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Synthesis, β-hematin inhibition studies and antimalarial evaluation of dehydroxy isotebuquine derivatives against *Plasmodium berghei*

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ABSTRACT

Diverse dehydroxy-isotebuquine derivatives were prepared by using a five step synthetic sequence in good yields. All these new 4-aminoquinolines were evaluated as inhibitors of haemozoin formation, where most of them showed a significant inhibition value (% IHF >97). The best inhibitors were tested in vivo as potential antimalarials in mice infected with *Plasmodium berghei* ANKA chloroquine susceptible strain, three of them (**11b**, **11d** and **11h**) displayed an antimalarial activity comparable to that of chloroquine.

1. Introduction

Malaria remains as one of the major causes of illness for humans, with approximately 250 million of reported clinical cases around the world annually, particularly in poor or developing countries.[1] *Plasmodium falciparum* malaria causes most of severe cases and deaths, where near 80% of all reported cases and 90% of malaria-attributed deaths occur in Africa.[1] Outside of Africa, *Plasmodium vivax* is the predominant species, mainly in Asia, including the Middle East and the Western Pacific, and in Central and South America.[2] In Venezuela, *P. vivax* infections are very common, accounting for 70% of the total malaria confirmed cases reported to the World Health Organization in 2013 (from 78.643 total cases of malaria, 30% were caused by *P. falciparum* infections).[1]

Chloroquine **1**, a 4-amino-quinoline antimalarial, usually is the first choice drug in the fight against *P. falciparum* and *P. vivax*, but its efficacy has been eroded by the emergence of resistant parasites. The spread of chloroquine resistance has prompted the re-investigation of the chemistry and pharmacology of alternative 4-aminoquinoline antimalarials such as amodiaquine **2**, which proved to be effective against chloroquine-resistant strains.[3–5] Amodiaquine is effective against many chloroquine resistant strains of *P. falciparum*.[6] However, clinical use of amodiaquine has been severely restricted because of associations with hepatotoxicity and agranulocytosis.[7,8] Toxicity of amodiaquine has been related to the reactive metabolites formed by oxidation of its phenolic ring, particularly to the formation of a quinone-imine intermediate by cytochrome P-450-catalyzed biological oxidation.[9] It has been found that amodiaquine is excreted in bile exclusively as the 5′-thioether conjugates (glutathione and cysteinyl) in rats.[10] This observation indicates that the parent drug undergoes extensive biotransformation in vivo to form an amodiaquine quinone-imine or semiquinone-imine with subsequent conjugation of glutathione.[11] Structure activity relationship (SAR) studies on amodiaquine had previously shown that wide variations in the side chain are accommodated with retention of antimalarial activity. Blocking of bioactivation pathways by removal of the phenolic group or introduction of non reactive substituents has been the main strategy.[5,12–14] From SAR studies it has been found that in the amodiaquine and tebuquine **3** series of 4-aminoquinoline analogs, the presence of the 4′-hydroxy group within the aromatic ring imparts greater inherent antimalarial activity against chloroquine resistant parasites than the corresponding dehydroxy analogs.[5] Interchange of the hydroxy group and the Mannich side chain provides a means of preventing oxidation to toxic metabolites while retaining possible important bonding interactions with the aromatic hydroxyl function generated an amodiaquine regioisomer called isoquine **4** which cannot form toxic metabolites by simple oxidation and is potent against chloroquine resistant parasites

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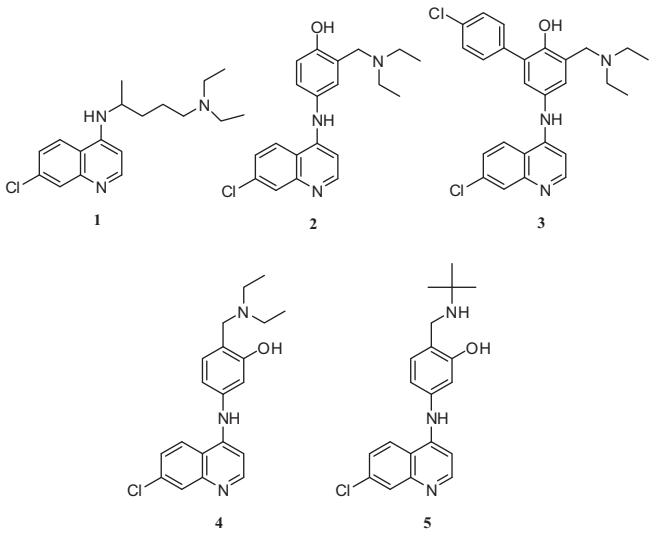
in vitro. Isoquine itself has been reported to possess potent in vitro and oral in vivo antimalarial activity in experimental animal models and it does not



undergo in vivo biotransformation to quinone-imine metabolites.[5] Apart from an excellent antiparasitic profile, isoquine and its different side-chain analogs, like the recently developed *N-tert*-butyl isoquine **5** are rather inexpensive antimalarials to synthesize and were expected to represent new leads for development of a safe, cheap, affordable, and effective antimalarials for both prophylaxis and treatment of malaria.[15,16] Unfortunately, *N-tert*-butyl isoquine **5** development was terminated[16] but recently, it was retaken for phase II clinical trials in Mali to study its efficacy in uncomplicated *P. falciparum* malaria.[35] Intrigued by the importance of the 3′-OH on the side chain of isoquine for the antimalarial activity, we decided to synthesize a group of 3′-dehydroxy analogs of isotebuquine **11a–h** and test them for both their β-hematin inhibition and preliminary antimalarial activity.

2. Results and discussion

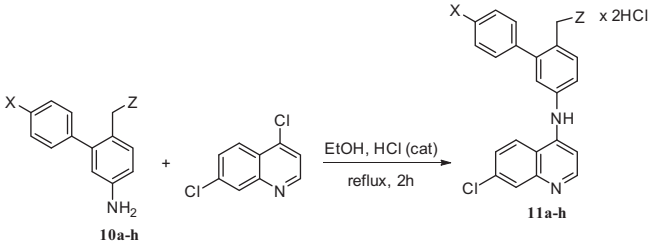
2.1. Chemistry

The synthesis of title compounds **11a–h** is depicted on Scheme 1. First, the corresponding nitro-biphenyl compounds **7a–b** were obtained by the Suzuki–Miyaura cross coupling reaction between different phenyl boronic acids with the starting material, 2-bromo-4-nitrotoluene **6**. Next, the benzylic bromination of those biphenyl intermediates followed by their corresponding nucleophilic substitution with different secondary amines and a subsequent reduction of the nitro group using tin in hydrochloric acid, afforded the required anilines **10a–h** in good yields. Finally, the heteroaromatic substitution of 4,7-dichloroquinoline on position 4 with the above mentioned anilines in refluxing ethanol employing concd HCl, gave desired 4-aminoquinolines **11a–h** as their hydrochloride salts (Table 1), which were further biologically tested (Tables 2 and 3).

