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Variedly connected 1,8-naphthalimide-7-chloroquinoline conjugates: Synthesis, anti-mycobacterial and cytotoxic evaluation

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ABSTRACT

Recent disclosures about anti-bacterial and anti-tubercular potential of naphthalimide and quinoline core respectively propelled us to synthesize a library of 1,8-naphthalimide-7-chloroquinoline hybrids. Different modes of linkage between two pharmacophoric units viz. simple alkyl chains and induction of amide bond were used and the substituents on the naphthalimide core were varied in order to determine Structure-Activity-Relationship (SAR). Our findings demonstrated that simple alkyl chain linked conjugates showed better activity profiles without any cytotoxicity, while the inclusion of amide bond enhanced the cytotoxic tendency. An interesting behaviour of conjugates in terms of activity and cytotoxicity was observed *via* switching over the nature of linker between two pharmacophores.

1. Introduction

Mycobacterium tuberculosis (*Mtb*); the causative agent of Tuberculosis (TB), remains a global health concern with huge impact particularly in Asian and African continents. According to WHO Global Tuberculosis report 2018, TB has become one of the top 10 causes of death worldwide. Moreover, co-morbidity with HIV/AIDS has further left the matter a grave concern as there is recorded 1.3 million deaths among HIV-negative people and additional 0.3 million deaths among HIV-positive people [1]. Recently, clinical prognoses have worsened due to the emergence of multi-drug resistant (MDR) and extensive-drug resistant (XDR) TB which causes non-compliance among TB patients with conventional drug regimens [1]. Multiple factors such as long treatment duration, increased toxicity, cost of treatment of MDR TB have made the fight against this epidemic more arduous than ever. In order to overcome the multitude of continuously arising challenges in the fight against TB, there is particular interest in the Research and Development sector where new compounds can be introduced with better efficacy and less severe side effects [2].

Quinoline is a privileged entity which acts as a pharmacophore in various drugs owing to its antimalarial, anticancer, antimicrobial, antifungal, antitubercular, antileishmanial properties [3]. There are some anti-TB drugs which already have been commercialized *viz* ciprofloxacin, ofloxacin, levofloxacin, all of which contain quinoline core [4]. Besides, the success in its exploration is evident from bedaquiline; a diaryl quinoline based scaffold that has been approved by United States Food and Drug Administration (FDA) and European Medicine Agency (EMA) and is one of only two anti-TB drugs successfully developed in the last 50 years. This drug is under advance phase of clinical trial and some countries have already started its usage as a regimen for MDR-TB [5]. However, clinical applications have been restricted by a black box warning due to serious adverse effects, such as increased mortality and QT (electrical depolarization and repolarization of the ventricles) prolongation [6]. In the endeavour to explore the potential of quinoline, researchers are being motivated to design and produce new molecules with improved safety and pharmacological profiles which can overcome these persisting limitations. There are a number of reports showing quinoline based derivatives and molecular hybrids having significant anti-mycobacterial potency, among which quinoline diamines proved to be a considerable precursor to produce a novel anti-TB entity [7]. The diamine core also finds its importance from Ethambutol, one of the first line drugs to treat TB, Fig. 1.

Heterocyclic naphthalimides have emerged as useful molecular framework because of its extensive potentiality in medicinal applications. Few reports have displayed naphthalimides as anticancer, antibacterial, antifungal, antiviral, anti-inflammatory, antidepressant agents [8]. The π-deficient conjugated planar structure that enables naphthalimide derivatives to feasibly interact with various biological cations, anions, small molecules and macromolecules like DNA, enzymes & receptors *via* non-covalent bonds, eventually allows us to

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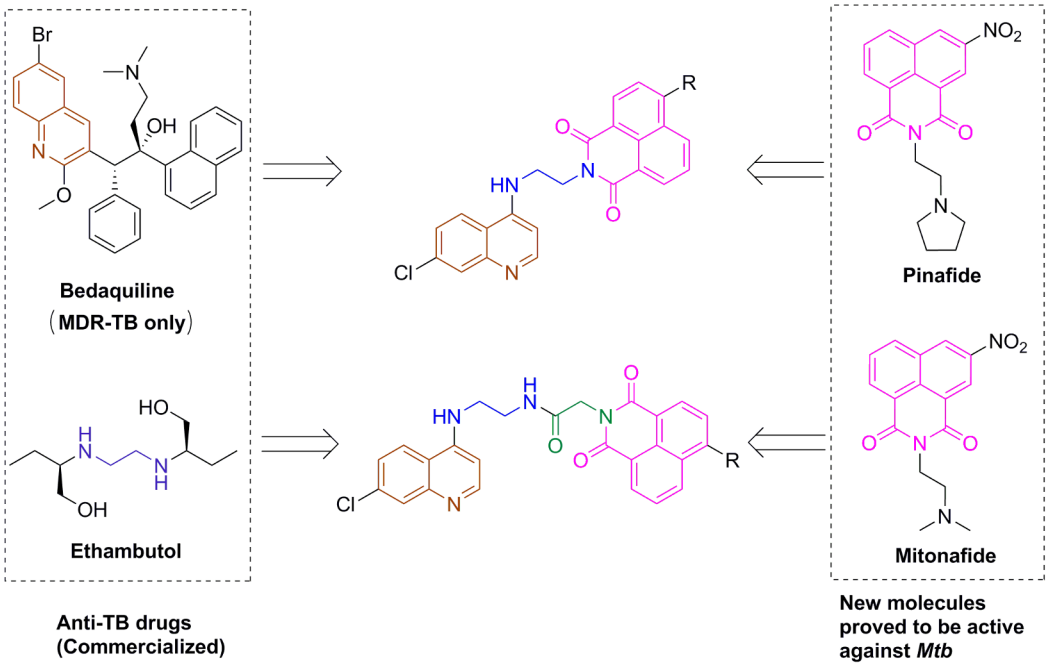


Fig. 1. Schematic diagram showing the design strategy for choosing the molecular fragments in the synthesis of 1,8-naphthalimde-7-chloroquinoline hybrids.

exploit these properties in current medicinal research [9]. Currently, naphthalimide based drugs *viz*. Amonafide and Mitonafide, are under clinical trials for their anticancer potential while Pinafide and Mitonafide have been recognized for their promising anti-TB activities [10], Fig. 1.

The nature of linker has great influence on the pharmacokinetic behaviour of the drug while designing a hybrid compound [11]. Using this concept, 7-chloroquinoline and 1,8-naphthalic anhydride, chosen as pharmacophoric units were connected through simple alkyl chains (using diamines) and *via* introducing amide bond. Thus, in the pursuit of our efforts to develop new hybrid compounds with biological potential [12], we herein report the synthesis, anti-mycobacterial and cytotoxic evaluation of 4-substituted 1,8-naphthalimide-7-chloroquinoline conjugates connected through varied linkers in order to study their structure-activity relationship.

2. Results and discussion

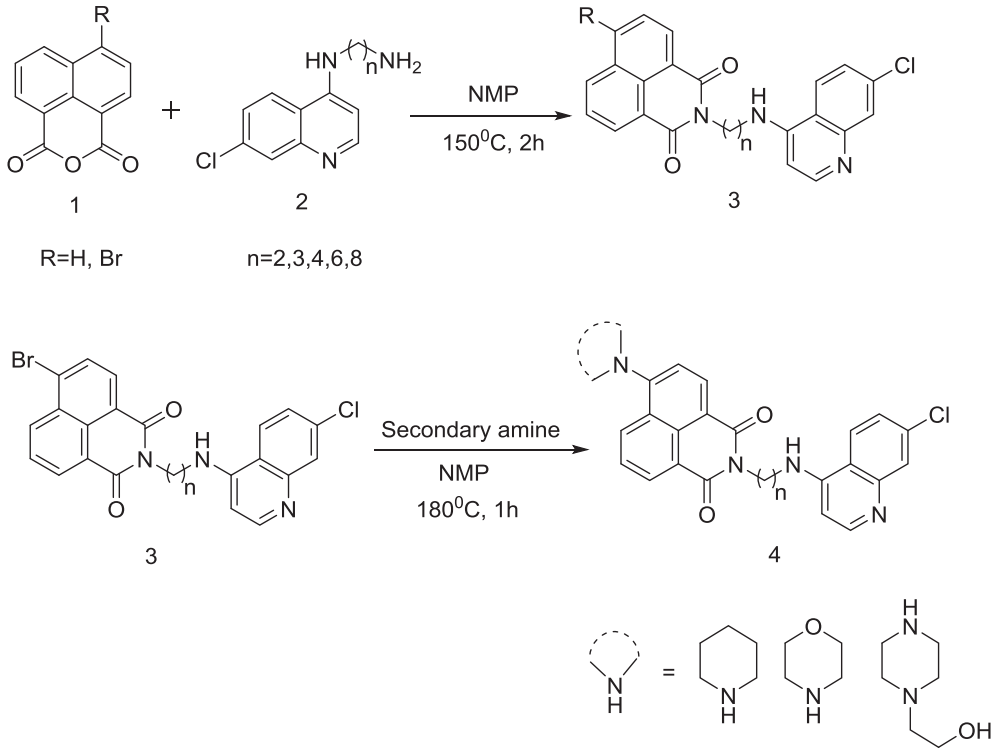
2.1. Chemistry

In the synthesis of the first set of target compounds, with alkyl chains as linker between two pharmacophoric units, 1,8-naphthalic anhydride **1** was heated with quinoline diamine **2**, prepared from reported methodology [13], at 150 °C using anhydrous *N*-methyl-2-pyrrolidone (NMP) as solvent. The compounds containing bromo substituted naphthalimide were further used as precursors to synthesize other target molecules. The synthesis of **4** was done by heating **3** with a secondary amine (piperidine/morpholine/hydroxyethyl piperazine) at 180 °C keeping NMP as solvent, Scheme 1. The structures of the synthesized hybrids were assigned on the basis of spectral data and analytical evidences. For illustration, the compound **3a,** showed molecular ion peak at *m/z* 402.0945 $[M + 1]^+$ in its HRMS. Its ^{1}H NMR spectrum showed doublets at δ 6.63 (J = 5.1 Hz) and 8.11 (J = 8.9 Hz) corresponding to quinoline ring protons and requisite peaks of naphthalimide core as well. Appearance of absorption peak at δ 164.15 in ^{13}C NMR spectrum of **3a** representing carbonyl carbon further corroborated the assigned structure.

Another set of target compounds with an amide functionality as a linker, was synthesized by initially heating 1,8-naphthalic anhydride **1** with glycine **5** in the presence of triethyl amine as base and anhydrous *N*,*N*-Dimethylformamide (DMF) as solvent. The product obtained **6** was further reacted with quinoline-diamines **2** in the presence of 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), *N*-hydroxybenzotriazole (HOBt) and *N*,*N*-Diisopropylethylamine (DIEA) using anhydrous DMF as solvent to yield **7**. Heating of **7** in the presence of secondary amine (piperidine/morpholine/hydroxyethyl piperazine) at 180 °C using NMP as solvent afforded the amide-linked 4-aminoquinoline-naphthalimides **8** as depicted in Scheme 2. The structures of the synthesized hybrids were assigned again on the basis of spectral data and analytical evidences. Taking an example of compound **7b**, which showed molecular ion peak at *m/z* 473.1328 $[M + 1]^+$ in its HRMS while the appearance of desired peaks in ^{1}H and ^{13}C NMR spectrum further confirmed the structure. Its ^{1}H NMR spectrum showed doublets at δ 6.55 (J = 5.4 Hz), 7.78 (J = 1.7 Hz) and 8.12 (J = 9.0 Hz) corresponding to quinoline ring along with another pair of doublets at δ 8.25 (J = 7.8 Hz) and 8.34 (J = 7.8 Hz) representing naphthalimide ring protons. Appearance of peaks at δ 163.27 and 163.32 representing carbonyl carbons in ^{13}C NMR spectrum further confirmed the structure.

2.2. In-vitro anti-mycobacterial and cytotoxic evaluation

Both the series of synthesized compounds were evaluated for anti-mycobacterial and cytotoxic evaluation against mc^{2}6230 and Vero kidney cell lines, respectively. Among the series where the pharmacophores were connected *via* alkyl linkage (**3a-j** and **4a-o**), the MIC_{99} value ranges from 6.25 to 200 μg/mL, with most of the compounds proven to be active against *Mtb*. For contemplation of Structure-Activity-Relationship (SAR) among the synthesized series, spacer lengths and substituents on the naphthalimide core were varied. For the hybrids having unsubstituted naphthalimide core **3a-e**, activity increases on increasing the spacer length with **3d** (n = 6) and **3e** (n = 8) being the most active with MIC_{99} of 12.5 μg/mL. Similar trend in activity was observed on introducing the bromo substituent at C-4 position of naphthalimide core among conjugates **3f-j** with the most potent hybrid, **3i**, having hexyl spacer showing MIC_{99} 12.5 μg/mL. On replacing bromo with piperidine, an enhancement in the anti-mycobacterial activity was observed with **4b** (n = 3) and **4c** (n = 4), **4d** (n = 6) being the potent hybrids with MIC_{99} values 12.5 and 6.25 μg/mL respectively.



Scheme 1. Synthesis of 1,8-naphthalimide-7-chloroquinoline hybrids **4** connected *via* simple alkyl chains.

The replacement of bromo with morpholine and/or hydroxyethyl piperazine did not produce significant changes in the activity profiles. The conjugates with longer chain length viz. **4i**, **4n** (n = 6) and **4j**, **4o** (n = 8) showed MIC_{99} 12.5 μg/mL. The evaluation of the conjugates for their cytotoxicity assay demonstrated a general trend among the whole series. Irrespective of the substituents on the naphthalimide core, the scaffolds with longer chain lengths i.e. with hexyl and octyl spacers showed IC_{50} values > 100 μg/mL (except **4n**), proving their non-cytotoxicity as evident from **3d**, **3e**, **3i**, **3j**, **4d**, **4e**, **4i**, **4j**, and **4o**. On analysing the second series of hybrids, where the pharmacophores were connected *via* induction of an amide bond along with variable chain lengths as well as substituents, loss of anti-TB activity was observed with MIC_{99} values ranges from 12.5 to 200 μg/mL. For hybrids with unsubstituted naphthalimides *viz*. **7a-e**, the one with butyl spacer, **7c**, showed good activity with MIC_{99} 12.5 μg/mL. The introduction of bromo substituent further decreased the anti-mycobacterial activities, irrespective of the length of alkyl chain as evident from **7g** (n = 3) and **7j** (n = 8) with MIC_{99} of > 200 μg/mL. The replacement of bromo with a secondary amine *viz*. piperidine enhanced the activity profiles with all the corresponding conjugates except **8e** exhibiting MIC_{99} of 12.5 μg/mL. On the other hand, morpholine and/or hydroxyethyl piperazine substituted conjugates did not show any significant anti-tubercular activities as evident from the hybrids **8f-8o**. For cytotoxicity assay, most of compounds of the synthesized series, except **7j**, **8e** and **8j**, displayed significant IC_{50} values, clearly proving their cytotoxic tendency. Selectivity Index (SI) of all the synthesized hybrids were also calculated as a function of the IC_{50}/MIC and was observed to be of moderate to good values, Table 1.

