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Design, synthesis, and biological evaluation of new mitonafide derivatives as potential antitumor drugs

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ABSTRACT

A series of potential DNA-binding antitumor agents, 2-[ω-(alkylamino)alkyl]-6-{[ω-(alkylamino)alkyl]amino}-1*H*-benzo[*de*]isoquinolin-1,3(2*H*)-diones and 1,7-bis{6-[(ω-(dimethylamino)alkyl)amino]-1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl}-4-methyl-4-azaheptanes, have been prepared as mitonafide derivatives. Their DNA-binding ability and cytotoxic activity have been evaluated. Some of the target compounds have shown high DNA affinity as well as relevant cytotoxic properties.

1. Introduction

A planar or semi-planar chromophore portion, capable of intercalation into DNA, is the common characteristic feature of DNA-intercalating anticancer drugs. Our research group has been interested to the acridine ring system since it constitutes a versatile building block for compounds useful in antitumor strategies. Many classes of acridine as well as bis acridine derivatives have been synthesized and studied by us.[1–6] For many of these compounds not only the chromophore structure is important, but also the nature of the substituents. In particular, the number and the feature of the pendant basic side chains are determinant for antitumor properties.[1] In Table 1 is reported the increment in cytotoxic activity due to a second basic side chain for three pairs of acridine derivatives: the pyrimido[5,6,1-de]acridines (**1**), the 9-acridone-4-carboxamides (**2**), and the acridine-4-carboxamides (**3**). The increment is already relevant, since the difference in activity varies about 40−300 times in the pairs reported.

On the other hand, there is a remarkable structural correlation (Fig. 1) between the pyrimido[5,6,1-de]acridindione chromophore of compounds **1** and the benzo[*de*]isoquinolindione chromophore of mitonafide (**4a**) and amonafide (**4b**), compounds that underwent clinical trials as anticancer drugs.[7,8] Since a second basic side chain on benzo[*de*]isoquinolindione nucleus could not only increment DNA affinity, but also affect chemical-physical characteristics and solubility of the benzo[*de*]isoquinolindione derivatives, likewise we have found with pyrimido[5,6,1-de]acridines (**1**),[1] we planned to synthesize the new derivatives **5**, attempting to increment the already noticeable antitumor properties of related compounds **4a,b**.

In particular, compounds **5a**–**f** (X = H) have been prepared to verify the effect of side chain homologation on biological properties; **5g** (R′ = $NHCH_2CH_2OH$) has been designed with a side chain like that of anticancer drug mitoxantrone; **5h,i** (X = NO_2) and **5k,l** (X = NH_2) allow to study the effect of five substituent groups corresponding to that of mitonafide and amonafide, respectively; finally, **5m** (R = R′ = NH_2), with two side chains similar to that of pixantrone an antitumor agent in advanced clinical trials,[9] has been obtained from the intermediate **5j** [R = R′ = $NHCOOC(CH_3)_3$].

Moreover, two planar intercalating moieties connected with an appropriate linker yield a bis derivative, which generally presents higher DNA affinity and prolonged drug residence times in DNA with respect to the monomer. Based on this rationale, we have prepared acridine-based families of potential bis-intercalators possessing noticeable antitumor properties.[2,3,6] Furthermore, successful examples of bis benzo[*de*]isoquinolindione analogues have also been described: elinafide and bisnafide (Fig. 1, LU 79553 and DMP 840, respectively) are very promising anticancer drugs.[10,11] Hence, we have designed and synthesized as their congeners the bis benzo[*de*]isoquinolindiones **6** with an additional basic side chains; in these derivatives the chromophores are connected by the polyamine linker found to be the most appropriate for our bis 'acridinic' derivatives.[2,3,6]

2. Chemistry

Scheme 1 shows the synthetic pathway leading to the target derivatives **5** and **6**. Thus, the 4-chloro-1,8-naphthalic anhydride

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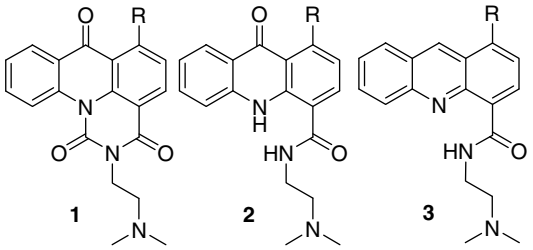
Table 1
Structures and cytotoxic activity of pyrimido[5,6,1-de]acridines (**1**), 9-acridone-4-carboxamides (**2**), and acridine-4-carboxamides (**3**) pairs with one or two basic side chains



	R	IC_{50} (nM)[a]	CI[b]
1a	Cl	6700	
1b	$NH(CH_2)_2N(CH_3)_2$	22	305
2a	H	1700	
2b	$NH(CH_2)_2N(CH_3)_2$	44	39
3a	H	990	
3b	$NH(CH_2)_2N(CH_3)_2$	7	141

[a] Data from Ref. 1.
[b] CI represents the cytotoxicity increment due to the second basic side chain calculated as the IC_{50} pair ratio (**a**/**b**).

(**7**) was allowed to react either with 2-dimethylaminoethylamine or with 3-dimethylaminopropylamine in $CHCl_3$ at room temperature to afford the desired intermediates **8a,b**, respectively. Compounds **8a,b** have been already synthesized in a different way.[12] The suitable imide **8** was diluted in the appropriate alkylaminoalkylamine under stirring at 100 °C to yield the target compounds **5a**–**g**. As previously described, the nitration of 4-chloro-1,8-naphthalic anhydride (**7**) was performed with a mixture of HNO_3/H_2SO_4 at room temperature to give the 4-chloro-3-nitro-1,8-naphthalic anhydride (**9**).[13] Compound **9** was reacted with the opportune alkylaminoalkylamine in $CHCl_3$ at room temperature to produce the target compounds **5h,i** and the intermediate **5j**. The reduction of **5h,i** with H_2 and Pd/C in CH_3OH and HCl at room temperature yielded the final amino derivatives **5k,l**, respectively. The deprotection of BOC protected intermediate **5j** with HCl in dioxane at room temperature gave the final compound **5m**. Finally, **7** was reacted with bis(3-aminopropyl)methylamine in toluene under reflux to afford the bis dichloro intermediate **10**, which by treatment either with 2-dimethylaminoethylamine or 3-dimethylaminopropylamine, used as reagent/solvent under stirring at 100 °C, produced target compounds **6a,b**, respectively.

