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# Synthesis, Antiprotozoal, Antimicrobial, β-Hematin Inhibition, Cytotoxicity and Methemoglobin (MetHb) Formation Activities of Bis(8-aminoquinolines)

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## Abstract

In continuing our search of potent antimalarials based on 8-aminoquinoline structural framework, three series of novel bis(8-aminoquinolines) using convenient one to four steps synthetic procedures were synthesized. The bisquinolines were evaluated for in vitro antimalarial (*P. falciparum*), antileishmanial (*L. donovani*), antimicrobial (a panel of pathogenic bacteria and fungi), cytotoxicity, β-hematin inhibitory and methemoglobin (MetHb) formation activities. Several compounds exhibited superior antimalarial activities compared to parent drug primaquine. Selected compounds (**41, 61** and **79**) when tested for in vivo blood-schizontocidal antimalarial activity (*P. berghei*) displayed potent blood-schizontocial activities. The bisquinolines showed negligible MetHb formation (0.2 – 1.2%) underlining their potential in the treatment of glucose-6-phosphate dehydrogenase deficient patients. The bisquinoline analogues (**36, 73** and **79**) also exhibited promising in vitro antileishmanial activity, and antimicrobial activities (**43, 44** and **76**) against a panel of pathogenic bacteria and fungi. The results of this study provide evidence that bis(8-aminoquinolines), like their bis(4-aminoquinolines) and artemisinin dimers counterparts, are a promising class of antimalarial agents.

## Introduction

Malaria is a serious parasitic disease, which ranks third among the major infectious diseases in causing deaths after pneumococcal acute respiratory infections, and tuberculosis. Approximately 1.5–2.5 million people die of malaria every year, accounting for about 5% of all fatalities in the world.[1] Uncontrolled and irrational use of existing blood-schizontocidal

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(for example, chloroquine) and limited use of newer blood-schizontocidal antimalarials (for example, artemisinin and its derivatives) has limited the clinical options to treat malaria.[2] 8-Aminoquinolines exemplified by primaquine (PQ, **1**, Fig. 1) display activity against all four species of *Plasmodium* that infect human. PQ, a tissue schizontocidal antimalarial, is the only clinical drug available for the treatment of relapsing cases of malaria. Although PQ is effective against all life cycle stages of human malaria parasite, its use is often associated with serious adverse effects as a consequence of its toxic metabolites.[3-6] Its clinical use as a tissue-schizontocide is limited by side effects such as severe hematotoxicities in patients with the glucose-6-phosphate dehydrogenase (G6PD) deficiency.[7] PQ is also known to have poor pharmacokinetic properties with a short half life of 4-6 h.[8] It has been shown that in rodents, the PQ is rapidly metabolized, and two major metabolites start appearing in the blood in about 30 min, one of which has been identified as 4-(6-methoxyquinolin-8-ylamino)pentanoic acid (carboxyprimaquine, **2**, Fig. 1), a product also reported by the microbiological degradation of PQ.[9] Modulation of PQ structure has resulted in less toxic and promising blood-schizontocides.[3,9,10] We have recently shown that appropriate substitution on the quinoline ring and on the side-chain results in PQ analogues exhibiting promising antimicrobial and antileishmanial activities, in addition to potent antimalarial activities.[11-17]

Dimer of known antimalarial agents has been an area of active interest. For example, a number of bis(4-aminoquinolines)[18-21] and bis-artemisinins[22-24] are known to display activities superior to their monomeric counterparts. Superior activity of artemisinin dimers is attributed to their dual functionality and increased number of peroxide linkage which is essential for expression of activity. The higher efficacy of the bis(4-aminoquinolines) against chloroquine resistant parasites is explained by the greater number of protonation sites compared with chloroquine resulting in their accumulation to a higher degree in the face of a decreased pH gradient in the chloroquine resistant parasites. Although, dimers of 4-aminquinolines and artemisinin are extensively investigated, the potential of bis(8-aminoquinolines) in antimalarial drug discovery is not fully explored. Blanton et al. reported the synthesis of varied chain length amino group containing dimeric 8-aminoquinolines; however, all analogues were inactive as blood-schizontocides.[25,26]

The importance of dimers in 4-aminoquinoline and artemisinin classes prompted us to examine the unexplored potential of bis(8-aminoquinolines) in antimalarial chemotherapy. The synthesis of bis(8-aminoquinolines) can be justified due to following facts: i) It is known that rapid metabolism of 8-aminoquinolines results in the removal of side-chain amino group to yield inactive metabolites, including **2**. We believe that the side-chain primary amino group present as an amide or secondary amine in the synthesized bis(8-aminoquinolines) could prevent metabolic degradation resulting in increased activity; ii) due to increase in steric bulk, the bis(8-aminoquinolines) are expected to penetrate less into the red blood cell that may not allow destabilization of red cell membrane inducing hemolysis, the main cause of toxicity. Thus, synthesis of bis(8-aminoquinolines) appears to be an attractive strategy to develop analogues with improved blood-schizontocidal activity and reduced MetHb toxicity. We report herein, synthesis, detailed antiprotozoal, and antimicrobial activities of bis(8-aminoquinolines) linked through their side-chain using a set of linkers, including amino acids.

## Results and Discussion

### Chemistry

We have earlier observed that the cationic amino acid conjugates of 8-aminoquinolines exhibit promising biological activities.[14] The 8-aminoquinolines conjugated to Lys, Arg, and Orn provide two free amino groups (α- and side-chain $NH_2$ or guanidino group), which

can be manipulated to synthesize a series of bis(8-aminoquinolines). In order to maintain the structural similarity with earlier reported monomers,[14] conjugates of 8-aminoquinolines were coupled through their free α-$NH_2$ group with PQ (**1**), 2-*tert*-butylprimaquine (**3**, Fig. 1) to obtain bis(8-aminoquinolines) linked through cationic amino acids (**35-44**, Scheme 1). Starting material, PQ (**1**) was obtained commercially, while its analogues, **3** and (4-ethyl-5-pentyloxy)primaquine (**4**, Fig. 1) were synthesized following previously published procedure.[12,13] The reaction of **1** or **3** with suitably orthogonally protected D/L-amino acids in the presence of 1,3-diisopropylcarbodiimide (DIC) readily provided α- and side-chain $NH_2$ protected amino acid conjugates **5-14**. Depending upon its nature, α-$NH_2$ protecting group in **5-14** was removed under acidic or basic conditions to afford **15-24**. The compounds **15-24** upon coupling reaction with **1** or **3** in the presence of 1,1′-carbonyldiimidazole (CDI) afforded protected bis(8-quinolinmaines) **25-34**. The side-chain amino protecting groups in the compounds **25-34** were removed by either acidolysis or hydrogenolysis to afford desired bis(8-aminoquinolines) **35-44**.

The anionic amino acids, Asp and Glu contain three groups, which provide suitable functionalities for the synthesis of bis(8-aminoquinolines). We have earlier observed that the presence of free $NH_2$ group in the side-chain is essential for the activity of 8-aminoquinolines.[14] Therefore, in this series we decided to synthesize bis(8-aminoquinolines) (**60-64**, Scheme 2) by first coupling 8-aminoquinolines with Asp or Glu residues through their α-$CO_2H$ group followed by linking another molecule of 8-aminoquinolines to the side-chain β/γ-$CO_2H$ group. The coupling reaction of **1** or **3** with suitable orthogonally protected D/L-amino acids using DIC afforded **45-49**. The benzyl ester or *tert*-butyl ester group in compounds **45-49** was cleaved using Pd-C/$H_2$ or acidolysis to provide analogues **50-54**. The latter compounds, **50-54** upon coupling reaction with **1** or **3** using DIC provided side-chain protected analogues **55-59**. The *t*-Boc group in compounds **55-58** was removed by acidolysis to provide **60-63**, while Fmoc group in the compound **59** was cleaved with a 20% solution of piperidine to give analogue **64**.

We also report one-pot synthesis of bis(8-aminoquinolines) **66-85** attached via both aliphatic and aromatic linkers to examine their effect on the antimalarial activity (Scheme 3). Bisquinolines **66-85** were obtained by covalent attachment of **1, 3** and **4** through the linker at the side-chain primary amino group. The 8-aminoquinolines **1, 3** and **4** upon reaction with various electrophiles **65** either under neat conditions in the presence of excess triethylamine ($Et_3N$), or in anhydrous tetrahydrofuran (THF), or dichloromethane (DCM) in the presence of catalytic $Et_3N$ afforded analogues **66-85** in good yields. Depending upon the relative reactivities of the electrophile, temperature varying from 0 °C to 70 °C and reaction time of 3 h to 24 h was used. Coupling reactions with 1,1′-carbonyldiimidazole (CDI), 1,1′-thiocarbonyldiimidazole (TCDI), bis(2-chloroethyl)amine and 2,6/3,4/3,5-pyridinedicarbonyl chloride were carried out at ambient temperature. The electrophiles like *N*-(chlorocarbonyl)isocyanate, chloromethyl chloroformate, oxalyl chloride and chlorocarbonylsulfenyl chloride gave products at 0 °C; while, condensation reaction using chloroacetic acid was achieved in THF at 70 °C.

## Antimalarial activity, cytotoxicity, inhibition of *β*-hematin (BH) and MetHb formation

Determination of in vitro antimalarial activity was based on the assay of plasmodial LDH activity.[27] The antimalarial activities of all synthesized analogues are reported as $IC_{50}$ values against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *P. falciparum* in Tables 1-3.

The bis(8-aminoquinolines) **35-44** linked through amino acids (Series 1) were active against both strains of plasmodium, except **41**. Among the series, **44** [R = $C(CH_3)_3$, $R_1$ = D-Orn] was the most active and exhibited $IC_{50}$ values of 0.34 and 0.30 $\mu$g/mL against D6 and W2

strains, respectively. While, analogue **43** showed $IC_{50}$ of 1.6 and 1.3 $\mu$g/mL against D6 and W2 strains, respectively. The $IC_{50}$ values of remaining analogues were in the range of 2.4 – 4.2 $\mu$g/mL for D6 strain and 1.5-3.2 $\mu$g/mL for W2 strain. The bis(8-aminoquinolines) **60-64** (Series 2) were less active with $IC_{50}$ values in the range of 2.7 – 4.76 $\mu$g/mL (D6 strain) and 2.3 – 3.8 $\mu$g/mL (W2 strain). In Series 3, the most promising analogue **84**, a bisquinoline derivative linked through pyridine-3,4-dicarbonyl linker displayed $IC_{50}$ of 1.5 $\mu$g/mL for D6 clone and 0.87 $\mu$g/mL for W2 clone. Analogues **70** and **73** showed $IC_{50}$ of 1.7 and 1.5 $\mu$g/mL for D6 clone, respectively, and 1.3 $\mu$g/mL for W2 clone. While, analogue **79**, displayed $IC_{50}$ of 2.6 $\mu$g/mL, and 1.5 $\mu$g/mL for D6 and W2 strains, respectively. Remaining analogues were also active ($IC_{50}$ values in the range of 1.7 – 4.76 $\mu$g/mL) with the exception of **41, 62, 71-72, 75, 77-78**, and **83**.

In vitro cytotoxicity of all the analogues was determined against mammalian kidney cell line (Vero) up to a highest concentration of 10 $\mu$g/mL by neutral red assay.[28,29] None of the compounds were found cytotoxic indicating a selectivity of antimalarial action.

Blocking heme detoxification is one of the main mechanism of antimalarial action of quinolines, and we have recently reported that potent blood-schizontocidal antimalarial activity of **3** is possibly due to its inhibition of heme crystallization.[30] In another report, effects of amino acids on the formation of $\beta$-hematin (BH) was investigated, and results showed that BH formation was significantly inhibited by basic amino acids due to their abilities to complex with heme.[31] Therefore, synthesized bisquinolines were assayed for inhibition of BH formation according to a procedure reported earlier.[32] Analogue **44** (Series 1) inhibited BH formation with a lower $IC_{50}$ value (10.8 $\mu$M) in comparison to several other analogues of the series ($IC_{50}$ values ranging between 45 and 162 $\mu$M). The compounds **60-64** of the Series 2 inhibited BH formation with $IC_{50}$ values in the range of 85 – 185 $\mu$M. Among bisquinolines **66-85** of Series 3, analogues **70, 73** and **84** displayed $IC_{50}$ values of 80, 75 and 70 $\mu$M, respectively compared to $IC_{50}$ of 80 $\mu$M for standard drug chloroquine (Table 1-3). It has been proposed that pKa of the side chain amino function contributes substantially to antimalarial activity of chloroquine. Therefore, it is possible that reduced basicity of these bisquinolines due to decreased pKa values is responsible for moderate $\beta$-hematin inhibition.

Hematotoxicity by 8-aminoquinolines is caused due to their metabolism to the toxic metabolites, which are unstable and difficult to isolate. The synthesized analogues were also tested for metabolism-linked methemoglobin (MetHb) toxicity in vitro and % MetHb formation was calculated at 20 $\mu$g/mL of the test compound, in comparison to vehicle control.[33] A number of analogues showed lower MetHb formation (Series1: 0.2 – 7.9%, Series 2: 1.55 – 13.35%, and Series 3: 0.25 – 20.3%) compared to PQ (10%) as shown in Tables 1-3. Analogues **42** (Series 1), **62** (Series 2), **68, 69, 83**, and **85** (Series 3) induced substantially lower MetHb formation (0.2 – 1.2%) than others, including standard drug, PQ.

Selected analogues were evaluated in vivo for the blood-schizontocidal antimalarial activity against *P. berghei* (sensitive strain) in a rodent malaria model using procedure described earlier.[12] The analogues **44, 61** and **79** exhibited significantly high activity and cured 100% mice at a dose of 25 mg/kg, and were suppressive at the lowest tested dose of 10 mg/kg. The PQ dimer, **36**, linked through L-Arg was curative at 100 mg/kg, while analogues **43** and **76** were suppressive at the same concentration with 5/6 mice surviving on day 60. Remaining of the tested analogues **37, 62, 63, 66, 68**, and **74** were inactive at the highest test dose of 100 mg/kg.

## Antileishmanial activities

Antileishmanial activities of the bisquinolines were evaluated in vitro against *L. donovani* promastigotes by Alamar Blue assay.[34,35] From Series 1, **36** (R = H, $R_1$ = L-Arg) exhibited better antileishmanial activity ($IC_{50}$ = 3.1 $\mu$g/mL, and $IC_{90}$ = 7.2 $\mu$g/mL) compared to other compounds of the series ($IC_{50}$ in the range of 14 – 21 $\mu$g/mL, and $IC_{90}$ in the range of 33 – 40 $\mu$g/mL) as shown in Table 1. Among the analogues of Series 2, **60** (R = H, n = 1) and **63** [R = $C(CH_3)_3$, n = 2] were more active ($IC_{50}$ = 6.2 and 3.9 $\mu$g/mL and $IC_{90}$ = 30 and 10.7 $\mu$g/mL, respectively) than others (Table 2). Several bisquinolines of Series 3 also exhibited antileishmanial activities to a considerable extent (Table 3). Of these analogues, **70, 73** and **79** were most potent with $IC_{50}$ values of 3.5, 2.9, and 2.99 $\mu$g/mL and $IC_{90}$ of 7.0, 7.8, and 27 $\mu$g/mL, respectively, compared to the $IC_{50}$ of 1 $\mu$g/mL and $IC_{90}$ of 3.8 $\mu$g/mL for standard drug, pentamidine. Analogues **67**, and **69** were also promising ($IC_{50}$ = 4 – 9 $\mu$g/mL and $IC_{90}$ = 20 – 31 $\mu$g/mL).

