DOI: http://dx.doi.org/10.4172/2161-0401.1000144

Organic Chemistry
Current Research

Noro et al., Organic Chem Curr Res 2015, 4:3
http://dx.doi.org/10.4172/2161-0401.1000144

Research Article Open Access

Evaluation of New Naphthalimides as Potential Anticancer Agents against Breast Cancer MCF-7, Pancreatic Cancer BxPC-3 and Colon Cancer HCT-15 Cell Lines

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Abstract

New 1,8-naphthalimido derivatives with 2,3 and 4 carbon chains bearing a number of different functionalities were synthesized and tested against a panel of breast cancer MCF-7, colon cancer HCT-15 and pancreatic cancer BxPC-3 cell lines. Generally structures with shorter alkyl chains were more active, with the one exception of the amide containing a p-nitrophenyl group. GI50 values (μM) were determined for the most active compounds. Three compounds exhibited GI50 values below 5 μM, two with MCF-7 cells, and one other with HCT-15. Compounds with different functionalities demonstrated cell line specificity: the MCF-7 cell line was more sensitive to an urea derivative (6f), the growth of HCT-15 cells were most affected by a triazole (9d), while the BxPC-3 cell line was inhibited in a higher extend by a guanidine (4a).

Keywords: Toxicity; Tetrabromide; Naphthalimide; Isocyanate; Membrane permeabilization

Introduction

Naphthalimide and bisnaphthalimide are groups of aromatic compounds that have generated intense interests for a number of years by scientists around the world and this is due to their diverse applications in the medical and environmental sciences [1-3]. For example due to their inherent intense UV and fluorescence properties, the naphthalimido derivatives had been developed as highly selective fluoride ion sensors [4], molecular probes [5] and dyes [6]. However, the main application to date for the naphthalimides, is their potential as therapeutic agents [7] especially in the area of cancer therapy. The flat aromatic structural of the naphthalimido ring and its ability to bind to DNA by intercalation are attractive features that had attracted much attention in the development of new mono naphthalimides [8-9] and bisnaphthalimido [10-11] derivatives for enhanced anticancer activities and aqueous solubility. Among the mononaphthalimido derivatives, Mitonafide and analogues had reached clinical trial but failed to progress due to unpredicted neurological toxicity side effects [12]. The first generation of mononaphthalmides developed by Brana et al. had a short linker chain from the naphthalimido ring with a tertiary amino group [1]. Since then other workers have made many changes to the naphthalimido rings especially with substitution at positions 5 and 6. More recently Qian et al. have been working on 6 substituted naphthalimido derivatives and demonstrated that these compounds exhibited their toxicities due to a multi targets approach that involve inhibition of topoisomerase II and induction lysosomal membrane permeabilization leading eventually to apoptosis and cell death [13]. Here in this work we have focused our attention in the length of linker chains and the terminal groups. The linker alkyl chain was modified with 2, 3 and 4 carbons and the terminal groups include amino, imino, pyrrole, nitrobenzene, ureas. These compounds were screened against breast cancer MCF-7, colon cancer HCT-15 and pancreatic cancer BxPC-3 cell lines.

Results and Discussion

Chemistry

1,8-Naphtalimide compounds bearing 2,3 and 4 carbon chains, and different terminal functional groups were synthesized by methodologies based on well-established chemistry. Scheme 1 depicts the synthesis of naphthlimido alkylamine and alkyl alcohol products 1a-f in excellent yields. This was achieved by the reaction of naphthalic anhydride with corresponding diamines and amino alcohols in excess [14].

Scheme 1: Synthesis of naphthalimidoalkylamines 1a-c and naphthalimidoalkyl alcohols 1d-f: Amines 1a-c: 1,8-naphthalic anhydride, amine (2-6 equiv.), ethanol; 20 min-1 h, r.t.; Alcohols 1d-f: 1,8-naphthalic anhydride, amino-alcohol (10 equiv.), DBU (1 equiv.), 4-7 h, 75°C. Naphthalimidoalkylamines 1a-c were conjugated with a series of functional groups as shown in scheme 2, yielding compounds 2a-i, 4a-c, 5a-i and 6a-i. Reaction of the amines with heteroaromatic aldehydes occur by refluxing in ethanol furnishing the corresponding stable imines 2a-i. From the furan and thiophene imines derivatives (2f,g) bearing a three-carbon chain linking to naphthalimido group were reduced under $NaBH_4$ to give the respective amines 3f,g. Subsequent hydrochloride salts were obtained by bubbling HCl gas through a solution of the amines in dichloromethane. The reactions of naphthalimidoalkylamines 1a-c with small excess of 2-methylisothiouronium iodide [15], gave guanidines 4a-c as the iodide salt in good yields. Reactions of 1a-c with cyclic or open

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Received: June 25, 2015; **Accepted:** July 13, 2015; **Published**: July 20, 2015

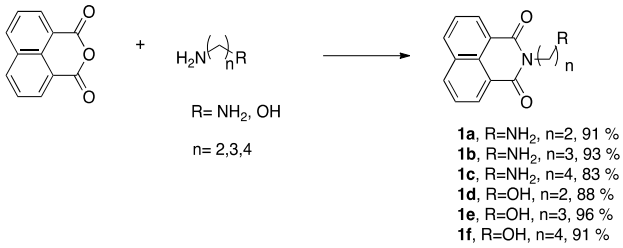
Citation: Noro J, Maciel J, Duarte D, Olival ACD, Baptista C, et al. (2015) Evaluation of New Naphthalimides as Potential Anticancer Agents against Breast Cancer MCF-7, Pancreatic Cancer BxPC-3 and Colon Cancer HCT-15 Cell Lines. Organic Chem Curr Res 4: 144. doi:10.4172/2161-0401.1000144

chain anhydrides gave amides 5a-l. Ureas 6a-l were obtained by the reaction of 1a-c with excess isocyanates under anhydrous atmosphere. Naphthalimidoalkyl 1,2,3-triazolo derivatives were obtained from the naphthamidoalkylalcohols 1d-f as depicted in Scheme 3. Briefly the hydroxyl group from 1d-f was functionalized into their respective bromides, 7d-f using carbon tetrabromide and triphenylphosphine. Subsequently these bromo intermediates were converted to the corresponding azides by Appel reaction. The azides 8d-f were submitted to click chemistry [16] conditions with phenylacetylene giving the corresponding 1,4-substituted 1,2,3-triazoles 9d-f.

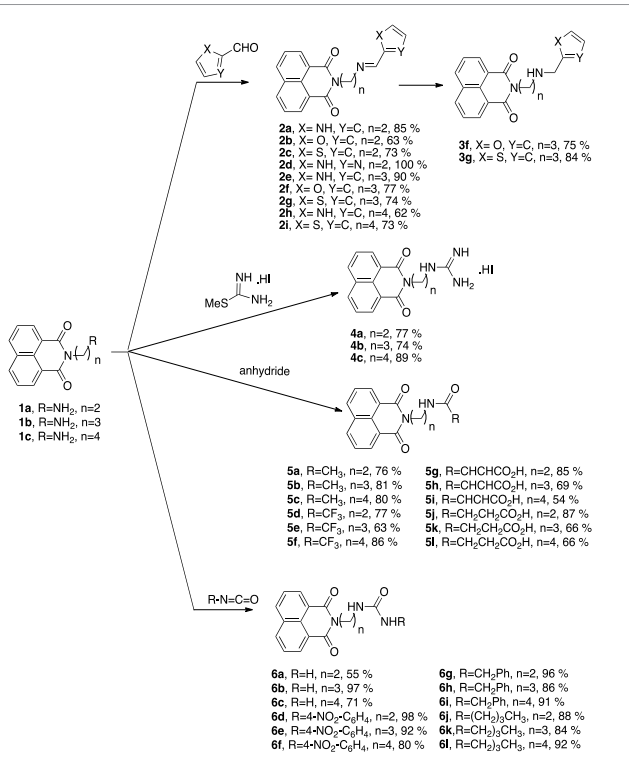
Scheme 2- Synthesis of naphthalimidoalkyl derivatives 2a-i, 3f,g, 4a-c, 5a-l, 6a-l Derivatives 2a-i : 1,8-naphthalimide 1a-c, aldehyde, ethanol; reflux for 20 min - 7 h; 61.7-100 %. Derivatives 3f,g: imine 2f,g, THF:MeOH, 1:1; $NaBH_4$ (2 equiv.), r.t., 4-12 h; than HCl(g); 53.4-55.2%. Derivatives 4a-c: 1,8-naphthalimide 1a-c, 2-methylisothiouronium iodide (1.3 equiv.); r.t.; 74.1-89.2%, Derivatives 5a-l: 1,8-naphthalimide 1a-c, anhydride (1.1-2.0 equiv.), ethanol or dichloromethane, reflux, 1 h-2 h 30 min.; 54-87 %. Derivatives 6a-l: 1,8-naphthalimide 1a-c, dry toluene, isocyanate (1.0-2.5 equiv.), addition at 0°C, followed by refluxing at 130°C, 4-6 h; 55-98%.

Biological activity

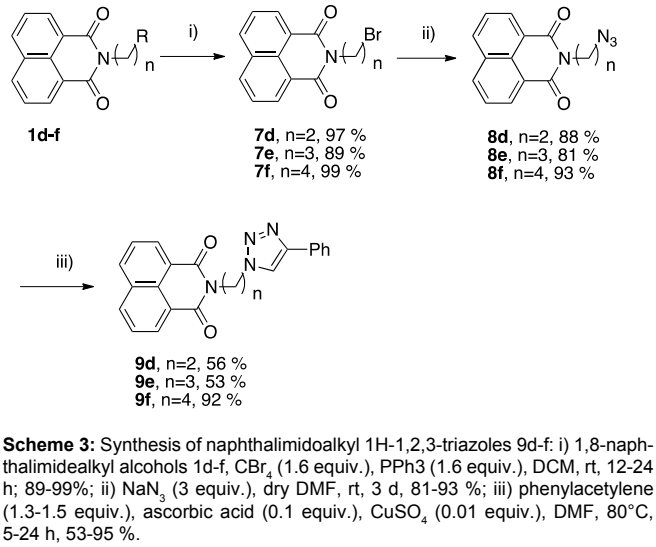
Toxicity of the compounds to tumor cells: All the naphthalimido compounds described above were firstly screened for activity in a human breast tumor cell line (MDA-MB-231) at concentrations range of 0-40 μM using the MTT assay (data not shown). Compounds that presented potential as anticancer agents were further analyzed by the sulforhodamine B assay on three human tumor cell lines: MCF-7 (breast cancer), HCT-15 (colon cancer) and BxPC-3 (pancreatic cancer). Results are presented in (Table 1). The GI_{50} values were determined for the most active compounds, i.e. those showing ≥ 50% of cell growth inhibition in 5 μM concentration (Table 2). The breast cancer cell line MCF-7 showed to be more sensitive to the naphthalimidoethylamine 1a (GI_{50} of 4.23 ± 0.50 μM), and to the urea 6f, which presented the lowest GI_{50} of all compounds tested (2.44 ± 0.25 μM). Concerning the other tumor cell lines HCT-15 and BxPC-3, the triazolo compound 9d (scheme 3) was the most active against HCT-15 (GI_{50} of 3.60 ± 0.25 μM), whereas the guanidine compound 4a (scheme 2) was the most potent against BxPC-3. GI_{50} of compound 4a was not determined due to solubility limitation, but a 42.4 ± 1.6 % of cell growth inhibition in the presence of 5 μM of the compound suggested a GI_{50} value slightly over 5 μM. Results show means ± SE of at least three independent experiments performed in duplicate. NA: no activity shown. Structure-activity relationship of the compounds against the three tumor cell lines showed that generally compounds with a 2 carbon linker (n=2) provided the best inhibition. The simplest compound, amine 1a inhibited MCF-7 cell growth in a higher extend (62.4 ± 4.4 %), followed by triazole 9d (46.5 ± 5.9 % inhibition), imine 2c (45.5 ± 12.1 % inhibition), guanidine 4a (29.0 ± 1.4 % inhibition), and urea 6d (14.0 ± 5.9 % inhibition). In addition, the activity of 1a compound was lost when increasing the chain length from 2 to 3 carbon atoms (1b with 19.9 ± 3.3 % inhibition), suggesting that the shorter carbon chain (n=2) increase the effect of amine type 1 compounds against the MCF-7 cell line. For the colon cancer HCT-15 cell line, compound 9d bearing a 4-phenyl-1*H*-1,2,3-triazole unit showed higher activity (64.9 ± 7.7 % inhibition) than any other 2 carbon-atoms linker compounds. Again, the cellular growth inhibition was lost when the triazole group was combined with a 3 atoms carbon chain; inhibition with 9e is only 8.2 ± 8.0 %. In the pancreatic cancer BxPC-3 cell line, the guanidine compound 4a showed to be the most active compound with 42.4 ±



Scheme 1: Synthesis of naphthalimidoalkylamines.