To examine the DNA-binding properties and the antineoplastic activity of these agents, the target derivatives **5** and **6** isolated as free base forms were converted to their water-soluble hydrochloride salts by usual methods.

3. DNA-binding

'Apparent' binding constant (K_{app}) values have been determined using a competitive fluorometric ethidium displacement method that has been used extensively for other DNA-binding ligands, particularly intercalants.[14–16] In this assay, the relative K_{app} affinity for CT-DNA (calf thymus DNA) is defined by K_{app} (drug) = 1.26/$C_{50} \times K_{app}$ (ethidium), where 1.26 is the concentration (μmol) of ethidium in ethidium–DNA complex, C_{50} represents the concentration (μmol) of added compound that is required to reduce the fluorescence of the ethidium–DNA complex by 50%, and the K_{app} (ethidium) binding constant is taken as 10^7 M^{-1}.[14,15] In the present

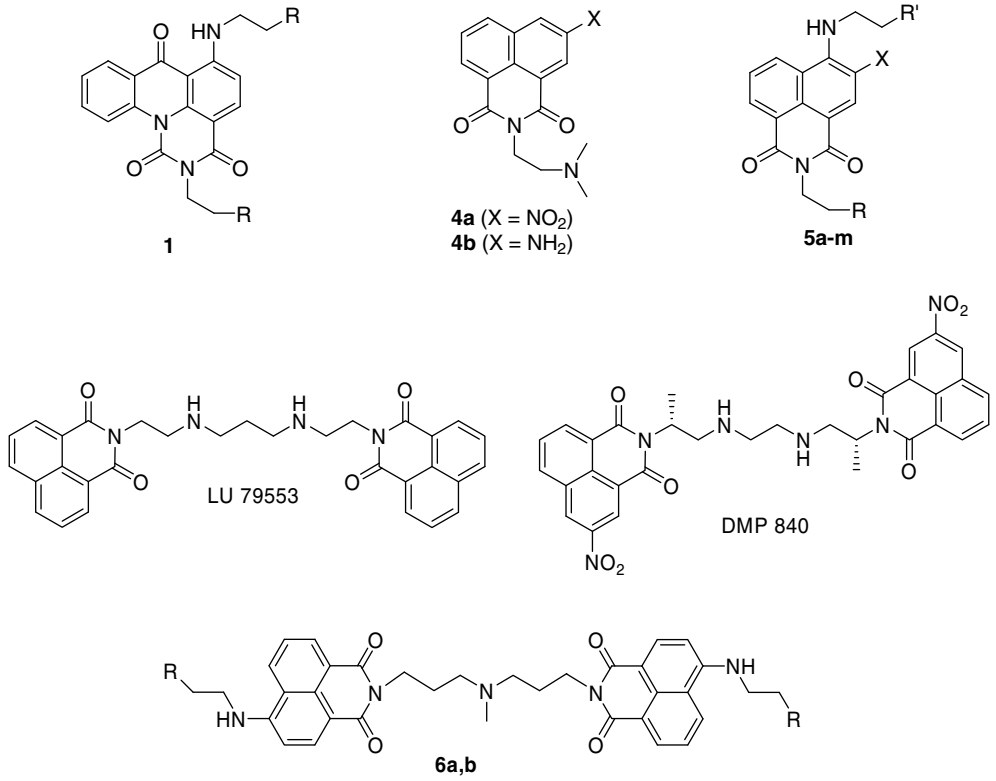
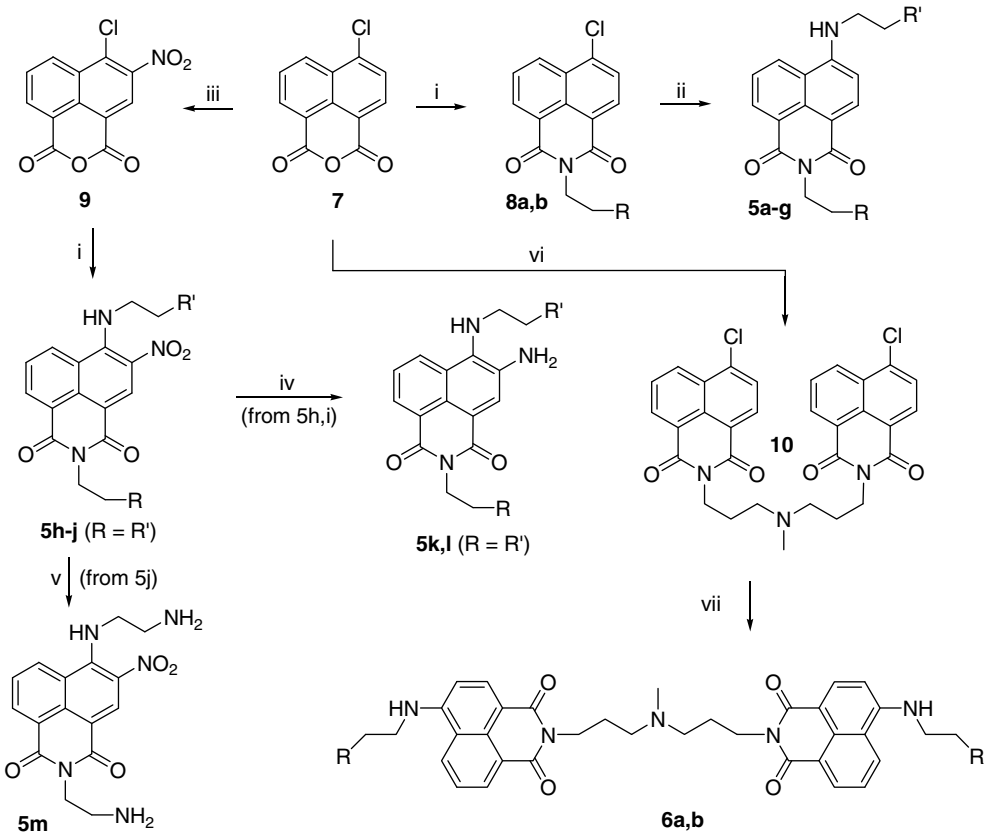


Figure 1. Design and structures of target derivatives **5** and **6** with a second basic side chain in position 6 of the benzo[*de*]isoquinolindione nucleus. R = $N(CH_3)_2$ for **5a,b,e–h,k** and **6a**; R = $CH_2N(CH_3)_2$ for **5c,d,i,l** and **6b**; R′ = $N(CH_3)_2$ for **5a,c,h,k**; R′ = $CH_2N(CH_3)_2$ for **5b,d,i,l**; R′ = $N(C_2H_5)_2$ for **5e**; R′ = $CH_2N(C_2H_5)_2$ for **5f**; R′ = $NH(CH_2)_2OH$ for **5g**; R = R′ = $NHCOOC(CH_3)_3$ for **5j**; R = R′ = NH_2 for **5m**; X = H for **5a–g**; X = NO_2 for **5h–j,m**; X = NH_2 for **5k,l**.