## Antimicrobial activities

Bisquinolines were also tested for their antibacterial properties against *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* ATCC 43300 (MRSA), *Mycobacterium intracellulare* ATCC 23068, *Escherichia coli* ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853. Susceptibility testing is performed using a modified version of the CLSI (formerly NCCLS) methods.[36-40] *M. intracellulare* is tested using a modified method of Franzblau et al.[41] None of the analogues were active against *E. coli* and *P. aeruginosa* (data not shown). Analogues **43** and **44** were active against *S. aureus*. Their $IC_{50}$ values were 1.78 – 2.73 $\mu$g/mL and they showed a bactericidal activity at 5 $\mu$g/mL, for both strains of *S. aureus*. Analogues **36**-**42** also possessed moderate activity against MRSA with $IC_{50}$ values in the range of 6.5 – 15 $\mu$g/mL and MIC of 20 $\mu$g/mL. All were bactericidal at 20 $\mu$g/mL, except analogues **38** and **40**. Activities were also observed against *M. intracellulare* with **36, 42, 43**, and **44** ($IC_{50}$ ranged between 7.12 – 15 $\mu$g/mL, MIC = 20 $\mu$g/mL, and MBC = 20 $\mu$g/mL, for analogue **36**). The bis(8-aminoquinolines) of Series 2 were also active against MRSA; **62** and **63** being most active with $IC_{50}$ values of 6.5 and 8.5 $\mu$g/mL, MIC of 10 and 20 $\mu$g/mL, respectively, and bactericidal at 20 $\mu$g/mL. Analogues of Series 3 also exhibited antibacterial activity against MRSA with the exception of **79**. Of these, **67, 71** and **84** were bactericidal ($IC_{50}$ = 3 – 15 $\mu$g/mL, MIC = 5 – 20 $\mu$g/mL, and MBC = 10 – 20 $\mu$g/mL). Analogue **67** also exhibited promising activity against *M. intracellulare* ($IC_{50}$, MIC and MBC of 4.5, 10 and 20 $\mu$g/mL, respectively) (Table 5).

The antifungal activities of the bis(8-aminoquinolines) against the opportunistic fungi *Candida albicans* ATCC 90028, *Cryptococcus neoformans* ATCC 90113, and *Aspergillus fumigatus* ATCC 90906, along with the positive control amphotericin B are reported in Table 6. All compounds were inactive at 20 $\mu$g/mL against *C. glabrata*, *C. krusei*, and *A. fumigatus* (data not shown). The bis(8-aminoquinolines) of all three series showed activities against *C. neoformans* to a variable extent and were found to be fungicidal except **67** (Table 6). The bis(8-aminoquinolines) **60, 61**, and **62** (Series 2) showed moderate activity against *C. neoformans*. The analogue **60** produced $IC_{50}$ value of 7.5 $\mu$g/mL, and MIC and MFC values of 10 $\mu$g/mL; while, analogues **61** and **62** exhibited the $IC_{50}$ values of 15 and 10 $\mu$g/mL, respectively, and were fungicidal at 20 $\mu$g/mL. Among the bis(8-aminoquinolines) of Series 3, analogues **67, 71, 76** and **79** were active against *C. neoformans* with $IC_{50}$ values ranging between 4.5 and 15 $\mu$g/mL. The promising analogue **76** exhibited $IC_{50}$ of 4.5 $\mu$g/mL, MIC of 5 $\mu$g/mL, and was also fungicidal at 5 $\mu$g/mL.

## Conclusions

We have synthesized three series of bisquinolines based upon 8-aminoquinolines structural framework. The bis(8-aminoquinolines) produced promising antimalarial activity in vitro

against drug-sensitive and drug-resistant strains of *P. falciparum*, and potent activity in the rodent malaria model in vivo. Inhibition of *β*-hematin formation by bisquinolines, although moderate, indicated it as a plausible pathway of their antimalarial activity. MetHb formation was observed for a considerably lower extent by several analogues compared to PQ, thereby offering new avenues for the treatment of patients suffering from severe hematotoxicities due to the deficiency of cytosolic enzyme G6PD. Most promising analogue **44** not only produced promising in vitro and in vivo antimalarial activity but also exhibited promising in vitro antimicrobial activities. Analogues **36, 70** and **73** exhibited best antileishmanial activities, while analogue **76** exhibited highest antifungal activity (against *C. neoformans*). The combination of broad spectrum of activities without any cytotoxic effects to the mammalian cells added to substantially reduced MetHb formation capabilities makes bis(8-aminoquinolines) as a promising new structural class of compounds.

## Experimental

### Material and Methods

The synthesized bisquinolines were checked for their purity on pre-coated silica gel $G_{254}$ TLC plates (Merck) and the spots were visualized under UV light and by exposing them to iodine vapors. Column chromatographic purification was carried out on Merck silica gel (100-200 mesh). Melting points were recorded on a capillary melting point apparatus and are uncorrected. All solvents used for synthesis were of analytical grade and used without any further purification unless otherwise stated. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a 300 MHz Bruker FT-NMR (Avance DPX 300) spectrometer using tetramethylsilane as internal standard and the chemical shifts are reported in δ units. Mass spectra were recorded on a Finnigan Mat LCQ spectrometer (APCI/ESI). Elemental analyses were recorded on Elementar Vario EL spectrometer. The elemental analyses of all final compounds were within ± 0.4% of the expected values, unless otherwise stated. All reagents were purchased from Aldrich Chemicals Ltd.

### General method for the synthesis of protected bis(8-quinolinamines) (25-34)

A mixture of **15-24** (0.58 mmol) and CDI (0.64 mmol) in anhydrous $CH_2Cl_2$ (5 mL) was stirred at ambient temperature for 30 min. At that time, TLC showed the absence of starting material. 8-Quinolinamine (**1** or **3**, 0.58 mmol) was added and the reaction mixture was stirred for another 5 h. The reaction mixture was diluted with $CH_2Cl_2$ (20 mL) and washed with water (3 × 10 mL) followed by brine solution (5 mL). The organic layer was dried ($Na_2SO_4$) and concentrated to afford crude product. Purified by column chromatography on silica gel (100-200 mesh) using 1.5% $CH_3OH$ in $CH_2Cl_2$ to give **25-34** as viscous oil.

### Benzyl{(5*R*)-6-({4-[(6-methoxyquinolin-8-yl)amino]pentyl}amino)-5-[({4-[(6-methoxy-quinolin-8-yl)amino]pentyl}carbamoyl)amino]-6-oxohexyl}carbamate (25)

Yield: 85%; oil; IR ($CH_2Cl_2$): 3338, 1696 $cm^{-1;}$ $^{1}H$ NMR ($CDCl_3$): δ 8.51 (d, 2H, $J$ = 4.4 Hz), 7.89 (d, 2H, $J$ = 8.1 Hz), 7.30 (m, 7H), 6.87 (bs, 2H), 6.32 (m, 2H), 6.25 (m, 2H), 6.02 (bs, 2H), 5.05 (s, 2H), 4.96 (bs, 2H), 4.17 (t, 1H, $J$ = 5.1 Hz), 3.87 (s, 6H), 3.55 (m, 2H), 3.26 (m, 6H), 1.61 (m, 14H), 1.25 (d, 6H, $J$ = 6.0 Hz); $^{13}C$ NMR ($CDCl_3$): δ 174.8, 159.4, 156.4, 144.9, 144.3, 136.6, 135.3, 129.8, 128.4, 128.0, 121.8, 96.7, 91.6, 66.5, 55.2, 54.9, 51.9, 47.8, 42.1, 40.6, 34.6, 29.6, 23.4, 22.7, 20.5; MS (ESI): *m/z* 807 (M+1); anal. for $C_{45}H_{58}N_8O_6$ (806.5), calcd: C, 66.97; H, 7.24; N, 13.89; found: C, 67.07; H, 7.15; N, 13.73.

**Benzyl(*N′*-[(benzyloxy)carbonyl]-*N*-{(4*R*)-5-({4-[(6-methoxyquinolin-8-yl)amino]-pentyl}amino)-4-[({4-[(6-methoxyquinolin-8-yl)amino]pentyl}carbamoyl)amino]-5-oxopentyl}carbamimidoyl)carbamate (26)**

Yield: 77%; oil; IR ($CH_2Cl_2$): 3436, 1693 $cm^{-1;}$ $^1H$ NMR ($CDCl_3$): δ 9.32 (bs, 2H), 8.49 (d, 2H, *J* = 4.1 Hz), 7.87 (d, 2H, *J* = 8.5 Hz), 7.31 (m, 12H), 6.83 (bs, 2H), 6.35 (m, 2H), 6.28 (m, 2H), 5.98 (bs, 2H), 5.11 (s, 2H), 5.09 (s, 2H), 5.02 (bs, 2H), 4.27 (t, 1H, *J* = 5.8 Hz), 3.86 (s, 6H), 3.62 (m, 2H), 3.22 (m, 6H), 1.69 (m, 12H), 1.30 (d, 6H, *J* = 6.5 Hz); $^{13}C$ NMR ($CDCl_3$): δ 171.2, 160.1, 159.6, 156.8, 144.9, 143.9, 135.6, 134.8, 129.9, 128.8, 128.5, 127.9, 122.2, 120.5, 96.8, 92.1, 65.7, 55.1, 47.9, 45.8, 41.3, 39.7, 39.2, 33.7, 32.8, 28.3, 25.2, 21.7; MS (APCI): *m/z* 969 (M+1); anal. for $C_{53}H_{64}N_{10}O_8$ (968.4), calcd: C, 65.68; H, 6.66; N, 14.45; found: C, 65.75; H, 6.72; N, 14.32.

**Benzyl{(4*R*)-5-({4-[(6-methoxyquinolin-8-yl)amino]pentyl}amino)-4-[({4-[(6-methoxyquinolin-8-yl)amino]pentyl}carbamoyl)amino]-5-oxopentyl}carbamate (27)**

Yield: 79%; oil; IR ($CH_2Cl_2$): 3338, 1695 $cm^{-1;}$ $^1H$ NMR ($CDCl_3$): δ 8.52 (d, 2H, *J* = 4.2 Hz), 7.92 (d, 2H, *J* = 8.6 Hz), 7.34 (m, 7H), 6.70 (bs, 2H), 6.31 (m, 2H), 6.25 (m, 2H), 5.95 (bs, 2H), 5.11 (s, 2H), 4.78 (bs, 1H), 4.68 (bs, 1H), 4.30 (t, 1H, *J* = 5.3 Hz), 3.88 (s, 6H), 3.53 (m, 2H), 3.21 (m, 4H), 3.14 (t, 2H, *J* = 5.9 Hz), 1.78-1.53 (m, 12H), 1.32 (d, 6H, *J* = 6.3 Hz); $^{13}C$ NMR ($CDCl_3$): δ 173.0, 159.4, 159.3, 156.5, 144.9, 144.4, 135.8, 135.3, 134.8, 129.9, 122.0, 121.8, 96.7, 92.1, 91.6, 65.2, 55.1, 47.9, 40.8, 40.3, 33.8, 33.7, 31.2, 30.9, 28.3, 26.0, 20.7; MS (APCI): *m/z* 793 (M+1); anal. for $C_{44}H_{56}N_8O_6$ (792.3), calcd: C, 66.64; H, 7.12; N, 14.13; found: C, 66.58; H, 7.24; N, 14.27.

***tert*-Butyl{(5*S*)-6-({4-[(6-methoxyquinolin-8-yl)amino]pentyl}amino)-5-[({4-[(6-methoxyquinolin-8-yl)amino]pentyl}carbamoyl)amino]-6-oxohexyl}carbamate (28)**

Yield: 75%; oil; IR ($CH_2Cl_2$): 3334, 1705, 1665 $cm^{-1;}$ $^1H$ NMR ($CDCl_3$): δ 8.69 (m, 2H), 8.08 (m, 2H), 7.47 (m, 2H), 7.00 (bs, 1H), 6.49 (m, 2H), 6.42 (m, 2H), 5.87 (bs, 1H), 5.78 (bs, 1H), 5.33 (bs, 1H), 5.23 (bs, 1H), 4.39 (t, 1H, *J* = 5.7 Hz), 4.04 (s, 6H), 3.70 (m, 2H), 3.36 (m, 6H), 1.91 (m, 14H), 1.57 (s, 9H), 1.41 (m, 6H); $^{13}C$ NMR ($CDCl_3$): δ 173.7, 158.8, 156.8, 145.4, 144.8, 135.8, 135.4, 122.4, 97.3, 92.1, 81.2, 55.7, 54.2, 48.3, 48.2, 40.7, 39.7, 34.5, 34.3, 32.7, 32.4, 30.1, 28.9, 27.4, 26.6, 23.2, 21.0; MS (APCI): *m/z* 773 (M+1); anal. for $C_{42}H_{60}N_8O_6$ (772.4), calcd: C, 65.26; H, 7.82; N, 14.50; found: C, 65.34; H, 7.74; N, 14.63.

***N*-{4-[(6-methoxyquinolin-8-yl)amino]pentyl}-$N^2$-({4-[(6-methoxyquinolin-8-yl)amino]pentyl}carbamoyl)-$N^5$-{*N*-[(2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-chromen-6-yl)sulfonyl]carbamimidoyl}-L-ornithinamide (29)**

Yield: 64%; oil; IR ($CH_2Cl_2$): 3031, 1665, 1386 $cm^{-1;}$ $^1H$ NMR ($CDCl_3$): δ 8.72 (bs, 2H), 8.42 (d, 2H, *J* = 2.8 Hz), 7.84 (d, 2H, *J* = 7.8 Hz), 7.22 (dd, 2H, *J* = 2.8 and 7.8 Hz), 6.25 (d, 2H, *J* = 2.1 Hz), 6.18 (d, 2H, *J* = 2.1 Hz), 5.62 (bs, 2H), 4.25 (t, 1H, *J* = 6.4 Hz), 3.91 (s, 6H), 3.50 (m, 2H), 3.21 (m, 6H), 2.49 (m, 11H), 1.72 (m, 12H), 1.21 (m, 12H); $^{13}C$ NMR ($CDCl_3$): δ 172.1, 158.3, 156.6, 155.4, 152.6, 143.8, 134.4, 134.1, 133.8, 128.9, 123.1, 120.8, 117.0, 95.8, 90.7, 54.1, 46.7, 43.9, 39.6, 38.5, 32.9, 31.7, 25.7, 25.0, 24.5, 24.1, 23.2, 20.3, 19.3, 17.5, 16.4; MS (ESI): *m/z* 989.1 (M+23); anal. for $C_{51}H_{70}N_{10}O_7S$ (966.5), calcd: C, 63.33; H, 7.29; N, 14.48; found: C, 63.24; H, 7.35; N, 14.65.

