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Short communication

Urea/oxalamide tethered β-lactam-7-chloroquinoline conjugates: Synthesis and *in vitro* antimalarial evaluation

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ABSTRACT

The manuscript pertains to the synthesis of urea/oxalamide tethered β-lactam-7-chloroquinoline conjugates with well modulated chain lengths and their antimalarial evaluation. The results reveal the dependence of activity profiles on the *N*-1 substituent of the β-lactam ring, the nature of the linker as well as the length of the alkyl chain. The most potent of the tested compounds showed an IC_{50} of 34.97 nM against chloroquine resistant W2 strain of *Plasmodium falciparum*.

1. Introduction

Malaria is one of the most widespread and lethal infectious disease in the world [1]. It is caused by five species of *Plasmodium*, the most lethal form being *Plasmodium falciparum*, which affects 216 million people and causes over 655,000 deaths worldwide in 2010 [2]. The 4-aminoquinolines were among the most important and widely used class of drugs for the treatment of malaria. Structure–activity relationship (SAR) studies on aminoquinoline antimalarials suggest that the 7-chloro-4-aminoquinoline nucleus is obligatory for its antimalarial activity, particularly for the inhibition of β-hematin formation and accumulation of the drug in the parasite digestive vacuole [3,4]. However, the most extensively used chloroquine (CQ) as a standard therapeutic drug for *falciparum* malaria is no longer appropriate in nearly all areas because of the dissemination of CQ-resistant strains, strongly linked to mutations in the gene *pfcrt*, a putative drug transporter located in the digestive vacuole (DV) membrane [5–8]. With no vaccine currently available, and with resistance of plasmodium to clinically used chemotherapeutic agents becoming increasingly common [9], novel, effective, safe and inexpensive drugs are greatly needed to control malaria.

Evidence from SAR in the 4-aminoquinoline series has further demonstrated that CQ resistance can be overcome by subtly altering the length [10–13] and the basicity of the side chain [14]. Although few comprehensive and meticulous modifications of the CQ side chain have been reported to date, it has been recognized that altering separation of the two aliphatic amino groups to 2–3 or 10–12 carbon atoms can improve activity against chloroquine-resistant (CQR) strains [11,13,15,16].

Since their introduction, β-lactam antibiotics have continued to be chemotherapeutics of unparalleled effectiveness combining a broad spectrum of activity with low toxicity [17]. Interest in this class has led to the development of classical β-lactam substrates such as penicillins and cephalosporins together with the non-classical compounds such as carbapenems and monobactams [18]. Past few years have witnessed a renewed interest on the synthesis and modification of the β-lactam ring to obtain compounds with diverse pharmacological activities such as cholesterol absorption inhibitory activity; human tryptase, thrombin and chymase inhibitory activity; vasopressin V1a antagonist activity; and antidiabetic, anti-inflammatory, antiparkinsonian, anti-tubercular and anti-HIV activity [19]. Recent report from our lab

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has shown the exploration of antiplasmodial potential of C-3 functionalized β-lactams [20]. This was further extended toward the synthesis of 1*H*-1,2,3-triazole-tethered β-lactam-7-chloroquinoline conjugates and their evaluation against chloroquine-resistant strain of *P. falciparum*. The observed activity profiles showed dependence on the nature of substituent at N-1 position of β-lactam ring and was further validated *via* docking studies [21].

In continuation with our interest in the synthesis of novel molecular frameworks with biological implications [22], the present work describes the synthesis of β-lactam-4-aminoquinoline conjugates having urea/oxalamide linkers and a well modulated alkyl chain length, along with their antimalarial activities. The introduction of groups like amides, sulphonamides, ureas and thioureas has shown to improve the pharmacological profiles against both CQ sensitive and CQ resistant strains of *P. falciparum* [23]. Moreover, the introduction of an oxalamide group in 4-aminoquinolines enhanced their antimalarial activity, probably due to its stronger hydrogen bonding ability compared to urea [24,25].

2. Result and discussions

2.1. Chemistry: synthesis

The precursors for the synthesis of the desired hybrids namely 3-carbamic acid ethyl ester **2** and 3-oxalamic acid ethyl ester-2-azetidinone **3** were synthesized diastereoselectively from *cis*-3-amino-2-azetidinones **1**, prepared according to our recently reported protocol [26]. For the synthesis of **2**, the 3-amino-2-azetidinone **1** was treated with ethyl chloroformate while the reaction of **1** with ethyl oxalyl chloride was carried out for the preparation of **3** (Scheme 1).

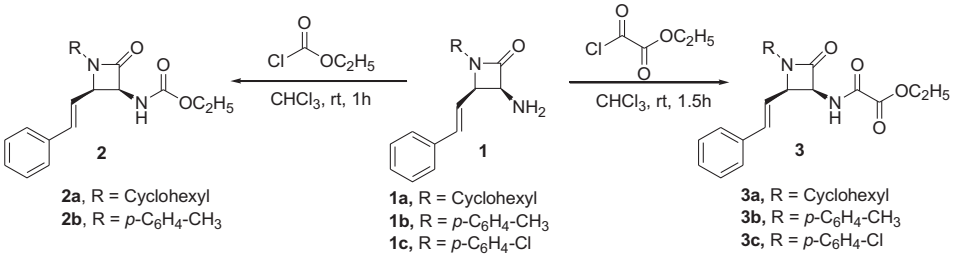
The desired urea based bifunctional hybrids **5** were prepared by refluxing a solution of **2** with **4**, prepared *via* heating 4,7-dichloroquinoline with an excess of corresponding diamine at 135 °C [15], in dry methanol for 8 h (Scheme 2). The similar synthetic protocol, as mentioned above, was followed for the synthesis of oxalamide based molecular hybrids **6** as elucidated in Scheme 3.

2.2. In vitro antiplasmodial activity of compounds *5a–h* and *6a–h*

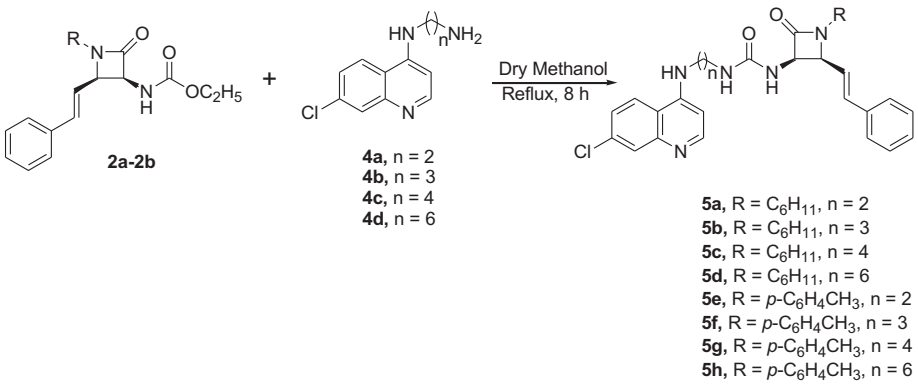
The antiplasmodial activity of synthesized conjugates was measured versus the CQ-R W2 strain using standardized methods as described in Experimental section. The IC_{50} values were calculated and compared to those for the standard drugs namely chloroquine (CQ), artemisinin (ART), desethylamodiaquine (DAQ) and quinine (QI). As depicted in Table 1, although the synthetic precursors **2a**,**b** and **3a–c** were proved to be inactive to inhibit the growth of *P. falciparum*, the introduction of chloroquinoline nucleus considerably improved the activity profiles of the synthesized conjugates. Among the compounds of urea tethered series **5a–h**, four compounds showed IC_{50} ranging from 42.38 nM to 53.11 nM while the remaining showed IC_{50} ranging from 73.02 nM to 193.15 nM. Interestingly, the variation of alkyl chain length and the presence of a substituent on *N*-1 of the β-lactam ring has been shown to influence the antiplasmodial profile and helped to establish a clear SAR for the synthesized compounds. The compounds with longer alkyl chain length ($n = 6$) displayed better antiplasmodial profiles compared to their short alkyl chain counter parts. Comparing the bifunctional hybrids with *N*-cyclohexyl substituent at *N*-1 of β-lactam ring **5a–d**, compound **5d**, with the longest alkyl chain length, displayed an IC_{50} value of 52.09 nM. The trend continued in the case of *N*-aryl substituted scaffolds **5e–h**, with activity considerably increasing with increasing alkyl chain length. The conjugate **5h** showed the best $IC_{50} = 42.38$ nM among the urea tethered conjugates.