Scheme 1. Reagents and conditions: (i) $H_2N(CH_2)_2R$, r.t.; (ii) $H_2N(CH_2)_2R'$, 100 °C; (iii) HNO_3/H_2SO_4, r.t.; (iv) H_2, Pd/C, 30 psi, r.t.; (v) H^+, r.t.; (vi) $H_2N(CH_2)_3N(CH_3)(CH_2)_3NH_2$, 110 °C; (vii) $H_2N(CH_2)_2R$, 100 °C. Substituents: R = $N(CH_3)_2$ for **8a**; R = $CH_2N(CH_3)_2$ for **8b**; for target compounds **5** and **6** see Figure 1 and Table 2.

study, fluorescence displacement assays were performed at pH 7 to enable comparison in biological conditions. On these bases, the K_{app} values can be regarded as indicative of the strength and extent of binding to this 'pseudo-random' DNA sequence, but not of the mode of interaction (e.g., intercalation and/or groove binding mechanism).

In Table 2 are reported the K_{app} values of novel derivatives **5a**–**m** and **6a,b**. The results indicate that target compounds possess excellent DNA affinity, greater (compounds **5**) or much greater (compounds **6**) than that of ethidium. Moreover, the second pendent side chain in position 6 of the benzo[*de*]isoquinolindione nucleus highly enhances the binding capacity of the new derivatives, K_{app} values of **5** and **6** being, respectively, one or two orders of magnitude superior than that of parent compound **4a**.

Some considerations can be drawn about the CT-DNA binding. (1) In the **5a**–**g** subseries, compounds without a substituent in

Table 2
Substituents, DNA binding, and cytotoxic activity of compounds **5**, **6**, and mitonafide (**4a**)

Compound	R	R′	X	K_{app} (M^{-1})[a] CT–DNA	IC_{50} (SD)[b] HT29
5a	$N(CH_3)_2$	$N(CH_3)_2$	H	2.3×10^7	0.16 (0.071)
5b	$N(CH_3)_2$	$CH_2N(CH_3)_2$	H	1.9×10^7	0.93 (0.58)
5c	$CH_2N(CH_3)_2$	$N(CH_3)_2$	H	1.5×10^7	1.7 (0.44)
5d	$CH_2N(CH_3)_2$	$CH_2N(CH_3)_2$	H	1.3×10^7	1.4 (1.1)
5e	$N(CH_3)_2$	$N(C_2H_5)_2$	H	2.3×10^7	2.1 (0.082)
5f	$N(CH_3)_2$	$CH_2N(C_2H_5)_2$	H	1.9×10^7	0.89 (0.098)
5g	$N(CH_3)_2$	$NH(CH_2)_2OH$	H	2.4×10^7	0.77 (0.41)
5h	$N(CH_3)_2$	$N(CH_3)_2$	NO_2	7.7×10^7	0.12 (0.083)
5i	$CH_2N(CH_3)_2$	$CH_2N(CH_3)_2$	NO_2	7.0×10^7	0.14 (0.046)
5j	$NHCOOC(CH_3)_3$	$NHCOOC(CH_3)_3$	NO_2	NT[c]	NT[c]
5k	$N(CH_3)_2$	$N(CH_3)_2$	NH_2	4.5×10^7	0.81 (0.15)
5l	$CH_2N(CH_3)_2$	$CH_2N(CH_3)_2$	NH_2	3.9×10^7	16.8 (2.1)
5m	NH_2	NH_2	NO_2	5.3×10^7	0.86 (0.27)
6a	$N(CH_3)_2$	—	—	2.3×10^8	0.060 (0.021)
6b	$CH_2N(CH_3)_2$	—	—	1.1×10^8	0.10 (0.072)
4a	—	—	—	3.4×10^6	0.22

[a] $K_{app} = 1.26/C_{50} \times 10^7$ in which 1.26 is the concentration (μM) of ethidium in ethidium–DNA complex, C_{50} is drug concentration (μM) to effect 50% drop in fluorescence of bound ethidium, and 10^7 is the value of K_{app} assumed for ethidium in the complex.

[b] IC_{50} is the drug concentration (μM) required to inhibit cell growth by 50%. SD = standard deviation. All assays were performed in triplicate.

[c] NT = not tested.

position 5, it may be evinced that the nature of side chains in positions 2 and 6 affects the DNA affinity in a restricted range (1.3–2.4×10^7). The K_{app} values appear to be correlated with the distance between nitrogen atoms of the side chain: a distance of two methylene units (compounds **5a,e,g**) leads to greatest affinity; there is a similar decrement in binding efficiency for compounds **5b,f**, in which the distance is three methylene units in the side chain in position 6; compound **5c**, in which the distance is three methylene units in the side chain in position 2, and compound **5d**, in which the distance is three methylene units in both side chains, present a more marked decrement. (2) In the **5h**–**m** subseries, compounds substituted in position 5, it may be underlined that the five substitution always enhances DNA affinity as can be seen from the K_{app} values of the unsubstituted/substituted corresponding pairs **5a**↔**5h**, **5d**↔**5i**, **5a**↔**5k**, and **5d**↔**5l**. Moreover, comparison of K_{app} values of nitro/amino five substituted corresponding pairs, **5h**↔**5k** and **5i**↔**5l**, shows that the nitro group is more efficacious than amino for DNA binding. As before, for DNA binding the optimal distance between nitrogen atoms of side chains is two methylene units. Compound **5j** was not tested being only a precursor of **5m**, which possesses a relevant K_{app} comprised between that of **5h** and **5k**. (3) As expected, the bis derivatives **6a,b** have shown the highest values of K_{app} among the target compounds. A distance of two methylene units between nitrogen atoms of side chains in positions 6,6′ is confirmed to be preferable for DNA affinity.

