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Short communication

Microwave-promoted facile access to 4-aminoquinoline-phthalimides: Synthesis and anti-plasmodial evaluation

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ABSTRACT

Microwave promoted high yielding synthesis of 4-aminoquinoline-phthalimides was developed with an aim to evaluate their anti-plasmodial potential. The scaffolds with longer spacer length (n = 6, 8) between two pharmacophores and a halogen substituent on the phthalimide ring displayed good anti-plasmodial activity. Compound **5w**, with an optimum combination of hexyl chain as spacer along with a tetra-bromophthalimide ring proved to be most potent and non-cytotoxic among the series exhibiting an IC_{50} value of 0.10 μM.

1. Introduction

Malaria caused by *Plasmodium falciparum*, is a rapidly spreading parasitic disease responsible for 214 million cases worldwide in 2015 along with 438,000 deaths mostly in sub-Saharan African region [1]. Chloroquine was the most accessible, effective and safe drug for the treatment of falciparum malaria for at least five decades and is still considered as a drug of choice against uncomplicated malaria caused by *P. vivax* [2]. The emergence of drug resistant parasites and the lack of an effective malaria vaccine are the major obstacles in the control and eventual elimination of the disease. The development of resistance to the quinoline antimalarials has propelled the development of artemisinin (ART) and its semisynthetic derivatives as fast-acting antimalarials [3]. However, recent reports on the emergence of resistance to artemisin combination therapy (ACT) in South-East Asia has provided strong impetus for the development of new antimalarial scaffolds with low incidence of resistance [4].

4-aminoquinoline hybridization, involving re-engineering and repositioning of the quinoline core with known drug families, has emerged as an important strategy for the development of new pharmacological templates with a potential to enhance antimalarial efficacy and be cost-effective [5–8]. Several potential antimalarials *viz.* trioxoferroquines [5], trioxaquines [9], artemisinin-quinine [10], tetraoxanes [11] and clotrimazole-4-aminoquinoline [12] have been developed using this strategy.

Derivatives of cyclic imide having the general structure –(CO–N(R)-CO)- are an important class of bioactive molecules showing numerous biological properties including antimicrobial, antimalarial, antihypertensive, antiviral and herbicidal activities [13]. Rathi et al. recently reported a series of phthalimides functionalized with cyclic amines and evaluated for their *in vitro* antimalarial activities against the 3D7 strain of *P. falciparum* as well as for falcipain-2 (FP2) inhibitory activity. The combination of the most potent compound with ART resulted in enhanced killing of *P. falciparum.* Phthalimides and their derivatives, thus emerged as potential candidates, which can be used in combination with ART or with other antimalarials to reduce the problem of drug resistance [14].

Recent disclosures from our lab have shown the antiplasmodial potential of amalgamating a 4-aminoquinoline with a β-lactam (cyclic amide) core tethered *via* diverse linkers. The synthesized conjugates have shown comparable antiplasmodial activity to that of chloroquine with IC_{50} values of the most potent conjugates being 35 nM and 42 nM [15]. In continuation with our interest in the synthesis and bio-evaluation of new molecular scaffolds with

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biological relevance [16], the present report describes the synthesis, antiplasmodial and cytotoxic evaluation of 4-aminoquinoline-phthalimides. The length of the linker as well as the nature of substituents at the C3/C4 position of phthalimide was varied so as to study the structure-activity relationship (SAR) of synthesized scaffolds.

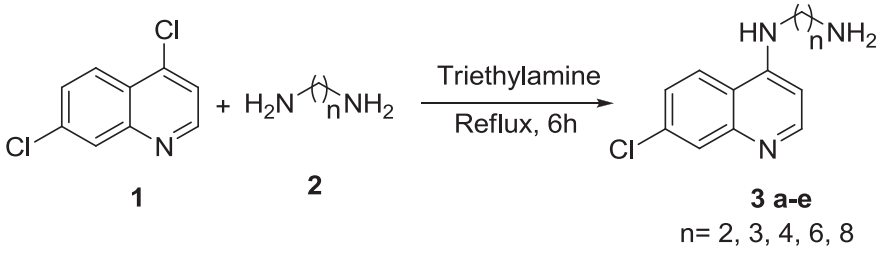
2. Results and discussion

The synthetic methodology involved an initial treatment of precursor **3a**, prepared by reacting 4,7-dichloroquinoline **1** with ethylene diamine [17] (Scheme 1), with phthalic anhydride **4a**. The reaction was carried out in a range of solvents at varied temperature and resulted in poor to moderate yields of the corresponding 4-aminoquinoline-phthalimide **5a**. The best results in terms of yields were obtained by heating in acetic acid at 110 °C for 6h. In order to circumvent the poor yields observed in the conventional heating, it was considered worthwhile to attempt the microwave-promoted synthesis of 4-aminoquinoline-phthalimides. Microwave heating has emerged as a powerful tool for the synthesis of various heterocycles and a number of publications have appeared lately describing its potential in phthalimide synthesis [18]. The reaction between phthalic anhydride **4a** and 4-aminoquinoline-diamine **3a** was chosen as a model reaction for optimizing reaction conditions. A range of solvents under different temperatures and time were screened so as to optimize the reaction conditions. The choice of solvent proved to be crucial on the reaction outcome and the best result in terms of yield was obtained using 0.5 mL of DMSO at 160 °C for 2 min (see Table 1) (Scheme 2). Having determined suitable reaction conditions, the developed strategy was next explored with a variety of phthalimide substrates and 4-aminoquinolines affording facile synthesis of a library of 4-aminoquinoline-phthalimides **5a-y** in excellent yields. The structure of the synthesized scaffolds was assigned on the basis of spectral data and analytical evidences. The compound **5a**, for example, analyzed as $C_{19}H_{14}ClN_3O_2$, showed a molecular ion peak at *m/z* 351.0781 $[M]^+$ in its high resolution mass spectrum. The salient features of its ^1H NMR spectrum included the appearance of a multiplet at δ 3.52–3.57 and a triplet at 3.78 (J = 6.2 Hz) corresponding to methylene protons and a multiplet at δ 7.77–7.82, because of aromatic protons of the phthalimide ring. The presence of absorptions at δ 168.5 corresponding to phthalimide carbonyls along with the appearance of methylene carbons at δ 36.3 and 40.6 as confirmed by ^{13}C NMR (DEPT) spectra further substantiated the assigned structure (see Scheme 3).