The antimalarial evaluation data of 2-azetidinone-7-chloro-4-aminoquinoline conjugates linked through an oxalamide group **6-6h** revealed an improvement in their activity profiles compared to the urea-tethered counter parts. In case of *N*-cyclohexyl substituted conjugates, compound **6d** with *n*-hexyl chain length, displayed an IC_{50} of 34.97 nM while **6b**, with *n*-propyl chain length proved to be the least active compound, with an $IC_{50} = 120.65$ nM. A similar analysis in the case of an *N*-aryl substituent revealed that compounds **6e** and **6h**, with $n = 2$ and $n = 6$, respectively had similar activity profiles, with **6e** showing a slightly better IC_{50} value (44.78 nM) compared to **6h** (50.82 nM). A careful analysis of the effect of the *N*-substituent on the β-lactam ring clearly points out that an *N*-aryl substituent has a profound effect at shorter chain lengths ($n = 2,3$), while the effect reverses in favor of a cyclohexyl substituent when higher alkyl chain lengths are considered ($n = 4,6$). The compound **6d**, with an optimum combination of inhibitory components, including an oxalamide linker, a cyclohexyl substituent and alkyl chain length of $n = 6$, displayed the best antimalarial activity profile among the test compounds. The lipophilicity of the tested compounds was also calculated and the results are depicted in Table 1. As evident, the observed activity profiles of the tested compounds do not correlate well with the lipophilicity data, however the potent compounds, in general have shown to possess higher lipophilicity values.

The most potent compounds **5h** and **6d** were then further evaluated for their cytotoxicity against HeLa cells so as to ascertain whether the observed activity profiles are due to their intrinsic antimalarial activity or the cytotoxicity. Test compounds **5h** and **6d** consistently showed 90% viability each, compared with untreated and DMSO-treated cells. The same cells were tested with bleomycin (54–85% viability) and doxorubicin (62–83% viability). It should be noted that the results of viability studies with both of these standard drugs on cultured HeLa cells can be variable. Taking this into account, these assays were carried out on three independent days with multiple trials in each experiment. Interestingly, both the compounds had consistently comparable toxicities to untreated and DMSO-treated HeLa cells suggestive of the fact that the synthesized β-lactam-7-chloroquinoline conjugates can act as an ideal starting point for the synthesis of new pharmacological templates against *P. falciparum*.



Scheme 1. Synthesis of β-lactam precursors.



Scheme 2. Synthesis of urea-tethered β-lactam-7-chloroquinoline conjugates **5a**–**h**.

In conclusion, this manuscript describes the synthesis of urea/oxalamide linked β-lactam-7-chloroquinoline hybrids with well modulated alkyl chain length along with their antimalarial activities. The presence of a substituent on *N*-1 of the β-lactam ring, the nature of the linker as well as the alkyl chain length influenced the activity profiles of the synthesized hybrids. The non-cytotoxic compound **6d**, with an IC_{50} of 34.97 nM against *P. falciparum* can be considered as a good hit.

3. Experimental section

Melting points were determined by open capillary using a Veego Precision Digital Melting Point apparatus (MP-D) and are uncorrected. IR spectra were recorded on a Shimadzu D-8001 spectrophotometer. ^{1}H NMR spectra were recorded in deuterochloroform and dimethylsulfoxide-d_6 with a Jeol 300 (300 MHz) spectrometer using TMS as an internal standard. 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 Jeol 300 (75 MHz) in dimethylsulfoxide using TMS as internal standard. High resolution mass spectra were recorded on Bruker-micrOTOF-Q II spectrometer.

3.1. General procedure for the synthesis of compounds *2* and *3*

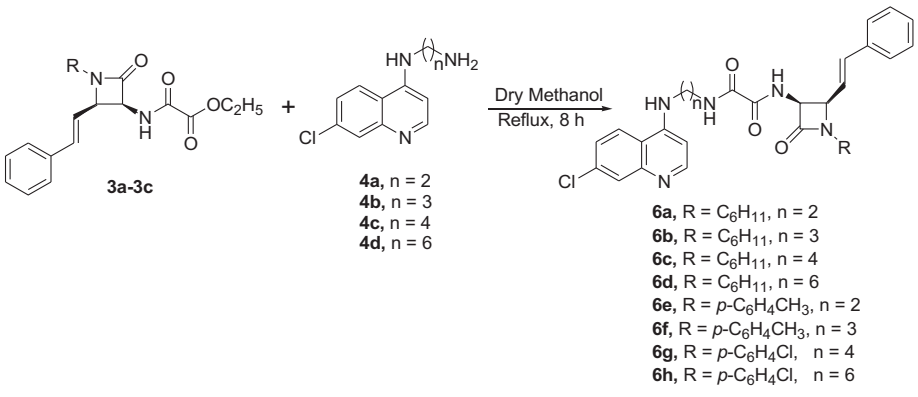
To the well stirred solution of **1** in dry DCM, was added 1.1 equiv. of ethyl chloroformate (for **2**) or 1.1 equiv. of ethyl 2-chloro-2-oxoacetate (for **3**). The reaction mixture was stirred at room temperature for 2 h. Upon completion of reaction, as evidenced by TLC, a saturated solution of sodium bicarbonate was added and the solution was stirred vigorously until it turned slightly alkaline in nature. The reaction mixture was then extracted with DCM and the organic layer was dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure resulting in the isolation of crystalline white solid. The structure was assigned on the basis of spectral data and analytical evidences.