4. Cytotoxic activity

In vitro cytotoxic potencies, as IC_{50} values, of target benzo[*de*]isoquinolindiones **5** and bis benzo[*de*]isoquinolindiones **6** in comparison with parent compound mitonafide (**4a**) against human colon adenocarcinoma cell line HT29 have been reported in Table 2. The results indicate that many of the target derivatives possess potent cytotoxic activity, with IC_{50} values in the sub μM range. Some are in the high nM range, being remarkably more potent than mitonafide.

The following remarks can be made:

(1) **5a**–**g** subseries (compounds without a substituent in position 5): these derivatives demonstrate that a second side chain in position 6 of benzo[*de*]isoquinolindione chromophore is important for activity. In fact, **5a**–**g** present a good cytotoxic activity despite lacking the nitro group in position 5 believed essential for antitumor activity of mitonafide. As for DNA binding, the optimal distance between nitrogen atoms of the side chain in position 2 is two methylene units, in fact **5c,d**, in which the distance is of three methylene units, are less active than homologues **5a,b**. Also the nature of side chain in position 6 influences the activity, but there is not an optimal distance between nitrogen atoms of side chain. In the homologues pairs **5a**↔**5b**, **5c**↔**5d**, and **5e**↔**5f** the prolongation of side chain leads to contrasting results in activity compare: **5a** more active than **5b** and **5c,e** less active than **5d,f**, respectively. Regarding the steric hindrance, more bulky substituents (ethyl instead of methyl) on distal nitrogen atom, **5e,f** compared with **5a,b**, respectively, gave different results with **5e** being much less active than **5a** and **5f** almost active as **5b**. Anyway, a distance of two methylene units between nitrogen atoms of both side chains, together with methyl substituents on both distal nitrogen atoms, (compound **5a**) leads to the highest activity in the subseries. The IC_{50} value of **5a** is 160 nM better than that of parent **4a**. Finally, **5g**, with a side chain in position 6 equal to that of mitoxantrone, possesses a relevant cytotoxic activity.

(2) **5h**–**m** subseries (compounds with a substituent in position 5). (a) X = NO_2 compounds **5h,i,m**: generally, we can say that the nitro group in position 5 confers excellent cytotoxic activity. Considering the corresponding unsubstituted/substituted pairs, there is a small increment in activity for the pair **5a**↔**5h** (IC_{50} from 0.16 to 0.12 μM), high for the pair **5d**↔**5i** (IC_{50} from 1.4 to 0.14 μM). Regarding the side chains we can say that the distance between the nitrogen atoms of the side chain is not so important for the activity as in the case of 5 unsubstituted derivatives: in fact, the IC_{50} values of homologue 5-nitro derivatives **5h,i**, are very similar (0.12 and 0.14 μM, respectively). Concerning **5m**, with side chains similar to that of pixantrone, the IC_{50} is in the sub μM range, but higher than that of **5h,i**. (b) X = NH_2 compounds **5k,l**: the reduction of nitro to amino group lead to a decrement in activity of about 7 times for the corresponding pair **5h**↔**5k** and of 120 times for the corresponding pair **5i**↔**5l**. In this case, the distance between the nitrogen atoms of the side chain is again important for the activity, compound **5k** having an IC_{50} value of 0.81 μM, which is about 20 times smaller than that of **5l** (16.8 μM).

(3) As expected, the bis derivatives **6a,b** show the highest cytotoxic activity among the new mitonafide derivatives with IC_{50} values in the nM range. The increment in activity between the corresponding mono and bis derivatives, pairs **5a**↔**6a** and **5d**↔**6b**, is of about 3 and 14 times, respectively. Again, the distance between the nitrogen atoms of the side chains in positions 6,6′ influences cytotoxic activity.

(4) There is not a simple linear correlation between cytotoxic activity and DNA binding. Considering compounds **5a**–**g** it can be remarked that **5a,g** are the most active and with the highest K_{app} values in the sub series, but **5e** with a similar value of K_{app} is the least active among this sub class. For the other target derivatives **5** and **6** there is, at least, a qualitative correlation between K_{app} and cytotoxic potency.

5. Conclusions

It can be asserted that the 2-[ω-(alkylamino)alkyl]-6-{[ω-(alkylamino)alkyl]amino}-1*H*-benzo[*de*]isoquinolin-1,3(2*H*)-diones (**5**) and the 1,7-bis{6-[(ω-(dimethylamino)alkyl)amino]-1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl}-4-methyl-4-azaheptanes (**6**) constituted a new class of potential intercalating agents endowed of excellent anti-proliferative and DNA-binding capacities. The benzo[*de*]isoquinoline chromophore is an interesting constituent of potential anticancer drugs for bis derivatives as well as monomeric derivatives. In particular, the 2-[2-(dimethylamino)ethyl]-6-{[2-(dimethylamino)-ethyl]amino}-5-nitro-1*H*-benzo[*de*]isoquinolin-1,3(2*H*)-dione (**5h**) and the 1,7-bis{6-[(2-(dimethylamino)ethyl)amino]-1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl}-4-methyl-4-azaheptane (**6a**) can become new leads in the continual search of novel specific and potent anticancer drugs.

6. Experimental

6.1. Chemistry

Reaction progress was monitored by thin-layer chromatography (TLC) accomplished using plates precoated with silica gel 60F-254 (Merck). All the target derivatives **5** and **6** isolated as free base forms were converted to their water-soluble hydrochloride salts by usual methods. **5k**–**m** were directly isolated as hydrochlorides. Melting points were determined on a Büchi 540 apparatus and are uncorrected. All ^{1}H NMR spectra were recorded on a Varian

VXR 300 instrument. Chemical shifts are reported as δ values (ppm) downfield from internal Me_4Si in the solvent shown. The following NMR abbreviations are used: br (broad), s (singlet), d (doublet), t (triplet), m (multiplet), ar (aromatic proton), and ex (exchangeable with D_2O). Elemental analyses were performed on an EA1108CHAZ-O elemental analyzer (Fisons Instruments).