2.2. Biological

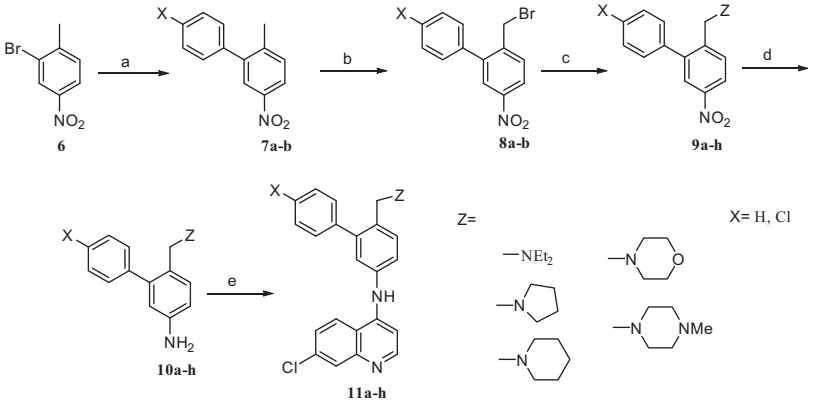
Different quinoline antimalarials are thought to target ferriprotoporphyrin IX (FP IX) heme within the digestive vacuole of the

Table 1
Yields of the last step for the synthesis of dehydroxy-isotebuquine derivatives **11 a–h**



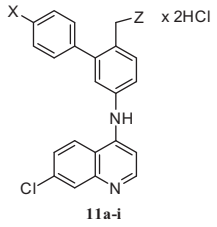
Compd No.	X	Z	Yield (%)
11a	H	$-NEt_2$	82[a]
11b	H		90
11c	H		79
11d	H		87
11e	Cl		38
11f	Cl		84
11g	Cl		86
11h	Cl		72

[a] Compound **11a** was obtained as the monohydrochloride salt.

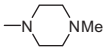


Scheme 1. Synthesis of novel dehydroxy-isotebuquine derivatives. Conditions: (a) phenylboronic acid (1.2 equiv)/toluene, $Pd(PPh_3)_4$ (5%), K_2CO_3 (2.5 equiv)/EtOH–H_2O (1:1), 100 °C, 24 h; (b) NBS (1.2 equiv)/CCl_4, $(PhCO)_2O_2$ (0.05 equiv), light, reflux/24 h; (c) dialkylamine (5 equiv)/toluene, reflux, 6 h; (d) Sn/HCl, 70 °C, 2 h; (e) 4,7-dichloroquinoline (1.2 equiv)/EtOH, concd HCl (1 equiv), reflux, 6 h.

Table 2
Percentage of inhibition of haemozoin formation (% IHF) for compounds **11a–h**



11a-i

Compd No.	X	Z	% IHF (±SD)[a,b]
11a	H	$-NEt_2$	98.450 (0.008)[d]
11b	H		97.080 (0.002)
11c	H		98.040 (0.015)
11d	H		98.490 (0.014)
11e	Cl		1.610 (0.061)
11f	Cl		97.210 (0.054)
11g	Cl		2.030 (0.040)
11h	Cl		97.250 (0.021)
Chloroquine[c]	—	—	98.570 (0.015)

[a] SD: standard deviation.
[b] % IHF: percentage of inhibition of haemozoin formation.
[c] Control.
[d] Monohydrochloride.

parasite, once it is released upon hemoglobin digestion.[17,18] Free FP IX is toxic and the malaria parasite sequesters FP IX as nontoxic crystalline haemozoin.[19,20] Heme detoxification in haemozoin is believed to be the main target of quinoline containing antimalarials.[21–23] It is not well known how quinoline drugs target heme to inhibit haemozoin formation in vivo, and even nor is it understood whether different quinoline antimalarials inhibit haemozoin through similar or different pathways.[21] Despite there is still some controversy, it has been shown that the inhibition of haemozoin growth will depend on drug accumulation inside the parasite digestive vacuole, where it can target the soluble forms of hematin monomer or dimer[24–26] and/or the haemozoin crystal face {0.01}.[27,28] Recently, it has been shown for quinoline related drugs (including chloroquine) that in vitro drug–heme interactions leading to the inhibition of β-hematin correlated with parasite growth inhibition (cytostatic activity) and suggested they do not necessarily affect the cytocidal potency.[29–31]

The antimalarial activity of synthesized 4-amino-quinolines **11a–h** was evaluated through the test of the ability of the compounds to inhibit β-hematin (synthetic haemozoin) formation, considering that haemozoin can be formed spontaneously under acid and low oxygen conditions found in the food vacuole of the parasite.[32] Results showing more than 50% of inhibition of haemozoin formation were considered significant (compounds **11a–d**, **11f**, **11h**; see Table 2).

Quinolines **11a–d**, **11f**, **11h** were then selected and tested in mice infected with *Plasmodium berghei* ANKA, a chloroquine-susceptible strain of murine malaria (Table 3). Mice were given the compound (chloroquine in 25 mg kg^{-1} or **11a–d**, **11f** and **11h** in 20 mg kg^{-1}, ip once daily) for 4 consecutive days (days 0–4 post-infection). At day fourth post-infection, the parasitemia was determined, the survival days were monitored and compared with control mice receiving saline (untreated mice).[34] Control mice died between days 6 and 7 post-infection. In general, compounds

Table 3
The effect of dehydroxy-isotebuquine derivatives **11a–d**, **11f**, **11h** on *P. berghei* infected mice (20 mg kg^{-1})

Compd No.	Post-infection days of survival (±SD)[a]	% *P* (±SD)[b]
11a	25.20 (3.00)*	3.20 (1.30)
11b	28.40 (1.60)*	1.80 (0.45)
11c	23.20 (3.17)*	6.50 (0.60)
11d	28.60 (1.40)*	2.10 (0.30)
11f	26.00 (2.75)*	7.30 (0.45)
11h	27.30 (1.40)**	2.00 (0.54)
Control treated[c]	29.40 (0.40)	0.60 (0.08)
Control untreated	6.80 (0.70)	65.20 (2.58)

[a] Results are expressed as the media ± standard deviation (SD). $n = 6$ (number of treated mice).
[b] % *P*: percentage of parasitemia.
[c] Positive control treated with chloroquine (25 mg kg^{-1}).
* $P < 0.05$ comparing to control treated group.
** $P < 0.01$ comparing to control treated group.

11a–d, **11f**, **11h** were all capable of increase significantly the survival time in more than 20 days post-infection (Table 3), but particularly three compounds **11b**, **11d** and **11h** increased the survival rates comparable to chloroquine, respectively. As can be seen, there is a good correlation between inhibition of haemozoin formation, the reduction of the parasitemia and the survival time of infected mice for these last three most active compounds. Despite some derivatives (**11a**, **11c**, **11f**) were very effective as inhibitors of the haemozoin formation, they were not able to extent the survival rate of infected mice over 27 days, perhaps this result is related to the pharmacokinetics and bioavailability of these compounds in vivo. Both, the *N*-piperazino substituted quinoline **11e** and the *N*-morpholino analog **11g**, respectively, were the two least active compounds of the series as inhibitors of the haemozoin formation. We believe this could be related, in the case of compound **11e**, to the pK_a of the *N*-benzyl nitrogen when interacting with the heme group affecting it (calculated pK_a = 3.8, value obtained from the ChemBioDraw Ultra suite),[33] a hypothesis that should be still investigated. The reasons for the strong difference encountered between compounds **11c** and **11g** as inhibitors of haemozoin formation (Table 2) are unknown, the calculated pK_a for both is close (7.5 and 7.4, respectively) and **11g** (*C*log*P* = 7.2) seemed to be more lipophilic than **11c** (*C*log*P* = 6.5).[33] Based on recent suggestions,[21] we consider important to test further the comparison of % of β-hematin inhibition of most active compounds with their cytostatic (IC_{50}) versus the cytocidal (LD_{50}) effects in order to better understand their possible mechanism of action. Our results are still preliminary and require also further evaluation to discard toxic compounds (such as the selectivity index, SI),[36] however from the in vivo test, no apparently toxicity was seen on mice for most active compounds The quinolines **11b**, **11d** and **11h** should merely be evaluated as antimalarial compounds over *P. falciparum* chloroquine susceptible and resistance strains, their high activity over *P. berghei* chloroquine sensitive parasites showed that the 3′-OH substitution is not full necessary in this case for an effective antimalarial potency, a remarkable difference when compared to amodiaquine analogs which lacks the 4′-OH group.[5]

3. Conclusions

Eight dehydroxy-isotebuquine derivative **11a–h** were prepared by using a five step synthetic sequence in good yields. All these new 4-aminoquinolines were evaluated as inhibitors of haemozoin formation, but only six (**11a–d**, **11f** and **11h**) showed a significant inhibition value (% IHF >97). Compounds **11a–d**, **11f** and **11h** were tested in vivo as potential antimalarials in mice infected with *P.*

berghei ANKA chloroquine susceptible strain, three of them (**11b**, **11d** and **11h**) displayed an antimalarial activity comparable to that of chloroquine, demonstrating that the 3′-hydroxy group for these dehydroxy-isotebuquine analogs is not necessary for a potent antimalarial effect.