On comparing the observed anti-mycobacterial profiles of both the series of 1,8-naphthalimide-7-chloroquinoline conjugates, the hybrids having alkyl chain as spacer proved to be active and non-cytotoxic while the induction of amide linkage not only reduced the anti-mycobacterial efficacy, but also resulted in increase in cytotoxicity. The most potent of the synthesized hybrids, **4d** with hexyl chain length and piperidine substituent on the naphthalimide core, proved to be the most potent and non-cytotoxic with MIC_{99} of 6.25 μg/mL and selectivity index of > 16. The SAR of the synthesized compounds has been diagrammatically represented in Fig. 2.

3. Experimental section

3.1. General information

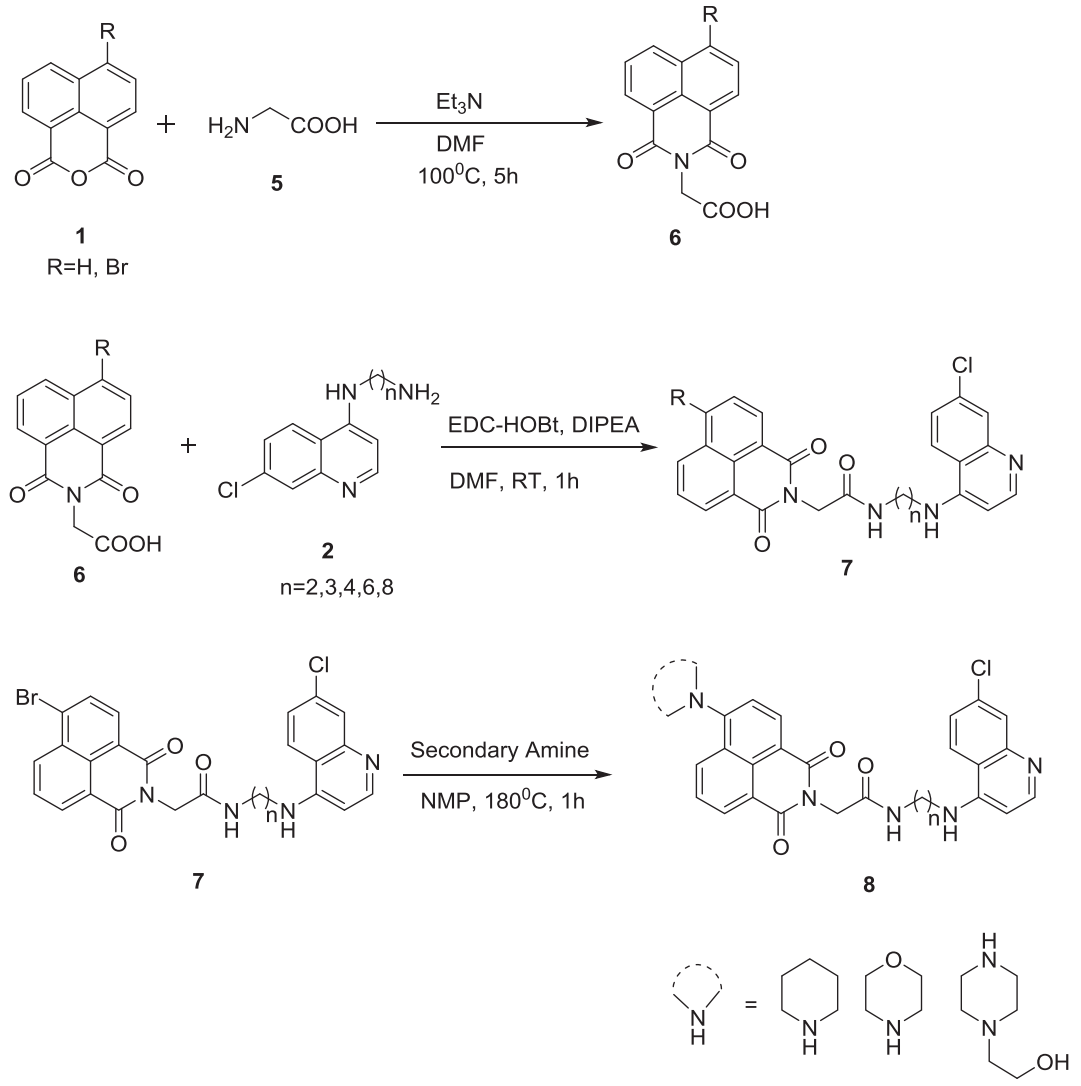
Stuart Digital Melting Point apparatus (SMP10) and an open capillary was used to determine melting points and are uncorrected. ^{1}H NMR spectra were recorded in DMSO-d_6 and $CDCl_3$ with a BRUKER AVANCE II (500 MHz) and JEOL (400 MHz) spectrometer. ^{13}C NMR spectra were recorded in DMSO-d_6 and $CDCl_3$ with a BRUKER AVANCE II (125 MHz) and JEOL (100 MHz). Chemical shift values are expressed as parts per million (ppm) downfield from TMS (used as reference) and the coupling constants represented by *J* values are in hertz. Splitting patterns are indicated as s: singlet, d: doublet, t: triplet, m: multiplet, dd: double doublet, dt: doublet of a triplet and br: broad peak. Mass spectra were recorded on a BRUKER high resolution mass spectrometer (micrOTOF-QII).

3.2. Procedure for synthesis of conjugates *3a-j*

To a well stirred solution of 1,8-naphthalic anhydride **1** (1 mmol) in anhydrous NMP as solvent, was added quinoline diamine **2** (1 mmol) and the reaction mixture was heated at 150 °C for 2 h. On reaction completion, as evident from TLC, ice cold water was added to the reaction mixture. The desired product **3**, got precipitated out which was then filtered, dried and recrystallized in ethanol.

3.2.1. 2-(2-((7-chloroquinolin-4-yl)amino)ethyl)-1H-benzo[de] isoquinoline-1,3(2H)-dione (*3a*)

Yield-86%, pale yellow solid, M.P = 130–135 °C, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.61–3.65 (m, 2H, CH_2), 4.30 (t, *J* = 6.8 Hz, 2H, CH_2), 6.83 (d, *J* = 5.1 Hz, 1H, H^2), 7.41 (dd, *J* = 1.4, 8.8 Hz, 1H, H^4), 7.70 (t, *J* = 5.6 Hz, 1H, NH-exchangeable with D_2O), 7.78 (s, 1H, H^5), 7.83–7.86 (m, 2H, H^7 + H^{10}), 8.11 (d, *J* = 8.9 Hz, 1H, H^3),



Scheme 2. Synthesis of 1,8-naphthalimide-7-chloroquinoline hybrids **8** connected *via* amide bond.

8.42–8.53 (m, 5H, $H^1 + H^6 + H^8 + H^9 + H^{11}$). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 164.15, 152.87, 145.45, 143.10, 136.45, 134.79, 131.71, 131.14, 127.63, 127.50, 125.95, 125.15, 122.85, 122.45, 117.50, 99.17, 40.85, 38.22. HRMS calcd for $C_{23}H_{16}ClN_3O_2$ $[M + 1]^+$ 402.0931, found 402.1024.

3.2.2. 2-(3-((7-chloroquinolin-4-yl)amino)propyl)-1H-benzo[de] isoquinoline-1,3(2H)-dione (3b)

Yield-90%, pale yellow solid, M.P = 165–170 °C, ^1H NMR (DMSO-d_6, 400 MHz): δ 2.00–2.07 (m, 2H, CH_2), 3.42–3.46 (m, 2H, CH_2), 4.13 (t, J = 6.8 Hz, 2H, CH_2), 6.57 (d, J = 6.1 Hz, 1H, H^2), 7.41 (d, J = 2.1, 9.0 Hz, 1H, H^4), 7.74–7.78 (m, 3H, $H^5 + H^7 + H^{10}$), 8.17 (s, 1H, NH-exchangeable with D_2O), 8.24 (d, J = 9.1 Hz, 1H, H^3), 8.34–8.36 (m, 5H, $H^1 + H^6 + H^8 + H^9 + H^{11}$). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 164.03, 152.63, 148.57, 145.06, 135.89, 134.75, 131.70, 131.15, 127.80, 127.64, 125.58, 125.06, 124.38, 122.50, 117.04, 99.16, 41.30, 38.34, 26.83. HRMS calcd for $C_{24}H_{18}ClN_3O_2$ $[M + 1]^+$ 416.1088, found 416.1183.

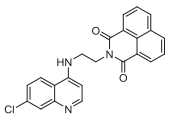
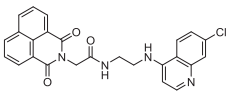
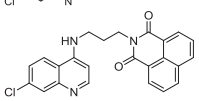
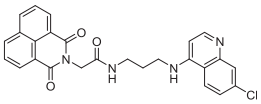
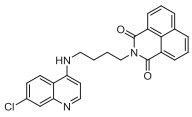
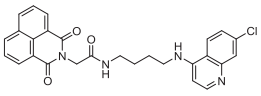
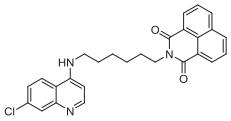
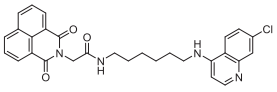
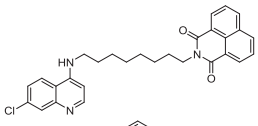
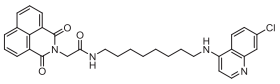
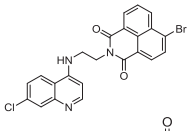
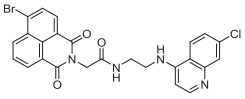
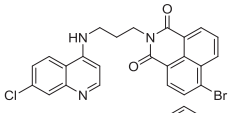
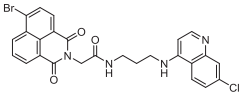
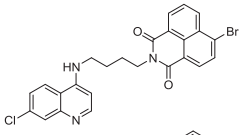
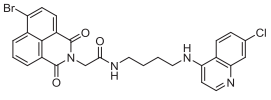
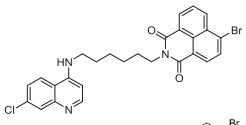
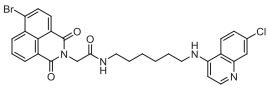
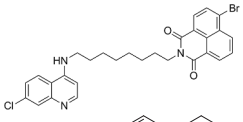
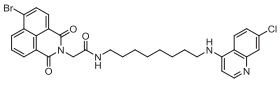
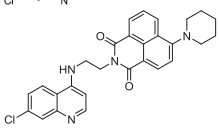
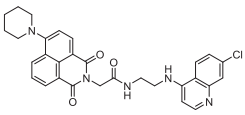
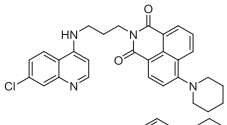
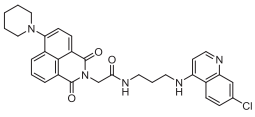
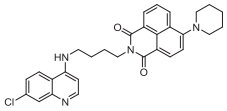
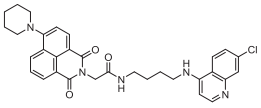
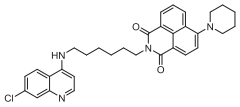
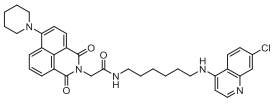
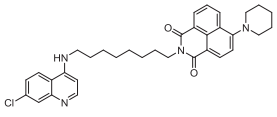
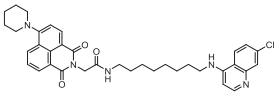
3.2.3. 2-(4-((7-chloroquinolin-4-yl)amino)butyl)-1H-benzo[de] isoquinoline-1,3(2H)-dione (3c)

Yield-89%, grey solid, M.P = 130–135 °C, ^1H NMR (DMSO-d_6, 500 MHz): δ 1.74–1.78 (m, 4H, $(CH_2)_2$), 3.45–3.46 (m, 2H, CH_2), 4.08 (t, J = 5.0 Hz, 2H, CH_2), 6.68 (d, J = 6.45 Hz, 1H, H^2), 7.53 (dd, J = 1.8, 8.9 Hz, 1H, H^4), 7.78–7.81 (m, 2H, $H^7 + H^{10}$), 7.87 (d, J = 1.6 Hz, 1H, H^5), 8.37–8.40 (m, 5H, $H^1 + H^6 + H^8 + H^9 + H^{11}$), 8.44 (d, J = 9.1 Hz, 1H, H^3), 8.76 (s, 1H, NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 163.87, 153.78, 146.25, 142.56, 136.62, 134.72, 131.61, 131.10, 127.65, 127.57, 126.07, 125.54, 122.33, 122.30, 116.47, 98.95, 43.07, 25.65, 25.62. HRMS calcd for $C_{25}H_{20}ClN_3O_2$ $[M + 1]^+$ 430.1244, found 430.1337.

3.2.4. 2-(6-((7-chloroquinolin-4-yl)amino)hexyl)-1H-benzo[de] isoquinoline-1,3(2H)-dione (3d)

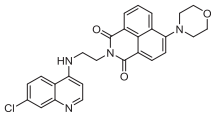
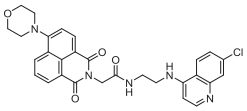
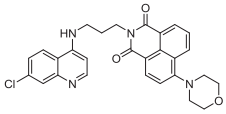
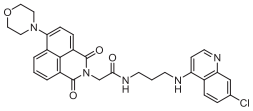
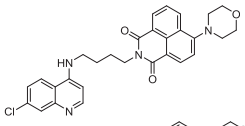
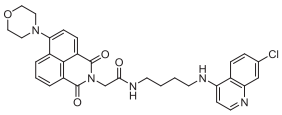
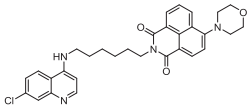
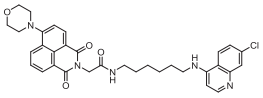
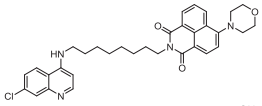
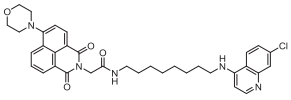
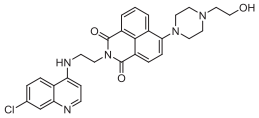
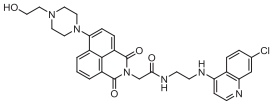
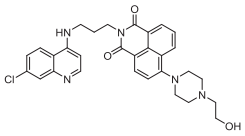
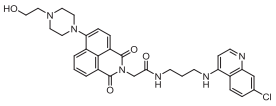
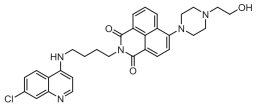
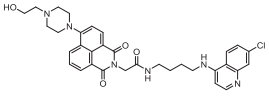
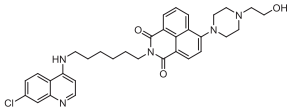
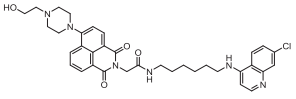
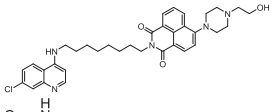
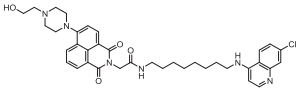
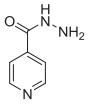
Yield-88%, light brown solid, M.P = 185–190 °C, ^1H NMR (DMSO-d_6, 500 MHz): δ 1.38–1.44 (m, 4H, $(CH_2)_2$), 1.63–1.69 (m, 4H, $(CH_2)_2$), 3.41–3.45 (m, 2H, CH_2), 3.99–4.04 (m, 2H, CH_2), 6.73 (d, J = 6.75 Hz, 1H, H^2), 7.63 (dd, J = 1.7, 8.9 Hz, 1H, H^4), 7.81–7.85 (m, 2H, $H^7 + H^{10}$), 7.95 (d, J = 1.6 Hz, 1H, H^5), 8.39–8.45 (m, 5H, $H^1 + H^6 + H^8 + H^9 + H^{11}$), 8.53 (d, J = 9.0 Hz, 1H, H^3), 9.02 (s, 1H, NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 163.80, 154.53, 145.15, 141.30, 137.26, 134.73, 131.69, 131.11, 127.73, 127.63, 126.50, 125.83, 122.42, 121.31, 116.29, 98.97, 43.41, 27.91, 27.87, 26.71, 26.59. HRMS calcd for $C_{27}H_{24}ClN_3O_2$ $[M + 1]^+$ 458.1557, found 458.1655.

