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Novel chiral naphthalimide-cycloalkanediamine conjugates: Design, synthesis and antitumor activity

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ABSTRACT

A novel series of enantiopure naphthalimide-cycloalkanediamine conjugates were designed, synthetized and evaluated for *in vitro* cytotoxicity against human colon adenocarcinoma (LoVo), human lung adenocarcinoma (A549), human cervical carcinoma (Hela) and human promyelocytic leukemia cell lines (HL-60). The cytotoxicity of the compounds was highly dependent on size and relative stereochemistry of the cycloalkyl ring as well as length of the spacer. By contrast, any kind of enantioselection was observed for each pair of enantiomers. Flow cytometric analysis indicated that compounds **22** and **23** could effectively induce G_2/M arrest in the four previous cell lines despite a mild apoptotic effect.

1. Introduction

The 1,8-naphthalimide unit has spawned a broad family of DNA intercalating agents with several applications as DNA targeting binders, cellular imaging agents as fluorescent dyes and anticancer compounds [1a–e]. The leading compounds of this family, namely amonafide and mitonafide (**1**–**2**, Fig. 1) have demonstrated to inhibit topoisomerase II and exhibit high activity against a variety of tumor cells [2a,b]. Particularly, **1** was the first naphthalimide derivative tested in clinical studies and failed to enter phase III trials due to dose-limiting myelosuppression. **1** is metabolized in humans (in variable extent between individuals) by the enzyme *N*-acetyltransferase 2 (NAT2) to *N*-acetilamonafide, a metabolite with unpredictable toxicities [3]. Similarly, **2** suffered from severe toxicity issues, especially central nervous system (CNS) toxicity and it was given up in for clinical purposes. In spite of the limitations arising of the poor therapeutic index and side effects, the naphthalimide unit remains an attractive scaffold for novel therapeutic agents. Thus, a plethora of derivatives covering mono and bis 1,8-naphthalimides decorated with polyamines, [4] amino acids and peptides, [5] heterocycles, [6a,b] among others [7] have been reported in the last decade (Fig. 1). Some of these compounds, like UNBS5162 (**3**) or NNM-25 (**4**) displayed a distinct mechanism of action regarding **1** and **2**, notably inducing autophagy and senescence in cancer cells, and tackling previous toxicological hurdles [8]. However, and despite of the impressive number of existing analogues, only a few contain chiral units attached to the naphthalimide chromophore, mainly amino acids and a few polyamines [5,9] In this regard, chiral polyamines are molecules with a broad range of applications ranging from asymmetric catalysis to molecular recognition. Particularly, chiral cyclohexane-1,2-diamine and cyclopentane-1,2-diamine are among the most widely used diamines in modern chemistry [10]. These cyclic diamines have unique structural features for the induction of a chiral environment (chiral ligands) as well as for the development of new synthetic strategies, taking advantage of their geometrical preorganization. Herein we report a series of bioactive naphthalimide-cycloalkanediamine conjugates and the corresponding studies of antitumor activity against several tumor cells. Preliminary studies about mechanism of action and toxicity were also addressed.

2. Results and discussion

2.1. Chemistry and cytotoxicity studies

The knowledge gathered from previous structure–activity relationship (SAR) studies on naphthalimides **1** and **2** allowed to identify key structural features for the antitumor activity. Thus, the presence of a terminal amino group in the side chain and an alkylic spacer longer than two or three methylene units from the imide moiety turned out to be critical for the biological activity [1,11]. Bearing these facts in mind, a set of eight naphthalimide-cycloalkanediamine derivatives was designed with the focus on the diamine moiety. The conjugates **6**–**13**

