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Article

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Antimalarial Activity of 4-Amidinoquinoline and 10-Amidinobenzonaphthyridine Derivatives

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Abstract

Chloroquine (CQ) has been used as first line malaria therapeutic drug for decades. Emergence of CQ drug-resistant *Plasmodium falciparum* malaria throughout endemic areas of the world has limited its clinical value. Mefloquine (MQ) has been used as an effective malaria prophylactic drug, due to its long acting and high potency against blood stage *Plasmodium falciparum (Pf)*. However, serious CNS toxicity of MQ has compromised its clinical value as prophylaxis drug. Therefore, new and inexpensive antimalarial drugs with no cross-resistance to CQ or CNS toxicity are urgently needed to combat this deadly human disease. In this study, a series of new 4-amidinoquinoline (4-AMQ) and 10-amidinobenzonaphthyridine (10-AMB) derivatives were designed, prepared and assessed to search for new therapeutic agents to replace CQ and MQ. The new derivatives displayed high activity in vitro and in vivo, with no cross-resistance to CQ, and none were toxic in mice up to 160 mpk x 3. The best compound shows IC_{50} < 1 ng/mL against D6, W2 and C235 *Pf* clones, low inhibitory activity in hERG K^+ channel blockage test, negative in Ames test and 5/5 cure @ < 15 mpk x 3 in mice infected with *P. berghei*. In addition to these desirable pharmacological profiles, compound **13b,** one of the most active compounds, is metabolically stable in both human and mouse liver microsomal preparations and has plasma $t_{1/2}$ of 50 hours in mice which made it a good MQ replacement candidate.

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INTRODUCTION

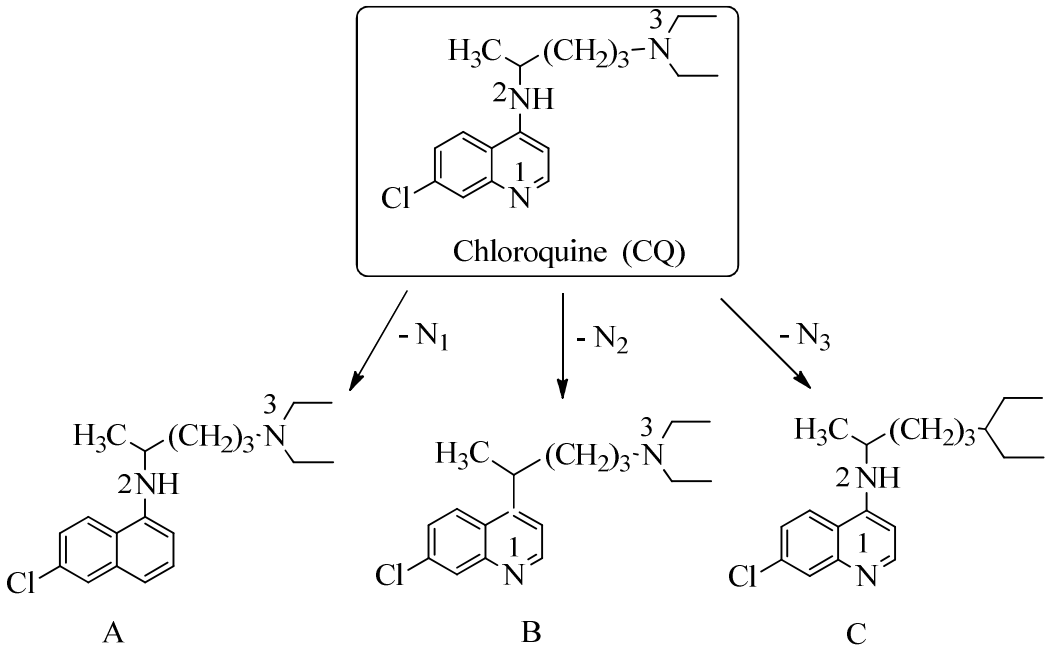
Chloroquine (CQ), one of the 4-aminoquinoline antimalarial agents, has been used as the first line antimalarial drug since War World II.[1-2] However, the wide spread of chloroquine-resistant *Plasmodium falciparum (Pf)* malaria is severely limiting its therapeutic value to combat this deadly human disease.[3-5] Mefloquine (MQ) was commonly used as a malaria prophylactic drug,[6] but drug resistance, high cost and CNS toxicity have greatly compromised its clinical value as an antimalarial drug. Thus, there remains an urgent need for new and inexpensive antimalarial agents.[7]

Chloroquine is believed to exert its activity by inhibiting hemozoin formation in the digestive vacuoles of the malaria parasites,[8-13] although some oppose this view[14] and other possibilities have also been postulated.[15-17] O'Brien and Hahnla[18-20] have offered a model to account for the antimalarial activity of chloroquine and its congeners. They suggested that: (1) these compounds exert their antimalarial effect by intercalation with the parasite DNA, and that the activity of a given compound depends on the stability of its complex with DNA; (2) high activity requires an electronegative group attached to the 7-position of the quinoline ring; and (3) the diamino side chain attached to the 4-position of quinoline ring bridges the two DNA strands by electrostatic interactions between the two side chain nitrogens and the DNA phosphate groups.

Additionally, the potency of chloroquine to inhibit parasite growth is greatly enhanced by its concentration within the parasites, possibly because of the high acidity in parasitic food vacuoles and the basicity of CQ molecule, resulting in protonation of CQ and decreasing its efflux from parasites.[21] The cause of chloroquine resistance is unknown, but it is clearly associated with alteration in membrane-associated transport processes causing a reduction of drug uptake into parasites and/or an increased efflux of the drug out of the bugs.[22-23]

Existing structure-activity relationships of 4-aminoquinoline (4-AQ) antimalarials clearly indicate that not only both side chain nitrogens (N_2 and N_3), but all 3 nitrogen atoms of the CQ molecule play an important role in CQ activity and/or toxicity. Replacement of any one of the three CQ nitrogen atoms with a carbon results in dramatic decrease or complete abolishment of the antimalarial activity of compounds A, B and C.[24-26] (Figure 1).

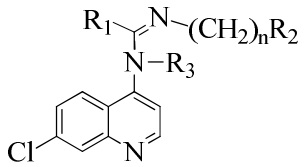
Figure 1. All 3 Amino Groups of CQ Are Essential for CQ Activity



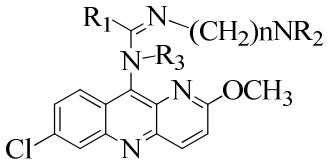
All three compounds A, B, and C are either inactive or weakly active

Although large numbers of 4-aminoquinoline derivatives were prepared in search for better CQ analogs during the past decades, the majority of the medicinal chemistry efforts focused mainly on modifications of the chain length between the two amino groups (N^2 and N^3) and the size of substituents on the terminal amino group (N^3).[27-30] Although some attempts have been made to change the pKa of either ring nitrogen (N^1) or 4-amino (N^2) of CQ, no attempt has been made to change the basicity of both N^1 and N^2 nitrogens of CQ at the same time.[24] In this study, a series of new 4-amidinoquinoline (4-AMQ) and 10-amidinobenzonaphthyridine (10-AMB) derivatives were designed, prepared and assessed for antimalarial activities in an attempt to explore the effect of changing the N^1 and N^2 basicity to the antimalarial activities (Figure 2). The main difference between the new 4-AMQ derivatives and CQ is that the 4-amino group of CQ is replaced by an amidine (4-NHCR=NH) functional group. Through conjugation with the amidine group, the basicity of ring nitrogen (N^1) and 4-amino group (N^2) of the new molecules will be altered significantly as shown in Figure 3. Furthermore, the new 4-AMQ analogs add an additional amino group (N^4) which could provide a potential additional binding site to the drug's biological receptors, resulting in significant changes in pharmacological profiles from chloroquine and its congeners. Another advantage of the new 4-amidino analogs over the CQ is

Figure 2: Structures of 4-Amidinoquinolines and 10-Amidinobenzonaphthyridines

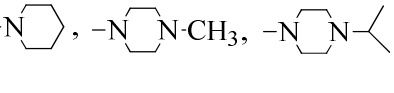


4-Amidinoquinolines (AMQ)



10-Amidinobenzonaphthyridines (10-AMB)

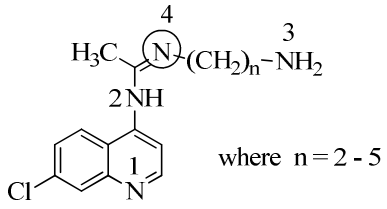
n = 2 - 5 R_1 = -CH_3, -$CH(CH_3)_2$,  or  X = -H, or -Cl,

R_2 = -$N(CH_3)_2$, -$N(CH_2CH_3)_2$, 

R_3 = H, or -$(CH_2)nR_2$

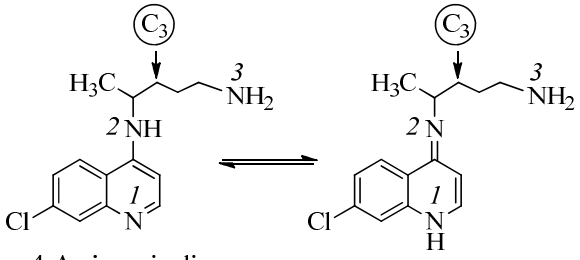
that amidine is a stronger base than the 4-aminoquinoline.[31-32] Higher basicity of amidines (N^2 and N^4) may also lead to more stable DNA intercalation, and/or render the new amidines better inhibitors of hemozoin formation, both of which are believed to be responsible for CQ antimalarial activity.

Figure 3: Rationale for the Synthesis of 4-Amidinoquinoline Derivatives as Antimalarial Agents

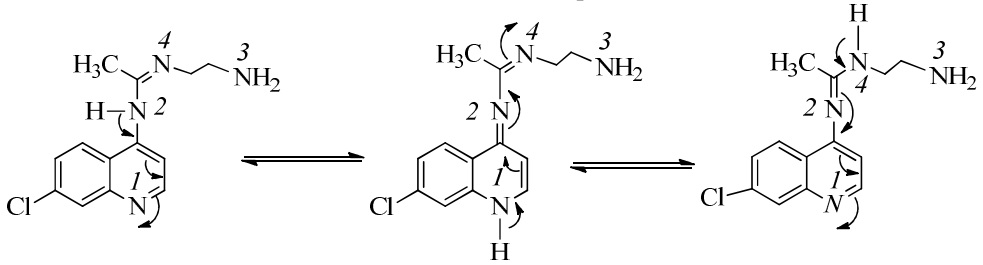


where n = 2 - 5

4-Amidinoquinolines



4-Aminoquinolines
chloroquine

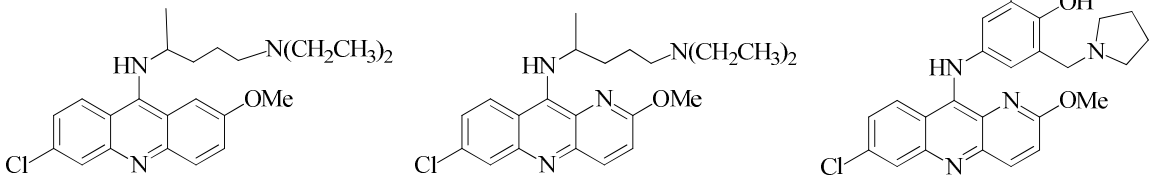


1). N^1, N^2 and N^3 of CQ are critical for antimalarial activity.

2). Replacement of C^3 of CQ with a "N^4" will change drastically the basicity of N^1 and N^2, add another binding site (N^4) and alter the pharmacological proflie of CQ.

Extending the same approach, a series of new 10-amidinobenzonaphthyridine derivatives (10-AMB) was also prepared and tested in this study (Figure 2). The difference between 10-AMB and the known antimalarial azacrin is that the 10-amino group of the latter is replaced with an amidine function. Antimalarial activities of 10-aminobenzonaphthyridine derivatives, such as azacrin and pyronaridine, have been described previously[33-37] (Figure 4). Clinical use of azacrin was abandoned because its efficacy was not superior to mepacrine or CQ.[34b] Pyronaridine has been used clinically for treatment of CQ-resistant *P. falciparum* infections in Africa continent.[35] However, pyronaridine is a Mannich base antimalarial with a benzonaphthyridine instead of a quinoline ring, like those found in amodiaquine, amopyroquine, tebuquine and isotebuquine.[38-42] Mannich base antimalarials have attracted vast attention in malaria chemotherapy for decades, because they are more potent than and showed no cross-resistance to CQ. Nevertheless, the clinical use of Mannich bases has been severely restricted because of chronic hepatoxicity and agranulocytosis side effects associated with its long term use.[43-45] Recent reports indicate the amodiaquine is metabolized by cytochrome P-450 to form a reactive quinoneimine metabolite with subsequent conjugation of the metabolite to glutathione or cysteinyl functions of enzymes.[46-48] The report suggests that alkylation of key macromolecules, such as DNA, RNA, glutathione and enzymes, by the reactive quinoneimine metabolite is the possible mechanism of action and/or toxicity of Mannich base antimalarials. The new 10-AMB derivatives described in this study, while they do contain a benzonaphthyridine ring, are not Mannich base compounds and thus are not likely to share the same hepatotoxicity of pyronaridine.

Figure 4: Structure of Mepacrine, Azacrin and Pyronaridine



Mepacrine Azacrin Pyronaridine

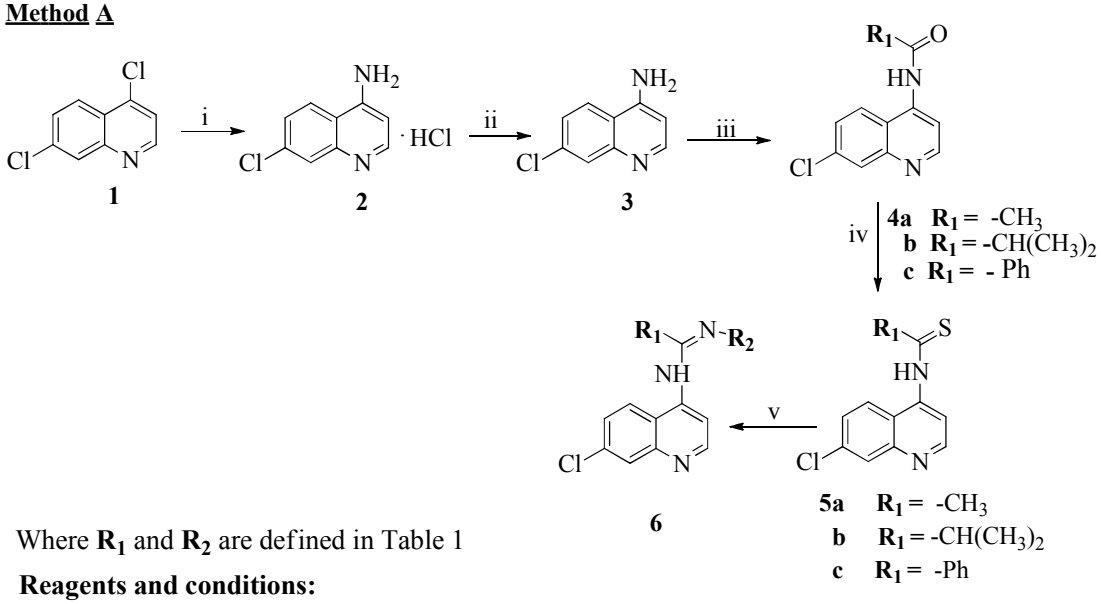
CHEMISTRY

4-Amidinoquinolines derivatives **6a-s** were prepared according to the method shown in Scheme 1. The key starting material, 4-amino-7-chloroquinoline (**3**), was prepared by two approaches. The first approach involved heating 4,7-dichloroquinoline (**1**) with ammonia in phenol at 165 ^{0}C to give compound **3** in 40% yields. The second approach used ammonium carbonate/phenol as ammonia source to give the desired product **3** in > 90% yields. The products prepared by both methods are identical in NMR and MS, but the second approach gave much better yields than the first. N-(7-Chloroquinolin-4-yl)-acetamide (**4**) was prepared in 72% yields by heating 4-amino-7-chloroquinoline (**3**) in acetic anhydride at 140 ^{o}C for 2 hrs. The key intermediate, N-(7-chloroquinolin-4-yl)-ethanethioamide (**5**), was prepared by refluxing acetamide **4** and Lawesson reagent [2,4-bis-(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane 2,4-disulfide][49] in toluene. Lawesson reagent is the most commonly used thiation reagent and is available commercially. Initially, thiation of amide **4** failed to afford the thioacetamide **5a** by heating **4a** and Lawesson's reagent in benzene or THF. However, at higher temperature, thiation of acetamide **4a** with Lawesson reagent proceeded smoothly when the reaction was carried out in boiling toluene to give **5a** in 25 - 45% yields. Product **5a** is not very stable at room temperature and thus was used soon after it was prepared. The instability of the product **5a** may account for the low and variable yields of thiation reactions.

Two different catalysts, HgO and DCC (N, N'-dicyclohexylcarbodiimide), were used in the preparation of the final products **6a-**s (Table 1). Coupling reactions of thioacetamide **5** with appropriate amines proceeded smoothly at room temperature under HgO catalysis to afford the desired 4-amidinoquinoline derivatives **6** in 70 - 90 % yields. Without HgO as catalyst, the thioacetamide failed to couple with amines even at elevated temperature. Due to high toxicity of HgO, non-toxic DCC was employed as an alternative catalyst. The latter catalyst, however, gave lower yields than HgO, due to tedious separation of the desired product from the thiourea byproduct. Although HgO gave cleaner reactions and superior yields than DCC, the latter is a preferred catalyst for the final step coupling reactions, in view of the strict FDA regulations on residual heavy metals in foods and drugs.

Scheme 1: Synthesis of 4-Amidinoquinolines Derivatives

Method A



Where $\mathbf{R_1}$ and $\mathbf{R_2}$ are defined in Table 1

Reagents and conditions:

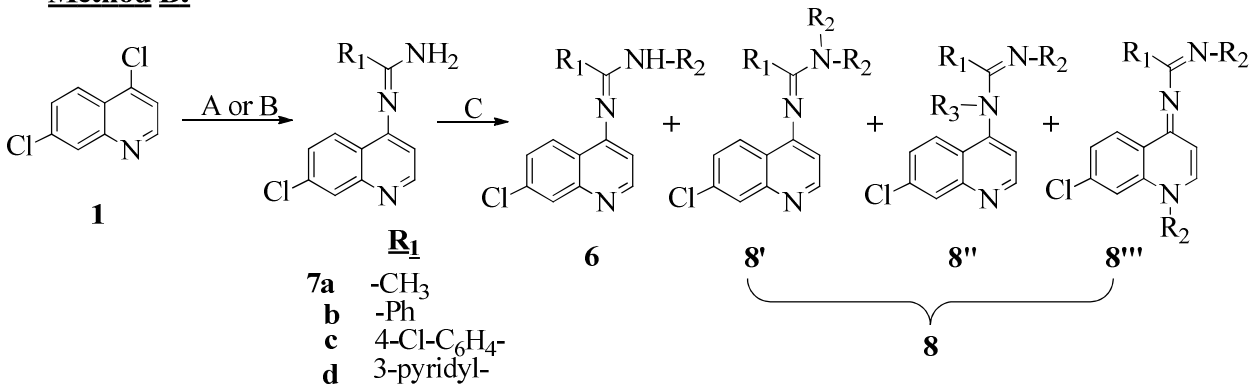
i) Ammonium carbonate, Phenol, 110 ^{0}C, 3h; ii) 10% NaOH, $CHCl_3$, 89.0 %; iii) Ac_2O, reflux, 2h, 72.6%; iv) Lawson's reagent, Toluene, 24h reflux, 60 %; v) Amine, HgO/DMF, Et_3N, rt, 8h, 90%; vi) Succinic acid, CH_3CN/MeOH, rt, 3h, 74%.

The Method A for preparation of **5a** was successfully applied to prepare (7-chloroquinolin-4-yl)-thioisobutyramide (**5b**). However, thiation of quinolin-4-yl-benzamide (**4c**) using Lawesson's reagent[49] failed to give the corresponding thioamide **5c.** Therefore, an alternative Method B with shorter route and better yields was developed to prepare benzamidine intermediates **7a-d** and the final products **6** and **8** as shown in Scheme 2.

