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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 (*Plasmodium falciparum*), antileishmanial (*Leishmania 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 (**44**, **61** and **79**) when tested for in vivo blood-schizontocidal antimalarial activity (*Plasmodium 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.

1. 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] The uncontrolled and irrational use of existing blood-schizontocidal (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] The 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] The 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 the PQ analogues exhibiting promising antimicrobial and antileishmanial activities, in addition to potent antimalarial activities.[11–17]

The dimers 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 the 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 the chloroquine resulting in

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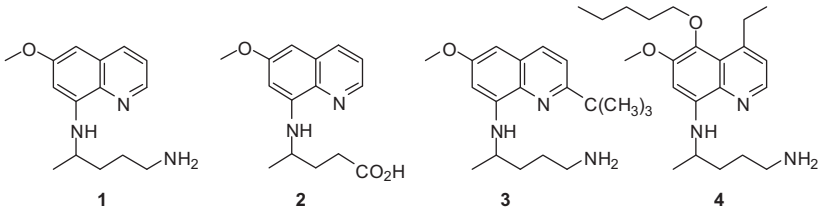


Figure 1. 8-Aminoquinolines.

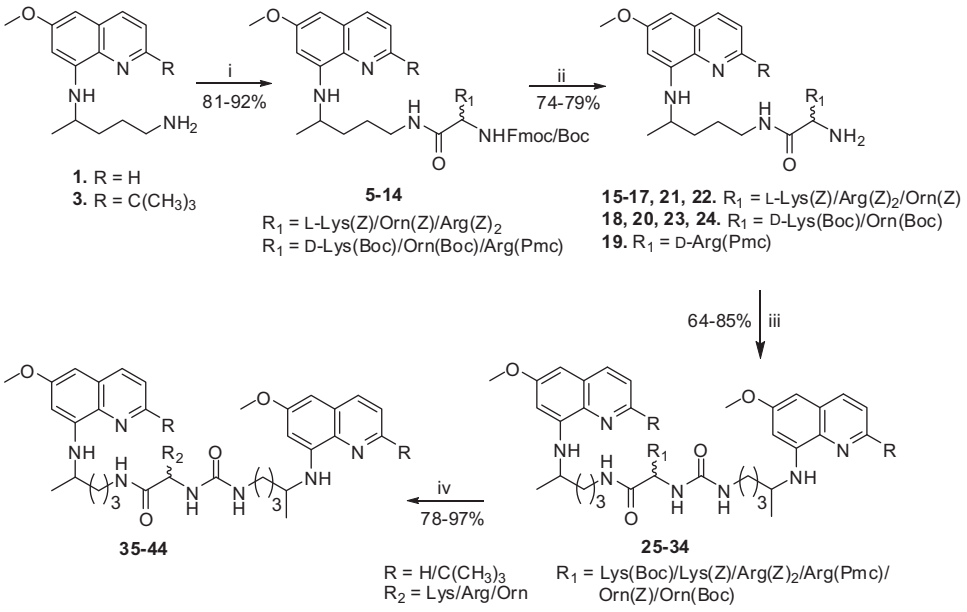
their accumulation to a higher degree in the face of a decreased pH gradient in the chloroquine resistant parasites. Although, dimers of 4-aminquinolines and artemisinin are extensively investigated, the potential of bis(8-aminoquinolines) in antimalarial drug discovery is not fully explored. Blanton 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.

2. Results and discussion

2.1. 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**), and 2-*tert*-butylprimaquine (**3**, Fig. 1) to obtain bis(8-aminoquinolines) linked through cationic amino acids (**35–44**, Scheme 1). The 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**.



Scheme 1. Reagents and conditions: (i) DIC, Fmoc/Boc-NH–CH(R_1)–CO_2H, DCM, 0 °C–rt, 4 h; (ii) 4 N 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, 4 h or 4 N HCl in MeOH, rt, 45 min or 8 N HCl in MeOH, rt, 8 h.