4. Experimental

4.1. Chemistry

Melting points were determined in a Fischer-Johns micro hot-stage apparatus and are uncorrected. NMR spectra were obtained using a JEOL Eclipse Plus or JEOL Eclipse spectrometers in either deuterated chloroform ($CDCl_3$) or hexadeuterated dimethylsulfoxide (DMSO-d_6). ^{1}H NMR were recorded at 400 or 270 MHz, ^{13}C NMR at 100 or 67.9 MHz, respectively. Chemical shifts (δ) are given in ppm downfield from the residual $CHCl_3$ or DMSO (^{1}H NMR, ^{13}C NMR). Coupling constants are given in hertz. The IR spectra were recorded as KBr disks (for solids) or liquid films in NaCl cells (for oils) using a FT-IR Nicolet Magna Spectrometer. GC–MS spectra were recorded using an Agilent Gas Chromatograph model 7898A using an EI ionization (70 eV). Elemental analyses of new synthesized compounds were performed on a Perkin Elmer 2400 CHN analyzer; results fell in the range of ±0.4% of the required theoretical values. Silica gel plates ALUGRAM® SIL G/UV254 (Macherey-Nagel GmbH & Co., Germany) were used for TLC testing. Column chromatography was performed, when required, with Kieselsgel 60 silica (70–230 mesh, Merck, Darmstadt, Germany) and hexanes–ethyl acetate as eluent. Reagents were obtained from Aldrich (Milwaukee, MI, USA) or Merck (Darmstadt, Germany) and used without further purification.

4.1.1. General procedure for the preparation of 2-methyl-5-nitro-1,1′-biphenyls 7a–b

To a mixture of 2-bromo-4-nitrotoluene (14 mmol), substituted phenylboronic acid (16.8 mmol, 1.2 equiv) and tetrakis(triphenylphosphine)palladium(0) (0.81 g, 0.7 mmol, 5%) in toluene (50 mL) was added potassium carbonate (35 mmol, 2.5 equiv) dissolved in a water–ethanol (1:1) solution (30 mL). The mixture was heated (100 °C) with stirring under argon for 24 h, cooled at room temperature and filtered, washed with water (3 × 20 mL), the organic phase treated with anhydrous magnesium sulfate, filtered and evaporated under vacuo to give a brown crude solid which was further purified by column chromatography using hexanes–ethyl acetate (9:1).

4.1.1.1. 2-Methyl-5-nitro-1,1′-biphenyl 7a. Yellow solid, 78%, mp: 74–76 °C. FT-IR (ν, cm^{-1}): 1571 (st, NO_2), 1478 (st, NO_2). ^{1}H NMR (270 MHz, $CDCl_3$, δ, cm^{-1}): 8.08 (m, 2H), 7.39 (m, 6H), 2.35 (s, 3H, CH_3). ^{13}C NMR (67.9 MHz, $CDCl_3$, δ, cm^{-1}): 146.4, 143.2, 139.7, 131.2, 129.0 (2C), 128.6 (2C), 128.0, 124.7, 122.2, 20.9. GC–MS (60 eV, m/z): 213 (M$^+$, 100%). Anal. Calcd for $C_{13}H_{11}NO_2$: C, 73.23; H, 5.20; N, 6.57. Found: C, 73.30; H, 5.14; N, 6.52.

4.1.1.2. 4′-Chloro-2-methyl-5-nitro-1,1′-biphenyl 7b. White solid, 81%, mp: 108–110 °C. FT-IR (ν, cm^{-1}): 1564 (st, NO_2), 1477 (st, NO_2). ^{1}H NMR (270 MHz, $CDCl_3$, δ, cm^{-1}): 8.10 (dd, 1H, J = 8.4 Hz; J = 2.5 Hz), 8.06 (d, 1H, J = 2.2 Hz), 7.42 (m, 2H), 7.24 (m, 2H, 2H), 2.34 (s, 3H, CH_3). ^{13}C NMR (60 MHz, $CDCl_3$, δ, cm^{-1}): 146.3, 143.5, 141.9, 138.0, 134.2, 131.4, 130.4 (2C), 128.9 (2C), 124.6, 122.5, 20.8 (CH_3). GC–MS (60 eV, m/z): 247 (M$^+$, 100%). Anal. Calcd for $C_{13}H_{10}NO_2Cl$: C, 63.04; H, 4.07; N, 5.66. Found: C, 63.10; H, 4.11; N, 5.60.

4.1.2. General procedure for the preparation of 2-(bromomethyl)-5-nitro-1,1′-biphenyls 8a–b

To a solution of the corresponding 2-methyl-5-nitro-1,1′-biphenyl **7a–b** (16 mmol) in carbon tetrachloride (90 mL) was added *N*-bromosuccinimide (3.7 g, 20.8 mmol) and benzoyl-peroxide (0.19 g, 0.8 mmol). The reaction was refluxed with stirring for 24 h under light irradiation. The mixture was then cooled at room temperature, the solid succinimide filtered and the solvent evaporated under vacuo to give a crude material which was purified by column chromatography using hexanes.

4.1.2.1. 2-(Bromomethyl)-5-nitro-1,1′-biphenyl 8a. Yellow oil, 75%. FT-IR (ν, cm^{-1}): 1571 (st, NO_2), 1477 (st, NO_2). ^{1}H NMR (270 MHz, $CDCl_3$, δ, cm^{-1}): 8.18 (dd, 1H, J = 8.4 Hz, J = 2.5 Hz), 8.12 (d, 1H J = 2.5 Hz), 7.70 (d, 1H, J = 8.4 Hz), 7.47 (m, 5H), 4.44 (s, 2H CH_2). ^{13}C NMR (67.9 MHz, $CDCl_3$, δ, cm^{-1}): 147.5, 143.4, 142.4, 138.0, 132.1, 128.9, 128.6, 125.4, 122.8, 29.8. Anal. Calcd for $C_{13}H_{10}BrNO_2$: C, 53.45; H, 3.45; N, 4.79. Found: C, 53.54; H, 3.49; N, 4.84.

4.1.2.2. 2-(Bromomethyl)-4′-chloro-5-nitro-1,1′-biphenyl 8b. White solid, 70%, mp 114–116 °C. FT-IR (ν, cm^{-1}): 1476 (st, NO_2). ^{1}H NMR (270 MHz, $CDCl_3$, δ, cm^{-1}): 8.20 (dd, 1H, J = 8.4 Hz, J = 2.5 Hz), 8.10 (d, 1H, J = 2.5 Hz), 7.69 (d, 1H, J = 8.4 Hz), 7.47 (d, 2H, J = 8.2 Hz), 7.38 (d, 2H, J = 8.2 Hz), 4.39 (s, 2H CH_2). ^{13}C NMR (67.9 MHz, $CDCl_3$, δ, cm^{-1}): 147.2, 142.2, 142.1, 136.3, 134.9, 132.2, 130.2, 129.1, 125.3, 123.1, 29.2. GC–MS (60 eV, m/z): 325 (M$^+$, 100%). Anal. Calcd for $C_{13}H_9BrClNO_2$: C, 47.81; H, 2.78; N, 4.29. Found: C, 47.69; H, 2.82; N, 4.32.