The Method B is a new and facile procedure to synthesize the target compounds with wider choice of substituents at R1 position than Method A. In addition, Method B also avoided the use of hazardous HgO or tedious DCC as catalyst. The approach started with N-arylation of substituted amidines [R_1-C(=NH)NH2] with 4,7-dichloroquinoline (1) to give **7a-d**, followed by N alkylation with appropriate N,N-disubstituted 3-chloropropylamines (Scheme-2, Method-B). Copper-catalyzed arylation of amidines with aryl iodides as coupling partners was reported, providing either arylated amidines[50] or anilines[51]. The latter was formed as a result of hydrolysis of amidines **6** or **7**. However, with an active chloride, 4, 7-dichloroquinoline (**1**) does not require copper catalyst for arylation reaction. In all coupling reactions, small amount

Scheme 2: Synthesis of 4-Amidinoquinolines Antimalarials

Method B:



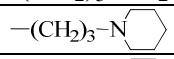
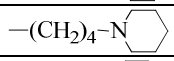
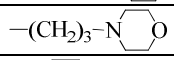
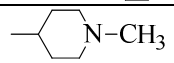
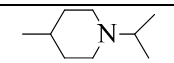
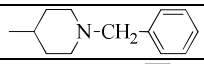
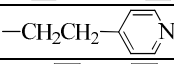
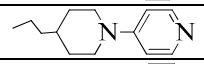
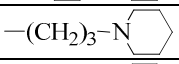
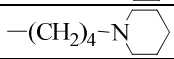
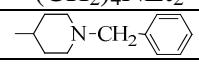
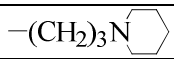
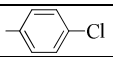
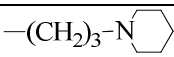
Where $\mathbf{R_1}$, $\mathbf{R_2}$ and $\mathbf{R_3}$ are defined in Table 1,

Reagents and conditions: (A) $R_1C(=NH)NH_2 \times HCl$, K_2CO_3, DMSO, 120 °C, 4-24 h; (B) $R_1C(=NH)NH_2 \times HCl$, NaH, DMSO, rt, 68 h; (C) $RN(CH_2)nCl$, NaH, DMSO, 100 °C, 2-6 h.

of unreacted starting material 4, 7-dichloroquinine was recovered, and in some cases, formation of 4-amino-7-chloroquinoline was observed, a result of base hydrolysis of the amidines formed. At higher temperatures or longer reaction times, formation of multiple unknown side products was observed. Treatment of **7a-d** and N, N-disubstituted 3-chloropropylamines in anhydrous DMSO and K_2CO_3 or NaH gave final products **6** and **8** in various yields, depending on amidine **7** and the ratio of the reagents used. On 1: 1 ratio of the reagents, the coupling reactions provided predominantly product **6**, whereas higher ratio of halide and amidine **7** yielded mainly the bis-alkylation product **8**. While alkylation of acetamidine **7a** and isobutyrimidamide **7b** preceded smoothly using K_2CO_3 or NaH as base catalyst, alkylation of benzamidine (**7c-d**) required the use of NaH; use of K_2CO_3 as catalyst provided poor yields of desired product **6**. Since compounds **6w** and **6x** contain an electron-withdrawing 4-chlorophenyl and pyridine ring, respectively, they are more prone to be hydrolyzed under the strong base reaction conditions than analogs **6t-v**, resulting in lower yields of **6w-x** than **6t-v**.

N-alkylation of amidines **7a** with N, N-diethyl-3-chloropropylamine can produce two isomeric products with N-alkylation taking place at either terminal nitrogen (N^1) to give **6b** or

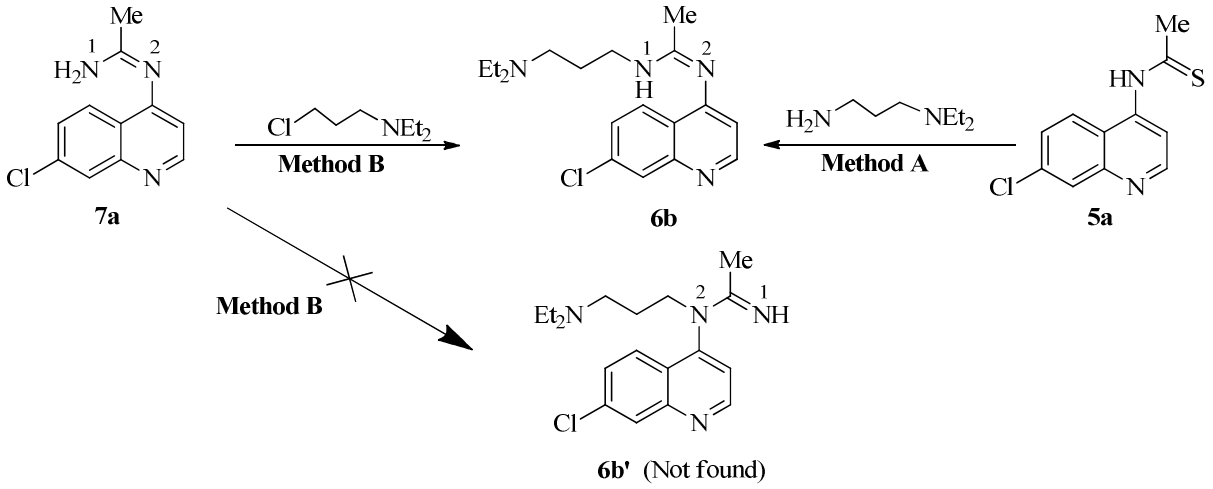
Table 1: List of 4-Amidinoquinoline Derivatives

Compound #	R_1	R_2	R_3	Method	% Yield
6a	$-CH_3$	$-(CH_2)_2NEt_2$	H	**A**	81
6b	$-CH_3$	$-(CH_2)_3NEt_2$	H	**A** and **B**	90
6c	$-CH_3$	$-(CH_2)_4NEt_2$	H	**A**	86
6d	$-CH_3$	$-(CH_2)_5NEt_2$	H	**A**	92
6e	$-CH_3$		H	**A**	72
6f	$-CH_3$		H	**A**	77
6g	$-CH_3$		H	**A**	64
6h	$-CH_3$		H	**A**	73
6i	$-CH_3$		H	**A**	80
6j	$-CH_3$		H	**A**	85
6k	$-CH_3$		H	**A**	74
6l	$-CH_3$		H	**A**	87
6m	$-CH(CH_3)_2$		H	**A**	87
6n	$-CH(CH_3)_2$		H	**A**	94
6p	$-CH(CH_3)_2$	$-(CH_2)_2NEt_2$	H	**A**	71
6q	$-CH(CH_3)_2$	$-(CH_2)_3NEt_2$	H	**A**	
6r	$-CH(CH_3)_2$	$-(CH_2)_4NEt_2$	H	**A**	98
6s	$-CH(CH_3)_2$		H	**A**	70
6t		$-(CH_2)_3NMe_2$	H	B	50
6u		$-(CH_2)_3NEt_2$	H	B	50
6v			H	B	49
6w		$-(CH_2)_3NMe_2$	H	B	9
6x		$-(CH_2)_3NEt_2$	H	B	25
8a	$-CH_3$	$[-(CH_2)_3NEt_2]_2$	-	B	13
8b		$-(CH_2)_3NEt_2$	$-(CH_2)_3NEt_2$	B	8
8c		$-(CH_2)_3NMe_2$	$-(CH_2)_3NMe_2$	B	4
8d				B	12
8e		$-(CH_2)_3NEt_2$	$-(CH_2)_3NEt_2$	B	$^{+}3$
8f		$-(CH_2)_3NEt_2$	$^{*}-(CH_2)_3NEt_2$	B	$^{+}14$

$^{+}$by-product of **6x** synthesis; $^{*}2^{nd}$ alkyl side chain on ring nitrogen.

the substituted nitrogen (N^2) to produce **6b'** (Scheme 3). Unambiguous identification of **6b** from **6b'** based on NMR data is difficult, due to similarity in NMR spectrum. To positively confirm the structure of the alkylation product, both Method A and Method B were used to prepare compound **6** as shown in Scheme 3. Method-A can provide only N_1 –alkylation product **6b**, but Method B produces either N_1- or N_2- alkylation product, **6b** or **6b'**. Since NMR and LC/MS of the compounds prepared by both methods are identical, N-alkylation reaction of **7a** with N,N-diethyl-3-chloropropylamine at ratio of 1: 1 should have occurred predominantly at terminal nitrogen N^1 to yield **6b**, not **6b'**.

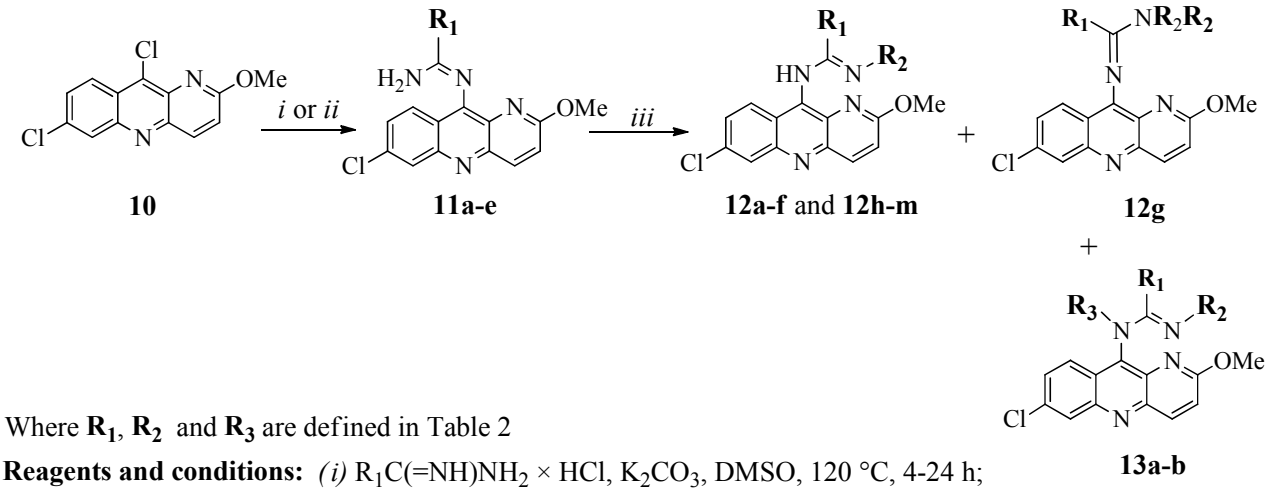
Scheme 3: Confirmation of compound **6b** prepared by both **Methods A** and **B**



As described above (Scheme 2), treatment of compound **7a** with 3-chloro-N,N-disubstituted- propylamine in 1: 1 ratio yielded desired product **6b** in 80% yields, but a minor bis-alkylation product **8a** was also isolated in 13 % yields. Other bis-aminoalkyl analogs **8b-f** were, likewise, isolated from the synthesis of the corresponding benzamidine analogs **6t, 6u**, **6v** and **6x,** respectively (Table 1). When the ratio of N, N-disubstituted 3-chloropropylamines to amidines **7** was increased to 2: 1 or higher, the N, N'-bis-alkylation products **8a-e** were the major products of the coupling reactions. Depending upon the substituent at R_1 position, the follow-up alkylation of **6** could take place at either amidine nitrogen (N_1, N_2) or ring nitrogen to give bis-alkylation products **8a-f** (Table 1)**.** While compound **6b** (R_1 = -CH_3) gave bis-alkylation product **8a** with the first and the 2nd alkyl side chains attached at the same terminal nitrogen (N_1) as in **8'**, the second alkylation of the other 3 benzamidine analogs **6t-v** (R_1 = Ph) occurred at N_2

nitrogen as in **8"** to give **8b-d,** respectively. Compound **6x** (R_1 = 3-pyridyl), however, gave two isomeric bis-alkylation products **8e** and **8f** with the 2nd aminoalkyl side chain located at N_2 (**8"**) and quinoline ring nitrogen (**8"'**), respectively. Assignment of structures **8a-f** was based on ^1H and ^{13}C NMR spectra. There is only one set of ^1H and ^{13}C signals for both side chains of compound **8a** (**8'**); but both aminoalkyl chains of **8b-f** display as two different sets of signals. The difference in location of the 2nd aminoalkyl side chain of **8e** and **8f** was reflected in changes in chemical shifts of 2-H and 3-H of quinoline ring. The chemical shift of 2-H was up field shifted from 8.35 ppm for **8e** to 7.01 ppm for **8f**. Similarly, the proton at 3 position of **8e** was up field shifted from 6.12 ppm for **8e** to 5.50 ppm for compound **8f**.

Scheme 4: Synthesis of Benzonaphthyridine Derivatives **12** and **13**

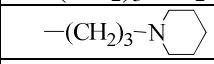
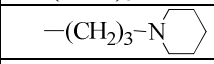
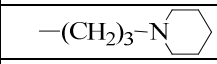
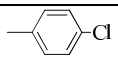
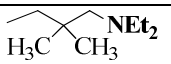


10 **11a-e** **12a-f** and **12h-m** **12g** **13a-b**

Where $\mathbf{R_1}$, $\mathbf{R_2}$ and $\mathbf{R_3}$ are defined in Table 2

Reagents and conditions: *(i)* $R_1C(=NH)NH_2 \times HCl$, K_2CO_3, DMSO, 120 °C, 4-24 h; *(ii)* $R_1C(=NH)NH_2 \times HCl$, NaH, DMSO, rt, 68 h; *(iii)* $RN(CH_2)nCl$, NaH, DMSO, 100 °C, 2-6 h.

Method B used for the synthesis of 4-amidinoquinoline derivatives was adapted to prepare 10-amidinobenzonaphthyridine analogs **12** and **13** as shown in Scheme 4. Like the synthesis of 4-amidinoquinolines, treatment of 7,10-dichlorobenzonaphthyridine (**10**) with amidines in dried DMSO using K_2CO_3 or NaH as catalyst gave good yields of amidines **11a-e,** which on treatment with N,N-disubstituted 3-chloropropylamine provided final products **12** and **13** in various yields and products distribution, depending upon the conditions and ratio of starting materials and reagents used. Alkylation of amidine **11a-e** gave predominantly mono-alkylation products **12a-m** with alkylation took place at the terminal nitrogen N_1 of amidine plus

Table 2: List of 10-Amidinobenzonaphthyridine Derivatives					
Compound #	**R_1**	**R_2**	**R_3**	**Method**	**% Yield**
12a	$-CH_3$	$-(CH_2)_3NMe_2$	-H	B	43
12b	$-CH_3$	$-(CH_2)_3NEt_2$	-H	B	47
12c	$-CH_3$		-H	B	44
12d	$-CH(CH_3)_2$	$-(CH_2)_3NEt_2$	-H	B	70
12e	$-CH(CH_3)_2$	$-(CH_2)_3NMe_2$	-H	B	61
12f	$-CH(CH_3)_2$		-H	B	61
12g	$-CH(CH_3)_2$	$-[(CH_2)_3NEt_2]_2$	-	B	8
12h		$-(CH_2)_3NMe_2$	-H	B	51
12i		$-(CH_2)_3NEt_2$	-H	B	49
12j			-H	B	23
12k		$-(CH_2)_3NEt_2$	-H	B	25
12l		$-(CH_2)_3NMe_2$	-H	B	28
12m			-H	B	63
13a	$-CH_3$	$-(CH_2)_3NMe_2$	$-(CH_2)_3NMe_2$	B	*20
13b	$-CH_3$	$-(CH_2)_3NEt_2$	$-(CH_2)_3NEt_2$	B	**11
* by-product of **12a** synthesis**,** ** by-product of **12b** synthesis					

minor bis-alkylation products with which the 2nd alkylation occurred at either N_1 or N_2 nitrogen of amidine to give **12g** or **13**, respectively. Like the quinoline analogs **8a-f**, assignments of the structures **12g** and **13a-b** were based on ^1H and ^{13}C NMR spectra. Compound **12g** possesses only one set of signals for both side chain protons, indicating both aminoalkyl side chains are located on N_1 amidine nitrogen. The protons and carbons of the two aliphatic chains of analogs **13a-b** appear as two different sets of signals, indicative of different alkylation sites for both alkylamino side chains as shown in Scheme 4. The new 10–amidinobenzonaphthyridine (10-AMB) compounds are listed in Table 2.

RESULTS AND DISCUSSION:

Each of the two new classes of 4-amidinoquinoline (4-AMQ) and 10-amidinobenzo-naphthyridine (10-AMB) antimalarial contains acetimidamide, isobutyrimidamide and benzimidamide analogs (Figure 2, $\mathbf{R_1}$ = $-CH_3$, $-CH(CH_3)_2$, -Ph, 4-Cl-Ph and 3-pyridyl). The majorities of the 4-acetimidamidoquinoline derivatives (**6b-l**) are metabolically stable in both

human and mouse microsomal preparations with $t_{1/2}$ > 60 min, except **6a, 6e, 6g,** and **6k.** However, among the 6 isobutyrimidamide analogs **6m-s**, only compound **6q** is metabolically stable and all 5 benzimidamide analogs (**6t-x**) are metabolically unstable. Piperidine, morpholine, and pyridine rings which are vulnerable to microsomal oxidation are partially responsible for the metabolic instability of the new compounds **6e, 6g,** and **6k**. It is interesting to note that compounds **8a-f** with bis-alkyl side chains are metabolically stable. Steric hindrance may play a role in reducing the susceptibility of **8a-f** to microsomal hydroxylation and subsequent degradation of the hydroxylated metabolites (Table 3).

Most of the new compounds exhibited moderate to potent cell growth inhibition in both CQ sensitive (D6) and CQ resistant *P. falciparum* clones (W-2 and C-235) with IC_{50} <100 ng/mL, except **6g**, **6k**, **8e** and **8f** which contain morpholine or pyridine rings. Benzimidamide derivatives (**6t-w**) are more active than the isobutyrimidamide and acetimidamide analogs. The IC_{50}s of the most active compounds, **6t** and **6u**, are less than 1 ng/mL against the CQ sensitive clone D6, with a W2/D6 ratio of 4. This is much improved compared to that of CQ, which has a W2/D6 ratio of 35, suggesting that compounds of this series have little or no cross resistance to chloroquine. Against the CQ resistant clone W-2, compounds **6t** and **6u** are 50-60 folds more active than CQ with IC_{50}s of 3.4 and 2.8 ng/mL, respectively. Furthermore, the 4-chlorophenyl analog **6w** is equally potent against both CQ sensitive and CQ resistant cell lines, D6, W2 and C235, with IC_{50}s of 1.1, 1.5 and 0.59 ng/mL, respectively. Although metabolically stable and highly active against the CQ sensitive cell line D6, compounds **8a-d** with bis-aminoalkyl side chains showed significant CQ cross resistance with W2/D6 ratio of 8 – 17.

Table 3: In vitro Biological Activities of 4-Amidinoquinoline Derivatives

#	*P. falciparum* IC_{50}: *ng/mL(nM)*				HepG2 IC_{50} *(ng/mL)*	Met. Stab. *($t_{1/2}$, min)*		Clog-P	*hERG %@ 10 μM
	D6	W2	C235	W2/D6		*HLM	*MLM		
CQ	4.8 (15)	168 (525)	66 (206)	35	12020	>60	>60	3.73	71
6a	60 (189)	94 (296)	58 (182)	1.6	1322	26	18	2.94	#NA
6b	46 (138)	75 (226)	44 (133)	1.63	>10000	>60	>60	3.12	NA
6c	8.6 (24.9)	34 (98.3)	9.0 (26)	3.95	2683	>60	>60	3.53	NA
6d	45 (125)	164 (455)	63 (175)	3.64	12469	56	>60	3.96	NA
6e	8.6 (25)	12.5 (36)	4.9 (14)	1.45	23273	>60	25	3.14	27.67
6f	22.3 (62)	45 (126)	23 (64)	2.02	9814	>60	>60	3.66	NA

6g	113 (327)	102 (295)	200 (578)	0.9	>30300	25	22	2.07	NA
6h	24 (76)	87 (275)	28 (89)	3.6	>30300	>60	>60	2.24	NA
6i	37 (108)	57 (166)	46 (134)	1.54	5710	>60	>60	3.01	NA
6j	22 (56)	78 (199)	34 (87)	3.54	8072	>60	>60	3.96	NA
6k	298 (920)	200 (617)	233 (719)	0.67	11270	47	13	3.02	NA
6l	14.8 (36.4)	78.6 (193)	25.9 (64)	5.3	6726	>60	>60	3.74	NA
6m	12.7 (34)	34 (91)	21 (56)	2.7	>24242	29	43	4.38	74.5
6n	2.9 (7.5)	7.2 (18.6)	4.7 (12)	2.5	5715	29	31	4.90	33
6p	18.6 (54)	33.7 (97)	18 (52)	1.8	12699	>60	12	4.18	NA
6q	7.9 (22)	15.3 (42)	9.3 (25.8)	1.93	>24242	>60	>60	4.24	NA
6r	23 (61)	95.2 (254)	47 (125)	4.14	5008	55	42	4.76	54.6
6s	6.3 (15)	24.7 (58.7)	13 (30.9)	3.92	5154	25	30	5.24	51.8
6t	0.79 (2.1)	3.4 (9.2)	1.25 (3.4)	4.3	5130	>60	52	4.06	90.6
6u	0.71 (1.8)	2.84 (7.2)	1.3 (3.3)	4	5085	21	16	4.77	100
6v	2.02 (5.2)	6.4 (15.7)	2.3 (5.7)	3.2	5123	12	18	4.91	93
6w	1.07 (2.0)	1.5 (2.9)	0.59 (1.1)	1.4	1926	48	35	4.66	115
6x	15.4 (38.9)	80.4 (203)	31.5 (79.5)	5.22	7867	36	>60	3.56	101
8a	6.8 (15)	54 (121)	15.1 (33.9)	7.94	3002	>60	>60	3.97	16.2
8b	3.1 (6.1)	39 (76.8)	17.1 (33.7)	12.5	4092	>60	>60	5.82	21.9
8c	3.2 (7.1)	54.7 (121)	20.5 (45.4)	17	5773	>60	>60	4.39	16.4
8d	1.0 (1.9)	8.5 (16)	3.7 (6.95)	8.5	2449	31	>60	6.09	21.7
8e	13.7 (34.8)	195 (383)	147.5 (290)	14	2944	>60	>60	4.60	NA
8f	984 (2497)	1994 (3917)	1356 (2664)	2.0	14106	>60	>60	5.09	NA
*HLM = human liver microsomes, MLM = mouse liver microsomes; #NA = not tested									

Among the 4-amidinoquinolines, the in vitro activity and Clog-P of the new compounds appear to correlate with the side chain length of the molecules. As the side chain length of the 4-AMQ increases from compounds **6a** (Scheme 1, n = 2), to **6b** (n = 3) and **6c** (n = 4), the lipophilicity increases and so does their cell growth inhibitory activity (Table 3). However, as the chain length of **6c** (n = 4) increases further to **6d** (n = 5), the Clog-P increases from 3.58 to 3.99, and its activity decreases more than 5 fold. Thus, it appears that the optimal side chain length for activity of this 4-AMQ is n = 3 – 4. To further explore the relationship of lipophilicity and antimalarial activity, a series of new analogs with side chain length fixed at n = 3 and the methyl group of **6a-l** ($\mathbf{R_1}$ = -CH_3 in Scheme 1 and 2) being replaced with isopropyl (**6m-s**), phenyl (**6t-w** and **8c-d**) or pyridyl (**6x** and **8e-f**) groups were prepared and evaluated. The results confirm the previous observation that lipophilicity plays an important role in antimalarial activity (Table 3). Notably, replacement of the piperidine group of **6e** with a

morpholine ring resulted in analog **6g** which has a lower Clog-P as well as diminished antimalarial activity. Compounds **6g-i**, and **6k** with low Clog-P (< 3), showed weak in vitro antimalarial activity (IC50 > 100 ng/mL). When the side chain length of the isopropyl and phenyl analogs (**6m-6x**) are fixed at n = 3, the relationships of Clog-P and activity of methyl (**6b**), isopropyl (**6q**), and phenyl (**6u**) analogs are rather clear and straightforward. As the methyl group of **6b** (R_1 = -Me) was replaced with a larger isopropyl [**6q,** R_1 **=** $-CH(CH_3)_2$] or phenyl (**6u,** R_1 = Ph) group, Clog-P increased substantially from 3.12 to 4.24 and 4.77 for **6b, 6q** and **6u,** respectively. Parallel to Clog-P, antimalarial activity of the three analogs increased 6 fold from methyl **6b** to isopropyl **6q** analog and 11 fold from isopropyl **6q** to phenyl **6u** analogs. Introduction of 4-Cl to the phenyl ring resulted in 4-chlorophenyl analog **6w** which is equally active to phenyl analog **6u** against CQ sensitive D6**,** but is almost twice as active as **6u** against CQ resistant W-2 and C235 clones. However, when phenyl group of **6u** was replaced with a 3-pyridyl ring, the Clog-P and activity of the resulting pyridine analog **6x** decrease simultaneously. Despite the fact that compounds with bis-aminoalkyl side chains **8a-c** show good activity against D-6 with IC_{50} < 10 ng/ml, they are much less active against W-2 clone with IC50 > 40 ng/ml, indicating cross-resistance to CQ. The pyridyl analog **8f**, in which the 2nd aminoalkyl side chain is attached to the ring nitrogen, loses nearly all of its antimalarial activity. The result is of no surprise from the SAR point of view, since the aminoalkyl side chain of CQ is attached to the 4-amino group, not on ring nitrogen. Nevertheless, it is a surprise to note that compound **6k,** an analog of **6a** with the terminal amino group being replaced with a pyridine ring nitrogen, instead of disubstituted amino group, shows moderate growth inhibitory activity against both CQ-sensitive and CQ-resistant *Pf* clones with IC_{50} in the range of 200 - 300 ng/ml, making it a potential new lead warranting further exploration.