The synthesized 4-aminoquinoline-phthalimides were evaluated for their antiplasmodial activity against the chloroquine (CQ)-resistant and mefloquine-sensitive W2 strain of *P. falciparum* and the activities are listed in Table 2. As evident, although the scaffolds are not as active as standard drugs *viz*. CQ and ART; most of the compounds displayed good antiplasmodial activity. A careful analysis of structure-activity relationship (SAR) of the synthesized compounds revealed the dependence of activity both on the nature of substituents at the phthalimide ring as well as the alkyl chain length, introduced as spacer. Analysis of antiplasmodial activities among compounds **5a-e** (R = H) revealed an improvement in activity with increase in chain length as evident from compounds **5d** (n = 6, 0.11 μM) and **5e** (n = 8, 0.14 μM). Introducing a fluoro or nitro substituent at C-3, C-4 position resulted in the reduction of antiplasmodial efficacy at shorter alkyl chain lengths (ethyl and propyl) while the activity improved considerably at longer alkyl chain lengths as evident by **5i**, **5j**, **5n**, **5o** and **5y**. Introducing a tetrabromo and tetrachloro-phthalimide substantially improved the activity profiles even at shorter alkyl chain lengths. The compounds **5n**, **5w** and **5y** with an optimum combination of hexyl chain length as linker and halogen substituents on the phthalimide ring proved to be most potent among the series, exhibiting IC_{50} values of 0.12, 0.10 and 0.15 μM, respectively.

Cytotoxicity of the potent scaffolds *viz*. **5d**, **5j**, **5n** and **5w** was determined using J774 murine macrophage cells in order to ascertain whether the observed activities are due to their antiplasmodial efficacy or cytotoxicity (Table 3). As evident, the compounds were non-cytotoxic to J774 murine macrophage cells and their Selectivity index ranges from 126 to 291.

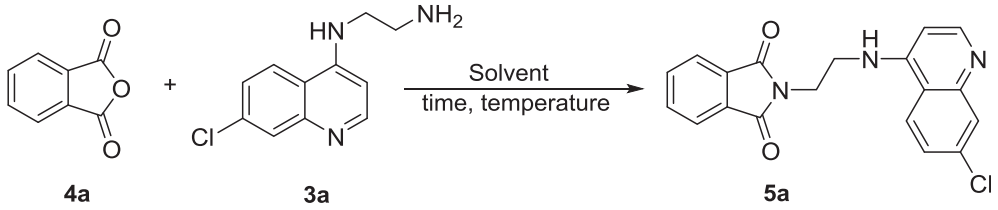
In conclusion, a high yielding microwave-promoted synthesis of 4-aminoquinoline-phthalimides was developed with the aim of studying SAR against *P. falciparum*. Most of the synthesized scaffolds were non-cytotoxic and displayed good antiplasmodial profiles with the activities being dependent upon the nature of substituent on the phthalimide ring as well as the length of alkyl chain, introduced as spacer. Higher values of selectivity index of most potent compounds among the series is suggestive of the fact



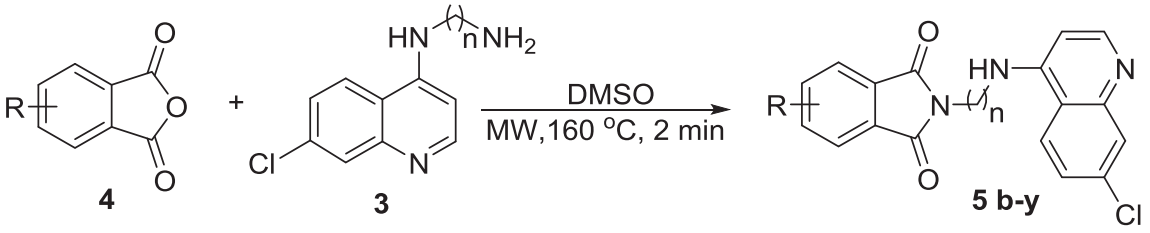
Scheme 1. Synthesis of 4-aminoquinoline based diamines.

Table 1
Synthesis of 4-aminoquinoline-phthalimide **5a**, both under conventional and microwave heating.

Conventional heating				Microwave heating			
Solvent	Time	Temperature	% yield	Solvent	Time	Temperature	% yield
CH_3CN	6h	80	22%	CH_3CN	3min	80	39%
DMF	6h	130	31%	DMF	5min	130	81%
DMSO	8h	160	51%	DMSO	2min	160	89%
C_2H_5OH	6h	80	34%	CH_3COOH	5min	110	76%
CH_3COOH	6h	110	50%				



Scheme 2. Synthesis of 4-aminoquinoline-phthalimide **5a.**



Entry	R	n	Yield
5b	H	3	86%
5c	H	4	74%
5d	H	6	87%
5e	H	8	90%
5f	3-F	2	78%
5g	3-F	3	82%
5h	3-F	4	90%
5i	3-F	6	79%
5j	3-F	8	86%
5k	4-F	2	89%
5l	4-F	3	78%
5m	4-F	4	77%

Entry	R	n	Yield
5n	4-F	6	84%
5o	4-F	8	88%
5p	3,4,5,6-Cl	2	92%
5q	3,4,5,6-Cl	3	91%
5r	3,4,5,6-Cl	4	90%
5s	3,4,5,6-Cl	6	85%
5t	3,4,5,6-Br	2	83%
5u	3,4,5,6-Br	3	81%
5v	3,4,5,6-Br	4	79%
5w	3,4,5,6-Br	6	74%
5x	3-NO_2	2	90%
5y	3-NO_2	6	82%

Scheme 3. Microwave promoted synthesis of 4-aminoquinoline-phthalimides (**5b-5y**).

that these compounds can act as starting points for the synthesis of new pharmacological templates against *P. falciparum*.

3. Experimental section

Melting points were determined by open capillary using a Veego Precision Digital Melting Point apparatus (MP-D) and are uncorrected. ^{1}H NMR spectra were recorded in DMSO-d_6 with Bruker 500 (500 MHz) spectrometers using TMS as internal standard. Microwave reactions were carried out in a Biotage® Initiator + instrument using sealed 2–5 mL process vials. Reaction times refer to irradiation time at the target temperature, not the total irradiation time. The temperature was measured with an IR sensor. Chemical shift values are expressed as parts per million downfield from TMS, and J values are in hertz. Splitting patterns are indicated as s: singlet, d: doublet, t: triplet, m: multiplet, dd: double doublet, ddd: doublet of a doublet of a doublet, and br: broad peak. ^{13}C NMR spectra were recorded on Bruker 500 MHz spectrometers in DMSO-d6 using TMS as internal standard. High resolution mass spectra were recorded on a Bruker-micrOTOF-Q II spectrometer.

*3.1. General procedure for synthesis of 4-aminoquinoline-phthalimide **5a** under conventional heating*

To a stirred solution of phthalic anhydride **4a** (1.0 eq.) in 5 mL of acetic acid was added 4-aminoquinoline diamine **3a** (1.0 eq.). The reaction mixture was refluxed for 6h at 110 °C and the progress was monitored by TLC. The crude product was poured in water (20 mL) and extracted with ethyl acetate (2 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Pure product was obtained by re-crystallisation using absolute ethanol.

