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Molecular design, chemical synthesis, and biological evaluation of '4-1' pentacyclic aryl/heteroaryl-imidazonaphthalimides

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Abstract—A novel series of '4-1' pentacyclic naphthalimides, where the chromophore consists of a naphthalimide moiety, fused to an imidazole ring containing an unfused aryl or heteroaryl ring, were synthesized and evaluated for in vitro antitumor activity. In general, the new derivatives showed an improved cytotoxic activity over amonafide. DNA binding experiments supported that this class of compounds behaves as effective DNA-intercalating agents.

1. Introduction

DNA-intercalating agents, containing a planar chromophore with two to four fused aromatic rings, represent a large family of antitumor drugs.[1] They exhibit cytostatic activity through DNA intercalation, which causes enzymatic blockade and reading errors during the replication process.[2] Naphthalimides are typically DNA-intercalating agents[3] and two lead compounds of naphthalimides, amonafide[4] and elinafide,[5] were selected for phase I and II clinical trials (Fig. 1).

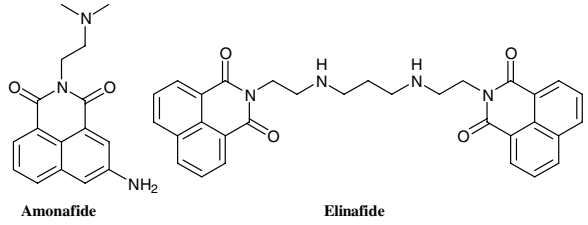

Figure 1. The lead compounds of naphthalimides: Amonafide (left) and Elinafide (right).

Over the past decade, a great deal of work has been devoted toward the modification of the chromophore of naphthalimide in order to enhance the potency of naphthalimides.[6–8] Recently, increasing attention has been focused on the design of new naphthalimides where heterocyclic systems have been 'fused' to the naphthalimide chromophore.[6e,7,8] Brana and co-workers synthesized several series of naphthalimides, where the chromophore consisted of a naphthalimide moiety, fused to an imidazole,[7a] pyrazine,[7b] furan, or thiophene ring,[7c] and some of them showed significant improvement in cellular cytotoxic activity over amonafide. More recently, another series of naphthalimides, where an unfused benzene or furan ring was introduced, was reported by the same research group and exhibited extraordinary cytotoxicity and growth delays for cancer cell lines in vitro.[7d]

Our research group also developed several series of naphthalimides with extended heteroaromatic systems as effective artificial photonucleases and antitumor agents.[8] In an attempt to further explore structurally novel DNA-intercalating agents, we herein wish to report the design, synthesis, and biological evaluation of a series of '4-1' pentacyclic naphthalimides, where the chromophore consists of a naphthalimide moiety, fused to an imidazole ring containing an unfused aryl or heteroaryl ring (Fig. 2). The introduction of a fused

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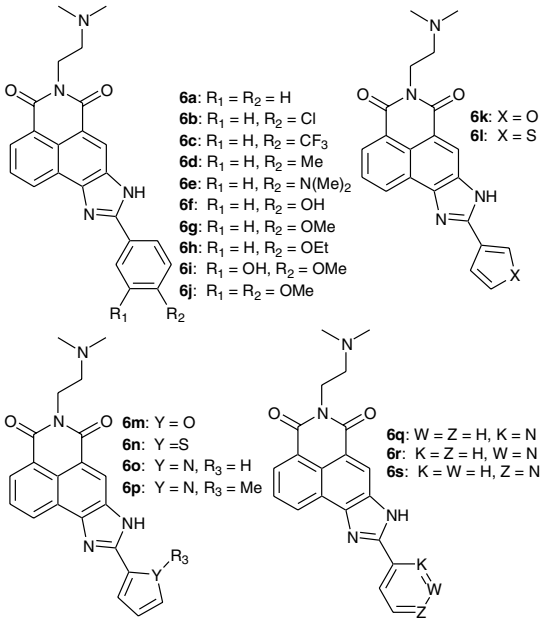


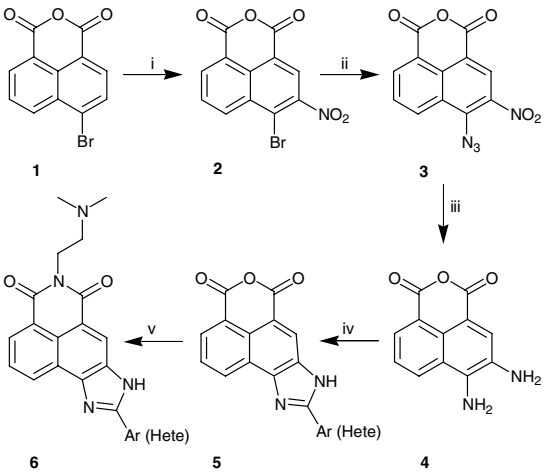
Figure 2. Molecular structures of aryl/heteroaryl-imidazonaphthalimides.

imidazole ring can lead to the formation of a larger aromatic system and consequently to a higher affinity for the DNA molecular, and must have an effect on the electrostatic properties of the chromophore.[7a] The conformationally flexible, unfused aryl or heteroaryl ring not only further extends the conjugated system, but also was expected to amplify the antitumor activity[9] and to be relatively nontoxic to human cells, as has been shown for other unfused aromatic compounds.[10]

2. Results and discussion

2.1. Chemistry

3,4-Diamino-naphthalene-1,8-dicarboxylic anhydride was the key intermediate for the synthesis of targeted compounds and could be prepared using the method of Brana and co-workers.[7b]



Scheme 1. Reagents and conditions: (i) HNO_3, AcOH, 0–5 °C, 85%; (ii) NaN_3, H_2O, DMF, 100 °C, 97%; (iii) Pd/C, H_2, DMF, rt, 96%; (iv) Ar/heteroaryl-CHO, $NaHSO_3$, DMF, 100 °C, 67–79%; (v) $NH_2(CH_2)_2N(CH_3)_2/C_2H_5OH$, refluxed, 70–77%.

An alternatively shorter route for the synthesis of diamine **4** was explored and is outlined in Scheme 1. Nitration of commercially available 4-bromo-naphthalene-1,8-dicarboxylic anhydride **1** and treatment of the resulting **2** with sodium azide in DMF at 100 °C gave the compound **3**, which was further reduced by hydrogenation over Pd/C to afford the desired diamine **4** with 79% overal yield. The last two steps in new three-step procedure were achieved in nearly quantitative yield without any purification. To the best of our knowledge, this is the first example of the preparation of diamine by catalytic hydrogenation of nitro and azide groups at the same time.