Table 1
In-vitro anti-mycobacterial and cytotoxic evaluation of the compounds against *M. tuberculosis* mc^{2}6230 and Vero kidney cell line.

Code	Structure	MIC_{99}[a]	IC_{50}[b]	SI[c]	Code	Structure	MIC_{99}[a]	IC_{50}[b]	SI[c]
3a		200	> 100	> 0.5	**7a**		100	5.287	0.052
3b		25	1.305	0.052	**7b**		200	1.144	0.005
3c		25	3.261	0.130	**7c**		12.5	1.41	0.112
3d		12.5	> 100	> 8	**7d**		25	1.713	0.068
3e		12.5	> 100	> 8	**7e**		25	2.374	0.094
3f		50	3.747	0.074	**7f**		50	2.008	0.040
3g		25	5.296	0.211	**7g**		> 200	2.03	0.010
3h		25	7.096	0.283	**7h**		50	11.55	0.231
3i		12.5	> 100	> 8	**7i**		25	2.074	0.082
3j		25	> 100	> 4	**7j**		> 200	> 100	> 0.5
4a		50	61.33	1.226	**8a**		12.5	2.138	0.171
4b		12.5	> 100	> 8	**8b**		12.5	5.41	0.432
4c		6.25	17.35	2.776	**8c**		12.5	1.51	0.120
4d		6.25	> 100	> 16	**8d**		12.5	22.84	1.827
4e		50	> 100	> 2	**8e**		50	> 100	2

(*continued on next page*)

Table 1 (*continued*)

Code	Structure	MIC_{99} [a]	IC_{50} [b]	SI [c]	Code	Structure	MIC_{99} [a]	IC_{50} [b]	SI [c]
4f		50	13.04	0.260	**8f**		100	6.369	0.063
4g		50	34.58	0.691	**8g**		50	46.23	0.924
4h		25	6.434	0.257	**8h**		50	11.11	0.222
4i		12.5	> 100	> 8	**8i**		50	27.83	0.556
4j		12.5	> 100	> 8	**8j**		200	> 100	0.5
4k		100	23.97	0.239	**8k**		200	5.852	0.029
4l		50	35.23	0.704	**8l**		200	7.007	0.035
4m		25	17.81	0.712	**8m**		200	6.023	0.030
4n		12.5	7.684	0.614	**8n**		200	9.594	0.047
4o		12.5	> 100	8.416	**8o**		200	6.009	0.030
INH [d]		0.01	> 100						

[a] MIC_{99} – Minimum inhibitory concentration (in μg/mL).
[b] IC_{50} – Inhibitory concentration or Cytotoxicity (in μg/mL).
[c] SI- Selectivity index (IC_{50}/MIC).
[d] INH-Reference drug (Isoniazid).

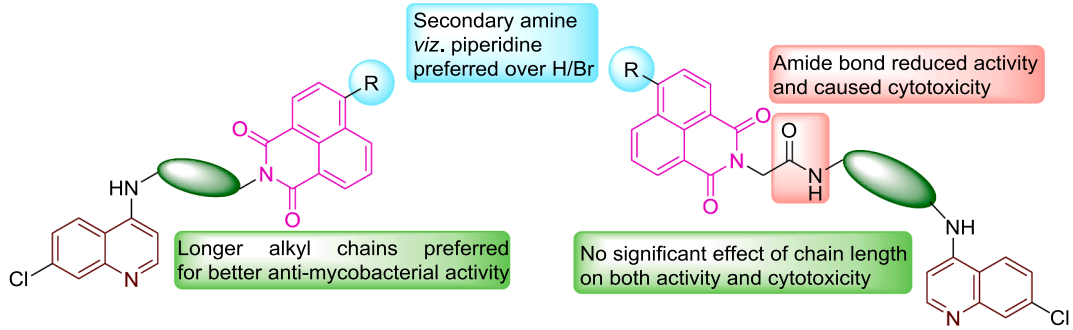


Fig. 2. Diagrammatic representation of SAR of alkyl chain versus amide bond tethered 4-substituted 1,8-naphthalimide-7-chloroquinoline hybrids.

*3.2.5. 2-(8-((7-chloroquinolin-4-yl)amino)octyl)-1H-benzo[de] isoquinoline-1,3(2H)-dione (**3e**)*

Yield-88%, grey solid, M.P = 105–110 °C, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.31 (s, 8H, $(CH_2)_4$), 1.61–1.62 (m, 4H, $(CH_2)_2$), 3.21–3.22 (m, 2H, CH_2), 4.00 (t, J = 7.2 Hz, 2H, CH_2), 6.42 (d, J = 5.4 Hz, 1H, H^2), 7.34 (s, 1H, NH- exchangeable with D_2O), 7.41 (dd, J = 1.7, 8.8 Hz, 1H, H^4), 7.75 (s, 1H, H^5), 7.80–7.83 (m, 2H, H^7 + H^{10}), 8.26 (d, J = 9.0 Hz, 1H, H^3), 8.36 (d, J = 5.1 Hz, 1H, H^1), 8.39–8.45 (m, 4H, H^6 + H^8 + H^9 + H^{11}). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 163.77, 151.90, 150.75, 149.03, 134.68, 133.97, 131.70, 131.11, 127.74, 127.60, 127.50, 124.61, 124.48, 122.44, 117.79, 99.00, 42.90, 29.17, 29.15, 28.19, 27.89, 27.05, 26.94. HRMS calcd for $C_{29}H_{28}ClN_3O_2$ $[M + 1]^+$ 486.1870, found 486.1961.

*3.2.6. 6-bromo-2-(2-((7-chloroquinolin-4-yl)amino)ethyl)-1H-benzo[de] isoquinoline-1,3(2H)-dione (**3f**)*

Yield-84%, pale yellow solid, M.P = 240–245 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.56–3.58 (m, 2H, CH_2), 4.20 (t, J = 6.5 Hz, 2H, CH_2), 6.62 (d, J = 6.4 Hz, 1H, H^2), 7.56 (dd, J = 1.9, 9.0 Hz, 1H, H^4), 7.89 (d, J = 1.8 Hz, 1H, H^5), 7.92 (dd, J = 8.0, 7.9 Hz, 1H, H^9), 8.12 (d, J = 7.6 Hz, 1H, H^7), 8.25 (d, J = 7.5 Hz, 1H, H^6), 8.40 (d, J = 6.3 Hz, 1H, H^1), 8.45–8.50 (m, 3H, H^3 + H^8 + H^{10}), 8.65 (s, 1H, NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 163.20, 163.15, 153.10, 146.40, 137.10, 133.10, 132.45, 132.10, 131.45, 131.20, 130.15, 129.24, 128.98, 128.65, 125.90, 125.35, 123.50, 122.03, 121.70, 116.23, 98.90, 40.75, 38.20. HRMS calcd for $C_{23}H_{15}BrClN_3O_2$ $[M + 1]^+$ 480.0036, found 480.0136 and $[M + 3]^+$ 482.0036, found 482.0146.

*3.2.7. 6-bromo-2-(3-((7-chloroquinolin-4-yl)amino)propyl)-1H-benzo[de] isoquinoline-1,3(2H)-dione (**3g**)*

Yield-83%, peach white solid, M.P = 115–120 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.98–2.01 (m, 2H, CH_2), 3.40–3.43 (m, 2H, CH_2), 4.10 (t, J = 6.7 Hz, 2H, CH_2), 6.61 (d, J = 6.5 Hz, 1H, H^2), 7.55 (dd, J = 2.0, 9.1 Hz, 1H, H^4), 7.88 (d, J = 1.9 Hz, 1H, H^5), 7.91 (dd, J = 7.8, 8.0 Hz, 1H, H^9), 8.11 (d, J = 7.5 Hz, 1H, H^7), 8.24 (d, J = 7.4 Hz, 1H, H^6), 8.41 (d, J = 6.4 Hz, 1H, H^1), 8.44–8.49 (m, 3H, H^3 + H^8 + H^{10}), 8.64 (s, 1H, NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 163.96, 163.90, 152.45, 145.10, 136.65, 133.31, 132.35, 132.01, 131.10, 131.35, 130.10, 129.15, 128.10, 127.98, 125.92, 125.45, 123.40, 122.15, 121.68, 115.85, 99.10, 40.92, 38.30, 25.99. HRMS calcd for $C_{24}H_{17}BrClN_3O_2$ $[M + 1]^+$ 494.0193, found 494.0343 and $[M + 3]^+$ 496.0193, found 496.0353.

*3.2.8. 6-bromo-2-(4-((7-chloroquinolin-4-yl)amino)butyl)-1H-benzo[de] isoquinoline-1,3(2H)-dione (**3h**)*

Yield-85%, Off-white solid, M.P = 240–245 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.70–1.75 (m, 4H, $(CH_2)_2$), 3.41–3.43 (m, 2H, CH_2), 4.05 (t, J = 6.5 Hz, 2H, CH_2), 6.67 (d, J = 6.5 Hz, 1H, H^2), 7.60 (dd, J = 1.8, 9.0 Hz, 1H, H^4), 7.89 (d, J = 1.9 Hz, 1H, H^5), 7.95 (dd, J = 8.0, 8.1 Hz, 1H, H^9), 8.17 (d, J = 7.6 Hz, 1H, H^7), 8.27 (d, J = 7.5 Hz, 1H, H^6), 8.40 (d, J = 6.4 Hz, 1H, H^1), 8.45–8.50 (m, 3H, H^3 + H^8 + H^{10}), 8.68 (s, 1H, NH- exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 163.21, 163.10, 153.59, 146.26, 135.71, 133.20, 132.12, 131.42, 131.25, 131.10, 130.10, 129.72, 129.15, 127.74, 125.98, 125.62, 122.63, 121.65, 121.36, 116.42, 99.05, 43.06, 25.89, 25.21. HRMS calcd for $C_{25}H_{19}BrClN_3O_2$ $[M + 1]^+$ 508.0349, found 508.0459 and $[M + 3]^+$ 510.0349, found 510.0449.

*3.2.9. 6-bromo-2-(6-((7-chloroquinolin-4-yl)amino)hexyl)-1H-benzo[de] isoquinoline-1,3(2H)-dione (**3i**)*

Yield-84%, Off-white solid, M.P = 220–225 °C, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.41–1.45 (m, 4H, $(CH_2)_2$), 1.64–1.70 (m, 4H, $(CH_2)_2$), 3.39–3.43 (m, 2H, CH_2), 4.02 (t, J = 7.3 Hz, 2H, CH_2), 6.69 (d, J = 6.45 Hz, 1H, H^2), 7.60 (dd, J = 1.7, 9.1 Hz, 1H, H^4), 7.90 (d, J = 1.8 Hz, 1H, H^5), 7.96 (dd, J = 7.9, 7.9 Hz, 1H, H^9), 8.18 (d, J = 7.8 Hz, 1H, H^7), 8.28 (d, J = 7.8 Hz, 1H, H^6), 8.44 (d, J = 6.2 Hz, 1H, H^1), 8.46–8.52 (m, 3H, H^3 + H^8 + H^{10}), 8.69 (s, 1H, NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 163.32, 163.27, 153.86, 146.44, 136.68, 133.04, 131.99, 131.80, 131.39, 131.13, 130.25, 129.55, 129.24, 128.74, 126.16, 125.57, 123.20, 122.46, 122.43, 116.57, 99.01, 43.33, 27.94, 27.80, 26.70, 26.62. HRMS calcd for $C_{27}H_{23}BrClN_3O_2$ $[M + 1]^+$ 536.0662, found 536.0802 and $[M + 3]^+$ 538.0662, found 538.0782.

*3.2.10. 6-bromo-2-(8-((7-chloroquinolin-4-yl)amino)octyl)-1H-benzo[de] isoquinoline-1,3(2H)-dione (**3j**)*

Yield-83%, light brown solid, M.P = 100–105 °C, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.33 (s, 8H, $(CH_2)_4$), 1.62–1.66 (m, 4H, $(CH_2)_2$), 3.39–3.40 (m, 2H, CH_2), 3.99 (t, J = 7.0 Hz, 2H, CH_2), 6.69 (d, J = 6.4 Hz, 1H, H^2), 7.60 (d, J = 8.9 Hz, 1H, H^4), 7.90 (s, 1H, H^5), 7.96 (dd, J = 8.0, 7.6 Hz, 1H, H^9), 8.17 (d, J = 7.8 Hz, 1H, H^7), 8.28 (d, J = 7.8 Hz, 1H, H^6), 8.44–8.52 (m, 4H, H^1 + H^3 + H^8 + H^{10}), 8.67 (s, 1H, NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 163.29, 163.24, 153.85, 146.48, 136.67, 133.02, 131.99, 131.80, 131.39, 131.13, 130.25, 129.54, 129.23, 128.73, 127.64, 126.14, 125.58, 123.19, 122.42, 116.58, 98.98, 43.37, 40.23, 29.10, 29.05, 28.07, 27.81, 26.89, 26.85. HRMS calcd for $C_{29}H_{27}BrClN_3O_2$ $[M + 1]^+$ 564.0975, found 564.1075 and $[M + 3]^+$ 566.0975, found 566.1075.

*3.3. Procedure for synthesis of conjugates **4a-o***

To a well stirred solution of **3** (for **3f-j**, when R = Br) (1 mmol) in anhydrous NMP (1–2 mL), was added different secondary amines (2 mmol) (piperidine/morpholine/hydroxyethyl piperazine) and the reaction mixture was heated at 180 °C for 1 h. On completion of the reaction, as monitored from TLC, ice cold water was added to the reaction mixture. The solid got precipitated out of the reaction mixture which was then filtered, washed with water and recrystallized in ethanol to afford the desired compounds **4a-o**.