Table 4: In vitro Biological Activities of Benzonaphthyridine Derivatives

#	*P. falciparum* IC_{50}: ng/mL (nM)				Metab. Stab. ($t_{1/2}$, min)		HepG2 IC_{50} (ng/ml)	Clog-P	hERG %@ 10 μM
	D6	W-2	C235	W2/D6	*HLM	*MLM			
CQ	4.8 (15)	168 (525)	66 (206)	35	>60	>60		3.73	71
12a	10.7 (26)	41 (100)	21 (51.3)	8.5	>60	42	18052	3.91	91.45
12b	7.3 (17.6)	17 (41)	11 (26.6)	2.3	33	29	2057	3.93	100
12c	4.8 (8.0)	15 (24.9)	7.9 (13.1)	3.1	14	15	2809	4.07	94.55
12d	0.9 (2.0)	3.9 (8.8)	1.67 (3.8)	4.3	20	22	2063	5.14	86.53
12e	0.9 (2.2)	4 (9.7)	1.78 (4.3)	4.4	33	46	3562	4.43	72.12

12f	0.5 (1.1)	1.8 (3.96)	0.88 (1.94)	3.6	9	8	2007	5.28	69.61
12g	0.62 (1.1)	3.4 (6.1)	1.8 (3.2)	5.7	>60	>60	668	6.2	32.46
12h	0.8 (1.8)	0.9 (1.98)	0.78 (1.7)	1.1	18	44	5778	5.04	48.89
12i	0.8 (1.7)	2.7 (5.6)	1.34 (2.8)	3.4	16	26	3699	5.75	NA
12j	2.6 (5.1)	1.6 (3.3)	2.2 (4.3)	0.62	9	12	2564	5.89	80
12k	3.5 (7.2)	10 (20.6)	4.00 (8.2)	2.9	32	29	8603	4.54	63
12l	1.3 (2.8)	3.2 (6.6)	2.1 (4.3)	2.4	32	>60	2510	5.65	77
12m	11 (21.8)	30.8 (61)	15 (29.7)	2.7	>60	>60	>24242	6.73	10
13a	5.5 (11.7)	38 (80.8)	24 (51)	6.9	>60	>60	1696	3.52	18.26
13b	0.7 (1.3)	5.1 (9.5)	2.7 (5.1)	7.3	>60	>60	3045	4.94	13.78

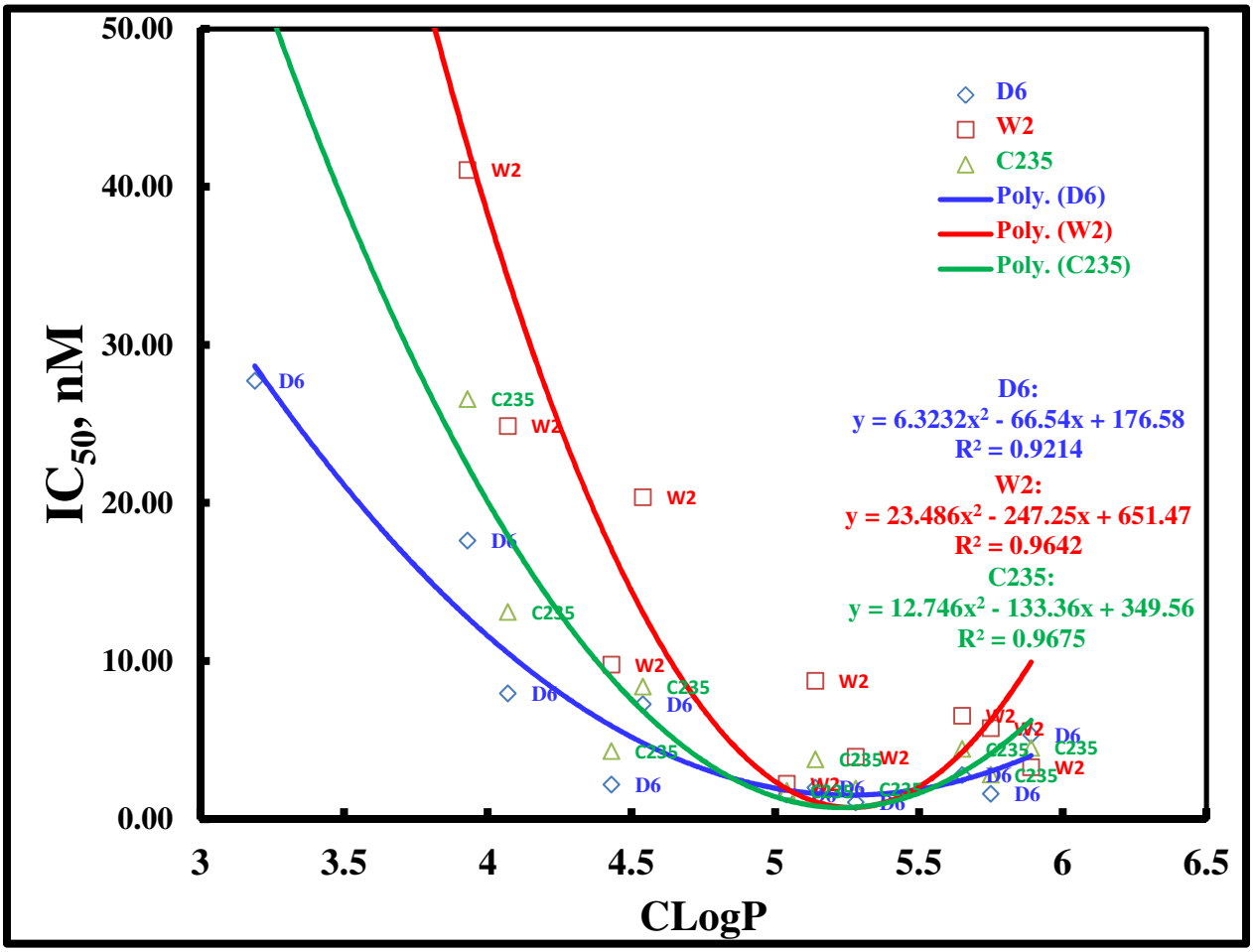
*HLM = human liver microsomes, MLM = mouse liver microsomes.

A relationship between lipophilicity and antimalarial activity exists not only in the 4-AMQ class, but also among 10-AMB derivatives. In general, 10-AMB derivatives are more lipophilic and more active than 4-AMQ derivatives in tests against *Pf* clones. In comparison to 4-A MQ derivative **6b,** the corresponding 10-AMB analog **12b** is 6.3 times more active and the Clog-P is also more than one log higher than **6b**. The same relationship also exists between **6q** and **12d,** with the latter about 8 fold more active than the former and more than a log higher in Clog-P. The relationship between Clog-P and activity appears to diminish when the R_1 is a phenyl group, as in the case of **6u** and **12i**. Even though Clog-P of **12i** is still a log higher than **6u,** the in vitro activities of both compounds are about equal with IC50 ~ 0.8 ng/mL. Figure 5 shows the correlation between Clog-P of 10-AMB derivatives and their in vitro antimalarial activity (IC_{50}) against Pf clones, W2, D6 and C235. As the Clog-P of the 10-AMB analogs increases, so does their antimalarial activity (smaller IC_{50}) until an optimal Clog-P value is reached around 5 – 5.5, at which point the compounds show maximal antimalarial activity and minimum CQ cross-resistance.

Five of the methyl analogs (R1 = -Me) of 4-AMQ (**6c**, **6e**, **6f**, 6i, and **6j**) with good metabolic stability (t1/2 > 60 min) and potent in vitro activity were selected for assessment in mice infected with CQ resistant P. berghei-infected erythrocytes (ANKA strain). Chloroquine was used as a positive control which gave 5/5 cures at 160 MPK (milligram per kilogram). The results are shown in Table 5. All 5 test compounds are active at 160 mg/kg x 3, po. The treated mice were parasite free 2-4 days after treatment, except **6e** which did not completely clear parasites during the treatment, but showed 95.4% parasitemia reduction 2 days after the treatment. None of the five methyl analogs cured mice at 160 mpk, but the treated mice stayed parasite free for 5 to 7 days before recrudescence took place. Among the 5 compounds tested,

compounds **6c**, **6f**, **6i** and **6j** are metabolically stable with t1/2 > 60 minutes and showed comparable in vivo and in vitro antimalarial activity. The 5th compound **6e** is the most active (IC_{50}, 8.6 ng/mL) among the five compounds tested, but is the least active in the Thompson test and the least stable metabolically. The poor metabolic stability of 6e in mice may have led to shorter plasma half-life and consequently weaker in vivo activity than analogs 6c, 6f, 6i and 6j.

Figure 5: Correlations of Clog-P and In vitro Antimalarial Activities of 10-AMB



Five isopropyl analogs **6m, 6n, 6p, 6q**, and **6r** [R_1 = -$CH(CH_3)_2$] were also selected for assessment in mice. All showed better efficacy than methyl analogs **6c**, **6e, 6f, 6i** and **6j** with cures at 160 mpk x 3, po, although the in vitro activity of these isopropyl analogs are at best comparable to that of the above mentioned 5 methyl analogs. Since Clog-P values (> 4.2) of

isopropyl analogs are more than one log higher than the average methyl analogs tested (Table 3), positive correlations between Clog-P and in vivo activity in mice are apparent. However, no correlation between the metabolic stability and in vivo antimalarial activity was observed. Metabolically more stable compounds are expected to maintain longer plasma half-life and thus should be more active than those with shorter plasma half-life. However, the data in Tables 5

Table 5: Antimalarial Activity of New Compounds in Mice Infected with *P. berghei*

Compd. #	Oral Dose *(mpk x 3)*	Results *# Mice (Day Died)*	Log-P	Met. Stab *(min)*		Remark
				*HLM	*MLM	
6c	160	5/5 (D14)	3.53	>60	>60	active
6e	160	2/5 (D10), 2/5 (D12), 1/5 (D17)	3.14	>60	25	suppressive
6f	160	2/5 (D14), 2/5 (D17), 1/5 (D21)	3.66	>60	>60	active
6i	160	17 (3/5), 19 (1/5), 21 (1/5)	3.01	>60	>60	active
6j	160	4/5 (D17), 1/5 (D19)	3.96	>60	>60	active
6m	160	3/5C, 1/5 (D19), 1/5 (D21)	4.38	29	43	cure
6n	120	1/5C, 4/5 (D18)	4.90	29	31	cure
6p	160	2/5C, 3/5 (D7)	4.18	>60	12	cure/toxic
6q	160	4/5C, 1/5 (D24)	4.24	>60	>60	cure
6r	160	5/5C	4.76	55	42	cure
12a	120	1/5 (D5), 1/5 (D19), 1/5 (20), 1/5 (D24), 1/5 (D26)	3.91	>60	42	active
12b	120	1/5 (D4), 1/5 (D6), 1/5 (D10), 1/5 (D14), 1/5 (D21)	3.93	33	29	active
12c	120	2/5C, 1/5 (D19), 2/5 (D21)	4.07	14	15	cure
12h	120	4/5C, 1/5 (D5)	5.04	18	44	cure
	60	5/5C		18	44	cure
12j	120	5/5C	5.85	9	12	cure
12l	60	5/5C	5.65	32	>60	cure
	15	5/5C		32	>60	cure
12m	60	5/5C	6.73	>60	>60	cure
	15	1/5C, 1/5 (D19), 1/5 (D24), 1/5 (D25), 1/5 (D26)	6.73	>60	>60	cure
13b	120	5/5C	4.94	>60	>60	cure
	60	4/5C, 1/5 (D24)		>60	>60	cure
CQ	160	5/5C	3.73	>60	>60	valid
Vehicle	0	2/5 (D6), 2/5 (D7), 1/5 (D11)				valid

*HLM = human liver microsomes, MLM = mouse liver microsomes.

showed that metabolically stable methyl analogs **6c, 6e, 6f, 6i** and **6j** displayed much weaker in vivo activity than the metabolically less stable isopropyl analogs **6m, 6n, 6p, 6q,** and **6r,** implying that metabolites may also contribute to the observed antimalarial activity. 10-Amidinobenzonaphthyridines (10-AMB) are higher in Clog-P and more active in vitro and in vivo than the corresponding 4-amidinoquinoline (4-AMQ) derivatives. However, the former are generally less stable metabolically than the latter, except compounds with branched aminoalkyl (**12m**) and bis-aminoalkyl side chains (**12g**, **13a** and **13b**). This observation indicates that steric hindrance may play a role in reducing the susceptibility of **12m**, **12g**, **13a** and **13b** to microsomal hydroxylation and subsequent degradation of the hydroxylated metabolites.

A clear correlation between Clog-P and in vivo antimalarial activities exists not only among 4-AMQ derivatives, but also among the 10-AMB analogs. As shown in Table 5, none of the compounds with Clog-P < 4 showed cure in the Thompson test and all compounds with Clog-P > 4 showed cures at 15, 60, 120 or 160 mpk x 3 by oral dosing, and this is the case for both 4-AMQ or 10-AMB derivatives. Like 4-AMQ, the metabolic stability of the 10-AMB derivatives does not correlate with their antimalarial activity, suggesting again that metabolites may be, at least partially, responsible for the observed antimalarial activity.

One of the major goals of this study is to search for a potent, safe and long acting antimalarial compound to replace mefloquine as malaria prophylactic drug. Among the 10-AMB analogs, compound **13b** showed potent in vitro (IC_{50} < 1 ng/mL) and in vivo activities (TT: 4/5c at 60 mpk), good metabolic stability ($t_{1/2}$ >60 min.) and a very long plasma half-life ($t_{1/2}$ ~ 50 hr) in mouse PK studies (Table 6). Furthermore, compound **13b** also showed weak inhibitory activity in hERG (the **h**uman ***E**ther-à-go-go*-**R**elated **G**ene) K^+ channel blockage test and was negative in an Ames mutagenicity study (data not shown, assays performed by WuXi App Tech, Inc., St. Paul, MN; see also further discussion below on hERG inhibition testing). The results indicate that **13b** is a promising potential candidate to replace mefloquine.

Table 6: Compounds **6q** and **13b** PK parameters after single PO at 80 mg/kg in mice plasma

Compound #	Regimen	Half-life (hr)	T_{max} (hr)	C_{max} (ng/ml)	AUC_{last} (hr*ng/ml)	AUC_{INF_obs} (hr*ng/ml)	Vz/F_obs (ml/kg)	Cl/F_obs (ml/hr/kg)	MRT_{last} (hr)
6q	oral-1	6.74	0.5	695.7	3805.73	3916.64	198725	20425.7	7.73
	oral-2	6.58	0.5	696.8	3222.6	3315.58	228918	24128.5	7.55
	Mean	**6.66**	0.5	696.3	3514.2	3616.107	213822	22277.1	7.641
	SD	0.118	0	0.778	412.332	425.014	21349.7	2618.31	0.133
	CV%	1.8	0	0.1	11.7	11.8	10	11.8	1.7
13b	oral-1	40.6	2	279.3	5815.35	8374.75	559467	9552.53	23.65
	oral-2	54.43	2	163.8	4043.45	9517.05	660128	8405.97	20.61
	Mean	**47.515**	2	221.6	4929.4	8945.898	609797	8979.25	22.127
	SD	9.785	0	81.67	1252.923	807.727	71177.5	810.738	2.149
	CV%	20.6	0	36.9	25.4	9	11.7	9	9.7

The hERG potassium channels are essential for normal electrical activity in the heart. Inherited mutations in the hERG gene cause long QT syndrome, a disorder that predisposes individuals to life-threatening arrhythmias. Arrhythmia can also be induced by blockage of hERG channels by a surprisingly diverse group of drugs [52-53], including antimalarial agents, such as quinidine [54-56] and Halofantrine [57]. This side effect is a common reason for drug failure in preclinical safety trials. Chloroquine [58] and mefloquine [59-60] also exhibited the same cardiac toxicity as quinidine, but to a lesser extent. Selected 4-AMQ and 10-AMB analogs were submitted for hERG channel inhibition test and the results are shown in Tables 3 and 4. Among the fourteen 4-AMQ derivatives tested, the mono-alkylated analogs [R_1 = -Me or $–CH(CH_3)_2$, **6e**, **6m**, **6n**, **6q**, and **6r**] showed comparable to or weaker inhibition than CQ at 10 μM concentration. While all 5 aromatic analogs (R_1 = Ph or pyridyl, **6t**, **6u**, **6v**, **6w**, and **6x**), showed higher hERG inhibitory activity than CQ, another four analogs with bis-alkyl side chains, **8a-d,** displayed much weaker hERG inhibitory activity (6 – 20 %) than chloroquine (71 [Tables 3 and 4]. At the same concentration, mefloquine showed 95%, whereas Halofantrine and quinidine showed 100% inhibition. Likewise, among the 10-AMB derivatives tested, mono-alkylated analogs (**12a-c** and **12h**) have stronger proarrhythmic potential than CQ, while bis-alkylated compounds (**12g**, **12k**, **13a** and **13b**) displayed much weaker potential than CQ. Bis-alkylated analogs of 4-AMQ and 10-AMB are not only much less toxic in the hERG test, but are also more

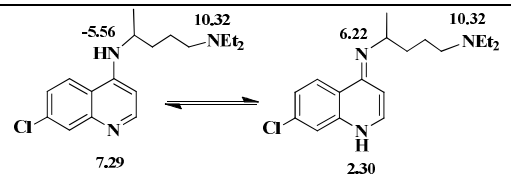
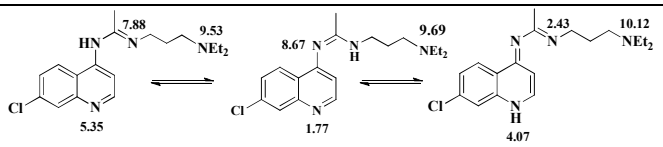
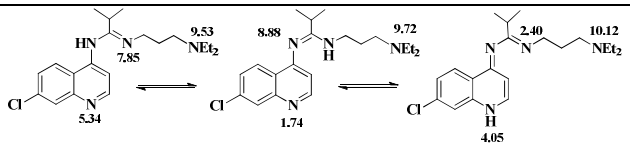
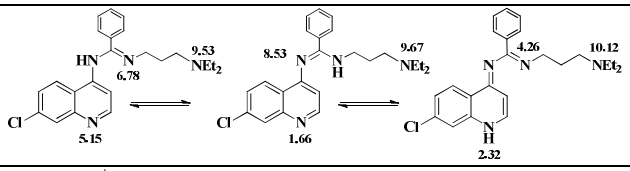
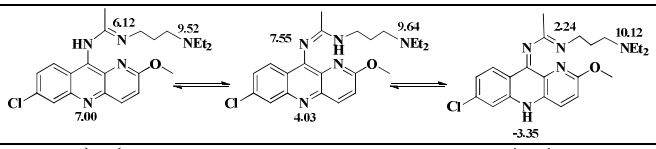
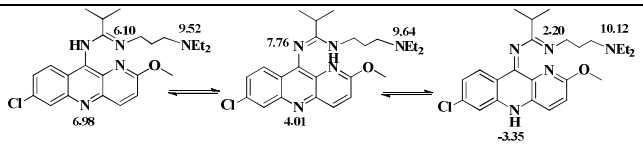
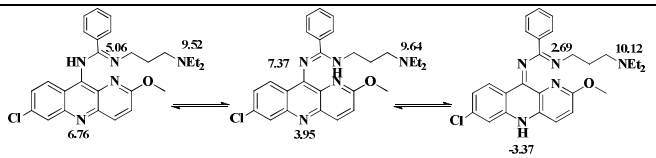
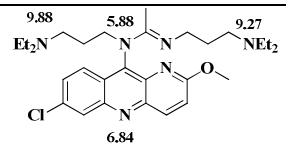
active than mono-alkylated analogs. Furthermore, the new compounds showed no in vitro toxicity in HepG2 (human hepatocellular carcinoma) with IC_{50} > 2000 ng/mL and no host toxicity in mice up to 160 mpk/day x 3 days. More encouraging discovery on toxicity of the amidine analogs is that all 4 selected compounds (**6t**, **6w**, **12l** and **13b**) are negative in Ames mutagenicity test performed by WuXi App Tech, Inc., St. Paul, MN.