The next step in the synthesis involved the formation of aryl/heteroaryl-imidazole ring system. Condensation of diamine **4** and aldehydes using sodium bisulfite as the oxidant in presence of DMF at 100 °C gave the corresponding aryl/heteroaryl-naphthalene-1,8-dicarboxylic anhydrides **5a**–**s** with 67–79% yields.

In the final step, aryl/heteroaryl-imidazonaphthalimides **6a**–**s** were obtained by treatment of the corresponding anhydrides **5a**–**s** with *N*,*N*-dimethylethane-1,2-diamine in ethanol at reflux temperature with 70–77% yields.

2.2. Biological evaluation

The aryl/heteroaryl-imidazonaphthalimides were evaluated for in vitro cytotoxicity against P388 (murine leukemia), A-549 (human lung cancer), SMMC-7721 (human hepatoma), HeLa (human cervical carcinoma), and HL-60 (human acute promyelocytic leukemia) cell lines. The results are summarized in Table 1 and compared with the activities of amonafide. In general, the new derivatives showed an improved cytotoxic activity over amonafide. Among them, arylimidazonaphthalimides **6g** and **6j**, bearing a methoxyl group and two methoxyl groups, were found to be the most effective compounds against HeLa and SMMC-7721, with IC_{50} values of 0.21 μM and 0.22 μM, respectively. Heteroaryl-imidazonaphthalimides **6l**, **6p**, and **6q** exhibit the highest activities against P388, A-549, and HL-60, presenting values that are 28.5-fold, 31.7-fold, and 5.4-fold lower than the values found for amonafide under the same experimental conditions, respectively.

2.3. DNA binding properties

To evaluate the DNA binding properties of aryl/heteroaryl-imidazonaphthalimides, the melting temperature (T_m) measurements were performed with calf thymus DNA (CT-DNA). It is known that thermal denaturation profiles provide the simplest means for detecting binding and asserting relative binding strength. As shown in Table 1, binding of any of the compounds tested resulted in T_m being raised, indicative of stabilization of DNA double helix. Obviously, the stabilization of DNA double helix may be contributing to the inhibition of DNA replication,

Table 1. Growth-inhibitory properties for aryl/heteroaryl-imidazonaphthalimides

Entry	Compound	Ar/heteroaryl	P388[a] IC_{50} (μM)	A-549[b] IC_{50} (μM)	SMMC-7721[c] IC_{50} (μM)	Hela[d] IC_{50} (μM)	HL-60[e] IC_{50} (μM)	ΔT_m[f] (°C)
1	**6a**	Phenyl	0.86	7.54	3.36	0.40	0.86	7.2
2	**6b**	4′-Cl-phenyl	0.95	9.13	0.27	0.28	3.28	7.1
3	**6c**	4′-CF_3-*phenyl*	0.71	26.2	1.63	0.40	4.90	4.8
4	**6d**	4′-Me-phenyl	5.50	5.53	0.58	0.32	1.04	6.6
5	**6e**	4′-N(Me)$_2$-*phenyl*	1.68	8.50	2.86	0.25	1.08	12.1
6	**6f**	4′-OH-phenyl	1.10	17.8	>100	13.1	23.0	6.3
7	**6g**	4′-OMe-phenyl	3.72	2.16	1.35	0.21	0.46	7.0
8	**6h**	4′-OEt-phenyl	2.48	14.7	1.02	0.52	4.58	10.5
9	**6i**	3′-OMe-4′-OH-phenyl	1.92	5.08	>100	>100	50.5	7.8
10	**6j**	3′,4′-(OMe)$_2$-phenyl	1.07	0.60	0.22	5.19	10.4	13.3
11	**6k**	Furan-3-yl	0.71	7.48	0.88	13.9	5.21	6.7
12	**6l**	Thiophen-3-yl	0.16	8.59	0.93	0.54	0.58	8.4
13	**6m**	Furan-2-yl	0.65	7.23	1.30	1.73	6.24	6.8
14	**6n**	Thiophen-2-yl	4.15	6.67	3.27	1.34	0.59	6.9
15	**6o**	1H-pyrrol-2-yl	0.21	0.45	2.99	3.30	3.03	5.7
16	**6p**	1-Me-1H-pyrrol-2-*yl*	0.21	0.41	2.90	7.98	3.66	6.2
17	**6q**	Pyridin-2-yl	0.77	9.33	3.36	2.71	0.30	3.8
18	**6r**	Pyridin-3-yl	2.31	9.97	1.29	1.16	1.33	4.0
19	**6s**	Pyridin-4-yl	5.65	60.9	1.76	5.35	2.66	4.8
20	Amonafide		4.56	13.0	4.15	1.40	1.63	NT[g]

[a] Murine leukemia cell line.
[b] Human lung cancer cell line.
[c] Human hepatoma cell line.
[d] Human cervical carcinoma cell line.
[e] Human acute promyelocytic leukemia cell line.
[f] Variation in melting temperature ($\Delta T_m = T_m^{complex} - T_m^{DNA}$). T_m measurements were performed in BPE buffer, pH 7.1 (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA), using 10 μM compound and 20 μM calf thymus DNA (CT-DNA) at 260 nm with a heating rate of 1 °C/min T_m of CT-DNA = 69.0 °C.
[g] NT, not test.

thus resulting in antitumor activity. In some cases, the compounds that were found to bind strongly to DNA correspond to the most cytotoxic agent. For example, compounds **6e**, **6l**, and **6j**, which are highly cytotoxic, gave ΔT_m values of >8 °C with CT-DNA. However, there is no direct relationship between DNA binding and cytotoxicity. It indicated that DNA is a potential, but not a unique, target for antitumor activity.

The DNA binding properties of compound **6a** as a model of aryl-imidazonaphthalimide and **6m** as a model of heteroaryl-imidazonaphthalimide were further studied by viscosimetric titration and UV–visible spectrometry with CT-DNA. Viscosity measurement is regarded as the least ambiguous and the most critical tests of a binding model in solution in the absence of X-ray and NMR structural data. A classical intercalation model demands that the DNA helix lengthens as base pairs are separated to accommodate the bound ligand, leading to the increase of DNA viscosity.[11] The viscosity of DNA increases steadily with increasing concentration of the compounds, and the experiment results suggested that compounds bind to DNA through a classical intercalation mode (Fig. 3). Addition of CT-DNA caused the absorptions for free **6a** at 396 nm and **6m** at 408 nm (λ_{max}) to decrease in intensity, and exhibited 8 nm and 5 nm red shifts, respectively (Fig. 4). The well-defined spectral changes also further confirmed that they behave as DNA-intercalating agents.[12]