*3.3.1. 2-(2-((7-chloroquinolin-4-yl)amino)ethyl)-6-(piperidin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4a**)*

Yield-78%, green solid, M.P > 250 °C, ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.62–1.70 (m, 2H, CH_2-**CH_2**-CH_2), 1.78–1.88 (m, 4H, **CH_2**-CH_2-**CH_2**), 3.15–3.18 (m, 4H, **CH_2**-CH_2-**CH_2**), 3.58–3.60 (m, 2H, CH_2), 4.40 (t, J = 7.1 Hz, 2H, CH_2), 5.90 (s, 1H, NH-exchangeable with D_2O), 6.30 (d, J = 5.3 Hz, 1H, H^2), 7.05 (d, J = 8.0 Hz, 1H, H^7), 7.21 (dd, J = 1.8, 8.8 Hz, 1H, H^4), 7.58 (dd, J = 7.8, 8.1 Hz, 1H, H^9), 7.80–7.84 (m, 2H, H^3 + H^5), 8.30 (d, J = 8.1 Hz, 1H, H^6), 8.35 (d, J = 5.1 Hz, 1H, H^1), 8.40 (d, J = 8.0 Hz, 1H, H^{10}), 8.45 (d, J = 7.6 Hz, 1H, H^8). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 165.01, 164.79, 158.19, 150.70, 150.54, 135.36, 132.91, 131.33, 131.23, 130.85, 129.88, 127.52, 126.33, 125.56, 125.35, 122.80, 121.76, 117.10, 115.32, 114.72, 98.65, 54.45, 40.62, 38.16, 26.30, 24.27. HRMS calcd for $C_{28}H_{25}ClN_4O_2$ $[M + 1]^+$ 485.1666, found 485.1756.

*3.3.2. 2-(3-((7-chloroquinolin-4-yl)amino)propyl)-6-(piperidin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4b**)*

Yield-76%, pale yellow solid, M.P = 190–195 °C, ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.70–1.73 (m, 2H, CH_2-**CH_2**-CH_2), 1.85–1.91 (m, 4H, **CH_2**-CH_2-**CH_2**), 2.11–2.17 (m, 2H, CH_2), 3.23–3.25 (m, 4H, **CH_2**-CH_2-**CH_2**), 3.35–3.40 (m, 2H, CH_2), 4.32 (t, J = 7.7 Hz, 2H, CH_2), 6.35 (s, 1H, NH-exchangeable with D_2O), 6.40 (d, J = 6.7 Hz, 1H, H^2), 7.16 (d, J = 10.0 Hz, 1H, H^7), 7.37 (dd, J = 2.7, 11.1 Hz, 1H, H^4), 7.68 (dd, J = 9.1, 10.5 Hz, 1H, H^9), 7.90 (d, J = 2.6 Hz, 1H, H^5), 7.97 (d, J = 11.2 Hz, 1H, H^3), 8.39 (d, J = 10.5 Hz, 1H, H^6), 8.47 (d, J = 6.8 Hz, 1H, H^1), 8.51 (d, J = 10.1 Hz, 1H, H^{10}), 8.59 (d, J = 9.1 Hz, 1H, H^8). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 165.28, 164.79, 157.90, 151.98, 149.92, 134.87, 133.29, 131.53, 131.27, 130.11, 128.52, 127.17, 126.25, 125.49, 125.34, 122.73, 121.76, 117.56, 115.24, 114.82, 98.67, 54.64, 39.71, 37.39, 26.64, 26.27, 24.40. HRMS calcd for

$C_{29}H_{27}ClN_4O_2$ [M + 1]$^+$ 499.1823, found 499.1840.

*3.3.3. 2-(4-((7-chloroquinolin-4-yl)amino)butyl)-6-(piperidin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione **(4c)***

Yield-77%, pale yellow solid, M.P = 125–130 °C, ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.65–1.68 (m, 2H, CH_2-**CH_2**-CH_2), 1.78–1.87 (m, 8H, **CH_2**-CH_2-**CH_2** + $(CH_2)_2$), 3.16–3.18 (m, 4H, **CH_2**-CH_2-**CH_2**), 3.37–3.40 (m, 2H, CH_2), 4.20 (t, *J* = 7.0 Hz, 2H, CH_2), 5.91 (s, 1H, NH-exchangeable with D_2O), 6.33 (d, *J* = 5.5 Hz, 1H, H^2), 7.10 (d, *J* = 8.1 Hz, 1H, H^7), 7.25 (dd, *J* = 1.9, 8.9 Hz, 1H, H^4), 7.61 (dd, *J* = 7.5, 8.1 Hz, 1H, H^9), 7.83–7.85 (m, 2H, H^3 + H^5), 8.32 (d, *J* = 8.3 Hz, 1H, H^6), 8.37 (d, *J* = 5.5 Hz, 1H, H^1), 8.42 (d, *J* = 8.1 Hz, 1H, H^{10}), 8.49 (d, *J* = 7.1 Hz, 1H, H^8). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 164.83, 164.34, 157.61, 150.72, 150.54, 135.29, 132.90, 131.38, 131.19, 130.95, 129.96, 127.47, 126.21, 125.42, 125.38, 122.86, 121.81, 117.03, 115.48, 114.74, 98.80, 54.55, 42.94, 39.36, 26.21, 25.80, 25.35, 24.33. HRMS calcd for $C_{30}H_{29}ClN_4O_2$ [M + 1]$^+$ 513.1979, found 513.1991.

*3.3.4. 2-(6-((7-chloroquinolin-4-yl)amino)hexyl)-6-(piperidin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione **(4d)***

Yield-76%, light brown solid, M.P = 105–110 °C, ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.40–1.44 (m, 4H, $(CH_2)_2$), 1.65–1.67 (m, 6H, CH_2-**CH_2**-CH_2 + $(CH_2)_2$), 1.85–1.90 (m, 4H, **CH_2**-CH_2-**CH_2**), 3.23–3.25 (m, 4H, **CH_2**-CH_2-**CH_2**), 3.30–3.32 (m, 2H, CH_2), 4.10 (t, *J* = 7.4 Hz, 2H, CH_2), 4.48 (s, 1H, NH-exchangeable with D_2O), 6.38 (d, *J* = 5.2 Hz, 1H, H^2), 7.12 (d, *J* = 8.0 Hz, 1H, H^7), 7.31 (dd, *J* = 1.9, 8.9 Hz, 1H, H^4), 7.61–7.68 (m, 2H, H^3 + H^9), 7.90 (d, *J* = 1.8 Hz, 1H, H^5), 8.46 (d, *J* = 8.2 Hz, 1H, H^6), 8.49 (d, *J* = 8.1 Hz, 1H, H^{10}), 8.52 (d, *J* = 5.1 Hz, 1H, H^1), 8.56 (d, *J* = 7.0 Hz, 1H, H^8). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.75, 164.10, 157.30, 152.15, 134.76, 132.52, 131.10, 130.90, 130.50, 129.90, 128.65, 126.93, 126.81, 125.42, 125.30, 123.15, 120.56, 117.25, 115.85, 114.69, 99.10, 54.60, 43.35, 40.65, 27.92, 27.76, 26.70, 26.50, 26.25, 24.50. HRMS calcd for $C_{32}H_{33}ClN_4O_2$ [M + 1]$^+$ 541.2292, found 541.2235.

*3.3.5. 2-(8-((7-chloroquinolin-4-yl)amino)octyl)-6-(piperidin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione **(4e)***

Yield-75%, dark green solid, M.P = 95–100 °C, ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.44–1.48 (m, 8H, $(CH_2)_4$), 1.74–1.79 (m, 6H, CH_2-**CH_2**-CH_2 + $(CH_2)_2$), 1.89–1.91 (m, 4H, **CH_2**-CH_2-**CH_2**), 3.24 (s, 4H, **CH_2**-CH_2-**CH_2**), 3.30–3.34 (m, 2H, CH_2), 4.18 (t, *J* = 7.5 Hz, 2H, CH_2), 5.07 (s, 1H, NH- exchangeable with D_2O), 6.43 (d, *J* = 5.3 Hz, 1H, H^2), 7.18 (d, *J* = 8.1 Hz, 1H, H^7), 7.36 (dd, *J* = 2.0, 8.8 Hz, 1H, H^4), 7.67–7.71 (m, 2H, H^3 + H^9), 7.97 (d, *J* = 1.7 Hz, 1H, H^5), 8.40 (d, *J* = 8.4 Hz, 1H, H^6), 8.50 (d, *J* = 8.1 Hz, 1H, H^{10}), 8.54 (d, *J* = 5.3 Hz, 1H, H^1), 8.58 (d, *J* = 7.1 Hz, 1H, H^8). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 164.63, 164.16, 157.32, 152.02, 134.79, 132.64, 131.17, 130.98, 130.60, 129.93, 128.77, 126.93, 126.29, 125.35, 125.22, 123.16, 120.88, 117.13, 115.95, 114.71, 99.08, 54.55, 43.23, 40.15, 29.01, 28.98, 28.76, 27.93, 26.95, 26.86, 26.23, 24.34. HRMS calcd for $C_{34}H_{37}ClN_4O_2$ [M + 1]$^+$ 569.2605, found 569.2698.

*3.3.6. 2-(2-((7-chloroquinolin-4-yl)amino)ethyl)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione **(4f)***

Yield-75%, dark green solid, M.P = 165–170 °C, ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.24–3.26 (m, 4H, CH_2-N-CH_2), 3.64–3.67 (m, 2H, CH_2), 3.98–4.00 (m, 4H, CH_2-O-CH_2), 4.64–4.67 (m, 2H, CH_2), 6.36 (d, *J* = 5.4 Hz, 1H, H^2), 6.49 (s, 1H, NH-exchangeable with D_2O), 7.21 (d, *J* = 8.0 Hz, 1H, H^7), 7.36 (dd, *J* = 1.8, 8.9 Hz, 1H, H^4), 7.68–7.76 (m, 2H, H^3 + H^9), 7.86 (s, 1H, H^5), 8.41 (d, *J* = 8.4 Hz, 1H, H^6), 8.44 (d, *J* = 5.5 Hz, 1H, H^1), 8.56 (d, *J* = 8.1 Hz, 1H, H^{10}), 8.62 (d, *J* = 7.3 Hz, 1H, H^8). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.58, 164.21, 156.10, 151.46, 149.95, 134.85, 133.10, 131.42, 130.40, 129.67, 128.30, 127.10, 126.23, 125.89, 125.15, 122.85, 121.64, 117.71, 116.25, 115.10, 98.52, 66.45, 52.98, 41.01, 37.90. HRMS calcd for $C_{27}H_{23}ClN_4O_3$ [M + 1]$^+$ 487.1459, found 487.1525.

*3.3.7. 2-(3-((7-chloroquinolin-4-yl)amino)propyl)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione **(4g)***

Yield-75%, light green solid, M.P = 135–140 °C, ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.18–2.20 (m, 2H, CH_2), 3.31 (s, 4H, CH_2-N-CH_2), 3.42–3.43 (m, 2H, CH_2), 4.05 (s, 4H, CH_2-O-CH_2), 4.37 (t, *J* = 6.0 Hz, 2H, CH_2), 6.33 (s, 1H, NH-exchangeable with D_2O), 6.43 (d, *J* = 5.4 Hz, 1H, H^2), 7.26 (d, *J* = 8.1 Hz, 1H, H^7), 7.39 (d, *J* = 9.0 Hz, 1H, H^4), 7.75 (dd, *J* = 7.9, 7.8 Hz, 1H, H^9), 7.92 (s, 1H, H^5), 7.97 (d, *J* = 9.0 Hz, 1H, H^3), 8.46 (d, *J* = 8.4 Hz, 1H, H^6), 8.50 (d, *J* = 5.3 Hz, 1H, H^1), 8.59 (d, *J* = 8.0 Hz, 1H, H^{10}), 8.64 (d, *J* = 7.2 Hz, 1H, H^8). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 165.01, 164.53, 156.15, 151.75, 149.90, 134.89, 133.03, 131.58, 130.59, 129.95, 128.32, 127.08, 126.10, 125.92, 125.30, 122.90, 121.59, 117.42, 116.53, 115.01, 98.64, 66.93, 53.47, 39.80, 37.46, 26.60. HRMS calcd for $C_{28}H_{25}ClN_4O_3$ [M + 1]$^+$ 501.1615, found 501.4631.

*3.3.8. 2-(4-((7-chloroquinolin-4-yl)amino)butyl)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione **(4 h)***

Yield-74%, light green solid, M.P = 130–135 °C, ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.67–1.71 (m, 4H, $(CH_2)_2$), 3.12 (s, 4H, CH_2-N-CH_2), 3.24–3.25 (m, 2H, CH_2), 3.84 (s, 4H, CH_2-O-CH_2), 4.10 (t, *J* = 5.6 Hz, 2H, CH_2), 6.10 (s, 1H, NH- exchangeable with D_2O), 6.40 (d, *J* = 5.6 Hz, 1H, H^2), 7.25 (d, *J* = 8.2 Hz, 1H, H^7), 7.35 (d, *J* = 8.9 Hz, 1H, H^4), 7.72 (dd, *J* = 7.9, 8.0 Hz, 1H, H^9), 7.90 (s, 1H, H^5), 7.95 (d, *J* = 9.0 Hz, 1H, H^3), 8.45 (d, *J* = 8.3 Hz, 1H, H^6), 8.50 (d, *J* = 5.5 Hz, 1H, H^1), 8.55 (d, *J* = 8.1 Hz, 1H, H^{10}), 8.61 (d, *J* = 7.8 Hz, 1H, H^8). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.06, 163.53, 155.85, 151.74, 150.83, 148.79, 134.09, 132.61, 131.11, 130.94, 129.48, 127.36, 126.50, 125.64, 124.61, 124.60, 122.94, 117.73, 116.21, 115.44, 98.08, 66.70, 53.51, 42.70, 25.84, 25.81. HRMS calcd for $C_{29}H_{27}ClN_4O_3$ [M + 1]$^+$ 515.1772, found 515.1872.

*3.3.9. 2-(6-((7-chloroquinolin-4-yl)amino)hexyl)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione **(4i)***

Yield-74%, yellow solid, M.P = 90–95 °C, ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.40–1.44 (m, 4H, $(CH_2)_2$), 1.65–1.67 (m, 4H, $(CH_2)_2$), 3.21–3.25 (m, 6H, CH_2-N-CH_2 + CH_2), 3.91 (s, 4H, CH_2-O-CH_2), 4.05 (t, *J* = 6.1 Hz, 2H, CH_2), 6.32 (s, 1H, NH-exchangeable with D_2O), 6.40 (d, *J* = 5.5 Hz, 1H, H^2), 7.21 (d, *J* = 8.1 Hz, 1H, H^7), 7.32 (d, *J* = 8.8 Hz, 1H, H^4), 7.65–7.71 (m, 2H, H^3 + H^9), 7.93 (s, 1H, H^5), 8.42 (d, *J* = 8.2 Hz, 1H, H^6), 8.51–8.54 (m, 2H, H^1 + H^{10}), 8.62 (d, *J* = 7.3 Hz, 1H, H^8). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.16, 163.95, 155.60, 151.83, 150.61, 147.96, 134.45, 132.60, 131.15, 130.90, 129.45, 127.10, 126.17, 125.60, 124.75, 124.56, 122.83, 117.95, 116.18, 115.21, 99.10, 66.65, 53.50, 42.81, 39.85, 28.12, 28.07, 26.83, 26.80. HRMS calcd for $C_{31}H_{31}ClN_4O_3$ [M + 1]$^+$ 543.2085, found 543.2205.