*3.2. General procedure for microwave promoted synthesis of 4-aminoquinoline-phthalimides (**5a-5y**)*

To substituted phthalic anhydride (1.0 eq.) in 0.5 mL of DMSO was added 4-aminoquinoline based diamines (1.0 eq.) in a microwave reaction vial. The vessel was sealed with a PTFE cap and heated to 160° C for 2 min in a microwave reactor. After completion of the reaction as evident from TLC, the vessel contents were poured in water (20 mL) and extracted with ethyl acetate (2 × 30 mL). The organic layers were combined, dried over

Table 2
Antiplasmodial activities of tested compounds against the CQ-resistant W2 strain of *P. falciparum*.

Compound	IC_{50} (μM) ± SD	Compound	IC_{50} (μM) ± SD
5a	1.11 ± 0.01	**5o**	0.17 ± 0.007
5b	1.51 ± 0.02	**5p**	0.21 ± 0.05
5c	0.37 ± 0.05	**5q**	0.57 ± 0.04
5d	0.11 ± 0.004	**5r**	0.27 ± 0.000002
5e	0.14 ± 0.01	**5s**	0.46 ± 0.00003
5f	4.73 ± 0.8	**5t**	0.40 ± 0.03
5g	3.96 ± 0.17	**5u**	3.76 ± 0.02
5h	0.64 ± 0.11	**5v**	0.19 ± 0.03
5i	0.24 ± 0.01	**5w**	0.10 ± 0.006
5j	0.12 ± 0.0001	**5x**	7.55 ± 0.30
5k	4.56 ± 0.46	**5y**	0.15 ± 0.02
5l	1.19 ± 0.16	**CQ**	0.077 ± 0.004
5m	0.42 ± 0.01	**ART**	0.007 ± 0.0007
5n	0.12 ± 0.07		

Table 3
Cytotoxicity of selected compounds on J774 murine macrophage cells and their Selectivity index.

Compound	IC_{50} (μM)	Cytotoxicity (μM)	Selectivity Index (SI)
5d	0.11	14.34	130.36
5j	0.12	28.24	235.33
5n	0.12	15.16	126.33
5w	0.10	29.11	291.1

anhydrous Na_2SO_4 and concentrated under reduced pressure to yield the crude product which was re-crystallized using absolute ethanol.

*3.2.1. 2-(2-(7-chloroquinolin-4-ylamino)ethyl)isoindoline-1,3-dione **(5a)***

Light yellow solid, M.P = 159-160 ^{O}C ^{1}H NMR (DMSO-d_6, 400 MHz): 3.52–3.57 (m, 2H, CH_2), 3.78 (t, J = 6.2 hz, 2H, CH_2), 6.60 (d, J = 5.4 hz, 1H, H^2), 7.38 (dd, J = 9.0 hz, 2.2 hz, 1H, H^2), 7.48 (t, J = 4.8 hz, 1H, NH-exchangeable with D_2O), 7.74 (d, J = 2.2 hz, 1H, H^5), 7.77–7.82 (m, 4H, Ar-H), 8.00 (d, J = 9.0 hz, 1H, H^3), 8.37 (d, J = 5.4 hz, 1H, H^1). ^{13}C NMR (DMSO-d_6, 100 MHz): 36.3, 40.6, 99.1, 118.0, 123.5, 124.3, 124.8, 127.9, 132.3, 134.0, 134.8, 149.5, 150.4, 152.3, 168.5. $C_{19}H_{14}ClN_3O_2$ $[M]^+$ 351.0775. Found 351.0781.

*3.2.2. 2-(3-(7-chloroquinolin-4-ylamino)propyl)isoindoline-1,3-dione **(5b)***

Light yellow solid, M.P = 130-131 ^{O}C ^{1}H NMR (DMSO-d_6, 400 MHz): 2.00–2.07 (m, 2H, CH_2), 3.53–3.58 (m, 2H, CH_2), 3.69 (t, J = 6.8 Hz, 2H, CH_2), 6.83 (d, J = 7.2 Hz, 1H, H^2), 7.69 (dd, J = 9.0 Hz, 2.2 Hz, 1H, H^4), 7.75–7.80 (m, 4H, Ar-H), 8.01 (d, J = 1.9 Hz, 1H, H^5), 8.49 (d, J = 9.1 Hz, 1H, H^3), 8.55 (d, J = 5.3 Hz, 1H, H^1), 9.43 (t, J = 5.5 Hz, 1H, NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 100 MHz): 26.9, 35.8, 41.6, 99.2, 115.9, 119.5, 123.4, 126.2, 127.3, 132.1, 134.8, 138.4, 138.9, 143.3, 155.8, 168.5. $C_{20}H_{16}ClN_3O_2$ $[M]^+$ 365.0931. Found 365.0920.

*3.2.3. 2-(4-(7-chloroquinolin-4-ylamino)butyl)isoindoline-1,3-dione **(5c)***

Light yellow solid, M.P = 115-116 ^{O}C ^{1}H NMR (DMSO-d_6, 400 MHz): 1.62–1.71 (m, 4H, $2CH_2$), 3.48–3.52 (m, 2H, CH_2), 3.58 (t, J = 6.2 Hz, CH_2), 6.83 (d, J = 7.2 Hz, 1H, H^2), 7.33 (dd, J = 9.0 Hz, 2.1 Hz, 1H, H^4), 7.77–7.80 (m, 4H, Ar-H), 7.99 (d, J = 2.1 Hz, 1H, H^5), 8.46 (d, J = 7.1 Hz, 1H, H^1), 8.57 (d, J = 9.1 Hz, 1H, H^3), 9.49 (t, J = 5.6 Hz, 1H, NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 100 MHz): 25.4, 25.9, 37.6, 43.1, 99.1, 115.9, 119.5, 123.4, 126.2, 127.2, 132.0, 134.9, 138.4, 138.9, 143.2, 155.8, 168.5. $C_{21}H_{18}ClN_3O_2$ $[M]^+$ 379.1088. Found 379.1091.

*3.2.4. 2-(6-(7-chloroquinolin-4-ylamino)hexyl)isoindoline-1,3-dione **(5d)***

Light yellow solid, M.P = 109-110 ^{O}C ^{1}H NMR (DMSO-d_6, 400 MHz): 1.25–1.40 (m, 4H, $2CH_2$), 1.52–1.64 (m, 4H, $2CH_2$), 3.43–3.48 (m, 2H, CH_2), 3.52 (t, J = 7.0 Hz, 2H, CH_2), 6.80 (d, J = 7.1 Hz, 1H, H^2), 7.70 (dd, J = 9.0 Hz, 1.2 Hz, 1H, H^4), 7.76–7.81 (m, 4H, Ar-H), 8.01 (d, J = 1.7 Hz, 1H, H^5), 8.46 (d, J = 7.1 Hz, 1H, H^1), 8.60 (d, J = 9.1 Hz, 1H, H^3) 9.49 (t, J = 4.7 Hz, 1H, NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 100 MHz): 26.4, 26.5, 27.9, 28.3, 37.8, 43.5, 99.0, 115.9, 119.4, 123.4, 126.3, 127.2, 132.0, 134.9, 138.3, 139.0, 143.0, 155.7, 168.4. $C_{23}H_{22}ClN_3O_2$ $[M]^+$ 407.1401. Found 407.1412.