4.1.3. General procedure for the preparation of 1-((5-nitro-[1,1′-biphenyl]-2-yl)methyl)amines 9a–h

To a solution of the corresponding 2-(bromomethyl)-5-nitro-1,1′-biphenyl **8** (2 mmol) in toluene (30 mL) was added the dialkylamine (10 mmol, 5 equiv) and the reaction stirred under reflux for 6 h. The mixture was then cooled at room temperature, the solvent evaporated under vacuo and the unreacted dialkylamine eliminated through vacuum distillation. The remaining residue was treated with sodium hydroxide (aq 10%) and the obtained solid filtrated and recrystallized (EtOH) to give yellow-orange colored solids.

4.1.3.1. *N*-Ethyl-*N*-((5-nitro-[1,1′-biphenyl]-2-yl)methyl)ethanamine 9a. Yellow oil, 67%. FT-IR (ν, cm^{-1}): 1475 (st, NO_2). ^{1}H NMR (270 MHz, $CDCl_3$, δ, cm^{-1}): 8.18 (dd, 1H, J = 8.5 Hz, J = 2.5 Hz), 8.07 (d, 1H, J = 2.5 Hz), 8.02 (br s, 1H), 7.27–7.47 (m, 5H), 3.59 (s, 2H, CH_2), 2.45 (q, 4H, CH_2), 0.88 (t, 6H, CH_3). ^{13}C NMR (67.9 MHz, $CDCl_3$, δ, cm^{-1}): 148.2, 144.9, 137.7, 137.6, 133.5, 129.4 (2C), 129.3 (2C), 129.2, 125.4, 123.5, 51.5 (CH_2-*N*), 46.1 (2C, CH_2), 8.2 (2C, CH_3). GC–MS (60 eV, m/z): 284 (M$^+$, 100%). Anal. Calcd for $C_{17}H_{20}N_2O_2$: C, 71.81; H, 7.09; N, 9.85. Found: C, 71.89; H, 7.03; N, 9.79.

4.1.3.2. 1-((5-Nitro-[1,1′-biphenyl]-2-yl)methyl)piperidine 9b. Yellow solid, 87%, mp 60–62 °C. IR (KBr, ν cm^{-1}): 1583, 1497 (st. N═O, nitro). ^{1}H NMR (270 MHz, δ ppm, $CDCl_3$): 8.17 (dd, 1H, J = 8.1 Hz, J = 1.9 Hz), 8.10 (d, 1H), 7.90 (d, 1H), 7.41–7.51 (m, 3H), 7.27–7.29 (m, 2H), 3.85 (s, 2H, CH_2-*N*), 2.49 (br s, 4H, CH_2-*N*), 1.69 (br s, 4H, CH_2), 1.44 (br s, 2H, CH_2). ^{13}C NMR (100 MHz, δ ppm, $CDCl_3$): 147.3, 144.4, 138.6, 132.0, 132.0, 129.3 (2C), 128.8 (2C), 128.4, 125.0, 12.6, 58.5 (CH_2-N), 54.0 (2C, CH_2-N), 24.5 (CH_2), 23.3 (CH_2). GC–MS (60 eV, m/z): 296 (M$^+$, 100%). Anal. Calcd for $C_{18}H_{20}N_2O_2$: C, 72.95; H, 6.80; N, 9.45. Found: C, 72.88; H, 6.84; N, 9.50.

4.1.3.3. 4-((5-Nitro-[1,1′-biphenyl]-2-yl)methyl)morpholine 9c. Yellow solid, 91%, mp 118–120 °C. IR (KBr, ν cm^{-1}): 1570, 1495 (st. N=O, nitro). ^{1}H NMR (270 MHz, δ ppm, $CDCl_3$): 8.22 (d, 1H, J = 6.7 Hz), 8.13 (br s, 1H), 7.41–7.52 (m, 3H), 7.30–7.28 (m, 2H), 3.80 (br s, 6H, CH_2-*O*, CH_2-*N*), 2.54 (br s, 4H, CH_2-*N*). ^{13}C NMR (100 MHz, δ ppm, $CDCl_3$): 147.5, 144.5, 138.4, 132.1, 130.0, 129.2 (2C), 128.9 (2C), 128.6, 125.2, 122.6, 65.6 (2C, CH_2-O), 58.4 (2C, CH_2-N), 52.79 (CH_2-N). GC–MS (60 eV, *m*/*z*): 298 (M$^+$, 100%). Anal. Calcd for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.51; H, 6.12; N, 9.32.

4.1.3.4. 1-((5-Nitro-[1,1′-biphenyl]-2-yl)methyl)pyrrolidine 9d. Orange solid, 81%, mp 92–94 °C. IR (KBr, ν cm^{-1}): 1570, 1465 (st. N=O, nitro). ^{1}H NMR (270 MHz, δ ppm, $CDCl_3$): 8.26 (s, 1H), 8.13 (m, 2H); 7.50–7.24 (m, 5H), 3.99 (s, 2H, CH_2-*N*), 2.86 (br s, 4H, CH_2-N), 1.87 (br s, 4H, CH_2). ^{13}C NMR (100 MHz, δ ppm, $CDCl_3$): 148.2, 144.4, 137.7, 134.4, 133.0, 129.5 (2C), 129.2 (2C), 129.2, 125.3, 123.6, 54.1 (CH_2-N), 53.8 (2C, CH_2-N), 23.0 (CH_2). GC–MS (60 eV, *m*/*z*): 281 ((M−1)$^+$, 100%). Anal. Calcd for $C_{17}H_{18}N_2O_2$: C, 72.32; H, 6.43; N, 9.92. Found: C, 72.39; H, 6.39.; N, 9.85.

4.1.3.5. 1-((4′-Chloro-5-nitro-[1,1′-biphenyl]-2-yl)methyl)-4-methylpiperazine 9e. Yellow solid, 90%, mp 100–102 °C. IR (KBr, ν cm^{-1}): 1582, 1497 (st. N=O, nitro). ^{1}H NMR (270 MHz, δ ppm, $CDCl_3$): 8.17 (dd, 1H, J = 8.5 Hz, J = 2.5 Hz), 8.08 (d, 1H, J = 2.5 Hz), 7.70 (d, 1H, J = 8.6 Hz), 7.41 (d, 2H, J = 8.4 Hz), 7.29 (d, 2H, J = 8.4 Hz), 3.43 (s, 2H, CH_2-*N*), 2.46 (br s, 8H, CH_2-N), 2.33 (3H, CH_3). ^{13}C NMR (100 MHz, δ ppm, $CDCl_3$): 146.9, 143.5, 142.7, 137.4, 134.3, 130.9, 130.6 (2C), 128.7 (2C), 125.0, 122.3, 59.4 (CH_2-*N*), 55.0 (2C, CH_2-*N*), 52.4 (2C, CH_2-*N*), 45.71 (*N*-CH_3). Anal. Calcd for $C_{18}H_{20}ClN_3O_2$: C, 62.52; H, 5.83; N, 12.15. Found: C, 62.46; H, 5.86; N, 12.19.