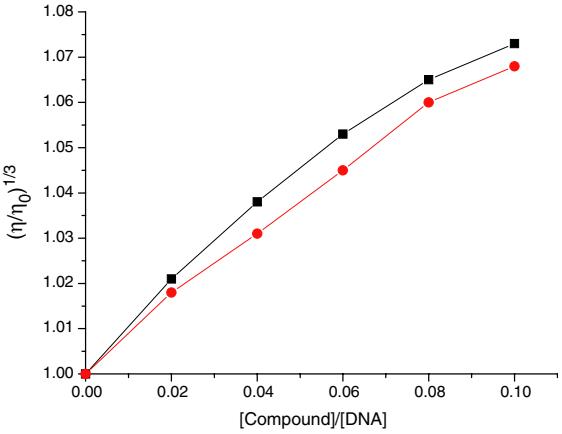


Figure 3. Effect of increasing amounts of compounds **6a** (black curve) and **6m** (red curve) on the relative viscosities of CT DNA at 25 (±0.1) °C. [DNA] = 20 μM. η is the viscosity of DNA in the presence of compound and η_0 is the viscosity of DNA in the absence of compound.

3. Conclusions

In summary, we developed a novel series of '4-1' pentacyclic DNA-intercalating agents based on an aryl/heteroaryl-imidazonaphthalimide system. The present work also opens up new perspectives for further development of DNA-targeted antitumor agents. Further studies to carry out structure–activity relationships (SARs) for cellular growth inhibition and stabilization of ternary complex DNA-naphthalimide-Topo II as well

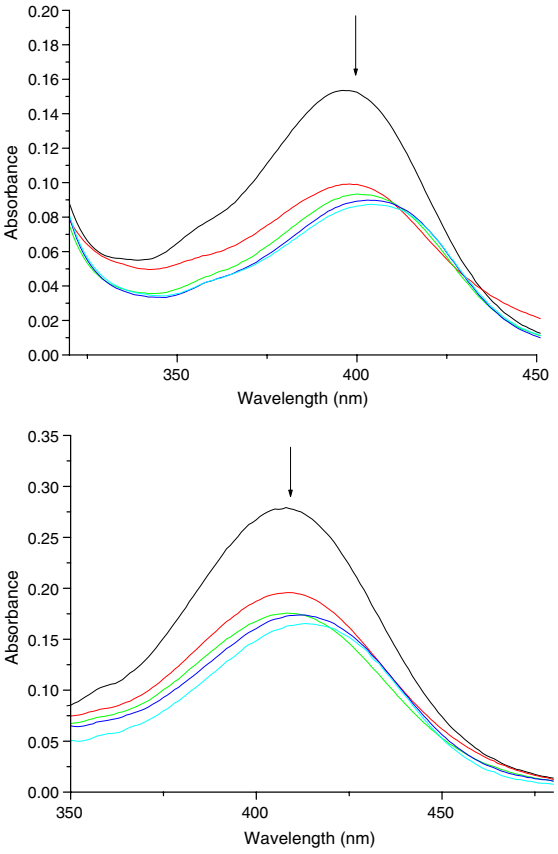


Figure 4. Absorption spectra of compounds **6a** (top) and **6m** (bottom) in Tris–HCl buffer upon addition of CT DNA with subtraction of the DNA absorbance ([Compound] = 20 μM; [DNA]/[Compound] = 0, 2, 4, 6, 8).

as to select a part of compounds for in vivo antitumoral assays are currently under way.

4. Experimental

4.1. Chemistry

Melting points were determined by a X-6 micro-melting point apparatus and are uncorrected. Infrared (IR) spectra were measured on a Nicolet 20DXB FR-I infrared spectrometer, with samples analyzed as KBr disks. High-resolution mass spectra (HRMS) were obtained on a HPLC-Q-Tof MS (Micro) spectrometer. The purity of compounds was checked by ascending TLC on Merck's silica gel plates (0.25 mm) with fluorescent baking. NMR measurements (data reported in ppm) were performed on a Varian INOVA spectrometer operating at 400 MHz or a AVANCE spectrometer operating at 500 MHz, respectively. Chemical shifts δ are reported in ppm downfield from tetramethylsilane, J values are in hertz, and the splitting patterns were designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad. UV spectra were recorded on HP 8453 UV–visible spectrophotometer.

4.1.1. 3-Bromo-4-nitro-naphthalene-1,8-dicarboxylic anhydride (2). To a suspension of 4-bromo-naphthalene-1,8-dicarboxylic anhydride **1** (15.0 g, 54.3 mmol) in AcOH (130 ml) at 0–5 °C was added slowly a mixture of 65% HNO_3 (5.8 g, 59.7 mmol), keeping the temperature at 0–5 °C. The mixture was allowed to stand at room temperature for 2 h, and the solution was poured into water and ice. The precipitate formed was filtered, washed with water, and dried. Recrystallization from AcOH gave **2** (14.8 g, 85%) as a yellow solid, mp 233–234 °C (lit.[13] 231–232 °C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.92 (d, J = 8.4, 1H, ArH), 8.82 (m, 2H, ArH), 8.16 (t, J = 8.0, 1H, ArH), 8.06 (t, J = 8.8, 1H, ArH).

4.1.2. 3-Azido-4-nitro-naphthalene-1,8-dicarboxylic anhydride (3). To a suspension of **2** (10.0 g, 31.2 mmol) in DMF (30 ml) was added a suspension of sodium azide (2.2 g, 34.3 mmol) in water (0.5 ml). The mixture was heated to 100 °C for 10 min and then poured into water and ice. The precipitate formed was filtered, washed with water, and dried. Compound **3** (8.6 g, 97%) was obtained as a yellow solid, mp 217–218 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.90 (d, J = 8.8, 1H, ArH), 8.86 (s, 1H, ArH), 8.69 (d, J = 8.8, 1H, ArH), 8.06 (t, J = 8.8, 1H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.9, 159.1, 139.7, 137.9, 135.3, 131.7, 131.4, 129.3, 127.4, 126.2, 119.8, 115.7; IR (KBr, cm^{-1}) 1770.1, 1740.5; HRMS-EI (70 eV) m/z calcd for $C_{12}H_4N_2O_5$ 284.0182, found 284.0187.

4.1.3. 3,4-Diamino-naphthalene-1,8-dicarboxylic anhydride (4). A mixture of **3** (7.0 g, 24.6 mmol) and 10% Pd/C (300 mg) in DMF (100 ml) was shaken in a Parr hydrogenator under hydrogen at 50 PSI pressure for 24 h. The catalyst was then filtered off and washed with DMF. The filtrate was concentrated, and water was added. The precipitate was then filtered, washed with water, and dried. Compound **4** (5.4 g, 96%) was obtained as a brown solid, mp > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.78 (d, J = 8.4, 1H), 8.43 (d, J = 6.8, 1H, ArH), 8.28 (s, 1H, ArH), 7.76 (dd, J = 8.4, 6.8, 1H, ArH).