*3.2.5. 2-(8-(7-chloroquinolin-4-ylamino)octyl)isoindoline-1,3-dione **(5e)***

Yellow Semisolid Liquid, ^{1}H NMR (DMSO-d_6, 500 MHz): 1.23–1.38 (m, 8H, $4CH_2$), 1.55–1.66 (m, 4H, $2CH_2$), 3.27–3.29 (m, 2H, CH_2), 3.56 (t, J = 5.7 Hz, 2H, CH_2), 6.52 (d, J = 5.8 Hz, 1H, H^2), 7.49 (dd, J = 8.9 Hz, 2.2 Hz, 1H, H^4), 7.63 (t, J = 6.0 Hz, 1H, NH-exchangeable with D_2O), 7.79 (d, J = 2.2 Hz, 1H, H^5), 7.79–7.88 (m, 4H, Ar-H), 8.31 (d, J = 9.0 Hz, 1H, H^3), 8.40 (d, J = 5.6 Hz, 1H, H^1). ^{13}C NMR (DMSO-d_6, 125 MHz): 26.7, 26.9, 28.1, 28.3, 29.0, 29.5, 37.8, 43.3, 99.0, 116.9, 123.5, 123.6, 125.8, 132.0, 134.9, 136.1, 144.3, 147.8, 153.2, 168.4. $C_{25}H_{26}ClN_3O_2$ $[M]^+$ 435.1714. Found 435.1721.

*3.2.6. 2-(2-(7-chloroquinolin-4-ylamino)ethyl)-4-fluoroisoindoline-1,3-dione **(5f)***

Light yellow solid, M.P = 160-161 ^{O}C ^{1}H NMR (DMSO-d_6, 500 MHz): 3.57–3.61 (m, 2H, CH_2), 3.80 (t, J = 6.2 Hz, 2H, CH_2), 6.64 (d, J = 5.4Hz, 1H, H^2), 7.43 (dd, J = 9.0 Hz, 2.2 Hz, 1H, H^4), 7.50 (t, J = 6.1 Hz, 1H, NH-exchangeable with D_2O), 7.64–7.71 (m, 2H, Ar-H), 7.79 (d, J = 2.3 Hz, 1H, H^5), 7.85–7.89 (m, 1H, Ar-H), 8.04 (d, J = 9.0 Hz, 1H, H^3), 8.43 (d, J = 5.4 Hz, 1H, H^1). ^{13}C NMR (DMSO-d_6, 125 MHz): 36.3, 40.3, 99.0, 117.9 (d, J = 12.4 Hz), 118.0, 120.0 (d, J = 3.1 Hz), 122.8 (d, J = 19.5 Hz), 124.2, 124.7, 127.8, 134.6 (d, J = 1.2 Hz), 137.8 (d, J = 7.7 Hz), 149.4, 150.3, 152.2, 157.0 (d, J = 258.8 Hz), 165.2, 167.3 (d, J = 2.7 Hz). $C_{19}H_{13}ClFN_3O_2$ $[M]^+$ 369.0680. Found 369.0688.

*3.2.7. 2-(3-(7-chloroquinolin-4-ylamino)propyl)-4-fluoroisoindoline-1,3-dione **(5g)***

Light yellow solid, M.P = 117-118 ^{O}C ^{1}H NMR (DMSO-d_6, 400 MHz): 1.94–2.01 (m, 2H, CH_2), 3.27–3.30 (m, 2H, CH_2), 3.65 (t, J = 6.8 Hz, 2H, CH_2), 6.43 (d, J = 5.5 Hz, 1H, H^2), 7.29 (t, J = 5.4 Hz, 1H, NH-exchangeable with D_2O), 7.38 (dd, J = 8.8 Hz, 2.2 Hz, 1H, H^4), 7.55–7.63 (m, 2H, Ar-H), 7.71 (d, J = 2.2 Hz, 1H, H^5), 7.77–7.82 (m, 1H, Ar-H), 8.16 (d, J = 9.0 Hz, 1H, H^3), 8.33 (d, J = 5.4 Hz, 1H, H^1). ^{13}C NMR (DMSO-d_6, 100 MHz): 27.0, 36.3, 40.6, 99.3, 117.8, 118.0 (d, J = 12.7 Hz), 119.9 (d, J = 2.7 Hz), 122.8 (d, J = 19.6 Hz), 124.5, 124.6, 127.7, 134.2 (d, J = 56.8 Hz), 137.7 (d, J = 7.7 Hz), 149.1, 150.6, 152.1, 157.0 (d, J = 259.8 Hz), 165.3, 167.4 (d, J = 2.4 Hz). $C_{20}H_{15}ClFN_3O_2$ $[M]^+$ 383.0837. Found 383.0841.

*3.2.8. 2-(4-(7-chloroquinolin-4-ylamino)butyl)-4-fluoroisoindoline-1,3-dione **(5h)***

Light yellow solid, M.P = 107-108 ^{O}C ^{1}H NMR (DMSO-d_6, 400 MHz): 1.56–1.60 (m, 4H, $2CH_2$), 3.26–3.29 (m, 2H, CH_2), 3.83 (t, J = 6.4 Hz, 2H, CH_2), 6.61 (d, J = 5.4 Hz, 1H, H^2), 7.43 (dd, J = 9.1 Hz, 2.2 Hz, 1H, H^2), 7.50 (t, J = 6.2 Hz, 1H, NH-exchangeable with D_2O), 7.64–7.71 (m, 2H, Ar-H), 7.79 (d, J = 2.2 Hz, 1H, H^5), 7.83–7.88 (m, 1H, Ar-H), 8.05 (d, J = 9.1 Hz, 1H, H^3), 8.45 (d, J = 5.4 Hz, 1H, H^1). ^{13}C NMR (DMSO-d_6, 100 MHz): 25.2, 26.5, 37.4, 42.2, 99.1, 117.8 (d, J = 12.0 Hz), 118.2, 120.0 (d, J = 3.3 Hz), 122.7 (d, J = 20.0 Hz), 124.2, 124.5, 127.8, 133.7, 134.4 (d, J = 1.2 Hz), 137.8 (d, J = 7.7 Hz), 149.5,

150.0, 152.2, 157.3 (d, J = 260.1 Hz), 165.1, 167.3 (d, J = 2.5 Hz). $C_{21}H_{17}ClFN_3O_2$ $[M]^+$ 397.0993. Found 397.0989.