*3.3.10. 2-(8-((7-chloroquinolin-4-yl)amino)octyl)-6-morpholino-1H-benzo[de]isoquinoline-1,3(2H)-dione **(4j)***

Yield-72%, light brown solid, M.P = 85–90 °C, ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.44–1.47 (m, 8H, $(CH_2)_4$), 1.76–1.79 (m, 4H, $(CH_2)_2$), 3.27–3.29 (m, 4H, CH_2-N-CH_2), 3.30–3.34 (m, 2H, CH_2), 4.03–4.04 (m, 4H, CH_2-O-CH_2), 4.18 (t, *J* = 7.6 Hz, 2H, CH_2), 5.08 (s, 1H, NH-exchangeable with D_2O), 6.43 (d, *J* = 5.3 Hz, 1H, H^2), 7.24 (d, *J* = 8.0 Hz, 1H, H^7), 7.36 (d, *J* = 8.9 Hz, 1H, H^4), 7.69–7.73 (m, 2H, H^3 + H^9), 7.96 (s, 1H, H^5), 8.44 (d, *J* = 8.3 Hz, 1H, H^6), 8.53–8.55 (m, 2H, H^1 + H^{10}), 8.60 (d, *J* = 7.3 Hz, 1H, H^8). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 164.44, 163.99, 155.63, 151.95, 134.83, 133.90, 132.49, 131.14, 130.04, 129.87, 128.72, 126.93, 126.16, 125.84, 125.24, 123.36, 120.89, 117.20, 117.11, 114.96, 99.07, 66.96, 53.45, 43.23, 40.22, 29.01, 28.99, 28.76, 27.92, 26.96, 26.86. HRMS calcd for $C_{33}H_{35}ClN_4O_3$ [M + 1]$^+$ 571.2398, found 571.2488.

*3.3.11. 2-(2-((7-chloroquinolin-4-yl)amino)ethyl)-6-(4-(2-hydroxyethyl) piperazin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4 k**)*

Yield-70%, dark green solid, M.P > 250 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.54 (t, J = 5.6 Hz, 2H, **CH$_2$**-CH$_2$-OH), 2.74 (s, 4H, CH$_2$-N-CH$_2$), 3.25 (s, 4H, CH$_2$-N-CH$_2$), 3.57 (t, J = 5.8 Hz, 2H, CH$_2$-**CH$_2$**-OH), 3.60–3.62 (m, 2H, CH$_2$), 4.27 (t, J = 6.5 Hz, 2H, CH$_2$), 4.50 (s, 1H, OH– exchangeable with D$_2$O), 6.35 (d, J = 5.6 Hz, 1H, H^2), 7.20–7.25 (m, 2H, H^7 + NH- exchangeable with D$_2$O), 7.35 (dd, J = 2.1, 9.0 Hz, 1H, H^4), 7.71 (d, J = 2.0 Hz, 1H, H^5), 7.73 (dd, J = 7.6, 8.1 Hz, 1H, H^9), 8.21 (d, J = 8.9 Hz, 1H, H^3), 8.25–8.28 (m, 2H, H^1 + H^6), 8.35 (d, J = 8.2 Hz, 1H, H^{10}), 8.40 (d, J = 7.3 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 164.81, 164.35, 156.12, 151.75, 150.20, 134.72, 132.65, 131.18, 130.46, 129.75, 128.24, 126.96, 126.10, 125.26, 124.90, 122.74, 121.85, 117.30, 116.30, 114.15, 99.10, 60.56, 59.15, 53.61, 53.10, 40.95, 37.80. HRMS calcd for $C_{29}H_{28}ClN_5O_3$ [M + 1]$^+$ 530.1881, found 530.1990.

*3.3.12. 2-(3-((7-chloroquinolin-4-yl)amino)propyl)-6-(4-(2-hydroxyethyl)piperazin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4 l**)*

Yield-70%, yellow solid, M.P = 145–150 °C, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 2.04–2.06 (m, 2H, CH$_2$), 2.53–2.54 (m, 2H, **CH$_2$**-CH$_2$-OH), 2.74 (s, 4H, CH$_2$-N-CH$_2$), 3.21 (s, 4H, CH$_2$-N-CH$_2$), 3.39–3.41 (m, 2H, CH$_2$), 3.58 (t, J = 5.9 Hz, 2H, CH$_2$-**CH$_2$**-OH), 4.17 (t, J = 6.8 Hz, 2H, CH$_2$), 4.50 (s, 1H, OH– exchangeable with D$_2$O), 6.44 (d, J = 5.4 Hz, 1H, H^2), 7.25 (d, J = 8.2 Hz, 1H, H^7), 7.28 (t, J = 5.1 Hz, 1H, NH-exchangeable with D$_2$O), 7.35 (dd, J = 2.0, 8.9 Hz, 1H, H^4), 7.73–7.77 (m, 2H, H^5 + H^9), 8.13 (d, J = 9.0 Hz, 1H, H^3), 8.31 (d, J = 8.1 Hz, 1H, H^6), 8.35 (d, J = 8.5 Hz, 1H, H^1), 8.38 (d, J = 8.5 Hz, 1H, H^{10}), 8.41 (d, J = 6.9 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 164.14, 163.61, 156.12, 152.27, 150.38, 149.42, 134.69, 133.74, 132.61, 131.02, 129.57, 127.83, 126.31, 125.67, 124.38, 124.29, 122.99, 117.88, 115.86, 115.31, 99.11, 60.66, 59.06, 53.60, 53.13, 40.85, 38.17, 26.98. HRMS calcd for $C_{30}H_{30}ClN_5O_3$ [M + 1]$^+$ 544.2037, found 544.1860.

*3.3.13. 2-(4-((7-chloroquinolin-4-yl)amino)butyl)-6-(4-(2-hydroxyethyl) piperazin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4m**)*

Yield-68%, brown solid, M.P = 140–145 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.68–1.72 (m, 4H, (CH$_2$)$_2$), 2.55 (t, J = 5.5 Hz, 2H, **CH$_2$**-CH$_2$-OH), 2.75 (s, 4H, CH$_2$-N-CH$_2$), 3.20 (s, 4H, CH$_2$-N-CH$_2$), 3.30–3.35 (m, 2H, CH$_2$), 3.55 (t, J = 5.8 Hz, 2H, CH$_2$-**CH$_2$**-OH), 4.10 (t, J = 5.1 Hz, 2H, CH$_2$), 4.50 (s, 1H, OH– exchangeable with D$_2$O), 6.35 (d, J = 5.6 Hz, 1H, H^2), 7.21–7.25 (m, 2H, H^7 + NH-exchangeable with D$_2$O), 7.35 (dd, J = 2.1, 9.0 Hz, 1H, H^4), 7.71–7.75 (m, 2H, H^5 + H^9), 8.21 (d, J = 9.1 Hz, 1H, H^3), 8.25–8.30 (m, 2H, H^1 + H^6), 8.35 (d, J = 8.3 Hz, 1H, H^{10}), 8.40 (d, J = 7.5 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 164.61, 164.12, 156.09, 151.69, 150.10, 134.67, 132.68, 131.17, 130.53, 129.81, 128.17, 126.95, 126.04, 125.65, 124.97, 122.94, 121.94, 117.29, 116.31, 114.91, 98.84, 59.56, 58.02, 53.21, 53.01, 42.80, 39.46, 25.73, 25.44. HRMS calcd for $C_{31}H_{32}ClN_5O_3$ [M + 1]$^+$ 558.2194, found 558.2113.

*3.3.14. 2-(6-((7-chloroquinolin-4-yl)amino)hexyl)-6-(4-(2-hydroxyethyl) piperazin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4n**)*

Yield-68%, brown solid, M.P = 85–90 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.36 (s, 4H, (CH$_2$)$_2$), 1.57–1.62 (m, 4H, (CH$_2$)$_2$), 2.48–2.49 (m, 2H, **CH$_2$**-CH$_2$-OH), 2.68 (s, 4H, CH$_2$-N-CH$_2$), 3.16 (s, 6H, CH$_2$-N-CH$_2$ + CH$_2$), 3.53 (t, J = 6.0 Hz, 2H, CH$_2$-**CH$_2$**-OH), 3.97 (t, J = 7.1 Hz, 2H, CH$_2$), 4.46 (s, 1H, OH– exchangeable with D$_2$O), 6.37 (d, J = 5.4 Hz, 1H, H^2), 7.22–7.24 (m, 2H, H^7 + NH-exchangeable with D$_2$O), 7.36 (dd, J = 2.2, 8.9 Hz, 1H, H^4), 7.70–7.74 (m, 2H, H^5 + H^9), 8.20 (d, J = 9.0 Hz, 1H, H^3), 8.27–8.31 (m, 2H, H^1 + H^6), 8.34 (d, J = 8.4 Hz, 1H, H^{10}), 8.38 (d, J = 7.4 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 164.05, 163.52, 156.18, 152.40, 150.57, 149.57, 133.83, 132.70, 131.11, 131.10, 129.60, 127.94, 126.47, 125.75, 124.63, 124.45, 123.04, 117.94, 115.93, 115.44, 99.10, 60.73, 59.10, 53.67, 53.18, 42.85, 39.89 28.13, 28.02, 26.85, 26.83. HRMS calcd for $C_{33}H_{36}ClN_5O_3$ [M + 1]$^+$ 586.2507, found 586.2585.

*3.3.15. 2-(8-((7-chloroquinolin-4-yl)amino)octyl)-6-(4-(2-hydroxyethyl) piperazin-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4o**)*

Yield-67%, dark green solid, M.P = 85–90 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.35 (s, 8H, (CH$_2$)$_4$), 1.60–1.64 (m, 4H, (CH$_2$)$_2$), 2.46 (t, J = 5.8 Hz, 2H, **CH$_2$**-CH$_2$-OH), 2.70 (s, 4H, CH$_2$-N-CH$_2$), 3.15 (s, 4H, CH$_2$-N-CH$_2$), 3.37–3.39 (m, 2H, CH$_2$), 3.55 (t, J = 6.0 Hz, 2H, CH$_2$-**CH$_2$**-OH), 4.01 (t, J = 6.9 Hz, 2H, CH$_2$), 4.52 (s, 1H, OH– exchangeable with D$_2$O), 6.36 (d, J = 5.1 Hz, 1H, H^2), 7.21–7.25 (m, 2H, H^7 + NH-exchangeable with D$_2$O), 7.36 (dd, J = 2.1, 8.8 Hz, 1H, H^4), 7.71–7.75 (m, 2H, H^5 + H^9), 8.22 (d, J = 8.5 Hz, 1H, H^3), 8.25–8.31 (m, 2H, H^1 + H^6), 8.30 (d, J = 8.2 Hz, 1H, H^{10}), 8.35 (d, J = 7.1 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 163.94, 163.53, 155.76, 151.91, 150.15, 149.14, 133.72, 132.61, 131.45, 131.10, 129.15, 127.90, 126.45, 125.81, 125.01, 124.60, 123.10, 117.84, 115.90, 115.25, 98.91, 60.84, 59.15, 53.72, 53.20, 43.29, 40.25, 29.10, 28.96, 28.45, 27.96, 26.90, 26.56. HRMS calcd for $C_{35}H_{40}ClN_5O_3$ [M + 1]$^+$ 614.2820, found 614.2889.

*3.4. Procedure for synthesis of conjugates **7a-j***

The synthesis was initiated by heating 1,8-naphthalic anhydride **1**, with glycine **5** in dry DMF at 100 °C in the presence of triethyl amine as base. The product obtained **6** (1 mmol) was then reacted with quinoline diamine **2** (1 mmol) using coupling reagents EDC (1.1 eq), HOBt (1.2 eq) and DIEA (2 eq) in anhydrous DMF for 1 h. The reaction was monitored using TLC and after its completion, water was added to quench the reaction. The desired products precipitated out of the reaction mixture which was filtered, washed with water and recrystallized in ethanol to yield the desired conjugates.

*3.4.1. N-(2-((7-chloroquinolin-4-yl)amino)ethyl)-2-(1,3-dioxo-1H-benzo [de]isoquinolin-2(3H)-yl)acetamide (**7a**)*

Yield-90%, Off-white solid, M.P > 250 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.37 (s, 2H, CH$_2$), 3.62 (s, 2H, CH$_2$), 4.64 (s, 2H, CH$_2$), 6.51 (d, J = 5.4 Hz, 1H, H^2), 7.30 (s, 1H, NH- exchangeable with D$_2$O), 7.34 (dd, J = 2.2, 9.0 Hz, 1H, H^4), 7.74 (d, J = 2.2 Hz, 1H, H^5), 7.83–7.86 (m, 2H, H^7 + H^{10}), 8.09 (d, J = 9.1 Hz, 1H, H^3), 8.36 (d, J = 5.4 Hz, 1H, H^1), 8.44–8.46 (m, 5H, H^6 + H^8 + H^9 + H^{11} + NH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 168.01, 163.89, 152.47, 150.51, 149.53, 135.10, 133.91, 131.87, 131.40, 128.04, 128.02, 127.80, 124.64, 124.40, 122.47, 117.91, 99.16, 43.04, 42.53, 37.91. HRMS calcd for $C_{25}H_{19}ClN_4O_3$ [M + 1]$^+$ 459.1146, found 459.1198.

*3.4.2. N-(3-((7-chloroquinolin-4-yl)amino)propyl)-2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)acetamide (**7b**)*

Yield-90%, Off-white solid, M.P = 125–130 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.80–1.82 (m, 2H, CH$_2$), 3.18–3.20 (m, 2H, CH$_2$), 3.25–3.28 (m, 2H, CH$_2$), 4.62 (s, 2H, CH$_2$), 6.50 (d, J = 5.3 Hz, 1H, H^2), 7.27 (s, 1H, NH- exchangeable with D$_2$O), 7.35 (dd, J = 2.1, 8.9 Hz, 1H, H^4), 7.73 (d, J = 2.0 Hz, 1H, H^5), 7.82–7.85 (m, 2H, H^7 + H^{10}), 8.10 (d, J = 9.0 Hz, 1H, H^3), 8.37 (d, J = 5.3 Hz, 1H, H^1), 8.45–8.47 (m, 5H, H^6 + H^8 + H^9 + H^{11} + NH-exchangeable with D$_2$O). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 167.85, 163.31, 151.98, 150.40, 148.73, 135.01, 133.65, 131.87, 130.33, 128.10, 128.02, 127.80, 124.64, 124.40, 122.47, 116.91, 99.62, 41.85, 36.94, 28.31. HRMS calcd for $C_{26}H_{21}ClN_4O_3$ [M + 1]$^+$ 473.1302, found 473.1387.