3.1.1. (1-Cyclohexyl-2-oxo-4(SR)-styryl-azetidin-3(SR)-yl)-carbamic acid ethyl ester (*2a*)

White solid, Yield 82%; m.p. 170–172 °C; IR (KBr) ν_{max}: 3017, 1742, 1715 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.08–1.95 (m, 13H, 10H cyclohexyl ring + 3H, $-CH_3$), 3.56 (m, 1H, cyclohexyl ring), 4.30 (q, J = 7.2 Hz, 2H, $-CH_2-$), 4.56 (dd, J = 5.1, 8.1 Hz, 1H, H^a), 5.24 (dd, J = 5.1, 8.7 Hz, 1H, H^b), 6.03 (dd, J = 8.7, 15.9 Hz, 1H, H^c), 6.67 (d, J = 15.9 Hz, 1H, H^d), 7.26–7.38 (m, 5H, Ar–H), 7.67 (d, J = 7.8 Hz, 1H, lactam–NH, exchangeable with D_2O); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C 13.8, 22.2, 27.2, 30.8, 47.1, 59.5, 59.9, 63.6, 123.3, 126.2, 127.4, 127.7, 128.4, 134.9, 156.7, 162.3. MS *m*/*z* 343 (M$^+$); Analysis calculated for $C_{20}H_{26}N_2O_3$: C, 70.15; H, 7.65; N, 8.18. Found: C, 70.11; H, 7.61; N, 8.11.

3.1.2. (2-Oxo-4(SR)-styryl-1-p-tolyl-azetidin-3(SR)-yl)-carbamic acid ethyl ester (*2b*)

White solid, Yield 80%; m.p. 155–157 °C; IR (KBr) ν_{max}: 3022, 1738, 1712 cm^{-1}, ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.28 (t, J = 7.2 Hz, 3H, $-CH_3$), 2.31 (s, 3H, $-CH_3$), 4.32 (q, J = 7.4 Hz, 2H, $-CH_2-$), 5.01 (dd, J = 5.1, 8.1 Hz, 1H, H^a), 5.28 (dd, J = 5.1, 8.7 Hz, 1H, H^b), 6.21 (dd, J = 8.7, 15.9 Hz, 1H, H^c), 6.76 (d, J = 15.9 Hz, 1H, H^d), 7.23–7.41 (m, 9H, Ar–H), 7.87 (d, J = 8.4 Hz, 1H, lactam–NH, exchangeable with D_2O); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C 13.8, 23.2, 59.6, 60.0, 63.7, 118.4, 121.0, 126.7, 128.7, 128.8, 129.3, 129.9, 135.1, 135.6, 137.3, 156.5, 159.4, 162.5. MS *m*/*z* 371 (M$^+$); Analysis calculated for



Scheme 3. Synthesis of oxalamide-tethered β-lactam-7-chloroquinoline conjugates **6a**–**h**.

Table 1
Antimalarial activity results of tested compounds.

Compound	IC_{50} (nM) for *P. falciparum* CQ-R W2 strain	C Log P[a] [29]
2a	>1000	2.98
2b	>1000	3.58
3a	>1000	2.43
3b	>1000	3.04
3c	>1000	3.11
5a	157.25	3.89
5b	73.025	3.99
5c	193.15	4.45
5d	52.09	5.28
5e	53.11	4.49
5f	51.02	4.60
5g	149.45	5.05
5h	42.38	5.88
6a	61.34	3.34
6b	120.65	3.45
6c	68.63	3.90
6d	34.97	4.74
6e	44.78	3.95
6f	88.70	4.05
6g	82.19	4.51
6h	50.82	5.34
Artemisinin (ART)	10.63	
Chloroquine (CQ)	59.09	
Quinine (QI)	18.67	
Desethylamodiaquine (DAQ)	400.7	

[a] Calculated using Chem Draw Ultra 10.0.

$C_{20}H_{19}ClN_2O_3$: C, 64.78; H, 5.16; N, 7.55. Found: C, 64.74; H, 5.12; N, 7.51.

3.1.3. *N-(1-Cyclohexyl-2-oxo-4(SR)-styryl-azetidin-3(SR)-yl)-oxalamic acid ethyl ester (**3a**)*

White solid, Yield 82%; m.p. 170–172 °C; IR (KBr) ν_{max}: 3032, 1742, 1715, 1702 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$): δ_H 1.10–1.94 (m, 13H, 10H cyclohexyl ring + 3H, $-CH_3$), 3.56 (m, 1H, cyclohexyl ring), 4.31 (q, J = 7.4 Hz, 2H, $-CH_2-$), 4.54 (dd, J = 5.2, 8.4 Hz, 1H, H^a), 5.23 (dd, J = 5.2, 8.7 Hz, 1H, H^b), 6.02 (dd, J = 8.7, 15.9 Hz, 1H, H^c), 6.67 (d, J = 15.9 Hz, 1H, H^d), 7.23–7.41 (m, 5H, Ar–H), 7.69 (d, J = 8.4 Hz, 1H, lactam–NH, exchangeable with D_2O); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C 13.9, 22.1, 27.3, 30.7, 47.2, 59.6, 59.9, 63.7, 123.5, 126.3, 127.2, 127.5, 128.6, 135.0, 156.7, 159.5, 162.5. MS *m/z* 371 (M^+); Analysis calculated for $C_{21}H_{26}N_2O_4$: C, 68.09; H, 7.07; N, 7.56. Found: C, 68.03; H, 7.01; N, 7.51.

3.1.4. *N-(2-Oxo-4(SR)-styryl-1-p-tolyl-azetidin-3(SR)-yl)-oxalamic acid ethyl ester (**3b**)*

White solid, Yield 81%; m.p. 188–190 °C; IR (KBr) ν_{max}: 3028, 1742, 1718, 1700 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$): δ_H 1.28 (t, J = 7.4 Hz, 3H, CH_3), 2.29 (s, 3H, $-CH_3$), 4.31 (q, J = 7.4 Hz, 2H, $-CH_2-$), 5.01 (dd, J = 5.1, 8.1 Hz, 1H, H^a), 5.26 (dd, J = 5.1, 8.7 Hz, 1H, H^b), 6.21 (dd, J = 8.7, 15.9 Hz, 1H, H^c), 6.73 (d, J = 15.9 Hz, 1H, H^d), 7.22–7.42 (m, 9H, Ar–H), 7.83 (d, J = 7.8 Hz, 1H, lactam–NH, exchangeable with D_2O); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C 13.6, 59.5, 59.9, 63.6, 118.6, 121.1, 126.5, 128.3, 128.7, 129.4, 129.9, 135.2, 135.6, 137.4, 156.5, 159.5, 162.3. MS *m/z* 379 (M^+); Analysis calculated for $C_{22}H_{22}N_2O_4$: C, 69.83; H, 5.86; N, 7.40. Found: C, 69.79; H, 5.81; N, 7.35.

3.1.5. *N-[1-(4(SR)-Chloro-phenyl)-2-oxo-4-styryl-azetidin-3(SR)-yl]-oxalamic acid ethyl ester (**3c**)*

White solid, Yield 82%; m.p. 185–187 °C; IR (KBr) ν_{max}: 3025, 1742, 1715, 1704 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ_H 1.27 (t, J = 7.4 Hz, 3H, $-CH_3$), 4.31 (q, J = 7.4 Hz, 2H, $-CH_2$), 5.03 (dd, J = 5.1, 8.1 Hz, 1H, H^a), 5.27 (dd, J = 5.1, 8.7 Hz, 1H, H^b), 6.20 (dd, J = 8.7, 15.9 Hz, 1H, H^c), 6.75 (d, J = 15.9 Hz, 1H, H^d), 7.21–7.39 (m, 9H, Ar–H), 7.82 (d, J = 7.8 Hz, 1H, lactam–NH, exchangeable with D_2O); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C 13.7, 20.9, 59.6, 59.9, 63.7, 118.5, 121.2, 126.6, 128.4, 128.6, 129.5, 129.8, 135.1, 135.5, 137.6, 156.7, 159.5, 162.3. MS *m/z* 399 (M^+); Analysis calculated for $C_{21}H_{19}ClN_2O_4$: C, 63.24; H, 4.80; N, 7.02. Found: C, 63.19; H, 4.73; N, 6.96.