Rationale for the preparation of amidine analogs in this study, as stated in the introductory section of this report, is that replacement of the 4-amino group of CQ with an amidine function will alter or enhance the basicity of both ring nitrogen (N^1) and the 4-amino group (N^2) of CQ, resulting in changes in pharmacological profiles and antimalarial activities of the new amidine analogs (Figure 3). As was postulated, the pKa of the ring nitrogen (N^1) and the 4-amino (N^2) or 10-amino (N^2) groups of the amidine analogs are much higher than that of CQ (Table 7). However, no or little changes in pKa (9.27 – 10.32) of the disubstituted aminoalkyl side chain (N^3) of CQ and the amidine analogs were observed and the side chain amino groups (N^3) remain the most basic nitrogen in CQ and all amidine analogs; thus, the results ruled out the possibility that pKa change of amidine analogs is the primary cause of their better antimalarial activities than CQ against the CQ-resistant *P. falciparum* clone (W2). On the other hand, the pKa of the amino group at the 4-position of 4-AMQ and 10-position of 10-AMB are substantially higher than that of the 4-amino group of CQ, from -5.56 for CQ to average of 8 and 6 for 4-AMQ and 10-AMB, respectively. The results indicate a clear correlation between pKa of the 4- or 10-amino group of the amidine analogs and their antimalarial activity against CQ resistant cell line. While there is little change in pKa of the 10-AMB and CQ ring nitrogen, substantial differences in pKa of the ring nitrogen between 4-AMQ and CQ were observed. The relationship between the observed differences in pKa of the ring nitrogen between 4-AMQ and 10-AMB and their antimalarial activity is not clear.

The mechanism of action of CQ has been the subject of extensive studies in many laboratories around the world. The literature reports on this subject are complex and far from clear,[8-26] including DNA intercalation, drug accumulation in the food vacuole of the parasites, inhibition of hemozoin formation or *Pf*CRT (*P. falciparum* Chloroquine-Resistance Transporter). Since all 3 amino groups of CQ are essential for its antimalarial activity, alteration of pKa of N_1 and N_2 of amidine analogs will result in alteration of binding affinity of the new analogs to the listed targets and change the antimalarial activity. The antimalarial activity results confirm our

postulation. However, it is not the intention of this paper to determine the mechanism of action of this new class of antimalarial which is in progress and the results will be reported elsewhere.

Table 7: *pKa, Clog-P and in Vitro Activity of Selected Compounds

Compd	Structure and pKa of Amino groups	Clog-P	IC_{50} (ng/ml)		
			D6	W2	RI= W2/D6
CQ		3.73	4.8	168	35
6b		3.00	46	75	1.6
6q		4.24	7.9	15.3	1.9
6u		4.77	0.7	2.8	4
12b		4.22	7.3	17.0	2.3
12d		5.14	0.9	3.9	4.3
12i		5.75	0.8	2.7	3.4
13b		4.94	0.7	5.1	7.3

*pKa and Clog-P values were calculated using physico-chemical property predictors from ChemAxon's Calculators and Calculator Plugins, ChemAxon, Cambridge, MA. http://www.chemaxon.com/products/calculator-plugins/property-predictors/

CONCLUSION

4-AMQ and 10-AMB derivatives prepared in this study represent two new classes of antimalarial agents with potent in vitro growth inhibitory activity against CQ resistant *Pf* clones W-2 and C235 and demonstrated cures in mice infected with *P. berghei*. The high activity of the new amidine analogs against CQ resistant parasites correlates well with the increase in pKa of the nitrogen at 4- or 10-position of the 4-AMQ and 10-AMB. Further, the results also indicate a positive correlation between the Clog-p and the antimalarial activity of the new compounds prepared in this study. No overt toxicity was observed in HepG2 culture or in mice. Preliminary data from hERG channel blockage testing also showed much weaker hERG inhibitory activity than CQ and are negative in Ames test, indicating analogs of this new class have good potential to be developed as CQ replacement drugs to combat malaria infection. Furthermore, compound **13b**, a 10-AMB analog with bis-aminoalkyl side chains, showed not only potent activity in mice, low in vitro and in vivo toxicity, negative in Ames test and also possessed a long plasma half-life of about 50 hours in mouse PK studies, suggesting it could be a good potential candidate to replace mefloquine as a weekly or biweekly malaria prophylactic drug.

EXPERIMENTAL SECTION

Melting points were determined in open capillary tubes on an OptiMelt melting point apparatus (Standard Research Systems, USA) and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker Avance-600 and Bruker Avance 300 spectrometers (Bruker Instrument, Inc., Wilmington, DE). Chemical shifts are given in ppm (δ) relative to tetramethylsilane (TMS) as internal standard. Analytical thin layer chromatography (TLC) was performed using HPLC-HLF normal phase 150 μm silica gel plates (Analtech, Newark, DE). Visualization of the developed chromatogram was performed with UV254 lamp or iodine chamber. Flash chromatography was conducted with silica gel 60, 0.060-0.2mm (70-230 mesh) from Sigma-Aldrich Co. Solvents and reagents obtained from commercial sources were used without purification unless noted. Reactions were carried out under an inert atmosphere of nitrogen. Elemental analysis was performed by Atlantic Microlab, Inc. (Norcross, GA). Where

analyses are indicated by symbols of the elements, the analytical results obtained were within ± 0.4% of the theoretical values. An LC/UV – Vis/Trap MS was employed for purity analysis and chromophore properties. The system consisted of an Agilent 1100 series LC-UV/VIS system online with a ThermoFinnigan (now Thermo-Fisher Scientific, Waltham, MA) LCQ MS equipped with electro spray ionization (ESI) source. Samples were analyzed using shallow CH_3CN:1% $HCOOH/H_2O$ gradients (pH 2.5) at low rate. The purity of the final products is ≥ 95%. Log- P values are obtained from ChemDraw Ultra software version 12.0.

A. Chemistry

***Synthesis of 4-Amino-7-Chloroquinoline* (3)[61]:** 4,7-Dichloroquinoline (10.0g, 50 mmol) was dissolved in phenol (47.51g, 0.5 mol.) with stirring and heating at 110.^{0}C. Ammonium carbonate (24.2g, 0.25 mol.) was added in portions as rapidly as the frothing would permit. After a further 2hr at 165 ^{0}C, the mixture was cooled and macerated with diethyl ether (500mL). The crystalline white HCl salt compound **2** was collected, washed with ether, and dried. The free amine was obtained by shaking a suspension of **2** (HCl salt) for an hr in an excess of 10% NaOH solution. The free amine **3** was collected and recrystallized from EtOH to give 8.0 g (89.0%) of the desired product as bright white needles, mp 148 ^{0}C (lit [62], mp 148.5-149.5 ^{0}C). ^{1}H NMR (300 MHz, CD_3OD): δ 6.63 (d, *J* = 5.4 Hz, 1 H), 7.41 (dd, *J* = 9.0, and 2.1 Hz, 1 H), 7.79 (d, *J* = 2.1, 1 H), 8.09 (d, *J* = 9.0, 1 H), 8.29 (d, *J* = 5.4 Hz, 1 H). ^{13}C NMR (75 MHz, CD_3OD): δ 102.51, 116.92, 123.51, 124.36, 126.02, 135.07, 148.61, 150.54, 152.90. MS (ESI): *m/z* 179.09 $[M + 1]^+$.

***Synthesis of N-(7-chloroquinolin-4-yl)-acetamide* (4a):** A solution of 4-amin-7-chloroquinoline (**3**) (5.0 g, 28 mmol) in acetic anhydride (20 mL) was refluxed for 2h. After cooling to rt, the reaction mixture was treated with brine to precipitate the product, which was filtered off and re-dissolved in water. The aqueous solution was basified with 10% NaOH and extracted with EtOAc (50 mL x 3). The extracts were combined, dried over anhydrous Na_2SO_4 and evaporated to afford 3.58g (72.6 %) of the desired product as white crystals, mp, 195.6 ^{0}C (lit [63], mp 195 ^{0}C). ^{1}H NMR (600 MHz, CD_3OD): δ 2.34 (s, 3 H), 7.62 (dd, *J* = 9.0 and 2.4 Hz, 1 H), 8.01 (d, *J* = 2.4 Hz, 1 H), 8.18 (d, *J* = 4.8 Hz, 1 H), 8.27 (d, *J* = 9.0 Hz, 1 H), 8.77 (d, *J* =

4.8 Hz, 1 H); ^{13}C NMR (150 MHz, CD_3OD): δ 24.32, 113.86, 121.08, 124.90, 128.36, 128.56, 137.03, 144.11, 150.23, 153.08, 172.72. MS (ESI): *m/z* 221.10 $[M + 1]^+$.

Synthesis of N-(7-chloroquinolin-4yl)-isobutyramide **(4b):** A solution of 4-amino-7-chloroquinoline (**3**), (1.0g, 5.6 mmol) in butyric anhydride (4.64 mL, 28 mmol) was refluxed for 4h. After cooling to rt the reaction mixture was poured into ice cold water and extracted with ethyl acetate (20 x 3). The combined extracts were dried over anhydrous Na_2SO_4 and evaporated to afford the product as a light yellow solid which was recrystallized from EtOH to give pure product as white solid (1.0g, 71.83%), mp 173.9 ^{0}C. ^{1}H NMR (300 MHz, CD_3OD): δ 1.31 (d, *J* = 6.60 Hz, 6H), 2.95 (m, 1H), 7.65 (dd, *J* = 9.00 and 2.10 Hz, 1H) 8.03 (d, *J* = 2.10 Hz, 1H), 8.16 (d, *J* = 5.40 Hz, 1H), 8.26 (d, *J* =9.00Hz, 1H), 8.80 (d, *J* = 5.40 Hz, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 20.04, 37.12, 114.46, 121.51, 125.05, 128.40, 128.58, 137.07, 144.28, 150.30, 153.06, 179.60. MS (ESI): *m/z* 249.08 $[M + 1]^+$.

Synthesis of N-(7-chloroquinolin-4-yl)-ethanethioamide **(5a):** A mixture consisting Lawesson reagent[49] (0.91 g, 2.3 mmol), N-(7-chloroquinolin-4-yl)-acetamide (**4a,** 0.5g, 2.3 mmol) and toluene (15 mL) was refluxed with stirring for 24 hr. The solvent was removed under reduced pressure. The residue was purified with a flash silica gel column using CCl_3: MeOH (20 : 1 v/v) as eluent to give 0.32g (60%) of thioamide **5a** as a yellow gum. ^{1}H NMR (300 MHz, CD_3OD): δ 2.78 (s, 3 H), 7.61 (dd, *J* = 9.0 and 2.4 Hz, 1 H) 7.88 (d, *J* = 2.4 Hz, 1 H), 8.01 (d, *J* = 4.8 Hz, 1 H), 8.05 (d, *J* =9.0 Hz, 1H), 8.89 (d, *J* = 4.8 Hz, 1 H). ^{13}C NMR (75 MHz, CD_3OD): δ 34.8, 120.1, 124, 126, 128.7, 129.1, 137.4, 146.3, 150.7, 153, 205.9. MS (ESI): *m/z* 237.0 $[M + 1]^+$.

Synthesis of N-(7-chloro-quinolin-4yl)-2-methylpropanethioamide **(5b):** To a stirred solution of N-(7-chloro-quinolin-4yl)-isobutyramide (**4b**), (0.5 g, 2 mmol) in 1,4-dioxane (10mL) was added Lawson's reagent[49] (2.43g, 6.0308 mmol). The reaction mixture was refluxed with stirring for 24h, concentrated under reduced pressure and purified by flash chromatography ($CHCl_3$: MeOH 20: 1 v/v) to provide thioamide as yellow gum (0.35g, 66% yields). ^{1}H NMR (300 MHz, CD_3OD): δ 1.42 (d, *J* = 6.60 Hz, 6H), 3.28 (m, 1H), 7.65 (dd, *J* = 9.00 and 2.10 Hz, 1H) 7.82 (d, *J* = 2.10 Hz, 1H), 7.99 (d, *J* = 5.40 Hz, 1H), 8.10 (d, *J* =9.00 Hz, 1H), 8.93 (d, *J* = 5.40 Hz, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 23.62, 44.92, 120.70, 124.43, 126.11, 128.74, 129.03, 137.34, 146.51, 150.68, 153.05, 216.96. MS (ESI): m/z 264.98 $[M + 1]^+$

***Synthesis of (Z)-N-(7-chloroquinolin-4-yl)-N'-[2-(diethylamino)-ethyl]-acetimidamide* (6a):** Triethylamine (0.6 mL, 5.8 mmol) was added drop wise to a stirring suspension consisting N, N-diethylethylenediamine (0.1 g, 0.93 mmol), thioamide **5a** (0.2 g, 0.85 mmol), HgO (0.2 g, 0.92 mmol) and anhydrous DMF (10 mL). The stirring was continued at rt. for 10 hr after the triethylamine addition was completed. The mixture was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure. Water (15 mL) was added to the residue and the mixture was extracted with $CHCl_3$ (10 mL) 3 times. The organic extracts were combined, washed with brine, dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The crude product was purified with a silica gel flash column and eluted with 10% MeOH in $CHCl_3$ to give a colorless gum (0.22 g, 81.4% yields). ^{1}H NMR (600 MHz, CD_3OD): δ 1.08 (t, *J* = 7.2 Hz, 6 H), 1.79 (s, 3 H), 2.64 (m, 4 H), 2.73 (t, *J* = 7.2 Hz , 2 H), 3.52 (t, *J* = 7.2 Hz , 2 H), 6.75 (d, *J* = 4.8 Hz, 1 H) 7.43 (dd, *J* = 9.0 and 2.1 Hz, 1 H), 7.87 (d, *J* = 2.1 Hz, 1 H), 8.0 (d, *J* = 9.0 Hz, 1 H), 8.55 (d, *J* = 4.8 Hz, 1 H). ^{13}C NMR (150 MHz, CD_3OD): δ 10.19, 16.10, 38.13, 46.73, 50.75, 112.25, 123.69, 125.78, 125.93, 126.14, 135.24, 148.71, 151.22, 157.02, 157.81. MS (ESI): *m/z* 319.3 $[M + 1]^+$. Anal ($C_{17}H_{23}ClN_4$): C, H, Cl, N.

The same procedure was used to prepare new compounds **6b-s.** The yield, ^{1}HNMR, ^{13}CNMR and MS data of the new analogs were described as follows.

***Synthesis of (Z)-N-(7-chloroquinolin-4-yl)-N'-[3-(diethylamino)-propyl]-acetimidamide* (6b):** The title compound was prepared by the same procedure for **6a** from **5a** and 3-diethylamino-propylamine to give **6b** as a colorless gum (0.27 g, 90.0% yields). ^{1}H NMR (600 MHz, CD_3OD): δ 1.11 (t, *J* = 7.2 Hz, 6 H), 1.83 (s, 3 H), 1.9 (m, 2 H), 2.67 (m, 6 H), 3.45 (t, *J* = 6.6 Hz, 2 H), 6.81 (d, *J* = 4.8 Hz, 1 H) 7.48 (dd, *J* = 9.0 and 2.1 Hz, 1 H), 7.92 (d, *J* = 2.1 Hz, 1 H), 8.04 (d, *J* = 9.0 Hz, 1 H), 8.6 (d, *J* = 4.80 Hz, 1 H). ^{13}C NMR (150 MHz, CD_3OD): δ 9.91, 16.10, 25.23, 39.40, 46.32, 50.16, 112.54, 123.74, 125.84, 125.92, 126.10, 135.29, 148.69, 151.24, 157.92. MS (ESI): *m/z* 333.22 $[M + 1]^+$. Anal ($C_{18}H_{25}ClN_4$): C, H, Cl, N.

***Synthesis of (Z)-N-(7-chloroquinolin-4-yl)-N'-[3-(diethylamino)-propyl]acetimidamide succinate salt*.** The gum **6b** (0.2g, 0.6 mmol) was dissolved in CH_3CN (5mL) and treated with a solution of succinic acid (71 mg, 0.6 mmol) in MeOH (0.5 mL) and CH_3CN (2 mL) mixed solvent to give white succinate salt of **6b** (0.2g, 74.0% yields), mp 116.5 ^{0}C. ^{1}H NMR (600 MHz, CD_3OD): δ 1.30 (t, *J* = 7.20 Hz, 6 H), 1.89 (s, 3 H), 2.18 (m, 2 H), 2.55 (s, 12 H, C$\underline{H_2}$ of 3

succinic acid), 3.24 (m, 6 H), 3.56 (t, *J* = 6.6 Hz, 2 H), 6.85 (d, *J* = 4.8 Hz, 1 H), 7.51 (dd, *J* = 9.0 and 2.1 Hz, 1 H), 7.95 (d, *J* = 2.1 Hz, 1H), 8.04 (d, *J* = 9.0 Hz, 1 H), 8.62 (d, *J* = 4.8 Hz, 1 H). ^{13}C NMR (150 MHz, CD_3OD): δ 7.59, 16.21, 23.42, 29.63, 38.13, 46.58, 49.45, 112.17, 123.44, 125.07, 126.02, 126.33, 136.08, 147.28, 150.00, 158.32, 158.55, 175.97. MS (ESI): *m/z* 333.22 $[M + 1]^+$. Anal ($C_{30}H_{43}ClN_4O_{12}$).0.1 H_2O: C, H, Cl, N.

***Synthesis of (Z)-N-(7-chloroquinolin-4-yl)-N'-[4-(diethylamino)-butyl]-acetimidamide* (6c):** Compound **6c** was prepared by the same procedure from **5a** and 4-(diethylamino)-butylamine to give a colorless gum (0.25 g, 86.2% yields). ^{1}H NMR (600 MHz, CD_3OD): δ 1.04 (t, *J* = 7.2 Hz, 6 H), 1.62 (m, 4 H), 1.82 (s, 3 H), 2.53 (t, *J* = 8.40 Hz , 2 H), 2.58 (m, 4 H), 3.41 (t, *J* = 6.6 Hz, 2 H), 6.76 (d, *J* = 4.8 Hz, 1 H) 7.44 (dd, *J* = 9.0 and 2.1 Hz, 1 H), 7.87 (d, *J* = 2.1 Hz, 1 H), 7.98 (d, *J* = 9.0 Hz, 1 H), 8.55 (d, *J* = 4.8 Hz, 1 H). ^{13}C NMR (150 MHz, CD_3OD): δ 11.31, 17.63, 24.89, 28.27, 42.28, 47.76, 53.59, 114.07, 125.26, 127.32, 127.40, 127.62, 136.74, 150.19, 152.72, 158.75, 159.44. MS (ESI): *m/z* 347.31 $[M + 1]^+$. Anal ($C_{19}H_{27}ClN_4$).0.7 H_2O: C, H, Cl, N.

***Synthesis of (Z)-N-(7-chloroquinolin-4-yl)-N'-[5-(diethylamino)pentyl]-acetimidamide* (6d):** Compound **6d** was prepared by the same procedure from compound **5a** and 5-diethylaminopentylamine to give 0.7 g (92.1%) of the desired product as colorless gum. ^{1}H NMR (300 MHz, CD_3OD): δ 1.08 (t, *J* = 7.20 Hz, 3H), 1.49 (m, 2H), 1.59 (m, 2H), 1.75 (m , 2H), 1.84 (s, 3H), 2.61 (m, 6H), 3.45 (t, *J* = 6.90 Hz, 2H), 6.82 (d, *J* = 4.8 Hz, 1H), 7.49 (dd, *J* = 9.0 and 2.1 Hz, 1H), 7.93 (d, *J* = 2.1 Hz, 1 H), 8.04 (d, *J* = 9.0 Hz, 1H), 8.61 (d, *J* = 4.8 Hz, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 11.32, 17.66, 26.39, 26.96, 30.06, 42.37, 47.82, 53.80, 114.06, 125.31, 127.34, 127.43, 127.69, 136.77, 150.27, 152.75, 158.75, 159.50. MS (ESI): *m/z* 361.46 $[M + 1]^+$. Anal ($C_{20}H_{29}ClN_4$): C, H, Cl, N.