6.2. 6-Chloro-2-[2-(dimethylamino)ethyl]-1*H*-benzo[*de*]-isoquinoline-1,3(2*H*)-dione (8a)

To a suspension of 4-chloro-1,8-naphthalic anhydride (**4**, 0.5 g, 2.15 mmol) in $CHCl_3$ (20 mL) a solution of *N,N*-dimethylethylendiamine (0.7 mL, 6.45 mmol) in $CHCl_3$ (10 mL) was added dropwise. After stirring at room temperature for 20 min, the mixture was partitioned between $CHCl_3/CH_3OH$ (7:3 v/v) and aqueous 1 M Na_2CO_3. The organic layer was worked up to give a solid residue, which was washed with Et_2O and such as used for the next step (0.52 g, 80%). ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 6H, 2× CH_3), 2.62 (t, J = 5.77 Hz, 2H, CH_2), 4.28 (t, J = 5.77 Hz, 2H, CH_2), 7.75–7.88 (m, 2H, ar), 8.45 (d, J = 7.97 Hz, 1H, ar), 8.52–8.67 (m, 2H, ar).

6.3. 6-Chloro-2-[3-(dimethylamino)propyl]-1*H*-benzo[*de*]-isoquinoline-1,3(2*H*)-dione (8b)

To a suspension of 4-chloro-1,8-naphthalic anhydride (**4**, 0.5 g, 2.15 mmol) in $CHCl_3$ (20 mL) a solution of *N,N*-dimethylpropylen-1,3-diamine (0.8 mL, 6.45 mmol) in $CHCl_3$ (10 mL) was added dropwise. After stirring at 60 °C for 30 min, the mixture was partitioned between $CHCl_3/CH_3OH$ (7:3 v/v) and aqueous 1 M Na_2CO_3. The organic layer was worked up to give a residue, which was flash chromatographed on silica gel column eluted with $CHCl_3$/CH_3OH (19:1 v/v) to yield a solid (0.58 g, 85%) used such as for the next step. ^{1}H NMR ($CDCl_3$) δ 1.87 (m, 2H, CH_2), 2.23 (s, 6H, 2× CH_3), 2.43 (t, J = 5.77 Hz, 2H, CH_2), 4.22 (t, J = 5.77 Hz, 2H, CH_2), 7.78–7.89 (m, 2H, ar), 8.50 (d, J = 7.97 Hz, 1H, ar), 8.55–8.68 (m, 2H, ar).

6.4. 2-[2-(Dimethylamino)ethyl]-6-{[2-(dimethylamino)ethyl]-amino}-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (5a). Example of general procedure for the preparation of 5a–g

The 6-chloro-2-[2-(dimethylamino)ethyl]-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**8a**, 100 mg, 0.33 mmol) in *N,N*-dimethylethylendiamine (1.5 mL) was stirred at 100 °C for 1.5 h. The mixture was partitioned between $CHCl_3$ (3× 20 mL) and aqueous 1 M Na_2CO_3 (20 mL). The organic layer was worked up to give a residue, which was flash chromatography on a silica gel column eluted first with $CHCl_3$/MeOH (4:1 v/v) and then with $CHCl_3$/MeOH (1:1 v/v) containing 32% aqueous NH_3 (10 mL for 1 L of eluent) to obtain **5a** (90 mg, 77%): mp 119–120 °C (dec); hydrochloride mp 124–125 °C (dec); ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 6H, 2× CH_3), 2.36 (s, 6H, 2× CH_3), 2.65 (t, J = 5.77 Hz, 2H, CH_2), 2.72 (m, 2H, CH_2), 3.35 (m, 2H, CH_2), 4.30 (t, J = 5.77 Hz, 2H, CH_2), 6.31 (m, 1H, NH ex), 6.64 (d, J = 8.54 Hz, 1H, ar), 7.60 (t, J = 7.32 Hz, 1H, ar), 8.14 (d, J = 7.33 Hz, 1H, ar), 8.43 (d, J = 8.24 Hz, 1H, ar), 8.56 (d, J = 6.40 Hz, 1H, ar). Anal. Calcd. For $C_{20}H_{26}N_4O_2$: C, 67.77; H, 7.39; N, 15.81. Found: C, 67.54; H, 7.11; N, 15.94.

Compounds **5b**–**g** were prepared in the same experimental conditions from the appropriate intermediate **8** and the suitable diamine.

6.5. 2-[2-(Dimethylamino)ethyl]-6-{[3-(dimethylamino)propyl]-amino}-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (5b)

Yield 82%; mp 188–189 °C; hydrochloride mp 275–277 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.97 (m, 2H, CH_2), 2.35 (s, 6H, 2× CH_3), 2.46 (s, 6H, 2× CH_3), 2.62–2.82 (m, 4H, 2× CH_2), 3.45 (m, 2H, CH_2), 4.18 (t, J = 5.77 Hz, 2H, CH_2), 6.82 (d, J = 8.49 Hz, 1H, ar), 7.72 (t, J = 7.31 Hz, 1H, ar), 8.04 (m, 1H, NH ex), 8.28 (d, J = 8.24 Hz, 1H, ar), 8.45 (d, J = 7.34 Hz, 1H, ar), 8.73 (d, J = 6.40 Hz, 1H, ar). Anal. Calcd. For $C_{21}H_{28}N_4O_2$: C, 68.45; H, 7.66; N, 15.21. Found: C, 68.58; H, 7.81; N, 15.01.

6.6. 2-[3-(Dimethylamino)propyl]-6-{[2-(dimethylamino)ethyl]-amino}-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (5c)

Yield 66%; mp 119–120 °C; hydrochloride mp 278–279 °C (dec); ^{1}H NMR ($CDCl_3$) δ 1.90 (m, 2H, CH_2), 2.25 (s, 6H, 2× CH_3), 2.35 (s, 6H, 2× CH_3), 2.44 (t, J = 5.77 Hz, 2H, CH_2), 2.75 (t, J = 4.21 Hz, 2H, CH_2), 3.38 (m, 2H, CH_2), 4.21 (t, J = 5.77 Hz, 2H, CH_2), 6.28 (m, 1H, NH ex), 6.67 (d, J = 8.52 Hz, 1H, ar), 7.62 (t, J = 7.32 Hz, 1H, ar), 8.15 (d, J = 7.33 Hz, 1H, ar), 8.43 (d, J = 8.24 Hz, 1H, ar), 8.58 (d, J = 6.41 Hz, 1H, ar). Anal. Calcd. For $C_{21}H_{28}N_4O_2$: C, 68.45; H, 7.66; N, 15.21. Found: C, 68.23; H, 7.54; N, 15.52.