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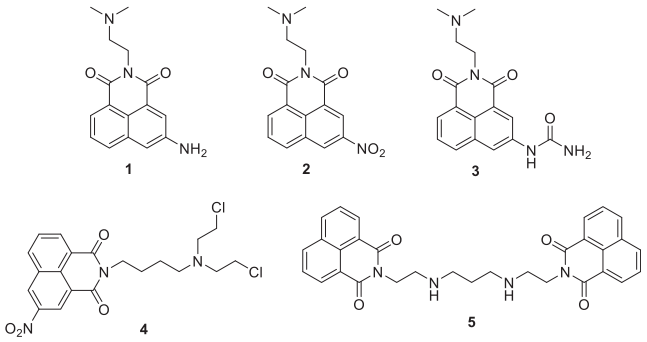
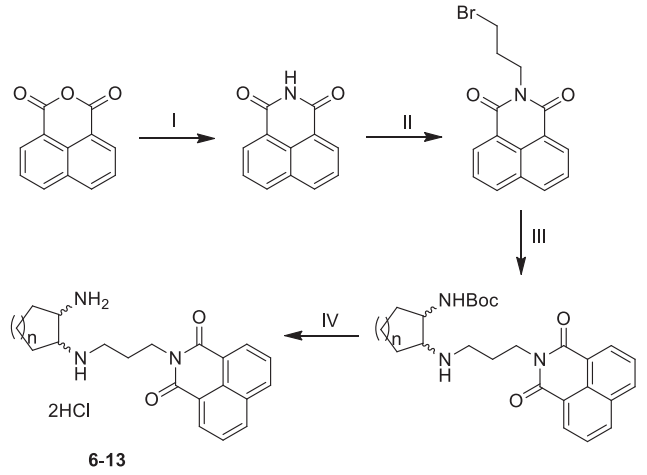


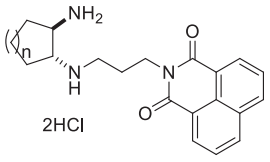
Fig. 1. Representative naphtalimide-based antitumor compounds.



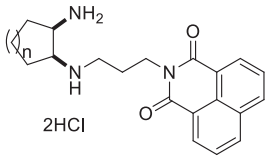
Scheme 1. Synthesis of naphthalimide-cycloalkanediamine conjugates **6**–**13**. (I) aq NH_3, ethanol, reflux, 12 h; (II) 1,3-dibromopropane, K_2CO_3, CH_3CN, 80 °C, 8 h; (III) *N*-Boc-monoprotected cycloalkane-1,2-diamine, K_2CO_3, CH_3CN, 80 °C, 8 h; (IV) 3 N aq HCl, reflux, 5 h.

were synthesized in a four-step sequence according to Scheme 1. The commercially available 1,8-naphthalic anhydride was reacted with aqueous ammonia in ethanol to afford 1,8-naphthalimide (step I). This intermediate was treated, without further purification, with 1,3-dibromopropane in the presence of potassium carbonate to reach *N*-(3-bromopropyl)-1,8-naphthalimide (step II). Further nucleophilic substitution on the bromo-derivative with various *N*-Boc-monoprotected cycloalkanediamines led to stable intermediates (step III) which provided, after *N*-Boc cleavage (step IV), the target **6**–**13** as dihydrochloride salts (50–60% yield). The structure of the resulting products was unambiguously confirmed by spectroscopic techniques (^1H, ^{13}C NMR, MS).

Table 1
Percentage growth inhibition (GI, %) of *in vitro* subpanel tumor cell lines at 10 μMconcentration of compounds **6**–**13**.[a]



(*R*,*R*)-**6**, n=1
(*S*,*S*)-**7**, n=1
(*R*,*R*)-**8**, n=2
(*S*,*S*)-**9**, n=2



(*R*,*S*)-**10**, n=1
(*S*,*R*)-**11**, n=1
(*R*,*S*)-**12**, n=2
(*S*,*R*)-**13**, n=2

Compound	GI (%) LoVo	HL-60	A-549	HeLa
6	**L**	23.0	**89.4**	75.2
7	**98.9**	13.7	**90.0**	72.7
8	39.9	5.3	68.9	25.6
9	25.1	6.7	62.4	16.8
10	1.4	13.8	39.2	5.0
11	9.6	4.4	33.7	8.6
12	15.2	16.3	50.3	14.1
13	25.8	10.7	53.4	11.2