***tert*-Butyl{(4*S*)-5-({4-[(6-methoxyquinolin-8-yl)amino]pentyl}amino)-4-[({4-[(6-methoxyquinolin-8-yl)amino]pentyl}carbamoyl)amino]-5-oxopentyl}carbamate (30)**

Yield: 71%; oil; IR ($CH_2Cl_2$): 3436, 1681 $cm^{-1;}$ $^1H$ NMR ($CDCl_3$): δ 8.51 (m, 2H), 7.91 (m, 2H), 7.30 (m, 2H), 6.32 (m, 2H), 6.24 (m, 2H), 4.31 (t, 1H, *J* = 6.1 Hz), 3.86 (s, 6H), 3.51 (m, 2H), 3.16 (m, 6H), 1.75 (m, 12H), 1.38 (s, 9H), 1.28 (m, 6H); $^{13}C$ NMR ($CDCl_3$): δ

172.3, 159.2, 157.8, 145.6, 143.8, 135.3, 135.0, 122.7, 97.2, 92.1, 80.8, 55.7, 54.2, 48.8, 48.2, 40.6, 38.8, 34.7, 32.8, 32.4, 30.1, 28.9, 27.4, 23.6, 23.2, 21.0; MS (APCI): *m/z* 759 (M +1); anal. for $C_{41}H_{58}N_8O_6$ (758.4), calcd: C, 64.88; H, 7.70; N, 14.76; found: C, 64.75; H, 7.64; N, 14.81.

**Benzyl{(5*R*)-6-({4-[(2-*tert*-butyl-6-methoxyquinolin-8-yl)amino]pentyl}amino)-5-[({4-[(2-*tert*-but-yl-6-methoxyquinolin-8-yl)amino]pentyl}carbamoyl)amino]-6-oxohexyl}carbamate (31)**

Yield: 80%; oil; IR ($CH_2Cl_2$): 3436, 1691 $cm^{-1;}$ $^1H$ NMR ($CDCl_3$): δ 7.85 (d, 2H, *J* = 8.5 Hz), 7.42 (d, 2H, *J* = 8.5 Hz), 7.37 (m, 5H), 6.29 (d, 2H, *J* = 1.5 Hz), 6.24 (d, 2H, *J* = 1.5 Hz), 5.07 (s, 2H), 4.11 (t, 1H, *J* = 6.0 Hz), 3.85 (s, 6H), 3.51 (m, 2H), 3.22 (m, 6H), 1.84 (m, 14H), 1.41 (s, 18H), 1.28 (d, 6H, *J* = 6.2 Hz); $^{13}C$ NMR ($CDCl_3$): δ 173.8, 163.8, 159.3, 158.8, 158.4, 157.2, 145.4, 137.1, 135.5, 134.1, 129.0, 128.6, 128.5, 128.0, 119.3, 97.1, 91.9, 67.0, 57.4, 55.6, 54.2, 48.4, 48.3, 41.0, 40.8, 39.9, 38.2, 34.6, 32.8, 30.8, 29.9, 29.7, 27.4, 26.4, 23.1, 21.1; MS (APCI): *m/z* 919 (M+1); anal. for $C_{53}H_{74}N_8O_6$ (918.5), calcd: C, 69.25; H, 8.11; N, 12.19; found: C, 69.33; H, 8.20; N, 12.05.

**Benzyl{(4*R*)-5-({4-[(2-*tert*-butyl-6-methoxyquinolin-8-yl)amino]pentyl}amino)-4-[({4-[(2-*tert*-but-yl-6-methoxyquinolin-8-yl)amino]pentyl}carbamoyl)amino]-5-oxopentyl}carbamate (32)**

Yield: 77%; oil; IR ($CH_2Cl_2$): 3385, 1663 $cm^{-1;}$ $^1H$ NMR ($CDCl_3$): δ 7.85 (d, 2H, *J* = 8.5 Hz), 7.41 (d, 2H, *J* = 8.5 Hz), 7.29 (m, 5H), 6.54 (bs, 2H), 6.29 (d, 2H, *J* = 2.7 Hz), 6.23 (d, 2H, *J* = 2.7 Hz), 6.09 (bs, 2H), 5.85 (bs, 1H), 5.04 (s, 2H), 4.87 (bs, 1H), 3.85 (s, 6H), 3.54 (m, 2H), 3.20 (m, 7H), 1.61 (m, 12H), 1.40 (s, 18H), 1.28 (m, 6H); $^{13}C$ NMR ($CDCl_3$): δ 172.8, 163.4, 163.3, 158.8, 158.7, 144.8, 139.2, 134.9, 133.5, 128.4, 128.0, 127.4, 118.7, 114.0, 96.6, 91.4, 66.6, 55.1, 53.1, 47.9, 47.6, 40.0, 39.3, 37.6, 34.0, 33.7, 31.6, 30.2, 29.6, 29.3, 29.1, 28.9, 26.7, 25.9, 22.6, 20.6; MS (MALDI): *m/z* 905 (M+1); anal. for $C_{52}H_{72}N_8O_6$ (904.5), calcd: C, 69.00; H, 8.02; N, 12.38; found: C, 69.07; H, 8.17; N, 12.51.

***tert*-Butyl{(5*S*)-6-({4-[(2-*tert*-butyl-6-methoxyquinolin-8-yl)amino]pentyl}amino)-5-[({4-[(2-*tert*-butyl-6-methoxyquinolin-8-yl)amino]pentyl}carbamoyl)amino]-6-oxohexyl}carbamate (33)**

Yield: 84%; oil; IR ($CH_2Cl_2$): 3360, 1673 $cm^{-1;}$ $^1H$ NMR ($CDCl_3$): δ 7.85 (d, 2H, *J* = 8.5 Hz), 7.42 (d, 2H, *J* = 8.5 Hz), 6.48 (bs, 1H), 6.30 (m, 2H), 6.22 (m, 2H), 6.11 (bs, 2H), 5.06 (bs, 2H), 4.21 (t, 1H, *J* = 5.8 Hz), 3.87 (s, 6H), 3.56 (m, 2H), 3.19 (m, 6H), 1.65 (m, 14H), 1.41 (m, 27H), 1.28 (d, 6H, *J* = 6.2 Hz); $^{13}C$ NMR ($CDCl_3$): δ 174.3, 169.0, 163.4, 157.5, 145.4, 135.5, 134.1, 128.0, 119.3, 97.1, 91.9, 71.2, 55.7, 48.4, 38.2, 34.2, 30.8, 30.1, 28.9, 21.2, 21.1; MS (APCI): *m/z* 885 (M+1); anal. for $C_{50}H_{76}N_8O_6$ (884.5), calcd: C, 67.84; H, 8.65; N, 12.66; found: C, 67.79; H, 8.74; N, 12.54.

***tert*-Butyl{(4*S*)-5-({4-[(2-*tert*-butyl-6-methoxyquinolin-8-yl)amino]pentyl}amino)-4-[({4-[(2-*tert*-butyl-6-methoxyquinolin-8-yl)amino]pentyl}carbamoyl)amino]-5-oxopen-tyl}carbamate (34)**

Yield: 80%; oil; IR ($CH_2Cl_2$): 3378, 1686 $cm^{-1;}$ $^1H$ NMR ($CDCl_3$): δ 7.86 (d, 2H, *J* = 8.5 Hz), 7.42 (d, 2H, *J* = 8.5 Hz), 6.66 (bs, 1H), 6.30 (m, 4H), 6.11 (bs, 2H), 5.20 (bs, 1H), 4.73 (bs, 1H), 3.86 (s, 6H), 3.56 (m, 3H), 3.18 (m, 6H), 1.64 (m, 12H), 1.41 (m, 27H), 1.28 (m, 6H); $^{13}C$ NMR ($CDCl_3$): δ 172.9, 163.4, 163.3, 158.8, 158.7, 144.9, 139.3, 134.9, 133.5, 118.7, 114.0, 96.6, 91.4, 80.6, 66.6, 55.1, 53.1, 47.9, 40.0, 39.3, 37.6, 33.7, 31.6, 29.3, 22.6, 21.2; MS (APCI): *m/z* 871 (M+1); anal. for $C_{49}H_{74}N_8O_6$ (870.5), calcd: C, 67.56; H, 8.56; N, 12.86; found: C, 67.46; H, 8.51; N, 12.94.

### General method for the synthesis of *N*-{4-[(6-methoxy-2-substituted-quinolin-8-yl)amino]-pentyl}-$N^2$-({4-[(6-methoxy-2-substituted-quinolin-8-yl)amino]pentyl}-carbamoyl)-D/L-lysinamide/argininamide/ornithinamide (35-44)

**a) Removal of Z group—**To a mixture of **25-27, 31** and **32** (0.12 mmol) and 10% Pd-C (0.04 g) in glacial acetic acid (1 mL) and $CH_3OH$ (20 mL) was bubbled $H_2$ gas for 4 h. The catalyst was removed and solvent evaporated to obtain the product as oily syrup, which upon treatment with ethereal HCl (2N solution) provided **35-37, 41** and **42** as hydrochloride salts.

**b) Removal of t-Boc and Pmc groups—**A solution of **28-30, 31** and **32** (0.43 mmol) in 6N methanolic HCl (10 mL) was stirred at ambient temperature for 45 min (for *t*-Boc group) or 8N HCl in MeOH (10 mL) for 8 h (for Pmc group). The solvent was removed to afford **38-40, 41** and **42** as hygroscopic salts.

### *N*-{4-[(6-methoxyquinolin-8-yl)amino]pentyl}-$N^2$-({4-[(6-methoxyquinolin-8-yl)-amino]pentyl}carbamoyl)-D-lysinamide·3HCl (35)

Yield: 81%; hygroscopic solid; IR (KBr): 3338, 1664 $cm^{-1;}$ $^1H$ NMR ($CD_3OD$): δ 8.84 (m, 4H), 7.90 (m, 2H), 6.81 (m, 4H), 4.25 (t, 1H, *J* = 5.1 Hz), 3.97 (s, 6H), 3.76 (m, 2H), 3.35 (m, 4H), 2.96 (t, 2H, *J* = 5.6 Hz), 1.74 (m, 14H), 1.25 (d, 6H, *J* = 6.5 Hz); $^{13}C$ NMR ($CD_3OD$): δ 172.1, 161.9, 158.9, 145.3, 140.2, 139.3, 133.0, 122.6, 106.7, 97.4, 56.1, 50.9, 41.0, 39.9, 33.3, 32.1, 27.4, 26.8, 26.1, 23.3, 19.8; MS (APCI): *m/z* 673 (M+1).

### *N*-{4-[(6-methoxyquinolin-8-yl)amino]pentyl}-$N^2$-({4-[(6-methoxyquinolin-8-yl)-amino]pentyl}carbamoyl)-D-argininamide·3HCl (36)

Yield: 78%; hygroscopic solid; IR (KBr): 3435 $cm^{-1;}$ $^1H$ NMR (free base, $CDCl_3$): δ 8.48 (d, 2H, *J* = 4.0 Hz), 7.87 (d, 2H, *J* = 8.2 Hz), 7.71 (bs, 1H), 7.42 (bs, 1H), 7.24 (dd, 2H, *J* = 4.0 and 8.2 Hz), 6.98 (bs, 1H), 6.28 (m, 2H), 6.21 (m, 2H), 5.89 (bs, 2H), 4.13 (t, 1H, *J* = 6.0 Hz), 3.82 (s, 6H), 3.46 (m, 2H), 3.05 (m, 6H), 1.51 (m, 12H), 1.25 (d, 6H, *J* = 6.0 Hz); $^{13}C$ NMR (free base, $CDCl_3$): δ 173.5, 159.3, 158.9, 157.3, 144.8, 144.3, 135.2, 134.9, 129.9, 121.9, 96.9, 91.7, 55.2, 53.4, 47.4, 40.8, 40.2, 39.4, 33.9, 29.7, 26.8, 25.9, 25.0, 20.3; MS (APCI): *m/z* 701 (M+1).

### *N*-{4-[(6-methoxyquinolin-8-yl)amino]pentyl}-$N^2$-({4-[(6-methoxyquinolin-8-yl)-amino]pentyl}carbamoyl)-D-ornithinamide·3HCl (37)

Yield: 83%; hygroscopic solid; IR (KBr): 3383 $cm^{-1;}$ $^1H$ NMR ($CD_3OD$): δ 8.86 (m, 2H), 8.80 (m, 2H), 7.92 (m, 2H), 7.04 (m, 2H), 6.91 (m, 2H), 4.10 (t, 1H, *J* = 5.7 Hz), 3.98 (m, 6H), 3.81 (m, 2H), 2.99 (m, 6H), 1.98-1.74 (m, 12H), 1.36 (m, 6H); $^{13}C$ NMR ($CD_3OD$): δ 173.8, 170.1, 158.6, 145.9, 141.3, 134.0, 123.5, 98.1, 92.7, 56.7, 54.9, 50.2, 40.8, 40.4, 34.6, 30.4, 29.9, 27.2, 25.4, 24.2, 20.1; MS (APCI): *m/z* 659 (M+1).

### *N*-{4-[(6-methoxyquinolin-8-yl)amino]pentyl}-$N^2$-({4-[(6-methoxyquinolin-8-yl)-amino]pentyl}carbamoyl)-L-lysinamide·3HCl (38)

Yield: 97%; hygroscopic solid; IR (KBr): 3404, 1648 $cm^{-1;}$ $^1H$ NMR ($CD_3OD$): δ 8.96 (m, 6H), 7.89 (m, 2H), 7.61 (m, 2H), 4.19 (t, 1H, *J* = 5.2 Hz), 3.96 (s, 6H), 3.73 (m, 2H), 3.35 (m, 4H), 3.01 (t, 2H, *J* = 6.0 Hz), 1.71 (m, 14H), 1.32 (m, 6H); $^{13}C$ NMR ($CD_3OD$): δ 176.0, 162.8, 161.1, 145.9, 141.3, 140.3, 135.7, 133.9, 120.9, 107.1, 97.4, 57.0, 54.2, 41.4, 40.9, 34.3, 28.4, 28.2, 27.3, 24.2, 20.1; MS (APCI): *m/z* 673 (M+1).

### *N*-{4-[(6-methoxyquinolin-8-yl)amino]pentyl}-$N^2$-({4-[(6-methoxyquinolin-8-yl)-amino]pentyl}carbamoyl)-L-argininamide·3HCl (39)

Yield: 86%; hygroscopic solid; IR (KBr): 3433 $cm^{-1;}$ $^1H$ NMR ($CD_3OD$): δ 8.88 (m, 2H), 8.73 (m, 2H), 7.81 (m, 2H), 7.52 (m, 4H), 4.15 (t, 1H, *J* = 5.7 Hz), 3.87 (s, 6H), 3.64 (m, 2H), 3.15 (m, 6H), 2.02-1.75 (m, 12H), 1.24 (d, 6H, *J* = 5.3 Hz); $^{13}C$ NMR ($CD_3OD$): δ 175.6, 162.9, 161.9, 161.5, 159.0, 146.7, 146.2, 141.2, 140.4, 139.2, 138.8, 135.7, 134.0, 120.9, 97.4, 92.1, 57.1, 42.4, 41.6, 34.4, 34.1, 33.9, 31.1, 28.1, 27.5, 26.8, 22.7, 20.3; MS (APCI): *m/z* 701 (M+1).