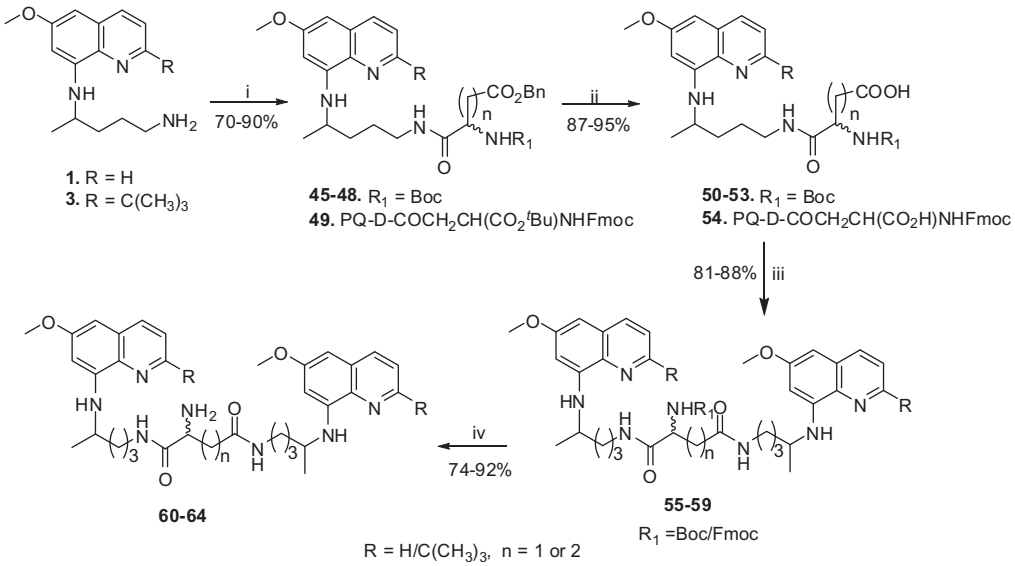
The anionic amino acids, Asp and Glu contain three groups, which provide suitable functionalities for the synthesis of bis(8-aminoquinolines). We have earlier observed that the presence of free NH_2 group in the side-chain is essential for the activity of 8-aminoquinolines.[14] Therefore, in this series 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). The 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 range varying from 0 °C to 70 °C and reaction time of 3 h to 24 h was used. The 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.

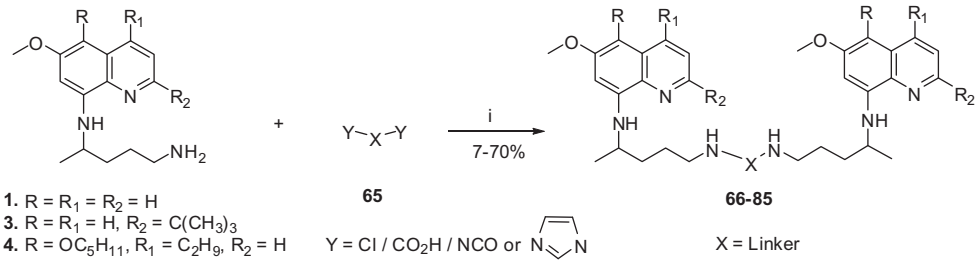
2.2. Antimalarial activity, cytotoxicity, inhibition of β-hematin (BH) and MetHb formation

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 *Plasmodium 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 μg/mL against D6 and W2 strains, respectively. While, analogue **43** showed IC_{50} of 1.6 and 1.3 μg/mL against D6 and W2 strains, respectively. The IC_{50} values of remaining analogues were in the range of 2.4–4.2 μg/mL for D6 strain and 1.5–3.2 μg/mL for W2 strain. The bis(8-aminoquinolines) **60–64** (Series 2) were less active with