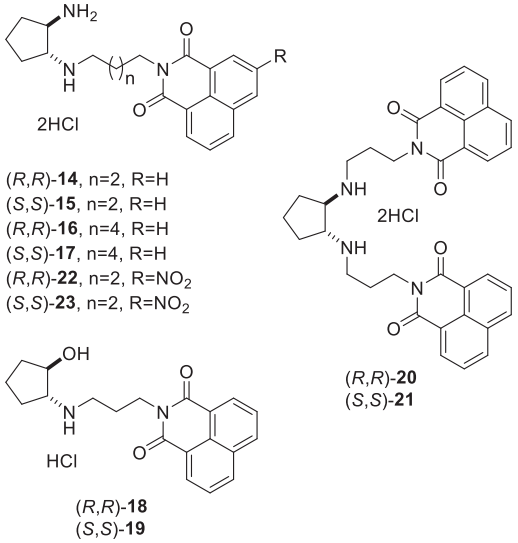
[a] Prominent GI values are bolded. L, compounds proved lethal to the cancer cell line.

As a first approach to assess the potential of these compounds, the *in vitro* antitumor activity against four cell lines was measured at 10 μM and their output reported as a mean graph of the percent growth of treated cells presented as percentage growth inhibition (GI %). As shown in Table 1, the pair of enantiomers (*R*,*R*)-**6** and (*S*,*S*)-**7** displayed significant growth inhibition, especially in colon (LoVo) and lung (A549) tumor cells meanwhile the cyclohexylic homologues (*R*,*R*)-**8** and (*S*,*S*)-**9** showed a more attenuated cytotoxicity against the four cell lines. Likewise, the *cis*-isomers **10**–**13** exhibited low potency and were significant less active than each *trans*-counterpart. In fact, only **12** and **13** reached 50% inhibition for the cell line A549. Accordingly, the SAR emerged from this study revealed that the combination of 1,8-naphthalimide and cycloalkane-1,2-diamine units was well tolerated for *in vitro* anticancer activity. In one hand, it was found that both stereochemistry and size of the cycloalkyl ring influence decisively, the *trans*-cyclopentyl moiety being the optimal for the activity. On the other hand, no significant differences were found between each pair of enantiomers.

Next, we focus the SAR study on the carbon spacer starting from the optimized core of **6** and **7**. Thus, we designed analogues increasing the chain length of the linker connecting the naphthalimide to cyclopentanediamine from three to four and six carbons (**14**–**17**, figure of Table 1). Besides, and following the methodology depicted in Scheme 1, 18 and 19 were prepared by replacing the previous cyclopentanediamine by an aminocyclopentanol unit. Finally, and based on the fact that some bisnaphthalimides like elinafide (**5**, Fig. 1) have demonstrated improved cytotoxicity and binding affinity to DNA compared to their mononaphthalimide counterparts, the dimeric analogues **20** and **21** bearing C_2 symmetry were also synthesized [1b,12,13]. The novel compounds were evaluated for *in vitro* antitumor activity against the previous cell lines. As can be concluded from Table 2, the pair of enantiomers **14** and **15** bearing an alkylic chain of 4 carbons was the most active of the series. Both proved to be lethal to colon cancer cell line LoVo and exhibited inhibition>80% for lung cancer cell line A549 and cervical cancer cell line HeLa, meanwhile leukemia cancer cells HL-60 showed again low sensitivity towards **14** and **15** (as observed above with compounds of Table 1). Conversely, the elongation of the linker with a 6 carbon-alkylic chain led to complete loss of activity (**16** and **17**). Similarly, the substitution of the primary amino group by a hydroxyl group (**18** and **19**) resulted in a drastic reduction of cytotoxicity. Regarding the dimeric compounds **20** and **21**, they were slightly less active than **14** and **15** against LoVo, A549 and HeLa, but **20** was interestingly the only compound reaching 50% inhibition against HL-60. Once identified **14** and **15** as the most active of the series, we designed two analogues, namely **22** and **23**, by introducing a nitro group in the 3-position of the chromophore moiety. For this, the synthetic route was identical to that depicted in Scheme 1. As expected according to previous reports, this functionalization led to an enhanced cytotoxicity against the four tumor cell lines.