Scheme 2: Synthesis of naphthalimidoalkyl derivatives.



Scheme 3: Synthesis of naphthalimidoalkyl 1H-1,2,3-triazoles 9d-f: i) 1,8-naphthalimidealkyl alcohols 1d-f, CBr_4 (1.6 equiv.), PPh3 (1.6 equiv.), DCM, rt, 12-24 h; 89-99%; ii) NaN_3 (3 equiv.), dry DMF, rt, 3 d, 81-93 %; iii) phenylacetylene (1.3-1.5 equiv.), ascorbic acid (0.1 equiv.), $CuSO_4$ (0.01 equiv.), DMF, 80°C, 5-24 h, 53-95 %.

Compound	Inhibition of cell growth (%)		
(5 μM)	MCF-7	HCT-15	BxPC-3
1a	**62.4 ± 4.4**	37.0 ± 7.7	33.8 ± 2.6
1b	19.9 ± 3.3	18.6 ± 0.9	10.1 ± 3.7
2a	19.7 ± 3.6	9.3 ± 3.7	17.6 ± 3.2
2b	30.1 ± 5.5	28.2 ± 5.5	33.5 ± 7.8
2c	**45.5 ± 12.1**	**46.2 ± 9.3**	15.9 ± 8.8
2d	20.9 ± 2.4	12.4 ± 4.4	15.4 ± 1.4
2e	18.7 ± 2.4	17.2 ± 1.8	12.2 ± 6.7
2f	19.7 ± 8.8	15.8 ± 4.2	3.5 ± 6.2
2g	15.4 ± 3.7	18.1 ± 3.5	10.9 ± 3.0
2h	27.1 ± 3.0	25.2 ± 5.7	15.0 ± 5.1
2i	9.4 ± 0.6	16.7 ± 5.3	2.6 ± 3.2
3f	NA	NA	NA
3g	NA	NA	NA
4a	29.0 ± 1.4	11.8 ± 3.3	**42.4 ± 1.6**
4b	NA	9.1 ± 4.4	NA
6d	14.0 ± 5.9	10.7 ± 1.7	3.9 ± 1.7
6e	28.3 ± 4.1	15.4 ± 1.5	9.5 ± 1.9
6f	**84.5 ± 3.2**	42.8 ± 3.0	28.5 ± 6.8
6g	9.1 ± 1.4	9.4 ± 6.4	NA
6h	6.9 ± 3.1	7.3 ± 2.8	NA
6i	10.8 ± 0.6	8.8 ± 6.6	NA
6l	8.6 ± 3.1	8.2 ± 2.6	NA
9d	**46.5 ± 5.9**	**64.9 ± 7.7**	32.9 ± 7.9
9e	31.5 ± 6.4	NA	23.7 ± 5.2
Doxorubicin	99.5 ± 0.3	73.6 ± 4.4	161.6 ± 5.7

Table 1: Effect of naphthalimide compounds 1a,b, 2a-l, 3f,g, 4a,b, 6d-l, 9d,e compared to doxorubicin on the growth of MCF-7, HCT-15, and BxPC-3 human tumor cell lines.

1.6 % cell growth inhibition. Within the three types of tumor cell lines tested, BxPC-3 turned out to be the most sensitive to the compound 4a. Interestingly a different trend was observed with the ureas, in particular compound bearing a *p*-nitrophenyl group. Urea 6f bearing a 4 carbon atoms chain showed to be the most active compound against MCF-7 cell line, displaying 84.5 ± 3.2 % inhibition of cellular growth. Shorter carbon chain resulted in the loss of activity particularly on MCF-7 cell line; this is evident when comparing the inhibition activity of compounds 6f with 6e (n=3; 28.3 ± 4.1 %), and 6d (n=2; 14.0 ± 5.9 %). The nature of terminal groups in the urea compounds was also found to be important. This was evident when comparing the inhibitory activity of the three ureas 6f (84.5 ± 3.2 %), 6i (10.8 ± 0.6 %) and 6l (8.6 ± 3.1 %), all possessing 4-carbon atoms chains. Certainly, compound 6i incorporates an extra methylene within the benzyl, extending the length of the compound, but as in the benzyl series (6g, 6h, 6i) the length of the carbon chain does not appear to have a relationship between activity and the structure of the molecules. However the relevant structural feature in terms of activity appears to be related with the presence of the nitro group attached to the phenyl group.

[a]GI_{50}, concentration of compound required to cause 50% cell growth inhibition after a continuous exposure for 48h. Results are presented as means ± SE of at least three independent experiments performed in duplicate. ND: not determined. Further analysis of the results obtained from the heterocylic imine compounds revealed that the 2c compound bearing a thiophene unit together with a 2 atom carbon chain presented better activity than its furan and pyrrole counterparts on MCF-7 (45.5 ± 12.1 % inhibition), and HCT-15 (46.2 ± 9.3 % inhibition) cell lines. BxPC3 cell line was more sensitive to the furan compound 2b (33.5 ± 7.8 % inhibition). Compound 2a bearing a pyrrole ring was the least active among the 2 carbon atoms chain imine compounds. The amino compounds in its hydrochloride salts (3f and 3g), obtained from the heterocyclic imines 2f and 2g, showed no activity on any of the analyzed tumor cell lines. For the MCF-7 cell the best inhibitor compound obtained was the compound 6f with a percentage of cell growth inhibition of 84.5% at 5 μM and a GI_{50} of 2.44 μM, close to the one found for the reference drug, doxorubicin, with 99.5% and a GI_{50} of 0.024 μM. HCT-15 was more susceptible to the compound 9d with 64.9% of cell growth inhibition at 5 μM, and a GI_{50} of 3.6 μM, similar to doxorubicin which presented 73.6% and a GI_{50} of 1.07 μM. These two compounds can be proposed as leaders for modification by medical chemistry.

Toxicity of the compounds to non-tumor cells: The toxicity of the synthesized compounds was evaluated on THP-1 differentiated macrophages and compounds 1a, 2c, 6f and 9d were further tested on mouse bone marrow-derived macrophages (BMMØ) by the MTT assay. No toxicity was observed for a 10 μM concentration from most of the compounds on THP1 differentiated macrophages after 72h incubation (data not shown). The exception occurred with compound 6f, which presented 61.1 ± 3.0 % of cell viability, value below the minimum considered as non-toxic in the screening of new compounds (70 %). However, toxicity assays performed on BMMØ with 5 μM and 50 μM compound concentrations revealed no toxicity of compound 6f for any of the concentrations tested (Table 3). Results are presented as means ± SE of two independent experiments performed in triplicate. This contradictory result may be related with the fact that THP1 differentiated macrophages derived from an immortalized cell line, which although not tumorigenic is derived from the peripheral blood of a patient with acute monocytic leukemia in contrast with the primary non-tumor BMMØ. Also, no toxicity was observed on BMMØ for compounds 1a, 2c and 9d at a concentration of 5 μM. It is noteworthy to mention that at 50 μM concentration, compound 1a induced a decrease in cell viability to 46.2 ± 12.9 % and compound 2c to 67.3 ±

Compound	GI_{50} (μM)[a]	
	MCF-7	HCT-15
1a	4.23 ± 0.50	ND
2c	> 5	> 5
6f	2.44 ± 0.25	ND
9d	> 5	3.60 ± 0.06
Doxorubicin	0.024 ± 0.007	1.07 ± 0.18

Table 2: Determination of the GI_{50} concentration of the most active naphthalimide compounds on different human tumor cell lines.

Compound	% relative cell viability	
	5 μM	50 μM
1a	88.2 ± 4.5	**46.2 ± 12.9**
2c	108.9 ± 7.9	**67.3 ± 16.6**
6f	118.5 ± 11.5	111.6 ± 8.2
9d	85.8 ± 1.6	93.3 ± 10.2

Table 3: Toxicity of compounds 1a, 2c, 6f, and 9d on BMMØ.

16.6 %. These values suggest some toxicity of these two compounds only at a concentration tenfold higher than the concentration used in our assays (5 μM).

Conclusions

Several naphthalimides were synthetized in good yields and showed very good GI_{50} values towards MCF-7, HCT-15, and BxPC-3 cancer cell lines. By changing the alkyl chain length between the naphthalimido group and the functionality at the end of the chain, it was possible to find the best structural features from 1,8-naphthalimido derivatives to achieve enhanced anticancer activity, either generically speaking either against each type of cancer cell line. From these results new perspectives can be drawn to improve the activity of naphthalimides as anticancer agents.

Experimental

Chemistry

General: All starting materials were purchased from Sigma-Aldrich, were of research-grade quality and were used without further purification. Compounds were purified by dry flash chromatography, using silica 60 <0.063 mm and water pump vacuum. TLC plates (silica gel 60 F_{254}, Macherey-Nagel) were visualized either at UV lamp or with I_2. ^{1}H NMR and ^{13}C NMR were carried out on a Varian Unity Plus 300 (300 MHz) and Brucker Avance III 400 (400 MHz) spectrometers. Infrared spectra were recorded on a Bomem MB 104. Samples were run as nujol mulls and oils as thin films. MS spectra were recorded on a VG Autospec M. spectrometer. Microanalyses were performed in a LECO-CHNS-932 analyzer. Melting points (m.p.) were determined on a Gallenkamp block and are uncorrected. The synthesis of compounds 1a-c were described before.[9a] The yields were improved by increasing the number of equivalents of diamine, and by diminishing the coupling reaction time. Alcohols 1d-f were prepared by analogy to the synthesis reported for *N*-(3-propanol)-1,8-naphthalimide (1e)[9b]. The ammonium chloride salts were obtained by dissolving the amines in dichloromethane and treatment with the solution with HCl gas, produced by heating a concentrated solution of HCl, and passing the gas through H_2SO_4.

Synthesis of naphthalimide precursors with a terminal amino group, compounds 1a-c[9a]

General procedure: To a solution of 1,8-naphthalic anhydride (1.00-2.00 g, 5.05-10.09 mmol) in ethanol (150-200 mL) was added the amine (2-6 equiv.). The reaction mixture was refluxed for 20 min-1 h, cooled down to r.t., and the precipitated solid filtered off. The solution was concentrated in the rotary evaporator, washed with diethyl ether, to give yellow solids 1a-c (83-93 %).

Synthesis of 2-(2-aminoethyl)-1*H*-benzo(*de*)isoquinoline-1,3(2*H*)-dione (1a): 1,8-Naphthalic anhydride (1.00 g, 5.05 mmol); ethanol (150 mL); 1,2-diaminoethane (6 equiv.); 1 h; yellow solid 1a (91 %); m.p.: 124-127°C; δ_H (400 MHz, $CDCl_3$) 2.95 (2H, t, *J*=6.6 Hz), 4.11 (2H, t, *J*=6.6 Hz), 7.53 (2H, t, *J*=7.8 Hz), 7.98 (2H, dd, *J*=8.4, 0.8 Hz), 8.34 (2H, dd, *J*=7.6, 0.8 Hz) ppm.

Synthesis of 2-(3-aminopropyl)-1*H*-benzo(*de*)isoquinoline-1,3(2*H*)-dione (1b): 1,8-Naphthalic anhydride (2.00 g, 10.09 mmol); ethanol (200 mL); 1,3-diaminopropane (2 equiv.); 20 min; yellow solid 1b (93 %); m.p.: 126-130°C; ν_{max} (Nujol) 1655, 3346 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.89 (2H, quint, *J*=6.8 Hz), 2.76 (2H, t, *J*=6.6 Hz), 4.27 (2H, t, *J*=7.0 Hz), 7.73 (2H, dd, *J*=8.0, 7.2 Hz), 8.19 (2H, dd, *J*=8.4, 1.2 Hz), 8.57 (2H, dd, *J*=7.4, 1.0 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 32.1, 37.7, 39.4, 122.5, 126.9, 128.1, 131.2, 131.5, 133.9, 164.2 ppm.