4.2. General procedure for the preparation of anhydride and the corresponding naphthalimide

A mixture of diamine **4**, aldehyde and $NaHSO_3$ in DMF was heated at 100 °C until the reaction was completed (TLC). After the solution was cooled, water was added and then the precipitate was filtered. Recrystallization from DMF gave the corresponding anhydride as a solid.

A suspension of the corresponding anhydride was treated with an excess of the *N*,*N*-dimethylethane-1,2-diamine in absolute EtOH. The mixture was heated at reflux temperature until the reaction was completed (TLC). After removal of organic solvent under reduced pressure, the crude mixture was purified by flash chromatography (silica gel, $CHCl_3$/CH_3OH, 10:1, v/v) to afford the pure naphthalimide. In all cases, no peak of impurity was detected by ^{1}H NMR spectrum of the products.

4.2.1. 5-[2-(Dimethylamino)ethyl]-9-phenyl-5,8-dihydrobenz[*de*]imidazo[4,5-*g*]isoquinoline-4,6-dione (6a). From **4** (200 mg, 0.88 mmol) and benzaldehyde (116 mg, 1.10 mmol), and $NaHSO_3$ (137 mg, 1.32 mmol) in DMF (15 ml) yielded **5a** (212 mg, 77%) as a yellow solid, mp > 300 °C. From **5a** (150 mg, 0.48 mmol) and *N*,*N*-dimethylethane-1,2-diamine (103 mg, 1.17 mmol) in absolute EtOH (15 ml) yielded **6a** (136 mg, 74%) as a yellow solid, mp 275–276 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.86 (d, J = 8.0, 1H, ArH), 8.61 (s, 1H, ArH), 8.42 (d, J = 6.8, 1H, ArH), 8.28 (d, J = 6.8, 2H, ArH), 7.89 (dd, J = 8.0, 6.8, 1H, ArH), 7.61 (d, J = 6.8, 2H, ArH), 7.60 (m, 1H, ArH), 4.15 (t, J = 6.4, 2H, CH_2NCO), 2.54 (t, J = 6.4, 2H, CH_2N), 2.23 (s, 6H, 2× CH_3); IR (KBr, cm^{-1}) 3250.3, 1691.6, 1642.0; HRMS-EI (70 eV) *m*/*z* calcd for $C_{23}H_{20}N_4O_2$ 384.1586, found 384.1584.

4.2.2. 5-[2-(Dimethylamino)ethyl]-9-(4-chlorophenyl)-5,8-dihydrobenz[*de*]imidazo[4,5-*g*]isoquinoline-4,6-dione (6b). From **4** (200 mg, 0.88 mmol) and 4-chloro-benzaldehyde (148 mg, 1.05 mmol), and $NaHSO_3$ (137 mg, 1.32 mmol) in DMF (15 ml) yielded **5b** (223 mg, 73%) as a yellow solid, mp > 300 °C. From **5b** (150 mg, 0.43 mmol) and *N*,*N* – dimethylethane-1,2-diamine (103 mg, 1.17 mmol) in absolute EtOH (15 ml) yielded **6b** (137 mg, 76%) as a yellow solid, mp 274–275 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.81 (d, J = 8.0, 1H, ArH), 8.57 (s, 1H, ArH), 8.40 (d, J = 7.6, 1H, ArH), 8.23 (d, J = 8.4, 2H, ArH), 7.88 (dd, J = 8.0, 7.6, 1H, ArH), 7.66 (d, J = 8.4, 2H, ArH), 4.15 (t, J = 6.4, 2H, CH_2NCO), 2.55 (t, J = 6.4, 2H, CH_2N), 2.25 (s, 6H, 2× CH_3); IR (KBr, cm^{-1}) 3274.7, 1682.5, 1647.7; HRMS-EI (70 eV) *m*/*z* calcd for $C_{23}H_{19}ClN_2O_4$ 418.1197, found 418.1198.

4.2.3. 5-[2-(Dimethylamino)ethyl]-9-[4-(trifluoromethyl)phenyl]-5,8-dihydrobenz[*de*]imidazo[4,5-*g*]isoquinoline-4,6-dione (6c). From **4** (200 mg, 0.88 mmol) and 4-trifluoromethyl-benzaldehyde (168 mg, 0.97 mmol), and $NaHSO_3$ (137 mg, 1.32 mmol) in DMF (15 ml) yielded **5c** (226 mg, 74%) as a yellow solid, mp > 300 °C. From **5c** (150 mg, 0.39 mmol) and *N*,*N*-dimethylethane-1,2-diamine (103 mg, 1.17 mmol) in absolute EtOH (15 ml) yielded **6c** (135 mg, 76%) as a yellow solid, mp 268–270 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.67 (d, J = 8.4, 1H, ArH), 8.40 (s, 1H, ArH), 8.34 (d, J = 8.0, 2H, ArH), 8.31 (d, J = 7.6, 1H, ArH), 7.90 (d, J = 8.0, 2H, ArH), 7.80 (dd, J = 8.4, 7.6, 1H, ArH), 4.08 (t, J = 6.8, 2H, CH_2NCO), 2.51 (t, J = 6.8, 2H, CH_2N), 2.25 (s, 6H, 2× CH_3); IR (KBr, cm^{-1}) 3254.8, 1700.4, 1642.7; HRMS-EI (70 eV) *m*/*z* calcd for $C_{24}H_{19}F_3N_4O_2$ 452.1460, found 452.1464.

4.2.4. 5-[2-(Dimethylamino)ethyl]-9-[4-(methyl)phenyl]-5,8-dihydrobenz[*de*]imidazo[4,5-*g*]isoquinoline-4,6-dione (6d). From **4** (200 mg, 0.88 mmol) and 4-methyl-benzaldehyde (117 mg, 0.97 mmol), and $NaHSO_3$ (137 mg, 1.32 mmol) in DMF (15 ml) yielded **5d** (241mg, 76%) as a yellow solid, mp > 300 °C. From **5d** (150 mg, 0.46 mmol) and *N*,*N*-dimethylethane-1,2-diamine (103 mg, 1.17 mmol) in absolute EtOH (15 ml) yielded **6d** (138 mg, 72%) as a yellow solid, mp 269–271 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.84 (d, J = 8.8, 1H, ArH), 8.58 (s, 1H, ArH), 8.40 (d, J = 7.2, 1H, ArH), 8.15 (d, J = 8.0, 2H, ArH), 7.88 (dd, J = 8.8, 7.2, 1H, ArH), 7.41 (d, J = 8.0, 2H, ArH), 4.15 (t, J = 6.4, 2H, CH_2NCO), 2.54 (t, J = 6.4, 2H, CH_2N), 2.23 (s, 6H, 2× CH_3); IR (KBr, cm^{-1}), 3294.0, 1692.7, 1638.6; HRMS-EI (70 eV) *m*/*z* calcd for $C_{24}H_{22}N_4O_2$ 398.1743, found 398.1745.

