Souza, Cytotoxic activity of polysubstituted 7-chloro-4-quinolinylhydrazone derivatives, Lett. Drug Des. Discov. 9 (2012) 251–256.
[10] F.A.R. Rodrigues, I. da S. Bomfim, B.C. Cavalcanti, C. do Ó. Pessoa, J.L. Wardell, S.M.S.V. Wardell, A.C. Pinheiro, C.R. Kaiser, T.C.M. Nogueira, J.N. Low, L.R. Gomes, M.V.N. de Souza, Design, synthesis and biological evaluation of (e)-2-(2-arylhydrazinyl)quinoxalines, a promising and potent new class of anticancer agents, Bioorg. Med. Chem. Lett. 24 (2014) 934–939.
[11] F.A.R. Rodrigues, I. da S. Bomfim, B.C. Cavalcanti, C. Pessoa, R.S.B. Goncalves, J.L. Wardell, S.M.S.V. Wardell, M.V.N. de Souza, Mefloquine-Oxazolidine derivatives: a new class of anticancer agents, Chem. Biol. Drug Des. 83 (2014) 126–131.
[12] R. Delvecchio, L.M. Higa, P. Pezzuto, A.L. Valadao, P.P. Garcez, F.L. Monteiro, E.C. Loiola, S. Rehen, L. Campanati, R.S. de Aguiar, A. Tanuri, Chloroquine inhibits zika virus infection in different cellular models, Cold Spring Harb. Labs Journals (2016). http://dx.doi.org/10.1101/051268. in press.
[13] D. Musso, D.J. Gubler, Zika virus, Clin. Microbiol. Rev. 29 (2016) 487–524.
[14] D. Musso, C. Roche, E. Robin, T. Nhan, A. Teissier, V.M. Cao-Lormeau, Potential sexual transmission of zika virus, Emerg. Infect. Dis. 21 (2015) 359–361.
[15] D.T. Deckard, W.M. Chung, J.T. Brooks, J.C. Smith, S. Woldai, M. Hennessey, N. Kwit, P. Mead, Male-to-male sexual transmission of zika virus, Morb. Mortal. Wkly. Rep. 65 (2016) 372–374.
[16] E. D'Ortenzio, S. Matheron, X. de Lamballerie, B. Hubert, G. Piorkowski, M. Maquart, D. Descamps, F. Damond, Y. Yazdanpanah, I. Leparc-Goffart, Evidence of sexual transmission of zika virus. N. Engl, J. Med. 374 (2016) 2195–2198.
[17] R.S. Lanciotti, O.L. Kosoy, J.J. Laven, J.O. Velez, A.J. Lambert, A.J. Johnson, S.M. Stanfield, M.R. Duffy, Genetic and serologic properties of zika virus associated with an epidemic, Emerg. Infect. Dis. 14 (2008) 1232–1239.
[18] WHO. WHO report| Zika Situation Report.
[19] WHO. WHO report| WHO Director-general Summarizes the Outcome of the Emergency Committee Regarding Clusters of Microcephaly and Guillain-barré Syndrome http://www.who.int/mediacentre/news/statements/2016/emergency-committee-zika-microcephaly/en/(Accessed 30 August 2016).
[20] C.Q. Sacramento, G.R. de Melo, N. Rocha, L.V.B. Hoelz, M. Mesquita, C.S. de Freitas, N. Fintelman-Rodrigues, A. Marttorelli, A.C. Ferreira, G. Barbosa-Lima, M.M. Bastos, E. de M. Volotao, D.A. Tschoeke, L. Leomil, F.A. Bozza, P.T. Bozza, N. Boechat, F.L. Thompson, A.M.B. de Filippis, K. Bruning, T. Souza, The clinically approved antiviral drug sofosbuvir impairs brazilian zika virus replication, Cold Spring Harb. Labs Journals (2016) in press, http://dx.doi.org/10.1101/061671.
[21] M. Onorati, Z. Li, F. Liu, A.M.M. Sousa, N. Nakagawa, M. Li, M.T. Dell'Anno, F.O. Gulden, S. Pochareddy, A.T.N. Tebbenkamp, W. Han, M. Pletikos, T. Gao, Y. Zhu, C. Bichsel, L. Varela, K. Szigeti-Buck, S. Lisgo, Y. Zhang, A. Testen, X.B. Gao, J. Mlakar, M. Popovic, M. Flamand, S.M. Strittmatter, L.K. Kaczmarek, E.S. Anton, T.L. Horvath, B.D. Lindenbach, N. Sestan, Zika virus disrupts phospho-tbk1 localization and mitosis in human neuroepithelial stem cells and radial glia, Cell Rep. 16 (2016) 1–17, http://dx.doi.org/10.1016/j.celrep.2016.08.038.