### *N*-{4-[(6-methoxyquinolin-8-yl)amino]pentyl}-$N^2$-({4-[(6-methoxyquinolin-8-yl)-amino]pentyl}carbamoyl)-L-ornithinamide·3HCl (40)

Yield: 91%; hygroscopic solid; IR (KBr): 3400, 1631 $cm^{-1;}$ $^1H$ NMR ($CD_3OD$): δ 8.86 (m, 2H), 8.74 (m, 2H), 7.81 (m, 2H), 6.90 (m, 2H), 6.85 (m, 2H), 4.13 (t, 1H, *J* = 6.0 Hz), 3.87 (s, 6H), 3.65 (m, 2H), 3.22 (m, 4H), 2.90 (t, 2H, *J* = 6.4 Hz), 1.62 (m, 12H), 1.29 (m, 6H); $^{13}C$ NMR ($CD_3OD$): δ 171.4, 160.9, 144.6, 143.8, 139.7, 138.5, 133.9, 132.1, 121.7, 121.6, 119.1, 97.6, 93.1, 55.2, 53.4, 39.5, 38.7, 32.5, 29.5, 29.1, 28.7, 26.5, 25.6, 23.9, 23.6, 21.5; MS (APCI): *m/z* 659 (M+1).

### *N*-{4-[(2-*tert*-butyl-6-methoxyquinolin-8-yl)amino]pentyl}-$N^2$-({4-[(2-*tert*-butyl-6-methoxyquinlin-8-yl)amino]pentyl}carbamoyl)-D-lysinamide·3HCl (41)

Yield: 84%; hygroscopic solid; IR (KBr): 3391, 1643 $cm^{-1;}$ $^1H$ NMR (free base, $CDCl_3$): δ 7.85 (d, 2H, *J* = 7.8 Hz), 7.42 (d, 2H, *J* = 7.8 Hz), 6.76 (bs, 1H), 6.29 (m, 2H), 6.22 (m, 2H), 6.10 (bs, 2H), 4.67 (bs, 2H), 4.23 (t, 1H, *J* = 6.4 Hz), 3.85 (s, 6H), 3.53 (m, 2H), 3.21 (m, 4H), 2.67 (t, 2H, *J* = 5.3 Hz), 2.22 (bs, 2H), 1.60 (m, 14H), 1.40 (s, 18H), 1.27 (d, 6H, *J* = 5.7 Hz); $^{13}C$ NMR (free base, $CDCl_3$): δ 174.3, 169.0, 163.2, 157.7, 145.4, 144.0, 135.5, 134.1, 128.0, 119.3, 97.1, 91.9, 55.7, 53.0, 48.4, 38.2, 34.6, 30.8, 30.2, 21.1; MS (APCI): *m/z* 785 (M+1).

### *N*-{4-[(2-*tert*-butyl-6-methoxyquinolin-8-yl)amino]pentyl}-$N^2$-({4-[(2-*tert*-butyl-6methoxyquinolin-8-yl)amino]pentyl}carbamoyl)-D-ornithinamide·3HCl (42)

Yield: 79%; hygroscopic solid; IR (KBr): 3403, 1678 $cm^{-1;}$ $^1H$ NMR ($CD_3OD$): δ 8.37 (d, 2H, *J* = 8.1 Hz), 7.81 (d, 2H, *J* = 8.1 Hz), 7.20 (m, 2H), 7.18 (m, 2H), 4.10 (t, 1H, *J* = 5.1 Hz), 3.98 (s, 6H), 3.86 (m, 2H), 3.25 (m, 6H), 1.77 (m, 12H), 1.49 (s, 18H), 1.28 (d, 6H, *J* = 6.1 Hz); $^{13}C$ NMR ($CD_3OD$): δ 175.1, 169.3, 158.2, 145.0, 144.8, 137.9, 132.4, 121.9, 119.3, 97.1, 91.9, 60.1, 56.8, 54.9, 40.7, 39.3, 32.1, 30.8, 30.4, 27.5, 26.6, 25.0, 21.1; MS (APCI): *m/z* 771 (M+1).

### *N*-{4-[(2-*tert*-butyl-6-methoxyquinolin-8-yl)amino]pentyl}-$N^2$-({4-[(2-*tert*-butyl-6methoxyquinolin-8-yl)amino]pentyl}carbamoyl)-L-lysinamide·3HCl (43)

Yield: 92%; hygroscopic solid; IR (KBr): 3360, 1686 $cm^{-1;}$ $^1H$ NMR ($CD_3OD$): δ 8.34 (d, 2H, *J* = 8.8 Hz), 7.81 (d, 2H, *J* = 8.8 Hz), 7.71 (m, 2H), 7.59 (m, 2H), 4.12 (t, 1H, *J* = 7.2 Hz), 3.99 (s, 6H), 3.23 (m, 2H), 3.14 (m, 4H), 2.96 (t, 2H, *J* = 7.2 Hz), 1.81-1.69 (m, 14H), 1.49 (s, 18H), 1.37 (d, 6H, *J* = 6.4 Hz); $^{13}C$ NMR ($CD_3OD$): δ 176.0, 169.4, 160.9, 158.5, 144.7, 137.9, 136.6, 130.2, 122.2, 97.3, 91.6, 57.0, 41.0, 40.9, 39.6, 32.4, 30.8, 30.5, 28.5, 27.9, 24.2, 21.3; MS (APCI): *m/z* 785 (M+1).

### *N*-{4-[(6-methoxyquinolin-8-yl)amino]pentyl}-$N^2$-({4-[(6-methoxyquinolin-8-yl)-amino]pentyl}carbamoyl)-L-lysinamide·3HCl (44)

Yield: 94%; hygroscopic solid; IR (KBr): 3398, 1678 $cm^{-1;}$ $^1H$ NMR ($CD_3OD$): δ 8.34 (d, 2H, *J* = 8.7 Hz), 7.81 (d, 2H, *J* = 8.7 Hz), 7.56 (m, 2H), 7.46 (m, 2H), 4.08 (t, 1H, *J* = 5.7 Hz), 3.98 (s, 6H), 3.22 (m, 2H), 3.00 (m, 4H), 2.70 (t, 2H, *J* = 7.0 Hz), 1.79 (m, 12H), 1.48

(s, 18H), 1.36 (m, 6H); $^{13}$C NMR ($CD_3OD$): δ 172.3, 162.3, 161.8, 154.3, 145.6, 135.2, 134.7, 128.3, 120.5, 98.2, 91.6, 55.5, 39.2, 38.9, 37.9, 30.5, 29.0, 26.1, 25.2, 23.6, 21.6; MS (APCI): *m/z* 771 (M+1).

### General method for the synthesis of protected bis(8-quinolinamines) (55-59)

**a) Removal of benzyl ester group—**To a mixture of **45-48** (0.1 mmol), 10% Pd-C (0.04 g) in glacial acetic acid (1 mL) and $CH_3OH$ (20 mL), $H_2$ gas was bubbled for 4 h. The catalyst was filtered and solvent removed to afford **50-53** as oily syrup

**b) Removal of tert-butyl ester group—**A solution of **49** in 6N HCl (5 mL) was stirred for 5 h at ambient temperature. The solvent was removed to afford HCl salt, which was dissolved in water (5 mL) and neutralized by drop wise addition of 25% $NH_4OH$ solution. The mixture was extracted with $CH_2Cl_2$ (3 × 20 mL). The organic layer washed with brine (5 mL) and dried ($Na_2SO_4$). The solvent was removed to afford **54** as oil. The formation of intermediate products **50-54** was confirmed by TLC and mass spectral analysis, which were used for the next step without any purification.

To an ice cooled stirred solution of 8-quinolinamine (**1** or **3**, 0.24 mmol) and **50-54** (0.24 mmol) in anhydrous $CH_2Cl_2$ (5 mL), DIC (0.26 mmol) was added. The reaction mixture was allowed to attain room temperature and stirring was continued for another 4 h. The solvent was removed yielding the crude product, which was purified by column chromatography on silica gel (100-200 mesh) using 1.2-2% $CH_3OH$ in $CH_2Cl_2$ to afford **55-59** as oil.

### *tert*-Butyl[(2*S*)-1,4-bis({4-[(6-methoxyquinolin-8-yl)amino]pentyl}amino)-1,4-dioxobutan-2-yl]carbamate (55)

Yield: 81%; oil; IR ($CH_2Cl_2$): 3338, 1656 cm$^{-1;}$ $^1$H NMR ($CDCl_3$): δ 8.52 (d, 2H, *J* = 3.9 Hz), 7.92 (d, 2H, *J* = 8.0 Hz), 7.31 (dd, 2H, *J* = 3.9 and 8.0 Hz), 6.86 (bs, 2H), 6.54 (bs, 2H), 6.32 (m, 2H), 6.25 (m, 2H), 5.98 (bs, 1H), 4.38 (m, 1H), 3.87 (s, 6H), 3.57 (m, 2H), 3.22 (m, 4H), 2.65 (m, 2H), 1.58 (m, 8H), 1.42 (s, 9H), 1.27 (d, 6H, *J* = 6.1 Hz); $^{13}$C NMR ($CDCl_3$): δ 171.7, 170.5, 159.4, 155.5, 144.9, 144.3, 135.3, 134.7, 121.8, 114.0, 96.7, 91.6, 79.3, 55.2, 50.6, 47.7, 42.2, 39.5, 39.4, 36.1, 33.7, 31.9, 29.7, 28.2, 26.1, 22.6; MS (APCI): *m/z* 716 (M +1).

### *tert*-Butyl[(2*S*)-1,5-bis({4-[(6-methoxyquinolin-8-yl)amino]pentyl}amino)-1,5-dioxopentan-2-yl]carbamate (56)

Yield: 83%; oil; IR ($CH_2Cl_2$): 3323, 1735, 1657 cm$^{-1;}$ $^1$H NMR ($CDCl_3$): δ 8.51 (d, 2H, *J* = 3.9 Hz), 7.92 (d, 2H, *J* = 8.2 Hz), 7.29 (dd, 2H, *J* = 3.9 and 8.2 Hz), 6.32 (m, 3H), 6.27 (m, 2H), 5.81 (bs, 2H), 5.74 (bs, 2H), 4.07 (t, 1H, *J* = 5.9 Hz), 3.87 (s, 6H), 3.59 (m, 2H), 3.23 (m, 4H), 2.88 (t, 2H, *J* = 6.3 Hz), 2.17 (m, 2H), 1.89 (m, 8H), 1.39 (s, 9H), 1.27 (d, 6H, *J* = 6.0 Hz); $^{13}$C NMR ($CDCl_3$): δ 173.4, 172.1, 163.1, 159.9, 145.4, 144.8, 135.8, 135.4, 130.4, 122.4, 97.4, 92.4, 80.4, 55.7, 54.1, 48.3, 40.1, 37.0, 34.5, 33.2, 31.9, 30.2, 28.8, 26.6, 21.0; MS (APCI): *m/z* 730 (M+1).

### *tert*-Butyl[(2*S*)-1,4-bis({4-[(2-*tert*-butyl-6-methoxyquinolin-8-yl)amino]pentyl}-amino)-1,4-dioxobutan-2-yl]carbamate (57)

Yield: 88%; oil; IR ($CH_2Cl_2$): 3308, 1645 cm$^{-1;}$ $^1$H NMR ($CDCl_3$): δ 7.85 (d, 2H, *J* = 7.8 Hz), 7.42 (d, 2H, *J* = 7.8 Hz), 6.99 (bs, 2H), 6.30 (m, 2H), 6.23 (m, 2H), 6.11 (bs, 1H), 5.87 (bs, 2H), 4.39 (m, 1H), 3.86 (s, 6H), 3.56 (m, 2H), 3.24 (m, 4H, *J* = 5.9 Hz), 2.63 (m, 2H), 1.58 (m, 8H), 1.41 (m, 27H), 1.28 (d, 6H, *J* = 5.7 Hz); $^{13}$C NMR ($CDCl_3$): δ 170.9, 163.2, 158.7, 144.8, 134.9, 133.5, 127.4, 118.8, 96.6, 91.4, 80.6, 55.1, 47.8, 39.5, 37.6, 33.8, 30.2, 28.3, 26.1, 23.5, 20.5; MS (APCI): *m/z* 828 (M+1).

### *tert*-Butyl[(2*S*)-1,5-bis({4-[(2-*tert*-butyl-6-methoxyquinolin-8-yl)amino]pentyl}-amino)-1,5-dioxopentan-2-yl]carbamate (58)

Yield: 85%; oil; IR ($CH_2Cl_2$): 3383, 1700, 1657 $cm^{-1;}$ $^1H$ NMR ($CDCl_3$): δ 7.86 (d, 2H, *J* = 8.6 Hz), 7.43 (d, 2H, *J* = 8.6 Hz), 6.80 (bs, 1H), 6.66 (bs, 1H), 6.31 (d, 2H, *J* = 2.1 Hz), 6.25 (d, 2H, *J* = 2.1 Hz), 6.12 (bs, 1H), 5.99 (bs, 1H), 5.68 (bs, 1H), 4.01 (t, 1H, *J* = 6.0 Hz), 3.87 (s, 6H), 3.56 (m, 2H), 3.29 (m, 4H), 2.02 (t, 2H, *J* = 6.5 Hz), 1.78-1.62 (m, 10H), 1.42 (m, 27H), 1.31 (d, 6H, *J* = 6.1 Hz); $^{13}C$ NMR ($CDCl_3$): δ 171.4, 163.3, 158.8, 153.6, 144.8, 134.9, 133.5, 127.4, 118.7, 96.7, 91.4, 79.7, 55.1, 53.1, 47.8, 47.1, 43.1, 39.7, 37.6, 34.0, 31.6, 30.2, 28.9, 28.2, 26.1, 22.3, 20.7; MS (ESI): *m/z* 842 (M+1).

### 9*H*-Fluoren-9-ylmethyl[(2*R*)-1,4-bis({4-[(6-methoxyquinolin-8-yl)amino]pentyl}-amino)-1,4-dioxobutan-2-yl]carbamate (59)

Yield: 87%; oil; IR ($CH_2Cl_2$): 3356, 1709, 1675 $cm^{-1;}$ $^1H$ NMR ($CDCl_3$): δ 8.51 (d, 2H, *J* = 3.9 Hz), 7.91 (d, 2H, *J* = 7.5 Hz), 7.74 (d, 2H, *J* = 7.4 Hz), 7.57 (d, 2H, *J* = 6.6 Hz), 7.39 (m, 6H), 6.93 (bs, 2H), 6.38 (bs, 2H), 6.32 (m, 2H), 6.25 (m, 2H), 5.97 (bs, 1H), 4.39 (d, 2H, *J* = 6.5 Hz), 4.18 (m, 2H), 3.86 (s, 6H), 3.57 (m, 2H), 3.21 (m, 4H), 2.73 (m, 2H), 1.59 (m, 8H), 1.26 (d, 6H, *J* = 6.1 Hz); $^{13}C$ NMR ($CDCl_3$): δ 172.1, 169.9, 169.6, 158.4, 153.1, 143.9, 142.7, 140.2, 134.3, 133.8, 128.9, 126.7, 124.0, 118.9, 95.8, 95.7, 90.7, 90.6, 66.1, 54.1, 50.7, 46.7, 46.1, 38.5, 37.0, 32.7, 29.8, 28.6, 25.1, 25.0, 24.9, 21.0; MS (APCI): *m/z* 838 (M+1).

### General method for the synthesis of $N^1$,$N^4$-bis{4-[(6-methoxy-2-substituted-quinolin-8-yl)amino]pentyl}-D/L-aspartamide/glutamamide (60-64)

A solution of *t*-Boc group protected bis(8-aminoquinolines) (**55**-**58**, 0.30 mmol) was stirred at ambient temperature in HCl (5 mL, 6N solution) for 45 min. The removal of solvent provides salts of **60**-**63** in good yields. In case of Fmoc group, to 8-aminoquinoline (**59**, 0.54 mmol) a solution of 20% piperidine in $CH_2Cl_2$ (10 mL) was added and reaction mixture was stirred for 20 min. The solvent was removed and the crude product was purified by silica gel (100-200 mesh) column chromatography eluting with 3% $CH_3OH$ in $CH_2Cl_2$ to afford **64**, which upon treatment with HCl (2N in ether) provided its hydrochloride salt.