6.7. 2-[3-(Dimethylamino)propyl]-6-{[3-(dimethylamino)-propyl]amino}-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (5d)

Yield 71%; mp 110–111 °C (dec); hydrochloride mp 258–260 °C (dec); ^{1}H NMR ($CDCl_3$) δ *1.82–2.08* (m, 4H, 2× CH_2), 2.27 (s, 6H, 2× CH_3), 2.41 (s, 6H, 2× CH_3), 2.46 (t, J = 5.76 Hz, 2H, CH_2), 2.63 (m, 2H, CH_2), 3.48 (m, 2H, CH_2), 4.20 (t, J = 5.78 Hz, 2H, CH_2), 6.58 (d, J = 8.53 Hz, 1H, ar), 7.57 (t, J = 7.30 Hz, 1H, ar), 7.98 (d, J = 7.35 Hz, 1H, ar), 8.43 (d, J = 8.24 Hz, 1H, ar), 8.50–8.62 (m, 2H, ar+NH ex). Anal. Calcd. For $C_{22}H_{30}N_4O_2$: C, 69.08; H, 7.91; N, 14.65. Found: C, 69.32; H, 7.75; N, 14.55.

6.8. 2-[2-(Dimethylamino)ethyl]-6-{[2-(diethylamino)ethyl]-amino}-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (5e)

Yield 63%; mp 110–112 °C (dec); hydrochloride mp 288–290 °C (dec); ^{1}H NMR ($CDCl_3$) δ 1.08 (t, J = 5.45 Hz, 6H, 2× CH_3), 2.33 (s, 6H, 2× CH_3), 2.55–2.69 (m, 6H, 3× CH_3), 2.85 (m, 2H, CH_2), 3.34 (m, 2H, CH_2), 4.31 (t, J = 5.77 Hz, 2H, CH_2), 6.53 (m, 1H, NH ex), 6.62 (d, J = 8.54 Hz, 1H, ar), 7.61 (t, J = 7.32 Hz, 1H, ar), 8.12 (d, J = 7.33 Hz, 1H, ar), 8.43 (d, J = 8.24 Hz, 1H, ar), 8.55 (d, J = 6.40 Hz, 1H, ar). Anal. Calcd. For $C_{22}H_{30}N_4O_2$: C, 69.08; H, 7.91; N, 14.65. Found: C, 69.27; H, 7.85; N, 14.50.

6.9. 2-[2-(Dimethylamino)ethyl]-6-{[3-(diethylamino)propyl]-amino}-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (5f)

Yield 63%; mp 82–83 °C; hydrochloride mp 73–75 °C (dec); ^{1}H NMR ($CDCl_3$) δ 1.10 (t, J = 5.44 Hz, 6H, 2× CH_3), 1.90 (m, 2H, CH_2); 2.33 (s, 6H, 2× CH_3), 2.58–2.80 (m, 8H, 4× CH_2), 3.42 (m, 2H, CH_2), 4.28 (t, J = 5.77 Hz, 2H, CH_2), 6.53 (d, J = 8.50 Hz, 1H, ar), 7.50 (t, J = 7.31 Hz, 1H, ar), 8.12 (d, J = 7.33 Hz, 1H, ar), 8.40 (d, J = 8.26 Hz, 1H, ar), 8.55 (d, J = 6.41 Hz, 1H, ar), 8.63 (m, 1H, NH ex). Anal. Calcd. For $C_{23}H_{32}N_4O_2$: C, 69.67; H, 8.13; N, 14.13. Found: C, 69.72; H, 8.25; N, 14.05.

6.10. 2-[2-(Dimethylamino)ethyl]-6-({2-[(2-hydroxyethyl)-amino]ethyl}amino)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (5g)

Yield 74%; mp 130–131 °C (dec); hydrochloride mp 270–272 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.18 (s, 6H, 2× CH_3), 2.43–2.55 (m, 2H, CH_2); 2.65 (t, J = 5.44 Hz, 2H, CH_2), 2.86 (t, J = 5.78 Hz, 2H, CH_2), 3.40–3.53 (m, 4H, 2× CH_2), 4.12 (t, J = 5.78 Hz, 2H, CH_2), 4.53 (br s, 1H, NH ex); 6.81 (d, J = 8.51 Hz, 1H, ar), 7.62–7.82 (m, 2H, ar+NH ex), 8.26 (d, J = 8.22 Hz, 1H, ar), 8.42 (d, J = 7.33 Hz, 1H, ar), 8.70 (d, J = 6.89 Hz, 1H, ar). Anal. Calcd. For $C_{20}H_{26}N_4O_3$: C, 64.84; H, 7.07; N, 15.12. Found: C, 64.75; H, 7.21; N, 14.95.

6.11. 2-[2-(Dimethylamino)ethyl]-6-{[2-(dimethylamino)ethyl]amino}-5-nitro-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (5h). Example of general procedure for the preparation of 5h–j

A solution of *N,N*-dimethylethylendiamine (0.6 mL, 5.4 mmol) in $CHCl_3$ (10 mL) was added dropwise to a suspension of 4-chloro-3-nitro-1,8-naphthalic anhydride (**9**, 0.5 g, 1.8 mmol) in $CHCl_3$ (20 mL). The resulting mixture was stirred at room temperature for 3 h, then partitioned between $CHCl_3$ (3× 20 mL) and aqueous 1 M Na_2CO_3 (20 mL). The organic layer was worked up to give a residue, which was chromatography on a silica gel column eluted with $CHCl_3$/MeOH (19:1 v/v) to obtain 5 h (300 mg, 42%): mp 218–219 °C (dec); hydrochloride mp 268–269 °C (dec); ^{1}H NMR ($CDCl_3$) δ 2.34 (s, 6H, 2× CH_3), 2.39 (s, 6H, 2× CH_3), 2.57–2.66 (m, 4H, 2× CH_2), 3.93 (m, 2H, CH_2), 4.28 (t, J = 5.80 Hz, 2H, CH_2), 7.67 (t, J = 7.43 Hz, 1H, ar), 8.65 (m, 2H, ar), 9.26 (s, 1H, ar), 10.32 (m, 1H, NH ex). Anal. Calcd. For $C_{20}H_{25}N_5O_4$: C, 60.14; H, 6.31; N, 17.53. Found: C, 60.34; H, 6.11; N, 17.34.