Synthesis of 2-(4-aminobutyl)-1*H*-benzo(*de*)isoquinoline-1,3(2*H*)-dione (1c): 1,8-Naphthalic anhydride (2.00 g, 10.09 mmol); ethanol (200 mL); 1,4-diaminobutane (2 equiv.); 1 h; yellow solid 1c (83 %); m.p.: 105-109°C; δ_H (400 MHz, $CDCl_3$) 1.47-1.55 (2H, m), 1.64-1.74 (2H, m), 2.70 (2H, t, *J*=7.0 Hz), 4.09 (2H, t, *J*=7.4 Hz), 7.61 (2H, dd, *J*=8.2, 7.4 Hz), 8.06 (2H, dd, *J*=8.4, 1.2 Hz), 8.43 (2H, dd, *J*=7.2, 0.8 Hz) ppm.

Synthesis of naftalimide precursors with a terminal hydroxyl group, compounds 1d-f

General procedure: To a solution of 1,8-naphthalic anhydride (1.00-2.03 g, 5.05-10.25 mmol), in DMF (12-24 mL) was added the amino-alcohol (10 equiv.) and DBU (1 equiv.). The reaction mixture was stirred in an oil bath at 75°C, for 4-7 h. Dichloromethane (100 mL) was added, the solution washed with water (5x60 mL) and the organic phase, dried over magnesium sulfate, filtered and concentrated in the rotary evaporator to give compounds 1d-f (88-96 %) as pale yellow solids.

Synthesis of 2-(2-hydroxy-ethyl)-1*H*-benzo(*de*)isoquinoline-1,3(2*H*)-dione (1d): 1,8-Naphthalic anhydride (2.03 g, 10.13 mmol); DMF (24 mL); 2-aminoethanol (10 equiv.); DBU (1 equiv.); 7 h; yellow-brown solid 1d (88 %); m.p.: 164-167°C; ν_{max} (Nujol) 1650, 3482 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 3.99 (2H, t, *J*=5.4 Hz), 4.47 (2H, t, *J*=5.2 Hz), 7.76 (2H, dd, *J*=8.4, 7.2 Hz), 8.22 (2H, dd, *J*=8.4, 0.8 Hz), 8.60 (2H, dd, *J*=7.4, 1.0 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 42.8, 61.7, 122.4, 127.0, 128.2, 131.5, 131.5, 134.2, 165.1 ppm. MS: *m/z* 242 $(M+1)^+$.

Synthesis of 2-(3-hydroxypropyl)-1*H*-benzo(*de*)isoquinoline-1,3(2*H*)-dione (1e) [9b]**:** 1,8-Naphthalic anhydride (2.03 g, 10.25 mmol); DMF (24 mL); 3-aminopropanol (10 equiv.); DBU (1 equiv.); 7 h; pale yellow solid 1e (96 %); m.p.: 115-118°C; δ_H (400 MHz, $CDCl_3$) 1.96-2.04 (2H, m), 3.14 (1H, t, *J*=7.0 Hz), 3.60 (2H, q, *J*=6.0 Hz), 4.36 (2H, t, *J*=6.2 Hz), 7.77 (2H, dd, *J*=8.2, 7.4 Hz), 8.24 (2H, dd, *J*=8.2, 1.0 Hz), 8.62 (2H, dd, *J*=7.2, 1.2 Hz) ppm. MS: *m/z* 256 $(M+1)^+$.

Synthesis of 2-(4-hydroxybutyl)-1*H*-benzo(*de*)isoquinoline-1,3(2*H*)-dione (1f): 1,8-Naphthalic anhydride (1.00 g, 5.05 mmol); DMF (12 mL); 4-aminobutanol (10 equiv.); DBU (1 equiv.); 4 h; pale yellow solid 1f (91 %); m.p.: 109-111°C; ν_{max} (Nujol) 1655, 3510 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.66-1.76 (2H, m), 1.80-1.90 (2H, m), 3.75 (2H, t, *J*=6.4 Hz), 4.23 (2H, dd, *J*=8.0, 6.8 Hz), 7.75 (2H, dd, *J*=8.2, 7.4 Hz), 8.20 (2H, dd, *J*=8.4, 0.8 Hz), 8.59 (2H, dd, *J*=7.4, 1.0 Hz) ppm; δ_C (100

MHz, $CDCl_3$) 24.5, 29.9, 39.9, 62.5, 122.6, 126.9, 128.1, 131.2, 131.5, 133.9, 164.2 ppm. MS: *m/z* 270 $(M+1)^+$.

Synthesis of imines 2a-i

General procedure: To a solution of 1,8-naphthalimide 1a-c (92-413 mg, 0.38-1.63 mmol) in ethanol (5-12 mL) was added the aldehyde (1-1.2 eq.), and the mixture refluxed for 20 min-7 h. A solid precipitated out at rt or by cooling at - 20°C. The solid formed was filtrated, washed with ethanol, to give the respective imine (compounds **2a-i**; 61.7-100 %).

Synthesis of (*E*)-2-(2-(((1H-pyrrol-2-yl)methylene)amino)ethyl -1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (2a): Compound 1a (247 mg, 1.03 mmol); pyrrol-2-carboxaldehyde (1.2 equiv.); ethanol (10 mL); 1 h; salmon solid (85.2 %); m.p.: 195-198°C; ν_{max} (Nujol) 3180, 1695, 1657, 1639 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 3.89 (2H, t, *J*=6.6 Hz), 4.50 (2H, t, *J*=6.8 Hz), 6.18 (1H, dd, *J*=3.2, 2.4 Hz), 6.40 (1H, dd, *J*=3.6, 1.6 Hz), 6.88 (1H, dd, *J*=2.0, 1.2 Hz), 7.75 (2H, dd, *J*=8.4, 7.6 Hz), 8.06 (1H, d, *J*=0.4 Hz), 8.21 (2H, dd, *J*=8.4, 1.2 Hz) 8.60 (2H, dd, *J*=7.2, 1.2 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 40.9, 57.9, 109.6, 114.5, 122.0, 122.6, 126.9, 128.2, 130.0, 131.3, 131.6, 133.9, 153.1, 164.2 ppm; HRMS (FAB): calcd for $C_{19}H_{15}N_3O_2$: 318.1237 (M+1); obtained: 318.1234.

Synthesis of (*E*)-2-(2-((furan-2-ylmethylene)amino)ethyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (2b): Compound **1a** (142 mg, 0.59 mmol); furfural (1 equiv.); ethanol (6 mL); 4 h 50 min; brown solid (62.8 %); m.p.: 149-151°C; ν_{max} (Nujol) 1653, 1584 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 3.94 (2H, t, *J*=8.2 Hz), 4.55 (2H, t, *J*=8.2 Hz), 6.47 (1H, dd, *J*=3.6, 2.0 Hz), 6.76 (1H, dd, *J*=3.6, 0.4 Hz), 7.52 (1H, d, *J*=1.6 Hz), 7.77 (2H, dd, *J*=8.4, 7.2 Hz), 8.19 (1H, d, *J*=1.2 Hz), 8.23 (2H, dd, *J*=8.4, 1.2 Hz), 8.63 (2H, dd, *J*=7.2, 1.2 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 40.8, 58.6, 111.6, 114.3, 122.6, 126.9, 128.2, 131.3, 131.6, 133.9, 144.8, 151.3, 151.5, 164.1 ppm. Anal. Cald for $C_{19}H_{14}N_2O_3$: C, 71.69, H, 4.43, N, 8.80. Found: C, 71.14, H, 4.36, N, 8.68.

Synthesis of (*E*)-2-(2-((thiophen-2-ylmethylene)amino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (2c): Compound 1a (92 mg, 0.38 mmol); thiophene-2-carboxaldehyde (1 equiv.); ethanol (5 mL); 6 h; brown solid (72.6 %); m.p.: 176-178°C; ν_{max} (Nujol) 1692, 1656, 1634 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 3.94 (2H, t, *J*=7.0 Hz), 4.53 (2H, t, *J*=6.8 Hz), 7.04 (dd, *J*=5.2, 4.0 Hz, 1H), 7.27 (1H, dd, *J*=3.6, 1.2 Hz), 7.38 (1H, dt, *J*=5.2, 1.2 Hz), 7.76 (2H, dd, *J*=8.4, 7.2 Hz), 8.22 (2H, dd, *J*=8.0, 0.8 Hz), 8.45 (1H, d, *J*=1.2 Hz), 8.62 (2H, dd, *J*=7.6, 1.2 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 40.7, 58.2, 122.7, 126.9, 127.2, 128.2, 129.1, 130.5, 131.3, 131.6, 133.9, 142.4, 155.9, 164.1 ppm. Anal. Cald for $C_{19}H_{14}N_2O_2S$: C, 68.24, H, 4.22, N, 8.38, S, 9.59. Found: C, 68.13, H, 4.20, N, 8.34, S, 9.36.

Synthesis of (*E*)-2-(2-(((1H-imidazol-2-yl)methylene)amino) ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (2d): Compound 1a (99 mg, 0.41 mmol); imidazol-2-carboxaldehyde (1 equiv.); ethanol (5 mL); 20 min; white solid (quant.); m.p.: 221-222°C; ν_{max} (Nujol) 3148, 1696, 1649 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 4.01 (2H, t, *J*=6.4 Hz), 4.57 (2H, t, *J*=6.4 Hz), 7.09 (1H, br s), 7.13 (1H, br s), 7.76 (2H, dd, *J*=8.4, 7.6 Hz), 8.19 (1H, t, *J*=1.2 Hz), 8.22 (2H, dd, *J*=8.4 , 0.8 Hz), 8.60 (2H, dd, *J*=7.2, 1.2 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 40.6, 58.3, 118.0, 122.5, 126.9, 128.2, 131.4, 131.6, 134.1, 144.8, 153.0, 164.3 ppm. Anal. Calcd for $C_{18}H_{14}N_4O_2$: C, 67.91, H, 4.43, N, 17.60. Found; C, 67.28, H, 4.33, N, 17.60.

Synthesis of (*E*)-2-(3-(((1*H*-pyrrol-2-yl)methylene)amino) propyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (2e): Compound 1b (136 mg, 0.54 mmol); pyrrol-2-carboxaldehyde (1 equiv.); ethanol (12 mL); 50 min; salmon solid (90.3 %); m.p.: 170-172°C; ν_{max} (Nujol) 3351, 1693, 1656, 1642 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 2.13 (2H, quint, *J*=6.8 Hz), 3.65 (2H, dt, *J*=6.8, 1.2 Hz), 4.28 (2H, t, *J*=7.2 Hz), 6.18 (1H, dd, *J*=3.6, 2.4 Hz), 4.44 (1H, dd, *J*=3.6, 1.2 Hz), 6.82 (1H, dd, *J*=1.2, 0.4 Hz), 7.74 (2H, dd, *J*=8.4, 7.6 Hz), 8.06 (1H, d, *J*=0.4 Hz), 8.19 (2H, dd, *J*=8.4, 0.8 Hz), 8.58 (2H, dd, *J*=7.6, 1.2 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 29.3, 38.7, 58.2, 109.5, 114.6, 122.0, 122.6, 126.9, 128.1, 129.8, 131.2, 131.5, 133.9, 152.2, 164.2 ppm; HRMS (FAB): calcd for $C_{20}H_{17}N_3O_2$: 332.1389 (M+1); found: 332.1394.

Synthesis of (*E*)-2-(3-((furan-2-ylmethylene)amino)propyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (2f)

Compound **1b** (174 mg, 0.68 mmol); furfural (1 equiv.); ethanol (5 mL); 3h 20 min; brown solid (76.5 %); m.p.: 98-100°C; ν_{max} (Nujol) 1690, 1651 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 2.20 (2H, quint. *J*=6.8 Hz), 3.71 (2H, dt, *J*=6.8, 1.2 Hz), 4.31 (2H, t, *J*=6.8 Hz), 6.40 (1H, dd, *J*=3.6, 2.0 Hz), 6.65 (1H, d, *J*=3.6 Hz), 7.41 (1H, d, *J*=1.6 Hz), 7.73 (2H, dd, *J*=8.0, 7.2 Hz), 8.12 (1H, d, *J*=1.2 Hz), 8.19 (2H, dd, *J*=8.0, 1.2 Hz), 8.57 (2H, dd, *J*=7.2, 1.2 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 29.3, 38.6, 59.5, 111.4, 113.6, 122.6, 126.8, 128.1, 131.1, 131.5, 133.8, 144.5, 150.1, 151.5, 164.2 ppm. HRMS: calcd for $C_{20}H_{17}O_3N_2$: 333.1234 (M+1); found: 333.1234.