4.1.3.6. 1-((4′-Chloro-5-nitro-[1,1′-biphenyl]-2-yl)methyl)piperidine 9f. Orange solid, 84%, mp 106–108 °C. IR (KBr, ν cm^{-1}): 1480 (st. N=O, nitro). ^{1}H NMR (270 MHz, δ ppm, J Hz, $CDCl_3$): 8.21 (dd, 1H, H5, J = 8.64; 2.22); 8.08 (d, 1H, H3, J = 2.2); 7.94 (s, 1H, H6); 7.41 (d, 2H, 2H3′, J = 8.40); 7.28 (d, 2H, 2H2′, J = 8.40); 3.48 (s, 2H, CH_2-N); 2.33 (s, 4H, CH_2-N); 1.58 (sa, 4H, CH_2); 1.43 (sa, 2H, CH_2). ^{13}C NMR (100 MHz, δ ppm, $CDCl_3$): 147.8, 142.9, 137.3, 134.6, 131.8, 130.7, 128.9, 124.9, 122.8, 59.3 (CH_2-*N*), 54.2 (2C, CH_2-*N*), 25.1 (2C, CH_2), 23.5 (CH_2). Anal. Calcd for $C_{18}H_{19}ClN_2O_2$: C, 65.35; H, 5.79; N, 8.47. Found: C, 65.41; H, 5.83; N, 8.41.

4.1.3.7. 4-((4′-Chloro-5-nitro-[1,1′-biphenyl]-2-yl)methyl)morpholine 9g. Beige solid, 89%, mp 95–96 °C. IR (KBr, ν cm^{-1}): (st. N=O, nitro). ^{1}H NMR (270 MHz, δ ppm, $CDCl_3$): 8.23 (dd, 1H, J = 8.7 Hz), 8.11 (d, 1H, J = 2.2 Hz), 7.86 (br s, 1H), 7.47 (d, 2H, J = 8.4 Hz), 7.38 (d, 2H, J = 8.4 Hz), 3.79 (br s, 4H, 2CH_2-O), 3.71 (s, 2H, CH_2-*N*), 2.75 (s, 4H, CH_2-*N*). ^{13}C NMR (100 MHz, δ ppm, $CDCl_3$): 147.3, 143.0, 137.1, 134.7, 131.7 (2C), 130.6 (2C), 129.0, 125.1, 122.7, 66.2 (2C, CH_2-*O*), 63.8 (CH_2-*N*), 53.1 (2C, CH_2-*N*).

Anal. Calcd for $C_{17}H_{17}ClN_2O_3$: C, 61.36; H, 5.15; N, 8.42. Found: C, 61.43; H, 5.11; N, 8.47.

4.1.3.8. 1-((4′-Chloro-5-nitro-[1,1′-biphenyl]-2-yl)methyl)pyrrolidine 9h. Orange solid, 87%, mp 102–104 °C. IR (KBr, ν cm^{-1}): 1479 (st. N=O, nitro). ^{1}H NMR (270 MHz, δ ppm, $CDCl_3$): 8.24 (dd, 1H, J = 8.4 Hz), 8.16 (br s, 1H), 8.09 (d, 1H, J = 1.8 Hz), 7.45 (d, 2H, J = 8.2 Hz), 7.27 (d, 2H, J = 8.4 Hz), 3.88 (s, 2H, CH_2-*N*), 2.71 (br s, 4H, CH_2-*N*), 1.87 (br s, 4H, CH_2). ^{13}C NMR (400 MHz, δ ppm, $CDCl_3$): 147.3, 142.4, 136.9, 134.9, 131.7, 130.6, 129.1, 125.0, 123.1, 55.9 (CH_2-*N*), 53.9 (CH_2-*N*), 23.4 (2C, CH_2). Anal. Calcd for $C_{17}H_{17}ClN_2O_2$: C, 64.45; H, 5.41; N, 8.84. Found: C, 64.50; H, 5.37; N, 8.90.

4.1.4. General procedure for the preparation of anilines 10a–h

A mixture of the corresponding 1-((5-nitro-[1,1′-biphenyl]-2-yl)methyl)amine **9** (2 mmol), tin powder (4 mmol) and concentrated hydrochloric acid (20 mmol) was heated (70 °C) with stirring for 2 h. The mixture was then cooled at room temperature and neutralized carefully with a solution of NaOH (aq 10%), extracted with dichloromethane (3 × 20 mL), the organic phase washed with distilled water, dried ($MgSO_4$), filtered and the solvent evaporated under vacuo to give a solid aniline product which was purified by column chromatography (Hex/EtOAc 7:3).

4.1.4.1. 6-((Diethylamino)methyl)-[1,1′-biphenyl]-3-amine 10a. Yellow solid, 74%, mp 61–63 °C. IR (KBr, ν cm^{-1}): 3352, 3162 (st. N–H). ^{1}H NMR (270 MHz, δ ppm, $CDCl_3$): 8.18 (dd, 1H, J = 8.5 Hz, J = 2.5 Hz), 8.07 (d, 1H, J = 2.5 Hz), 8.02 (d, 1H, J = 2.5 Hz), 7.27–7.47 (m, 5H), 3.59 (s, 2H, CH_2-*N*), 2.45 (c, 4H, CH_2-*N*), 0.88 (t, 6H, CH_3). ^{13}C NMR (100 MHz, δ ppm, $CDCl_3$): 144.8, 140.5, 133.6, 129.6, 129.4, 128.8, 127.8, 116.4, 115.2, 51.9 (CH_2-*N*), 46.2 (CH_2-*N*), 8.7 (CH_3). GC–MS (60 eV, *m*/*z*): 284 (M$^+$, 100%).

Anal. Calcd for $C_{17}H_{22}N_2$: C, 80.27; H, 8.72; N, 11.01. Found: C, 80.34.50; H, 8.69; N, 11.05.

4.1.4.2. 6-(Piperidin-1-ylmethyl)-[1,1′-biphenyl]-3-amine 10b. Yellow solid, 84%, mp 120–122 °C. IR (KBr, ν cm^{-1}): 3422, 3311 (st. N–H). ^{1}H NMR (400 MHz, δ ppm, $CDCl_3$): 7.30–7.37 (m, 6H), 6.66 (dd, 1H, J = 9.5 Hz, J = 2.5 Hz), 6.58 (d, 1H, J = 2.5 Hz), 3.65 (s, 2H, CH_2-*N*), 3.30 (s, 2H, NH_2), 2.25 (br s, 4H, CH_2-*N*), 1.51 (br s, 4H, CH_2), 1.36 (2H, CH_2). ^{13}C NMR (100 MHz, δ ppm, $CDCl_3$): 145.0, 143.9, 141.8, 131.6, 129.6, 127.8, 126.8, 116.7, 114.1, 59.8 (CH_2-*N*), 54.0 (CH_2-*N*), 25.9 (CH_2), 24.4 (CH_2). Anal. Calcd for $C_{18}H_{22}N_2$: C, 81.16; H, 8.32; N, 10.52. Found: C, 81.10; H, 8.36; N, 10.47.

4.1.4.3. 6-(Morpholinomethyl)-[1,1′-biphenyl]-3-amine 10c. Yellow solid, 85%, mp 128–130 °C. IR (KBr, ν cm^{-1}): 3428, 3314 (est. N–H). ^{1}H NMR (270 MHz, δ ppm, $CDCl_3$): 7.31–7.38 (m, 6H), 6.67 (dd, 1H, J = 2.5 Hz, J = 8.1 Hz), 6.58 (d, 1H, J = 2.5 Hz), 3.97 (t, 4H, CH_2-*O*), 3.50 (br s, 2H, CH_2-*N*), 2.41 (br s, 4H, CH_2-*N*). ^{13}C NMR (100 MHz, δ ppm, $CDCl_3$): 145.9, 144.4, 141.3, 132.2, 129.5, 128.2, 127.2, 116.7, 114.3, 66.2 (2C, CH_2-*O*), 59.1 (2C, CH_2-*N*), 52.5 (*N*-CH_2). Anal. Calcd for $C_{17}H_{20}N_2O$: C, 76.09; H, 7.51; N, 10.44. Found: C, 76.13; H, 7.54; N, 10.48.