***Synthesis of (Z)-N-(7-chloroquinolin-4-yl)-N'-[3-(piperidin-1-yl)-propyl]-acetimid-amide* (6e):** The title compound was prepared by the same procedure from **5a** and N-(3-aminopropyl)-piperidine to give **6e** in 72.1% yields as colorless gum. ^{1}H NMR (600 MHz, CD_3OD): δ 1.08 (m, 2 H), 1.61 (m, 4 H), 1.79 (s, 3 H), 1.88 (m, 2 H), 2.47 (m, 6 H), 3.28 (t, *J* = 1.8 Hz, 2 H), 6.77 (d, *J* = 4.8 Hz, 1 H), 7.44 (dd, *J* =9.0 and 2.1 Hz, 1 H), 7.88 (d, *J* = 2.1 Hz, 1 H), 8.0 (d, *J* = 9.0 Hz, 1 H), 8.56 (d, *J* = 4.8 Hz, 1 H). ^{13}C NMR (150 MHz, CD_3OD): δ 16.12,

23.82, 25.18, 25.49, 39.45, 54.15, 56.84, 112.50, 123.74, 125.84, 125.95, 126.13, 135.28, 148.71, 151.23, 157.19, 157.92. MS (ESI): *m/z* 345.1 $[M + 1]^+$. Anal ($C_{19}H_{25}ClN_4$): C, H, Cl, N.

***Synthesis of (Z)-N-(7-chloroquinolin-4-yl)-N'-[4-(piperidin-1-yl)-butyl]-acetimidamide* (6f):** The title compound was prepared from **5b** and 4-piperidin-1-yl-butylamine in 77.0% yields as colorless gum. ^{1}H NMR (300 MHz, CD_3OD): δ 1.51 (m, 2 H), 1.61 (m, 8 H), 1.84 (s, 3 H), 2.45 (m, 6 H), 3.46 (t, *J* = 1.8 Hz, 2 H), 6.81 (d, *J* = 4.8 Hz, 1 H), 7.49 (dd, *J* = 9.0 and 2.1 Hz, 1 H), 7.93 (d, *J* = 2.1 Hz, 1 H), 8.03 (d, *J* = 9.0 Hz, 1 H), 8.61 (d, *J* = 4.8 Hz, 1 H). ^{13}C NMR (75 MHz, CD_3OD): δ 16.16, 23.84, 25.11, 26.85, 40.78, 54.09, 58.81, 112.56, 123.77, 125.84, 125.91, 126.17, 135.26, 148.75, 151.24, 157.25, 157.96. MS (ESI): *m/z* 359.24 $[M + 1]^+$. Anal ($C_{20}H_{27}ClN_4$): C, H, Cl, N.

***Synthesis of (Z)-N-(7-chloroquinolin-4-yl)-N'-[3-(morpholino)-propyl]-acetimidamide* (6g):** Compound **6g** was prepared by the same procedure from **5a** and 3-morpholinopropylamine in 64.3% yields as colorless gum. ^{1}H NMR (300 MHz, CD_3OD): δ 1.80 (s, 3 H), 1.86 (m, 2 H), 2.50 (m, 6 H), 3.45 (t, *J* = 6.90 Hz, 2 H), 3.70 (m, 4 H), 6.77 (d, *J* = 4.8 Hz, 1 H), 7.45 (dd , *J* = 9.0 and 2.1 Hz, 1 H), 7.89 (d, *J* = 2.1 Hz, 1 H), 8.01 (d, *J* = 9.0 Hz, 1 H), 8.57 (d, *J* = 4.8 Hz, 1 H). ^{13}C NMR (75 MHz, CD_3OD): δ 16.20, 25.25, 39.31, 53.42, 56.50, 66.29, 78.15, 112.47, 123.76, 125.85, 125.97, 126.17, 135.27, 148.71, 151.24, 157.15, 157.88. MS (ESI): *m/z* 347.16 $[M + 1]^+$. Anal ($C_{18}H_{23}ClN_4O$): C, H, Cl, N.

***Synthesis of (Z)-N-(7-chloroquinolin-4-yl)-N'-(1-methylpiperidin-4-yl)-acetimidamide* (6h):** Compound **6h** was prepared from **5a** and 4-amino-1-methylpiperidine in 73.0% yields as light brown solid, mp 168.7 ^{0}C. ^{1}H NMR (600 MHz, CD_3OD): δ 1.62 (m, 2 H), 1.78 (s, 3 H), 2.16 (m, 4 H), 2.28 (s, 3 H), 2.89 (m, 2 H), 3.92 (m, 1 H), 6.77 (d, *J* = 4.8 Hz,1 H), 7.46 (dd, *J* = 9.0 and 2.1 Hz, 1 H), 7.89 (d, *J* = 2.1 Hz, 1 H), 7.96 (d, *J* = 9.0 Hz, 1 H), 8.57 (d, *J* = 4.8 Hz, 1 H). ^{13}C NMR (150 MHz, CD_3OD): δ 16.24, 30.92, 50.50, 52.06, 62.68, 112.42, 123.70, 125.86, 125.89, 126.21, 127.05, 127.92, 129.37, 135.27, 137.08, 148.75, 151.22, 156.27, 157.88. MS (ESI): *m/z* 317.25 $[M + 1]^+$. Anal ($C_{17}H_{21}ClN_4$): C, H, Cl, N.

***Synthesis of (Z)-N-(7-chloroquinolin-4-yl)-N'-(1-isopropylpiperidin-4-yl)-acetimidamide* (6i):** The title compound was prepared from **5a** and 4-amino-1-isopropylpiperidine in 80% yields as light white solid. mp 177 ^{0}C. ^{1}H NMR (300 MHz, CD_3OD): δ 1.14 (d, J = 6.6 Hz, 6

H), 1.66 (m, 2 H), 1.81 (s, 3 H), 2.20 (m, 2 H), 2.48 (m, 2 H), 2.87 (m, 1 H), 3.03 (m, 2 H), 3.96 (m, 1 H), 6.79 (d, *J* = 4.8 Hz,1 H), 7.48 (dd, *J* = 9.0 and 2.1 Hz, 1 H), 7.91 (d, *J* = 2.1 Hz, 1 H), 7.98 (d, *J* = 9.0 Hz, 1 H), 8.59 (d, *J* = 4.8 Hz, 1 H). ^{13}C NMR (75 MHz, CD_3OD): δ 16.15, 16.85, 30.65, 38.76, 46.07, 55.07, 112.50, 123.67, 125.86, 125.93, 128.46, 135.31, 137.08, 148.69, 151.25, 156.41, 157.85. MS (ESI): *m/z* 345.95 $[M + 1]^+$. Anal ($C_{19}H_{25}ClN_4$): C, H, Cl, N.

***Synthesis of (Z)-N-(1-benzylpiperidin-4-yl)-N'-(7-chloroquinolin-4-yl))-acetimidamide* (6j):** Compound **6j** was prepared from **5a** and 4-amino-1-benzylpiperidine in 84.8% yields as light brown gum. ^{1}H NMR (600 MHz, CD_3OD): δ 1.71 (m, 2 H), 1.87 (s, 3 H), 1.79 (s, 3 H), 2.19 (m, 2 H), 2.28 (t, *J* = 10.8 Hz, 2 H), 3.01 (m, 2 H), 3.63 (s, 2 H), 4.01 (m, 1 H), 6.85 (d, J = 4.8 Hz,1 H), 7.34 (dd, *J* = 9.0 and 2.1 Hz, 1 H), 7.41 (m, 4 H), 7.53 (dd, *J* = 9.0 and 2.1 Hz, 1 H), 7.98 (d, *J* = 2.1 Hz, 1 H), 8.03 (d, *J* = 9.0 Hz, 1 H), 8.65 (d, *J* = 4.8 Hz, 1 H). ^{13}C NMR (150 MHz, CD_3OD): δ 16.24, 30.92, 50.50, 52.06, 62.68, 112.42, 123.70, 125.86, 125.89, 126.21, 127.05, 127.92, 129.37, 135.27, 137.08, 148.75, 151.22, 156.27, 157.88. MS (ESI): *m/z* 393.29 $[M + 1]^+$. Anal ($C_{23}H_{25}ClN_4$): C, H, Cl, N.

***Synthesis of (Z)-N-(7-chloroquinolin-4-yl)-N'-[2-(pyridin-4-yl)-ethyl]-acetimidamide* (6k).** Compound **6k** was prepared by the same procedure from **5a** and 2-(pyridin-4-yl)-ethylamine to give the product as brown solid which was recrystallized from EtOH as colorless needles (0.2 g, 74.0% yields), mp 165.0 ^{0}C. ^{1}H NMR (300 MHz, CD_3OD): δ 1.80 (s, 3H), 3.00 (t, *J* = 6.90 Hz, 2H), 3.77 (t, *J* = 6.90 Hz, 2H), 6.75 (d, *J* = 4.8 Hz, 1H), 7.44 (d, *J* = 6.00 Hz, 2H), 7.48 (dd, *J* = 9.0 and 2.1 Hz, 1H), 7.82 (d, *J* = 9.0 Hz, 1H), 7.92 (d, *J* = 2.1 Hz, 1H), 8.49 (d, *J* = 6.0 Hz, 2H), 8.60 (d, *J* = 4.8 Hz, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 16.02, 34.33, 40.98, 112.35, 123.80, 124.80, 125.80, 125.87, 126.11, 135.28, 148.52, 150.51, 151.20, 157.00, 157.72. MS (ESI): *m/z* 325.25 $[M + 1]^+$. Anal ($C_{18}H_{17}Cl\ N_4$): C, H, Cl, N.

***Synthesis of (Z)-N-(7-chloroquinolin-4-yl)-N'-2-[1-(pyridin-4-yl)-piperidin-4-yl]ethyl-acetimidamide* (6l):** Compound **6l** was prepared from **5a** and 2-[1-(pyridin-4-yl)-piperidin-4-yl]-ethylamine as brown solid which was recrystallized from EtOH to give in 87.2 % yields as colorless needles, mp 196.5 ^{0}C. ^{1}H NMR (300 MHz, CD_3OD): δ 1.33 (m, 3H), 1.69 (m, 2H), 1.85 (s, 3H), 1.95 (m, 2H), 2.96 (m, 2H), 3.54 (m, 2H), 4.05 (m, 2H), 6.82 (d, *J* = 4.8 Hz, 1H), 6.85 (d, *J* = 6.9 Hz, 2H), 7.49 (dd, *J* = 9.0 and 2.1 Hz, 1H), 7.94 (d, *J* = 2.1 Hz, 1H), 8.04 (d, *J* =

9.0 Hz, 1H), 8.09 (d, *J* = 6.9 Hz, 2H), 8.61 (d, J = 4.8 Hz, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 16.17, 31.18, 33.54, 35.28, 38.35, 45.96, 107.95, 112.53, 123.77, 125.87, 125.91, 126.17, 135.29, 148.31, 148.72, 151.27, 155.21, 157.24, 157.95. MS (ESI): *m/z* 408.19 [M + 1] $^+$. Anal ($C_{23}H_{26}ClN_5$): C, H, Cl, N.

***Synthesis of (Z)-N-(7-chloroquinolin-4-yl)-N'-[3-(piperidin-1-yl)-propyl]-isobutyrimid-amide* (6m):** The title compound was prepared by the same procedure from **5b** and 3-(piperidin-1-yl)-propylamine to give 86.6% yields of the product as colorless gum. ^{1}H NMR (600 MHz, CD_3OD): δ 1.13 (d, *J* = 6.90 Hz, 6H), 1.52 (m, 2H), 1.66 (m, 4H), 1.93 (m, 2H), 2.56 (m, 7H), 3.43 (t, *J* = 6.90 Hz, 2H), 6.76 (d, *J* = 5.10 Hz, 1H) 7.49 (dd, *J* = 9.00 and 2.10 Hz, 1H), 7.93 (d, *J* = 2.10 Hz, 1H), 8.00 (d, *J* =9.00 Hz, 1H), 8.60 (d, *J* = 5.40 Hz, 1H). ^{13}CNMR (150 MHz, CD_3OD): δ 19.54, 23.76, 25.12, 25.28, 30.23, 39.33, 54.18, 56.92, 112.19, 123.65, 125.82, 125.93, 126.12, 135.34, 148.70, 151.24, 157.69 and 164.26. MS (ESI): *m/z* 373.29 [M + 1]$^+$. Anal ($C_{21}H_{29}ClN_4$): C, H, Cl, N.

***Synthesis of (Z)-N-(7-chloroquinolin-4-yl)-N'-[4-(piperidin-1-yl)-butyl]-isobutyrimid-amide* (6n):** The title compound was prepared by the same procedure from **5b** and 4-(piperidin-1-yl)-butylamine in 94% yields as colorless gum. ^{1}H NMR (600 MHz, CD_3OD): δ 1.13 (d, *J* = 6.90 Hz, 6H), 1.51 (m, 2H), 1.65 (m, 8H), 2.44 (m, 6H), 2.56 (m, 1H), 3.41 (t, *J* = 6.90 Hz, 2H), 6.75 (d, *J* = 5.10 Hz, 1H) 7.49 (dd, *J* = 9.00 and 2.10 Hz, 1H), 7.93 (d, *J* = 2.10 Hz, 1H), 7.99 (d, *J* =9.00 Hz, 1H), 8.59 (d, *J* = 5.40 Hz, 1H). ^{13}C NMR (150 MHz, CD_3OD): δ 19.53, 23.54, 23.80, 25.06, 26.88, 30.32, 40.51, 48.46, 54.08, 58.84, 112.16, 123.68, 125.78, 125.93, 126.16, 135.29, 148.72, 151.22, 157.75 and 164.20. MS (ESI): *m/z* 387.31 [M + 1]$^+$. Anal ($C_{22}H_{31}ClN_4$): C, H, Cl, N.

***Synthesis of (Z)-N-(7-chloroquinolin-4-yl)-N'-[2-(diethylamino)ethyl]-isobutyrimid-amide* (6p):** Compound **6p** was prepared by the same procedure from compound **5b** and N, N-diethylamino-ethylenediamine to give 71.4% yields of the desired product as colorless gum. ^{1}H NMR (300 MHz, CD_3OD): δ 1.11 (d, *J* = 6.90 Hz, 6H), 1.29 (t, J = 7.20 Hz, 6H), 2.62 (m, 1H), 2.72 (m, 4H), 2.88 (m, 2H), 3.56 (m, 2H), 6.77 (d, *J* = 5.10 Hz, 1H) 7.50 (dd, *J* = 9.00 and 2.10 Hz, 1H), 7.93 (d, *J* = 2.10 Hz, 1H), 8.00 (d, *J* =9.00 Hz, 1H), 8.60 (d, *J* = 5.10 Hz, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 10.36, 19.50, 30.12, 37.91, 46.85, 50.76, 111.95, 123.54,

125.78, 125.87, 126.15, 135.32, 148.72, 151.24, 157.58, and 163.98. MS (ESI): *m/z* 347.29 [M + 1]$^+$. Anal ($C_{19}H_{27}ClN_4$): C, H, Cl, N.

***Synthesis of (Z)-N-(7-chloroquinolin-4-yl)-N'-[3-(diethylamino)-propyl]-isobutyrimid-amide* (6q):** The title compound was prepared by the same procedure from **5b** and 3-(diethylamino)-propylamine to afford 88.2% yields of the desired compound as colorless oil. ^{1}HNMR (300 MHz, CD_3OD): δ 1.11 (m, 12H), 1.89 (m, 2H), 2.64 (m, 7H), 3.43 (m, 2H), 6.75 (d, *J* = 5.10 Hz, 1H) 7.49 (dd, *J* = 9.00 and 2.10 Hz, 1H), 7.93 (d, *J* = 2.10 Hz, 1H), 8.00 (d, *J* =9.00 Hz, 1H), 8.60 (d, *J* = 5.10 Hz, 1H). ^{13}CNMR (75 MHz, CD_3OD): δ 11.43, 21.09, 26.68, 31.76, 40.89, 47.93, 51.75, 113.71, 125.19, 127.33, 127.45, 127.72, 136.88, 150.28, 152.79, 159.24, and 165.76. MS (ESI): *m/z* 361.15 [M + 1]$^+$. Anal ($C_{20}H_{29}ClN_4$): C, H, Cl, N.

***Synthesis of (Z)-N-(7-chloroquinolin-4-yl)-N'-(4-diethylaminobutyl)-isobutyrimid-amide* (6r):** Compound **6r** was prepared by the same procedure from **5b** and 4-(diethylamino)-butylamine to give 98% yields of the desired product as colorless gum. ^{1}H NMR (600 MHz, CD_3OD): δ 1.14 (d, *J* = 6.90 Hz, 6H), 1.26 (t, J = 7.20 Hz, 6H), 1.78 (m, 4H), 1.93 (m, 2H), 2.62 (m, 1H), 3.00 (m, 6H), 3.46 (m, 2H), 6.76 (d, *J* = 5.10 Hz, 1H) 7.50 (dd, *J* = 9.00 and 2.10 Hz, 1H), 7.93 (d, *J* = 2.10 Hz, 1H), 7.99 (d, *J* =9.00 Hz, 1H), 8.60 (d, *J* = 5.10 Hz, 1H). ^{13}C NMR (150 MHz, CD_3OD): δ 8.37, 19.58, 21.85, 26.16, 30.30, 40.12, 46.85, 51.71, 112.19, 123.63, 125.87, 125.95, 126.18, 135.32, 148.72, 151.28, 157.65, 164.38. MS (ESI): *m/z* 375.32 [M + 1]$^+$. Anal ($C_{21}H_{31}ClN_4$): C, H, Cl, N.

***Synthesis of (Z)-N'-(1-benzylpiperidin-4-yl)-N-(7-chloroquinolin-4-yl)-isobutyrimid-amide* (6s):** The title compound was prepared by the same procedure from **5b** and 4-amino-1-benzyl-piperidine to give 71% yields of the desired product as colorless gum. ^{1}H NMR (600 MHz, CD_3OD): δ 1.09 (d, *J* = 6.90 Hz, 6H), 1.65 (m, 2H), 2.09 (m, 4H), 2.54 (m, 1H), 2.93 (m, 2H), 3.50 (s, 2H), 3.87 (m, 1H), 6.71 (d, *J* = 5.10 Hz, 1H) 7.29 (m, 5H), 7.46 (dd, *J* = 9.00 and 2.10 Hz, 1H), 7.94 (d, *J* =9.00 Hz, 1H), 8.57 (d, *J* = 5.40 Hz, 1H). ^{13}CNMR (150 MHz, CD_3OD): δ 19.40, 30.30, 30.70, 30.91, 52.31, 62.66, 112.04, 123.59, 125.86, 126.19, 127.09, 127.93, 129.43, 135.33, 136.98, 148.69, 151.23, 157.63, and 163.36. MS (ESI): *m/z* 421.24 [M + 1]$^+$. Anal ($C_{25}H_{29}ClN_4$): C, H, Cl, N.

***Synthesis of N'-(7-chloroquinolin-4-yl)-acetimidamide* (7a):** Solution of acetamidine (2.3 g, 12 mmol) in anhydrous DMSO (70 mL) was added dropwise to a suspension of NaH (576 mg, 24 mmol) in 20 mL of anhydrous DMSO. The mixture was stirred at rt for 1 h, and a suspension of 4,7-dichloroquinoline (1.98 g, 10 mmol) in anhydrous DMSO (80 mL) was added in one portion. Mixture was stirred at rt for 6 h, quenched with iced water (1.3 L), and the water solution was saturated with K_2CO_3 and NaCl. The product was collected, washed successively with water (4 × 50 mL) and $CHCl_3$ (5 × 30 mL), and dried under vacuum to give **7a** in 29 % yields as off-white solid, mp: 166-169 ºC, R_f = 0.20 ($CHCl_3$: MeOH, 9: 1 v/v) or 0.42 ($CHCl_3$: MeOH: NH_4OH, 10: 1: 0.1 v/v), MS (ESI): *m/z* 220 $[M + 1]^+$. ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.93 (s, 3H), 6.20-6.80 (br.s, 2H), 6.77 (d, *J* = 4.8 Hz, 1H), 7.48 (dd, *J* = 9.0 and 2.4 Hz, 1H), 7.92 (d, *J* = 2.4 Hz, 1H), 7.94 (d, *J* = 9.0 Hz, 1H), 8.63 (d, *J* = 4.8 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 20.14, 112.80, 123.37, 125.82, 126.67, 127.80, 134.02, 149.86, 152.69, 156.27, 157.97.

***Synthesis of N'-(7-chloroquinolin-4-yl)-benzimidamide* (7b):** The title compound was prepared by the same procedure for the synthesis of **7a** using benzamidine HCl as reagent to give **7b** as beige solid in 49% yield, mp 195-197 °C. R_f = 0.52 ($CHCl_3$: MeOH = 9: 1 v/v), ^{1}H NMR (600 MHz, DMSO-d_6): δ 6.70-7.20 (br.s, 2H), 6.90 (d, *J* = 4.8 Hz, 1H), 7.47-7.51 (m, 3H), 7.52-7.56 (m, 1H), 7.96 (d, *J* = 4.2 Hz, 1H), 7.97 (d, *J* = 2.4 Hz, 1H), 8.03 (d, *J* = 7.2 Hz, 2H), 8.70 (d, *J* = 4.8 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 112.80, 122.87, 126.00, 126.59, 127.87, 127.95, 128.68, 131.10, 134.11, 135.39, 149.95, 152.83, 155.89, 156.20. MS (ESI): *m/z* 282 $[M + 1]^+$.