3.2.9. 2-(6-(7-chloroquinolin-4-ylamino)hexyl)-4-fluoroisoindoline-1,3-dione (5i)

Light yellow solid, M.P = 94-95 ^{0}C ^{1}H NMR (DMSO-d_6, 400 MHz): 1.26–1.39 (m, 4H, $2CH_2$), 1.53–1.63 (m, 4H, $2CH_2$), 3.16–3.21 (m, 2H, CH_2), 3.48 (t, J = 6.5 Hz, 2H, CH_2), 6.39 (d, J = 5.5 Hz, 1H, H^2), 7.33–7.39 (m, 2H, H^4 + NH-exchangeable with D_2O), 7.60–7.64 (m, 2H, Ar-H), 7.70 (d, J = 2.2 Hz, 1H, H^5), 7.78–7.82 (m, 1H, Ar-H), 8.22 (d, J = 9.0 Hz, 1H, H^3), 8.31 (d, J = 5.4 Hz, 1H, H^1). ^{13}C NMR (DMSO-d_6, 100 MHz): 26.5, 26.6, 28.1, 28.3, 38.0, 42.8, 99.0, 117.8, 117.9 (d, J = 8.3 Hz), 120.0 (d, J = 2.8 Hz), 122.9 (d, J = 19.4 Hz), 124.5, 124.7, 127.4, 134.2 (d, J = 39.7 Hz), 137.8 (d, J = 7.5 Hz), 148.9, 150.8, 151.8, 157.0 (d, J = 260.1 Hz), 165.2, 167.4 (d, J = 2.5 Hz). $C_{23}H_{21}ClFN_3O_2$ $[M]^+$ 425.1306. Found 425.1311.

3.2.10. 2-(8-(7-chloroquinolin-4-ylamino)octyl)-4-fluoroisoindoline-1,3-dione (5j)

Yellow Semisolid, ^{1}H NMR (DMSO-d_6, 400 MHz): 1.18–1.28 (m, 8H, $4CH_2$), 1.51–1.65 (m, 4H, $2CH_2$), 3.40–3.44 (m, 2H, CH_2), 3.48 (t, J = 7.0 Hz, 2H, CH_2), 6.74 (d, J = 6.8 Hz, 1H, H^2), 7.59–7.67 (m, 3H, 2Ar-H + H^4), 7.80–7.85 (m, 1H, Ar-H), 7.91 (d, J = 2.2 Hz, 1H, H^5), 8.45 (d, J = 6.7 Hz, 1H, H^1), 8.50 (d, J = 9.1 Hz, 1H, H^3), 8.98 (t, J = 6.6 Hz, 1H, NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 100 MHz): 26.4, 26.8, 28.3, 28.8, 28.9, 29.3, 37.9, 43.2, 99.2, 117.5 (d, J = 12.4 Hz), 118.3, 120.3 (d, J = 3.3 Hz),122.5 (d, J = 12.2 Hz), 124.0, 124.2, 127.4, 133.3, 134.4 (d, J = 1.4 Hz), 137.5 (d, J = 7.7 Hz), 149.1, 150.3, 151.9, 157.5 (d, J = 259.8 Hz), 165.2, 167.0 (d, J = 2.2 Hz). $C_{25}H_{25}ClFN_3O_2$ $[M]^+$ 453.1619. Found 453.1625.

3.2.11. 2-(2-(7-chloroquinolin-4-ylamino)ethyl)-5-fluoroisoindoline-1,3-dione (5k)

Light yellow solid, M.P = 148-149 ^{0}C ^{1}H NMR (DMSO-d_6, 400 MHz): 3.16–3.21 (m, 2H, CH_2), 3.50 (t, J = 7.1 Hz, 2H, CH_2), 6.38 (d, J = 5.5 Hz, 1H, H^2), 7.24 (t, J = 5.3 Hz, 1H, NH-exchangeable with D_2O), 7.36 (dd, J = 9.0 Hz, 2.2 Hz, 1H, H^4), 7.56–7.59 (m, 1H, Ar-H), 7.62–7.65 (m, 1H, Ar-H), 7.70 (d, J = 2.2 Hz, 1H, H^5), 7.78–7.82 (m, 1H, Ar-H), 8.20 (d, J = 9.0 Hz, 1H, H^3), 8.31 (d, J = 5.4 Hz, 1H, H^1). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 36.4, 41.4, 99.1, 111.5 (d, J = 25.1 Hz), 117.5, 121.7 (d, J = 23.5 Hz), 125.3, 125.4, 126.3 (d, J = 9.5 Hz), 127.1, 128.4 (d, J = 2.6 Hz), 135.2 (d, J = 9.6 Hz), 137.7, 145.7, 149.7, 155.0, 166.1 (d, J = 235.8 Hz), 167.2 (d, J = 2.7 Hz), 167.4. $C_{19}H_{13}ClFN_3O_2$ $[M]^+$ 369.0680. Found 369.0688.

3.2.12. 2-(3-(7-chloroquinolin-4-ylamino)propyl)-5-fluoroisoindoline-1,3-dione (5l)

Light yellow solid, M.P = 139-140 ^{0}C ^{1}H NMR (DMSO-d_6, 400 MHz): 1.94–2.00 (m, 2H, CH_2), 3.27–3.30 (m, 2H, CH_2), 3.66 (t, J = 6.6 Hz, 2H, CH_2), 6.42 (d, J = 5.4 Hz, 1H, H^2), 7.32–7.39 (m, 2H, NH-exchangeable with D_2O + H^4), 7.53–7.58 (m, 1H, Ar-H), 7.62–7.64 (m, 1H, Ar-H), 7.71 (d, J = 1.1 Hz, 1H, H^5), 7.80–7.84 (m, 1H, Ar-H), 8.13 (d, J = 8.9 Hz, 1H, H^3), 8.31 (d, J = 5.2 Hz, 1H, H^1). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 27.0, 36.4, 40.7, 99.2, 111.3 (d, J = 25.0 Hz), 117.7, 121.5 (d, J = 25.01 Hz), 124.5, 124.7, 126.1 (d, J = 9.6 Hz), 127.3, 128.3 (d, J = 2.0 Hz), 134.2, 135.1 (d, J = 9.7 Hz), 148.8, 150.7, 151.8, 166.2 (d, J = 251.8 Hz), 167.2 (d, J = 1.8 Hz), 167.6. $C_{20}H_{15}ClFN_3O_2$ $[M]^+$ 383.0837. Found 383.0841.