4.1.4.4. 6-(Pyrrolidin-1-ylmethyl)-[1,1′-biphenyl]-3-amine 10d. Orange solid, 86%, mp 57–59 °C. IR (KBr, ν cm^{-1}): 3421, 3310 (st. N–H). ^{1}H NMR (270 MHz, δ ppm, $CDCl_3$): 7.45 (d, 1H, J = 8.1 Hz), 6.70 (dd, 1H, J = 8.1 Hz, J = 2.5 Hz); 6.57 (d, 1H, J = 2.5 Hz), 3.69 (s, 2H, CH_2-*N*), 3.58 (s, 2H, NH_2), 2.50 (br s, 4H, CH_2-*N*), 1.87 (br s, 4H, CH_2). ^{13}C NMR (100 MHz, δ ppm, $CDCl_3$): 147.1, 144.5, 140.3, 133.3, 129.3, 129.1, 128.9, 116.6, 115.5, 54.0 (CH_2-*N*), 52.3 (CH_2-*N*), 25.63 (CH_2). Anal. Calcd for $C_{17}H_{20}N_2$: C, 80.91; H, 7.99; N, 11.10. Found: C, 80.99; H, 8.04; N, 11.15.

4.1.4.5. 4′-Chloro-6-((4-methylpiperazin-1-yl)methyl)-[1,1′-biphenyl]-3-amine 10e. Yellow solid, 71%, mp 131–133 °C. IR (KBr, ν cm^{-1}): 3361, 3208 (st. N–H). ^{1}H NMR (270 MHz, δ ppm, $CDCl_3$): 7.33 (m, 4H), 7.20 (d, 1H, J = 8.1 Hz), 6.64 (dd, 1H, H5, J = 8.2 Hz, J = 2.2 Hz), 6.64 (d, 1H, J = 2.2 Hz), 3.68 (s, 2H, NH_2), 3.28 (s, 2H, CH_2-*N*), 2.45 (br s, 8H, CH_2-*N*), 2.31 (3H, CH_3). ^{13}C NMR (100 MHz, δ ppm, $CDCl_3$): 145.5, 142.9, 140.1, 133.0, 132.0, 130.9, 128.0, 116.7, 114.2, 59.2 (CH_2-*N*), 54.9 (CH_2-*N*), 51.6 (CH_2-*N*), 45.5 (CH_3).

Anal. Calcd for $C_{18}H_{22}ClN_3$: C, 68.45; H, 7.02; N, 13.30. Found: C, 68.51; H, 6.98; N, 13.27.

4.1.4.6. 4′-Chloro-6-(piperidin-1-ylmethyl)-[1,1′-biphenyl]-3-amine 10f. Light yellow solid, 70%, mp 145–147 °C. IR (KBr, ν cm^{-1}): 3422, 3307 (st. N–H). ^1H NMR (400 MHz, δ ppm, $CDCl_3$): 7.46 (d, 1H, J = 8.3 Hz), 7.34 (d, 2H, J = 8.2 Hz), 7.28 (d, 2H, J = 8.4 Hz), 6.68 (dd, 1H, J = 8.3 Hz, J = 2.5), 6.52 (d, 1H, J = 2.5 Hz), 3.74 (s, NH_2), 3.47 (s, 2H, CH_2-*N*), 2.37 (br s, 4H, CH_2), 1.61 (br s, 4H, CH_2), 1.39 (2H, CH_2). ^{13}C NMR (100 MHz, δ ppm, $CDCl_3$): 145.9, 143.0, 139.9, 132.6, 130.9, 128.3, 116.5, 114.6, 59.1 (CH_2-*N*), 53.5 (CH_2-*N*), 25.0 (CH_2), 23.8 (CH_2). Anal. Calcd for $C_{18}H_{21}ClN_2$: C, 71.87; H, 7.04; N, 9.31. Found: C, 71.94; H, 7.08; N, 9.27.

4.1.4.7. 4′-Chloro-6-(morpholinomethyl)-[1,1′-biphenyl]-3-amine 10g. Yellow solid, 79%, mp 105–107 °C. IR (KBr, ν cm^{-1}): 3314, 3212 (st. N–H). ^1H NMR (270 MHz, δ ppm, $CDCl_3$): 7.31 (m, 5H), 6.68 (dd, 1H, J = 8.2 Hz, J = 2.5 Hz), 6.54 (d, 1H, J = 2.5 Hz), 3.71 (s, 2H, CH_2-*O*), 3.64 (s, 2H, NH_2), 3.45 (s, 2H, CH_2-*N*), 2.42 (br s, 4H, CH_2-*N*). ^{13}C NMR (100 MHz, δ ppm, $CDCl_3$): 146.2, 143.2, 139.8, 133.3, 132.6, 130.8, 128.3, 116.5, 114.6, 66.2 (CH_2-*O*), 59.2 (CH_2-*N*), 52.6 (CH_2-*N*). Anal. Calcd for $C_{17}H_{19}ClN_2O$: C, 67.43; H, 6.32; N, 9.25. Found: C, 67.49; H, 6.37; N, 9.28.

4.1.4.8. 4′-Chloro-6-(pyrrolidin-1-ylmethyl)-[1,1′-biphenyl]-3-amine 10h. Yellow solid, 71%, mp 99–101 °C. IR (KBr, ν cm^{-1}): 3430, 3280 (est. N–H). ^1H NMR (270 MHz, δ ppm, $CDCl_3$): 7.29–7.33 (m, 4H), 6.67 (d, 1H, J = 8.2 Hz, J = 2.5 Hz), 6.53 (d, 1H, J = 3.0 Hz), 3.67 (s, 2H, CH_2-*N*), 2.41 (br s, 4H, CH_2-*N*), 1.70 (br s, 4H, CH_2). ^{13}C NMR (60 MHz, δ ppm, $CDCl_3$): 145.3, 142.0, 140.2, 132.9, 131.6, 130.9, 128.1, 116.4, 114.6, 56.5 (CH_2-*N*), 53.6 (CH_2-*N*), 23.4 (CH_2). Anal. Calcd for $C_{17}H_{19}ClN_2$: C, 71.19; H, 6.68; N, 9.77. Found: C, 71.24; H, 6.64; N, 9.80.

4.1.5. General procedure for the preparation of 4-amino-quinolines 11a–h

To a solution of 4,7-dichloroquinoline (1 mmol) in ethanol (20 mL) was added concentrated hydrochloric acid (1 equiv), followed by the corresponding aniline (**10a**–**h**) (1 mmol) previously dissolved in ethanol (10 mL). The reaction mixture was heated under reflux with continuous stirring for 6 h. The mixture was then cooled at room temperature and the precipitated solid was filtrated, washed with ethanol and dried under vacuo to yield the desired quinolines **11a**–**h** as their practically pure dihydrochloride salts.