3.2. *General procedure for the synthesis of compounds **5a–h***

To a solution of **2** was added 1.0 equiv. of amines **4a–d** in anhydrous methanol (MeOH). The resulting reaction mixture was refluxed for 8 h. The progress of the reaction was monitored by TLC. The solvent was removed under reduced pressure on the completion of the reaction and solid product was obtained which was purified by recrystallization using chloroform:methanol (80:20) mixture.

3.2.1. *1-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-3-(1-cyclohexyl-2-oxo-4(SR)-styryl-azetidin-3(SR)-yl)-urea (**5a**)*

White solid, Yield 80%; m.p. 172–174 °C; IR (KBr) ν_{max}: 1742, 1672, 1530 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ_H 1.18–1.80 (m, 10H, cyclohexyl ring), 2.97 (t, J = 6.0 Hz, 2H, $N-CH_2-$), 3.43 (t, J = 6.0 Hz, 2H, $N-CH_2-$), 3.56 (m, 1H, cyclohexyl ring), 4.52 (dd, J = 5.1, 9.3 Hz, 1H, H^b), 5.20 (dd, J = 5.1, 9.6 Hz, 1H, H^a), 6.41 (d, J = 5.7 Hz, 1H, H^e), 6.49 (dd, J = 9.0, 15.9 Hz, 1H, H^c), 6.67 (d, J = 15.9 Hz, 1H, H^d), 7.19–7.39 (m, 5H, Ar–H), 7.42 (dd, J = 2.1, 9.1 Hz, 1H, H^h), 7.75 (d, J = 2.1 Hz, 1H, H^g), 8.22 (d, J = 9.0 Hz, 1H, H^i), 8.35 (d, J = 5.4 Hz, 1H, H^f), 8.83 (t, J = 6.3 Hz, 1H, NH, exchangeable with D_2O), 9.40 (d, J = 9.6 Hz, 1H, lactam–NH, exchangeable with D_2O); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C 22.4, 27.3, 30.9, 47.2, 48.6, 51.4, 58.3, 59.0, 98.7, 117.4, 123.8, 124.1, 126.3, 126.6, 127.5, 127.9, 128.6, 133.4, 134.5, 136.2, 149.0, 149.9, 151.8, 160.2, 164.5. MS *m/z* 520 (M^+); Analysis calculated for $C_{29}H_{33}ClN_5O_2$: C, 67.10; H, 6.41; N, 13.49. Found: C, 67.04; H, 6.35; N, 13.43.

3.2.2. *1-[3-(7-Chloro-quinolin-4-ylamino)-propyl]-3-(1-cyclohexyl-2-oxo-4(SR)-styryl-azetidin-3(SR)-yl)-urea (**5b**)*

White solid, Yield 78%; m.p. 170–172 °C; IR (KBr) ν_{max}: 1738, 1680, 1528 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$): δ_H 1.20–1.82 (m, 12H, 10H, cyclohexyl ring + 2H, $-CH_2-$), 2.96 (t, J = 6.3 Hz, 2H, $N-CH_2-$), 3.41 (t, J = 6.2 Hz, 2H, $N-CH_2-$), 3.57 (m, 1H, cyclohexyl ring), 4.53 (dd, J = 5.2, 9.0 Hz, 1H, H^b), 5.12 (dd, J = 5.1, 9.3 Hz, 1H, H^a), 6.39 (d, J = 5.7 Hz, 1H, H^e), 6.52 (d, J = 9.3, 15.9 Hz, H^c), 6.66 (d, 1H, J = 15.9 Hz, H^d), 7.20–7.38 (m, 5H, Ar–H), 7.42 (dd, 1H, J = 2.1, 9.1 Hz, 1H, H^h), 7.74 (d, J = 2.1 Hz, 1H, H^g), 8.23 (d, J = 9.1 Hz, 1H, H^i), 8.35 (d, J = 5.4 Hz, 1H, H^f), 8.83 (t, J = 6.3 Hz, 1H, NH), 9.42 (d, J = 9.3 Hz, 1H, lactam–NH, exchangeable with D_2O); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C 22.4, 27.3, 30.9, 32.5, 47.2, 48.6, 51.4, 58.3, 59.0, 98.7, 117.4, 123.8, 124.1, 126.3, 126.6, 127.5, 127.9, 128.6, 133.4, 134.5, 136.2, 149.0, 149.9, 151.8, 160.2, 164.5. MS *m/z* 533 (M^+); Analysis calculated for $C_{30}H_{34}ClN_5O_2$: C, 67.72; H, 6.44; N, 13.16. Found: C, 67.65; H, 6.37; N, 13.11.

3.2.3. *1-[4-(7-Chloro-quinolin-4-ylamino)-butyl]-3-(1-cyclohexyl-2-oxo-4(SR)-styryl-azetidin-3(SR)-yl)-urea (**5c**)*

White solid, Yield 80%; m.p. 166–168 °C; IR (KBr) ν_{max}: 1742, 1682, 1526 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$): δ_H 1.20–1.82 (m, 14H, 10H, cyclohexyl ring + 4H, $-CH_2-CH_2-$), 2.96 (t, J = 6.0 Hz, 2H, $-N-CH_2-$), 3.44 (t, J = 6.0 Hz, 2H, $N-CH_2-$), 3.57 (m, 1H, cyclohexyl ring), 4.53 (dd, J = 5.1, 9.0 Hz, 1H, H^b), 5.21 (dd, J = 5.1 Hz, 9.6, 1H, H^a), 6.40 (d, J = 5.4 Hz, 1H, H^e), 6.49 (dd, J = 9.6, 15.9 Hz, 1H, H^c), 6.66 (d, J = 15.9 Hz, 1H, H^d), 7.17–7.36 (m, 5H, Ar–H), 7.41 (dd, J = 2.1, 9.3 Hz, 1H, H^h), 7.75 (d, J = 2.1 Hz, 1H, H^g), 8.23 (d, J = 9.3 Hz, 1H, H^i), 8.34 (d, J = 5.4 Hz, 1H, H^f), 8.82 (t, J = 6.3 Hz, 1H, NH, exchangeable with D_2O), 9.39 (d, J = 9.6 Hz, 1H, lactam–NH,

exchangeable with D_2O); ^{13}C NMR ($CDCl_3$, 75 Hz): δ_C 22.4, 27.3, 28.1, 28.4 30.9, 47.2, 48.6, 51.4, 58.3, 59.0, 98.7, 117.4, 123.8, 124.1, 126.3, 126.6, 127.5, 127.9, 128.6, 133.4, 134.5, 136.2, 149.0, 149.9, 151.8, 160.2, 164.5. MS *m/z* 547 (M^+); Analysis calculated for $C_{31}H_{36}ClN_5O_2$: C, 68.18; H, 6.64; N, 12.82. Found: C, 68.12; H, 6.59; N, 12.77.