Synthesis of (*E*)-2-(2-((thiophen-2-ylmethylene)amino)propyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (2g): Compound 1b (127 mg, 0.50 mmol); thiophene-2-carboxaldehyde (1 equiv.); ethanol (11 mL); 1:30 h, brown solid (74.1 %); m.p.: 132-134°C; ν_{max} (Nujol) 1688, 1652 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 1.98 (2H, quint. *J*=6.8 Hz), 3.59 (2H, t, *J*=6.8 Hz), 4.13 (2H, t, *J*=6.8 Hz), 7.05 (1H, dd, *J*=5.2, 3.6 Hz), 7.34 (1H, dd, *J*=3.6, 1.2 Hz), 7.52 (1H, dt, *J*=2.4, 1.2 Hz), 7.82 (2H, dd, *J*=8.4, 7.2 Hz), 8.40 (2H, dd, *J*=8.4, 1.2 Hz), 8.42 (1H, d, *J*=1.2 Hz), 8.45 (2H, dd, *J*=7.2, 1.2 Hz) ppm; δ_C (100 MHz, DMSO-d_6) 28.7, 38.2, 58.1, 122.1, 127.1, 127.4, 127.5, 129.1, 130.6, 130.9, 131.2, 134.2, 142.2, 154.7, 163.5 ppm; HRMS (FAB): calcd for $C_{20}H_{16}N_2O_2S$: 349.1004 (M+1); found: 349.1005.

Synthesis of (*E*)-2-(4-(((1*H*-pyrrol-2-yl)methylene)amino) butyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (2h): Compound 1c (151 mg, 0.56 mmol); pyrrol-2-carboxaldehyde (1 equiv.); ethanol (10 mL); 7 h; salmon solid (61.7 %); m.p.: 124-126°C; ν_{max} (Nujol) 3346, 1689, 1654, 1641 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.79-1.80 (4H, m), 3.59 (2H, t, *J*=6.4 Hz), 4.23 (2H, t, *J*=7.2 Hz), 6.20 (1H, dd, *J*=3.6, 2.8 Hz), 6.45 (1H, dd, *J*=3.6, 1.6 Hz), 6.87-6.88 (1H, m), 7.74 (2H, dd, *J*=8.0, 7.2 Hz), 8.05 (1H, d, *J*=0.8 Hz), 8.19 (2H, dd, *J*=8.4, 0.8 Hz), 8.59 (2H, dd, *J*=7.2, 1.2 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 25.8, 28.5, 40.1, 60.2, 109.5, 114.1, 121.8, 122.7, 126.9, 128.1, 130.1, 131.2, 131.5, 133.8, 151.8, 164.2 ppm; HRMS (FAB): calcd for $C_{21}H_{19}N_3O_2$: 346.1554 (M+1); found: 346.1550.

Synthesis of (*E*)-2-(4-((thiophen-2-ylmethylene)amino)butyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (2i): Compound 1c (413 mg, 1.63 mmol); thiopene-2-carboxaldehyde (1 equiv.); ethanol (10 mL); 2h 30 min, beige solid (72.6 %); m.p.: 103-105°C; ν_{max} (Nujol) 1696, 1657, 1635 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 1.63-1.66 (4H, m), 3.53 (2H, t, *J*=6.4 Hz), 4.05 (2H, t, *J*=6.8 Hz), 7.10 (1H, dd, *J*=4.8, 3.6 Hz), 7.40 (1H, dd, *J*=3.6, 1.2 Hz), 7.60 (1H, dt, *J*=3.6, 1.2 Hz), 7.82 (2H, dd, *J*=8.0, 7.2 Hz), 8.40 (2H, dd, *J*=8.4, 0.8 Hz), 8.43 (1H, s), 8.44 (2H, dd, *J*=7.2, 1.2 Hz) ppm; δ_C (100 MHz, DMSO-d_6) 25.4, 28.0, 56.0, 59.1, 122.0, 127.2, 127.3, 127.7, 129.2, 130.7, 131.0, 131.2, 134.2, 142.3, 154.5, 163.4 ppm; HRMS (FAB): calcd for $C_{21}H_{18}N_2O_2S$: 363.1171 (M+1); found: 363.1162.

Synthesis of amines 3f,g

General procedure: To a stirred solution of imine 2f/g (32-80 mg, 0.09-0.24 mmol) in a 1:1 mixture of THF (5 mL): MeOH (5 mL) was added $NaBH_4$ (2 equiv.), the reaction continued at r.t. for 4-12 h. The solvents were removed in the rotary evaporator, DCM (30 mL) was added, and the solution washed with water (3x30 mL), dried over $MgSO_4$, and the solvent removed in the rotary evaporator to give amines **3f**,**g** (75.0-83.9%) as oils. The respective oil (80-100 mg) was diluted in DCM (15 mL), HCl bubbled through for 2-4 h. The hydrochloride salt precipitated out of solution (53.4-55.2%).

Synthesis of 2-(3-((furan-2-ylmethyl)amino)propyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (3f): Imine **2f** (80 mg, 0.24 mmol); 4 h; orange oil (75.0 %); ν_{max} 3067, 1697, 1657 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.99 (2H, quint, *J*=6.8 Hz), 2.73 (2H, t, *J*=6.8 Hz), 3.83 (2H, s), 4.28 (2H, t, *J*=6.8 Hz), 6.19 (1H, dd, *J*=3.2, 0.8 Hz), 6.27 (1H, dd, *J*=3.2, 2.0 Hz), 7.31 (1H, dd, *J*=2.0, 0.8 Hz), 7.76 (2H, dd, *J*=8.4, 7.6 Hz), 8.22 (2H, dd, *J*=8.4, 0.8 Hz), 8.60 (2H, dd, *J*=7.2, 1.2 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 28.1, 38.2, 45.9, 46.1, 107.1, 110.1, 122.6, 126.9, 128.2, 131.3, 131.6, 133.9, 141.8, 153.6, 164.3 ppm. MS: *m/z* 334 $(M+1)^+$.

Hydrochloride ammonium salt: amine 3f (80 mg, 0.24 mmol); 2 h; beige solid (55.2 %); δ_H (400 MHz, D_2O) 2.06 (2H, quint, *J*=6.8 Hz), 3.09 (2H, t, *J*=7.6 Hz), 4.02 (2H, t, *J*=6.8 Hz) 4.33 (2H, s), 6.39 (1H, dd, *J*=3.2, 1.6 Hz), 6.60 (1H, d, *J*=3.6 Hz), 7.50 (1H, d, *J*=1.2 Hz), 7.62 (2H, t, *J*=7.6 Hz), 8.13 (2H, d, *J*=8.0 Hz), 8.16 (2H, d, *J*=7.2 Hz) ppm.

Synthesis of 2-(3-((thiophen-2-ylmethyl)amino)propyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (3g): Imine 2g (32 mg, 0.09 mmol); 12 h; orange oil (83.9 %); ν_{max} 3307, 1692, 1653 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 2.00 (2H, quint, *J*=6.8 Hz), 2.77 (2H, t, *J*=6.8 Hz), 4.03 (2H, s), 4.29 (2H, t, *J*=6.8 Hz), 6.92 (1H, dd, *J*=4.8, 3.6 Hz), 6.94-6.95 (1H, m), 7.17 (1H, dd, *J*=4.8, 1.2 Hz), 7.76 (2H, dd, *J*=8.4, 7.6 Hz), 8.22 (2H, dd, *J*=8.4, 0.8 Hz), 8.61 (2H, dd, *J*=7.6, 1.2 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 28.1, 38.2, 46.1, 48.1, 122.6, 124.3, 125.0, 126.6, 126.9, 128.2, 131.3, 131.6, 133.9, 143.8, 164.3 ppm. HRSM (FAB): calcd for $C_{20}H_{19}N_2O_2S$: 351.1162 (M+1); found: 351.1165.

Hydrochloride ammonium salt: amine 3g (100 mg, 0.29 mmol); 4 h; white solid (53.4 %); δ_H (400 MHz, D_2O) 2.05-2.10 (2H, m), 3.10 (2H, t, *J*=7.2 Hz), 4.03 (2H, *J*=6.0 Hz), 4.50 (2H, s), 6.97 (1H, dd, *J*=5.2, 3.6 Hz), 7.24 (1H, dt, *J*=3.6, 0.8 Hz), 7.42 (1H, dd, *J*=5.2, 1.2 Hz), 7.62 (2H, t, *J*=7.6 Hz), 8.13-8.17 (4H, m) ppm.

Synthesis of guanidine 4a-c

General procedure: To a solution of 1,8-naphthalimide 1a-c (0.13-0.41 g, 0.53-1.63 mmol) in THF (10 mL) was added 2-methylisothiouronium iodide[10] (1.3 equiv.) at r.t. The suspension was refluxed for 3h – 6h 30 min. A solid precipitated out from the reaction mixture by cooling at rt. Guanidines **4a-c** (74.1-89.2 %) were obtained as white solids.

Synthesis of 1-(2-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)ethyl)guanidine hydroiodide (4a): Compound **1a** (0.13 g, 0.53 mmol); THF (10 mL); 3 h; white solid (77.4 %); m.p.: 204-207°C. ν_{max} (Nujol) 3429, 3195, 3168, 1693, 1652 cm^{-1}. δ_H (400 MHz, DMSO-d_6) 3.09 (½ x2H, t, *J*=6.0 Hz)[a], 3.49 (½ x2H, t, *J*=5.6 Hz)[a], 4.19 (½ x2H, t, *J*=6.0 Hz)[b], 4.26 (½ x2H, t, *J*=6.0 Hz)[b], 6.2-7.7 (5H, br s), 7.88 (2H, t, *J*=7.2 Hz), 8.47 (2H, d, *J*=6.8 Hz), 8.49 (2H, d, *J*=7.2 Hz) ppm. δ_C (100 MHz, DMSO-d_6) 38.1[c], 38.4[c], 38.6[c], 38.8[c], 122.1, 122.2, 127.2, 127.5, 130.7, 131.3, 134.4, 156.8, 163.7, 164.0 ppm. HRSM (FAB): calcd for $C_{15}H_{14}N_4O_2$: 283.1186 (M+1); found: 283.1190.

[a],[b] Signals of methylene Hs at C-1 and C-2 in the two conformations of compound 4a (1:1 ratio), probably resulting from hydrogen bonding. [c] Signals related to the C-1 and C-2 of the two conformers.

Synthesis of 1-(3-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)propyl)guanidine hydroiodide (4b): Compound 1b (0.41 g, 1.63 mmol); THF (10 mL); 6 h; white solid (74.1 %); m.p.: 255-257°C; ν_{max} (Nujol) 3184, 3127, 3059, 1696, 1657 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 1.67-1.73 (2H, m, 20%)[a], 1.80-1.86 (2H, quint., *J*=6.8 Hz, 22%)[a], 1.93 (2H, quint, *J*=6.8 Hz, 58 %)[a], 2.87 (2H, t, *J*=7.6 Hz, 60.8 %)[b], 3.13 (2H, t, *J*=6.8 Hz, 19.6 %)[b], 3.19 (2H, t, *J*=6.8 Hz, 19.6%)[b], 4.07 (2H, t, *J*=6.8 Hz), 5.59-7.60 (5H, br s), 7.81 (2H, t, *J*=7.6 Hz), 8.40 (4H, d, *J*=7.6 Hz) ppm; δ_C (100 MHz, DMSO-d_6) 26.0[c], 27.4[a], 27.9[a], 37.0[c], 37.1[c], 38.2[b], 38.8[b], 121.9, 127.2, 127.3, 130.6, 131.2, 134.3, 156.6, 163.6 ppm. MS: *m/z* 297 $(M-I)^+$.

[a],[b] Signals of methylene Hs at C-2 and C-3, are relative to the two conformations (1:3 ratio) of compound **4b**, probably resulting from hydrogen bonding. [c] Signals for C-1, C-2 and C-3 of the major conformer.