The optimized nitro-derivatives **22** and **23** were further evaluated against the previous four tumor cell lines at a 5-log dose range. Mitonafide (**2**) activity, as a positive control, was also tested the cytotoxic experiments (Table 3). As can be seen in Table 3, 22 and 23 exhibited IC_{50} values in the μM range, being slightly higher to those reported for **2** (0.4 μM, 2.4 μM and 1.6 μM respectively, against LoVo, A549 and HL-60 cell lines) [14] Moreover, it is worth noting that there was not any kind of enantioselection, values being almost identical for both enantiomers. This fact was common to all the enantiomeric pairs tested throughout this study, in sharp contrast to a previous report of naphthalimide

Table 2
Percentage growth inhibition (GI, %) of *in vitro* subpanel tumor cell lines at 10 μM concentration of compounds **14**–**23**.[a]



Compound	GI (%)			
	LoVo	HL-60	A-549	HeLa
14	**L**	26.9	**93.3**	**80.4**
15	**L**	37.9	**99.4**	**84.3**
16	0.0	0.0	0.0	0.0
17	0.0	0.0	0.0	1.5
18	7.3	2.1	32.0	10.4
19	1.3	0.0	6.7	0.0
20	**99.0**	56.1	**86.3**	64.4
21	**94.6**	31.5	**79.9**	56.1
22	**L**	**90.4**	**L**	**99.3**
23	**L**	**93.6**	**L**	**82.6**

[a] Prominent GI values are bolded. L, compounds proved lethal to the cancer cell line.

Table 3
Antitumor activity of naphthalimide analogues **22** and **23**.

Compound	*In Vitro Citotoxicity* IC_{50}[a] (μM)			
	LoVo	HL-60	A-549	HeLa
22	4.53 ± 0.26	7.03 ± 0.54	3.49 ± 0.29	4.17 ± 0.45
23	4.46 ± 0.28	7.43 ± 0.35	3.34 ± 0.44	4.26 ± 0.53
2	1.30	1.02	0.78	0.97

[a] Dose of compound required to inhibit cell growth by 50% compared to untreated cell controls. Values are derived from IC_{50} graphs. All experiments were done in heptuplicate wells and each experiment was repeated twice except for mitonafide value.

intercalators bearing chiral pyrrolidines, which exhibited notably differences of cytotoxicity, DNA binding and photodamage [9]. The absence of enantioselection unveiled herein would agree with the known fact that helical grooves of DNA are the most pronounced chiral-inducing region meanwhile that between the achiral DNA bases is less susceptible to enantiorecognition [15].

2.2. Cell cycle analysis

In the search for naphthalimides free of unpredictable toxicity risks, novel derivatives such as **3** or **4** have been introduced, which exert distinct mechanisms of action [8,16]. To gain further knowledge on the mechanism of action of the novel analogues reported herein, the effect of **22** and **23** on the cell cycle was investigated by fluorescence activated cell sorting (FACS). LoVo, HL-60, A-549 and HeLa cell lines were used in the assay. Cells were treated with **22** and **23** and after 48 h were fixed and labelled with propidium iodide. Results collected in Fig. 2 together with the untreated control as well as the control treated with **2**, revealed that **22** and **23** induce significant cell cycle perturbation in all cell lines. 1) Preliminary apoptosis studies were performed at 5 μM for compounds **22** and **23** with no results of dead cell induction. Therefore, we decided to increase the drug concentration at 10 μM. The exposure of the four types of cells to 10 μmol of both compounds was associated with an almost disappearance of G_1 and S peaks and a very high increase in G_2/M fraction. In particular, the G_2/M population enhanced from 10 to 15% to 70–95% versus the untreated control in HL-60, LoVo and A-549 cells. Regarding HeLa cells, the G_2/M fraction significantly increased considering the diploid fraction (red histogram), but also the induction of aneuploids (yellow histogram) has been taken into account, especially the generation of octaploid cells. This fact was not observed in the other three cell lines. Moreover, the profile of cell cycle displayed by **22** and **23** was quite different to that observed with **2** which produced only a slight change with regard to the untreated control. In fact, the effect of **22** and **23** was very similar to that observed with **1** with HCT116 cells, which have been determined to induce G_2 arrest through inhibiting DNA topoisomerase II accompanied by Chk1 degradation [17]. Compounds **22** and **23** may display such a mechanism of action.