*3.2.4. 1-[6-(7-Chloro-quinolin-4-ylamino)-hexyl]-3-(1-cyclohexyl-2-oxo-4(SR)-styryl-azetidin-3(SR)-yl)-urea (**5d**)*

White solid, Yield 82%; m.p. 175–177 °C; IR (KBr) ν_{max}: 1748, 1679, 1530 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$): δ_H 1.18–1.90 (m, 18H, 10H, cyclohexyl ring + 8H, $-CH_2-CH_2-CH_2-CH_2-$), 2.95 (t, J = 6.1 Hz, 2H, $-N-CH_2-$), 3.46 (t, J = 6.1 Hz, 2H, $N-CH_2$), 3.56 (m, 1H, cyclohexyl ring), 4.52 (dd, J = 5.1, 9.3 Hz, 1H, H^b), 5.22 (dd, J = 5.1, 9.6 Hz, 1H, H^a), 6.41 (d, J = 5.7 Hz, 1H, H^e), 6.48 (dd, J = 9.6, 15.9 Hz, 1H, H^c), 6.67 (d, J = 15.9 Hz, 1H, H^d), 7.18–7.39 (m, 5H, Ar–H), 7.41 (dd, J = 2.1, 9.0 Hz, 1H, H^h), 7.73 (d, J = 2.1 Hz, 1H, H^g), 8.24 (d, J = 9.0 Hz, 1H, H^i), 8.33 (d, J = 5.4 Hz, 1H, H^f), 8.83 (t, J = 6.2 Hz, 1H, NH, exchangeable with D_2O), 9.39 (d, J = 9.3 Hz, 1H, lactam–NH, exchangeable with D_2O); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C 22.4, 27.3, 26.4, 26.8, 30.9, 31.2, 31.9, 47.2, 48.6, 51.4, 58.3, 59.0, 98.7, 117.4, 123.8, 124.1, 126.3, 126.6, 127.5, 127.9, 128.6, 133.4, 134.5, 136.2, 149.0, 149.9, 151.8, 160.2, 164.5. MS *m/z* 575 (M^+); Analysis calculated for $C_{33}H_{40}ClN_5O_2$: C, 69.03; H, 7.02; N, 12.20. Found: C, 68.92; H, 6.97; N, 12.13.

*3.2.5. 1-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-3-(2-oxo-4(SR)-styryl-1-p-tolyl-azetidin-3(SR)-yl)-urea (**5e**)*

White solid, Yield 78%; m.p. 155–157 °C; IR (KBr) ν_{max}: 1745, 1670, 1530 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$): δ_H 2.18 (s, 3H, $-CH_3$) 3.17–3.39 (m, 4H, $-N-CH_2-CH_2-N-$), 5.06 (dd, J = 5.2, 9.1 Hz, 1H, H^b), 5.41 (dd, J = 5.2, 9.3 Hz, 1H, H^a), 6.43 (dd, J = 9.3, 15.9 Hz, 1H, H^c), 6.54 (d, J = 5.5 Hz, 1H, H^e), 6.93 (d, J = 15.9 Hz, 1H, H^d), 7.19–7.40 (m, 9H, Ar–H, 1H, H^h), 7.75 (d, J = 2.1 Hz, 1H, H^g), 8.14 (d, J = 9.0 Hz, 1H, H^i), 8.35 (d, J = 5.5 Hz, 1H, H^f), 9.05 (s, 1H, NH, exchangeable with D_2O), 9.67 (d, J = 9.1 Hz, 1H, lactam–NH, exchangeable with D_2O); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C 20.3, 46.1, 53.6, 58.2, 59.1, 98.8, 117.5, 120.3, 123.6, 124.2, 126.2, 126.6, 127.4, 128.0, 128.5, 129.4, 133.5, 133.9, 134.4, 136.3, 137.7, 149.2, 149.8, 151.7, 159.6, 164.6.; MS *m/z* 527 (M^+); Analysis calculated for $C_{30}H_{28}ClN_5O_2$: C, 68.50; H, 5.37; N, 13.31. Found: C, 68.47; H, 5.32; N, 13.27.

*3.2.6. 1-[3-(7-Chloro-quinolin-4-ylamino)-propyl]-3-(2-oxo-4(SR)-styryl-1-p-tolyl-azetidin-3(SR)-yl)-urea (**5f**)*

White solid, Yield 75%; m.p. 152–154 °C; IR (KBr) ν_{max}: 1738, 1672, 1532 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$): δ_H 1.89 (m, 2H, $-CH_2-$), 2.20 (s, 3H, $-CH_3$) 3.18–3.40 (m, 4H, $-N-CH_2-CH_2-N-$), 5.05 (dd, J = 5.1, 9.6 Hz, 1H, H^b), 5.42 (dd, J = 5.1, 9.3 Hz, 1H, H^a), 6.42 (dd, J = 9.6, 15.9 Hz, 1H, H^c), 6.54 (d, J = 5.7 Hz, 1H, H^e), 6.94 (d, J = 15.9 Hz, 1H, H^d), 7.18–7.39 (m, 10H, 9H, Ar–H + 1H, H^h), 7.74 (d, J = 2.1 Hz, 1H, H^g), 8.13 (d, J = 9.0 Hz, 1H, H^i), 8.34 (d, J = 5.7 Hz, 1H, H^f), 9.07 (s, 1H, NH, exchangeable with D_2O), 9.65 (d, J = 9.6 Hz, 1H, lactam–NH, exchangeable with D_2O); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C 20.4, 30.1, 41.4, 43.6, 58.2, 59.1, 98.7, 117.5, 120.2, 123.7, 124.2, 126.1, 126.8, 127.4, 128.1, 128.6, 129.5, 133.3, 133.9, 134.5, 136.4, 137.7, 149.1, 149.7, 151.8, 159.6, 164.7. MS *m/z* 541 (M^+); Analysis calculated for $C_{31}H_{30}ClN_5O_2$: C, 68.94; H, 5.60; N, 12.97. Found: C, 68.89; H, 5.56; N, 12.93.