Synthesis of 1-(4-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)butyl)guanidine hydroiodide (4c): Compound **1c** (0.15 g, 0.56 mmol); THF (10 mL); 6h 30 min; white solid (89.2%); m.p.: 224-226°C; ν_{max} (Nujol) 3419, 3309, 3258, 3161, 1691, 1655 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 1.51-1.56 (2H, m), 1.65-1.69 (2H, m), 3.10-3.15 (2H, m), 4.07 (2H, t, *J*=7.2 Hz), 5.59-7.50 (5H, br s), 7.87 (2H, dd, *J*=8.4, 7.2 Hz), 8.46 (2H, dd, *J*=8.4, 1.2 Hz), 8.49 (2H, dd, *J*=7.2, 1.2 Hz) ppm; δ_C (100 MHz, DMSO-d_6) 24.8, 26.3, 39.2, 40.6, 121.9, 127.2, 127.3, 130.7, 131.3, 134.3, 156.6, 163.5 ppm. HRMS calcd for $C_{17}H_{19}O_2N_4$ 311.1503; found: 311.1501.

Synthesis of amides 5a-l

General procedure: To a solution of compound 1a-c (0.10-0.15 g, 0.37-0.62 mmol) in a solvent (12-15 mL) was added the anhydride (1.1-2.0 equiv.). The reaction mixture refluxed for 1 h-2 h 30 min., concentrated in the rotary evaporator and the resulting oil cooled to -20°C overnight. White, pale yellow, or brownish solids 5a-l was formed, filtered, and eventually recrystallized from ethanol (54-87 %).

Synthesis of *N*-(2-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl)ethyl)acetamide (5a)

Compound **1a** (0.15 g, 0.62 mmol); ethanol (14 mL); acetic anhydride (1.1 equiv.); 1 h; white solid **5a** (76 %); m.p.: 198-200°C; ν_{max} (Nujol) 1652, 1676, 3359 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.90 (3H, s), 3.66 (2H, q, *J*=5.4 Hz), 4.39 (2H, t, *J*=5.4 Hz), 6.23 (1H, br s), 7.76 (2H, dd, *J*=8.0, 7.2 Hz), 8.22 (2H, dd, *J*=8.4, 1.2 Hz), 8.59 (2H, dd, *J*=7.2, 1.2 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 23.2, 39.4, 39.6, 122.3, 127.0, 128.2, 131.5, 131.6, 134.3, 164.8, 170.4 ppm. HRMS (FAB): calcd for $C_{16}H_{14}N_2O_3$: 283.1077 (M+1); found: 283.1076.

Synthesis of *N*-(3-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl)propyl)acetamide (5b)

Compound **1b** (0.15 g, 0.59 mmol); ethanol (14 mL); acetic anhydride (1.1 equiv.); 1 h; white solid **5b** (81 %); m.p.: 170-173°C; ν_{max} (Nujol) 1659, 1696, 3293 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.96 (2H, quint, *J*=6.3 Hz), 2.05 (3H, s), 3.26 (2H, q, *J*=6.1 Hz), 4.27 (2H, t, *J*=6.4 Hz), 6.49 (1H, br s), 7.77 (2H, dd, *J*=8.4, 7.4 Hz), 8.42 (2H, dd, *J*=8.2, 1.0 Hz), 8.60 (2H, dd, *J*=7.4, 1.0 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 23.5, 27.9, 36.1, 37.5, 122.3, 127.0, 128.1, 131.5, 131.6, 134.2, 164.5, 170.2 ppm. HRMS (FAB): calcd for $C_{17}H_{16}N_2O_3$: 297.1234 (M+1); found: 297.1233.

Synthesis of *N*-(4-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl)butyl)acetamide (5c): Compound 1c (0.15 g, 0.56 mmol); ethanol (14 mL); acetic anhydride (1.1 equiv.); 1h 30 min; white solid 5c (80 %); m.p.: 199-201°C; ν_{max} (Nujol) 1658, 1695, 3301 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.63 (2H, quint, *J*=7.2 Hz), 1.79 (2H, quint, *J*=7.5 Hz), 1.98 (3H, s), 3.34 (2H, q, *J*=6.5 Hz), 4.18 (2H, t, *J*=7.4 Hz), 5.96 (1H, br s), 7.74 (2H, t, *J*=7.8 Hz), 8.20 (2H, d, *J*=8.4 Hz), 8.57 (2H, d, *J*=7.2 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 23.3, 25.4, 26.7, 39.1, 39.7, 122.5, 126.9, 128.1, 131.2, 131.5, 134.0, 164.2, 170.1 ppm. MS: *m/z* 333 $(M+Na)^+$.

Synthesis of *N*-(2-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl)ethyl)-2,2,2-trifluoroacetamide (5d) : Compound 1a (0.15 g, 0.62 mmol); dry DCM (15 mL); trifluoroacetic anhydride (2 equiv.); 2 h 30 min; recrystallization; white solid 5d (77 %); m.p.: 186-189°C; ν_{max} (Nujol) 1662, 1703, 3107 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 3.74-3.80 (2H, m), 4.47-4.52 (2H, m), 7.48 (1H, br s), 7.78 (2H, dd, *J*=8.2, 7.4 Hz), 8.25 (2H, dd, *J*=8.4, 0.8 Hz), 8.62 (2H, dd, *J*=7.4, 1.0 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 38.8, 40.4, 115.7 (3F, q, *J*=286 Hz), 122.0, 127.1, 128.2, 131.6, 131.8, 134.6, 157.6 (q, *J*=37 Hz), 165.1 ppm. HRMS (FAB): calcd for $C_{16}H_{12}O_3N_2F_3$: 337.0795 (M+1); found: 337.0797.

Synthesis of *N*-(3-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl)propyl)-2,2,2-trifluoroacetamide (5e): Compound 1b (0.15 g, 0.59 mmol); dry DCM (15 mL); trifluoroacetic anhydride (2 equiv.); 2 h 30 min; recrystallization; beige solid 5e (63 %); m.p.: 149-152°C; ν_{max} (Nujol) 1655, 1697, 3097 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 2.04 (2H, quint, *J*=6.0 Hz), 3.37 (2H, q, *J*=6.2 Hz), 4.29 (2H, t, *J*=6.0 Hz), 7.77 (1H, s), 7.80 (2H, d, *J*=7.6 Hz), 8.26 (2H, dd, *J*=8.0, 0.8 Hz), 8.63 (2H, dd, *J*=7.4, 1.0 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 27.4, 36.3, 37.1, 116.0 (3F, q, *J*=286 Hz), 126.9, 127.1, 128.2, 131.7, 133.9, 134.5, 157.9 (q, *J*=36 Hz) 164.9 ppm. MS: *m/z* 373 $(M+Na)^+$.

Synthesis of *N*-(4-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl)butyl)-2,2,2-trifluoroacetamide (5f): Compound 1c (0.15 g, 0.56 mmol); dry DCM (15 mL); trifluoroacetic anhydride (2 equiv.); 2 h 30 min; recrystallization; pale brown solid 5f (86 %); m.p.: 153-156°C; ν_{max} (Nujol) 1657, 1698, 3108 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.74 (2H, quint, *J*=6.8 Hz), 1.85 (2H, quint, *J*=7.1 Hz), 3.50 (2H, q, *J*=6.4 Hz), 4.22 (2H, t, *J*=7.2 Hz), 7.07 (1H, br s), 7.77 (2H, dd, *J*=8.2, 7.4 Hz), 8.24 (2H, dd, *J*=8.2, 1.0 Hz), 8.60 (2H, dd, *J*=7.2, 0.8 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 27.2, 25.8, 39.3, 39.4, 115.9 (3F, q, *J*=286 Hz), 122.3, 127.0, 128.1, 131.5, 131.6, 134.3, 157.5 (q, *J*=37 Hz), 164.5 ppm. MS: *m/z* 287 $(M+Na)^+$.

Synthesis of (*E*)-4-((2-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl)ethyl)amino)-4-oxobut-2-enoic acid (5g): Compound 1a (0.15 g, 0.62 mmol); ethanol (14 mL); maleic anhydride (1.1 equiv.); 1 h; light yellow solid 5g (85 %); m.p.: 212-215°C; ν_{max} (Nujol) 1625, 1664, 1695, 3284 cm^{-1}; δ_H (400 MHz, DMSO) 3.52 (2H, q, *J*=6.0 Hz), 4.20 (2H, t, *J*=6.0 Hz), 6.18 (2H, d, *J*=12.4 Hz), 6.25 (2H, d, *J*=12.4 Hz), 7.85 (2H, dd, *J*=8.4, 7.2 Hz), 8.46 (4H, ddd, *J*=9.4, 7.8, 1.0 Hz), 9.22 (1H, t, *J*=5.8 Hz), 14.74 (1H, s) ppm; δ_C (100 MHz, DMSO) 37.0, 38.8, 122.1, 127.2, 127.5, 130.7, 134.3, 131.3, 131.5, 132.9, 163.7, 165.4, 165.7 ppm. HRMS (FAB): calcd for $C_{18}H_{14}O_5N_2Na$ 361.0795 (M+Na); found: 361.0794.

Synthesis of (*E*)-4-((3-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl)propyl)amino)-4-oxobut-2-enoic acid (5h): Compound 1b (0.15 g, 0.59 mmol); ethanol (14 mL); maleic anhydride (1.1 equiv.); 1 h; light yellow solid 5h (69 %); m.p.: 165-169°C; ν_{max} (Nujol) 1629, 1656, 1703, 3250 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 2.07 (2H, quint, *J*=6.1 Hz), 3.40 (2H, q, *J*=6.0 Hz), 4.30 (2H, t, *J*=6.2 Hz), 6.41 (2H, s), 7.81 (2H, t, *J*=7.8 Hz), 7.95 (1H, br s), 8.28 (2H, dd, *J*=8.0, 0.8 Hz), 8.63 (2H, dd, *J*=7.4, 1.0 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 27.2, 36.9, 37.3, 122.0, 127.2, 128.2, 131.1, 131.6, 131.8, 134.7, 136.7, 165.0, 165.0, 166.0 ppm. HRMS (FAB): calcd for $C_{19}H_{16}N_2O_5$: 351.0975 (M+1); found: 351.0970.

Synthesis of (*E*)-4-((4-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl)butyl)amino)-4-oxobut-2-enoic acid (5i)

Compound **1c** (0.10 g, 0.37 mmol); ethanol (12 mL); maleic anhydride (1.1 equiv.); 1 h; white solid **5i** (54 %); m.p.: 156-160°C; ν_{max} (Nujol) 1629, 1657, 1692, 3355 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.73 (2H, quint, *J*=6.8 Hz), 1.85 (2H, quint, *J*=7.2 Hz), 3.50 (2H, q, *J*=6.3 Hz), 4.20 (2H, t, *J*=7.2 Hz), 6.32 (1H, d, *J*=12.8 Hz), 6.47 (1H, d, *J*=12.8 Hz), 7.75 (2H, dd, *J*=8.2, 7.4 Hz), 8.17 (1H, br s), 8.22 (2H, dd, *J*=8.4, 1.2 Hz), 8.58 (2H, dd, *J*=7.4, 1.0 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 25.2, 25.4, 39.4, 39.9, 122.3, 127.0, 128.1, 131.5, 131.5, 131.6, 134.3, 136.1, 164.4, 165.6, 166.2 ppm. MS: *m/z* 389 $(M+Na)^+$.

Synthesis of 4-((2-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl)ethyl)amino)-4-oxobutanoic acid (5j): Compound 1a (0.15 g, 0.62 mmol); ethanol (14 mL); succinic anhydride (1.1 equiv.); 1 h 30 min; white solid 5j (87%); m.p.: 195-199°C; ν_{max} (Nujol) 1640, 1657, 1696, 3311 cm^{-1}; δ_H (400 MHz, DMSO) 2.19 (2H, t, *J*=6.8 Hz), 2.33 (2H, t, *J*=6.8 Hz), 3.36 (2H, t, *J*=6.0 Hz), 4.11 (2H, t, *J*=6.0 Hz), 7.85 (2H, dd, *J*=8.0, 7.2 Hz), 7.97 (1H, t, *J*=6.0 Hz), 8.43 (2H, dd, *J*=8.4, 1.2 Hz), 8.46 (2H, dd, *J*=7.6, 1.2 Hz), 12.00 (1H, s) ppm; δ_C (100 MHz, DMSO) 29.1, 30.2, 36.5, 39.4, 122.3, 127.2, 127.5, 130.6, 131.3, 134.2, 163.6, 171.2, 173.8 ppm. HRMS (FAB): calcd for $C_{18}H_{16}N_2O_5$: 339.0986 (M+1); found: 339.0979.