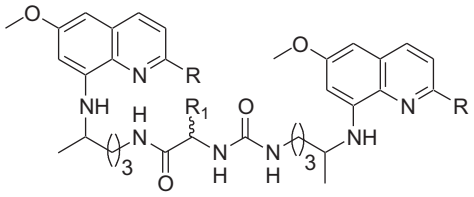


Scheme 2. Reagents and conditions: (i) DIC, R_1NH–$CHCOOH(CH_2)_n$–CO_2Bn, DCM, 0 °C–rt, 4 h; (ii) Pd–C/H_2, MeOH, rt, 4 h or 6 N HCl, rt, 5 h, 20% NH_4OH; (iii) DIC, **1** or **3**, DCM, 0 °C–rt, 4 h; (iv) 4 N HCl in MeOH, rt, 45 min or 20% piperidine in DCM, rt, 20 min.



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.

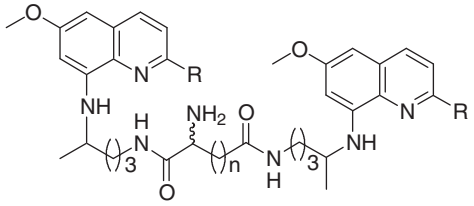
Table 1
In vitro antimalarial activity (*P. falciparum*), cytotoxicity, β-hematin (BH) inhibition, methemoglobin (MetHb) formation and in vitro antileishmanial activity (*L. donovani*) of bis(8-aminoquinolines) (**35**–**44**) (Series 1)



Compd No.	R	R_1	≀	*P. falciparum* (D6) 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 20 μg/mL)	*L. donovani* IC_{50} (μg/mL)	*L. donovani* IC_{90} (μg/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 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 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) 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 20 μg/mL)	*L. donovani* IC_{50} (μg/mL)	*L. donovani* IC_{90} (μg/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 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.

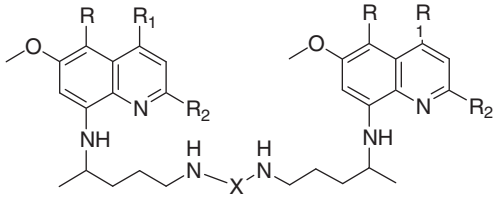
IC_{50} values in the range of 2.7–4.76 μg/mL (D6 strain) and 2.2–3.8 μg/mL (W2 strain).

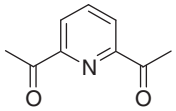
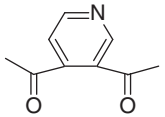
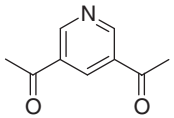
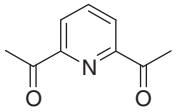
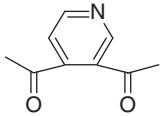
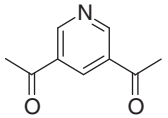
In Series 3, the most promising analogue **84**, a bisquinoline derivative linked through pyridine-3,4-dicarbonyl linker displayed IC_{50} of 1.5 μg/mL for D6 clone and 0.87 μg/mL for W2 clone. Analogues **70** and **73** showed IC_{50} of 1.7 and 1.5 μg/mL for D6 clone, respectively, and 1.3 μg/mL for W2 clone. While, analogue **79**, displayed IC_{50} of 2.6 μg/mL, and 1.5 μg/mL for D6 and W2 strains, respectively. Remaining analogues were also active (IC_{50} values in the range of 1.7–4.76 μg/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 μg/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 β-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 μM) in comparison to several other

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 μg/mL)	*L. donovani*	
					IC_{50} (μg/mL)	IC_{50} (μg/mL)	IC_{50} (μg/mL)	IC_{50} (μM)		IC_{50} (μg/mL)	IC_{90} (μg/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
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
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.

analogues of the series (IC_{50} values ranging between 45 and 162 μM). The compounds **60–64** of the Series 2 inhibited BH formation with IC_{50} values in the range of 85–185 μM. Among bisquinolines **66–85** of Series 3, analogues **70**, **73** and **84** displayed IC_{50} values of 80, 75 and 70 μM, respectively compared to IC_{50} of 80 μM for standard drug chloroquine (Tables 1–3). It has been proposed that pK_a 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 pK_a values is responsible for moderate β-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 μg/mL of the test compound, in comparison to vehicle control.[33] A number of analogues showed lower MetHb formation (Series 1: 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 *Plasmodium berghei* (sensitive strain) in a rodent malaria model using procedure described earlier (Table 4).[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.