*3.2.7. 1-[4-(7-Chloro-quinolin-4-ylamino)-butyl]-3-(2-oxo-4(SR)-styryl-1-p-tolyl-azetidin-3(SR)-yl)-urea (**5g**)*

White solid, Yield 78%; m.p. 145–147 °C; IR (KBr) ν_{max}: 1740, 1680, 1528 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$): δ_H 1.87 (m, 2H, $-CH_2-$), 1.63 (m, 2H, $-CH_2-$), 2.20 (s, 3H, CH_3), 3.18–3.40 (m, 4H, $-N-CH_2-CH_2-N-$), 5.05 (dd, J = 5.1, 9.1 Hz, 1H, H^b), 5.43 (dd, J = 5.1, 9.6 Hz, 1H, H^a), 6.44 (dd, J = 9.6, 15.9 Hz, 1H, H^c), 6.51 (d, J = 5.7 Hz, 1H, H^e), 6.95 (d, J = 15.9 Hz, 1H, H^d), 7.20–7.42 (m, 10H, 9H, Ar–H + 1H, H^h), 7.77 (d, J = 2.1 Hz, 1H, H^g), 8.14 (d, J = 9.0 Hz, 1H, H^i), 8.34 (d, J = 5.7 Hz, 1H, H^f), 9.06 (s, 1H, NH, exchangeable with D_2O), 9.64 (d, J = 9.1 Hz, 1H, lactam–NH, exchangeable with D_2O); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C 20.4, 28.9, 30.1, 41.3, 43.5, 58.4, 59.2, 98.7, 117.5, 120.3, 123.7, 124.4, 126.2, 126.8, 127.4, 128.1, 128.7, 129.6, 134.0, 134.5, 136.5, 137.9, 149.2, 149.8, 151.8, 159.8, 160.2, 164.5. MS *m/z* 555 (M^+); Analysis calculated for $C_{32}H_{32}ClN_5O_2$: C, 69.37; H, 5.82; N, 12.64. Found: C, 69.32; H, 5.76; N, 12.59.

*3.2.8. 1-[6-(7-Chloro-quinolin-4-ylamino)-hexyl]-3-(2-oxo-4(SR)-styryl-1-p-tolyl-azetidin-3(SR)-yl)-urea (**5h**)*

White solid, Yield 80%; m.p. 150–152 °C; IR (KBr) ν_{max}: 1742, 1681, 1531 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ_H 1.52 (m, 6H, 3× CH_2), 1.76 (m, 2H, $-CH_2-$), 2.21 (s, 3H, $-CH_3$), 3.17–3.38 (m, 4H, $-N-CH_2-CH_2-N-$), 5.06 (dd, J = 5.1, 9.3 Hz, 1H, H^b), 5.44 (dd, J = 5.1, 9.6 Hz, 1H, H^a), 6.43 (dd, J = 9.6, 15.9 Hz, 1H, H^c), 6.55 (d, J = 5.7 Hz, 1H, H^e), 6.92 (d, J = 15.9 Hz, 1H, H^d), 7.17–7.39 (m, 10H, 9H, Ar–H + 1H, H^h), 7.74 (d, J = 2.1 Hz, 1H, H^g), 8.13 (d, J = 9.0 Hz, 1H, H^i), 8.34 (d, J = 5.7 Hz, 1H, H^f), 9.05 (s, 1H, NH, exchangeable with D_2O), 9.64 (d, 1H, J = 9.0 Hz, lactam–NH, exchangeable with D_2O); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C 20.4, 26.4, 26.7, 28.9, 30.2, 41.3, 43.6, 58.6, 59.1, 98.8, 117.5, 120.5, 123.6, 124.4, 126.3, 126.8, 127.5, 128.1, 128.8, 129.5, 134.1, 134.7, 136.3, 137.7, 149.2, 149.7, 151.6, 159.8, 160.4, 164.7. MS *m/z* 583 (M^+); Analysis calculated for $C_{34}H_{36}ClN_5O_3$: C, 70.15; H, 6.23; N, 12.03. Found: C, 70.11; H, 6.19; N, 11.97.

*3.3. General procedure for the synthesis of compounds **6a–h***

To a solution of **3** was added 1.0 equiv. of amines **4a–d** in anhydrous methanol (MeOH). The resulting reaction mixture was refluxed for 8 h. The progress of the reaction was monitored by TLC. The solvent was removed under reduced pressure on the completion of the reaction and solid product was obtained which was purified by recrystallization using chloroform:methanol (80:20) mixture.

*3.3.1. N-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-N′-(1-cyclohexyl-2-oxo-4(SR)-styryl-azetidin-3(SR)-yl)-oxalamide (**6a**)*

White solid, Yield 80%; m.p. 188–190 °C; IR (KBr) ν_{max}: 1740, 1645, 1512 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): δ_H 1.18–1.80 (m, 10H, cyclohexyl ring), 2.96 (t, J = 6.0 Hz, 2H, $N-CH_2-$), 3.44 (t, 2H, J = 6.0 Hz, 2H, $N-CH_2-$), 3.56 (m, 1H, cyclohexyl ring), 4.53 (dd, J = 5.1, 9.3 Hz, 1H, H^a), 5.15 (dd, J = 5.1, 9.6 Hz, 1H, H^b), 6.53 (d, J = 5.4 Hz, 1H, H^e), 6.57 (dd, J = 9.6, 15.9 Hz, 1H, H^c), 6.67 (d, J = 15.9 Hz, 1H, H^d), 7.22–7.37 (m, 5H, Ar–H), 7.42 (dd, J = 2.1, 9.0 Hz, 1H, H^h), 7.77 (d, J = 2.4 Hz, 1H, H^g), 8.14 (d, J = 9.3 Hz, 1H, H^i), 8.36 (d, J = 5.4 Hz, 1H, H^f), 9.03 (t, J = 6.3 Hz, 1H, NH, exchangeable with D_2O), 9.48 (d, J = 9.3 Hz, 1H, lactam–NH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 75 MHz): δ_C 22.5, 27.2, 30.8, 47.3, 48.7, 51.6, 58.2, 59.1, 98.8, 117.5, 123.7, 124.2, 126.2, 126.7, 127.4, 128.0, 128.5, 133.6, 134.4, 136.3, 149.1, 149.8, 151.7, 159.6, 160.2, 164.5. MS *m/z* 547 (M^+); Analysis calculated for $C_{30}H_{32}ClN_5O_3$: C, 65.99; H, 5.91; N, 12.83. Found: C, 65.93; H, 5.87; N, 12.79.

*3.3.2. N-[3-(7-Chloro-quinolin-4-ylamino)-propyl]-N′-(1-cyclohexyl-2-oxo-4(SR)-styryl-azetidin-3(SR)-yl)-oxalamide (**6b**)*

White solid, Yield 80%; m.p. 184–186 °C; IR (KBr) ν_{max}: 1740, 1645, 1512 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ_H 1.20–1.82 (m, 12H, 10H, cyclohexyl ring + 2H, $-CH_2-$), 3.22 (t, J = 7.2 Hz, 2H, $-N-CH_2-$), 3.44 (t, J = 7.2 Hz, 2H, $N-CH_2-$), 3.57 (m, 1H, cyclohexyl ring), 4.54 (dd, J = 5.2, 9.0 Hz, 1H, H^a), 5.14 (dd, J = 5.1, 9.3 Hz, 1H, H^b), 6.40 (d, J = 5.4 Hz, 1H, H^e), 6.53 (dd, J = 9.3, 15.9 Hz, 1H, H^c),

6.67 (d, J = 15.9 Hz, 1H, H^d), 7.21–7.37 (m, 5H, Ar–H), 7.44 (dd, J = 2.1, 9.1 Hz, 1H, H^h), 7.75 (d, J = 2.1 Hz, 1H, H^h), 8.22 (d, J = 9.1 Hz, 1H, H^i), 8.36 (d, J = 5.4 Hz, 1H, H^f), 8.84 (t, J = 6.3 Hz, 1H, NH, exchangeable with D_2O), 9.43 (d, J = 9.3 Hz, 1H, lactam–NH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 75 MHz): δ_C 24.5, 24.9, 27.2, 30.1, 31.4, 36.7, 51.4, 58.3, 59.0, 98.6, 117.4, 123.9, 124.0, 126.3, 126.7, 127.3, 127.9, 128.7, 133.4, 134.5, 136.2, 148.9, 150.0, 151.7, 159.4, 160.2, 164.6. MS m/z 561 (M^+); Analysis calculated for $C_{31}H_{34}ClN_5O_3$: C, 66.48; H, 6.12; N, 12.50. Found: C, 66.41; H, 6.07; N, 12.43.