4.2.5. 5-[2-(Dimethylamino)ethyl]-9-[4-(dimethylamino)phenyl]-5,8-dihydrobenz[*de*]imidazo[4,5-*g*]isoquinoline-4,6-dione (6e). From **4** (200 mg, 0.88 mmol) and 4-dimethylamino-benzaldehyde (144 mg, 0.97 mmol), and $NaHSO_3$ (137 mg, 1.32 mmol) in DMF (15 ml) yielded 5e (248 mg, 75%) as a red solid, mp > 300 °C. From 5e (150 mg, 0.42 mmol) and *N*,*N*-dimethylethane-1,2-diamine (103 mg, 1.17 mmol) in absolute EtOH (15 ml) yielded 6e (128 mg, 77%) as a red solid, mp 272–273 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.82 (d, J = 7.6, 1H, ArH), 8.52 (s, 1H, ArH), 8.38 (d, J = 7.2, 1H, ArH), 8.07 (d, J = 8.8, 2H, ArH), 7.84 (dd, J = 7.6, 7.2, 1H, ArH), 6.86 (d, J = 8.8, 2H, ArH), 4.14 (t, J = 6.8, 2H, CH_2NCO), 3.18 (s, 6H, $N(CH_3)_2$), 2.51 (t, J = 6.8, 2H, CH_2N), 2.51 (s, 6H, 2× CH_3), 2.22 (s, 6H, 2× CH_3); IR (KBr, cm^{-1}) 3261.4, 1691.0, 1642.3; HRMS-EI (70 eV) *m*/*z* calcd for $C_{25}H_{25}N_5O_2$ 427.2008, found 427.1998.

4.2.6. 5-[2-(Dimethylamino)ethyl]-9-[4-(hydroxyl)phenyl]-5,8-dihydrobenz[*de*]imidazo[4,5-*g*]isoquinoline-4,6-dione (6f). From **4** (200 mg, 0.88 mmol) and 4-hydroxy-benzaldehyde (118 mg, 0.97 mmol), and $NaHSO_3$ (137 mg, 1.32 mmol) in DMF (15 ml) yielded **5f** (226 mg, 78%) as a yellow solid, mp > 300 °C. From **5f** (150 mg, 0.45 mmol) and *N*,*N*-dimethylethane-1,2-diamine (103 mg, 1.17 mmol) in absolute EtOH (15 ml) yielded **6f** (140 mg, 77%) as a yellow solid, mp > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.85 (d, J = 8.4, 1H, ArH), 8.58 (s, 1H, ArH), 8.41 (d, J = 7.2, 1H, ArH), 8.12 (d, J = 8.8, 2H, ArH), 7.88 (dd, J = 8.4, 7.2, 1H, ArH), 6.99 (d, J = 8.8, 2H, ArH), 4.16 (t, J = 6.8, 2H, CH_2NCO), 2.55 (t, J = 6.8, 2H, CH_2N), 2.25 (s, 6H, 2× CH_3); IR (KBr, cm^{-1}) 3442.0, 3190.8, 1687.3, 1646.6; HRMS-EI (70 eV) *m*/*z* calcd for $C_{23}H_{20}N_4O_3$ 400.1535, found 400.1534.

4.2.7. 5-[2-(Dimethylamino)ethyl]-9-(4-methoxylphenyl)-5,8-dihydrobenz[*de*]imidazo[4,5-*g*]isoquinoline-4,6-dione (6g). From **4** (200 mg, 0.88 mmol) and 4-methoxy-benzaldehyde (132 mg, 0.97 mmol), and $NaHSO_3$ (137 mg, 1.32 mmol) in DMF (15 ml) yielded **5g** (211 mg, 70%) as a yellow solid, mp > 300 °C. From **5g** (150 mg, 0.44 mmol) and *N*,*N*-dimethylethane-1,2-diamine (135 mg, 1.17 mmol) in absolute EtOH (15 ml) yielded **6g** (128 mg, 75%) as a yellow solid, mp 249–251 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.84 (d, J = 8.0, 1H, ArH), 8.57 (s, 1H, ArH), 8.40 (d, J = 6.8, 1H, ArH), 8.20 (d, J = 8.8, 2H, ArH), 7.87 (dd, J = 8.0, 6.8, 1H, ArH), 7.16 (d, J = 8.8, 2H, ArH), 4.15 (t, J = 6.4, 2H, CH_2NCO), 3.72 (s, 3H, OCH_3,) 2.54 (t, J = 6.4, 2H, CH_2N), 2.23 (s, 6H, 2× CH_3); IR (KBr, cm^{-1}) 3288.0, 1685.3, 1640.3; HRMS-EI (70 eV) *m*/*z* calcd for $C_{24}H_{22}N_4O_3$ 414.1692, found 414.1700.

4.2.8. 5-[2-(Dimethylamino)ethyl]-9-[4-(ethoxyl)phenyl]-5,8-dihydrobenz[*de*]imidazo[4,5-*g*]isoquinoline-4,6-dione (6h). From **4** (200 mg, 0.88 mmol) and 4-ethoxy-benzaldehyde (145 mg, 0.97 mmol) and $NaHSO_3$ (137 mg, 1.32 mmol) in DMF (15 ml) yielded **5h** (226 mg, 72%) as a yellow solid, mp > 300 °C. From **5h** (150 mg, 0.42 mmol) and *N*,*N*-dimethylethane-1,2-diamine (103 mg, 1.17 mmol) in absolute EtOH (15 ml) yielded **6h** (133 mg, 74%) as a yellow solid, mp 287–288 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.84 (d, J = 8.8, 1H, ArH), 8.58 (s, 1H, ArH), 8.40 (d, J = 7.2, 1H, ArH), 8.15 (d, J = 8.0, 2H, ArH), 7.88 (dd, J = 8.8, 7.2, 1H, ArH), 7.41 (d, J = 8.0, 2H, ArH), 4.15 (t, J = 6.4, 2H, CH_2NCO), 2.54 (t, J = 6.4, 2H, CH_2N), 2.23 (s, 6H, 2× CH_3); IR (KBr, cm^{-1}) 3257.4, 1690.2, 1642.6; HRMS-EI (70 eV) *m*/*z* calcd for $C_{25}H_{24}N_4O_3$ 428.1848, found 428.1849.