[22] N.J. Barrows, R.K. Campos, S.T. Powell, K.R. Prasanth, G. Schott-Lerner, R. Soto-Acosta, G. Galarza-Muñoz, E.L. McGrath, R. Urrabaz-Garza, J. Gao, P. Wu, R. Menon, G. Saade, I. Fernandez-Salas, S.L. Rossi, N. Vasilakis, A. Routh, S.S. Bradrick, S.S.M.A. Garcia-Blanco, A screen of fda-approved drugs for inhibitors of zika virus infection, Cell Host Microbe 20 (2016) 259–270.
[23] M. Xu, E.M. Lee, Z. Wen, Y. Cheng, W.K. Huang, X. Qian, J. Tcw, J. Kouznetsova, S.C. Ogden, C. Hammack, F. Jacob, H.N. Nguyen, M. Itkin, C. Hanna, P. Shinn, C. Allen, S.G. Michael, A. Simeonov, W. Huang, K.M. Christian, A. Goate, K.J. Brennand, R. Huang, M. Xia, G.L. Ming, W. Zheng, H. Song, H. Tang, Identification of small-molecule inhibitors of zika virus infection and induced neural cell death via a drug repurposing Screen, Nat. Med. 22 (2016) 1101–1109, http://dx.doi.org/10.1038/nm.4184.
[24] E.P. Acosta, C. Flexner, Antiviral agents (nonretroviral), in: Goodman & Gilman's - the Pharmacological Basis of Therapeutics, New York, 2011, pp. 1593–1622.
[25] C. Flexner, Antiretroviral agents and treatment of HIV infection, in: Goodman and Gilman's - the Pharmacological Basis of Therapeutics, New York, 2011, pp. 1623–1664.
[26] Data were collected at 100(2)K with Mo-Ka radiation using an Rigaku Saturn724+ (2x2 bin mode) of the UK National Crystallographic Service (NCS), based at the University of Southampton. Data collection, data reduction and unit cell refinement were achieved with CrystalClear-SM Expert 3.1 b27. Correction for absorption was achieved by an empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm. The program MERCURY were used in the preparation of the Figures. SHELXL97 and PLATON were used in the calculation of molecular geometry. The structures were solved by direct methods using SHELXS-97 and fully refined by means of the program SHELXL-97. Difference map peaks provided positions for water hydrogens and hydrogen attached to N(2). The coordinates, along with isotropic displacement parameters, were fully refined. All other hydrogen atoms were placed Calc. positions.
[27] (a) CrystalClear-SM Expert, Rigaku Corporation, Tokyo, Japan, 2012;
(b) Mercury 3.3.1 Cambridge Crystallographic Data Centre, UK, (c) G.M. Sheldrick, A short history of SHELX, Acta Crystallogr. A64 (2008) 112–122;
(d) A.L. Spek, Single-Crystal structure validation with the program PLATON, Appl. Crystallogr. 36 (2003) 7–13.
[28] Crystal data collected at 100(2)K; Colourless crystal: 0.11 × 0.08 × 0.05 mm; Formula: C19H24ClN3O4; M= 393.86; triclinic, P-1; a = 7.0634(14) Å, α = 99.875(3)°, b = 10.946(2) Å, β = 96.638(4)°, c = 12.855(3) Å, γ = 93.435(3)°, Z = 2, V= 969.4(3)Å3; 4411 independent reflections [R(int) = 0.0356]; 3827 observed reflections [I>2 s (I)]; data/restrainsts/parameters 4411/0/266; R1 = 0.035; largest diff. peak and hole 0.31 and -0.22 e.Å-3. Atomic coordinates, bond lengths, angles and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre, deposition number 1459439.
[29] L.J. Reed, H. Muench, A simple method of estimating fifty per cent endpoints, Am. J. Epidemiol. 27 (1938) 493–497.
[30] D.A. Scudiero, R.H. Shoemaker, K.D. Paull, A. Monks, S. Tierney, T.H. Nofziger, M.J. Currens, D. Seniff, M.R. Boyd, Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines, Cancer Res. 48 (1988) 4827–4833.