Compounds **5i**–**j** were prepared in the same experimental conditions from **9** and the suitable diamine.

6.12. 2-[3-(Dimethylamino)propyl]-6-{[3-(dimethylamino)propyl]amino}-5-nitro-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (5i)

Yield 41%; mp 112–115 °C (dec); hydrochloride mp 178–180 °C (dec); ^{1}H NMR ($CDCl_3$) δ *1.80–1.95* (m, 4H, 2× CH_2), 2.25 (s, 6H, 2× CH_3), 2.35 (s, 6H, 2× CH_3), 2.42 (t, J = 5.80 Hz, 2H, CH_2), 2.57 (t, J = 5.48 Hz, 2H, CH_2), 3.85 (m, 2H, CH_2), 4.20 (t, J = 5.80 Hz, 2H, CH_2), 7.68 (t, J = 7.41 Hz, 1H, ar), 8.52 (d, J = 8.01 Hz, 1H, ar), 8.63 (d, J = 7.39 Hz, 1H, ar), 9.17 (s, 1 H, ar), 10.28 (m, 1H, NH ex). Anal. Calcd. For $C_{22}H_{29}N_5O_4$: C, 61.81; H, 6.84; N, 16.38. Found: C, 61.62; H, 6.55; N, 16.55.

6.13. 2-{2-[(*tert*-Butoxycarbonyl)amino]ethyl}-6-({2-[(*tert*-butoxycarbonyl)amino]ethyl}amino)-5-nitro-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (5j)

Yield 32%; mp 208–210 °C (dec); hydrochloride mp 178–180 °C (dec); ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 9H, 3× CH_3), 1.45 (s, 9H, 3× CH_3), 3.50 (m, 4H, 2× CH_2), 3.38 (m, 2H, CH_2), 4.30 (t, J = 5.79 Hz, 2H, CH_2), 5.00 (m, 2H, 2× NH ex), 7.70 (t, J = 7.40 Hz, 1H, ar), 8.66 (m, 2H, ar), 9.18 (s, 1H, ar), 9.41 (m, 1H, NH ex).

6.14. 2-[2-(Dimethylamino)ethyl]-6-{[2-(dimethylamino)ethyl]amino}-5-amino-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (5k)

A mixture of 5-nitro derivative **5h** (0.2 g, 0.5 mmol), Pd/C (0.02 g, 5%), and aqueous HCl (1 mL of 37% w/w) in MeOH (30 mL) was stirred under hydrogen atmosphere (30 psi) for 1 h at room temperature. The reaction mixture was filtered and the filtrate evaporated to yield a residue which was crystallized (MeOH) to give **5k** 3HCl (40 mg, 42%): mp 246–247 °C (dec); ^{1}H NMR (DMSO-d_6) δ *2.85–2.95* (m, 12H, 4× CH_3), 3.37–3.53 (m, 4H, 2× CH_2), 3.73 (t, J = 5.48 Hz, 2H, CH_2), 4.38 (t, J = 5.71 Hz, 2H, CH_2), 6.85 (very br s, 5H, NH_3^++2× NH^+), 7.78 (t, J = 7.41 Hz, 1H, ar), 8.28–8.40 (m, 2H, ar), 8.72 (d, J = 6.98 Hz, 1H, ar), 10.03 (m, 1H, NH ex). Anal. Calcd. For $C_{20}H_{30}Cl_3N_5O_2$: C, 50.17; H, 6.31; N, 14.63. Found: C, 50.34; H, 6.11; N, 14.44.

6.15. 2-[3-(Dimethylamino)propyl]-6-{[3-(dimethylamino)propyl]amino}-5-amino-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (5l)

Compound **5l** 3HCl was prepared in the same experimental conditions from **5i**: yield 45%; mp 60–62 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ *1.95–2.20* (m, 4H, 2× CH_2), 2.76 (m, 12H, 4× CH_3), 3.09–3.21 (m, 4H, 2× CH_2), 3.45 (m, 2H, CH_2), 4.09 (t, J = 5.72 Hz, 2H, CH_2), 4.75 (very br s, 5H, NH_3^++2× NH^+), 7.74 (t, J = 7.39 Hz, 1H, ar), 8. 25 (s, 1H, ar), 8.31 (d, J = 6.44 Hz, 1H, ar), 8.60 (d, J = 7.83 Hz, 1H, ar), 10.26 (m, 1H, NH ex). Anal. Calcd. For $C_{22}H_{34}Cl_3N_5O_2$: C, 52.13; H, 6.76; N, 13.82. Found: C, 52.32; H, 6.55; N, 13.55.

6.16. 2-[2-(Amino)ethyl]-6-{[2-(amino)ethyl]amino}-5-nitro-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (5m)

A mixture of intermediate **5j** (80 mg, 0.15 mmol) in dioxane (10 mL) and aqueous HCl (0.5 mL of 37% w/w) was stirred at room temperature for 3 h. The reaction mixture was evaporated to yield a residue which was crystallized (EtOH) to give **5m** 2HCl (40 mg, 65%): mp 245–247 °C (dec); ^{1}H NMR (DMSO-d_6) δ *3.05–3.20* (m, 2H, CH_2); 3.28–3.44 (m, 2H, CH_2); 3.58–3.68 (m, 2H, CH_2), 4.28 (t, J = 5.76 Hz, 2H, CH_2), 7.89 (t, J = 7.61 Hz, 1H, ar), 8.02 (br s, 3H, NH_3^+ ex), 8.30 (br s, 3H, NH_3^+ ex), 8.60 (d, J = 7.27 Hz, 1H, ar), 8.75 (s, 1H, ar), 9.11 (m, 1H, NH ex), 9.22 (d, J = 7.58 Hz, 1H, ar). Anal. Calcd. For $C_{16}H_{19}Cl_2N_5O_4$: C, 46.17; H, 4.60; N, 16.82. Found: C, 46.02; H, 4.35; N, 16.65.