4.2.9. 5-[2-(Dimethylamino)ethyl]-9-(3-methoxy-4-hydroxyl-phenyl)-5,8-dihydrobenz[*de*]imidazo[4,5-*g*]iso-quinoline-4,6-dione (6i). From **4** (200 mg, 0.88 mmol) and 3-methyloxy-4-hydroxy-benzaldehyde (147 mg, 0.97 mmol), and $NaHSO_3$ (137 mg, 1.32 mmol) in DMF (15 ml) yielded 5i (246 mg, 78%) as a yellow solid, mp > 300 °C. From 5i (150 mg, 0.42 mmol) and *N*,*N*-dimethylethane-1,2-diamine (103 mg, 1.17 mmol) in absolute EtOH (15 ml) yielded 6i (126 mg, 70%) as an orange solid, mp > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.89 (d, J = 7.6, 1H, ArH), 8.60 (s, 1H, ArH), 8.44 (d, J = 6.8, 1H, ArH), 7.91 (dd, J = 7.6, 6.8, 1H, ArH), 7.83 (s, 1H, ArH), 7.74 (d, J = 8.0, 1H, ArH), 6.98 (d, J = 8.0, 1H, ArH), 4.19 (t, J = 6.4, 2H, CH_2NCO), 3.98 (s, 3H, OCH_3), 2.56 (t, J = 6.4, 2H, CH_2N), 2.22 (s, 6H, 2× CH_3); IR (KBr, cm^{-1}) 3369.8, 3189.8, 1683.5, 1648.7; HRMS-EI (70 eV) *m*/*z* calcd for $C_{24}H_{22}N_4O_4$ 430.1641, found 430.1641.

4.2.10. 5-[2-(Dimethylamino)ethyl]-9-[3,4-(dimethoxyl)-phenyl]-5,8-dihydrobenz[*de*]imidazo[4,5-*g*]isoquinoline-4,6-dione (6j). From **4** (200 mg, 0.88 mmol) and 3,4-dimethyloxy-benzaldehyde (160 mg, 0.97 mmol), and $NaHSO_3$ (137 mg, 1.32 mmol) in DMF (15 ml) yielded **5j** (260 mg, 79%) as a yellow solid, mp > 300 °C. From **5j** (150 mg, 0.40 mmol) and *N*,*N*-dimethylethane-1,2-diamine (103 mg, 1.17 mmol) in absolute EtOH (15 ml) yielded 6j (135 mg, 76%) as an orange solid, mp 247–249 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (d, J = 7.6, 1H, ArH), 8.46 (s, 1H, ArH), 8.33 (d, J = 7.2, 1H, ArH), 7.81 (dd, J = 7.6, 7.2, 1H, ArH), 7.78–7.77 (m, 2H, ArH), 7.12 (d, J = 8.4, 1H, ArH), 4.10 (t, J = 7.2, 2H, CH_2NCO), 3.92 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 2.52 (t, J = 6.4, 2H, CH_2N), 2.24 (s, 2× CH_3, 6H); IR (KBr, cm^{-1}) 3294.7, 1686.5, 1639.8; HRMS-EI (70 eV) *m*/*z* calcd for $C_{25}H_{24}N_4O_4$ 444.1798, found 444.1799.

4.2.11. 5-[2-(Dimethylamino)ethyl]-9-(3-furyl)-5,8-dihydrobenz[*de*]imidazo[4,5-*g*]isoquinoline-4,6-dione (6k). From **4** (200 mg, 0.88 mmol) and furan-3-carbaldehyde (93 mg, 0.97 mmol), and $NaHSO_3$ (137 mg, 1.32 mmol) in DMF (15 ml) yielded **5k** (205 mg, 77%) as a yellow solid, mp > 300 °C. From **5k** (150 mg, 0.49 mmol) and *N*,*N*-dimethylethane-1,2-diamine (103 mg, 1.17 mmol) in absolute EtOH (15 ml) yielded **6k** (138 mg, 75%) as a brown solid, mp 212–213 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.73 (d, J = 6.7, 1H), 8.51 (br s, 2H, ArH and FuranH), 8.36 (d, J = 7.0), 7.90 (s, 1H, FuranH), 7.83 (dd, J = 6.7, 7.0, 1H, ArH), 7.16 (s, 1H, FuranH), 4.12 (t, J = 6.7, 2H, CH_2NCO), 2.55 (t, J = 6.9, 2H, CH_2N), 2.24 (s, 6H, 2× CH_3); IR (KBr, cm^{-1}) 3284.9, 1686.7, 1646.0; HRMS-EI (70 eV) *m*/*z* calcd for $C_{21}H_{18}N_4O_3$ 374.1379, found 374.1377.

4.2.12. 5-[2-(Dimethylamino)ethyl]-9-(3-thienyl)-5,8-dihydrobenz[*de*]imidazo[4,5-*g*]isoquinoline-4,6-dione (6l). From **4** (200 mg, 0.88 mmol) and thiophene-3-carbaldehyde (108 mg, 0.97 mg), and $NaHSO_3$ (137 mg, 1.32 mmol) in DMF (15 ml) yielded **5l** (202 mg, 76%) as a brown solid, mp > 300 °C. From **5l** (150 mg, 0.47 mmol) and *N*,*N*-dimethylethane-1,2-diamine (103 mg, 1.17 mmol) in absolute EtOH (15 ml) yielded **6l** (140 mg, 72%) as a yellow solid, mp 249–250 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.76 (d, J = 7.8, 1H, ArH), 8.53 (s, 1H, ArH), 8.36 (d, J = 9.5, 1H, ArH), 8.35 (s, 1H, ThiopheneH), 7.85 (dd, J = 7.8, 9.5, 1H, ArH), 7.77 (q, 1H, ThiopheneH), 4.11 (t, J = 7.0, 2H, CH_2NCO), 2.51 (t, J = 6.9, 2H, CH_2N), 2.21 (s, 6H, 2× CH_3); IR (KBr, cm^{-1}) 3332.3, 1683.8, 1637.2; HRMS-EI (70 eV) *m*/*z* calcd for $C_{21}H_{18}N_4O_2S$ 390.1150, found 390.1147.