3.2.13. 2-(4-(7-chloroquinolin-4-ylamino)butyl)-5-fluoroisoindoline-1,3-dione (5m)

Light yellow solid, M.P = 121-122 ^{0}C ^{1}H NMR (DMSO-d_6, 500 MHz): 1.63–1.76 (m, 4H, $2CH_2$), 3.26–3.30 (m, 2H, CH_2), 3.62 (t, J = 6.4 Hz, 2H, CH_2), 6.44 (d, J = 5.4 Hz, 1H, H^2), 7.29 (t, J = 5.4 Hz, 1H, NH-exchangeable with D_2O), 7.42 (dd, J = 8.9 Hz, 2.3 Hz, 1H, H^4), 7.60–7.64 (m, 1H, Ar-H), 7.69–7.71 (m, 1H, Ar-H), 7.75 (d, J = 2.2 Hz, 1H, H^5), 7.88–7.90 (m, 1H, Ar-H), 8.23 (d, J = 9.0 Hz, 1H, H^3), 8.35 (d, J = 5.4 Hz, 1H, H^1). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 25.5, 26.0, 37.9, 42.3, 99.1, 111.2 (d, J = 25.0 Hz), 117.8, 121.5 (d, J = 23.5 Hz), 124.4, 124.5, 126.1 (d, J = 9.7 Hz), 127.8, 128.1 (d, J = 2.5 Hz), 133.8, 135.0 (d, J = 9.6 Hz), 149.3, 150.5, 152.2, 166.1 (d, J = 246.9 Hz), 167.1 (d, J = 1.9 Hz), 167.4. $C_{21}H_{17}ClFN_3O_2$ $[M]^+$ 397.0993. Found 397.0989.

3.2.14. 2-(6-(7-chloroquinolin-4-ylamino)hexyl)-5-fluoroisoindoline-1,3-dione (5n)

Light yellow solid, M.P = 112-113 ^{0}C ^{1}H NMR (DMSO-d_6, 500 MHz): 1.31–1.41 (m, 4H, $2CH_2$), 1.58–1.65 (m, 4H, $2CH_2$), 3.20–3.24 (m, 2H, CH_2), 3.56 (t, J = 7.0 Hz, 2H, CH_2), 6.42 (d, J = 5.5 Hz, 1H, H^2), 7.29 (t, J = 5.3 Hz, 1H, NH-exchangeable with D_2O), 7.41 (dd, J = 8.9 Hz, 2.2 Hz, 1H, H^4), 7.60–7.64 (m, 1H, Ar-H), 7.70–7.72 (m, 1H, Ar-H), 7.75 (d, J = 2.2 Hz, 1H, H^5), 7.88–7.91 (m, 1H, Ar-H), 8.25 (d, J = 9.0 Hz, 1H, H^3), 8.36 (d, J = 5.4 Hz, 1H, H^1). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 26.5, 26.6, 28.0, 28.3, 38.0, 42.8, 99.0, 111.3 (d, J = 25.0Hz), 117.7, 121.5 (d, J = 23.4 Hz), 124.5, 124.6, 126.1 (d, J = 9.7 Hz), 127.3, 128.2 (d, J = 2.5 Hz), 134.0, 135.0 (d, J = 9.6 Hz), 148.8, 150.8, 151.8, 166.1 (d, J = 251.5 Hz), 167.1 (d, J = 2.7 Hz), 167.4. $C_{23}H_{21}ClFN_3O_2$ $[M]^+$ 425.1306. Found 425.1311.

3.2.15. 2-(8-(7-chloroquinolin-4-ylamino)octyl)-5-fluoroisoindoline-1,3-dione (5o)

Yellow Semisolid liquid, ^{1}H NMR (DMSO-d_6, 400 MHz): 1.47–1.54 (m, 8H, $4CH_2$), 1.58–1.63 (m, 4H, $4CH_2$), 3.24–3.28 (m, 2H, CH_2), 3.50 (t, J = 7.1 Hz, 2H, CH_2), 6.51 (d, J = 5.9 Hz, 1H, H^2), 7.47 (dd, J = 8.9 Hz, 2.1 Hz, 1H, H^4), 7.57–7.62 (m, 1H, Ar-H), 7.69–7.72 (m, 1H, Ar-H), 7.77 (d, J = 2.1 Hz, 1H, H^5), 7.81 (t, J = 4.6 Hz, 1H, NH-exchangeable with D_2O), 7.86–7.89 (m, 1H, Ar-H), 8.30 (d, J = 9.1 Hz, 1H, H^3), 8.36 (d, J = 5.7 Hz, 1H, H^1). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 26.3, 26.7, 28.2, 28.9, 30.1, 30.3, 37.5, 42.1, 99.1, 111.2 (d, J = 24.6Hz), 117.8, 121.4 (d, J = 23.8 Hz), 124.3, 124.4, 126.0 (d, J = 9.8 Hz), 127.5, 128.1 (d, J = 2.5 Hz), 134.1, 135.0 (d, J = 9.8 Hz), 148.7, 150.6, 151.6, 166.0 (d, J = 251.2 Hz), 167.0 (d, J = 2.6 Hz), 167.5. $C_{25}H_{25}ClFN_3O_2$ $[M]^+$ 453.1619. Found 453.1625.

3.2.16. 4,5,6,7-Tetrachloro-2-(2-(7-chloroquinolin-4-ylamino)ethyl) isoindoline-1,3-dione (5p)

Light yellow solid, M.P = 154-155 ^{0}C ^{1}H NMR (DMSO-d_6, 500 MHz): 3.65–3.68 (m, 2H, CH_2), 3.88 (t, J = 6.1 Hz, 2H, CH_2), 6.73 (d, J = 5.6 Hz, 1H, H^2), 7.50 (dd, J = 2.0 Hz, 8.9 Hz, 1H, H^4), 7.69 (t, J = 5.3 Hz, 1H, NH-exchangeable with D_2O), 7.85 (d, J = 1.9 Hz, 1H, H^5), 8.33 (d, J = 8.0 Hz, 1H, H^3), 8.50 (d, J = 5.5 Hz, 1H, H^1). ^{13}C NMR (DMSO-d_6, 125 MHz): 36.7, 40.2, 99.0, 117.8, 124.3, 124.9, 127.1, 128.5, 128.6, 134.1, 136.6, 148.5, 150.8, 151.5, 163.9. $C_{19}H_{10}Cl_5N_3O_2$ $[M]^+$ 488.9186. Found 488.9178. $[M+2]^+$ 490.9157. Found 490.9148.

3.2.17. 4,5,6,7-Tetrachloro-2-(3-(7-chloroquinolin-4-ylamino) propyl)isoindoline-1,3-dione (5q)

Light yellow solid, M.P = 142-143 ^{0}C ^{1}H NMR (DMSO-d_6, 400 MHz): 1.59–1.64 (m, 2H, CH_2), 3.15–3.20 (m, 2H, CH_2), 3.51 (t, J = 6.9 Hz, 2H, CH_2), 6.79 (d, J = 5.3 Hz, 1H, H^2), 7.67 (dd, J = 1.8 Hz, 9.1 Hz, 1H, H^4), 7.98 (d, J = 2.1 Hz, 1H, H^5), 8.46 (d, J = 6.9 Hz, 1H, H^1), 8.60 (d, J = 9.4 Hz, 1H, H^3), 9.38 (t, J = 5.6 Hz, 1H, NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 100 MHz): 26.1, 37.3, 40.8, 99.4, 117.5, 124.4, 124.6, 127.1, 131.3, 131.4, 134.2, 136.6, 148.4, 150.7, 151.5, 164.2. $C_{20}H_{12}Cl_5N_3O_2$ $[M]^+$ 502.9343. Found 502.9349. $[M+2]^+$ 504.9313. Found 504.9119.