3.3.3. *N-[4-(7-Chloro-quinolin-4-ylamino)-butyl]-N′-(1-cyclohexyl-2-oxo-4(SR)-styryl-azetidin-3(SR)-yl)-oxalamide (**6c**)*

White solid, Yield 82%; m.p. 192–194 °C; IR (KBr) ν_{max}: 1745, 1644, 1518 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): δ_H 1.17–1.95 (m, 14H, 10H, cyclohexyl ring + 4H, $-CH_2-CH_2-$), 3.20 (t, J = 6.0 Hz, 2H, $-N-CH_2-$), 3.43 (t, J = 6.0 Hz, 2H, $N-CH_2-$), 3.57 (m, 1H, cyclohexyl ring), 4.52 (dd, J = 5.1, 9.3 Hz, 1H, H^a), 5.11 (dd, J = 5.1, 9.3 Hz, H^b), 6.41 (d, J = 5.7 Hz, 1H, H^e), 6.48 (dd, J = 9.0, 15.9 Hz, 1H, H^c), 6.66 (d, J = 15.9 Hz, 1H, H^d), 7.19–7.39 (m, 5H, Ar–H), 7.43 (dd, J = 2.1, 9.0 Hz, 1H, H^h), 7.75 (d, J = 2.1 Hz, 1H, H^g), 8.22 (d, J = 9.3 Hz, 1H, H^i), 8.35 (d, J = 5.4 Hz, 1H, H^f), 8.83 (t, J = 6.3 Hz, 1H, NH, exchangeable with D_2O), 9.39 (d, J = 9.6 Hz, 1H, lactam–NH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 75 MHz): δ_C 24.9, 25.4, 26.7, 30.5, 31.8, 42.4, 48.3, 51.8, 58.8, 59.5, 99.0, 117.8, 124.4, 124.5, 126.8, 127.2, 127.9, 128.4, 129.1, 133.7, 134.9, 136.6, 149.5, 150.5, 152.3, 159.7, 160.9, 165.0. MS m/z 575 (M^+); Analysis calculated for $C_{32}H_{36}ClN_5O_3$: C, 66.95; H, 6.32; N, 12.20. Found: C, 66.91; H, 6.26; N, 12.13.

3.3.4. *N-[6-(7-Chloro-quinolin-4-ylamino)-hexyl]-N′-(1-cyclohexyl-2-oxo-4(SR)-styryl-azetidin-3(SR)-yl)-oxalamide (**6d**)*

White solid, Yield 78%; m.p. 185–187 °C; IR (KBr) ν_{max}: 1748, 1647, 1522 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): δ_H 1.18–1.90 (m, 18H, 10H, cyclohexyl ring + 8H, $-CH_2-CH_2-CH_2-CH_2-$), 2.95 (t, J = 6.1 Hz, 2H, $-N-CH_2-$), 3.46 (t, J = 6.1 Hz, 2H, $N-CH_2-$), 3.56 (m, 1H, cyclohexyl ring), 4.52 (dd, J = 5.1, 9.3 Hz, 1H, H^a), 5.22 (dd, J = 5.1, 9.6 Hz, 1H, H^b), 6.41 (d, J = 5.7 Hz, 1H, H^e), 6.48 (dd, J = 9.6, 15.9 Hz, 1H, H^c), 6.67 (d, J = 15.9 Hz, 1H, H^d), 7.18–7.39 (m, 5H, Ar–H), 7.41 (dd, J = 2.1, 9.0 Hz, 1H, H^h), 7.73 (d, J = 2.1 Hz, 1H, H^g), 8.24 (d, J = 9.0 Hz, 1H, H^i), 8.33 (d, J = 5.4 Hz, 1H, H^f), 8.83 (t, J = 6.2 Hz, 1H, NH, exchangeable with D_2O), 9.39 (d, J = 9.3 Hz, 1H, lactam–NH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 75 MHz): δ_C 22.4, 27.3, 26.4, 26.8, 30.9, 31.2, 31.9, 47.2, 48.6, 51.4, 58.3, 59.0, 98.7, 117.4, 123.8, 124.1, 126.3, 126.6, 127.5, 127.9, 128.6, 133.4, 134.5, 136.2, 149.0, 149.9, 151.8, 160.2, 161.3, 164.5. MS m/z 603 (M^+); Analysis calculated for $C_{34}H_{40}ClN_5O_3$: C, 67.82; H, 6.70; N, 11.63. Found: C, 67.77; H, 6.63; N, 11.58.

3.3.5. *N-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-N′-(2-oxo-4(SR)-styryl-1-p-tolyl-azetidin-3(SR)-yl)-oxalamide (**6e**)*

White solid, Yield 80%; m.p. 181–183 °C; IR (KBr) ν_{max}: 1752, 1644, 1532 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): δ_H 2.19 (s, 3H, CH_3) 3.18–3.40 (m, 4H, $-N-CH_2-CH_2-N-$), 5.07 (dd, J = 5.1, 9.3 Hz, 1H, H^a), 5.42 (dd, J = 6.0, 9.3 Hz, 1H, H^b), 6.44 (dd, J = 9.3, 16.2 Hz, 1H, H^c), 6.55 (d, J = 5.7 Hz, 1H, H^e), 6.92 (d, J = 16.2 Hz, 1H, H^d), 7.22–7.42 (m, 10H, 9H, Ar–H + 1H, H^h), 7.76 (d, J = 2.1 Hz, 1H, H^g), 8.13 (d, J = 9.0 Hz, 1H, H^i), 8.34 (d, J = 6.2 Hz, 1H, H^f), 9.06 (s, 1H, NH, exchangeable with D_2O), 9.66 (d, J = 9.3 Hz, 1H, lactam–NH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 75 MHz): δ_C 20.4, 46.1, 53.6, 58.2, 59.1, 98.8, 117.5, 120.3, 123.7, 124.2, 126.2, 126.7, 127.4, 128.0, 128.5, 129.4, 133.6, 133.9, 134.4, 136.3, 137.7, 149.1, 149.8, 151.7, 159.6, 160.2, 164.5.; MS m/z 555 (M^+); Analysis calculated for $C_{31}H_{28}ClN_5O_3$: C, 67.20; H, 5.09; N, 12.64. Found: C, 67.17; H, 5.01; N, 12.61.