2.3. Antileishmanial activities

The antileishmanial activities of the bisquinolines were evaluated in vitro against *Leishmania 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 μg/mL, and IC_{90} = 7.2 μg/mL) compared to other compounds of the series (IC_{50} in the range of 14–21 μg/mL, and IC_{90} in the range of 33–40 μg/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 μg/mL and IC_{90} = 30 and 10.7 μg/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 μg/mL and IC_{90} of 7.0, 7.8, and 27 μg/mL, respectively, compared to the IC_{50} of 1 μg/mL and IC_{90} of 3.8 μg/mL for standard drug, pentamidine. The analogues **67**, and **69** were also promising (IC_{50} = 4–9 μg/mL and IC_{90} = 20–31 μg/mL).

2.4. Antimicrobial activities

The bisquinolines were also tested for their antibacterial properties against *Staphylococcus aureus*, methicillin-resistant *S. 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 μg/mL and they showed a bactericidal activity at 5 μg/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 μg/mL and MIC of 20 μg/mL. All were bactericidal at 20 μg/mL, except analogues **38** and **40**. The activities were also observed against *M. intracellulare* with **36**, **42**, **43**, and **44** (IC_{50} ranged between 7.12 and 15 μg/mL, MIC = 20 μg/mL, and MBC = 20 μg/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 μg/mL, MIC of 10 and 20 μg/mL, respectively, and bactericidal at 20 μg/mL. The 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 μg/mL, MIC = 5–20 μg/mL, and MBC = 10–20 μg/mL). Analogue **67** also exhibited promising activity against *M. intracellulare* (IC_{50}, MIC and MBC of 4.5, 10 and 20 μg/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 μg/mL against *Candida glabrata*, *Candida 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**

Table 5
In vitro antibacterial activities of bis(8-aminoquinolines)

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

Table 6
In vitro antifungal activities of bis(8-aminoquinolines)

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

(Series 2) showed moderate activity against *C. neoformans*. The analogue **60** produced IC_{50} value of 7.5 μg/mL, and MIC and MFC values of 10 μg/mL; while, analogues **61** and **62** exhibited the IC_{50} values of 15 and 10 μg/mL, respectively, and were fungicidal at 20 μg/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 μg/mL. The promising analogue **76** exhibited IC_{50} of 4.5 μg/mL, MIC of 5 μg/mL, and was also fungicidal at 5 μg/mL.

2.5. 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. The inhibition of β-hematin formation by bisquinolines, although moderate, indicated it as a plausible pathway of their antimalarial activity. The 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. The 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.

3. Experimental

3.1. 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. ^1H 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.

3.2. 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-aminoquinoline (**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.

3.2.1. 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}; ^1H 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 (br s, 2H), 6.32 (m, 2H), 6.25 (m, 2H), 6.02 (br s, 2H), 5.05 (s, 2H), 4.96 (br s, 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. Calcd for $C_{45}H_{58}N_8O_6$ (806.5): C, 66.97; H, 7.24; N, 13.89. Found: C, 67.07; H, 7.15; N, 13.73.

3.2.2. 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 (br s, 2H), 8.49 (d, 2H, J = 4.1 Hz), 7.87 (d, 2H, J = 8.5 Hz), 7.31 (m, 12H), 6.83 (br s, 2H), 6.35 (m, 2H), 6.28 (m, 2H), 5.98 (br s, 2H), 5.11 (s, 2H), 5.09 (s, 2H), 5.02 (br s, 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. Calcd for $C_{53}H_{64}N_{10}O_8$ (968.4): C, 65.68; H, 6.66; N, 14.45. Found: C, 65.75; H, 6.72; N, 14.32.