3.2.18. 4,5,6,7-Tetrachloro-2-(4-(7-chloroquinolin-4-ylamino) butyl)isoindoline-1,3-dione (5r)

Light yellow solid, M.P = 140-141 ^{0}C ^{1}H NMR (DMSO-d_6, 500 MHz): 1.66–1.79 (m, 4H, $2CH_2$), 3.25–3.29 (m, 2H, CH_2), 3.60 (t,

J = 6.4 Hz, 2H, CH_2), 6.42 (d, J = 5.5 Hz, 1H, H^2), 7.42 (dd, J = 2.2 Hz, 9.0 Hz, 1H, H^4) 7.47 (t, J = 5.5 Hz, 1H, NH-exchangeable with D_2O), 7.66 (d, J = 2.2 Hz, 1H, H^5), 8.19 (d, J = 9.1 Hz, 1H, H^3), 8.32 (d, J = 5.2 Hz, 1H, H^1). ^{13}C NMR (DMSO-d_6, 125 MHz): 24.8, 25.3, 38.6, 42.2, 99.2, 117.5, 124.4, 124.6, 127.4, 128.2, 128.4, 134.0, 138.4, 148.8, 150.4, 151.7, 163.9. $C_{21}H_{14}Cl_5N_3O_2$ $[M]^+$ 516.9499. Found 516.9492. $[M+2]^+$ 518.9470. Found 518.9462.

3.2.19. 4,5,6,7-Tetrachloro-2-(6-(7-chloroquinolin-4-ylamino) hexyl)isoindoline-1,3-dione ***(5s)***

Light yellow solid, M.P = 128-129 ^{O}C ^1H NMR (DMSO-d_6, 400 MHz): 1.25–1.38 (m, 4H, $2CH_2$), 1.43–1.66 (m, 4H, $2CH_2$), 3.15–3.20 (m, 2H, CH_2), 3.43 (t, J = 6.4 Hz, 2H, CH_2), 6.76 (d, J = 6.6 Hz, 1H, H^2), 7.63 (dd, J = 2.2 Hz, 8.9 Hz, 1H, H^4), 7.94 (d, J = 2.2 Hz, 1H, H^5), 8.42 (d, J = 5.4 Hz, 1H, H^1), 8.54 (d, J = 9.4 Hz, 1H, H^3), 9.20 (t, J = 5.3 Hz, 1H, NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 100 MHz): 26.3, 26.5, 27.9, 28.0, 38.6, 43.5, 99.0, 116.1, 126.0, 126.1, 126.8, 128.3, 128.4, 132.9, 137.8, 148.8, 150.5, 151.4, 163.9. $C_{23}H_{18}Cl_5N_3O_2$ $[M]^+$ 544.9812 Found 544.9821. $[M+2]^+$ 546.9783. Found 546.9788.

3.2.20. 4,5,6,7-Tetrabromo-2-(2-(7-chloroquinolin-4-ylamino) ethyl)isoindoline-1,3-dione ***(5t)***

Light yellow solid, M.P = 173-174 ^{O}C ^1H NMR (DMSO-d_6, 400 MHz): 3.26–3.30 (m, 2H, CH_2), 3.67 (t, J = 6.6 hz, 2H, CH_2), 6.37 (d, J = 5.5 hz, 1H, H^2), 7.22 (t, J = 4.6 hz, 1H, NH-exchangeable with D_2O), 7.28 (dd, J = 2.0 hz, 9.0 hz, 1H, H^4), 7.65 (d, J = 2.2 hz, 1H, H^5), 8.02 (d, J = 9.0 hz, 1H, H^3), 8.30 (d, J = 5.4 hz, 1H, H^1). ^{13}C NMR (DMSO-d_6, 100 MHz): 37.2, 40.8, 99.3, 117.5, 120.6, 124.3, 124.5, 127.0, 131.2, 134.1, 136.5, 148.3, 150.6, 151.4, 164.1. $C_{19}H_{10}Br_4ClN_3O_2$ $[M]^+$ 666.7154. Found 666.7149. $[M+2]^+$ 668.7134. Found 668.7125.

3.2.21. 4,5,6,7-Tetrabromo-2-(3-(7-chloroquinolin-4-ylamino) propyl)isoindoline-1,3-dione ***(5u)***

Light yellow solid, M.P = 161-162 ^{O}C ^1H NMR (DMSO-d_6, 500 MHz): 2.03–2.08 (m, 2H, CH_2), 3.33–3.37 (m, 2H, CH_2), 3.72 (t, J = 6.6 hz, 2H, CH_2), 6.42 (d, J = 5.4 hz, 1H, H^2), 7.28 (t, J = 6.6 hz, 1H, NH-exchangeable with D_2O), 7.32 (dd, J = 9.0 hz, 2.0 hz, 1H, H^4), 7.69 (d, J = 2.2 hz, 1H, H^5), 8.06 (d, J = 8.9 hz, 1H, H^3), 8.34 (d, J = 5.5 hz, 1H, H^1). ^{13}C NMR (DMSO-d_6, 125 MHz): 26.0, 37.3, 40.7, 99.4, 117.6, 120.6, 124.2, 124.5, 127.3, 131.3, 134.0, 136.5, 148.8, 150.4, 151.8, 164.1. $C_{20}H_{12}Br_4ClN_3O_2$ $[M]^+$ 680.7311. Found 680.7320. $[M+2]^+$ 682.7290. Found 682.7295.

3.2.22. 4,5,6,7-Tetrabromo-2-(4-(7-chloroquinolin-4-ylamino) butyl)isoindoline-1,3-dione ***(5v)***

Light yellow solid, M.P = 155-156 ^{O}C ^1H NMR (DMSO-d_6, 400 MHz): 1.69–1.75 (m, 4H, $2CH_2$), 3.20–3.25 (m, 2H, CH_2), 3.52 (t, J = 6.3 Hz, 2H, CH_2), 6.87 (d, J = 5.5 Hz, 1H, H^2), 7.73 (dd, J = 9.4 Hz, 2.0 Hz, 1H, H^4), 7.99 (d, J = 2.0 Hz, 1H, H^5), 8.54 (d, J = 9.1 Hz, 1H, H^3), 8.59 (d, J = 5.6 Hz, 1H, H^1), 9.46 (t, J = 5.2 Hz, 1H, NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 100 MHz): 25.0, 25.4, 38.7, 42.3, 99.2, 117.6, 120.7, 124.5, 124.9, 127.5, 131.2, 134.1, 136.7, 148.9, 150.8, 151.8, 164.3. $C_{21}H_{14}Br_4ClN_3O_2$ $[M]^+$ 694.7467. Found 694.7461. $[M+2]^+$ 696.7447. Found 696.7440.