3.3.6. *N-[3-(7-Chloro-quinolin-4-ylamino)-propyl]-N′-(2-oxo-4(SR)-styryl-1-p-tolyl-azetidin-3(SR)-yl)-oxalamide (**6f**)*

White solid, Yield 80%; m.p. 192–194 °C; IR (KBr) ν_{max}: 1752, 1644, 1532 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): δ_H 1.89 (m, 2H, $-CH_2-$), 2.20 (s, 3H, CH_3) 3.18–3.40 (m, 4H, $-N-CH_2-CH_2-N-$), 5.06 (dd, J = 5.1, 9.0 Hz, 1H, H^a), 5.43 (dd, J = 6.0, 9.3 Hz, H^b), 6.43 (dd, J = 9.3, 15.9 Hz, 1H, H^c), 6.55 (d, J = 5.7 Hz, 1H, H^e), 6.93 (d, J = 15.9 Hz, 1H, H^d), 7.21–7.40 (m, 10H, 9H, Ar–H + 1H, H^h), 7.75 (d, J = 2.1 Hz, 1H, H^g), 8.12 (d, J = 9.0 Hz, 1H, H^i), 8.34 (d, J = 6.2 Hz, 1H, H^f), 9.06 (s, 1H, NH, exchangeable with D_2O), 9.65 (d, J = 9.0 Hz, 1H, lactam–NH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 75 MHz): δ_C 20.4, 30.1, 41.4, 43.6, 58.2, 59.1, 98.8, 117.4, 120.2, 123.6, 124.3, 126.1, 126.8, 127.5, 128.1, 128.6, 129.5, 133.4, 133.9, 134.5, 136.4, 137.8, 149.0, 149.8, 151.9, 159.7, 160.1, 164.6. MS m/z 569 (M^+); Analysis calculated for $C_{32}H_{30}ClN_5O_3$: C, 67.66; H, 5.32; N, 12.33. Found: C, 67.61; H, 5.27; N, 12.29.

3.3.7. *N-[1-(4-Chloro-phenyl)-2-oxo-4(SR)-styryl-azetidin-3(SR)-yl]-N′-[4-(7-chloro-quinolin-4-ylamino)-butyl]-oxalamide (**6g**)*

White solid, Yield 82%; m.p. 177–179 °C; IR (KBr) ν_{max}: 1742, 1645, 1528 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): δ_H 1.89 (m, 2H, $-CH_2-$), 1.62 (m, 2H, $-CH_2-$), 3.18–3.40 (m, 4H, $-N-CH_2-CH_2-N-$), 5.05 (dd, J = 5.2, 9.1 Hz, 1H, H^a), 5.44 (dd, J = 5.2, 9.3 Hz, H^b), 6.43 (dd, J = 9.3, 15.9 Hz, 1H, H^c), 6.53 (d, J = 5.4 Hz, 1H, H^e), 6.94 (d, J = 15.9 Hz, 1H, H^d), 7.19–7.41 (m, 10H, 9H, Ar–H + 1H, H^h), 7.76 (d, J = 2.1 Hz, 1H, H^g), 8.13 (d, J = 9.0 Hz, 1H, H^i), 8.33 (d, J = 5.4 Hz, 1H, H^f), 9.06 (s, 1H, NH, exchangeable with D_2O), 9.65 (d, J = 9.3 Hz, 1H, lactam–NH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 75 MHz): δ_C 28.9, 30.1, 41.3, 43.5, 58.4, 59.2, 98.7, 117.5, 120.3, 123.7, 124.4, 126.2, 126.8, 127.4, 128.1, 128.7, 129.6, 133.5, 134.0, 134.5, 136.5, 137.9, 149.2, 149.8, 151.8, 159.8, 160.2, 164.5. MS m/z 602 (M^+); Analysis calculated for $C_{32}H_{29}Cl_2N_5O_3$: C, 63.79; H, 4.85; N, 12.62. Found: C, 63.73; H, 4.81; N, 12.56.

3.3.8. *N-[1-(4-Chloro-phenyl)-2-oxo-4(SR)-styryl-azetidin-3(SR)-yl]-N′-[6-(7-chloro-quinolin-4-ylamino)-hexyl]-oxalamide (**6h**)*

White solid, Yield 82%; m.p. 186–188 °C; IR (KBr) ν_{max}: 1745, 1647, 1526 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6): δ_H 1.51 (m, 6H, 3× $-CH_2-$), 1.77 (m, 2H, $-CH_2-$), 3.18–3.40 (m, 4H, $-N-CH_2-CH_2-N-$), 5.03 (dd, J = 5.1, 9.0 Hz, 1H, H^a), 5.43 (dd, J = 5.1, 9.3 Hz, 1H, H^b), 6.44 (dd, J = 9.3, 15.9 Hz, 1H, H^c), 6.54 (d, J = 5.7 Hz, 1H, H^e), 6.93 (d, J = 15.9 Hz, 1H, H^d), 7.20–7.42 (m, 10H, 9H, Ar–H + 1H, H^h), 7.75 (d, J = 2.1 Hz, 1H, H^g), 8.12 (d, J = 9.0 Hz, 1H, H^i), 8.33 (d, J = 5.4 Hz, 1H, H^f), 9.06 (s, 1H, NH, exchangeable with D_2O), 9.65 (d, J = 9.0 Hz, 1H, lactam–NH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, 75 MHz): δ_C 26.4, 26.8, 28.9, 30.1, 41.3, 43.5, 58.5, 59.1, 98.8, 117.4, 120.5, 123.6, 124.5, 126.3, 126.7, 127.5, 128.0, 128.8, 129.5, 133.4, 134.1, 134.6, 136.4, 137.7, 149.3, 149.7, 151.7, 159.9, 160.4, 164.7. MS m/z 630 (M^+); Analysis calculated for $C_{34}H_{33}Cl_2N_5O_3$: C, 64.76; H, 5.27; N, 11.11. Found: C, 64.71; H, 5.23; N, 11.07.

3.4. *Methods for assessment of antimalarial activity of the compounds*

The W2 strains of *P. falciparum* were cultured in RPMI-1640 medium with 10% human serum, following standard methods, and parasites were synchronized with 5% D-sorbitol [27]. Beginning at this ring stage, microwell cultures were incubated with different concentrations of compounds for 48 h. The compounds were added from the DMSO stocks: the maximum concentration of DMSO used was 0.1%. Controls without inhibitors included 0.1% DMSO. After 48 h, once the control has progressed to new rings, the culture medium was removed; cultures were incubated for 48 h with 1% formaldehyde in PBS, pH 7.4, at room temperature. Fixed parasites were then transferred into 0.1% Triton X-100 in PBS containing

1 nM YOYO-1 dye (Molecular Probes). Parasitemia was determined from dot plots (forward scatter versus fluorescence) acquired on a FACSort flow cytometer using Cell Quest software (Beckton Dickinson). IC_{50} values for the growth inhibition were determined from plots of percent control parasitemia over inhibitor concentration using prism 3.0 programme, (GraphPad Software), with data from the duplicate experiments fitted by non linear regression [28].

3.5. Cytotoxic evaluation of **5h** and **6d**

The HeLa cells were maintained in Dulbecco's Modified Eagle Medium that contained 1% penicillin/streptomycin and 10% fetal bovine serum in a humified 5% CO_2 atmosphere at 37 °C. 1 μM Doxorubicin, 10 μM bleomycin, and 10 μM of **5h**/**6d** were added to the medium of cells 24 h after culture. A trypan blue assay was used 24 h after drug treatment to calculate cell viability. This was done in three separate trials to ensure that consistent results were found.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.10.079.

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