4.2.13. 5-[2-(Dimethylamino)ethyl]-9-(2-furyl)-5,8-dihydrobenz[*de*]imidazo[4,5-*g*]isoquinoline-4,6-dione (6m). From **4** (200 mg, 0.88 mmol) and furan-2-carbaldehyde (93 mg, 0.97 mmol), and $NaHSO_3$ (137 mg, 1.32 mmol) in DMF (15 ml) yielded **5m** (200 mg, 75%) as a brown solid, mp > 300 °C. From **5m** (135 mg, 0.49 mmol) and *N*,*N*-dimethylethane-1,2-diamine (103 mg, 1.17 mmol) in absolute EtOH (15 ml) yielded **6m** (128 mg, 73%) as an orange solid, mp 240–241 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.77 (d, J = 8.1, 1H, ArH), 8.52 (s, 1H, ArH), 8.37 (d, J = 7.2, 1H, ArH), 8.01 (br s, 1H, FuranH), 7.83 (dd, J = 8.1, 7.2, 1H, ArH), 7.34 (d, J = 3.4, 1H, FuranH), 6.78 (q, 1H, FuranH), 4.14 (t, J = 6.9, 2H, CH_2NCO), 2.56 (t, J = 4.7, 2H, CH_2N), 2.25 (s, 6H, 2× CH_3); IR (KBr, cm^{-1}) 3113.3, 1690.3, 1651.2; HRMS-EI (70 eV) *m*/*z* calcd for $C_{21}H_{18}N_4O_3$ 374.1379, found 374.1375.

4.2.14. 5-[2-(Dimethylamino)ethyl]-9-(2-thienyl)-5,8-dihydrobenz[*de*]imidazo[4,5-*g*]isoquinoline-4,6-dione (6n). From **4** (200 mg, 0.88 mmol) and thiophene-2-carbaldehyde (108 mg, 0.97 mg), and $NaHSO_3$ (137 mg, 1.32 mmol) in DMF (15 ml) yielded **5n** (222 mg, 79%) as a yellow solid, mp > 300 °C. From **5n** (150 mg, 0.47 mmol) and *N*,*N*-dimethylethane-1,2-diamine (103 mg, 1.17 mmol) in absolute EtOH (15 ml) yielded **6n** (129 mg, 70%) as a yellow solid, mp 248–250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.80 (d, J = 8.0, 1H, ArH), 8.57 (s, 1H, ArH), 8.41 (d, J = 6.4, 1H, ArH), 7.95 (d, J = 3.2, 1H, ThiopheneH), 7.87 (dd, J = 8.0, 6.4, 1H, ArH), 7.82 (d, J = 4.0, 1H, ThiopheneH), 7.30 (d, J = 3.2, 4.0, 1H, ThiopheneH), 4.16 (t, J = 6.8, 1H, ArH), 2.54 (t, J = 7.2, 2H, CH_2N), 2.24 (s, 6H, 2× CH_3); IR (KBr, cm^{-1}) 3287.3, 1685.1, 1615.2; HRMS-EI (70 eV) *m*/*z* calcd for $C_{21}H_{18}N_4O_2S$ 390.1150, found 390.1147.

4.2.15. 5-[2-(Dimethylamino)ethyl]-9-(2-pyrrolyl)-5,8-dihydrobenz[*de*]imidazo[4,5-*g*]isoquinoline-4,6-dione (6o). From **4** (200 mg, 0.88 mmol) and 1*H*-pyrrole-2-carbaldehyde (92 mg, 0.97 mmol), and $NaHSO_3$ (137 mg, 1.32 mmol) in DMF (15 ml) yielded **5o** (178 mg, 67%) as a yellow solid, mp > 300 °C. From **5o** (150 mg, 0.50 mmol) and *N*,*N*-dimethylethane-1,2-diamine (103 mg, 1.17 mmol) in absolute EtOH (15 ml) yielded **6o** (138 mg, 75%) as a brown solid, mp 247–249 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.82 (d, J = 8.1, 1H, ArH), 8.57 (s, 1H, ArH), 8.41 (d, J = 7.3, 1H, ArH), 7.87 (dd, J = 8.1, 7.3, 1H, ArH), 7.03 (br s, 1H, PyrioleH), 7.00 (d, J = 2.8, 1H, PyrroleH), 6.27 (dd, J = 3.5, 2.8, 1H, PyrroleH), 4.15 (t, J = 7.0, 2H, CH_2NCO), 2.51 (t, J = 7.1, 2H, CH_2N), 2.21 (s, 6H, 2× CH_3); IR (KBr, cm^{-1}) 3311.5, 1692.2, 1651.9; HRMS-EI (70 eV) *m*/*z* calcd for $C_{21}H_{19}N_5O_2$ 373.1539, found 373.1537.

4.2.16. 5-[2-(Dimethylamino)ethyl]-9-(*N*-methyl-2-pyrrolyl)-5,8-dihydrobenz[*de*]imidazo[4,5-*g*]isoquinoline-4,6-dione (6p). From **4** (200 mg, 0.88 mmol) and 1-methyl-1*H*-pyrrole-2-carbaldehyde (105 mg, 0.97 mmol), and $NaHSO_3$ (137 mg, 1.32 mmol) in DMF (15 ml) yielded **5p** (206 mg, 74%) as a yellow solid, mp >300 °C. From **5p** (150 mg, 0.47 mmol) and *N*,*N*-dimethylethane-1,2-diamine (103 mg, 1.17 mmol) in absolute EtOH (15 ml) yielded **6p** (136 mg, 73%) as a brown solid, mp 252–253 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.81 (d, J = 6.6, 1H, ArH), 8.53 (s, 1H, ArH), 8.40 (d, J = 6.2, 1H, ArH), 7.85 (dd, J = 6.6, 6.2, 1H, ArH), 7.09 (br s, 1H, PyrroleH), 7.02 (d, J = 2.8, 1H, PyrroleH), 6.23 (dd, J = 3.5, 2.8, 1H, PyrroleH), 4.19 (s, 3H, N–CH_3) 4.14 (t, J = 6.9, 2H, CH_2NCO), 2.51 (t, J = 7.2, 2H, CH_2N), 2.21 (s, 6H, 2× CH_3); IR (KBr, cm^{-1}) 3265.0, 1693.8, 1640.0; HRMS-EI (70 eV) *m*/*z* calcd for $C_{22}H_{21}N_5O_2$ 387.1695, found 387.1698.