*3.4.3. N-(4-((7-chloroquinolin-4-yl)amino)butyl)-2-(1,3-dioxo-1H-benzo [de]isoquinolin-2(3H)-yl)acetamide (**7c**)*

Yield-89%, Off-white solid, M.P = 125–130 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.51–1.55 (m, 2H, CH$_2$), 1.60–1.65 (m, 2H, CH$_2$), 3.10–3.14 (m, 2H, CH$_2$), 3.23–3.27 (m, 2H, CH$_2$), 4.61 (s, 2H, CH$_2$), 6.51 (d, J = 5.4 Hz, 1H, H^2), 7.29 (s, 1H, NH- exchangeable with D$_2$O),

7.34 (dd, J = 2.2, 9.0 Hz, 1H, H^4), 7.74 (d, J = 2.1 Hz, 1H, H^5), 7.83–7.85 (m, 2H, H^7 + H^{10}), 8.09 (d, J = 9.1 Hz, 1H, H^3), 8.35 (d, J = 5.3 Hz, 1H, H^1), 8.44–8.48 (m, 5H, H^6 + H^8 + H^9 + H^{11} + NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 168.10, 162.97, 152.47, 150.51, 149.53, 135.10, 133.59, 131.87, 131.40, 128.44, 128.02, 127.80, 124.64, 124.40, 123.47, 117.61, 98.45, 42.98, 42.50, 38.68, 27.25, 25.48. HRMS calcd for $C_{27}H_{23}ClN_4O_3$ $[M + 1]^+$ 487.1459, found 487.1501.

3.4.4. N-(6-((7-chloroquinolin-4-yl)amino)hexyl)-2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)acetamide (7d)

Yield-90%, Off-white solid, M.P = 230–235 °C, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.31–1.44 (m, 6H, $(CH_2)_3$), 1.62–1.68 (m, 2H, CH_2), 3.06–3.10 (m, 2H, CH_2), 3.22–3.26 (m, 2H, CH_2), 4.63 (s, 2H, CH_2), 6.44 (d, J = 5.5 Hz, 1H, H^2), 7.26 (t, J = 5.2 Hz, 1H, NH- exchangeable with D_2O), 7.40 (dd, J = 2.2, 9.0 Hz, 1H, H^4), 7.74 (d, J = 2.2 Hz, 1H, H^5), 7.84–7.87 (m, 2H, H^7 + H^{10}), 8.16 (t, J = 5.5 Hz, 1H, NH- exchangeable with D_2O), 8.25 (d, J = 9.0 Hz, 1H, H^3), 8.36 (d, J = 5.4 Hz, 1H, H^1), 8.46–8.48 (m, 4H, H^6 + H^8 + H^9 + H^{11}). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 166.90, 163.80, 152.33, 150.51, 149.52, 134.92, 133.75, 131.79, 131.20, 127.98, 127.89, 127.66, 124.54, 124.36, 122.49, 117.88, 99.03, 42.92, 42.74, 38.98, 29.50, 28.21, 26.70, 26.47. HRMS calcd for $C_{29}H_{27}ClN_4O_3$ $[M + 1]^+$ 515.1772, found 515.1825.

3.4.5. N-(8-((7-chloroquinolin-4-yl)amino)octyl)-2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)acetamide (7e)

Yield-89%, Off-white solid, M.P = 180–185 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.25–1.40 (m, 8H, $(CH_2)_4$), 1.62–1.64 (m, 4H, $(CH_2)_2$), 3.01–3.05 (m, 2H, CH_2), 3.21–3.25 (m, 2H, CH_2), 4.60 (s, 2H, CH_2), 6.50 (d, J = 5.3 Hz, 1H, H^2), 7.32 (s, 1H, NH- exchangeable with D_2O), 7.35 (dd, J = 2.1, 9.1 Hz, 1H, H^4), 7.74 (d, J = 2.0 Hz, 1H, H^5), 7.81–7.84 (m, 2H, H^7 + H^{10}), 8.10 (d, J = 9.0 Hz, 1H, H^3), 8.37 (d, J = 5.2 Hz, 1H, H^1), 8.45–8.47 (m, 5H, H^6 + H^8 + H^9 + H^{11} + NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 167.15, 162.80, 152.33, 151.75, 149.50, 134.82, 133.45, 131.79, 130.20, 127.98, 127.89, 127.10, 124.54, 124.35, 122.49, 117.72, 99.03, 42.95, 42.71, 39.10, 29.50, 29.22, 29.15, 28.25, 26.95, 26.68. HRMS calcd for $C_{31}H_{31}ClN_4O_3$ $[M + 1]^+$ 543.2085, found 543.2110.

3.4.6. 2-(6-bromo-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)-N-(2-((7-chloroquinolin-4-yl)amino)ethyl)acetamide (7f)

Yield-88%, light brown solid, M.P > 250 °C, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.36 (s, 4H, $(CH_2)_2$), 4.68 (s, 2H, CH_2), 6.55 (d, J = 5.4 Hz, 1H, H^2), 7.34 (s, 1H, NH- exchangeable with D_2O), 7.39 (dd, J = 1.5, 8.9 Hz, 1H, H^4), 7.78 (d, J = 1.7 Hz, 1H, H^5), 8.03 (dd, J = 7.7, 8.0 Hz, 1H, H^9), 8.12 (d, J = 9.0 Hz, 1H, H^3), 8.25 (d, J = 7.8 Hz, 1H, H^7), 8.34 (d, J = 7.8 Hz, 1H, H^6), 8.41 (d, J = 5.3 Hz, 1H, H^1), 8.47 (s, 1H, NH-exchangeable with D_2O), 8.58 (d, J = 7.2 Hz, 1H, H^{10}), 8.61 (d, J = 8.5 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 167.78, 163.32, 163.27, 152.21, 150.59, 149.25, 133.95, 133.41, 132.26, 131.94, 131.63, 130.41, 129.89, 129.39, 128.96, 127.77, 124.62, 124.33, 123.15, 122.36, 117.81, 99.11, 43.08, 42.55, 37.91. HRMS calcd for $C_{25}H_{18}BrClN_4O_3$ $[M + 1]^+$ 537.0251, found 537.0341 and $[M + 3]^+$ 539.0251, found 539.0346.

3.4.7. 2-(6-bromo-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)-N-(3-((7-chloroquinolin-4-yl)amino)propyl)acetamide (7g)

Yield-87%, light brown solid, M.P > 250 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.78–1.80 (m, 2H, CH_2), 3.15–3.18 (m, 2H, CH_2), 3.27–3.30 (m, 2H, CH_2), 4.54 (s, 2H, CH_2), 6.40 (d, J = 5.5 Hz, 1H, H^2), 7.31–7.35 (m, 2H, H^4 + NH-exchangeable with D_2O), 7.71 (d, J = 1.8 Hz, 1H, H^5), 7.90 (dd, J = 7.6, 8.2 Hz, 1H, H^9), 8.12 (d, J = 7.9 Hz, 1H, H^7), 8.17 (d, J = 9.0 Hz, 1H, H^3), 8.21–8.25 (m, 2H, H^6 + NH-exchangeable with D_2O), 8.28 (d, J = 5.4 Hz, 1H, H^1), 8.46–8.50 (m, 2H, H^8 + H^{10}). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 167.44, 164.15, 163.65, 157.32, 153.22, 150.51, 149.32, 134.86, 132.65, 131.19, 131.07, 129.81, 127.88, 126.35, 125.94, 124.61, 124.46, 123.03, 117.65, 115.45, 116.36, 98.75, 41.89, 36.96, 28.46. HRMS calcd for $C_{26}H_{20}BrClN_4O_3$ $[M + 1]^+$ 551.0407, found 551.0494 and $[M + 3]^+$ 553.0407, found 553.0487.

3.4.8. 2-(6-bromo-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)-N-(4-((7-chloroquinolin-4-yl)amino)butyl)acetamide (7h)

Yield-86%, light brown solid, M.P = 200–205 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.44–1.49 (m, 2H, CH_2), 1.55–1.60 (m, 2H, CH_2), 3.05–3.09 (m, 2H, CH_2), 3.18–3.23 (m, 2H, CH_2), 4.55 (s, 2H, CH_2), 6.41 (d, J = 5.6 Hz, 1H, H^2), 7.33–7.36 (m, 2H, H^4 + NH-exchangeable with D_2O), 7.70 (d, J = 1.9 Hz, 1H, H^5), 7.91 (dd, J = 7.7, 8.1 Hz, 1H, H^9), 8.13 (d, J = 7.8 Hz, 1H, H^7), 8.17 (d, J = 9.1 Hz, 1H, H^3), 8.20–8.23 (m, 2H, H^6 + NH-exchangeable with D_2O), 8.29 (d, J = 5.1 Hz, 1H, H^1), 8.45–8.48 (m, 2H, H^8 + H^{10}). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 167.15, 163.84, 163.25, 156.02, 152.19, 150.60, 133.85, 132.65, 131.14, 131.13, 129.62, 127.71, 126.56, 125.76, 124.87, 124.66, 124.47, 124.45, 123.05, 116.30, 115.51, 99.15, 42.87, 42.52, 38.70, 27.15, 25.42. HRMS calcd for $C_{27}H_{22}BrClN_4O_3$ $[M + 1]^+$ 565.0564, found 565.0656 and $[M + 3]^+$ 567.0564, found 567.0656.

3.4.9. 2-(6-bromo-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)-N-(6-((7-chloroquinolin-4-yl)amino)hexyl)acetamide (7i)

Yield-88%, light brown solid, M.P = 180–185 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.30–1.41 (m, 6H, $(CH_2)_3$), 1.60–1.62 (m, 2H, CH_2), 3.01–3.07 (m, 2H, CH_2), 3.18–3.24 (m, 2H, CH_2), 4.56 (s, 2H, CH_2), 6.42 (d, J = 5.6 Hz, 1H, H^2), 7.30–7.34 (m, 2H, H^4 + NH-exchangeable with D_2O), 7.71 (d, J = 1.9 Hz, 1H, H^5), 7.90 (dd, J = 7.5, 8.0 Hz, 1H, H^9), 8.10 (d, J = 7.8 Hz, 1H, H^7), 8.15 (d, J = 9.0 Hz, 1H, H^3), 8.21–8.25 (m, 2H, H^6 + NH-exchangeable with D_2O), 8.28 (d, J = 5.4 Hz, 1H, H^1), 8.43–8.46 (m, 2H, H^8 + H^{10}). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 168.01, 163.87, 163.15, 156.20, 151.79, 150.60, 132.85, 132.45, 131.25, 131.10, 129.62, 127.70, 126.66, 125.26, 124.87, 124.50, 124.47, 124.45, 122.10, 116.30, 115.21, 99.05, 42.95, 42.75, 38.95, 29.61, 28.25, 26.71, 26.45. HRMS calcd for $C_{29}H_{26}BrClN_4O_3$ $[M + 1]^+$ 593.0877, found 593.4530 and $[M + 3]^+$ 595.0877, found 595.4524.

3.4.10. 2-(6-bromo-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)-N-(8-((7-chloroquinolin-4-yl)amino)octyl)acetamide (7j)

Yield-88%, light brown solid, M.P = 145–150 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.20–1.35 (m, 8H, $(CH_2)_4$), 1.60–1.62 (m, 4H, $(CH_2)_2$), 3.01–3.04 (m, 2H, CH_2), 3.20–3.23 (m, 2H, CH_2), 4.56 (s, 2H, CH_2), 6.40 (d, J = 5.5 Hz, 1H, H^2), 7.30–7.35 (m, 2H, H^4 + NH-exchangeable with D_2O), 7.68 (d, J = 2.0 Hz, 1H, H^5), 7.90 (dd, J = 7.8, 8.1 Hz, 1H, H^9), 8.12 (d, J = 8.0 Hz, 1H, H^7), 8.16 (d, J = 9.0 Hz, 1H, H^3), 8.21–8.24 (m, 2H, H^6 + NH-exchangeable with D_2O), 8.28 (d, J = 5.3 Hz, 1H, H^1), 8.45–8.49 (m, 2H, H^8 + H^{10}). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 167.30, 163.98, 163.15, 157.28, 152.00, 150.72, 149.31, 133.94, 132.71, 131.15, 131.04, 128.81, 127.58, 126.62, 125.93, 124.61, 124.22, 123.00, 117.81, 115.46, 115.30, 99.10, 42.90, 42.71, 39.10, 29.45, 29.21, 29.10, 28.26, 27.02, 26.70. HRMS calcd for $C_{31}H_{30}BrClN_4O_3$ $[M + 1]^+$ 621.1190, found 621.1280 and $[M + 3]^+$ 623.1190, found 623.1285.

3.5. Procedure for synthesis of conjugates 8a-o

To a well stirred solution of **7** (for **7f-j**, when R = Br) (1 mmol) in anhydrous NMP (1–2 mL), was added different secondary amines (2 mmol) (piperidine/morpholine/hydroxyethyl piperazine) and the reaction mixture was heated at 180 °C for 1 h. On completion of the reaction, as monitored from TLC, ice cold water was added to the reaction mixture. The solid got precipitated out of the reaction mixture which was then filtered, washed with water and recrystallized in ethanol to afford the desired compounds **8a-o**.

*3.5.1. N-(2-((7-chloroquinolin-4-yl)amino)ethyl)-2-(1,3-dioxo-6-(piperidin-1-yl)-1H-benzo[de]isoquinolin-2(3H)-yl)acetamide (**8a**)*

Yield-81%, dark green solid, M.P = 225–230 °C, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.65 (s, 2H, CH_2-**CH_2**-CH_2), 1.81 (s, 4H, **CH_2**-CH_2-**CH_2**), 3.19 (s, 4H, CH_2-**N**-CH_2), 3.36 (s, 4H, $(CH_2)_2$), 4.65 (s, 2H, CH_2), 6.54 (d, J = 5.4 Hz, 1H, H^2), 7.28 (d, J = 8.2 Hz, 1H, H^7), 7.31 (s, 1H, NH-exchangeable with D_2O), 7.36 (dd, J = 1.9, 8.9 Hz, 1H, H^4), 7.76–7.81 (m, 2H, H^5 + H^9), 8.10 (d, J = 9.0 Hz, 1H, H^3), 8.33 (d, J = 8.1 Hz, 1H, H^6), 8.38–8.43 (m, 4H, H^1 + H^8 + H^{10} + NH). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 168.11, 163.99, 163.39, 157.35, 152.38, 150.45, 149.46, 133.81, 132.82, 131.23, 131.13, 129.82, 127.94, 126.24, 125.91, 124.54, 124.29, 122.89, 117.84, 115.32, 115.31, 99.09, 54.43, 42.80, 42.56, 37.86, 26.16, 24.31. HRMS calcd for $C_{30}H_{28}ClN_5O_3$ $[M + 1]^+$ 542.1881, found 542.1971.