4.1.5.1. 7-Chloro-*N*-(6-((diethylamino)methyl)-[1,1′-biphenyl]-3-yl)quinolin-4-amine monohydrochloride 11a. In this case, concentrated HCl was added as a catalyst (1 drop). Beige solid, 82%, mp 198–200 °C. IR (KBr, ν cm^{-1}): 3342 (st. N–H). ^1H NMR (400 MHz, δ ppm, DMSO-d_6): 9.11 (s, 1H, N–H), 8.44 (d, 1H, H2, J = 5.5 Hz), 8.38 (d, 1H, H6, J = 9.2 Hz), 7.88 (d, 1H, H8, J = 2.2 Hz), 7.58 (d, 1H, H5, J = 8.4 Hz), 7.53 (dd, 1H, J = 8.8 Hz, J = 2.2 Hz), 7.34–7.43 (m, 6H), 7.15 (d, 1H, J = 2.2 Hz), 7.01 (d, 1H, J = 5.5 Hz), 3.43 (s, 2H, CH_2-*N*), 2.34 (q, 4H, CH_2-*N*), 0.82 (t, 6H, CH_3). ^{13}C NMR (100 MHz, δ ppm, DMSO-d_6): 151.5, 149.8, 148.7, 143.4, 141.0, 138.7, 134.7, 133.5, 131.4, 129.7, 128.7, 127.8, 127.7, 125.7, 124.9, 124.0, 121.7, 118.8, 102.3, 54.2 (CH_2-*N*), 46.6 (CH_2-*N*), 11.9 (CH_3). Anal. Calcd (monohydrochloride salt) for $C_{26}H_{28}Cl_3N_3$: C, 69.03; H, 6.02; N, 9.29. Found: C, 69.12; H, 6.09; N, 9.35.

4.1.5.2. 7-Chloro-*N*-(6-(piperidin-1-ylmethyl)-[1,1′-biphenyl]-3-yl)quinolin-4-amine dihydrochloride 11b. Yellow solid, 90%, mp 252–254 °C. IR (KBr, ν cm^{-1}): 3524, 3446 (st. N–H). ^1H NMR (400 MHz, δ ppm, DMSO-d_6): 8.77 (d, 1H, J = 8.8 Hz), 8.54 (d, 1H, J = 6.8 Hz), 8.10 (d, 1H, J = 1.8 Hz), 8.06 (d, 1H, J = 8.4 Hz), 7.85 (d, 1H, J = 9.1 Hz, J = 2.2 Hz), 7.65 (d, 1H, J = 8.4 Hz, J = 2.2 Hz), 7.40–7.54 (m, 6H), 7.10 (d, 1H, J = 6.9 Hz), 4.31 (s, 2H, CH_2-*N*), 2.51 (br s, 4H, CH_2-*N*), 1.66 (6H, CH_2). ^{13}C NMR (60 MHz, δ ppm, DMSO-d_6): 155.0, 145.7, 144.6, 139.8, 139.2, 138.5, 133.7, 129.9, 129.5, 128.7, 128.2, 126.9, 126.5, 126.4, 124.5, 120.1, 116.8, 101.6, 56.2 (CH_2-*N*), 52.7 (CH_2-*N*), 22.6 (CH_2), 21.45 (CH_2). Anal. Calcd (dihydrochloride salt) for $C_{27}H_{28}Cl_3N_3$: C, 64.74; H, 5.63; N, 8.39. Found: C, 64.85; H, 5.70; N, 8.29.

4.1.5.3. 7-Chloro-*N*-(6-(morpholinomethyl)-[1,1′-biphenyl]-3-yl)quinolin-4-amine dihydrochloride 11c. Yellow solid, 79%, mp 232–234 °C. IR (KBr, ν cm^{-1}): 3528, 3446 (st. N–H). ^1H NMR (400 MHz, δ ppm, DMSO-d_6): 8.79 (d, 1H, J = 9.2 Hz), 8.54 (d, 1H, H2, J = 7.0 Hz), 8.07 (m, 2H), 7.86 (d, 1H, J = 9.2 Hz), 7.63 (d, 1H, J = 7.3 Hz) 7.42–7.53 (m, 6H), 7.10 (d, 1H, J = 7.0 Hz), 4.34 (br s, 4H, CH_2-*O*), 3.80 (br s, 2H, CH_2-*N*), 2.49 (br s, 4H, CH_2-*N*). ^{13}C NMR (100 MHz, δ ppm, DMSO-d_6): 155.2, 145.7, 144.2, 139.6, 139.3, 139.2, 138.5, 133.9, 129.9, 129.4, 128.7, 128.2, 127.0, 126.7, 126.3, 124.6, 119.9, 116.8, 101.5, 63.5 (CH_2-*O*), 56.6 (CH_2-*N*), 51.7 (CH_2-*N*). Anal. Calcd (dihydrochloride salt) for $C_{26}H_{26}Cl_3N_3O$: C, 62.10; H, 5.21; N, 8.36. Found: C, 62.18; H, 5.17; N, 8.42.

4.1.5.4. 7-Chloro-*N*-(6-(pyrrolidin-1-ylmethyl)-[1,1′-biphenyl]-3-yl)quinolin-4-amine dihydrochloride 11d. Yellow solid, 87%, mp 228–230 °C. IR (KBr, ν cm^{-1}): 3525, 3400 (st. N–H). ^1H NMR (400 MHz, δ ppm, DMSO-d_6): 8.72 (d, 1H, J = 9.5 Hz), 8.52 (d, 1H, J = 7.4 Hz), 8.05 (d, 1H, J = 2.2 Hz), 7.94 (d, 1H, J = 8.1 Hz), 7.85 (d, 1H, J = 9.5 Hz), 7.62 (dd, 1H, J = 7.0 Hz, J = 2.2 Hz), 7.40–7.55 (m, 6H), 7.07 (d, 1H, J = 7.0 Hz), 4.38 (s, 2H, CH_2-*N*), 2.49 (br s, 4H, CH_2-*N*), 1.80 (br s, 4H, CH_2). ^{13}C NMR (60 MHz, δ ppm, DMSO-d_6): 155.0, 145.0, 144.6, 139.9, 139.2, 139.0, 138.7, 132.9, 129.8, 129.5, 128.8, 128.2, 127.9, 126.9, 126.4, 124.6, 120.2, 116.8, 101.5, 54.1 (CH_2-*N*), 53.8 (CH_2-*N*), 22.6 (CH_2). Anal. Calcd (dihydrochloride salt) for $C_{26}H_{26}Cl_3N_3$: C, 64.14; H, 5.38; N, 8.63. Found: C, 64.23; H, 5.44; N, 8.57.

4.1.5.5. 7-Chloro-*N*-(4′-chloro-6-((4-methylpiperazin-1-yl)methyl)-[1,1′-biphenyl]-3-yl)quinolin-4-amine dihydrochloride 11e. Orange solid, 38%, mp 238–240 °C. IR (KBr, ν cm^{-1}): 3448 (est. N–H). ^1H NMR (400 MHz, δ ppm, DMSO-d_6): 11.27 (s, 1H), 8.90 (d, 1H, J = 6.4 Hz), 8.56 (d, 1H, J = 8.6 Hz), 8.16 (d, 1H, J = 1.8 Hz), 7.90 (dd, 1H, J = 1.8 Hz, J = 9.5 Hz), 7.51–760 (m, 6H), 7.42 (d, 1H, J = 1.5 Hz), 7.02 (d, 1H, J = 7.4 Hz), 3.51 (s, 2H, CH_2-*N*), 2.75 (m, 8H, CH_2-*N*), 2.09 (s, 3H, *N*-CH_3). ^{13}C NMR (100 MHz, δ ppm, DMSO-d_6): 155.3, 144.1, 143.5, 139.5, 139.2, 138.6, 137.3, 133.3, 132.9, 131.8, 129.2, 129.0, 128.2, 126.9, 126.6, 124.7, 119.8, 116.7, 101.3, 52.2 (CH_2-*N*), 49.0 (CH_2-*N*), 42.6 (CH_2-*N*), 31.2 (*N*-CH_3). Anal. Calcd (dihydrochloride salt) for $C_{27}H_{28}Cl_4N_4$: C, 58.93; H, 5.13; N, 10.18. Found: C, 58.99; H, 5.09; N, 10.25.