4.2.17. 5-[2-(Dimethylamino)ethyl]-9-(2-pyridinyl)-5,8-dihydrobenz[*de*]imidazo[4,5-*g*]isoquinoline-4,6-dione (6q). From **4** (200 mg, 0.88 mmol) and pyridine-2-carbaldehyde (103 mg, 0.97 mmol), and $NaHSO_3$ (137 mg, 1.32 mmol) in DMF (15 ml) yielded **5q** (216 mg, 78%) as a brown solid, mp > 300 °C. From **5q** (150 mg, 0.48 mmol) and *N*,*N*-dimethylethane-1,2-diamine (103 mg, 1.17 mmol) in absolute EtOH (15 ml) yielded **6q** (143 mg, 77%) as a yellow solid, mp 231–233 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.73 (m, 2H, ArH and PyridineH), 8.49 (s, 1H, ArH), 8.29 (m, 2H, ArH and PyridineH), 7.99 (t, J = 7.3, 1H, PyridineH), 7.77 (t, J = 7.7, 1H, ArH), 7.53 (q, 1H, PyridineH), 4.09 (t, J = 6.9, 2H, CH_2NCO), 2.56 (t, J = 6.8, 2H, CH_2N), 2.26 (s, 6H, 2× CH_3); IR (KBr, cm^{-1}) 3189.8, 1692.1, 1650.6; HRMS-EI (70 eV) *m*/*z* calcd for $C_{22}H_{19}N_5O_2$ 385.1539, found 385.1538.

4.2.18. 5-[2-(Dimethylamino)ethyl]-9-(3-pyridinyl)-5,8-dihydrobenz[*de*]imidazo[4,5-*g*]isoquinoline-4,6-dione (6r). From **4** (200 mg, 0.88 mmol) and pyridine-3-carbaldehyde (103 mg, 0.97 mmol), and $NaHSO_3$ (137 mg, 1.32 mmol) in DMF (15 ml) yielded **5r** (191 mg, 69%) as a yellow solid, mp > 300 °C. From **5r** (150 mg, 0.48 mmol) and *N*,*N*-dimethylethane-1,2-diamine (103 mg, 1.17 mmol) in absolute EtOH (15 ml) yielded **6r** (135 mg, 74%) as a brown solid, mp 243–244 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.31 (s, 1H, PyridineH), 8.67 (d, J = 4.3, 1H, PyridineH), 8.64 (d, J = 7.9, 1H, ArH), 8.45 (d, J = 7.10, 1H, ArH), 8.40 (s, 1H, PyridineH), 8.27 (d, J = 7.2, 1H, PyridineH), 7.76 (dd, J = 7.9, 7.1, 1H, ArH), 7.56 (q, 1H, pyridineH), 4.06 (t, J = 6.9, 2H, CH_2NCO), 2.55 (t, J = 7.0, 2H, CH_2N), 2.26 (s, 6H, 2× CH_3); IR (KBr, cm^{-1}) 3184.3, 1696.8, 1650.0; HRMS-EI (70 eV) *m*/*z* calcd for $C_{22}H_{19}N_5O_2$ 385.1539, found 385.1549.

4.2.19. 5-[2-(Dimethylamino)ethyl]-9-(4-pyridinyl)-5,8-dihydrobenz[*de*]imidazo[4,5-*g*]isoquinoline-4,6-dione (6s). From **4** (200 mg, 0.88 mmol) and pyridine-4-carbaldehyde (103 mg, 0.97 mmol), and $NaHSO_3$ (137 mg, 1.32 mmol) in DMF (15 ml) yielded **5s** (216 mg, 78%) as a brown solid, mp > 300 °C. From **5s** (139 mg, 0.48 mmol) and *N*,*N*-dimethylethane-1,2-diamine (103 mg, 1.17 mmol) in absolute EtOH (15 ml) yielded **6s** (128 mg, 75%) as an orange solid, mp 204–205 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.74 (d, J = 8.4, 2H, PyridineH), 8.67 (d, J = 8.0, 1H, ArH), 8.41 (s, 1H, ArH), 8.28 (d, J = 7.2, 1H, Ar), 8.07 (d, 2H, PyridineH), 7.77 (t, J = 8.0, 7.2, 1H, ArH), 4.07 (br s, 2H, CH_2NCO), 2.52 (br s, 2H, CH_2N), 2.28 (s, 6H, 2× CH_3); IR (KBr, cm^{-1}) 3332.3, 1692.4, 1645.5; HRMS-EI (70 eV) *m*/*z* calcd for $C_{22}H_{19}N_5O_2$ $[M+H]^+$ 386.1617, found 386.1626.

4.3. In vitro cytotoxicity assays

The prepared compounds were submitted to Shanghai Institute of Materia Medica and Dalian Medical University for in vitro cytotoxicity assays. Growth inhibitory effect on the cell lines (P388, SMMC-7721, HeLa, and HL-60) was measured by the MTT assay.[14] For A-549 cell lines, the growth inhibition was tested by the sulforhodamine B (SRB) assay.[15]

4.4. DNA binding experiments

4.4.1. DNA. Calf-thymus DNA was purchased from the Sino–American Biotechnology Company. Solutions of DNA in 30 mM Tris–HCl buffer (pH 7.2) and PBE buffer (pH 7.1) gave a ratio of UV absorbance at 260 and 280 nm of 1.8–1.9:1, indicating that the DNA was sufficiently free of protein. The concentration of calf-thymus DNA was determined spectrophotometrically assuming the molar absorption is 6600 M^{-1} cm^{-1} (260 nm).

4.4.2. UV–vis measurements. Spectroscopic titrations were carried out at room temperature to determine the binding affinity between DNA and each compound. Initially, solutions of the blank buffer were placed in the reference and sample cuvettes (1 cm path length), respectively, and then the first spectrum was recorded in the range 200–600 nm. During the titration, aliquots of buffered DNA solution were added to each cuvette to eliminate the absorbance of DNA itself, and the solutions were mixed by repeated inversion. After mixing for 10 min, the absorption spectra were recorded. The titration processes were repeated until there was no change

in the spectra for at least four titrations indicating binding saturation had been achieved. The UV–vis titrations for each sample were repeated at least three times.

4.4.3. Melting temperature measurements. Measurements were performed using 20 μM CT DNA in BPE buffer, pH 7.1 (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA). The temperature inside the cuvette was measured with a platinum probe; it was increased over the range 50–92 °C with a heating rate of 1 °C/min. The 'melting' temperature T_m was taken as the midpoint of the hyperchromic transition. Data are presented as T_m versus the temperature.

4.4.4. Viscometric titrations. Viscosity measurements were carried out using an Ubbelodhe viscometer maintained at a constant temperature at 25 (±0.1) °C in a thermostated bath. The DNA samples contained approximately 200 base pairs. Flow times were measured with a digital stopwatch and each sample was measured three times and an average flow time was calculated. Data are presented as $(\eta/\eta_0)^{1/3}$ versus the ratio of the concentration of compounds to that of DNA, where η is the viscosity of DNA in the presence of compound and η_0 is the viscosity of DNA in the absence of compound.

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