3.2.3. 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 (br s, 2H), 6.31 (m, 2H), 6.25 (m, 2H), 5.95 (br s, 2H), 5.11 (s, 2H), 4.78 (br s, 1H), 4.68 (br s, 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. Calcd for $C_{44}H_{56}N_8O_6$ (792.3): C, 66.64; H, 7.12; N, 14.13. Found: C, 66.58; H, 7.24; N, 14.27.

3.2.4. *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 (br s, 1H), 6.49 (m, 2H), 6.42 (m, 2H), 5.87 (br s, 1H), 5.78 (br s, 1H), 5.33 (br s, 1H), 5.23 (br s, 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. Calcd for $C_{42}H_{60}N_8O_6$ (772.4): C, 65.26; H, 7.82; N, 14.50. Found: C, 65.34; H, 7.74; N, 14.63.

3.2.5. *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 (br s, 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 (br s, 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. Calcd for $C_{51}H_{70}N_{10}O_7S$ (966.5): C, 63.33; H, 7.29; N, 14.48. Found: C, 63.24; H, 7.35; N, 14.65.

3.2.6. *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. Calcd for $C_{41}H_{58}N_8O_6$ (758.4): C, 64.88; H, 7.70; N, 14.76. Found: C, 64.75; H, 7.64; N, 14.81.

3.2.7. Benzyl{(5*R*)-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 (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. Calcd for $C_{53}H_{74}N_8O_6$ (918.5): C, 69.25; H, 8.11; N, 12.19. Found: C, 69.33; H, 8.20; N, 12.05.

3.2.8. Benzyl{(4*R*)-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-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 (br s, 2H), 6.29 (d, 2H, J = 2.7 Hz), 6.23 (d, 2H, J = 2.7 Hz), 6.09 (br s, 2H), 5.85 (br s, 1H), 5.04 (s, 2H), 4.87 (br s, 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. Calcd for $C_{52}H_{72}N_8O_6$ (904.5): C, 69.00; H, 8.02; N, 12.38. Found: C, 69.07; H, 8.17; N, 12.51.

3.2.9. *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 (br s, 1H), 6.30 (m, 2H), 6.22 (m, 2H), 6.11 (br s, 2H), 5.06 (br s, 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. Calcd for $C_{50}H_{76}N_8O_6$ (884.5): C, 67.84; H, 8.65; N, 12.66. Found: C, 67.79; H, 8.74; N, 12.54.

3.2.10. *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-oxopentyl}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 (br s, 1H), 6.30 (m, 4H), 6.11 (br s, 2H), 5.20 (br s, 1H), 4.73 (br s, 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. Calcd for $C_{49}H_{74}N_8O_6$ (870.5): C, 67.56; H, 8.56; N, 12.86. Found: C, 67.46; H, 8.51; N, 12.94.

3.3. 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 (2 N 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 6 N methanolic HCl (10 mL) was stirred at ambient temperature for 45 min (for *t*-Boc group) or 8 N 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.

3.3.1. *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).

3.3.2. *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 (br s, 1H), 7.42 (br s, 1H), 7.24 (dd, 2H, J = 4.0 and 8.2 Hz), 6.98 (br s, 1H), 6.28 (m, 2H), 6.21 (m, 2H), 5.89 (br s, 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).

3.3.3. *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).

3.3.4. *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).

3.3.5. *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).

3.3.6. *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).

3.3.7. *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 (br s, 1H), 6.29 (m, 2H), 6.22 (m, 2H), 6.10 (br s, 2H), 4.67 (br s, 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 (br s, 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).

3.3.8. *N*-{4-[(2-*tert*-Butyl-6-methoxyquinolin-8-yl)amino]pentyl}-N^2-({4-[(2-*tert*-butyl-6-methoxyquinolin-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).