*3.5.2. N-(3-((7-chloroquinolin-4-yl)amino)propyl)-2-(1,3-dioxo-6-(piperidin-1-yl)-1H-benzo[de]isoquinolin-2(3H)-yl)acetamide (**8b**)*

Yield-80%, light green solid, M.P = 165–170 °C, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.65 (s, 2H, CH_2-**CH_2**-CH_2), 1.78–1.81 (m, 6H, **CH_2**-CH_2-**CH_2** + CH_2), 3.19–3.21 (m, 6H, **CH_2**-N-**CH_2** + CH_2), 3.26–3.30 (m, 2H, CH_2), 4.64 (s, 2H, CH_2), 6.48 (d, J = 5.5 Hz, 1H, H^2), 7.29 (d, J = 8.2 Hz, 1H, H^7), 7.32 (s, 1H, NH- exchangeable with D_2O), 7.42 (dd, J = 2.2, 8.9 Hz, 1H, H^4), 7.76 (d, J = 2.1 Hz, 1H, H^5), 7.80 (dd, J = 7.5, 8.2 Hz, 1H, H^9), 8.23 (d, J = 9.0 Hz, 1H, H^3), 8.26 (t, J = 5.6 Hz, 1H, NH- exchangeable with D_2O), 8.36 (d, J = 8.1 Hz, 1H, H^6), 8.39 (d, J = 5.4 Hz, 1H, H^1), 8.41 (d, J = 8.4 Hz, 1H, H^{10}), 8.45 (d, J = 7.2 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 167.44, 164.05, 163.45, 157.32, 152.22, 150.53, 149.32, 133.86, 132.75, 131.19, 131.07, 129.85, 127.78, 126.27, 125.94, 124.51, 124.46, 123.00, 117.88, 115.45, 115.36, 99.15, 54.44, 42.86, 37.00, 28.32, 26.16, 24.30. HRMS calcd for $C_{31}H_{30}ClN_5O_3$ $[M + 1]^+$ 556.2037, found 556.2089.

*3.5.3. N-(4-((7-chloroquinolin-4-yl)amino)butyl)-2-(1,3-dioxo-6-(piperidin-1-yl)-1H-benzo[de]isoquinolin-2(3H)-yl)acetamide (**8c**)*

Yield-81%, pale yellow solid, M.P = 150–155 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.50–1.54 (m, 2H, CH_2), 1.60–1.67 (m, 4H, CH_2 + CH_2-**CH_2**-CH_2), 1.80 (s, 4H, **CH_2**-CH_2-**CH_2**), 3.10–3.14 (m, 2H, CH_2), 3.20–3.24 (m, 6H, CH_2 + **CH_2**-CH_2-**CH_2**), 4.60 (s, 2H, CH_2), 6.45 (d, J = 5.3 Hz, 1H, H^2), 7.30–7.34 (m, 2H, H^7 + NH-exchangeable with D_2O), 7.40 (dd, J = 2.0, 8.9 Hz, 1H, H^4), 7.80 (d, J = 1.9 Hz, 1H, H^5), 7.82 (dd, J = 7.5, 8.0 Hz, 1H, H^9), 8.16 (t, J = 5.5 Hz, 1H, NH-exchangeable with D_2O), 8.25 (d, J = 9.0 Hz, 1H, H^3), 8.35–8.37 (m, 2H, H^6 + H^1), 8.46 (d, J = 7.0 Hz, 1H, H^{10}), 8.49 (d, J = 8.5 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 167.08, 163.94, 163.39, 157.01, 152.18, 149.92, 132.85, 132.65, 131.14, 131.13, 129.76, 127.65, 126.56, 125.76, 124.61, 124.58, 124.47, 124.27, 123.07, 116.30, 115.61, 99.20, 54.10, 42.95, 42.50, 38.75, 27.21, 26.32, 25.56, 24.15. HRMS calcd for $C_{32}H_{32}ClN_5O_3$ $[M + 1]^+$ 570.2194, found 570.2289.

*3.5.4. N-(6-((7-chloroquinolin-4-yl)amino)hexyl)-2-(1,3-dioxo-6-(piperidin-1-yl)-1H-benzo[de]isoquinolin-2(3H)-yl)acetamide (**8d**)*

Yield-80%, pale yellow solid, M.P = 135–140 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.30–1.42 (m, 6H, $(CH_2)_3$), 1.61–1.68 (m, 4H, CH_2 + CH_2-**CH_2**-CH_2), 1.80 (s, 4H, **CH_2**-CH_2-**CH_2**), 3.05–3.09 (m, 2H, CH_2), 3.18 (s, 4H, **CH_2**-CH_2-**CH_2**), 3.21–3.25 (m, 2H, CH_2), 4.60 (s, 2H, CH_2), 6.45 (d, J = 5.4 Hz, 1H, H^2), 7.25 (d, J = 8.0 Hz, 1H, H^7), 7.29 (t, J = 5.2 Hz, 1H, NH-exchangeable with D_2O), 7.37 (dd, J = 2.0, 8.8 Hz, 1H, H^4), 7.75–7.80 (m, 2H, H^5 + H^9), 8.12 (t, J = 5.4 Hz, 1H, NH-exchangeable with D_2O), 8.26 (d, J = 8.9 Hz, 1H, H^3), 8.31–8.35 (m, 2H, H^6 + H^1), 8.41 (d, J = 8.4 Hz, 1H, H^{10}), 8.43 (d, J = 7.2 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 168.01, 163.84, 163.15, 157.10, 152.19, 151.32, 133.85, 132.65, 132.04, 131.89, 129.62, 127.45, 126.56, 125.76, 124.95, 124.66, 124.51, 124.29, 123.10, 116.30, 115.51, 99.25, 54.15, 42.93, 42.70, 38.90, 29.45, 28.25, 26.71, 26.45, 26.15, 24.60. HRMS calcd for $C_{34}H_{36}ClN_5O_3$ $[M + 1]^+$ 598.2507, found 598.2590.

*3.5.5. N-(8-((7-chloroquinolin-4-yl)amino)octyl)-2-(1,3-dioxo-6-(piperidin-1-yl)-1H-benzo[de]isoquinolin-2(3H)-yl)acetamide (**8e**)*

Yield-79%, pale yellow solid, M.P = 130–135 °C, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.24–1.39 (m, 10H, $(CH_2)_5$), 1.63–1.64 (m, 4H, CH_2 + CH_2-**CH_2**-CH_2), 1.81 (s, 4H, **CH_2**-CH_2-**CH_2**), 3.03–3.07 (m, 2H, CH_2), 3.17 (s, 4H, **CH_2**-CH_2-**CH_2**), 3.22–3.26 (m, 2H, CH_2), 4.59 (s, 2H, CH_2), 6.44 (d, J = 5.5 Hz, 1H, H^2), 7.27 (d, J = 8.1 Hz, 1H, H^7), 7.35 (s, 1H, NH- exchangeable with D_2O), 7.43 (dd, J = 2.1, 8.9 Hz, 1H, H^4), 7.76 (d, J = 2.0 Hz, 1H, H^5), 7.79 (dd, J = 7.6, 8.1 Hz, 1H, H^9), 8.11 (t, J = 5.4 Hz, 1H, NH- exchangeable with D_2O), 8.27 (d, J = 9.0 Hz, 1H, H^3), 8.35 (d, J = 8.1 Hz, 1H, H^6), 8.37 (d, J = 5.0 Hz, 1H, H^1), 8.39 (d, J = 8.4 Hz, 1H, H^{10}), 8.43 (d, J = 7.1 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 166.97, 163.98, 163.39, 157.28, 152.00, 150.72, 149.12, 133.94, 132.71, 131.15, 131.04, 129.81, 127.58, 126.26, 125.93, 124.61, 124.49, 123.00, 117.81, 115.46, 115.33, 98.70, 54.42, 42.92, 42.70, 39.04, 29.48, 29.21, 29.10, 28.23, 27.00, 26.69, 26.15, 24.29. HRMS calcd for $C_{36}H_{40}ClN_5O_3$ $[M + 1]^+$ 626.2820, found 626.2910.

*3.5.6. N-(2-((7-chloroquinolin-4-yl)amino)ethyl)-2-(6-morpholino-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)acetamide (**8f**)*

Yield-78%, mustard green solid, M.P = 185–190 °C, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.22–3.24 (m, 4H, **CH_2**-N-**CH_2**), 3.36 (s, 4H, $(CH_2)_2$), 3.91–3.93 (m, 4H, **CH_2**-O-**CH_2**), 4.66 (s, 2H, CH_2), 6.54 (d, J = 5.4 Hz, 1H, H^2), 7.34–7.36 (m, 2H, H^7 + NH-exchangeable with D_2O), 7.38 (dd, J = 2.1, 9.0 Hz, 1H, H^4), 7.78 (d, J = 2.1 Hz, 1H, H^5), 7.81 (dd, J = 7.8, 7.9 Hz, 1H, H^9), 8.11 (d, J = 9.0 Hz, 1H, H^3), 8.37 (d, J = 8.0 Hz, 1H, H^6), 8.40 (d, J = 5.3 Hz, 1H, H^1), 8.42 (s, 1H, NH-exchangeable with D_2O), 8.45 (d, J = 7.1 Hz, 1H, H^{10}), 8.51 (d, J = 8.4 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 168.07, 163.95, 163.38, 156.09, 152.21, 150.58, 149.24, 133.92, 132.75, 131.23, 129.76, 127.77, 126.56, 125.75, 124.61, 124.33, 122.96, 117.80, 116.17, 115.51, 99.10, 66.64, 53.51, 42.83, 42.57, 37.87. HRMS calcd for $C_{29}H_{26}ClN_5O_4$ $[M + 1]^+$ 544.1673, found 544.1765.

*3.5.7. N-(3-((7-chloroquinolin-4-yl)amino)propyl)-2-(6-morpholino-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)acetamide (**8g**)*

Yield-78%, light green solid, M.P = 175–180 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.80–1.82 (m, 2H, CH_2), 3.20–3.24 (m, 6H, **CH_2**-N-**CH_2** + CH_2), 3.25–3.28 (m, 2H, CH_2), 3.91–3.93 (m, 4H, **CH_2**-O-**CH_2**), 4.62 (s, 2H, CH_2), 6.47 (d, J = 5.4 Hz, 1H, H^2), 7.30 (d, J = 8.1 Hz, 1H, H^7), 7.31 (s, 1H, NH-exchangeable with D_2O), 7.40 (dd, J = 2.1, 8.8 Hz, 1H, H^4), 7.75 (d, J = 2.1 Hz, 1H, H^5), 7.81 (dd, J = 7.4, 8.1 Hz, 1H, H^9), 8.21 (d, J = 9.0 Hz, 1H, H^3), 8.25 (s, 1H, NH-exchangeable with D_2O), 8.35 (d, J = 8.0 Hz, 1H, H^6), 8.38 (d, J = 5.3 Hz, 1H, H^1), 8.40 (d, J = 8.0 Hz, 1H, H^{10}), 8.43 (d, J = 7.3 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 167.44, 164.10, 163.45, 157.12, 152.35, 150.53, 149.45, 133.86, 132.75, 131.74, 131.15, 130.05, 127.76, 126.17, 125.75, 124.57, 124.45, 122.95, 116.98, 115.45, 115.26, 98.95, 65.76, 52.62, 41.82, 36.92, 27.76. HRMS calcd for $C_{30}H_{28}ClN_5O_4$ $[M + 1]^+$ 558.1830, found 558.1841.

*3.5.8. N-(4-((7-chloroquinolin-4-yl)amino)butyl)-2-(6-morpholino-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)acetamide (**8 h**)*

Yield-76%, pale yellow solid, M.P = 175–180 °C, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.50–1.55 (m, 2H, CH_2), 1.62–1.68 (m, 2H, CH_2), 3.11–3.15 (m, 2H, CH_2), 3.21–3.23 (m, 4H, **CH_2**-N-**CH_2**), 3.25–3.29 (m, 2H, CH_2), 3.90–3.92 (m, 4H, **CH_2**-O-**CH_2**), 4.61 (s, 2H, CH_2), 6.47 (d, J = 5.4 Hz, 1H, H^2), 7.33–7.36 (m, 2H, H^7 + NH-exchangeable with D_2O), 7.42 (dd, J = 2.1, 8.9 Hz, 1H, H^4), 7.77 (s, 1H, H^5), 7.81 (dd, J = 7.6, 8.1 Hz, 1H, H^9), 8.18 (t, J = 5.6 Hz, 1H, NH-exchangeable with D_2O), 8.26 (d, J = 9.0 Hz, 1H, H^3), 8.36–8.38 (m, 2H, H^6 + H^1), 8.45 (d, J = 7.1 Hz, 1H, H^{10}), 8.50 (d, J = 8.4 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 167.08, 163.94, 163.39, 156.02, 152.18, 150.60, 133.85, 132.65, 131.14, 131.13, 129.76, 127.75, 126.56, 125.76,

124.57, 124.54, 124.47, 124.45, 123.07, 116.30, 115.51, 99.15, 66.64, 53.50, 42.78, 42.54, 38.80, 27.25, 25.60. HRMS calcd for $C_{31}H_{30}ClN_5O_4$ $[M + 1]^+$ 572.1986, found 572.2052.

*3.5.9. N-(6-((7-chloroquinolin-4-yl)amino)hexyl)-2-(6-morpholino-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)acetamide **(8i)***

Yield-76%, brown solid, M.P = 155–160 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.31–1.45 (m, 6H, $(CH_2)_3$), 1.62–1.68 (m, 2H, CH_2), 3.06–3.09 (m, 2H, CH_2), 3.21–3.25 (m, 6H, CH_2 + **CH_2**-N-**CH_2**), 3.90–3.92 (m, 4H, **CH_2**-O-**CH_2**), 4.62 (s, 2H, CH_2), 6.42 (d, J = 5.2 Hz, 1H, H^2), 7.27 (d, J = 8.1 Hz, 1H, H^7), 7.31 (t, J = 5.1 Hz, 1H, NH-exchangeable with D_2O), 7.36 (dd, J = 2.0, 9.0 Hz, 1H, H^4), 7.75–7.80 (m, 2H, H^5 + H^9), 8.16 (t, J = 5.4 Hz, 1H, NH-exchangeable with D_2O), 8.26 (d, J = 9.1 Hz, 1H, H^3), 8.32–8.35 (m, 2H, H^6 + H^1), 8.40 (d, J = 8.4 Hz, 1H, H^{10}), 8.42 (d, J = 7.2 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 167.10, 163.85, 163.51, 156.35, 152.18, 150.60, 133.85, 132.65, 132.14, 131.10, 129.76, 127.75, 126.16, 125.76, 124.57, 124.50, 124.41, 123.89, 123.07, 116.30, 115.35, 98.99, 66.49, 53.41, 42.70, 38.17, 29.91, 28.19, 26.74, 26.46. HRMS calcd for $C_{33}H_{34}ClN_5O_4$ $[M + 1]^+$ 600.2299, found 600.2390.