6.17. 1,7-Bis{6-chloro-1,3-dioxo-1*H*-benzo[*de*]isoquinoline-2(3*H*)-yl}-4-methyl-4-azaheptane (10)

N,N-Bis(3-aminopropyl)methylamine (0.18 mL, 1.1 mmol) was added to a suspension of 4-chloro-1,8-naphthalic anhydride (**4**, 0.5 g, 2.15 mmol) in toluene (20 mL). The mixture was refluxed for 1 h and water formed during the reaction was removed via a Dean–Stark separator. After cooling at room temperature, the mixture was partitioned between $CHCl_3$ (3× 20 mL) and aqueous 1 M Na_2CO_3 (20 mL). The organic layer was worked up to give a residue, which was flash chromatography on a silica gel column eluted with $CHCl_3$/CH_3OH (49:1 v/v) to obtain **10** (400 mg, 65%) enough pure to be used for the next step: mp 154–155 °C (dec); ^{1}H NMR ($CDCl_3$) δ *1.82–1.96* (m, 2H, 2× CH_2), 2.26 (s, 3H, CH_3), 2.52 (t, J = 5.75 Hz, 4H, 2× CH_2), 4.19 (t, J = 5.75 Hz, 4H, 2× CH_2), 7.71–7.89 (m, 4H, ar), 8.47 (d, J = 8.44 Hz, 2H, ar), 8.51–8.68 (m, 4H, ar).

6.18. 1,7-Bis{6-[(2-(dimethylamino)ethyl)amino]-1,3-dioxo-1*H*-benzo[*de*]isoquinoline-2(3*H*)-yl}-4-methyl-4-azaheptane (6a)

The compound **10** (100 mg, 0.17 mmol) in *N,N*-dimethylethylendiamine (1.5 mL) was refluxed for 1.5 h. After cooling at room temperature, the mixture was partitioned between $CHCl_3$ (3× 20 mL) and aqueous 1 M Na_2CO_3 (20 mL). The organic layer was worked up to give a residue, which was flash chromatography on a silica gel column eluted first with $CHCl_3$/MeOH (1:1 v/v) and then with $CHCl_3$/MeOH (1:1 v/v) containing 32% aqueous NH_3 (10 mL for 1 L of eluent) to obtain **6a** (40 mg, 35%): mp 110–113 °C (dec); hydrochloride mp 218–220 °C (dec); ^{1}H NMR ($CDCl_3$) δ *1.82–2.02* (m, 4H, 2× CH_2), 2.29 (s, 3H, CH_3), 2.34 (s, 12H, 4× CH_3), 2.55 (t, 4H, J = 6.01 Hz, 2× CH_2), 2.72 (t, J = 5.41 Hz, 4H, 2× CH_2), 3.37 (m, 4H, 2× CH_2), 4.19 (t, J = 6.01 Hz, 4H, 2× CH_2), 6.27 (m, 2H, 2× NH ex), 6.65 (d, J = 8.51 Hz, 2H, ar), 7.60 (t, J = 7.32 Hz, 2H, ar), 8.12 (d, J = 7.33 Hz, 2H, ar), 8.45 (d, J = 8.24 Hz, 2H, ar), 8.55 (d, J = 6.41 Hz, 2H, ar). Anal. Calcd. For $C_{39}H_{47}N_7O_4$: C, 69.10; H, 6.99; N, 14.46. Found: C, 69.23; H, 6.84; N, 14.55.

6.19. 1,7-Bis{6-[(3-(dimethylamino)propyl)amino]-1,3-dioxo-1*H*-benzo[*de*]isoquinoline-2(3*H*)-yl}-4-methyl-4-azaheptane (6b)

Compound **6b** was prepared in the same experimental conditions as **6a** from **10** and *N,N*-dimethyl-1,3-propylendiamine: yield 58%;

mp 139–140 °C (dec); hydrochloride mp 180–182 °C (dec); ^{1}H NMR ($CDCl_3$) δ *1.81–1.97* (m, 8H, 4× CH_2), 2.24 (s, 3H, CH_3), 2.38 (s, 12H, 4× CH_3), 2.50 (t, J = 5.99 Hz, 4H, 2× CH_2), 2.58 (t, J = 5.27 Hz, 4H, 2× CH_2), 3.45 (m, 4H, 2× CH_2), 4.16 (t, J = 6.00 Hz, 4H, 2× CH_2), 6.58 (d, J = 8.40 Hz, 2H, ar), 7.57 (t, J = 7.31 Hz, 2H, ar), 7.95 (d, J = 7.35 Hz, 2H, ar), 8.38 (d, J = 8.24 Hz, 2H, ar), 8.43 (m, 2H, 2× NH ex), 8.51 (d, J = 6.44 Hz, 2H, ar). Anal. Calcd. For $C_{41}H_{51}N_7O_4$: C, 69.76; H, 7.28; N, 13.89. Found: C, 69.55; H, 7.14; N, 14.06.

6.20. Fluorescence DNA-binding studies

The fluorometric assays have been described previously.[14] The C_{50} values for ethidium displacement from CT-DNA, were determined using aqueous buffer (10 mM Na_2HPO_4, 10 mM NaH_2PO_4, 1 mM EDTA, and pH 7.0) containing 1.26 μM ethidium bromide and 1 μM CT-DNA.[14–16]

All measurements were made in 10-mm quartz cuvettes at 20 °C using a Perkin-Elmer LS5 instrument (excitation at 546 nm; emission at 595 nm) following serial addition of aliquots of a stock drug solution (~5 mM in DMSO). The C_{50} values are defined as the drug concentrations which reduce the fluorescence of the DNA-bound ethidium by 50%, and are calculated as the mean from three determinations.

6.21. In vitro cytotoxicity assay

Establishment details of human colon adenocarcinoma cell line (HT29) used for cytotoxicity testing in vitro of target derivatives have been previously described.[17] Drug solutions of appropriate concentration were added to a culture containing HT29 cells at 2.5×10^4 cells/mL of medium and the drug exposure was protracted for 144 h. All assays were performed in triplicate, as previously described.[17]

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