### $N^1$,$N^4$-Bis{4-[(6-methoxyquinolin-8-yl)amino]pentyl}-L-aspartamide·3HCl (60)

Yield: 88%; hygroscopic solid; IR (KBr): 3435, 1638 $cm^{-1;}$ $^1H$ NMR ($CD_3OD$): δ 8.83 (m, 4H), 7.88 (m, 2H), 7.00 (m, 2H), 6.86 (m, 2H), 4.24 (m, 1H), 3.97 (m, 6H), 3.76 (m, 2H), 3.33 (m, 4H), 2.43 (m, 2H), 1.87 (m, 8H), 1.32 (m, 6H); $^{13}C$ NMR ($CD_3OD$): δ 172.3, 171.3, 161.9, 145.3, 140.3, 139.4, 133.0, 122.6, 96.4, 91.8, 66.3, 56.2, 39.9, 39.7, 36.3, 33.5, 26.4, 21.2; MS (APCI): *m/z* 616 (M+1).

### $N^1$,$N^5$-Bis{4-[(6-methoxyquinolin-8-yl)amino]pentyl}-L-glutamamide·3HCl (61)

Yield: 86%; hygroscopic solid; IR (KBr): 3391, 1673 $cm^{-1;}$ $^1H$ NMR ($CD_3OD$): δ 8.87 (m, 4H), 7.91 (m, 2H), 7.02 (m, 2H), 6.85 (m, 2H), 3.97 (m, 6H), 3.78 (t, 3H), 3.04 (m, 4H), 2.56 (t, 2H, *J* = 5.6 Hz), 2.19 (m, 2H), 1.89 (m, 8H), 1.26 (m, 6H); $^{13}C$ NMR ($CD_3OD$): δ 174.2, 169.0, 161.9, 145.3, 140.1, 139.1, 133.0, 122.6, 96.5, 92.4, 66.2, 56.2, 53.5, 51.0, 49.3, 49.0, 40.3, 39.9, 34.9, 33.4, 31.6, 27.9, 26.3, 26.1, 19.1; MS (APCI): *m/z* 630 (M+1).

### $N^1$,$N^4$-Bis{4-[(2-*tert*-butyl-6-methoxyquinolin-8-yl)amino]pentyl}-L-aspartamide·3HCl (62)

Yield: 90%; hygroscopic solid; IR (KBr): 3401, 1646 $cm^{-1;}$ $^1H$ NMR ($CD_3OD$): δ 8.40 (d, 2H, *J* = 7.8 Hz), 7.83 (d, 2H, *J* = 7.8 Hz), 7.71 (m, 2H), 7.50 (m, 2H), 4.12 (m, 1H), 3.97 (m, 6H), 3.51 (m, 2H), 3.36 (m, 4H), 2.05 (m, 2H), 1.76 (m, 8H), 1.51 (m, 18H), 1.32 (m, 6H); $^{13}C$ NMR ($CD_3OD$): δ 170.4, 168.7, 161.3, 157.4, 145.6, 144.3, 137.6, 135.3, 121.3,

118.4, 107.4, 94.5, 66.3, 59.6, 56.3, 51.1, 39.5, 38.7, 36.2, 31.3, 29.9, 26.0, 25.9, 22.0; MS (ESI): *m/z* 728 (M+1).

### $N^1$,$N^5$-Bis{4-[(2-*tert*-butyl-6-methoxyquinolin-8-yl)amino]pentyl}-L-glutamamide·3HCl (63)

Yield: 92%; hygroscopic solid; IR (KBr): 3438, 1658 $cm^{-1;}$ $^1$H NMR ($CD_3OD$): δ 8.34 (d, 2H, *J* = 8.6 Hz), 7.80 (d, 2H, *J* = 8.6 Hz), 7.59 (m, 2H), 7.42 (m, 2H), 4.10 (m, 1H), 3.97 (m, 6H), 3.78 (m, 2H), 3.31 (m, 4H), 2.00 (t, 2H, *J* = 5.6 Hz), 1.74 (m, 2H), 1.65 (m, 8H), 1.49 (s, 18H), 1.37 (m, 6H); $^{13}$C NMR ($CD_3OD$): δ 170.9, 169.8, 161.5, 157.5, 145.7, 138.0, 131.4, 129.4, 121.4, 118.3, 107.5, 93.1, 66.2, 59.5, 56.4, 53.4, 44.1, 38.7, 31.3, 29.9, 21.7, 20.4; MS (ESI): *m/z* 742 (M+1).

### $N^1$,$N^4$-Bis{4-[(6-methoxyquinolin-8-yl)amino]pentyl}-D-aspartamide·3HCl (64)

Yield: 74%; hygroscopic solid; IR (KBr): 3405, 1687 $cm^{-1;}$ $^1$H NMR (free base, $CDCl_3$): δ 8.53 (dd, 2H, *J* = 1.4 and 4.1 Hz), 7.92 (dd, 2H, *J* = 1.4 and 8.2 Hz), 7.30 (dd, 2H, *J* = 4.1 and 8.2 Hz), 6.82 (bs, 2H), 6.32 (d, 2H, *J* = 2.3 Hz), 6.26 (d, 2H, *J* = 2.3 Hz), 5.99 (bs, 2H), 3.88 (s, 6H), 3.59 (m, 3H), 3.25 (m, 4H), 2.59 (m, 2H), 2.09 (bs, 2H), 1.69 (m, 8H), 1.28 (d, 6H, *J* = 6.3 Hz); $^{13}$C NMR (free base, $CDCl_3$): δ 170.9, 169.2, 162.6, 140.9, 135.7, 123.0, 118.9, 97.1, 92.3, 56.5, 40.5, 40.3, 36.8, 34.2, 26.9, 23.7, 19.8; MS (APCI): *m/z* 616 (M+1).

## General method for the synthesis of 1,3-bis{4-[(6-methoxy-2/4,5-substituted-quinolin-8-yl)-amino]pentyl}urea/thiourea·2HCl (66-71)

A solution of CDI or TCDI (0.19 mmol) and 8-aminoquinoline (**1, 3**, or **4**, 0.39 mmol) in dry $CH_2Cl_2$ (5 mL) was stirred at ambient temperature for 24 h. The solvent was distilled off. The reaction mixture was dissolved in $CH_2Cl_2$ (20 mL) and washed with water (3 × 5 mL) followed by brine solution (5 mL). The organic layer was dried ($Na_2SO_4$) and concentrated to afford crude product, which was purified by column chromatography on silica gel (100-200 mesh) using 1.5% $CH_3OH$ in $CH_2Cl_2$ to provide product as viscous oil. Treatment with HCl solution (2N in ether) provided their HCl salts.

### 1,3-Bis{4-[(6-methoxyquinolin-8-yl)amino]pentyl}urea·2HCl (66)

Yield: 55%; hygroscopic solid; IR (free base, $CH_2Cl_2$): 3378, 1680 $cm^{-1;}$ $^1$H NMR (free base, $CDCl_3$): δ 8.52 (dd, 2H, *J* = 1.3 and 4.1 Hz), 7.92 (d, 2H, *J* = 8.0 Hz), 7.30 (dd, 2H, *J* = 4.1 and 8.0 Hz), 6.31 (d, 2H, *J* = 2.3 Hz), 6.27 (d, 2H, *J* = 2.3 Hz), 5.98 (bs, 2H), 3.86 (s, 6H), 3.52 (m, 4H), 3.14 (m, 2H), 1.67-1.59 (m, 8H), 1.27 (d, 6H, *J* = 6.3 Hz); $^{13}$C NMR (free base, $CDCl_3$): δ 172.2, 159.9, 145.4, 144.8, 135.8, 130.4, 128.2, 120.5, 97.3, 92.2, 55.7, 48.3, 42.7, 34.5, 26.7, 21.8; MS (APCI): *m/z* 545 (M+1).

### 1,3-Bis{4-[(6-methoxyquinolin-8-yl)amino]pentyl}thiourea·2HCl (67)

Yield: 52%; hygroscopic solid; IR (free base, $CH_2Cl_2$): 3374, 1615 $cm^{-1;}$ $^1$H NMR (free base, $CDCl_3$): δ 8.50 (d, 2H, *J* = 3.9 Hz), 7.89 (d, 2H, *J* = 7.8 Hz), 7.30 (dd, 2H, *J* = 3.9 and 7.8 Hz), 6.51 (d, 2H, *J* = 2.1 Hz), 6.45 (d, 2H, *J* = 2.1 Hz), 6.00 (bs, 2H), 3.87 (s, 6H), 3.55 (m, 4H), 3.14 (m, 2H), 1.65 (m, 8H), 1.25 (d, 6H, *J* = 6.1 Hz); $^{13}$C NMR (free base, $CDCl_3$): δ 183.6, 158.4, 145.8, 144.7, 134.8, 131.4, 128.6, 121.8, 97.8, 92.3, 55.1, 48.9, 41.2, 34.1, 26.8, 21.6; MS (ESI): *m/z* 583.1 (M+23).

### 1,3-Bis{4-[(2-*tert*-butyl-6-methoxyquinolin-8-yl)amino]pentyl}urea·2HCl (68)

Yield: 62%; hygroscopic solid; IR (free base, $CH_2Cl_2$): 3316, 1660 $cm^{-1;}$ $^1$H NMR (free base, $CDCl_3$): δ 7.84 (d, 2H, *J* = 8.6 Hz), 7.41 (d, 2H, *J* = 8.6 Hz), 6.30 (d, 2H, *J* = 1.9 Hz), 6.25 (d, 2H, *J* = 1.9 Hz), 6.12 (bs, 2H), 3.85 (s, 6H), 3.58 (m, 2H), 3.12 (m, 4H), 1.59 (m, 8H), 1.41 (s, 18H), 1.28 (d, 6H, *J* = 6.3 Hz); $^{13}$C NMR (free base, $CDCl_3$): δ 171.7, 162.5,

157.9, 143.8, 135.5, 129.0, 128.4, 119.3, 97.1, 92.0, 55.7, 48.4, 40.0, 34.4, 30.8, 28.9, 26.7, 21.1; MS (APCI): *m/z* 657 (M+1).

### 1,3-Bis{4-[(2-*tert*-butyl-6-methoxyquinolin-8-yl)amino]pentyl}thiourea·2HCl (69)

Yield: 60%; hygroscopic solid; IR (free base, $CH_2Cl_2$): 3363, 1651 $cm^{-1}$; $^1H$ NMR (free base, $CDCl_3$): δ 7.83 (d, 2H, *J* = 8.5 Hz), 7.41 (d, 2H, *J* = 8.5 Hz), 6.30-6.25 (m, 4H), 6.08 (bs, 1H), 5.64 (bs, 1H), 3.85 (m, 6H), 3.55 (m, 2H), 3.25 (m, 4H), 1.59 (m, 8H), 1.41 (s, 18H), 1.26 (d, 6H, *J* = 6.3 Hz); $^{13}C$ NMR (free base, $CDCl_3$): δ 181.4, 163.5, 158.8, 144.7, 135.0, 133.5, 127.5, 118.9, 97.0, 91.8, 55.2, 47.9, 44.3, 37.7, 33.8, 30.2, 25.4, 20.7; MS (APCI): *m/z* 673 (M+1).

### 1,3-Bis{4-[4-ethyl-6-methoxy-5-(pentyloxy)quinolin-8-ylamino]pentyl}urea·2HCl (70)

Yield: 39%; hygroscopic solid; IR (free base, $CH_2Cl_2$): 3376, 1669 $cm^{-1}$; $^1H$ NMR (free base, $CDCl_3$): δ 8.32 (d, 2H, *J* = 4.2 Hz), 7.55 (d, 2H, *J* = 4.2 Hz), 6.85 (bs, 2H), 6.37 (s, 2H), 3.89 (s, 6H), 3.83 (t, 4H, *J* = 6.9 Hz), 3.57 (m, 2H), 3.43 (m, 4H), 3.21 (q, 4H, *J* = 7.2 Hz), 1.98 (m, 20H), 1.55 (m, 18H); $^{13}C$ NMR (free base, $CDCl_3$): δ 169.3, 151.1, 141.8, 134.6, 134.5, 134.0, 128.5, 128.1, 122.4, 94.5, 56.9, 53.3, 50.8, 48.1, 38.1, 34.2, 29.6, 26.4, 26.2, 22.5, 20.7, 14.0; MS (APCI): *m/z* 773 (M+1).

### 1,3-Bis{4-[4-ethyl-6-methoxy-5-(pentyloxy)quinolin-8-ylamino]pentyl}-thiourea·2HCl (71)

Yield: 42%; hygroscopic solid; IR (free base, $CH_2Cl_2$): 3365, 1606 $cm^{-1}$; $^1H$ NMR (free base, $CDCl_3$): δ 8.36 (d, 2H, *J* = 4.2 Hz), 7.09 (d, 2H, *J* = 4.2 Hz), 6.44 (s, 2H), 5.88 (bs, 2H), 3.94 (s, 6H), 3.89 (t, 4H, *J* = 6.8 Hz), 3.61 (m, 2H), 3.33 (m, 4H), 3.25 (q, 4H, *J* = 7.2 Hz), 1.83 (t, 6H, *J* = 7.2 Hz), 1.64-1.55 (m, 20H), 1.43 (m, 12H); $^{13}C$ NMR (free base, $CDCl_3$): δ 182.3, 151.1, 144.3, 134.5, 134.1, 128.5, 128.1, 122.4, 94.6, 56.9, 50.8, 48.1, 39.3, 32.1, 28.5, 26.2, 20.7, 19.2, 13.9; MS (APCI): *m/z* 789 (M+1).

### General method for the synthesis of *N,N*$^2$-bis{4-[(6-methoxy-2-substituted-quinolin-8-yl)amino]pentyl}glycinamide·2HCl (72 and 73)

A mixture of 8-aminoquinoline (**1** or **3**, 1.08 mmol), chloroacetic acid (4.32 mmol) and $Et_3N$ (4.32 mmol) in anhydrous THF (15 mL) was refluxed for 24 h. The solvent was removed under reduced pressure. The residue was dissolved in $CH_2Cl_2$ (50 mL) and washed with water (3 × 10 mL) followed by brine solution (5 mL). The organic layer was dried ($Na_2SO_4$) and solvent was removed under reduced pressure. Column chromatographic purification of crude product on silica gel (100-200 mesh) using 2% $CH_3OH$ in $CH_2Cl_2$ gave product as viscous oil, which upon treatment with HCl solution (2N in ether) provided **72** and **73** as HCl salts.