2.3. Apoptosis studies

Induction of apoptosis or programmed cell death is a common mechanistic pathway of several antitumor agents [18]. To further find out about how these drugs display their antitumor activity, FACS analysis was carried out after double staining cells with propidium iodide and annexin V-FITC. As shown in Fig. 3, 22 and 23 induced, in general, a very slight apoptotic effect in the cell lines tested; Treatment of HL-60 and A-549 cells by 10 μM for 48 h resulted in a negligible effect respect to the untreated control. Regarding LoVo cells, treatment with **22** and **23** resulted in 31% and 27% of apoptotic cells, respectively, as compared to 16% in the untreated control. Finally, the induction in HeLa cells was more pronounced, particularly with **23** which increased the percent of apoptotic cells from 12% to 52%. In consequence, the target compounds induced a very slight apoptotic effect in comparison with **2** (77% and 89% of apoptotic cells in HL-60 and HeLa cells). Similar results were obtained when compound **22** and **2** were evaluated on PBMC (peripheral blood mononuclear cell), as a normal cell model (see details in the Supplementary Information).

2.4. DNA photocleavage studies

A substantial number of naphthalimides are well known DNA photocleavers [19]. Consequently, the ability of **22** and **23** to promote this effect was also studied, with amonafide (**1**) and mitonafide (**2**) as controls. Under non irradiation conditions, none of the compounds promoted DNA strand breaking, detecting just plasmid form I (Fig. 4a). However, after 2 h irradiation, all compounds except **1** could cleave the closed plasmid DNA into relaxed, open circular form (Form II) in a dose depending manner. There were no differences between isomers **22** and **23**, being **2** the most efficient one (Fig. 4b).

2.5. Maximum tolerated dose (MTD)

The limiting in vivo toxicity of the new naphthalimides was considered and the MTD for compound **23** conveniently established. Previous studies reported that the maximum tolerated dose for leading naphthalimides was around 40 mg/kg [20]. Accordingly 40 mg/kg was the starting dose for IP injection in mice. After such administration not significant side effects were observed. However, dose escalation to 80 mg/kg resulted in clinical signs of toxicity with loss of body weight and reduced mobility. Two deaths were reported in the days after administration. Therefore, 40 mg/kg was established as the maximum