Synthesis of 4-((3-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl)propyl)amino)-4-oxobutanoic acid (5k): Compound 1b (0.15 g, 0.59 mmol); ethanol (14 mL); succinic anhydride (1.1 equiv.); 1 h; beige solid 5k (66 %); m.p.: 165-168°C; ν_{max} (Nujol) 1644, 1658, 1695, 3293 cm^{-1}; δ_H (400 MHz, DMSO) 1.70 -1.80 (2H, m), 2.29 (2H, t, *J*=6.8 Hz), 2.39 (2H, t, *J*=7.2 Hz), 3.11 (2H, q, *J*=6.6 Hz), 4.11 (2H, t, *J*=7.2 Hz), 7.83 (2H, dd, *J*=8.0, 7.6 Hz), 7.89 (1H, t, *J*=5.6 Hz), 8.41 (2H, dd, *J*=8.4, 0.8 Hz), 8.45 (2H, dd, *J*=7.2, 0.8 Hz) ppm; δ_C (100 MHz, DMSO) 28.0, 29.5, 30.3, 36.6, 37.8, 122.0, 127.2, 127.3, 130.7, 131.3, 134.3, 163.4, 171.0, 174.0 ppm. HRMS (FAB): Calcd for $C_{19}H_{18}N_2O_5$: 353.1143 (M+1); found: 353.1139.

Synthesis of 4-((4-(1,3-dioxo-1*H*-benzo (*de*)isoquinolin-2(3*H*)-yl)butyl)amino)-4-oxobutanoic acid (5l): Compound 1c (0.15 g, 0.56 mmol); ethanol (14 mL); succinic anhydride (1.1 equiv.); 1 h; white solid 5l (66 %); m.p.: 169-171°C; ν_{max} (Nujol) 1648, 1666, 1699, 3188, 3356 cm^{-1}; δ_H (400 MHz, DMSO) 1.43 (2H, quint, *J*=7.4 Hz), 1.62 (2H, quint, *J*=7.5 Hz), 2.27 (2H, t, *J*=6.8 Hz), 2.38 (2H, t, *J*=7.2 Hz), 3.05 (2H, q, *J*=6.5 Hz), 4.02 (2H, t, *J*=7.2 Hz), 7.82 (1H, t, *J*=7.4 Hz), 7.84 (2H, t, *J*=7.8 Hz), 8.42 (2H, dd, *J*=7.8, 0.8 Hz), 8.46 (2H, dd, *J*=7.2, 0.8 Hz) ppm; δ_C (100 MHz, DMSO) 25.2, 26.8, 29.3, 30.1, 38.4, 39.4, 122.0, 127.2, 127.3, 130.7, 131.3, 134.3, 163.4, 170.8, 173.9 ppm. HRMS (FAB): calcd for $C_{20}H_{20}N_2O_5$: 367.1299 (M+1); found: 367.1295.

Synthesis of ureas 6a-l

General procedure: To a solution of compounds 1a-c (0.15-0.26 g, 0.56-0.83 mmol) in dry toluene (8-16 mL). Kept stirring under nitrogen atmosphere in an ice/water bath, was added dropwise the isocyanate (1.0-2.5 equiv.). After addition is complete the reaction mixture was stirred for 1 h at rt, and then refluxed at 130°C in an oil bath for 4-6 h. The reaction mixture was concentrated in the rotary evaporator, refrigerated at -20°C for 20 h to give white, yellow solids as products, which are eventually purified by column chromatography (silica; solvent), 6a-l (55-98 %).

Synthesis of 1-(2-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl)ethyl)urea (6a): Compound 1a (0.20 g, 0.83 mmol); dry toluene (15

mL); trimethylsilyl isocyanate (2.5 equiv.); 4 h; column (DCM:ethanol, 85:15); fluffy white solid 6a (55 %); m.p.: 217-219°C; ν_{max} (Nujol) 1653, 1693, 3370, 3493 cm^{-1}; δ_H (400 MHz, DMSO) 3.30 (2H, q, *J*=5.5 Hz), 4.10 (2H, t, *J*=6.2 Hz), 5.34 (2H, br s), 6.04 (1H, t, *J*=6.2 Hz), 7.85 (2H, dd, *J*=8.2, 7.4 Hz), 8.42 (2H, dd, *J*=8.4, 0.8 Hz), 8.46 (2H, dd, *J*=7.4, 1.0 Hz) ppm; δ_C (100 MHz, DMSO) 37.2, 40.1, 122.2, 127.1, 127.5, 130.6, 131.3, 134.1, 158.6, 163.5 ppm. MS: *m/z* 306 $(M+Na)^+$.

Synthesis of 1-(3-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl)propyl)urea (6b): Compound 1b (0.20 g, 0.79 mmol); dry toluene (15 mL); trimethylsilyl isocyanate (2.5 equiv.); 5 h; white solid 6b (97 %); m.p.: 205-208°C; ν_{max} (Nujol) 1659, 1702, 3328, 3449 cm^{-1}; δ_H (400 MHz, DMSO) 1.72 (2H, quint, *J*=7.0 Hz), 3.03 (2H, q, *J*=6.5 Hz), 4.04 (2H, t, *J*=7.2 Hz), 5.38 (2H, br s, NH_2, 25 %)[a], 5.46 (2H, br s, NH_2, 75 %)[a], 5.90 (1H, t, *J*=5.6 Hz, NH, 25 %)[a], 5.97 (1H, t, *J*=5.8 Hz, NH, 75 %)[a], 7.84 (2H, dd, *J*=7.2, 1.2 Hz), 8.42 (2H, dd, *J*=8.2, 1.0 Hz), 8.46 (2H, dd, *J*=7.2, 1.2 Hz) ppm; δ_C (100 MHz, DMSO) 28.9, 37.1, 37.7, 122.0, 127.2, 127.3, 130.7, 131.3, 134.3, 158.7, 163.5 ppm. HRMS (FAB) calcd for $C_{16}H_{16}N_3O_3$ 298.1186 (M+1); found: 298.1186.

[a] Duplication of peaks probably due to different hydrogen bonding structures.

Synthesis of 1-(4-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl)butyl)urea (6c): Compound 1c (0.20 g, 0.75 mmol); dry toluene (15 mL); trimethylsilyl isocyanate (2.5 equiv.); 5 h; white solid 6c (71 %); m.p.: 198-201°C; ν_{max} (Nujol) 1660, 1699, 3301, 3460 cm^{-1}; δ_H (400 MHz, DMSO) 1.41 (2H, quint, *J*=7.4 Hz), 1.61 (2H, quint, *J*=7.5 Hz), 2.97 (2H, q, *J*=6.5 Hz), 4.03 (2H, t, *J*=7.2 Hz), 5.34 (2H, br s), 5.91 (1H, t, *J*=5.6 Hz), 7.84 (2H, dd, *J*=8.0, 7.2 Hz), 8.42 (2H, dd, *J*=7.2, 0.8 Hz), 8.46 (2H, dd, *J*=8.4, 0.6 Hz) ppm; δ_C (100 MHz, DMSO) 25.2, 27.5, 27.7, 39.0, 122.0, 127.2, 127.3, 130.7, 131.3, 134.3, 158.7, 163.4 ppm. HRMS (FAB) calcd for $C_{17}H_{18}O_3N_3$: 312.1343 (M+1); found 312.1342.

Synthesis of 1-(2-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl) ethyl)-3(4-nitrophenyl)urea (6d): Compound 1a (0.20 g, 0.83 mmol); dry toluene (8 ml); 4-nitrophenyl isocyanate (1 equiv.) dissolved in dry toluene (8 ml); 4 h; light yellow solid 6d (98 %); m.p.: 242-244°C; ν_{max} (Nujol) 1329, 1555, 1659, 1700, 3391 cm^{-1}; δ_H (400 MHz, DMSO) 3.47 (2H, q, *J*=5.8 Hz), 4.21 (2H, t, *J*=5.8 Hz), 6.55 (1H, t, *J*=6.0 Hz), 7.48 (2H, d, *J*=9.2 Hz), 7.82 (2H, dd, *J*=8.2, 7.4 Hz), 8.02 (2H, d, *J*=9.2 Hz), 8.41 (2H, dd, *J*=8.4, 1.2 Hz), 8.44 (2H, dd, *J*=8.4, 1.2 Hz), 9.26 (1H, br s) ppm; δ_C (100 MHz, DMSO) 37.6, 39.7, 116.8, 122.2, 125.0, 127.1, 127.5, 130.7, 131.3, 134.2, 140.3, 147.2, 154.7, 163.7 ppm. Anal. Calcd for $C_{21}H_{16}N_4O_5$: C 62.37, H 3.99, N 13.86. Found: C 62.22, H 3.84, N 13.73.

Synthesis of 1-(3-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl)propyl)-3(4-nitrophenyl)urea (6e): Compound 1b (0.20 g, 0.79 mmol); dry toluene (8 ml); 4-nitrophenyl isocyanate (1 equiv.) dissolved in dry toluene (8 mL); 5 h; yellow solid 6e (92 %); m.p.: 221-224°C; ν_{max} (Nujol) 1376, 1564, 1693, 1701, 3310 cm^{-1}; δ_H (400 MHz, DMSO) 1.82 (2H, quint, *J*=6.7 Hz), 3.18 (2H, q, *J*=6.4 Hz), 4.09 (2H, t, *J*=6.8 Hz), 6.53 (1H, t, *J*=5.8 Hz), 7.58 (2H, d, *J*=9.2 Hz), 7.83 (2H, t, *J*=7.6 Hz), 8.09 (2H, d, *J*=9.2 Hz), 8.41 (2H, d, *J*=8.0 Hz), 8.46 (2H, d, *J*=7.2 Hz), 9.44 (1H, br s) ppm; δ_C (100 MHz, DMSO) 28.3, 37.0, 37.5, 116.7, 122.0, 125.1, 127.2, 127.4, 130.7, 131.3, 134.3, 140.3, 147.3, 154.4, 163.6 ppm. HRMS (FAB) calcd for $C_{22}H_{19}O_5N_4$: 419.1350 (M+1); found 419.1350.

Synthesis of 1-(4-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl) butyl)-3(4-nitrophenyl)urea (6f): Compound **1c** (0.15 g, 0.56 mmol); dry toluene (8 ml); 4-nitrophenyl isocyanate (1 equiv.) dissolved in dry toluene (8 ml); 5 h; column (DCM:etanol, 95:5); yellow solid **6f** (80%); m.p.: 232-235°C; ν_{max} (Nujol) 1376, 1558, 1638, 1696, 3330 cm^{-1}; δ_H (400 MHz, DMSO) 1.52 (2H, quint, *J*=7.2 Hz), 1.67 (2H, quint, *J*=7.4 Hz), 3.14 (2H, q, *J*=6.4 Hz), 4.06 (2H, t, *J*=7.2 Hz), 6.44 (1H, t, *J*=5.4 Hz), 7.56 (2H, d, *J*=9.2 Hz), 7.84 (2H, dd, *J*=7.8, 7.4 Hz), 8.08 (2H, d, *J*=9.2 Hz), 8.42 (2H, dd, *J*=8.4, 0.8 Hz), 8.46 (2H, dd, *J*=7.4, 1.0 Hz), 9.20 (1H, br s) ppm; δ_C (100 MHz, DMSO) 25.1, 27.2, 39.0, 39.4, 116.7, 122.0, 125.1, 127.2, 127.3, 130.7, 131.3, 134.3, 140.3, 147.2, 154.4, 163.4 ppm. HRMS (FAB) calcd for $C_{23}H_{21}O_5N_4$: 433.1506 (M+1); found 433.1506.