### *N,N*$^2$-Bis{4-[(6-methoxyquinolin-8-yl)amino]pentyl}glycinamide·2HCl (72)

Yield: 21%; hygroscopic solid; IR (free base, $CH_2Cl_2$): 3436, 1637 $cm^{-1}$; $^1H$ NMR (free base, $CDCl_3$): δ 8.53 (dd, 2H, *J* = 1.5 and 4.2 Hz), 7.94 (dd, 2H, *J* = 1.5 and 8.2 Hz), 7.33 (dd, 2H, *J* = 4.2 and 8.2 Hz), 6.34 (d, 2H, *J* = 2.3 Hz), 6.28 (d, 2H, *J* = 2.3 Hz), 5.96 (bs, 2H), 3.88 (s, 6H), 3.68 (m, 4H), 3.27 (t, 2H, *J* = 5.6 Hz), 2.30 (t, 2H, *J* = 6.4 Hz), 1.87-1.63 (m, 8H), 1.30 (d, 6H, *J* = 6.3 Hz); $^{13}C$ NMR (free base, $CDCl_3$): δ 173.4, 159.4, 144.9, 144.3, 135.3, 134.9, 129.9, 121.9, 96.9, 91.8, 62.3, 55.2, 47.8, 39.5, 34.0, 26.1, 20.6; MS (APCI): *m/z* 559 (M+1).

### *N,N*$^2$-Bis{4-[(2-*tert*-butyl-6-methoxyquinolin-8-yl)amino]pentyl}-glycinamide·2HCl (73)

Yield: 18%; hygroscopic solid; IR (free base, $CH_2Cl_2$): 3391, 1648 $cm^{-1}$; $^1H$ NMR (free base, $CDCl_3$): δ 7.87 (d, 2H, *J* = 8.5 Hz), 7.43 (d, 2H, *J* = 8.5 Hz), 6.50 (bs, 2H), 6.32 (s, 2H), 6.34 (s, 2H), 4.02 (s, 2H), 3.87 (s, 6H), 3.60 (m, 2H), 3.36 (m, 2H), 2.58 (t, 2H, *J* = 5.9

Hz), 1.79-1.60 (m, 8H), 1.42 (s, 18H), 1.30 (d, 6H, *J* = 6.1 Hz); $^{13}$C NMR (free base, $CDCl_3$): δ 170.4, 162.5, 157.6, 143.8, 134.0, 132.6, 126.4, 117.0, 95.6, 90.5, 61.1, 54.2, 46.8, 37.8, 36.6, 32.5, 29.2, 24.9, 20.9; MS (APCI): *m/z* 671 (M+1).

### General method for the synthesis of *N,N′*-Bis{4-[(6-methoxy-2-substituted-quinolin-8-yl)amino]pentyl}dicarbonimidic diamide·2HCl (74 and 75)

To an ice cooled stirred solution of **1** or **3** (1.31 mmol) and $Et_3N$ (0.65 mmol) in anhydrous $CH_2Cl_2$ (10 mL), *N*-(chlorocarbonyl)isocyanate (0.65 mmol) was added drop wise. The reaction mixture was allowed to attain room temperature and stirring continued for another 3 h. The solvent was then removed under reduced pressure. The residue was dissolved in $CH_2Cl_2$ (20 mL), and organic layer was washed with water (3 × 10 mL) followed by brine solution (5 mL). The organic layer was dried over $Na_2SO_4$ and concentrated to obtain crude product, which was purified by column chromatography on silica gel (100-200 mesh) using 2.5% $CH_3OH$ in $CH_2Cl_2$ to afford products, which were converted to their hydrogen chloride salts upon treatment with HCl solution (2N in ether).

### *N,N′*-Bis{4-[(6-methoxyquinolin-8-yl)amino]pentyl}dicarbonimidic diamide·2HCl (74)

Yield: 70%; hygroscopic solid; IR (free base, $CH_2Cl_2$): 3382, 1689, 1668 $cm^{-1;}$ $^1$H NMR (free base, $CDCl_3$): δ 8.77 (bs, 1H), 8.52 (bs, 1H), 8.06 (d, 2H, *J* = 4.2 Hz), 7.91 (d, 2H, *J* = 8.5 Hz), 7.22 (dd, 2H, *J* = 4.2 and 8.5 Hz), 6.32-6.27 (m, 4H), 3.87 (s, 6H), 3.62 (m, 2H), 3.24 (t, 4H, *J* = 6.1 Hz), 1.64 (m, 8H), 1.28 (d, 6H, *J* = 5.7 Hz); $^{13}$C NMR (free base, $CDCl_3$): δ 160.0, 157.6, 149.4, 145.5, 135.9, 135.2, 130.4, 122.3, 107.6, 97.3, 92.2, 55.7, 53.2, 48.4, 34.6, 27.1, 21.0; MS (APCI): *m/z* 588 (M+1).

### *N,N′*-Bis{4-[(2-*tert*-butyl-6-methoxyquinolin-8-yl)amino]pentyl}dicarbonimidic diamide·2HCl (75)

Yield: 38%; hygroscopic solid; IR (free base, $CH_2Cl_2$): 3374, 1682, 1665 $cm^{-1;}$ $^1$H NMR (free base, $CDCl_3$): δ 7.98 (d, 2H, *J* = 8.6 Hz), 7.85 (d, 2H, *J* = 8.6 Hz), 6.30 (s, 2H), 6.25 (s, 2H), 6.15 (bs, 2H), 3.86 (s, 6H), 3.59 (m, 2H), 3.27 (t, 4H, *J* = 6.2 Hz), 1.64 (m, 8H), 1.42 (s, 18H), 1.31 (d, 6H, *J* = 6.1 Hz); $^{13}$C NMR (free base, $CDCl_3$): δ 168.2, 159.4, 157.0, 145.5, 135.8, 135.4, 128.9, 123.7, 107.0, 96.9, 55.7, 48.5, 40.3, 34.7, 32.8, 30.5, 27.0, 21.1; MS (APCI): *m/z* 700 (M+1).

### General method for the synthesis of bisquinolines 76-78

To an ice cooled stirred solution of **1** (0.772 mmol) and $Et_3N$ (0.772 mmol) in anhydrous $CH_2Cl_2$ (5 mL), chloromethyl chloroformate or chlorocarbonylsulfenyl chloride or oxalyl chloride (0.386 mmol) was added drop wise. The reaction mixture was allowed to warm to ambient temperature and stirring continued for another 6 h. The reaction mixture was concentrated, and residue was dissolved in $CH_2Cl_2$ (20 mL). The organic layer was washed with water (3 × 10 mL) followed by brine solution (5 mL). Organic layer was dried over $Na_2SO_4$ and concentrated to obtain crude product. Pure product was isolated as viscous oil by column chromatography on silica gel (100-200 mesh) using 1.5% $CH_3OH$ in $CH_2Cl_2$. Treatment with HCl solution (2N in ether) provided **76-78** as HCl salts.

### ({4-[(6-Methoxyquinolin-8-yl)amino]pentyl}amino)methyl{4-[(6-methoxy-quinolin-8-yl)amino]pentyl}carbamate·2HCl (76)

Yield: 63%; hygroscopic solid; IR (free base, $CH_2Cl_2$): 3375, 1742 $cm^{-1;}$ $^1$H NMR (free base, $CDCl_3$): δ 8.53 (dd, 2H, *J* = 1.4 and 4.2 Hz), 7.93 (dd, 2H, *J* = 1.4 and 8.2 Hz), 7.32 (dd, 2H, *J* = 4.2 and 8.2 Hz), 6.34 (d, 2H, *J* = 2.3 Hz), 6.28 (d, 2H, *J* = 2.3 Hz), 6.00 (bs, 1H), 5.74 (s, 2H), 4.90 (bs, 1H), 3.88 (s, 6H), 3.64 (m, 2H), 3.26 (m, 4H), 1.72-1.62 (m, 8H), 1.31 (d, 6H, *J* = 6.3 Hz); $^{13}$C NMR (free base, $CDCl_3$): δ 159.9, 154.1, 145.4, 144.9,

135.8, 135.3, 130.4, 122.4, 97.3, 92.3, 71.0, 55.7, 48.3, 41.7, 34.3, 26.9, 21.1; MS (APCI): *m/z* 574.8 (M+1).

### ({4-[(6-Methoxyquinolin-8-yl)amino]pentyl}amino)[({4-[(6-methoxyquinolin-8-yl)amino]pentyl}amino)sulfanyl]methanone·2HCl (77)

Yield: 66%; hygroscopic solid; IR (free base, $CH_2Cl_2$): 3370, 1659 $cm^{-1;}$ $^1H$ NMR (free base, $CDCl_3$): δ 8.51 (m, 4H), 7.91 (m, 2H), 6.33 (m, 4H), 5.98 (bs, 1H), 5.49 (bs, 1H), 3.89 (s, 6H), 3.57 (m, 2H), 3.34 (m, 4H), 1.61 (m, 8H), 1.25 (d, 6H, *J* = 6.1 Hz); $^{13}C$ NMR (free base, $CDCl_3$): δ 162.1, 160.0, 148.4, 145.1, 135.4, 133.8, 130.5, 123.3, 97.4, 92.3, 55.7, 48.3, 41.6, 34.3, 26.9, 21.0; MS (APCI): *m/z* 577 (M+1).

### *N,N′*-Bis{4-[(6-methoxyquinolin-8-yl)amino]pentyl}ethanediamide·2HCl (78)

Yield: 7%; hygroscopic solid; IR (free base, $CH_2Cl_2$): 3381, 1659 $cm^{-1;}$ $^1H$ NMR (free base, $CDCl_3$): δ 8.52 (d, 2H, *J* = 3.2 Hz), 7.91 (d, 2H, *J* = 8.0 Hz), 7.30 (dd, 2H, *J* = 3.2 and 8.0 Hz), 6.32 (s, 2H), 6.27 (s, 2H), 3.87 (s, 6H), 3.63 (m, 2H), 3.33 (t, 4H, *J* = 5.5 Hz), 1.71 (m, 8H), 1.29 (d, 6H, *J* = 6.2 Hz); $^{13}C$ NMR (free base, $CDCl_3$): δ 160.4, 159.9, 145.4, 144.8, 135.9, 135.2, 130.4, 122.3, 97.3, 92.3, 55.7, 48.3, 40.2, 34.5, 26.6, 21.1; MS (APCI): *m/z* 573 (M+1).

### Synthesis of $N^1$,$N^{1\prime}$-(iminodiethane-2,1-diyl)bis[$N^4$-(6-methoxyquinolin-8-yl)pentane-1,4-diamine]·2HCl (79)

A mixture of **1** (0.965 mmol), $Et_3N$ (0.965 mmol) and bis(2-chloroethyl)amine (0.482 mmol) was stirred at room temperature for 14 h. At this stage, EtOAc (20 mL) was added and the separated $Et_3N$·HCl salt was filtered. The filtrate was concentrated and residue was purified by column chromatography on silica gel (100-200 mesh) using 7% $CH_3OH$ in $CH_2Cl_2$ to afford pure product, which was converted to HCl salt upon treating with 2N solution of HCl in ether. Yield: 82%; hygroscopic solid; IR (free base, $CH_2Cl_2$): 3428 $cm^{-1;}$ $^1H$ NMR (free base, $CDCl_3$): δ 8.52 (d, 2H, *J* = 3.2 Hz), 7.91 (d, 2H, *J* = 8.0 Hz), 7.32 (dd, 2H, *J* = 3.2 and 8.0 Hz), 6.32 (s, 2H), 6.26 (s, 2H), 5.97 (bs, 1H), 4.04 (bs, 1H), 3.87 (s, 6H), 3.63 (m, 2H), 3.33 (m, 12H), 1.71 (m, 8H), 1.29 (d, 6H, *J* = 6.2 Hz); $^{13}C$ NMR (free base, $CDCl_3$): δ 159.9, 145.3, 145.8, 135.8, 135.3, 130.4, 122.4, 97.5, 92.4, 55.7, 51.7, 48.2, 41.0, 34.1, 26.6, 20.8; MS (APCI): *m/z* 588 (M+1).

### General method for the synthesis of *N,N′*-bis{4-[(6-methoxy-2-substituted-quinolin-8-yl)amino]pentyl}pyridine-2,6/3,4/3,5-dicarboxamide·2HCl (80-85)

A mixture of 8-aminoquinoline (**1** or **3**, 0.980 mmol), $Et_3N$ (0.980 mmol) and pyridine-2,6/3,4/3,5-dicarbonyl chloride (0.490 mmol) was stirred in anhydrous THF (15 mL) at room temperature for 12 h and filtered. The filtrate was concentrated and residue was purified by column chromatography on silica gel (100-200 mesh) using 1% $CH_3OH$ in $CH_2Cl_2$ to afford **80**-**85** as viscous oil, which were converted to HCl salts upon treatment with a 2N solution of ethereal HCl.

### *N,N′*-Bis{4-[(6-methoxyquinolin-8-yl)amino]pentyl}pyridine-2,6-dicarboxamide·3HCl (80)

Yield: 63%; hygroscopic solid; IR (free base, $CH_2Cl_2$): 3391, 1659 $cm^{-1;}$ $^1H$ NMR (free base, $CDCl_3$): δ 8.48 (d, 2H, *J* = 4.0 Hz), 8.32 (d, 2H, *J* = 7.7 Hz), 7.97 (m, 3H), 7.28 (dd, 2H, *J* = 4.0 and 7.8 Hz), 6.30 (d, 2H, *J* = 2.1 Hz), 6.20 (d, 2H, *J* = 2.1 Hz), 5.93 (bs, 2H), 3.84 (s, 6H), 3.53 (m, 2H), 3.42 (t, 4H, *J* = 6.8 Hz), 1.60 (m, 8H), 1.26 (d, 6H, *J* = 6.2 Hz); $^{13}C$ NMR (free base, $CDCl_3$): δ 164.0, 159.9, 149.4, 145.3, 144.7, 139.3, 135.8, 135.4, 130.4, 125.4, 122.3, 97.4, 92.2, 55.7, 48.2, 40.0, 34.5, 26.8, 21.0; MS (APCI): *m/z* 650 (M +1).

### *N,N′*-Bis{4-[(6-methoxyquinolin-8-yl)amino]pentyl}pyridine-3,4-dicarboxamide·3HCl (81)

Yield: 35%; hygroscopic solid; IR (free base, $CH_2Cl_2$): 3392, 1634 $cm^{-1;}$ $^1H$ NMR (free base, $CDCl_3$): δ 8.76 (s, 1H), 8.63 (d, 1H, *J* = 5.0 Hz), 8.48 (d, 2H, *J* = 3.9 Hz), 7.91 (d, 2H, *J* = 7.4 Hz), 7.40 (d, 1H, *J* = 5.0 Hz), 7.29 (dd, 2H, *J* = 3.9 and 7.4 Hz), 6.31 (d, 2H, *J* = 1.8 Hz), 6.26 (d, 2H, *J* = 1.8 Hz), 5.98 (bs, 2H), 3.85 (s, 6H), 3.59 (m, 2H), 3.37 (t, 4H, *J* = 6.1 Hz), 1.70 (m, 8H), 1.28 (d, 6H, *J* = 6.3 Hz); $^{13}C$ NMR (free base, $CDCl_3$): δ 167.5, 159.9, 152.0, 149.9, 144.8, 142.0, 135.8, 135.3, 129.4, 122.6, 122.4, 97.4, 55.7, 48.3, 40.7, 34.3, 26.5, 21.1; MS (APCI): *m/z* 650 (M+1).