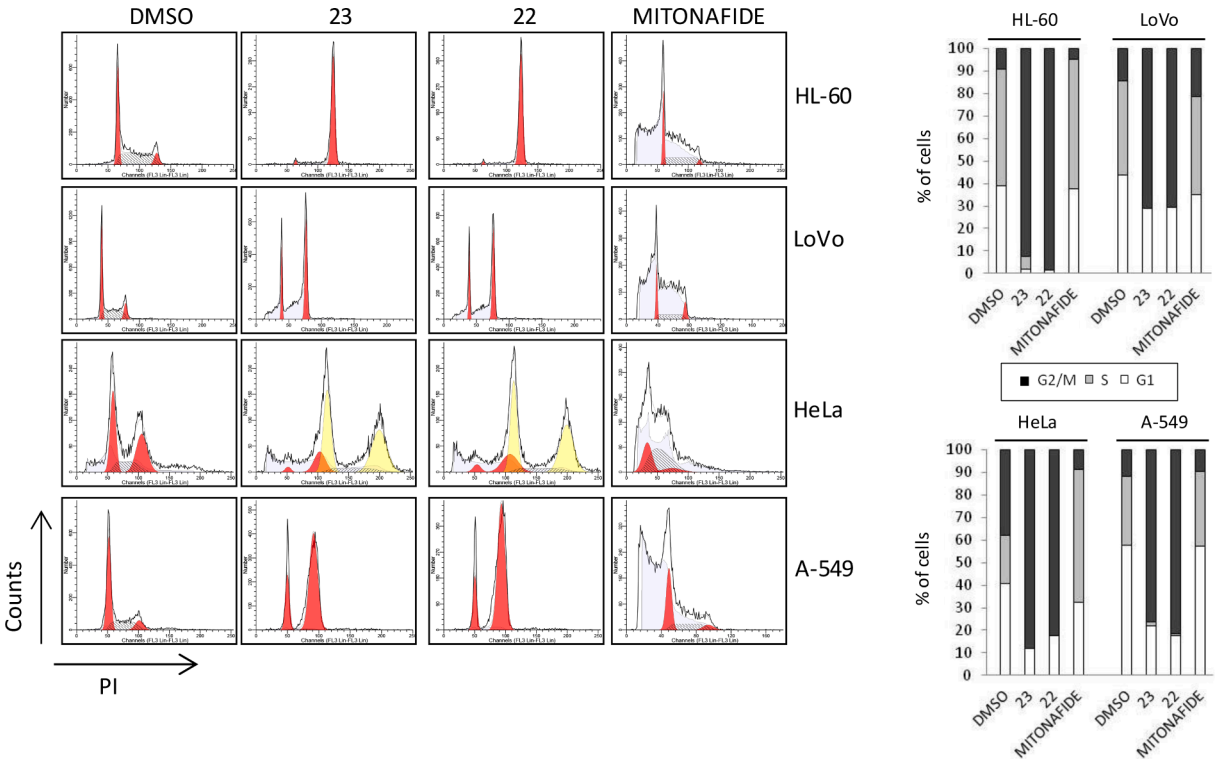


Fig. 2. Inhibition of cell cycle in HL-60, LoVo, HeLa and A-549 cells treated with **22**, **23** and mitonafide (**2**) after 48 h incubation at 10 μmol × L^{-1}.