4.1.5.6. 7-Chloro-*N*-(4′-chloro-6-(piperidin-1-ylmethyl)-[1,1′-biphenyl]-3-yl)quinolin-4-amine dihydrochloride 11f. Yellow solid, 84%, mp 260 °C *decomp*. IR (KBr, ν cm^{-1}): 3441 (st. N–H). ^1H NMR (270 MHz, δ ppm, DMSO-d_6): 8.42 (d, 1H, J = 8.8 Hz), 8.30 (d, 1H, J = 7.0 Hz), 7.91 (s, 1H), 7.74 (d, 1H, J = 8.2 Hz), 7.70 (d, 1H, J = 9.2 Hz), 7.54 (dd, 1H, J = 1.8 Hz, J = 8.2 Hz), 7.48 (d, 2H, J = 7.7 Hz), 7.33 (m, 3H), 7.03 (d, 1H, J = 7.0 Hz), 4.27 (s, 2H, CH_2-*N*), 2.66 (m, 4H, CH_2-*N*), 1.82 (br s, 4H, CH_2), 1.66 (br s, 2H, CH_2). ^{13}C NMR (100 MHz, δ ppm, DMSO-d_6): 155.2, 144.7, 143.7, 139.9, 139.2, 138.4, 137.5, 133.9, 133.6, 131.5, 129.4, 128.5, 126.8, 126.1, 125.4, 124.9, 119.8, 101.3, 55.4 (CH_2-*N*), 52.9 (CH_2-*N*), 22.5 (CH_2), 21.2 (CH_2). Anal. Calcd (dihydrochloride salt) for $C_{27}H_{27}Cl_4N_3$: C, 60.58; H, 5.08; N, 7.85. Found: C, 60.49; H, 5.03; N, 7.91.

4.1.5.7. 7-Chloro-*N*-(4′-chloro-6-(morpholinomethyl)-[1,1′-biphenyl]-3-yl)quinolin-4-amine dihydrochloride 11g. Yellow solid, 86%, mp 224–226 °C. IR (KBr, ν cm^{-1}): 3438 (st. N–H). ^1H NMR (400 MHz, δ ppm, DMSO-d_6): 11.02 (br s, 1H), 8.72 (d, 1H, J = 9.2 Hz), 8.51 (d, 1H, J = 6.6 Hz), 8.07 (d, 1H, J = 1.8 Hz), 7.98 (d, 1H, J = 8.4 Hz), 7.88 (d, 1H, J = 9.2 Hz), 7.63 (d, 1H, J = 9.2 Hz), 7.56 (d, 2H, J = 8.4 Hz), 7.47 (d, 2H, J = 8.1 Hz), 7.44 (d, 1H, J = 6.9 Hz), 7.07 (d, 1H, J = 6.9 Hz), 4.20 (s, 2H, CH_2-*N*), 3.79 (br s, 4H, CH_2-*O*), 2.90 (br s, 4H, CH_2-*N*). ^{13}C NMR (100 MHz, δ ppm, DMSO-d_6): 155.2, 144.6, 144.3, 139.5, 139.4, 138.6, 137.8, 133.9, 133.7, 131.8, 129.4, 128.3, 126.9, 126.3, 126.1, 124.9, 119.9, 116.7, 101.5, 63.6 (CH_2-*O*), 51.6 (CH_2-*N*), 40.0 (CH_2-*N*). Anal. Calcd (dihydrochloride) for $C_{26}H_{25}Cl_4N_3O$: C, 58.12; H, 4.69; N, 7.82. Found: C, 58.21; H, 4.62; N, 7.88.

4.1.5.8. 7-Chloro-*N*-(4′-chloro-6-(pyrrolidin-1-ylmethyl)-[1,1′-biphenyl]-3-yl)quinolin-4-amine dihydrochloride 11h. Yellow solid, 72%, mp 250–251 °C. IR (KBr, ν cm^{-1}): 3436 (st. N–H). ^1H NMR (270 MHz, δ ppm, DMSO-d_6): 8.91 (d, 1H, J = 8.8 Hz), 8.57 (d, 1H, J = 6.6 Hz), 8.20 (d, 1H, J = 8.1 Hz), 8.18 (d, 1H, J = 1.8 Hz), 7.84 (d, 1H, J = 9.2 Hz, J = 1.8 Hz), 7.64 (d, 1H, J = 8.2 Hz, J = 1.8 Hz), 7.57 (d, 2H, J = 8.4 Hz), 7.48 (d, 2H, J = 8.4 Hz), 7.45 (d, 1H, J = 6.6 Hz), 7.07 (d, 1H, J = 6.6 Hz), 4.36 (s, 2H, CH_2-*N*), 2.76 (br s, 4H, CH_2-*N*), 1.84 (br s, 4H, CH_2). ^{13}C NMR (100 MHz, δ ppm, DMSO-d_6): 154.9, 144.4, 143.3, 138.9, 138.3, 138.1, 133.6, 132.8, 132.3, 131.8, 129.3, 129.1, 128.3, 128.0, 126.9, 124.8, 120.1, 116.9, 101.5, 54.0 (CH_2-*N*), 53.5 (CH_2-*N*), 22.9 (CH_2). Anal. Calcd (dihydrochloride salt) for $C_{26}H_{25}Cl_4N_3$: C, 59.90; H, 4.83; N, 8.06. Found: C, 59.97; H, 4.87; N, 8.12.

4.2. Biological assays

4.2.1. Inhibition of haemozoin formation

The haemozoin formation assay was performed according to the literature.[32] Briefly, a solution of hemin chloride (50 μL, 4 mM), dissolved in DMSO (5.2 mg·mL^{-1}), was distributed in 96-well micro plates. Different concentrations (100–5 μM) of the quinoline compounds dissolved in DMSO, were added in triplicate in test wells (50 μL). Controls contained either water (50 μL) or DMSO (50 μL). β-Hematin formation was initiated by the addition Acetate buffer (100 μL 0.2 M, pH 4.4). Plates were incubated at 37 °C for 48 h to allow completion of the reaction and centrifuged (4000 rpm × 15 min, IEC-CENTRA, MP4R). After discarding the supernatant, the pellet was washed twice with DMSO (200 μL) and finally, dissolved in NaOH (200 μL, 0.2 N). The solubilized aggregates were further diluted 1:2 with NaOH (0.1 N) and absorbances recorded at 405 nm (Microplate Reader, BIORAD-550). The results were expressed as a percentage of inhibition of haemozin formation (% IHF).

4.2.2. Parasite, experimental host and strain maintenance

Male Balb-C mice, weighing 18–22 g were maintained on a commercial pellet diet and housed under conditions approved by Ethics Committee. *P. berghei* (ANKA strain), a rodent malaria parasite, was used for infection. Mice were infected by ip injection with 1×10^6 infected erythrocytes diluted in phosphate buffered saline solution (PBS, 10 mM, pH 7.4, 0.1 mL). Parasitemia was monitored by microscopic examination of Giemsa stained smears.

4.2.3. Four-days suppressive test

Balb-C mice (18–22 g) were infected iv (using caudal vein) with 10^6 infected RBC with *P. berghei* (n = 6). Two hours after infection, treatment began with the best compounds tested in the in vitro assays. These were dissolved in DMSO (0.1 M), diluted with Saline-Tween 20 solution (2%). Each compound (20 mg·kg^{-1}) was administered once by ip for 4 days. At day 4, the parasitemia was counted by examination of Giemsa stained smears. Chloroquine (25 mg·kg^{-1}) was used as a positive control. The survival time beyond the control group (saline treated) was recorded. The results were expressed as percentage of parasitemia (% of parasitemia) and survival days of each compound treated-group over the control (saline treated group).[34]

4.2.4. Data analysis

Data were statistically analyzed using one-way ANOVA and t-tests for specific group comparisons; assuming 95% of confidence according to GraphPad Prism 3.02.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.05.040.

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