### *N,N′*-Bis{4-[(6-methoxyquinolin-8-yl)amino]pentyl}pyridine-3,5-dicarboxamide·3HCl (82)

Yield: 68%; hygroscopic solid; IR (free base, $CH_2Cl_2$): 3369, 1645 $cm^{-1;}$ $^1H$ NMR (free base, $CDCl_3$): δ 9.00 (s, 2H), 8.49 (d, 2H, *J* = 4.0 Hz), 8.35 (s, 1H), 7.90 (d, 2H, *J* = 8.2 Hz), 7.29 (dd, 2H, *J* = 4.0 and 8.2 Hz), 6.30 (s, 2H), 6.25 (s, 2H), 5.94 (bs, 2H), 3.84 (s, 6H), 3.60-3.42 (m, 6H), 1.70 (m, 8H), 1.27 (d, 6H, *J* = 6.1 Hz); $^{13}C$ NMR (free base, $CDCl_3$): δ 165.5, 159.9, 151.0, 145.3, 144.8, 135.8, 135.4, 133.7, 130.4, 130.3, 122.4, 97.5, 92.4, 55.7, 48.3, 40.7, 34.5, 26.6, 21.1; MS (APCI): *m/z* 650 (M+1).

### *N,N′*-Bis{4-[(2-*tert*-butyl-6-methoxyquinolin-8-yl)amino]pentyl}pyridine-2,6-dicarboxamide. 3HCl (83)

Yield: 55%; hygroscopic solid; IR (free base, $CH_2Cl_2$): 3391, 1661 $cm^{-1;}$ $^1H$ NMR (free base, $CDCl_3$): δ 8.32 (d, 2H, *J* = 7.7 Hz), 8.01 (d, 1H, *J* = 7.7 Hz), 7.84 (d, 2H, *J* = 8.6 Hz), 7.41 (d, 2H, *J* = 8.6 Hz), 6.28 (d, 2H, *J* = 2.4 Hz), 6.22 (d, 2H, *J* = 2.3 Hz), 3.82 (s, 6H), 3.48 (m, 6H), 1.76 (m, 8H), 1.39 (s, 18H), 1.27 (d, 6H, *J* = 6.6 Hz); $^{13}C$ NMR (free base, $CDCl_3$): δ 162.8, 162.3, 157.7, 147.8, 143.8, 137.7, 133.9, 133.5, 126.4, 125.5, 117.7, 95.5, 90.4, 55.9, 54.0, 46.8, 36.6, 33.2, 25.8, 21.6; MS (APCI): *m/z* 762 (M+1).

### *N,N′*-Bis{4-[(2-*tert*-butyl-6-methoxyquinolin-8-yl)amino]pentyl}pyridine-3,4-dicarboxamide·3HCl (84)

Yield: 22%; hygroscopic solid; IR (free base, $CH_2Cl_2$): 3435, 1655 $cm^{-1;}$ $^1H$ NMR (free base, $CDCl_3$): δ 8.72 (s, 1H), 8.57 (d, 1H, *J* = 2.8 Hz), 7.83 (d, 2H, *J* = 8.5 Hz), 7.41 (d, 2H, *J* = 8.5 Hz), 7.33 (d, 1H, *J* = 2.8 Hz), 6.28 (s, 2H), 6.22 (s, 2H), 6.11 (bs, 2H), 3.86 (s, 6H), 3.67 (m, 6H), 1.70 (m, 8H), 1.40 (s, 18H), 1.29 (d, 6H, *J* = 6.2 Hz); $^{13}C$ NMR (free base, $CDCl_3$): δ 169.0, 167.9, 157.7, 152.9, 144.1, 143.4, 134.4, 134.0, 128.3, 124.1, 118.0, 96.0, 90.4, 56.0, 43.6, 35.8, 34.2, 30.9, 26.6, 21.5; MS (MALDI): *m/z* 762 (M+1).

### *N,N′*-Bis{4-[(2-*tert*-butyl-6-methoxyquinolin-8-yl)amino]pentyl}pyridine-3,5-dicarboxamide·3HCl (85)

Yield: 45%; hygroscopic solid; IR (free base, $CH_2Cl_2$): 3391, 1660 $cm^{-1;}$ $^1H$ NMR (free base, $CDCl_3$): δ 9.12 (d, 2H, *J* = 1.9 Hz), 8.43 (d, 1H, *J* = 1.9 Hz), 8.02 (d, 2H, *J* = 8.5 Hz), 7.60 (d, 2H, *J* = 8.5 Hz), 6.47 (d, 2H, *J* = 2.1 Hz), 6.42 (d, 2H, *J* = 2.1 Hz), 6.31 (bs, 2H), 4.02 (s, 6H), 3.69 (m, 6H), 1.95 (m, 8H), 1.59 (s, 18H), 1.50 (d, 6H, *J* = 6.6 Hz); $^{13}C$ NMR (free base, $CDCl_3$): δ 163.8, 162.4, 157.7, 149.2, 143.7, 134.0, 133.5, 132.0, 128.8, 117.8, 95.8, 90.7, 56.0, 54.1, 46.8, 36.6, 33.1, 30.5, 25.0, 21.6; MS (APCI): *m/z* 762 (M+1).

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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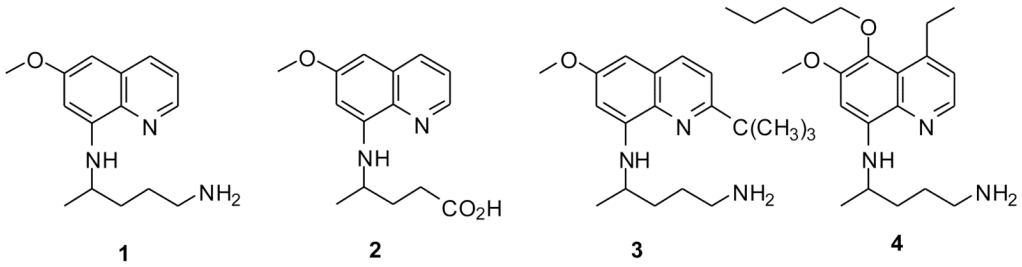
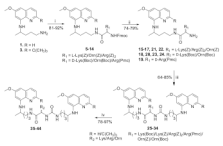
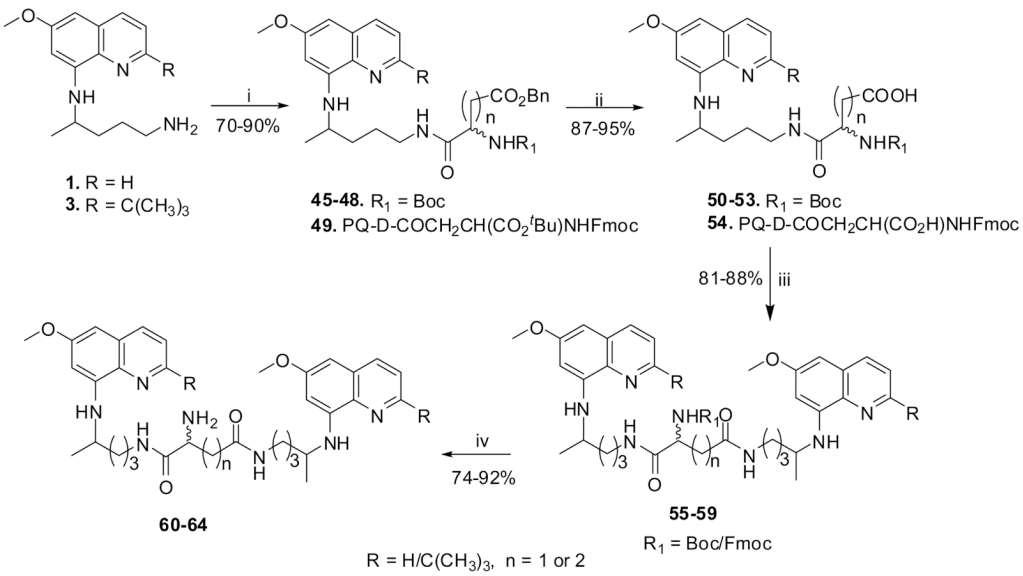



**Figure 1.**
8-Aminoquinolines



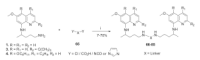
**Scheme 1.**

*Reagents and conditions:* (i) DIC, Fmoc-NH-CH($R_1$)-$CO_2$H, DCM, 0 °C - rt, 4 h; (ii) 4N HCl in MeOH, rt, 45 min, 20% $NH_4OH$ or 20% piperidine in DCM, 20 min, rt; (iii) CDI, **1** or **3**, DCM, rt, 5 h; (iv) Pd-C/$H_2$, MeOH, rt, 4h or 4N HCl in MeOH, rt, 45 min or 8N HCl in MeOH, rt, 8 h.




**Scheme 2.**

*Reagents and conditions:* (i) DIC, $R_1$NH-CHCOOH$(CH_2)_n$-$CO_2$Bn, DCM, 0 °C - rt, 4 h; (ii) Pd-C/$H_2$, MeOH, rt, 4h or 6N HCl, rt, 5h, 20%$NH_4$OH; (iii) DIC, **1** or **3**, DCM, 0 °C - rt, 4 h; (iv) 4N HCl in MeOH, rt, 45 min or 20% piperidine in DCM, rt, 20 min.



**Scheme 3.**

*Reagents and conditions:* (i) $Et_3N$, 0-70 °C, 4-24 h or $Et_3N$, 0-70 °C, THF/$CH_2Cl_2$, 4-24 h or $CH_2Cl_2$, rt, 24 h.

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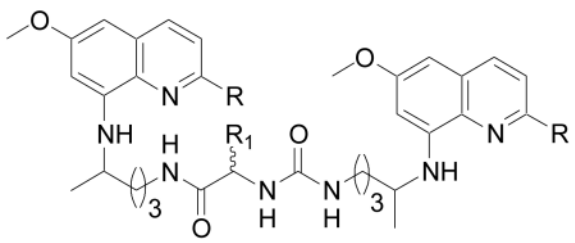
**Table 1**

In vitro antimalarial activity (*P. falciparum*), cytotoxicity, *β*-hematin (BH) inhibition, methemoglobin (MetHb) formation and in vitro antileishmanial activity (*L. donovani*) of bis(8-aminoquinolines) (**35-44**) (**Series 1**)

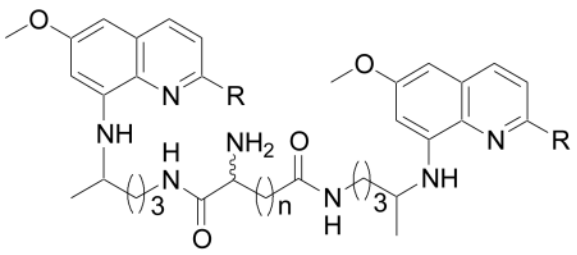


| Compd. No. | R | $R_1$ | | *P. falciparum* (D6) | *P. falciparum* (W2) | Cytotoxicity (Vero) | BH inhibition | MetHb toxicity (% MetHb formation at 20*μ*g/mL) | *L. donovani* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $IC_{50}$ (*μ*g/mL) | $IC_{50}$ (*μ*g/mL) | $IC_{50}$ (*μ*g/mL) | $IC_{50}$(*μ*M) | | $IC_{50}$ (*μ*g/mL) | $IC_{90}$ (*μ*g/mL) |
| **35** | H | Lys | (L) | 2.7 | 2.7 | NC | 80 | 3.75 | 18 | >40 |
| **36** | H | Arg | (L) | 3.2 | 1.8 | NC | 90 | 3.1 | 3.1 | 7.2 |
| **37** | H | Orn | (L) | 2.8 | 2.0 | NC | 88 | 7.9 | 14 | 40 |
| **38** | H | Lys | (D) | 3.6 | 2.8 | NC | 162 | 2.8 | 18.5 | 36 |
| **39** | H | Arg | (D) | 2.4 | 1.5 | NC | 88 | 5.5 | 19 | 36 |
| **40** | H | Orn | (D) | 4.2 | 2.8 | NC | 154 | 6.10 | 18 | 39 |
| **41** | $C(CH_3)_3$ | Lys | (L) | NA | NA | NC | >1000 | 1.1 | 19 | 38 |
| **42** | $C(CH_3)_3$ | Orn | (L) | 4.0 | 3.2 | NC | 152 | 0.2 | 18 | 33 |
| **43** | $C(CH_3)_3$ | Lys | (D) | 1.6 | 1.3 | NC | 45 | 2.25 | 21 | 36 |
| **44** | $C(CH_3)_3$ | Orn | (D) | 0.34 | 0.3 | NC | 10.8 | 2.3 | 20 | 34 |
| **PQ** | | | | 2.0 | 2.8 | NC | >1000 | 10 | 19.9 | NA |

$IC_{50}$ and $IC_{90}$ are the sample concentration that kills 50% and 90% cells compared to vehicle control. NC, not cytotoxic up to 10 *μ*g/mL. NA, Not active. Chloroquine: $IC_{50}$ = 0.014 *μ*g/mL (D6 clone); $IC_{50}$ = 0.1 *μ*g/mL, (W2 clone). Artemisinin: $IC_{50}$ = 0.015 *μ*g/mL (D6 clone); $IC_{50}$ = 0.009 *μ*g/mL (W2 clone). BH inhibition activity: Chloroquine: $IC_{50}$ = 80 *μ*M, BPQ: $IC_{50}$ = 2.9 *μ*M, PQ: $IC_{50}$ > 1000 *μ*M. Antileishmanial activity: Pentamidine: $IC_{50}$ = 1 *μ*g/mL, $IC_{90}$ = 3.8 *μ*g/mL. Amphotericin B: $IC_{50}$ = 0.19 *μ*g/mL, $IC_{90}$ = 0.35 *μ*g/mL.

**Table 2**

In vitro antimalarial activity (*P. falciparum*), cytotoxicity, *β*-hematin (BH) inhibition, methemoglobin (MetHb) formation and in vitro antileishmanial activity (*L. donovani*) of bis(8-aminoquinolines) (**60-64**) (**Series 2**)

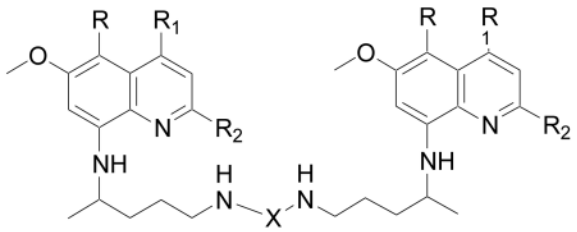


| Compd. No. | R | n | ≈ | *P. falciparum* (D6) | *P. falciparum* (W2) | Cytotoxicity (Vero) | BH inhibition | MetHb toxicity (% MetHb formation at 20$\mu$g/mL) | *L. donovani* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $IC_{50}$ ($\mu$g/mL) | $IC_{50}$ ($\mu$g/mL) | $IC_{50}$ ($\mu$g/mL) | $IC_{50}$($\mu$M) | | $IC_{50}$ ($\mu$g/mL) | $IC_{90}$ ($\mu$g/mL) |
| **60** | H | 1 | (L) | 4.76 | 3.8 | NC | 185 | 5.4 | 6.2 | 30 |
| **61** | H | 2 | (L) | 2.7 | 2.2 | NC | 85 | 13.35 | 16 | 35 |
| **62** | $C(CH_3)_3$ | 1 | (L) | NA | 3.2 | NC | 140 | 1.55 | 15 | 33 |
| **63** | $C(CH_3)_3$ | 2 | (L) | 4.7 | 2.3 | NC | >1000 | 3.3 | 3.9 | 10.7 |
| **64** | H | 1 | (D) | 3.3 | 3.6 | NC | 120 | 7.25 | 11.5 | 31 |
| **PQ** | | | | 2.0 | 2.8 | NC | >1000 | 10 | 19.9 | NA |

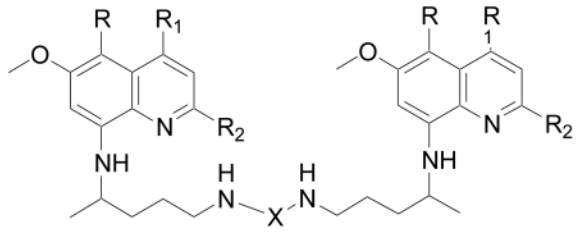
$IC_{50}$ and $IC_{90}$ are the sample concentration that kills 50% and 90% cells compared to vehicle control. NC, not cytotoxic up to 10 $\mu$g/mL. NA, Not active. Chloroquine: $IC_{50}$ = 0.014 $\mu$g/mL (D6 clone); $IC_{50}$ = 0.1 $\mu$g/mL (W2 clone). Artemisinin: $IC_{50}$ = 0.015 $\mu$g/mL (D6 clone); $IC_{50}$ = 0.009 $\mu$g/mL (W2 clone). BH inhibition activity: Chloroquine: $IC_{50}$ = 80 $\mu$M, BPQ: $IC_{50}$ = 2.9 $\mu$M, PQ: $IC_{50}$ > 1000 $\mu$M. Antileishmanial activity: Pentamidine: $IC_{50}$ = 1 $\mu$g/mL, $IC_{90}$ = 3.8 $\mu$g/mL. Amphotericin B: $IC_{50}$ = 0.19 $\mu$g/mL, $IC_{90}$ = 0.35 $\mu$g/mL.