3.3.9. *N*-{4-[(2-*tert*-Butyl-6-methoxyquinolin-8-yl)amino]pentyl}-N^2-({4-[(2-*tert*-butyl-6-methoxyquinolin-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).

3.3.10. *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).

3.4. 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 6 N 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-aminoquinoline (**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.

3.4.1. *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}; ^1H 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 (br s, 2H), 6.54 (br s, 2H), 6.32 (m, 2H), 6.25 (m, 2H), 5.98 (br s, 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).

3.4.2. *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}; ^1H 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 (br s, 2H), 5.74 (br s, 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).

3.4.3. *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}; ^1H NMR ($CDCl_3$): δ 7.85 (d, 2H, J = 7.8 Hz), 7.42 (d, 2H, J = 7.8 Hz), 6.99 (br s, 2H), 6.30 (m, 2H), 6.23 (m, 2H), 6.11 (br s, 1H), 5.87 (br s, 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).

3.4.4. *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 (br s, 1H), 6.66 (br s, 1H), 6.31 (d, 2H, J = 2.1 Hz), 6.25 (d, 2H, J = 2.1 Hz), 6.12 (br s, 1H), 5.99 (br s, 1H), 5.68 (br s, 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).

3.4.5. 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 (br s, 2H), 6.38 (br s, 2H), 6.32 (m, 2H), 6.25 (m, 2H), 5.97 (br s, 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).

3.5. 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, 6 N 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 (2 N in ether) provided its hydrochloride salt.

3.5.1. 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).

3.5.2. 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).

3.5.3. 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).

3.5.4. 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}; ^1H 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).

3.5.5. 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}; ^1H 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 (br s, 2H), 6.32 (d, 2H, J = 2.3 Hz), 6.26 (d, 2H, J = 2.3 Hz), 5.99 (br s, 2H), 3.88 (s, 6H), 3.59 (m, 3H), 3.25 (m, 4H), 2.59 (m, 2H), 2.09 (br s, 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).

3.6. 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 (2 N in ether) provided their HCl salts.

3.6.1. 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}; ^1H 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 (br s, 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).

3.6.2. 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}; ^1H 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 (br s, 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).

3.6.3. 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}; ^1H 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 (br s, 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).

3.6.4. 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 (br s, 1H), 5.64 (br s, 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).

3.6.5. 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 (br s, 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).

3.6.6. 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 (br s, 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).

3.7. 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 (2 N in ether) provided **72** and **73** as HCl salts.

3.7.1. *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 (br s, 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).

3.7.2. *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 (br s, 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).

3.8. 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 (2 N in ether).

3.8.1. *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}; ^1H NMR (free base, $CDCl_3$): δ 8.77 (br s, 1H), 8.52 (br s, 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).

3.8.2. *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}; ^1H 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 (br s, 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).

3.9. 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 (2 N in ether) provided **76–78** as HCl salts.

3.9.1. ({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}; ^1H 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 (br s, 1H), 5.74 (s, 2H), 4.90 (br s, 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).

3.9.2. ({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 (br s, 1H), 5.49 (br s, 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).

3.9.3. *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).

3.10. 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 2 N solution of HCl in ether. Yield: 82%; hygroscopic solid; IR (free base, CH_2Cl_2): 3428 cm^{-1}; ^{1}H 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 (br s, 1H), 4.04 (br s, 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).

3.11. 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 2 N solution of ethereal HCl.

3.11.1. *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}; ^{1}H 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 (br s, 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).

3.11.2. *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}; ^{1}H 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 (br s, 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).

3.11.3. *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}; ^{1}H 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 (br s, 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).

3.11.4. *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}; ^{1}H 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).

3.11.5. *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}; ^{1}H 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 (br s, 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).

3.11.6. *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}; ^{1}H 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 (br s, 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).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.11.036.

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