*3.5.10. N-(8-((7-chloroquinolin-4-yl)amino)octyl)-2-(6-morpholino-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)acetamide **(8j)***

Yield-75%, light brown solid, M.P = 145–150 °C, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.24–1.39 (m, 10H, $(CH_2)_5$), 1.62–1.66 (m, 2H, CH_2), 3.03–3.07 (m, 2H, CH_2), 3.20–3.26 (m, 6H, CH_2 + **CH_2**-N-**CH_2**), 3.89–3.91 (m, 4H, **CH_2**-O-**CH_2**), 4.60 (s, 2H, CH_2), 6.45 (d, J = 5.5 Hz, 1H, H^2), 7.32 (d, J = 8.0 Hz, 1H, H^7), 7.40 (t, J = 4.6 Hz, 1H, NH-exchangeable with D_2O), 7.44 (dd, J = 2.0, 8.9 Hz, 1H, H^4), 7.76 (d, J = 2.1 Hz, 1H, H^5), 7.80 (dd, J = 7.5, 8.1 Hz, 1H, H^9), 8.12 (t, J = 5.3 Hz, 1H, NH-exchangeable with D_2O), 8.27 (d, J = 9.0 Hz, 1H, H^3), 8.37–8.38 (m, 2H, H^6 + H^1), 8.45 (d, J = 7.2 Hz, 1H, H^{10}), 8.49 (d, J = 8.4 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 166.93, 163.92, 163.37, 156.00, 151.75, 150.85, 148.82, 134.06, 134.04, 132.63, 131.12, 129.74, 127.35, 126.54, 125.75, 124.63, 124.56, 123.05, 117.74, 116.29, 115.48, 98.10, 66.63, 53.49, 42.93, 42.72, 39.05, 29.48, 29.20, 29.10, 28.22, 27.00, 26.69. HRMS calcd for $C_{35}H_{38}ClN_5O_4$ $[M + 1]^+$ 628.2612, found 628.2690.

*3.5.11. N-(2-((7-chloroquinolin-4-yl)amino)ethyl)-2-(6-(4-(2-hydroxyethyl)piperazin-1-yl)-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)acetamide **(8k)***

Yield-71%, dark green solid, M.P = 180–185 °C, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 2.54 (t, J = 6.1 Hz, 2H, **CH_2**-CH_2-OH), 2.75 (s, 4H, **CH_2**-N-**CH_2**), 3.23 (s, 4H, **CH_2**-N-**CH_2**), 3.35–3.37 (m, 4H, $(CH_2)_2$), 3.58 (t, J = 6.1 Hz, 2H, CH_2-**CH_2**-OH), 4.65 (s, 2H, CH_2), 6.53 (d, J = 5.4 Hz, 1H, H^2), 7.30 (d, J = 8.2 Hz, 1H, H^7), 7.34 (s, 1H, NH-exchangeable with D_2O), 7.36 (dd, J = 2.1, 9.0 Hz, 1H, H^4), 7.76–7.80 (m, 2H, H^5 + H^9), 8.10 (d, J = 9.0 Hz, 1H, H^3), 8.34 (d, J = 8.1 Hz, 1H, H^6), 8.39 (d, J = 5.3 Hz, 1H, H^1), 8.41–8.44 (m, 3H, H^8 + H^{10} + NH-exchangeable with D_2O). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 168.11, 163.96, 163.38, 156.32, 152.25, 150.54, 149.28, 133.89, 132.76, 131.18, 131.17, 129.76, 127.79, 126.39, 125.72, 124.59, 124.30, 122.89, 117.80, 115.70, 115.36, 99.09, 60.63, 59.02, 53.57, 53.11, 42.81, 42.57, 37.86. HRMS calcd for $C_{31}H_{31}ClN_6O_4$ $[M + 1]^+$ 587.2095, found 587.2012.

*3.5.12. N-(3-((7-chloroquinolin-4-yl)amino)propyl)-2-(6-(4-(2-hydroxyethyl)piperazin-1-yl)-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)acetamide **(8l)***

Yield-71%, light green solid, M.P = 180–185 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.81–1.83 (m, 2H, CH_2), 2.53 (t, J = 6.0 Hz, 2H, **CH_2**-CH_2-OH), 2.74 (s, 4H, **CH_2**-N-**CH_2**), 3.19–3.24 (s, 6H, **CH_2**-N-**CH_2** + CH_2), 3.25–3.27 (s, 2H, CH_2), 3.58 (t, J = 6.1 Hz, 2H, CH_2-**CH_2**-OH), 4.63 (s, 2H, CH_2), 6.49 (d, J = 5.5 Hz, 1H, H^2), 7.30 (d, J = 8.1 Hz, 1H, H^7), 7.32 (s, 1H, NH-exchangeable with D_2O), 7.41 (dd, J = 2.1, 8.9 Hz, 1H, H^4), 7.75 (d, J = 2.1 Hz, 1H, H^5), 7.80 (dd, J = 7.6, 8.2 Hz, 1H, H^9), 8.21 (d, J = 8.8 Hz, 1H, H^3), 8.25 (s, 1H, NH-exchangeable with D_2O), 8.36 (d, J = 8.0 Hz, 1H, H^6), 8.40 (d, J = 5.4 Hz, 1H, H^1), 8.41 (d, J = 8.5 Hz, 1H, H^{10}), 8.43 (d, J = 7.2 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 167.34, 164.01, 163.65, 157.32, 152.22, 150.53, 149.32, 133.86, 132.84, 132.19, 131.21, 128.95, 127.76, 126.35, 125.91, 124.95, 124.48, 123.10, 117.81, 115.75, 115.37, 99.18, 60.26, 59.10, 53.21, 53.17, 43.02, 37.10, 27.86. HRMS calcd for $C_{32}H_{33}ClN_6O_4$ $[M + 1]^+$ 601.2252, found 601.2267.

*3.5.13. N-(4-((7-chloroquinolin-4-yl)amino)butyl)-2-(6-(4-(2-hydroxyethyl)piperazin-1-yl)-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)acetamide **(8 m)***

Yield-70%, light green solid, M.P = 165–170 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.51–1.55 (m, 2H, CH_2), 1.61–1.65 (m, 2H, CH_2), 2.53 (t, J = 6.0 Hz, 2H, **CH_2**-CH_2-OH), 2.75 (s, 4H, **CH_2**-N-**CH_2**), 3.10–3.14 (m, 2H, CH_2), 3.21–3.27 (s, 6H, **CH_2**-N-**CH_2** + CH_2), 3.57 (t, J = 6.1 Hz, 2H, CH_2-**CH_2**-OH), 4.58 (s, 2H, CH_2), 6.45 (d, J = 5.3 Hz, 1H, H^2), 7.31–7.35 (m, 2H, H^7 + NH-exchangeable with D_2O), 7.41 (dd, J = 2.0, 8.9 Hz, 1H, H^4), 7.75 (d, J = 2.1 Hz, 1H, H^5), 7.80 (dd, J = 7.7, 8.0 Hz, 1H, H^9), 8.15 (t, J = 5.5 Hz, 1H, NH-exchangeable with D_2O), 8.25 (d, J = 9.0 Hz, 1H, H^3), 8.35–8.37 (m, 2H, H^6 + H^1), 8.43 (d, J = 7.5 Hz, 1H, H^{10}), 8.49 (d, J = 8.1 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 167.08, 164.94, 163.39, 156.02, 152.18, 151.60, 133.85, 132.65, 131.14, 130.13, 129.76, 126.75, 126.56, 125.76, 124.95, 124.54, 124.47, 124.45, 123.18, 116.30, 116.51, 98.75, 60.56, 59.10, 53.60, 53.15, 42.75, 42.50, 38.75, 27.27, 25.66. HRMS calcd for $C_{33}H_{35}ClN_6O_4$ $[M + 1]^+$ 615.2408, found 615.2475.

*3.5.14. N-(6-((7-chloroquinolin-4-yl)amino)hexyl)-2-(6-(4-(2-hydroxyethyl)piperazin-1-yl)-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)acetamide **(8n)***

Yield-70%, yellow solid, M.P = 145–150 °C, ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.32–1.44 (m, 6H, $(CH_2)_3$), 1.61–1.67 (m, 2H, CH_2), 2.54 (t, J = 6.1 Hz, 2H, **CH_2**-CH_2-OH), 2.74 (s, 4H, **CH_2**-N-**CH_2**), 3.06–3.10 (m, 2H, CH_2), 3.20–3.26 (m, 6H, CH_2 + **CH_2**-N-**CH_2**), 3.58 (t, J = 6.2 Hz, 2H, CH_2-**CH_2**-OH), 4.61 (s, 2H, CH_2), 6.43 (d, J = 5.5 Hz, 1H, H^2), 7.26 (d, J = 8.1 Hz, 1H, H^7), 7.30 (t, J = 5.3 Hz, 1H, NH-exchangeable with D_2O), 7.38 (dd, J = 2.1, 8.9 Hz, 1H, H^4), 7.74–7.78 (m, 2H, H^5 + H^9), 8.15 (t, J = 5.5 Hz, 1H, NH-exchangeable with D_2O), 8.25 (d, J = 9.0 Hz, 1H, H^3), 8.33–8.36 (m, 2H, H^1 + H^6), 8.40 (d, J = 8.5 Hz, 1H, H^{10}), 8.42 (d, J = 7.3 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 167.06, 163.96, 163.38, 156.23, 152.06, 150.65, 149.19, 133.87, 132.64, 131.07, 129.75, 127.63, 126.37, 125.72, 124.55, 124.47, 124.42, 123.00, 117.80, 115.84, 115.33, 98.99, 60.64, 59.02, 53.57, 53.09, 42.71, 38.91, 29.50, 28.20, 26.62, 26.38. HRMS calcd for $C_{35}H_{39}ClN_6O_4$ $[M + 1]^+$ 643.2721, found 643.2782.

*3.5.15. N-(8-((7-chloroquinolin-4-yl)amino)octyl)-2-(6-(4-(2-hydroxyethyl)piperazin-1-yl)-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)acetamide **(8o)***

Yield-68%, light brown solid, M.P = 130–135 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.25–1.40 (m, 10H, $(CH_2)_5$), 1.61–1.65 (m, 2H, CH_2), 2.52 (t, J = 5.8 Hz, 2H, **CH_2**-CH_2-OH), 2.72 (s, 4H, **CH_2**-N-**CH_2**), 3.01–3.05 (m, 2H, CH_2), 3.21–3.27 (m, 6H, CH_2 + **CH_2**-N-**CH_2**), 3.56 (t, J = 5.9 Hz, 2H, CH_2-**CH_2**-OH), 4.62 (s, 2H, CH_2), 6.45 (d, J = 5.3 Hz, 1H, H^2), 7.33 (d, J = 8.1 Hz, 1H, H^7), 7.39 (t, J = 4.5 Hz, 1H, NH-exchangeable with D_2O), 7.45 (dd, J = 2.1, 8.8 Hz, 1H, H^4), 7.78 (d, J = 2.0 Hz, 1H, H^5), 7.81 (dd, J = 7.6, 8.0 Hz, 1H, H^9), 8.10 (t, J = 5.3 Hz, 1H, NH-exchangeable with D_2O), 8.28 (d, J = 9.0 Hz, 1H, H^3), 8.35–8.37 (m, 2H, H^6 + H^1), 8.46 (d, J = 7.5 Hz, 1H, H^{10}), 8.50 (d, J = 8.1 Hz, 1H, H^8). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 167.15, 163.98, 163.21, 157.28, 152.00, 150.70, 149.12, 133.94, 132.65, 131.15, 131.02, 129.81, 127.58, 126.26, 125.75, 124.61, 124.49, 123.02, 117.81, 115.46, 115.33, 98.95, 59.98, 59.10, 53.60, 53.10, 42.90, 42.70, 39.10, 29.50, 29.25, 29.12, 28.21, 27.02, 26.71. HRMS calcd for

$C_{37}H_{43}ClN_6O_4$ $[M + 1]^+$ 671.3034, found 671.3085.

3.6. Material and methods

3.6.1. Bacterial culture conditions

Mycobacterium tuberculosis strain mc^26230 was grown in Middlebrook 7H9 liquid broth (Difco) supplemented with 10% OADC enrichment, 0.025% Tyloxapol and 24 μg/mL pantothenic acid [14]. Bacteria were placed in culture flasks to an optical density at 600 nm (OD_{600}) of 0.05 and left to incubate at 37 °C for 1 week. Bacteria were harvested during the late exponential/early stationary phase and adjusted to OD_{600} of 0.01 prior to transfer into 96-well plates for compound screening.

3.6.2. Minimum inhibitory concentration (MIC)

Synthesized compounds were dissolved in DMSO (Dimethyl sulfoxide) and adjusted to a final concentration of 10 mg/mL. To examine the biological activity of synthetic compounds, serial dilutions were performed ranging from 200 μg to 0.39 μg/mL. Plates containing bacterial-synthetic compound dilutions were placed back into the incubator at 37 °C for 1 week. Following incubation, 10% (vol/vol) resazurin cell viability dye (0.025% stock concentration) was added to each well and left to incubate at 37 °C for 24 h prior to data acquisition using a fluorescent plate reader (excitation 540 nm, emission 590 nm). DMSO was included as a negative control, while INH was included as a positive control at the pre-designated concentrations.

3.6.3. Cell culture conditions

Vero kidney epithelial cells (ATCC® CCL-81™) were cultured in RPMI 1640 cell culture medium (Life technologies) supplemented with 10% foetal calf serum and incubated at 37 °C with 5% CO_2. Once the desired confluency was achieved, cells were detached from the flask surface using trypsin and centrifuged at 220 × *g* for 5 min at room temperature to pellet cells. The cell pellet was resuspended in 1 mL of culture medium and enumerated using a Malassez counting chamber. Cell density was adjusted to approximately 2×10^4 cells/well in 96-well plates and left to adhere overnight at 37 °C and 5% CO_2.

3.6.4. Cytotoxicity assay

To determine cytotoxicity of synthetic compounds, serial dilutions were performed ranging from 100 μg to 3.1 μg/mL. Cell cultures were placed back in the incubator at 37 °C and 5% CO_2 for 24 h. After incubation, 10% (vol/vol) resazurin was added to each well and left to incubate at 37 °C and 5% CO_2 prior to data acquisition using a fluorescent plate reader (excitation 540 nm, emission 590 nm). DMSO was included as a negative control, while 20% SDS was included as a positive control at the pre-designated concentrations.

3.6.5. Data analysis

Data was analysed using Graphpad Prism 5 (Graphphad software). The half maximal inhibitory concentration (IC_{50}) was calculated using a non-linear regression dose response curve. Selectivity Index (SI) was calculated as a function of the IC_{50}/MIC.

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Declaration of Competing Interest

The authors have declared no conflict of interest.

Appendix A. Supplementary material

Scanned copies of ^1H, ^{13}C and ^{13}C-DEPT NMR spectra of representative compounds *viz.* **3c**, **3d**, **3e**, **3i**, **3j**, **4c**, **4e**, **4g**, **4j**, **4l**, **4n** and **7a**, **7d**, **7f**, **8a**, **8b**, **8e**, **8h**, **8j**, **8k**, **8n** along with scanned spectra of HRMS of few representative compounds *viz.* **3c**, **3i**, **4g**, **4l**, **7i**, **8g**. Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103241.

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