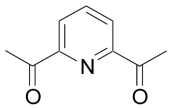
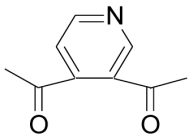
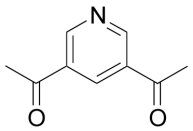
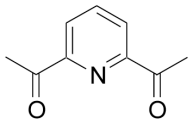
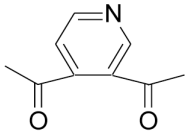
**Table 3**

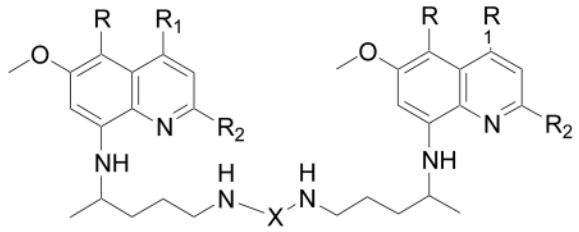
In vitro antimalarial activity (*P. falciparum*), cytotoxicity, *β*-hematin (BH) inhibition, methemoglobin (MetHb) formation and in vitro antileishmanial activity (*L. donovani*) of bis(8-aminoquinolines) (**66-85**) (**Series 3**)

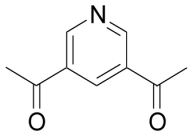


| Compd. No. | R | $R_1$ | $R_2$ | X | *P. falciparum* (D6) | *P. falciparum* (W2) | Cytotoxicity (Vero) | BH Inhibition | MetHb toxicity (% MetHb formation at 20$\mu$g/mL) | *L. donovani* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | $IC_{50}$ ($\mu$g/mL) | $IC_{50}$ ($\mu$g/mL) | $IC_{50}$ ($\mu$g/mL) | $IC_{50}$ ($\mu$M) | | $IC_{50}$ ($\mu$g/mL) | $IC_{90}$ ($\mu$g/mL) |
| **66** | H | H | H | CO | 4.76 | 4.76 | NC | >1000 | 9.8 | NA | NA |
| **67** | H | H | H | CS | 2.3 | 2.4 | NC | >1000 | 3.7 | 4 | 20 |
| **68** | H | H | $C(CH_3)_3$ | CO | 3.0 | 3.7 | NC | >1000 | 1.0 | NA | NA |
| **69** | H | H | $C(CH_3)_3$ | CS | 3.0 | 2.3 | NC | >1000 | 0.75 | 9 | 31 |
| **70** | $OC_5H_{11}$ | $C_2H_5$ | H | CO | 1.7 | 1.3 | NC | 80 | 4.7 | 3.5 | 7 |
| **71** | $OC_5H_{11}$ | $C_2H_5$ | H | CS | NA | 4.76 | NC | >1000 | 1.7 | 40 | NA |
| **72** | H | H | H | $COCH_2$ | NA | NA | NC | >1000 | 20.3 | 20 | NA |
| **73** | H | H | $C(CH_3)_3$ | $COCH_2$ | 1.5 | 1.3 | NC | 75 | 1.1 | 2.9 | 7.8 |
| **74** | H | H | H | CONHCO | 4.76 | 3.4 | NC | 155 | 2.25 | NA | NA |
| **75** | H | H | $C(CH_3)_3$ | CONHCO | NA | NA | NC | >1000 | 8.8 | 25 | >40 |
| **76** | H | H | H | $COOCH_2$ | 2.6 | 2.7 | NC | 101 | 5.3 | 20 | 38 |
| **77** | H | H | H | COS | NA | 4.76 | NC | 153 | 10.9 | 20 | 38 |
| **78** | H | H | H | COCO | NA | NA | NC | >1000 | 6.45 | 15 | >40 |
| **79** | H | H | H | $CH_2CH_2NHCH_2CH_2$ | 2.6 | 1.5 | NC | 180 | 8.4 | 2.99 | 27 |



| Compd. No. | R | $R_1$ | $R_2$ | X | *P. falciparum* (D6) $IC_{50}$ ($\mu$g/mL) | *P. falciparum* (W2) $IC_{50}$ ($\mu$g/mL) | Cytotoxicity (Vero) $IC_{50}$ ($\mu$g/mL) | BH Inhibition $IC_{50}$ ($\mu$M) | MetHb toxicity (% MetHb formation at 20$\mu$g/mL) | *L. donovani* $IC_{50}$ ($\mu$g/mL) | *L. donovani* $IC_{90}$ ($\mu$g/mL) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **80** | H | H | H |  | 2.2 | 2.3 | NC | 123 | 5.7 | 6.5 | NA |
| **81** | H | H | H |  | 3.0 | 1.7 | NC | 108 | 3.55 | NA | NA |
| **82** | H | H | H |  | 3.3 | 3.0 | NC | 110 | 5.5 | 18 | NA |
| **83** | H | H | $C(CH_3)_3$ |  | NA | 4.5 | NC | >1000 | 0.25 | NA | NA |
| **84** | H | H | $C(CH_3)_3$ |  | 1.5 | 0.87 | NC | 70 | 1.2 | 16 | 31 |




| Compd. No. | R | $R_1$ | $R_2$ | X | *P. falciparum* (D6) $IC_{50}$ (μg/mL) | *P. falciparum* (W2) $IC_{50}$ (μg/mL) | Cytotoxicity (Vero) $IC_{50}$ (μg/mL) | BH Inhibition $IC_{50}$ (μM) | MetHb toxicity (% MetHb formation at 20μg/mL) | *L. donovani* $IC_{50}$ (μg/mL) | *L. donovani* $IC_{90}$ (μg/mL) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **85** | H | H | $C(CH_3)_3$ |  | 2.7 | 1.8 | NC | 120 | 0.55 | 19 | NA |
| **PQ** | | | | | 2.0 | 2.8 | NC | >1000 | 10 | 19.9 | NA |

$IC_{50}$ and $IC_{90}$ are the sample concentration that kills 50% and 90% cells compared to vehicle control. NC, not cytotoxic up to 10 μg/mL. NA, Not active. Chloroquine: $IC_{50}$ = 0.014 μg/mL (D6 clone); $IC_{50}$ = 0.1 μg/mL (W2 clone). Artemisinin: $IC_{50}$ = 0.015 μg/mL (D6 clone); $IC_{50}$ = 0.009 μg/mL (W2 clone). BH inhibition activity: Chloroquine: $IC_{50}$ = 80 μM, BPQ: $IC_{50}$ = 2.9 μM, PQ: $IC_{50}$ > 1000 μM. Antileishmanial activity: Pentamidine: $IC_{50}$ = 1 μg/mL, $IC_{90}$ = 3.8 μg/mL. Amphotericin B: $IC_{50}$ = 0.19 μg/mL, $IC_{90}$ = 0.35 μg/mL.

**Table 4**

In vivo (*P. berghei*) antimalarial activity of selected bis(8-aminoquinolines)

| Compd. No. | *P. berghei* | | | |
|---|---|---|---|---|
| | **(10 mg/kg/day × 4, oral)** | **(25 mg/kg/day × 4, oral)** | **(50 mg/kg/day × 4, oral)** | **(100 mg/kg/day × 4, oral)** |
| **36** | – | – | (0/6) Inactive | (6/6) Curative |
| **37** | – | – | – | (0/6) Inactive |
| **43** | – | – | – | (5/6) Suppressive |
| **44** | (5/6) Suppressive | (6/6) Curative | (6/6) Curative | (6/6) Curative |
| **61** | (4/6) Suppressive | (6/6) Curative | (6/6) Curative | (6/6) Curative |
| **62** | – | – | – | (0/6) Inactive |
| **63** | – | – | – | (0/6) Inactive |
| **66** | – | – | – | (0/6) Inactive |
| **68** | – | – | – | (0/6) Inactive |
| **74** | – | – | – | (0/6) Inactive |
| **76** | – | – | – | (5/6) Suppressive |
| **79** | (3/6) Suppressive | (6/6) Curative | (6/6) Curative | (6/6) Curative |
| **PQ** | – | – | – | (0/6) Inactive |

The term ‘curative’ indicates complete elimination of malaria parasites from the body and animals survive up to day D+60. The term ‘suppressive’ indicates that all of the treated animals show negative parasitemia up to D+7. However, by D+60, some mice die, and some survive with complete elimination of parasitemia as indicated by numbers given in parentheses. The term ‘inactive’ indicates that the treated animals show positive parasitemia either on D+4 or D+7 and usually die by D+14. “–”, not tested.

**Table 5**

In vitro antibacterial activities of bis(8-aminoquinolines)

| Compd. No. | *S. aureus* | | | MRSA | | | *M. intracellulare* | | |
|---|---|---|---|---|---|---|---|---|---|
| | $IC_{50}$ ($\mu$g/mL) | MIC ($\mu$g/mL) | MBC ($\mu$g/mL) | $IC_{50}$ ($\mu$g/mL) | MIC ($\mu$g/mL) | MBC ($\mu$g/mL) | $IC_{50}$ ($\mu$g/mL) | MIC ($\mu$g/mL) | MBC ($\mu$g/mL) |
| **36** | – | – | – | 15 | 20 | 20 | 15 | 20 | 20 |
| **38** | – | – | – | 10 | 20 | NA | NA | NA | NA |
| **39** | – | – | – | 15 | 20 | 20 | NA | NA | NA |
| **40** | – | – | – | 10 | 20 | NA | NA | NA | NA |
| **41** | – | – | – | 8 | 20 | 20 | NA | NA | NA |
| **42** | – | – | – | 6.5 | 20 | 20 | 15 | 20 | NA |
| **43** | 2.58 | 5 | 5 | 1.97 | 5 | 5 | 7.12 | 20 | NA |
| **44** | 2.73 | 5 | 5 | 1.78 | 5 | 5 | 10.05 | 20 | NA |
| **60** | – | – | – | 15 | 20 | NA | 20 | NA | NA |
| **61** | – | – | – | 15 | 20 | NA | 15 | 20 | NA |
| **62** | – | – | – | 6.5 | 10 | 20 | NA | NA | NA |
| **63** | – | – | – | 8.5 | 20 | 20 | NA | NA | NA |
| **64** | – | – | – | 15 | NA | NA | 15 | 20 | NA |
| **67** | – | – | – | 3.0 | 5.0 | 10 | 4.5 | 10 | 20 |
| **70** | – | – | – | 8.5 | 20 | NA | NA | NA | NA |
| **71** | – | – | – | 3.5 | 5.0 | 10 | 15 | 20 | NA |
| **73** | – | – | – | 15 | 20 | NA | 15 | NA | NA |
| **79** | – | – | – | NA | NA | NA | 10 | 20 | 20 |
| **84** | – | – | – | 15 | 20 | 20 | NA | NA | NA |

$IC_{50}$ = the concentration ($\mu$g/mL) that affords 50% growth inhibition. MIC, minimum inhibitory concentration (the lowest concentration in $\mu$g/mL that allows no detectable growth). MBC, minimum bactericidal concentration (the lowest concentration in $\mu$g/mL that kills the organism). "–" not tested. NA, no activity at the highest test concentration of 20 $\mu$g/mL. Ciprofloxacin: $IC_{50}$ = 0.12 $\mu$g/mL, MIC = 0.50 $\mu$g/mL, MBC = 50 $\mu$g/mL (Sa); $IC_{50}$ = 0.09 $\mu$g/mL, MIC = 0.31 $\mu$g/mL, MBC = 2.5 $\mu$g/mL (MRSA); $IC_{50}$ = 0.3 $\mu$g/mL, MIC = 0.63 $\mu$g/mL, MBC = 2.5 $\mu$g/mL (Mi).

**Table 6**

In vitro antifungal activities of bis(8-aminoquinolines)

| Compd. No. | *C. albicans* | | | *C. neoformans* | | |
|---|---|---|---|---|---|---|
| | $IC_{50}$ ($\mu$g/mL) | MIC ($\mu$g/mL) | MFC ($\mu$g/mL) | $IC_{50}$ ($\mu$g/mL) | MIC ($\mu$g/mL) | MFC ($\mu$g/mL) |
| **36** | NA | NA | NA | 6.5 | 10 | 10 |
| **37** | NA | NA | NA | 15 | 20 | 20 |
| **38** | NA | NA | NA | 10 | 20 | 20 |
| **39** | NA | NA | NA | 15 | 20 | 20 |
| **40** | NA | NA | NA | 7 | 10 | 10 |
| **42** | 15 | NA | NA | 15 | 20 | 20 |
| **43** | 17.86 | 20 | NA | 6.45 | 10 | 10 |
| **44** | >20 | NA | NA | 9.97 | 20 | 20 |
| **60** | NA | NA | NA | 7.5 | 10 | 10 |
| **61** | NA | NA | NA | 15 | 20 | 20 |
| **62** | NA | NA | NA | 10 | 20 | 20 |
| **67** | 10 | 20 | NA | 15 | NA | NA |
| **71** | 10 | 20 | NA | 10 | 20 | 20 |
| **76** | NA | NA | NA | 4.5 | 5 | 5 |
| **79** | NA | NA | NA | 15 | 20 | 20 |

$IC_{50}$ = the concentration ($\mu$g/mL) that affords 50% growth inhibition. MIC, minimum inhibitory concentration (the lowest concentration in $\mu$g/mL that allows no detectable growth). MFC, minimum fungicidal concentration (the lowest concentration in $\mu$g/mL that kills the organism). NA, no activity at the highest test concentration of 20 $\mu$g/mL. Amphotericin B: $IC_{50}$ = 0.25 $\mu$g/mL, MIC = 0.63 $\mu$g/mL, MFC = 1.25 $\mu$g/mL (Ca); $IC_{50}$ = 0.75 $\mu$g/mL, MIC = 1.25 $\mu$g/mL, MFC = 1.5 $\mu$g/mL (Cn).