***Synthesis of 4-chloro-N'-(7-chloroquinolin-4-yl)-benzimidamide* (7c):** The title compound was obtained from **1** and 4-chlorobenzimidamide hydrochloride to give **7c** in 22% yields as white solid, mp 204-207 °C. R_f = 0.45 ($CHCl_3$: MeOH, 10: 1 v/v). ^{1}H NMR (600 MHz, DMSO-d_6): δ 6.70-7.15 (hump, 2H, NH_2), 6.91 (d, *J* = 4.8 Hz, 1H), 7.50 (dd, *J* = 9.0 and 1.8 Hz, 1H), 7.57 (d, *J* = 8.4 Hz, 2H), 7.94 (d, *J* = 9.0 Hz, 1H), 7.97 (d, *J* = 1.8 Hz, 1H), 8.06 (d, *J* = 8.4 Hz, 2H), 8.71 (d, *J* = 4.8 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 112.81, 122.75, 126.09, 127.97, 128.74, 129.77, 134.15, 134.22, 135.86, 149.92, 152.86, 154.83, 155.90. MS (ESI): *m/z* 316 $[M + 1]^+$.

***Synthesis of N'-(7-chloroquinolin-4-yl)-nicotinimidamide* (7d):** The title compound was obtained, using the same general procedure, from nicotinimidamide hydrochloride and compound **1** to give **7d** in 16% yield as white solid, mp 117-119 °C., R_f = 0.17 ($CHCl_3$: MeOH, 10: 1 v/v). ^{1}H NMR (600 MHz, DMSO-d_6): δ 6.94 (d, *J* = 4.8 Hz, 1H), 6.80-7.20 (br.s, 2H), 7.50 (dd, *J* = 9.0 and 1.8 Hz, 1H), 7.53 (dd, *J* = 7.8 and 4.8 Hz, 1H), 7.95-8.02 (m, 2H), 8.38 (d, *J* = 7.8 Hz, 1H), 8.70-8.75 (m, 2H), 9.19 (s, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 112.88, 122.69, 123.80, 126.17, 126.60, 127.97, 131.07, 134.20, 135.54, 148.95, 149.91, 151.81, 152.87, 154.15, 155.71. MS (ESI): *m/z* 282 $[M + 1]^+$.

***Synthesis of N-(7-chloroquinolin-4-yl)-N'-[3-(dimehylamino)-propyl]-benzimidamide* (6t).** The title compound was prepared by Method B described in Chemistry Section. Solution of N-(7-chloroquinolin-4-yl)-benzimidamide (**7b),** (1.127 g, 4 mmol) in anhydrous DMSO (60 mL) was added dropwise to a suspension of NaH (116 mg, 4.8 mmol) in anhydrous DMSO (20 mL). In a separate flask, a solution of 3-chloro-N,N-dimethylaminopropane hydrochloride (633 mg, 4 mmol) in anhydrous DMSO (30 mL) was added dropwise to a suspension of NaH (116 mg, 4.8 mmol) in anhydrous DMSO (20 mL). The resulting 1-chloro-3-N,N-dimethylaminopropane free base solution was added dropwise to the first reaction flask, heated at 100 °C under N_2 for 4.5 h, quenched with ice/water (1.3 L), saturated with K_2CO_3 and NaCl powder. The mixture was extracted with $CHCl_3$ (4 × 150 mL). The $CHCl_3$ extracts were combined, dried over Na_2SO_4, and evaporated to dryness under vacuum. The crude product was separated on a silica gel column (120 g) and eluted with mixed solvent ($CHCl_3$: MeOH: NH_4OH 10: 1: 0.1 v/v). Three compounds were isolated:

(a). starting material **7b** (314 mg, 28 %).

(b). Product compound **6t** (728 mg, 50 % yields) as yellow oil, R_f = 0.37 ($CHCl_3$: MeOH: NH_4OH 10: 1: 0.1 v/v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.87 (br.s, 2H), 2.22 (br.s, 6H), 2.49 (br.s, 2H), 3.67 (br.s, 2H), 6.16 (br.s, 1H, NH), 7.05 - 7.22 (m, 5H), 7.25-7.30 (m, 1H), 7.40 (dd, *J* = 9.0 and 1.8 Hz, 1H), 7.93 (d, *J* = 1.8 Hz, 1H), 8.26 (d, *J* = 9.0 Hz, 1H), 8.35 (br.s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 25.47, 42.49, 45.40, 58.92, 112.36, 124.06, 125.78, 125.93, 127.83, 127.85, 128.50, 129.88, 134.02, 134.80, 149.51, 151.59, 156.37, 158.61. MS (ESI): *m/z* 367 $[M + 1]^+$.

(c). Compound **8c** *(*68 mg, 4% yields): yellow oil, R_f = 0.21 ($CHCl_3$: MeOH: NH_4OH, 10: 1: 0.1 v/v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.68 (br.s, 2H), 1.85-2.15 (m, 10H), 2.06 (br.s, 6H), 2.42 (br.s, 2H), 3.18 (br.s, 2H), 3.75 (br.s, 2H), 6.08 (d, *J* = 4.8 Hz, 1H), 7.01 (d, *J* = 6.6 Hz, 1H), 7.11-7.20 (m, 3H), 7.37 (dd, *J* = 9.0 and 1.8 Hz, 1H), 7.88 (d, *J* = 1.8 Hz, 1H), 8.12 (d, *J* = 9.0 Hz, 1H), 8.29 (d, *J* = 4.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 25.60, 27.25, 45.29, 45.53, 48.23, 56.77, 57.41, 112.15, 124.10, 125.63, 125.88, 127.78, 127.82, 128.32, 129.05, 132.92, 134.60, 149.26, 151.44, 156.13, 160.40. MS (ESI): *m/z* 452 $[M + 1]^+$.

***Synthesis of N'-(7-chloroquinolin-4-yl)-N-[3-(diethylamino)-propyl]-benzimidamide* (6u).** The title compound was obtained using the same procedure for preparation of **6t** using compound **7b** and 1-chloro-3-*N,N*-diethylaminopropane as starting material to give 50 % yield of product **6u** as yellow oil. R_f = 0.53 ($CHCl_3$: MeOH: NH_4OH 10: 1: 0.1 v/v). **^{1}H NMR** (600 MHz, $CDCl_3$): 0.95 (t, *J* = 6.6. Hz, 6H), 1.89 (br.s, 2H), 2.53 (br.s, 4H), 2.68 (br.s, 2H), 3.72 (br.s, 2H), 6.19 (br.s, 1H), 7.16-7.22 (m, 4H), 7.42 (dd, *J* = 9.0 and 2.4 Hz, 1H), 7.64 (br.s, 1H), 7.96 (d, *J* = 1.8 Hz, 1H), 8.29 (d, *J* = 9.0 Hz, 1H), 8.38 (d, *J* = 4.8 Hz, 1H). **^{13}C NMR** (150 MHz, $CDCl_3$): 11.41, 24.76, 43.11, 46.62, 53.14, 112.43, 124.11, 125.73, 125.91, 127.82, 127.88, 128.43, 129.81, 134.19, 134.79, 149.54, 151.59, 156.42, 158.70. MS (ESI): *m/z* 395 $[M + 1]^+$.

***Synthesis of N'-(7-chloroquinolin-4-yl)-N-[3-(piperidin-1-yl)-propyl]-benzimidamide* (6v).** Title compound was obtained according to the same procedure using compound **7b** and 1-(3-chloropropyl)piperidine hydrochloride as reagents to give 49 % yield **6v** as yellow solid, mp 50-52 °C, R_f = 0.43 ($CHCl_3$: MeOH: NH_4OH 10: 1: 0.1 v/v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.36 (br.s, 6H), 1.88 (t, *J* = 5.4 Hz, 2H), 2.42 (br.s, 4H), 2.56 (t, *J* = 5.4 Hz, 2H), 3.71 (br.s, 2H), 6.18 (d, *J* = 5.4 Hz, 1H), 7.15-7.23 (m, 4H), 7.25-7.32 (m, 1H), 7.41 (dd, *J* = 9.0 and 1.8 Hz, 1H), 7.89 (br.s, 1H), 7.95 (d, *J* = 1.8 Hz, 1H), 8.30 (d, *J* = 8.4 Hz, 1H), 8.37 (d, *J* = 5.4 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 23.85, 24.22, 25.82, 43.43, 54.63, 59.04, 112.49, 124.16, 125.72, 127.88, 127.98, 128.39, 129.8,3 134.17, 134.78, 149.54, 151.62, 156.48, 158.80. MS (ESI): *m/z* 407 $[M + 1]^+$.

***Synthesis of 4-Chloro-N'-(7-chloroquinolin-4-yl)-N-3-(dimethylaminopropyl)-benzimid -amide* (6w):** Compound **6w** was obtained according to the same procedure using amidine **7c** and 1-chloro-3-*N,N*-dimethylaminopropane hydrochloride as reagents. The product after purified with a silica gel column was recrystallized from CH_3CN to give 9% yield of **6w** as yellow solid, mp 47-49 °C, R_f = 0.30 ($CHCl_3$: MeOH: NH_4OH, 10: 1: 0.1 v/v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.87 (br.s, 2H), 2.02 (s, 3H), 2.24 (br.s, 6H), 2.53 (br.s, 2H), 3.67 (br.s, 2H), 6.19 (br.s, 1H), 7.10-7.18 (m, 2H), 7.20 (d, *J* = 7.8 Hz, 2H), 7.25-7.32 (m, overlap with solvent), 7.43 (dd, *J* = 9.0 and 2.4 Hz, 1H), 7.98 (d, *J* = 1.8 Hz, 1H), 8.23 (d, *J* = 9.0 Hz, 1H), 8.43 (br.s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 1.97, 25.05, 43.05, 45.40, 59.25, 112.27, 116.43, 123.85, 125.73, 125.97, 128.05, 129.19, 132.50, 134.97, 135.94, 149.58, 151.71, 155.97, 157.29. MS (ESI): *m/z* 401 [M + 1]$^+$. Anal ($C_{21}H_{22}Cl_2N_4.1.5.C_4H_8O_4$): C, H, Cl, N.

***Synthesis of N'-(7-chloroquinolin-4-yl)-N-[3-(diethylamino)propyl)]-nicotinimidamide* (6x).** N'-(7-chloroquinolin-4-yl)-nicotinimidamide (**7d**) (373 mg, 1.32 mmol) in anhydrous DMSO (30 mL) was added to suspension of NaH (44 mg, 1.8 mmol) in anhydrous DMSO (10 mL). The mixture was stirred under nitrogen at rt for 1 h. 3-(N,N-diethylamino)-propyl chloride (217 mg, 1.45 mmol) was added dropwise to the mixture. At the end of the addition, the mixture was heated at 100 °C under nitrogen for 5.5 h, and quenched with iced water (0.3 L). The mixture was saturated with K_2CO_3 and NaCl, extracted with $CHCl_3$ (100 mL) 3 times. The combined extracts were dried over Na_2SO_4, evaporated to dryness under reduced pressure. The crude product was purified with a silica gel column (40 g) using CHCl3: MeOH: NH_4OH (10: 1: 0.1 v/v) as eluent. Three compounds **6x**, **8e** and **8f** were isolated from the mixture:

a). Title compound **6x** was isolated as amber oil (128 mg, 25 % yield), R_f = 0.28 ($CHCl_3$: MeOH: NH_4OH, 10: 1: 0.1 v/v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.91 (t, *J* = 7.2 Hz, 6H), 1.86 (br.s, 2H), 2.50 (q, *J* = 7.2 Hz, 4H), 2.67 (t, *J* = 5.4 Hz, 2H), 3.71 (br.s, 2H), 6.21 (d, *J* = 4.2 Hz, 1H), 7.36 (d, *J* = 7.5 Hz, 1H), 7.42 (dd, *J* = 8.7 and 1.8 Hz, 1H), 7.96 (d, *J* = 1.8 Hz, 1H), 8.20 (hump, 1H), 8.22 (d, *J* = 8.7 Hz, 1H), 8.41 (d, *J* = 4.8 Hz, 1H), 8.52 (d, *J* = 4.8 Hz, 1H), 8.56 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.49, 24.35, 43.73, 46.57, 53.47, 112.45, 123.08, 123.84, 125.66, 126.05, 128.03, 130.27, 135.02, 135.34, 148.48, 149.55, 150.82, 151.63, 155.60, 155.69. MS (ESI): *m/z* 396 [M + 1]$^+$.

b). ***N-(7-chloroquinolin-4-yl)-N,N'-bis[3-(diethylamino)-propyl]-nicotinimidamide*** **(8e).** The title compound was isolated from the product mixture as brown syrup (20 mg, 3 % yields), R_f = 0.11 ($CHCl_3$: MeOH: NH_4OH, 10: 1: 0.1 v/v). ^1H NMR (300 MHz, $CDCl_3$): δ 0.99 (br.s, 12H), 1.72 (br.s, 2H), 2.03 (br.s, 2H), 2.20-2.85 (m, 12H), 3.21 (br.s, 2H), 3.76 (br.s, 2H), 6.13 (d, *J* = 5.1 Hz, 1H), 7.09 (dd, *J* = 7.8 and 5.1 Hz, 1H), 7.25 - 7.35 (m, 1H, overlap with solvent), 7.40 (dd, *J* = 8.7 and 1.8 Hz, 1H), 7.91 (d, *J* = 1.8 Hz, 1H), 8.06 (d, *J* = 8.7 Hz, 1H), 8.35 (d, *J* = 4.8 Hz, 1H), 8.39 (d, *J* = 1.2 Hz, 1H), 8.45 (d, *J* = 4.8 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.61, 25.18, 26.92, 29.70, 46.73, 50.21, 112.17, 123.16, 123.79, 125.54, 125.99, 128.02, 129.11, 134.92, 135.11, 148.27, 149.34, 150.14, 151.51, 155.46, 157.27. MS (ESI): *m/z* 509 $[M + 1]^+$.

c). ***(3E,NE)-N-[7-chloro-1-(3-diethylaminopropyl)]quinolin-4(1H)-ylidene)-N'-[3-(diethylamino)propyl]-nicotinimidamide (8f).*** The title compound was isolated from the product mixture as brown syrup (93 mg, 14 % yields), R_f = 0.05 ($CHCl_3$: MeOH: NH_4OH, 10: 1: 0.1 v/v). ^1H NMR (300 MHz, $CDCl_3$): δ 0.98 (td, *J* = 6.9 and 1.2 Hz, 12H), 1.75-1.95 (m, 4H), 2.40 (t, *J* = 5.7 Hz, 2H), 2.45-2.60 (m, 10H), 3.29 (t, *J* = 7.2 Hz, 2H), 3.97 (t, *J* = 6.9 Hz, 2H), 5.51 (d, *J* = 7.8 Hz, 1H), 7.01 (d, *J* = 7.8 Hz, 1H), 7.18-7.30 (m, 2H), 7.38 (d, *J* = 1.5 Hz, 1H), 8.15 (d.t, *J* = 8.1 and 1.5 Hz, 1H), 8.48-8.58 (m, 2H), 9.00 (d, *J* = 1.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.49, 11.72, 26.26, 27.89, 46.42, 46.86, 47.64, 49.25, 50.25, 50.98, 102.09, 114.82, 122.29, 122.99, 124.03, 128.18, 132.56, 134.86, 137.66, 139.72, 140.36, 149.21, 150.28, 153.32, 162.07. MS (ESI): *m/z* 509 $[M + 1]^+$. Identity of compound **8f** was established based on up field shift of the proton signal at 2 and 3 position of quinoline ring as comparing to that of **8e.**

Synthesis of N'-(7-Chloroquinolin-4-yl)-N,N-bis-[3-(diethylamino)propyl]-acetimid-amide **(8a).** Title compound was isolated in 13 % yields as yellow oil from the products mixture of **6b** using method B. R_f = 0.33 ($CHCl_3$: MeOH: NH_4OH 10: 1: 0.1 v/v). ^1H NMR (600 MHz, $CDCl_3$): δ 0.96 (t, *J* = 7.2 Hz, 12H), 1.60-1.85 (m, 4H), 1.89 (s, 3H), 2.40-2.47 (m, 4H), 2.50 (q, *J* = 7.2 Hz, 8H), 3.25-3.55 (m, 4H), (d, *J* = 4.8 Hz, 1H), 7.30 (dd, *J* = 9.0 and 2.4 Hz, 1H), 7.85 (d, *J* = 9.0 Hz, 1H), 7.95 (d, *J* = 2.4 Hz, 1H), 8.60 (d, *J* = 4.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.62, 15.26, 24.99, 27.04, 46.77, 50.24, 111.67, 123.67, 125.62, 125.78, 127.89, 134.79, 149.71, 151.85, 156.14, 156.69. MS (ESI): *m/z* 446 $[M + 1]^+$.

***Synthesis of N-(7-Chloroquinolin-4-yl)-N,N'-bis[3-(diethylamino)propyl]-benzimid amide* (8b).** Title compound was isolated as a byproduct from the synthesis of **6u** in 8 % yields, R_f = 0.15 ($CHCl_3$: MeOH: NH_4OH 10: 1: 0.1 v/v). ^1H NMR (600 MHz, $CDCl_3$): δ 0.89 (br.s, 6H), 1.03 (br.s, 6H), 1.67 (br.s, 2H), 2.01 (br.s, 2H), 2.21 (br.s, 2H), 2.36 (br.s, 4H), 2.57 (br.s, 6H), 3.16 (br.s, 2H), 3.73 (br.s, 2H), 6.07 (d, *J* = 4.8 Hz, 1H), 7.01 (dd, *J* = 8.4 and 1.8 Hz, 2H), 7.12-7.20 (m, 3H), 7.36 (dd, *J* = 9.0 and 1.8 Hz, 1H), 7.87 (d, *J* = 2.4 Hz, 1H), 8.13 (d, *J* = 9.0 Hz, 1H), 8.29 (d, *J* = 4.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.64, 25.06, 26.73, 45.68, 46.59, 46.84, 48.30, 49.97, 50.53, 112.14, 124.15, 125.55, 125.92, 127.81, 128.28, 129.01, 133.01, 134.56, 149.30, 151.46, 156.16, 160.46. MS (ESI): *m/z* 508 $[M + 1]^+$.

***Synthesis of N-(7-chloroquinolin-4-yl)-N,N'-bis[3-(dimehylamino)propyl]benzimid-amide* (8c).** The title compound was isolated as a side product (68 mg, 4% yield) from synthesis of **6t** as yellow oil, R_f = 0.21 ($CHCl_3$: MeOH: NH_4OH, 10: 1: 0.1 v/v), ^1H NMR (600 MHz, $CDCl_3$): δ 1.68 (br.s, 2H), 1.85-2.15 (m, 10H), 2.06 (br.s, 6H), 2.42 (br.s, 2H), 3.18 (br.s, 2H), 3.75 (br.s, 2H), 6.08 (d, *J* = 4.8 Hz, 1H), 7.01 (d, *J* = 6.6 Hz, 1H), 7.11-7.20 (m, 3H), 7.37 (dd, *J* = 9.0 and 1.8 Hz, 1H), 7.88 (d, *J* = 1.8 Hz, 1H), 8.12 (d, *J* = 9.0 Hz, 1H), 8.29 (d, *J* = 4.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 25.60, 27.25, 45.29, 45.53, 48.23, 56.77, 57.41, 112.15, 124.10, 125.63, 125.88, 127.78, 127.82, 128.32, 129.05, 132.92, 134.60, 149.26, 151.44, 156.13, 160.40. MS (ESI): *m/z* 452 $[M + 1]^+$. Anal ($C_{26}H_{34}ClN_5$): C, H, N.

***Synthesis of N-(7-chloroquinolin-4-yl)-N,N'-bis(3-(piperidin-1-yl)propyl)-benzimid-amide* (8d).** Title compound was a byproduct from the synthesis of **6v** in 12 % yields as yellow oil, R_f = 0.20 ($CHCl_3$: MeOH: NH_4OH 10: 1: 0.1 v/v). ^1H NMR (600 MHz, $CDCl_3$): δ 1.35-1.50 (m, 8H), 1.55-1.65 (m, 4H), 1.65-1.82 (m, 4H), 1.95-2.15 (m, 4H), 2.15-2.30 (m, 4H), 2.35-2.55 (m, 6H), 3.19 (br.s, 2H), 3.76 (br.s, 2H), 6.09 (d, *J* = 4.8 Hz, 1H), 7.03 (dd, *J* = 8.4 and 1.8 Hz, 1H), 7.15-7.23 (m, 3H), 7.39 (dd, *J* = 9.0 and 2.4 Hz, 1H), 7.90 (d, *J* = 2.4 Hz, 1H), 8.14 (d, *J* = 9.0 Hz, 1H), 8.31 (d, *J* = 4.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 24.41, 24.92, 25.98, 26.39, 45.50, 48.17, 54.47, 54.68, 56.24, 56.93, 112.14, 124.13, 125.58, 125.92, 127.81, 127.85, 128.30, 129.00, 132.98, 134.58, 149.30, 151.46, 156.14, 160.46. MS (ESI): *m/z* 532 $[M + 1]^+$. Anal ($C_{32}H_{42}ClN_5$): C, H, N.

***N-(7-chloroquinolin-4-yl)-N,N'-bis[3-(diethylamino)-propyl]nicotinimidamide* (8e):** The title compound was isolated as a byproduct from the synthesis of **6x** as described above.