Synthesis of 1-benzyl-3-(2-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl)ethyl)urea (6g): Compound 1a (0.20 g, 0.83 mmol); dry toluene (15 mL); benzyl isocyanate (1 equiv.); 4 h; white solid **6g** (96 %); m.p.: 223-225°C; ν_{max} (Nujol) 1661, 1696, 3324 cm^{-1}; δ_H (400 MHz, DMSO) 3.39 (2H, q, *J*=6.1 Hz), 4.08 (2H, d, *J*=6.0 Hz), 4.14 (2H, t, *J*=6.0 Hz), 6.06 (1H, t, *J*=6.0 Hz), 6.26 (1H, t, *J*=6.0 Hz), 7.10-7.23 (5H, m), 7.85 (2H, dd, *J*=8.0, 7.2 Hz), 8.42 (2H, dd, *J*=8.2, 1.0 Hz), 8.47 (2H, dd, *J*=7.2, 1.2 Hz) ppm; δ_C (100 MHz, DMSO) 37.4, 40.2, 42.8, 122.2, 126.4, 126.8, 128.0, 127.2, 127.5, 130.6, 131.3, 134.1, 140.9, 158.1, 163.6 ppm. Anal. Calcd for $C_{22}H_{19}N_3O_3$: C 70.76, H 5.13, N 11.25. Found: C 70.72, H 4.92, N 11.32.

Synthesis of 1-benzyl-3-(3-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl)propyl)urea (6h)

Compound 1b (0.20 g, 0.79 mmol); dry toluene (16 mL); benzyl isocyanate (1 equiv.); 5 h; beige solid **6h** (86 %); m.p.: 203-205°C; ν_{max} (Nujol) 1654, 1695, 3311 cm^{-1}; δ_H (400 MHz, DMSO) 1.75 (2H, quint, *J*=7.0 Hz), 3.09 (2H, q, *J*=6.5 Hz), 4.04 (2H, t, *J*=7.2 Hz), 4.19 (2H, d, *J*=6.0 Hz), 5.95 (1H, t, *J*=5.8 Hz, NH, 21.4 %)[a], 6.01 (1H, t, *J*=5.8 Hz, NH, 78.6 %)[a], 6.34 (1H, t, *J*=6.0 Hz, NH, 21.4 %)[a], 6.44 (1H, t, *J*=6.0 Hz, NH, 78.6 %)[a], 7.16-7.32 (5H, m), 7.84 (2H, dd, *J*=7.4, 0.8 Hz), 8.42 (2H, dd, *J*=8.4, 0.8 Hz), 8.46 (2H, dd, *J*=7.2, 1.2 Hz) ppm; δ_C (100 MHz, DMSO) 28.9, 37.3, 37.7, 42.9, 122.0, 126.5, 126.9, 128.2, 127.2, 127.3, 130.7, 131.3, 134.3, 140.9, 158.0, 163.5 ppm. HRMS (FAB) calcd for $C_{23}H_{22}O_3N_3$: 388.1654 (M+1); found 388.1656.

[a] Duplication of peaks probably due to different hydrogen bonding structures.

Synthesis of 1-benzyl-3-(4-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl)butyl)urea (6i): Compound 1c (0.20 g, 0.75 mmol); dry toluene (15 mL); benzyl isocyanate (1 equiv.); 5 h; white solid 6i (91 %); m.p.: 216-218°C; ν_{max} (Nujol) 1667, 1702, 3310 cm^{-1}; δ_H (400 MHz, DMSO) 1.43 (2H, quint, *J*=7.3 Hz), 1.62 (2H, quint, *J*=7.5 Hz), 3.04 (2H, q, *J*=6.5 Hz), 4.04 (2H, t, *J*=7.4 Hz), 4.16 (2H, d, *J*=6.4 Hz), 5.93 (1H, t, *J*=5.6 Hz), 6.26 (1H, t, *J*=5.8 Hz), 7.12-7.31 (5H, m), 7.84 (2H, dd, *J*=7.4, 0.8 Hz), 8.43 (2H, dd, *J*=8.4, 0.8 Hz), 8.47 (2H, dd, *J*=7.2, 1.2 Hz) ppm; δ_C (100 MHz, DMSO) 25.2, 27.8, 39.2, 39.5, 42.8, 122.0, 126.4, 126.5, 126.9, 127.0, 128.1, 128.2, 127.2, 127.3, 130.7, 131.3, 134.3, 141.0, 158.1, 163.4 ppm. HRMS (FAB) calcd for $C_{24}H_{23}O_3NaN_3$: 424.1632 $(M+Na^+)$; found 424.1621.

Synthesis of 1-butyl-3-(2-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl)ethyl)urea (6j): Compound 1a (0.20 g, 0.83 mmol); dry toluene (16 mL); butyl isocyanate (1 equiv.); 5 h; white solid 6j (88 %); m.p.: 201-203°C; ν_{max} (Nujol) 1660, 1696, 3323 cm^{-1}; δ_H (400 MHz, DMSO) 0.74 (3H, t, *J*=7.0 Hz), 1.08-1.16 (4H, m), 2.81 (2H, q, *J*=6.4 Hz), 3.33 (2H, q, *J*=6.0 Hz), 4.09 (2H, t, *J*=6.0 Hz), 5.72 (1H, t, *J*=5.8 Hz), 5.87 (1H, t, *J*=6.0 Hz), 7.83 (2H, dd, *J*=8.2, 7.4 Hz), 8.40 (2H, dd, *J*=8.4, 1.2 Hz), 8.44 (2H, dd, *J*=7.4, 1.0 Hz) ppm; δ_C (100 MHz, DMSO) 13.6, 19.4, 32.0, 37.3, 38.9, 40.2, 122.2, 127.1, 127.4, 130.5, 131.3, 134.1, 158.1, 163.5 ppm. Anal. Calcd for $C_{19}H_{21}N_3O_3$: C 67.24, H 6.24, N 12.38; found: C 67.28, H 6.24, N 12.52.

Synthesis of 1-butyl-3-(3-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl)propyl)urea (6k): Compound 1b (0.20 g, 0.79 mmol); dry toluene (15 mL); butyl isocyanate (1.1 equiv.); 6 h; white solid 6k (84 %); m.p.: 199-202°C; ν_{max} (Nujol) 1665, 1699, 3319 cm^{-1}; δ_H (400 MHz, DMSO) 0.84 (3H, t, *J*=7.2 Hz), 1.20-1.33 (4H, m), 1.71 (2H, quint, *J*=7.0 Hz), 2.94 (2H, q, *J*=6.4 Hz), 3.04 (2H, q, *J*=6.5 Hz), 4.03 (2H, t, *J*=7.2 Hz), 5.82 (1H, t, *J*=5.8 Hz), 5.89 (1H, t, *J*=5.6 Hz), 7.84 (2H, dd, *J*=8.2, 0.8 Hz), 8.42 (2H, dd, *J*=8.4, 0.8 Hz), 8.45 (2H, dd, *J*=7.2, 1.2 Hz) ppm; δ_C (100 MHz, DMSO) 13.7, 19.6, 28.9, 32.2, 37.2, 37.8, 39.0, 122.1, 127.2, 127.4, 130.7, 131.3, 134.3, 158.1, 163.5 ppm. HRMS (FAB) calcd for $C_{20}H_{24}O_3N_3$: 354.1812 (M+1); found 354.1812.

Synthesis of 1-butyl-3-(4-(1,3-dioxo-1*H*-benzo(*de*)isoquinolin-2(3*H*)-yl)butyl)urea (6l)

Compound 1c (0.20 g, 0.75 mmol); dry toluene (16 mL); butyl isocyanate (1 equiv.); 5 h; white solid 6l (92%); m.p.: 204-207°C; ν_{max} (Nujol) 1666, 1700, 3323 cm^{-1}; δ_H (400 MHz, DMSO) 0.81 (3H, t, *J*=7.2 Hz), 1.15-1.32 (4H, m), 1.40 (2H, quint, *J*=7.4 Hz), 1.60 (2H, quint, *J*=7.5 Hz), 2.92 (2H, q, *J*=6.4 Hz), 2.99 (2H, q, *J*=6.5 Hz), 4.03 (2H, t, *J*=7.2 Hz), 5.68-5.77 (2H, m), 7.85 (2H, t, *J*=7.8 Hz), 8.43 (2H, d, *J*=8.0 Hz), 8.47 (2H, d, *J*=7.2 Hz) ppm; δ_C (100 MHz, DMSO) 13.7, 19.5, 25.2, 27.8, 32.1, 38.9, 39.0, 39.1, 122.0, 127.2, 127.3, 130.7, 131.3, 134.3, 158.1, 163.4 ppm. HRMS (FAB) calcd for $C_{21}H_{26}O_3N_3$: 368.1969 (M+1); found 368.1969.

Synthesis of compounds 9d-f

Conversion of alcohols 1d-f into bromides 7d-f

General procedure: To a solution of alcohols 1d-f (0.40-0.50 g, 1.49-2.07 mmol) in DCM (8 mL) kept stirring in an ice/water bath was added carbon tetrabromide (1.6 equiv.) and triphenylphosphine (1.6 equiv.). Stirring was prolonged at rt for 12 to 24 h, then concentrated in the rotary evaporator, and the residue purified by column chromatography (silica, DCM:ethanol, 95:5). Products 7d-f were obtained as white or pale yellow solids (89-99 %).

Synthesis of 2-(2-bromoethyl)-1*H*-benzo(*d,e*)-isoquinoline-1,3(2*H*)-dione (7d): Compound 1d (0.50 g, 2.07 mmol);12 h; pale yellow solid 7d (97 %); m.p.: 218-220°C; ν_{max} (Nujol) 1658 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 3.69 (2H, t, *J*=7.2 Hz), 4.63 (2H, t, *J*=7.2 Hz), 7.79 (2H, t, *J*=7.8 Hz), 8.25 (2H, dd, *J*=8.0, 0.8 Hz), 8.64 (2H, dd, *J*=7.2, 0.8 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 27.8, 41.2, 122.3, 127.0, 128.2, 131.5, 131.6, 134.3, 164.0 ppm. MS: *m/z* 326 (M+Na).

Synthesis of 2-(3-bromopropyl)-1*H*-benzo(*de*)-isoquinoline-1,3(2*H*)-dione (7e): Compound 1e (0.50 g, 1.96 mmol); 24 h; white solid 7e (89 %); m.p.: 134-137°C; ν_{max} (Nujol) 1661 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 2.34 (2H, quint, *J*=6.9 Hz), 3.51 (2H, t, *J*=6.8 Hz), 4.33 (2H, t, *J*=7.2 Hz), 7.76 (2H, dd, *J*=8.2, 7.4 Hz), 8.22 (2H, dd, *J*=8.2, 1.0 Hz), 8.60 (2H, dd, *J*=7.2, 1.2 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 30.5, 31.4, 39.3, 122.5, 127.0, 128.1, 131.3, 131.6, 134.1, 164.2 ppm. MS: *m/z* 340 $(M+Na)^+$.

Synthesis of 2-(4-bromobutyl)-1*H*-benzo(*de*)-isoquinoline-1,3(2*H*)-dione (7f): Compound 1f (0.40 g, 1.49 mmol); 20 h; white solid 7f (99 %); m.p.: 115-117°C; ν_{max} (Nujol) 1665 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.86-1.95 (2H, m), 1.96-2.03 (2H, m), 3.48 (2H, t, *J*=6.6 Hz), 4.22 (2H, t, *J*=7.0 Hz), 7.75 (2H, dd, *J*=8.2, 7.4 Hz), 8.20 (2H, dd, *J*=8.4, 1.2 Hz), 8.58 (2H, dd, *J*=7.2, 1.2 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 26.9, 30.2, 33.1, 39.3, 122.5, 126.9, 128.1, 131.2, 131.5, 133.9, 164.1 ppm. Anal. Calcd for $C_{16}H_{14}BrNO_2$: C 57.85, H 4.25, N 4.22. Found: C 57.62, H 4.28, N 4.01.

Conversion of bromides 7d-f into azides 8d-f

General procedure To a solution of compound 7d-7f (0.19-0.20 g, 0.58-0.66 mmol) in dry DMF (3 mL) kept stirring under nitrogen was added sodium azide (3 equiv.). The suspension was stirred at rt for 3 days. Dichloromethane (15 mL) was added and the mixture washed with water (6x10 mL). The combined organic layers were dried over magnesium sulfate, filtered and the solvent evaporated in the rotary evaporator to give products as white or yellow solids **8d-f** (81-93 %).