3.2.23. 4,5,6,7-Tetrabromo-2-(6-(7-chloroquinolin-4-ylamino) hexyl)isoindoline-1,3-dione ***(5w)***

Light yellow solid, M.P = 115-116 ^{O}C ^1H NMR (DMSO-d_6, 400 MHz): 1.28–1.41 (m, 4H, $2CH_2$), 1.52–1.65 (m, 4H, $2CH_2$), 3.34–3.40 (m, 2H, CH_2), 3.51 (t, J = 7.0 hz, 2H, CH_2), 6.63 (d, J = 6.3 hz, 1H, 7.57 (dd, J = 1.7 hz, 9.1 hz, 1H, H^4), 7.84 (d, J = 1.6 hz, 1H, H^5), 8.39–8.42 (m, 2H, $H^1 + H^3$). ^{13}C NMR (DMSO-d_6, 100 MHz): 26.1, 26.3, 27.7, 27.9, 37.6, 42.4, 98.6, 117.4, 119.6, 124.1, 124.3, 127.0, 133.6, 134.0, 137.4, 148.5, 150.4, 151.4, 164.8. $C_{23}H_{18}Br_4ClN_3O_2$ $[M]^+$ 722.7780. Found 722.7789. $[M+2]^+$ 724.7760. Found 724.7767.

3.2.24. 2-(2-(7-chloroquinolin-4-ylamino)ethyl)-4-nitroisoindoline-1,3-dione ***(5x)***

Light yellow solid, M.P = 111-112 ^{O}C ^1H NMR (DMSO-d_6, 500 MHz): 3.60–3.64 (m, 2H, CH_2), 3.83 (t, J = 6.1 Hz, 2H, CH_2), 6.69 (d, J = 5.5 Hz, 1H, H^2), 7.46 (dd, J = 8.9 Hz, 1.9 Hz, 1H, H^4), 7.64 (t, J = 6.3 Hz, 1H, NH-exchangeable with D_2O), 7.81 (d, J = 1.9 Hz, 1H, H^5), 8.03–8.06 (m, 2H, Ar-H), 8.15 (d, J = 7.4 Hz, 1H, Ar-H), 8.29 (d, J = 8.1 Hz, 1H, H^3), 8.46 (d, J = 5.5 Hz, 1H, H^1). ^{13}C NMR (DMSO-d_6, 125 MHz): 36.7, 40.4, 99.0, 117.8, 123.5, 124.3, 124.9, 127.1, 127.2, 128.5, 134.1, 134.3, 136.6, 144.7, 148.5, 150.8, 151.5, 163.9, 166.4. $C_{19}H_{13}ClN_4O_4$ $[M]^+$ 396.0625. Found 396.0618.

3.2.25. 2-(6-(7-chloroquinolin-4-ylamino)hexyl)-4-nitroisoindoline-1,3-dione ***(5y)***

Yellow Semisolid liquid, ^1H NMR (DMSO-d_6, 400 MHz): 1.28–1.33 (m, 4H, $2CH_2$), 1.52–1.61 (m, 4H, $2CH_2$), 3.25–3.29 (m, 2H, CH_2), 3.49 (t, J = 7.0 hz, 1H, CH_2), 6.53 (d, J = 6.1 hz, 1H, H^2), 7.44 (dd, J = 9.1 hz, 1.8 hz, 1H, H^4), 7.56–7.64 (m, 2H, NH-exchangeable with $D_2O + H^5$), 7.78–7.83 (m, 2H, Ar-H), 8.07 (d, J = 6.9 hz, 1H, Ar-H), 8.31–8.36 (m, 2H, $H^1 + H^3$). ^{13}C NMR (DMSO-d_6, 100 MHz): 26.5, 26.6, 28.0, 28.2, 38.0, 43.1, 99.0, 117.1, 122.8, 123.0, 124.0, 124.6, 125.2, 125.5, 134.4, 135.6, 137.8, 145.3, 148.7, 152.6, 155.7, 165.3, 167.4. $C_{23}H_{21}ClN_4O_4$ $[M]^+$ 452.1251.Found 452.1260.

4. Material and methods

4.1. Methods for assessment of anti-plasmodial activity of test compounds

The W2 strain of *P. falciparum* was cultured in RPMI-1640 medium with 10% human serum, following standard methods, and parasites were synchronized with 5% D-sorbitol [19]. Beginning at the ring stage, microwell cultures were incubated with different concentrations of compounds for 48 h. The compounds were added from DMSO stocks; the maximum concentration of DMSO used was 0.1%. Controls without inhibitors included 0.1% DMSO. After 48 h when control cultures had progressed to new rings, the culture medium was removed, and cultures were incubated for 48 h with 1% formaldehyde in PBS, pH 7.4, at room temperature. Fixed parasites were then transferred to 0.1% Triton X-100 in PBS containing 1 nM YOYO-1 dye (Molecular Probes). Parasitemia was determined from dot plots (forward scatter vs. fluorescence) acquired on a FACSort flow cytometer using Cell Quest software (Beckton Dickinson). IC_{50} values for growth inhibition were determined from plots of percent control parasitemia over inhibitor concentration using the Prism 3.0 program, (GraphPad Software), with data from duplicate experiments fitted by non-linear regression [20].

4.2. Cytotoxicity assay

Cell viability was determined using J774 murine macrophage cells which were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA), supplemented with 10% de-complemented fetal calf serum, under a 5% CO_2 atmosphere. Cells were seeded in 96-well plates at a density of 2×10^4 cells/well in 160 μL medium and incubated overnight at 37 °C to allow cells to adhere. Compounds (dissolved in DMSO) were freshly diluted to appropriate concentrations in DMEM, so as to allow addition of 20 μL volumes of the diluted compounds to the cells that resulted in final compound concentrations of 100 μg/mL, 50 μg/mL, 25 μg/mL, 12.5 μg/mL, 6.25 μg/mL and 3.125 μg/mL. The maximum final concentration of DMSO was 1% (v/v). After 24 h incubation at 37 °C, 20 μL of 1 mg/

mL resazurin (Sigma, Germany) was added to each well and the cells incubated for an additional 2 h at 37 °C. Fluorescence was measured in a Polarstar Omega fluorometer using appropriate filters (540 nm excitation and 590 nm emission wave length). Percentage survival was determined by dividing fluorescence values obtained in the compound containing wells by values obtained for control wells containing cells incubated with a dilution series of DMSO (1%, 0.5%, 0.25%, 0.125%, 0.0625%, and 0.03125% v/v). Finally the values obtained from three independent experiments were converted to μM and used to calculate IC50 values with the log (inhibitor) vs response nonlinear regression function of the GraphPad Prism software [21].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejmech.2017.11.033.

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