***(3E,NE)-N-7-chloro-1-[3-(diethylamino)-propylquinolin-4(1H)-ylidene]-N'-[3-(ethyl-amino)-propyl]-nicotinimidamide* (8f).** The title compound was isolated as a by-product from the synthesis of **6x as** described above.

***Synthesis of N'-(7-chloro-2-methoxybenzo[b][1,5]naphthyridin-10-yl)-acetamidamide* (11a).** 7,10-Dichloro-2-methoxybenzo[b][1,5]naphthyridine (**10**) (8.38 g, 30 mmol), K_2CO_3 (8.28 g, 60 mmol) and acetamidine hydrochloride (5.67 g, 60 mmol) were dissolved in anhydrous DMSO (200 mL) and stirred at 120 ºC in a sealed tube for 4.5 h. On cooling, the reaction mixture was poured into ice-water (2000 mL). The yellow precipitate was collected, washed with water (4 × 250 mL) and dried under vacuum to give 8.57 g (95 %) of the desired product as yellow solid, mp 205-207 ºC (dec.), R_f = 0.25 ($CHCl_3$: MeOH, 9: 1, v/v). ^{1}H NMR (600 MHz, DMSO-d_6): δ 1.90 (s, 3H), 3.98 (s, 3H), 6.50-7.20 (br.s, 2H), 7.28 (d, *J* = 9.0 Hz, 1H), 7.44 (d, *J* = 9.6 Hz, 1H), 7.99 (s, 1H), 8.19 (d, *J* = 9.6 Hz, 1H), 8.22 (d, *J* = 9.0 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 20.81, 53.52, 119.06, 121.77, 124.96, 127.34, 127.79, 129.95, 134.34, 140.91, 144.98, 147.85, 152.25, 159.48, 160.09. MS (ESI): *m/z* 301 $[M + 1]^+$.

***Synthesis of N'-(7-chloro-2-methoxybenzo[b][1,5]naphthyridin-10-yl)-isobutyrimid-amide* (11b).** The title compound was prepared according to the same procedure for the synthesis of **11a**, using isobutyrimidamide hydrochloride as reagent to give desired product in 67 % yield as a yellow solid, mp 219-221 °C, R_f = 0.15 ($CHCl_3$: MeOH 10: 1 v/v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.53 (d, *J* = 7.2 Hz, 6H), 2.82 (septet, *J* = 7.2 Hz, 1H), 4.08 (s, 3H), 5.21 (br.s, 2H), 6.89 (d, *J* = 9.6 Hz, 1H), 7.35 (dd, *J* = 9.0 and 1.2 Hz, 1H), 7.73 (s, 1H), 7.77 (d, *J* = 9.0 Hz, 1H), 8.08 (d, *J* = 9.0 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 21.16, 35.44, 53.73, 118.69, 120.86, 125.69, 126.09, 127.18, 129.29, 135.24, 139.29, 143.96, 147.19, 150.94, 160.54, 163.48. MS (ESI): *m/z* 329 $[M + 1]^+$.

***Synthesis of N'-(7-chloro-2-methoxybenzo[b][1,5]naphthyridin-10-yl)-benzimidamide* (11c).** The title compound was prepared according to the same procedure for the synthesis of

11a, using benzimidamide hydrochloride as reagent to give desired product in 43 % yield as yellow solid, mp 224-226 °C, R_f = 0.47 ($CHCl_3$: MeOH, 10: 1, v/v). ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.89 (s, 3H), 6.69 (br.s, 2H), 7.25 (d, *J* = 9.0 Hz, 1H), 7.46 (dd, *J* = 9.0 and 2.4 Hz, 1H), 7.47-7.57 (m, 3H), 8.02 (d, *J* = 1.8 Hz, 1H), 8.07 (dd, *J* = 9.0 and 1.8 Hz, 2H), 8.20 (d, *J* = 9.0 Hz, 1H), 8.24 (d, *J* = 9.0 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 53.54, 119.14, 121.67, 125.21, 127.48, 127.64, 127.94, 128.68, 129.70, 130.91, 134.32, 136.23, 140.82, 144.94, 147.81, 152.38, 156.71, 159.93. MS (ESI): *m/z* 363 $[M + 1]^+$.

***Synthesis of (Z)-4-chloro-N'-(7-chloro-2-methoxybenzo[b][1,5]naphthyridin-10-yl)-benzimidamide* (11d):** The title compound was prepared according to the same procedure for the synthesis of **11a**, using 4-chlorobenzimidamide hydrochloride as reagent to give desired product in 41 % yields as yellow solid, mp > 350 °C (dec.), R_f = 0.45 ($CHCl_3$: MeOH, 10: 1 v/v). ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.87 (s, 3H), 6.92 (hump, 2H), 7.28 (d, *J* = 9.0 Hz, 1H), 7.47 (dd, *J* = 9.0 and 1.8 Hz, 1H), 7.58 (d, *J* = 8.4 Hz, 2H), 8.04 (d, *J* = 1.8 Hz, 1H), 8.10 (d, *J* = 8.4 Hz, 2H), 8.21 (d, *J* = 9.0 Hz, 1H), 8.23 (d, *J* = 9.0 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 53.56, 119.27, 121.62, 125.33, 127.51, 127.60, 128.75, 129.63, 129.83, 134.36, 134.99, 135.66, 140.90, 145.00, 147.81, 152.04, 155.60, 160.00. MS (ESI): *m/z* 397 $[M + 1]^+$.

***Synthesis of (Z)-N'-(7-chloro-2-methoxybenzo[b][1,5]naphthyridin-10-yl)-nicotinimid-amide* (11e).** The title compound was prepared by the same procedure as described for the synthesis of **11a**, using nicotinimidamide hydrochloride as reagent. Compound **11e** was isolated as yellow solid in 22% yields, mp 244-246 °C. R_f = 0.50 ($CHCl_3$: MeOH, 10: 1 v/v). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 3.89 (s, 3H), 7.06 (br.s, 2H), 7.27 (d, *J* = 9.6 Hz, 1H), 7.48 (d, *J* = 9.0 Hz, 1H), 7.55 (dd, *J* = 7.8 and 4.8 Hz, 1H), 8.04 (s, 1H), 8.21 (d, *J* = 8.4 Hz, 1H), 8.25 (d, *J* = 9.0 Hz, 1H), 8.41 (d, *J* = 7.8 Hz, 1H), 8.27 (d, *J* = 4.8 Hz, 1H), 9.24 (s, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 53.58, 119.30, 121.59, 123.87, 125.45, 127.48, 127.62, 129.58, 131.85, 134.40, 135.57, 140.82, 144.94, 147.74, 148.94, 151.68, 151.79, 154.87, 160.10. MS (ESI): *m/z* 364 $[M + 1]^+$.

***Synthesis of N-(7-chloro-2-methoxybenzo[b][1,5]-naphthyridin-10-yl)-N'-(3-dimethyl-aminopropyl)-acetimidamide* (12a):** Solution of compound **11a** (1.20 g, 4 mmol) in anhydrous

DMSO (50 mL) was added dropwise to a suspension of NaH (116 mg, 4.8 mmol) in anhydrous DMSO (20 mL) in flask #1. In a separate flask (flask #2), a solution of 3-chloro-N,N-dimethylpropan-1-amine hydrochloride (696 mg, 4.4 mmol) in anhydrous DMSO (30 mL) was added dropwise to a suspension of NaH (110 mg, 4.6 mmol) in anhydrous DMSO (20 mL). After 1 h, the resulting solution of 3-chloro-N,N-dimethylpropan-1-amine (flask #2) was added dropwise to the reaction flask #1. The combined mixture was heated at 100 °C under nitrogen for 4 h, quenched with ice/water (1.5 L) and extracted with $CHCl_3$ (4 × 150 mL). The $CHCl_3$ extracts were combined, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The crude product **12a** was purified with a silica gel column using mixed solvent ($CHCl_3$: MeOH: NH_4OH, 10 : 1 : 0.1 v/v) to give 667 mg (43 %) of the desired product **12a** as yellow oil, R_f = 0.24 ($CHCl_3$: MeOH: NH_4OH, 10: 1: 0.1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.73 (br.s, 3H), 1.90 (br.s, 2H), 2.29 (s, 6H), 2.50 (br.s, 2H), 3.65-3.75 (br.s, 1H), 7.15 (d, *J* = 9.0 Hz, 1H), 7.35 (dd, *J* = 9.0 and 1.8 Hz, 1H), 8.07 (d, *J* = 1.8 Hz, 1H), 8.20 (d, *J* = 9.0 Hz, 1H), 8.21 (d, *J* = 9.0 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 19.87, 26.12, 41.40, 45.45, 53.25, 58.62, 118.59, 121.71, 125.15, 126.87, 127.41, 129.91, 135.21, 140.30, 144.76, 147.89 , 152.36, 157.17, 160.34. MS (ESI): *m/z* 387 $[M + 1]^+$. Anal ($C_{20}H_{24}ClN_5O_{\cdot}$ 1/3 H_2O): C, H, N.

Synthesis of N-(7-chloro-2-methoxybenzo[b][1,5]naphthyridin-10-yl)-N'-(3-diethyl-aminopropyl)-acetimidamide **(12b).** The title compound was prepared by the same general procedure using compound **11a** and 3-chloro-*N,N*-diethylpropan-1-amine as reagents to give 47 % yield of **12b** as brown oil, R_f = 0.43 ($CHCl_3$: MeOH: NH_4OH, 10: 1: 0.1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.08 (t, *J* = 7.2 Hz, 6H), 1.72 (s, 3H), 1.84 (br.s, 2H), 2.57 (br.s, 4H), 2.63 (br.s, 2H), 3.69 (br.s, 2H), 4.01 (s, 3H), 7.14 (d, *J* = 9.0 Hz, 1H), 7.18 (br.s, 1H), 7.34 (dd, *J* = 9.0 and 1.8 Hz, 1H), 8.06 (d, *J* = 1.8 Hz, 1H), 8.19 (d, *J* = 9.0 Hz, 1H), 8.24 (d, *J* = 9.0 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.05, 19.83, 25.24, 42.42, 47.00, 53.04, 53.16, 118.53, 121.82, 125.04, 126.95, 127.37, 129.95, 135.18, 140.31, 144.76, 147.92, 152.59, 157.15, 160.27. MS (ESI): *m/z* 414 $[M + 1]^+$. Anal ($C_{22}H_{28}ClN_5O$): C, H, N.

Synthesis of N-(7-chloro-2-methoxybenzo[b][1,5]naphthyridin-10-yl)-N'-[3-(piperidin-1-yl)-propyl]-acetimidamide **(12c).** Compound **12c** was prepared by the same general procedure using compound **11a** and 1-(3-chloropropyl)-piperidine as reagents to give 44 % yields of **12c** as

brown oil, R_f = 0.56 ($CHCl_3$: MeOH: NH_4OH, 10: 1: 0.1 v/v). ^1H NMR (600 MHz, $CDCl_3$): δ 1.50 (br.s, 2H), 1.63 (br.s, 4H), 1.76 (s, 3H), 1.89 (t, *J* = 6.0 Hz), 2.30-2.60 (m, 6H), 3.71 (br.s, 2H), 4.03 (s, 3H), 7.14 (br.s, 1H), 7.15 (d, *J* = 9.0 Hz, 1H), 7.35 (dd, *J* = 9.0, and 1.8 Hz, 1H), 8.08 (d, *J* = 1.8 Hz, 1H), 8.22 (d, *J* = 9.0 Hz, 1H), 8.24 (d, *J* = 9.0 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 19.94, 24.34, 24.68, 26.31, 42.36, 53.19, 54.67, 58.74, 118.52, 121.81, 125.09, 126.92, 127.45, 129.95, 135.21, 140.38, 144.80, 147.96, 152.52, 157.26, 160.29. MS (ESI): *m/z* 426 [M + 1]$^+$. Anal ($C_{23}H_{28}ClN_5O$ .1.5 $C_4H_6O_4$): C, H, N.

Synthesis of N'-(7-chloro-2-methoxybenzo[b][1,5]naphthyridin-10-yl)-N-[3-(diethyl-amino)propyl]-isobutyrimidamide **(12d).** The title compound was prepared by the same procedure for the preparation of **12a**, using compound **11b** and 3-chloro-*N,N*-diethylpropan-1-amine as reagents to give 70 % yields of the desired product **12d** as yellow solid, mp 75-78 °C, R_f = 0.33 ($CHCl_3$: MeOH: NH_4OH, 10: 1: 0.1, v/v). ^1H NMR (600 MHz, DMSO-d_6): δ 0.85-0.95 (m, 9H), 1.10 (d, *J* = 6.0 Hz, 3H), 1.75-1.85 (m, 2H), 2.22 (m, *J* = 6.0 Hz, 1H), 2.40-2.50 (m, 6H), 3.25-3.32 (m, 1H), 3.45-3.50 (m, 1H), 3.97 (s, 3H), 7.27 (d, *J* = 9.0 Hz, 1H), 7.37 (br.s, 1H), 7.43 (dd, *J* = 9.0 and 1.8 Hz, 1H), 7.98 (d, *J* = 1.8 Hz, 1H), 8.09 (d, *J* = 9.0 Hz, 1H), 8.21 (d, *J* = 9.0 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 12.03, 19.26, 21.51, 26.42, 32.76, 46.73, 50.81, 53.47, 119.08, 121.32, 124.91, 127.46, 127.52, 129.87, 134.41, 140.94, 144.78, 147.86, 153.09, 160.22, 164.46. MS (ESI): *m/z* 442 [M + 1]$^+$. Anal ($C_{24}H_{32}ClN_5O$): C, H, N.

Synthesis of N'-(7-chloro-2-methoxybenzo[b][1,5]naphthyridin-10-yl)-N-[3-(dimethyl-amino)-propyl]-isobutyrimidamide **(12e).** The title compound was prepared from **11b** and 3-chloro-N, N-dimethylpropan-1-amine, using the same procedure for the preparation of **12a**, to afford **12e** in 61 % yields as amber syrup, R_f = 0.51 ($CHCl_3$: MeOH: NH_4OH, 10: 1: 0.1, v/v). ^1H NMR (600 MHz, DMSO-d_6): δ 0.89 (d, *J* = 6.0 Hz, 3H), 1.08 (d, *J* = 6.0 Hz, 3H), 1.77-1.87 (m, 2H), 2.13 (s, 6H), 2.15-2.25 (m, 1H), 2.25-2.35 (m, 2H), 3.25-3.30 (m, 1H), 3.45-3.55 (m, 1H), 3.97 (s, 3H), 7.27 (d, *J* = 9.0 Hz, 1H), 7.39 (br.s, 1H), 7.44 (dd, *J* = 9.0 and 1.8 Hz, 1H), 7.98 (d, *J* = 1.8 Hz, 1H), 8.09 (d, *J* = 9.0 Hz, 1H), 8.21 (d, *J* = 9.0 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 19.17, 21.51, 26.81, 32.89, 39.79, 45.60, 53.48, 57.50, 119.09, 121.33, 124.97, 127.45, 127.52, 129.91, 134.43, 140.93, 144.78, 147.86, 153.11, 160.24, 164.51. MS (ESI): *m/z* 414 [M + 1]$^+$. Anal ($C_{22}H_{28}ClN_5O$): C, H, N.

***Synthesis of N'-(7-chloro-2-methoxybenzo[b][1,5]naphthyridin-10-yl)-N-[3-(piperidin-1-yl)-propyl]-isobutyrimidamide* (12f).** The title compound was prepared by the same general procedure using compound **11b** to couple with 1-(3-chloropropyl)-piperidine to provide **12f** in 61% yields as yellow solid, mp 158-160 °C, R_f = 0.68 ($CHCl_3$: MeOH: NH_4OH, 10: 1: 0.1, v/v). ^{1}H NMR (600 MHz, DMSO-d_6): δ 0.91 (d, *J* = 6.6 Hz, 3H), 1.10 (d, *J* = 6.6 Hz, 3H), 1.32-1.40 (m, 2H), 1.42-1.50 (m, 4H), 1.75-1.85 (m, 2H), 2.15-2.45 (m, 7H), 3.20-3.27 (m, 1H), 3.45-3.52 (m, 1H), 3.97 (s, 3H), 7.27 (d, *J* = 9.6 Hz, 1H), 7.36 (br.s, 1H), 7.44 (dd, *J* = 9.0 and 1.8 Hz, 1H), 7.98 (d, *J* = 1.8 Hz, 1H), 8.09 (d, *J* = 9.0 Hz, 1H), 8.21 (d, *J* = 9.6 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 19.30, 21.51, 24.53, 25.99, 26.09, 32.76, 53.50, 54.58, 57.11, 119.10, 121.29, 124.95, 127.45, 127.52, 129.89, 134.43, 140.93, 144.76, 147.85, 153.07, 160.23, 164.52. MS (ESI): *m/z* 454 $[M + 1]^+$. Anal ($C_{25}H_{32}ClN_5O$): C, H, N.

***Synthesis of N'-(7-chloro-2-methoxybenzo[b][1,5]naphthyridin-10-yl)-N,N-bis[3-(diethylamino)-propyl]-isobutyrimidamide* (12g).** Title compound was isolated as a side product from the synthesis of **12d** in 8 % yield as yellow oil, R_f = 0.17 ($CHCl_3$: MeOH, 1: 0.1 v/v), ^{1}H NMR (600 MHz, DMSO-d_6): 0.84 (br.s, 12H), 0.98 (d, *J* = 7.2 Hz, 3H), 1.04 (d, *J* = 7.2 Hz, 3H), 1.65-1.75 (m, 2H), 1.75-1.85 (m, 2H), 2.20-2.40 (m, 12H), 2.92 (septet, *J* = 7.2 Hz, 1H), 3.25-3.35 (m, 2H), 4.45-3.55 (m, 2H), 3.96 (s, 3H), 7.25 (d, *J* = 9.0 Hz, 1H), 7.39 (dd, *J* = 9.0, and 2.4 Hz, 1H), 7.92 (d, *J* = 1.8 Hz, 1H), 8.02 (d, *J* = 9.0 Hz, 1H), 8.17 (d, *J* = 9.0 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): 12.08, 20.65, 26.32, 32.44, 46.62, 47.71, 50.05, 53.50, 118.90, 119.69, 124.33, 127.28, 128.86, 134.34, 140.85, 144.40, 147.98, 152.06, 159.79, 162.50. MS (ESI): *m/z* 555 $[M + 1]^+$.

***Synthesis of N'-(7-chloro-2-methoxybenzo[b][1,5]naphthyridin-10-yl)-N-[3-(dimethyl-amino)-propyl]-benzimidamide* (12h).** The title compound was prepared by the same general procedure using compound **11c** and 3-chloro-N,N-dimethylpropan-1-amine as reagents to give **12h** in 51% yields as yellow oil, R_f = 0.52 ($CHCl_3$: MeOH: NH_4OH, 10: 1: 0.1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.93 (br.s, 2H), 2.26 (br.s, 6H), 2.55 (br.s, 2H), 3.70-3.80 (m, 2H), 3.99 (d, *J* = 9.6 Hz, 1H), 7.05-7.10 (m, 2H), 7.15-7.20 (m, 2H), 7.42 (dd, *J* = 9.0 and 1.8 Hz, 1H), 8.05-8.10 (m, 2H), 8.41 (d, *J* = 9.0 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 25.65, 42.84, 45.41,

53.25, 58.95, 118.34, 121.54, 125.22, 126.91, 127.02, 127.50, 128.23, 129.33, 129.87, 135.18, 135.71, 139.89, 144.66, 147.77, 152.79, 159.31, 159.89. MS (ESI): *m/z* 448 $[M + 1]^+$. Anal ($C_{25}H_{26}ClN_5O.$ 1/3 H_2O): C, H, N.

Synthesis of N'-(7-chloro-2-methoxybenzo[b][1,5]naphthyridin-10-yl)-N-[3-(diethyl-amino)-propyl]-benzimidamide **(12i).** The title compound was prepared by the same general procedure using compound **11c** and 3-chloro-*N,N*-diethylpropan-1-amine as reagents to give 49 % yield of **12i** as yellow oil, Rf = 0.78 (CHCl3: MeOH: NH4OH, 10: 1: 0.1 v/v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.96 (t, *J* = 6.6 Hz, 6H), 1.91 (br.s, 2H), 2.54 (q, *J* = 6.6 Hz, 4H), 2.67 (br.s, 2H), 3.74 (br.s, 2H), 3.99 (d, *J* = 9.6 Hz, 1H), 7.03 (t, *J* = 7.2 Hz, 2H), 7.13 (t, *J* = 9.0 Hz, 1H), 7.26 (d, *J* = 7.2 Hz, 2H), 7.40 (dd, *J* = 9.0 and 1.8 Hz, 1H), 7.71 (br.s, 1H), 8.04-8.08 (m, 2H), 9.42 (d, *J* = 9.0 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.36, 25.04, 43.36, 46.65, 53.00, 53.24, 118.31, 121.58, 125.13, 126.96, 127.03, 127.42, 128.11, 128.39, 129.80, 135.15, 135.77, 139.81, 144.63, 147.75, 153.00, 159.42, 159.86. MS (ESI): *m/z* 476 $[M + 1]^+$. Anal ($C_{27}H_{30}ClN_5O.$ 1/3 H_2O): C, H, N.