Synthesis of 2-(2-azidoethyl)-1*H*-benzo(*de*)-isoquinoline-1,3(2*H*)-dione (8d): Compound 7d (0.20 g, 0.66 mmol); yellow solid 8d (88%); m.p.: 149-152°C; ν_{max} (Nujol) 1657, 2102 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 3.68 (2H, t, *J*=3.2 Hz), 4.46 (2H, t, *J*=3.2 Hz), 7.77 (2H, dd, *J*=7.8, 1.2 Hz), 8.23 (2H, dd, *J*=8.2, 1.0 Hz), 8.62 (2H, dd, *J*=7.2, 1.2 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 38.8, 48.9, 122.3, 127.0, 128.2, 131.5, 131.6, 134.2, 164.2 ppm. MS: *m/z* 289 $(M+Na)^+$.

Synthesis of 2-(3-azidopropyl)-1*H*-benzo(*de*)-isoquinoline-1,3(2*H*)-dione (8e): Compound 7e (0.20 g, 0.63 mmol); white solid 8e (81 %); m.p.: 89-92°C; ν_{max} (Nujol) 1651, 2102 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 2.06 (2H, quint, *J*=7.0 Hz), 3.45 (2H, t, *J*=6.8 Hz), 4.30 (2H, t, *J*=7.0 Hz), 7.76 (2H, dd, *J*=8.2, 7.4 Hz), 8.23 (2H, dd, *J*=8.4, 1.2 Hz), 8.61 (2H, dd, *J*=7.2, 1.2 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 27.6, 37.9, 49.4, 122.5, 127.0, 128.1, 131.3, 131.6, 134.1, 164.2 ppm. MS: *m/z* 303 $(M+Na)^+$.

Synthesis of 2-(4-azidobutyl)-1*H*-benzo(*de*)-isoquinoline-1,3(2*H*)-dione (8f): Compound 7f (0.19 g, 0.58 mmol); pale yellow solid 7f (93 %); m.p.: 73-75°C; ν_{max} (Nujol) 1650, 2101 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.70-1.77 (2H, m), 1.80-1.90 (2H, m), 3.36 (2H, t, *J*=6.8 Hz), 4.23 (2H, t, *J*=7.2 Hz), 7.76 (2H, dd, *J*=8.2, 7.4 Hz), 8.22 (2H, dd, *J*=8.4, 0.8 Hz), 8.60 (2H, dd, *J*=7.4, 1.0 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 25.4, 26.5, 39.6, 51.2, 122.6, 126.9, 128.1, 131.3, 131.6, 134.0, 164.2 ppm. MS: *m/z* 317 $(M+Na)^+$.

Cycloadditions of the azides 8d-f with phenylacetylene

General procedure: To a solution of compounds 8d-f (0.11-0.28 g, 0.38-1.05 mmol) in DMF (3-4 mL), was added ascorbic acid (0.1 equiv.), copper sulfate (0.01 equiv.) and phenylacetylene (1.3-1.5 equiv.) [16]. The reaction mixture was kept stirring at 80°C in an oil bath for 5-24 h. The reaction mixture was cooled till rt, dichloromethane (15 mL) was added, and the resulting solution washed with water (6x10 mL). The combined organic phases were dried over magnesium sulfate, evaporated in the rotary evaporator to give white or yellow solids. In one case the crude product was purified by column chromatography (silica, solvent) to give compounds 9d-f (53-95 %).

Synthesis of 2-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethyl)-1*H*-benzo(*de*)isoquinoline-1,3(2*H*)-dione (9d): Compound 8d (0.28 g, 1.05 mmol); DMF (4 mL); phenylacetylene (1.5 equiv.); 5 h; column (DCM:ethanol, 95:5); white solid 9d (56 %); m.p.: 195-199°C; ν_{max} (Nujol) 1665, 2100, 3085 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 4.53 (2H, t, *J*=5.8 Hz), 4.75 (2H, t, *J*=6.0 Hz), 7.30 (1H, t, *J*=7.4 Hz), 7.40 (2H, t, *J*=7.6 Hz), 7.75 (2H, d, *J*=7.2 Hz), 7.84 (2H, t, *J*=7.8 Hz), 8.44 (4H, t, *J*=8.2 Hz), 8.64 (1H, s) ppm; δ_C (100 MHz, $CDCl_3$) 39.7, 47.4, 121.8, 122.0, 125.0, 127.2, 127.4, 127.7, 128.8, 130.8, 130.8, 131.3, 134.5, 146.2, 163.4 ppm. HRMS (FAB) calcd for $C_{22}H_{17}O_2N_4$: 369.1346 (M+1); found 369.1346.

Synthesis of 2-(3-(4-phenyl-1*H*-1,2,3-triazol-1-yl)propyl)-1*H*-benzo(*de*)isoquinoline-1,3(2*H*)-dione (9e): Compound **8e** (0.11 g, 0.40 mmol); DMF (4 mL); phenylacetylene (1.3 equiv.); 12 h; white solid **9e** (53 %); m.p.: 174-177°C; ν_{max} (Nujol) 1652, 2097, 3081 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 2.48 (2H, quint, *J*=6.9 Hz), 4.33 (2H, t, *J*=6.8 Hz),

4.55 (2H, t, *J*=7.0 Hz), 7.32 (1H, t, *J*=7.4 Hz), 7.41 (2H, t, *J*=6.8 Hz), 7.55 (2H, t, *J*=7.8 Hz), 7.80 (2H, d, *J*=7.2 Hz), 8.01 (1H, s), 8.16 (2H, dd, *J*=8.4, 0.8 Hz), 8.60 (2H, dd, *J*=7.4, 1.0 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 28.9, 37.6, 48.4, 120.0, 122.3, 125.7, 127.0, 128.0, 128.1, 128.7, 130.5, 131.4, 131.6, 134.2, 147.6, 164.3 ppm. HRMS (FAB) calcd for $C_{23}H_{19}O_2N_4$: 383.1502 (M+1); found 383.1502.

Synthesis of 2-(4-(4-phenyl-1*H*-1,2,3-triazol-1-yl)butyl)-1*H*-benzo(*de*)isoquinoline-1,3(2*H*)-dione (9f): Compound **8f** (0.11 g, 0.36 mmol); DMF (3 mL); phenylacetylene (1.5 equiv.); 24 h; yellow solid **9f** (95 %); m.p.: 137-139°C; ν_{max} (Nujol) 1658, 2096, 3080 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.85 (2H, quint, *J*=7.4 Hz), 2.09 (2H, quint, *J*=7.4 Hz), 4.28 (2H, t, *J*=7.2 Hz), 4.52 (2H, t, *J*=7.0 Hz), 7.33 (1H, t, *J*=7.4 Hz), 7.42 (2H, t, *J*=7.4 Hz), 7.60 (2H, t, *J*=7.8 Hz), 7.84 (2H, d, *J*=7.2 Hz), 7.89 (1H, s), 8.22 (2H, dd, *J*=8.2, 1.0 Hz), 8.60 (2H, dd, *J*=7.2, 1.2 Hz) ppm; δ_C (100 MHz, $CDCl_3$) 25.0, 27.8, 39.2, 49.9, 120.0, 122.4, 125.8, 127.0, 128.1, 128.8, 130.4, 131.3, 131.3, 131.6, 134.0, 134.1, 164.2 ppm. HRMS (FAB) calcd for $C_{24}H_{21}O_2N_4$: 397.1659 (M+1); found 397.1651.

Biological activity

Solutions of the compounds: Compound stock solutions were prepared in DMSO (Sigma-Aldrich) in a concentration of 1 mM and stored at -20°C. Doxorubicin (Sigma-Aldrich) was prepared in a stock concentration of 10 mM. All compounds were diluted to appropriate concentrations in cell culture medium (RPMI-1640 with Ultraglutamine I, Lonza) containing 5% heat-inactivated fetal bovine serum (FBS, Biowest) immediately before the assay.

Cell culture: Human tumor cell lines of breast (MCF-7), colorectal (HCT-15) and pancreatic (BxPC-3) adenocarcinomas were routinely maintained in RPMI-1640 with Ultraglutamine I, supplemented with 5% heat-inactivated FBS at 37°C in a humidified atmosphere containing 5% CO_2. The human leukemia monocyte THP1 cell line was differentiated into macrophages by incubating cells in the presence of 20 ng/ml phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich) for 18h at 37°C in 5% CO_2 humidified atmosphere, followed by a 24h incubation period in fresh medium with no PMA to induce maturation. Mouse bone marrow-derived macrophages (BMMØ) were obtained as previously described [17]. Briefly, bone marrow precursors recovered from femurs and tibias of Balb/c mice were cultured in complete macrophage medium (DMEM medium with glucose (4,5 g/L) (Lonza, Switzerland) and HEPES buffer supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin (BioWhittaker, Walkersville, MD), and added of 5% L-929 cell conditioned medium (LCCM) as source of macrophage colony-stimulating factor (M-CSF). After 4h of incubation, non-adherent cells were recovered and reseeded in complete medium containing 5% LCCM. Fully differentiated macrophages were obtained after 7 days of culture with a degree of purity higher than 90%.

Cell growth inhibition assay: The effect of the compounds on the growth of three different tumor cell lines, MCF-7, HCT-15 and BxPC-3, was evaluated using the sulforhodamine B (SRB, Sigma-Aldrich) assay according to the procedure adopted by the National Cancer Institute (USA) [18,9]. Briefly, cells were plated in 96-well plates at appropriate densities (5×10^4 cells/ml for MCF-7 and 1×10^5 cells/ml for HCT-15 and BxPC-3) and incubated for 24h. Cells were then treated for 48h with 5 µM of each compound or with a positive control (doxorubicin), fixed with 10% (wt/vol) trichloroacetic acid (TCA, Merck-Millipore), and stained with SRB (0.4% wt/vol in 1% acetic acid) for 30min. The protein-bound dye was solubilized in 10 mM Tris base solution (Sigma-Aldrich) and the absorbance measured at 510 nm in a microplate reader (Synergy HT - Biotek). The effect of the vehicle solvent (DMSO) on the growth of the cells was evaluated by exposing untreated cells to the same concentration of DMSO present in the compound solutions used in the assay (0.5%). No influence was found (data not shown). A dose-response curve (serial dilutions of the compounds ranging from 0.313 µM to 5 µM) and corresponding GI_{50} (the concentration of compound that inhibits growth in 50%) was determined for the most promising compounds as described by Monks et al. [20].

Cellular toxicity: The cellular toxicity of the compounds was evaluated on THP1 differentiated macrophages and BMMØ using the MTT assay. Briefly, cells were cultured in 96 well plates for 72h in the presence of the compounds at concentrations of 10 µM for THP1 differentiated macrophages or 5 µM and 50 µM for BMMØ at 37°C in 5% CO_2. At the end of the incubation period, the culture medium was removed and 0.5 mg/ml of MTT reagent (thiazolyl blue tetrazolium bromide, Sigma) was added to each well and put to incubate for 4h at 37°C. The formazan crystals resulting from the reduction of the tetrazolium salt were then solubilized by adding isopropanol and the optical density determined at 570 nm corrected for the background at 660 nm, using a micro plate reader. Results are presented as the percentage of viable cells compared with control non-treated cells [21,22].

Statistical analysis: All data is presented as mean ± standard error (SE). Three to six independent experiments were performed in duplicate to determine compounds activity on tumor cells. Cell toxicity assays were performed in triplicates in two independent experiments. The data was analyzed using two-sided unpaired Student's t-test. Differences in p values below 0.05 were considered statistically significant.

Acknowledgments

The research leading to these results has received funding from Fundação para a Ciência e a Tecnologia (FCT)/Ministério da Educação e Ciência (MEC) cofounded by FEDER, partnership agreement PT2020, through the Research Unit No. 4293. Joana Maciel was supported by a post-doctoral fellowship from the FCT project grant No. PTDC/BIA-MIC/118644/2010. Catarina Baptista is supported by a fellowship from the European Community's Seventh Framework Programme under grant agreements No. 603240 (project NMTrypI). Jennifer Noro grant was supported by FCT project ref PTDC/QEQ-MED/1671/2012. We thank FCT and FEDER for funding NMR spectrometer Bruker Avance III 400 as part of the National NMR Network, and the EPSRC UK National Mass Spectrometry Facility (NMSF) at Swansea University for all the HR analyses.

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Citation: Noro J, Maciel J, Duarte D, Olival ACD, Baptista C, et al.(2015) Evaluation of New Naphthalimides as Potential Anticancer Agents against Breast Cancer MCF-7, Pancreatic Cancer BxPC-3 and Colon Cancer HCT-15 Cell Lines. Organic Chem Curr Res 4: 144. doi:10.4172/2161-0401.1000144