Synthesis of N'-(7-chloro-2-methoxybenzo[b][1,5]naphthyridin-10-yl)-N-[3-(piperidin-1-yl)-propyl]-benzimidamide **(12j).** Compound **12j** was prepared by the same general procedure using compound **11c** and 1-(3-chloropropyl)-piperidine as reagents to give the title compound in 23 % yields as yellow oil, R_f = 0.62 ($CHCl_3$: MeOH: NH_4OH, 10: 1: 0.1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.35-1.55 (m, 6H), 1.90-.2.00 (m, 2H), 2.30-2.55 (m, 4H), 2.55-2.65 (m, 2H), 3.70-3.80 (m, 2H), 4.01 (s, 3H), 6.98 (d, *J* = 9.0 Hz, 1H), 7.04 (t, *J* = 7.2 Hz, 2H), 7.15 (t, *J* = 7.2 Hz, 1H), 7.25-7.30 (m, 2H), 7.41 (dd, *J* = 9.0 and 1.8 Hz, 1H), 7.79 (br.s, 1H), 8.04-8.08 (m, 2H), 8.43 (d, *J* = 9.0 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 24.22, 24.26, 25.80, 43.52, 53.27, 54.74, 58.99, 118.30, 121.62, 125.14, 126.96, 127.18, 127.48, 128.08, 129.43, 129.83, 135.13, 135.83, 139.87, 144.67, 147.78, 153.00, 159.57, 159.86. MS (ESI): *m/z* 488 $[M + 1]^+$. Anal ($C_{28}H_{30}ClN_5O.$. H_2O): C, H, N.

Synthesis of N'-(7-chloro-2-methoxybenzo[b][1,5]naphthyridin-10-yl)-N-[3-(diethyl-amino)-propyl]-nicotinimidamide **(12K).** The title compound was prepared by the same general procedure using compound **11e** and 3-chloro-*N,N*-diethylpropan-1-amine as reagents to give

12K in 54 % yields as brown sticky solid, mp 45-47 °C, R_f = 0.37 ($CHCl_3$: MeOH: NH_4OH, 10: 1: 0.1 v/v), ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.90-1.00 (m, 6H), 1.91 (br.s, 2H), 2.50-2.60 (m, 4H), 2.70 (br.s, 2H), 3.76 (br.s, 2H), 4.01 (s, 3H, CH_3O), 6.87-6.93 (m, 1H), 6.99 (d, *J* = 9.0 Hz, 1H), 7.42 (dd, *J* = 9.0 and 1.8 Hz, 2H), 8.03-8.08 (m, 2H), 8.22 (br.s, 1H), 8.35-8.42 (m, 2H), 8.68 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.44, 24.56, 43.88, 46.63, 53.25, 53.37, 118.58, 121.57, 122.77, 125.57, 126.63, 127.66, 129.04, 131.72, 134.45, 135.26, 140.08, 144.80, 147.74, 147.97, 150.67, 151.90, 156.69, 160.21. MS (ESI): *m/z* 477 $[M + 1]^+$. Anal ($C_{26}H_{29}ClN_6O_{.}$ 0.5 H_2O): C, H, N.

Synthesis of 4-chloro-N'-(7-chloro-2-methoxybenzo[b][1,5]naphthyridin-10-yl)-N-[3-(dimethylamino)-propyl]benzimidamide **(12l).** The title compound was prepared by the same general procedure using compound **11d** and 3-chloro-N,N-dimethylpropan-1-amine as reagents to give 28 % yield of **12l** as yellow oil, R_f = 040 ($CHCl_3$: MeOH: NH_4OH, 10: 1: 0.1 v/v). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 1.90 (br.s, 2H), 2.24 (br.s, 6H), 2.53 (br.s, 2H), 3.70 (br.s, 2H), 3.99 (s, 3H), 7.02 (d, *J* = 9.0 Hz, 1H), 7.06 (d, *J* = 7.8 Hz, 2H), 7.23 (d, *J* = 7.8 Hz, 2H), 7.39 (br.s, 1H, NH), 7.42 (dd, *J* = 9.0 and 1.8 Hz, 1H), 8.07 (d, *J* = 1.8 Hz, 1H), 8.09 (d, *J* = 9.0 Hz, 1H), 8.38 (d, *J* = 9.0 Hz, 1H). **^{13}C NMR** (150 MHz, $CDCl_3$): δ 25.31, 43.19, 45.41, 53.23, 59.14, 118.50, 121.46, 125.41, 126.75, 127.62, 128.37, 128.54, 129.1, 134.21, 135.2, 135.80, 140.03, 144.74, 147.78, 152.34, 158.21, 160.03. MS (ESI): *m/z* 482 $[M + 1]^+$. Anal ($C_{25}H_{25}Cl_2N_5O_{.}$ 1/3 H_2O): C, H, N.

Synthesis of N'-(7-chloro-2-methoxybenzo[b][1,5]naphthyridin-10-yl)-N-(3-(diethyl-amino)-2,2-dimethylpropyl)benzimidamide **(12m).** The title compound was prepared by the same method from **11c** (1.45 g, 4 mmol) and 3-chloro-N,N-diethyl-2,2-dimethylpropan-1-amine (782 mg, 4.4 mmol) as yellow solid in 63% yield (1.3 g). Recrystallization from CH_3CN gave dark yellow crystals, mp = 168-169 °C, R_f = 0.85 ($CHCl_3$: MeOH: NH_4OH, 10: 1: 0.1 v/v. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 0.90 (br.s, 6H), 1.05 (br.s, 3H), 1.16 (br.s, 3H), 2.35-2.65 (m, 6H), 3.60 (br.s, 2H), 4.00 (s, 3H, CH_3O), 6.95 (d, *J* = 9.3 Hz, 1H), 6.95-7.06 (m, 2H), 7.07-7.17 (m, 1H), 7.20-7.30 (m, 2H, overlap $CHCl_3$), 7.39 (dd, *J* = 9.3 and 1.8 Hz, 1H), 8.00-8.07 (m, 2H), 8.41 (d, *J* = 9.0 Hz, 1H), 8.53 (hump, 1H). **^{13}C NMR** (75 MHz, $CDCl_3$): δ 11.48, 25.09, 25.91, 34.39, 48.70, 53.20, 54.48, 66.60, 118.18, 121.80, 125.05, 126.95, 127.04, 127.60, 128.05,

129.57, 129.65, 135.03, 136.11, 139.98, 144.83, 147.92, 153.21, 159.53, 159.81. MS [ESI]: *m/z* 504 $[M + 1]^+$. Anal ($C_{29}H_{34}ClN_5O$): C, H, N.

***Synthesis of N-(7-chloro-2-methoxybenzo[b][1,5]naphthyridin-10-yl)-N,N'-bis[3-(dimethylamino)propyl]acetimidamide* (13a).** Title compound was isolated as side product in 20% yield from the synthesis of **12a** as yellow oil, R_f = 0.10 ($CHCl_3$: MeOH: NH_4OH, 10: 1: 0.1 v/v). ^{1}H NMR (600 MHz, CD_3OD): δ 1.85-2.15 (m, 4H), 1.93 (s, 3H), 2.22 (s, 6H), 2.30 (s, 6H), 2.45 (br.s, 4H), 3.40-3.70 (m, 3H), 3.90-4.00 (m, 1H), 4.03 (s, 3H), 7.24 (d, *J* = 9.0 Hz, 1H), 7.39 (dd, *J* = 9.0 and 1.8 Hz, 1H), 7.95 (d, *J* = 1.8 Hz, 1H), 8.16 (d, *J* = 9.0 Hz, 1H), 8.27 (d, *J* = 9.0 Hz, 1H). ^{13}C NMR (150 MHz, CD_3OD): δ 17.12, 24.89, 26.71, 44.09, 46.23, 47.28, 52.79, 56.24, 56.73, 118.77, 121.38, 124.33, 125.51, 127.24, 129.18, 135.51, 138.72, 144.08, 147.25, 152.97, 159.42, 160.41. MS (ESI): *m/z* 471 $[M + 1]^+$.

***Synthesis of N-(7-chloro-2-methoxybenzo[b][1,5]naphthyridin-10-yl)-N,N'-bis[3-(diethylamino)propyl]-acetimidamide* (13b).** Title compound was isolated as a side product from the synthesis of **12b** in 11% yield as yellow oil, R_f = 0.33 ($CHCl_3$: MeOH: NH_4OH, 10: 1: 0.1 v/v), ^{1}H NMR (600 MHz, DMSO-d_6): δ 0.80-0.90 (m, 6H), 0.90-1.00 (m, 6H), 1.70-1.83 (m, 2H), 1.84 (s, 3H, CH_3), 1.87-1.95 (m, 2H), 2.35-2.48 (m, 12H), 3.45-3.55 (m, 3H), 3.77-3.87 (m, 1H), 3.94 (s, 3H), 7.26 (d, *J* = 9.0 Hz, 1H), 7.40 (dd, *J* = 9.0 and 2.4 Hz, 1H), 7.96 (d, *J* = 2.4 Hz, 1H), 8.18 (d, *J* = 9.0 Hz, 1H), 8.20 (d, *J* = 9.0 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 11.97, 12.16, 18.23, 25.07, 27.05, 46.52, 46.70, 47.66, 49.90, 50.36, 53.39, 118.88, 121.65, 124.55, 127.28, 127.88, 129.28, 134.38, 140.97, 144.95, 147.94, 152.23, 159.37, 160.15. MS (ESI): *m/z* 527 $[M + 1]^+$. Anal ($C_{29}H_{43}ClN_6O$. 1/3 H_2O): C, H, N.

BIOLOGICAL STUDIES

All new compounds were assessed for metabolic stability in human and mouse liver microsomal preparations, and cell growth inhibitory activity in 3 clones of *Plasmodium falciparum* (D-6, W-2, and TM91C235).[64-67] Compounds with $t_{1/2}$ > 60 min in metabolic stability test and IC_{50} < 50 ng/ml were selected for Thompson test to evaluate their potency as blood schizonticides. Clog-P values were calculated using software from ChemDraw Pro version 12.0. A chloroquine resistant strain *P. berghei* was used in the screening and CQ was used as a

positive control. Selected 4-AMQ and 10-AMB analogs were submitted for hERG K^+ channel inhibition test to evaluate the proarrhythmic potential which leads to Q-T prolongation cardiac toxicity. Four compounds (**6t**, **6w**, **12l** and **13b**) were submitted to WuXi App Tech, Inc., St. Paul, MN for Ames mutagenicity study and were found to be negative.

(a). Metabolic Stability Studies: All samples were tested in human and mouse liver microsomal preparations. Sample stocks at 10 or 20 μM (depending on solubility) in DMSO were diluted to a final concentration of 1 μM with a mixture containing 0.5 mg/mL of pre-warmed pooled human or mouse liver microsomes (BD Gentest), 1.3 μM NADP (Sigma), 3.3 μM $MgCl_2$ (Sigma), and 0.1 M pH 7.4 PBS using a TECAN Genesis robotic liquid handler. The reaction was started with the addition of 1U/mL glucose-6-phosphate dehydrogenase (G6PD). The mixture was incubated on a shaking platform at 37 °C, and aliquots were taken and quenched with the addition of an equal volume of cold acetonitrile at 0, 10, 20, 30, and 60 min. Samples were centrifuged at 3700 rpm for 10 min at 20 °C to remove debris. Sample quantification was carried out by LC/MS, and metabolic half-life was calculated by log plots of the total ion chromatograph area remaining. The results are shown in Table 3 and Table 4**.**

(b). In Vitro Antimalarial Studies: The in vitro assays were conducted using a modification of the semiautomated microdilution technique of Desjardins and Milhous.[64-65] Three *P. falciparum* malaria parasite clones, Indochina (W-2), South East Asia (TM91C235), and Sierra Leone (D-6), were utilized in susceptibility testing. They were derived by direct visualization and micromanipulation [66] from patient isolates obtained by the Centers for Disease Control, Atlanta, GA. The patients had acquired infections either in Vietnam, in Sierra Leone or in South East Asia. The W2 clone is resistant to the antimalarials chloroquine, sulfadoxine, pyrimethamine, and quinine, but sensitive to mefloquine, whereas both D6 and TM91C235 clones are resistant to mefloquine but susceptible to chloroquine, quinine, sulfadoxine, and pyrimethamine.[67] Test compounds were initially dissolved in DMSO and diluted in RPMI 1640 culture medium with 10% human plasma to 400-fold. Drugs were subsequently further diluted using the Cetus Pro/Pette (Perkin-Elmer Corp., Norwalk, CT) over a range of (1.56-100) x 10^{-9}M. The final DMSO concentration in the culture medium is 0.25% - 0.5%, at which concentration DMSO showed no significant parasite growth inhibitory activity. Parasite inoculum (at 0.5% parasitemia and a 1% hematocrit) were incubated for 24 h and added to equimolar concentrations

of each test compound prior to the addition of [^{3}H]-hypoxanthine. After a further incubation of 18 h, particulate matter was harvested from each microtiter well by using an automated cell harvester (Skatron, Inc., Sterling, VA). Uptake of [^{3}H]-hypoxanthine was measured using a scintillation spectrophotometer (Model LS3801, Beckman Instruments, Irvine, CA). Concentration-response data were analyzed by nonlinear regression and the IC_{50} values (50% inhibitory concentrations) for each compound were calculated. The results are listed in Table 3 and Table4.

(c). In vitro Toxicity Assessment in HepG2 cells: The 384 well MTT cytotoxicity assay is a modification of the MTT method described by Ferrari et al (1990) [68] optimized for 384 well throughputs. The HepG2 target cells for this assay were cultured as follows: HepG2 (human hepatocellular carcinoma) cells were cultured in complete Minimal Essential Medium (Gibco-Invitrogen, #11090-099) prepared by supplementing MEM with 0.19% sodium bicarbonate (Gibco-BRL Cat #25080-094), 10% heat inactivated FBS (Gibco-Invitrogen #16000-036), 2 mM L-glutamine (Gibco-Invitrogen #25030-081), 0.1 mM MEM non-essential amino acids (Gibco-Invitrogen #11140-050), 0.009 mg/ml insulin (Sigma #I1882), 1.76 mg/ml bovine serum albumin (Sigma #A1470), 20 units/ml penicillin–streptomycin (Gibco-Invitrogen #15140-148), and 0.05 mg/ml gentamycin (Gibco-Invitrogen #15710-064). HepG2 cells cultured in complete MEM were first washed with 1X Hank's Balanced Salt Solution (Invitrogen #14175-095), trypsonized using a 0.25% trypsin/EDTA solution (Invitrogen #25200-106), assessed for viability using trypan blue, and resuspended at 250,000 cells/ml. Using a Tecan EVO Freedom robot, 38.3 µL of cell suspension were added to each well of clear, cell culture treated 384-well microtiter plates (Nunc Cat#164688) for a final concentration of 9570 liver cells per well, and plated cells were incubated overnight in 5% CO_2 at 37 ^oC. Drug plates were prepared with the Tecan EVO Freedom using sterile 96 well plates containing twelve duplicate 1.6-fold serial dilutions of each test compound suspended in DMSO. 4.25 µL of diluted test compound was then added to the 38.3 µL of media in each well providing a 10 fold final dilution of compound. Compounds were tested from a range of 57 - 10,000 ng/ml for all assays. Tryptanthrin[69] was used as control for all assays with average IC_{50} of 305.4 ng/mL. After a 48 hour incubation period, 8 µL of a 1.5 mg/ml solution of MTT diluted in complete MEM media was added to each well. All plates were subsequently incubated in the dark for 1 hour at room temperature. After incubation, the media and drugs in each well was removed by shaking plate over sink, the plates are then left to dry in hood for 15 minutes. Next, 30µL of isopropanol acidified by addition of

HCl at a final concentration of 0.36% was added to dissolve the formazan dye crystals created by reduction of MTT. Plates are put on a 3-D rotator for 15-30 minutes. Absorbance was determined in all wells using a Tecan iControl 1.6 Infinite plate reader. The 50% inhibitory concentrations (IC_{50}s) were then generated for each toxicity dose response test using GraphPad Prism (GraphPad Software Inc., SanDiego, CA) using the nonlinear regression (sigmoidal dose-response/variable slope) equation. The results are listed on Table 3 and Table 4.

(d). In Vivo Antimalarial Studies in Mice: The *in vivo* efficacies of the new compounds were determined by a modified method described by Peters.[70] The test measured the survivability of mice and parasite clearance following administration of the drug on days 3, 4, and 5 post-infection, instead of 4-day treatment in Peters' procedure. In brief, 5×10^6 of CQ resistant *P. berghei*-infected erythrocytes (ANKA strain) were inoculated with ½" 27 gauge of needle into the intra-peritoneal cavity of male mice that weigh 16 to 20 g. Each drug was administered once daily po from days 3, 4, and 5 post-infection. Five mice were used per each dose group. Chloroquine was used as positive control. Blood films were taken starting from day 3 and three times per week for 30 days. The volume of test drug suspension each mouse given depends on the weight of the mouse and the drug concentration of the suspension. In general, the tested negatives for parasitemia on day 30 post infection or surviving for 30 days were considered cured. The infected untreated control mice (negative controls) died on either day 6 or 7. Compounds are considered active when the survival time of the treated mice was greater than twice the control mice, i.e., 12-14 days. Mice losing >20% of their body weight were sacrificed. The results are shown in Table 5.

(e). Pharmacokinetic (PK) studies

Male 7-week-old ICR mice weighed from 23 to 28 grams (Charles River Labs. Inc. Raleigh, NC) were used for the PK evaluations. On arrival, the animals were acclimated for seven days in quarantine. The animals were housed in a cage maintained in a room with a temperature range of 64-79 °F, 34-68 % relative humidity and a 12-hr light/dark cycles. Food and water were provided *ad libitum* during quarantine and throughout the study. The animals were fed a standard rodent maintenance diet. All animal studies were performed under IACUC approved protocols. These protocols detail the experimental procedures and designs as well as

number of animals were used. All animal use, care, and handling were performed in accordance with the current "Guide for the Care and Use of Laboratory Animals" (8th Edition, 2011).

PK studies were performed using intragastric (IG) administration. For each time point to be acquired, three male ICR mice per time-point were dosed at single po dosing of 80 mg/kg. The drug vehicle was DD water, administered at 100 µL/20 g. At each time point, blood and plasma samples were collected. Whole blood was collected by cardiac puncture. Blood samples were collected in lithium heparin tubes. Following the separation of appropriate aliquots, plasma was obtained from the whole blood via centrifugation. All liquid and tissue samples were immediately preserved on dry ice and later stored at -80°C until analytical work was performed.

(f). PK parameter determination

Drug concentrations were generated for each sample taken from animals dosed with test compounds. A measured plasma drug concentration *vs*. time curve was produced, in graphic and tabular form, for each subject on both linear/linear and log/linear scales, for the parent compound. Mean plasma drug concentration vs. time curves were also prepared separately. Maximum plasma concentration (C_{max}), and time to maximum concentration (t_{max}) of **6q** and **13b** were obtained from the plasma drug concentration-time curves. The elimination half-life ($t_{1/2}$) was calculated from In2/k_{el}, which is the elimination rate constant calculated from the log concentration-time plot. The area under the curve (AUC) was determined by the linear trapezoidal rule with extrapolation to infinity based on the concentration of the last time point divided by the terminal rate constant. Mean residence time (MRT) was determined by dividing the area under the first moment curve (AUMC) by AUC. The volume of the central compartment (Vz) and volume of the tissue compartment (Vz/F) were calculated as the product of CL and MRT. The results are shown in Table 6.

(g). hERG IonWorks Electrophysiology Methods: The measurement of hERG channel inhibitory activity of the test compounds was performed by Essen BioScience Inc., Ann Arbor, MI. Basic principles and operation of the IonWorks platform have been described by Schroeder, et al.[71] The results are listed in Tables 3 and 4. Detailed protocol is available as Appendix 2.

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SUPPORTING INFORMATION:

The following supporting information is available free of charge via the internet at http://pub.acs.org: **Appendix 1**: Elemental Analysis of Compounds Prepared; **Appendix 2:** hERG IonWorks Electrophysiology Methods.

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ABBREVIATIONS USED

Abbreviations: CQ, chloroquine; MQ, mefloquine; 4-AMQ, 4-amidinoquinoline; 10AMB, 10-amidinobenzonaphthyridine; Lawesson reagent, 2,4-bis-(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide.

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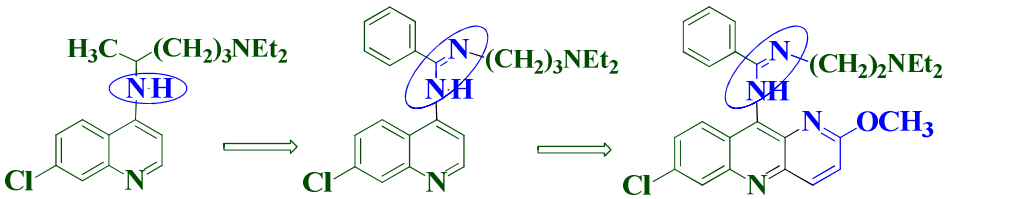
Table of Contents Graphic

Antimalarial Activity of 4-Amidinoquinoline and 10-Amidinobenzonaphthyridine Derivatives, Vasiliy Korotchenko, Ramadas Sathunuru, Gerena Lucia, Diana Caridha, Qigui Li, Mara KreishmanDeitrick, Philip L. Smith and Ai J. Lin*, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, Maryland 20910.



Clog-P	**3.73**	**4.77**	**5.75**
D6 IC_{50} (nM)	**15**	**1.8**	**1.7**
W2 IC_{50} (nM)	**525**	**7.2**	**5.6**
4 or 10-Amino pKa	**-5.6**	**8.53**	**7.37**