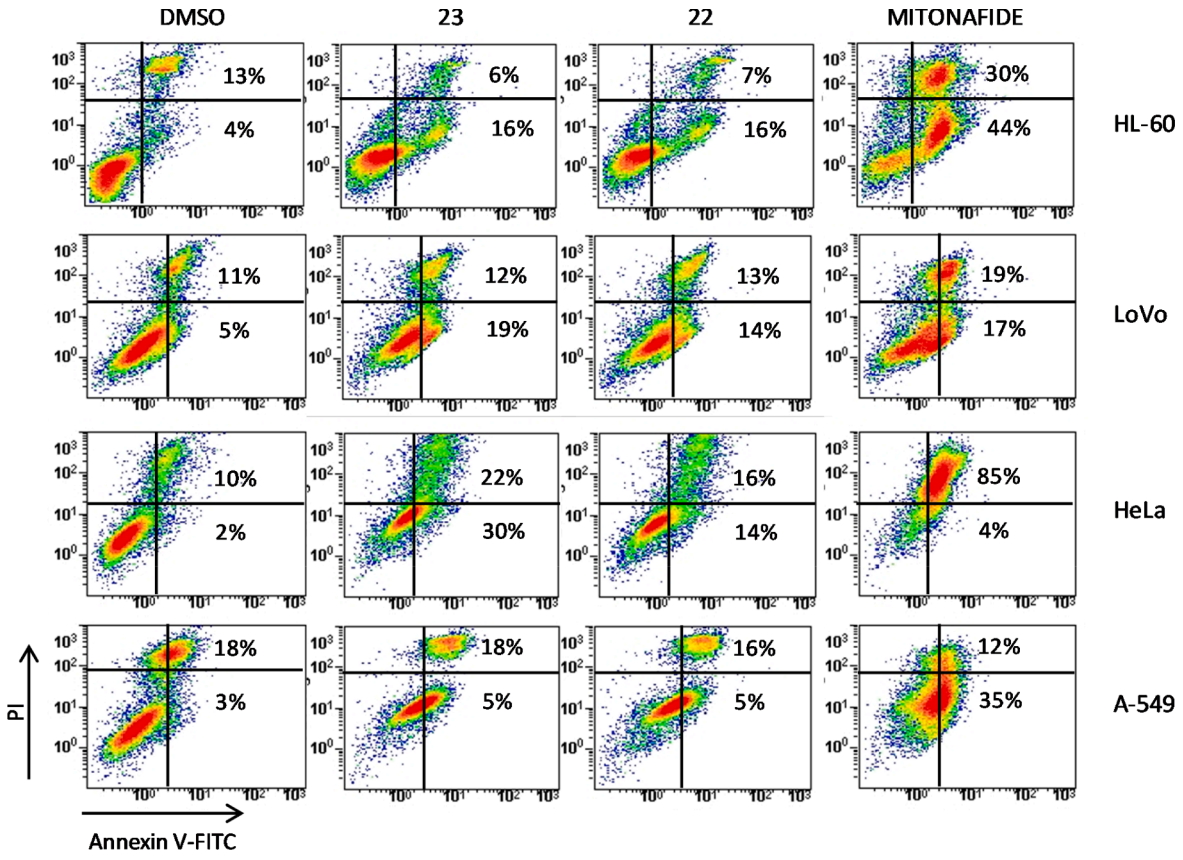


Fig. 3. Apoptosis studies of HL-60, LoVo, HeLa and A-549 cells treated with **22**, **23** and mitonafide (**2**) after 48 h incubation at 10 μmol × L^{-1}.

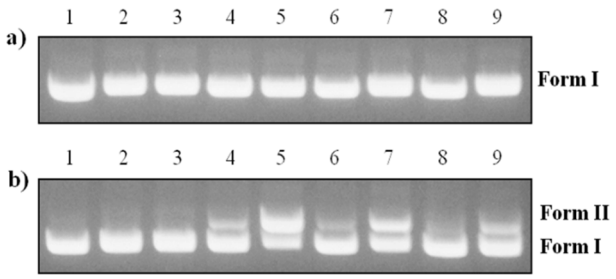


Fig. 4. DNA photocleavage of close pUC18 plasmid by different compounds under (a) no irradiaton or (b) 2 h irradiation. Lane 1: DNA alone; lane 2 and 3: DNA and amonafide (**1**) at 5 μM and 10 μM respectively; lane 4 and 5: DNA and mitonafide (**2**) at 5 μM and 10 μM, respectively; lane 6 and 7: DNA and compound **23** at 5 μM and 10 μM, respectively; lane 8 and 9: DNA and compound **22** at 5 μM and 10 μM, respectively.

tolerated dose for this new compound.

3. Conclusion

A series of chiral naphthalimide-cycloalkanediamine conjugates were designed and tested again four cancer cell lines *in vitro*. The size and relative stereochemistry of the cycloalkylic ring as well as the spacer turned out to be critical for the activity of the conjugates. Each pair of enantiomers did not show differences of cytotoxicity and the pair **22**–**23** exhibited the highest activity against all four tested cells, with values comparable to that measured with mitonafide. More interestingly, **22** and **23** induced a significant delay in exit from G_2/M, the final phase of cell cycle, together with a very mild apoptotic effect. These facts could be symptomatic of a predominant role of cytostasis over cytotoxicity, at least at the concentration fixed in the experiments.

4. Experimental protocols

All chemicals (reagent grade) used were commercially available. The set of enantiopure diamines and amino alcohols was available from the EntreChem S.L. catalogue. Thin-layer was performed on precoated TLC plates of Merck silica gel $60F_{254}$, using potassium permanganate as developing reagent. For column chromatography, Merck silica gel 60 (particle size, 40–63 μm) was used. Optical rotations were measured using a Perkin-Elmer 343 polarimeter. APCI-MS experiments were carried out to record mass spectra on a Hewlett-Packard 1100 HPLC/MS instrument. High-resolution mass spectra (HRMS) were recorded in ESI^+ mode. In both cases, M refers to the free molecule, without HCl. ^{1}H NMR and proton-decoupled ^{13}C NMR spectra (DMSO-d_6) were obtained using AV-300 or DPX-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz) spectrometers using the δ scale (ppm).

4.1. Synthesis of naphthalimide derivatives

4.1.1. General procedure for the synthesis of 6–17

Step I: a suspension of 1,8-naphthalic anhydride (10 mmol) in ethanol (20 mL) and aqueous ammonia (25 mL) was heated at 70 °C in a sealed tube overnight. After this time, the mixture was cooled, filtered, and the resulting solid washed with hexane to afford pure 1,8-naphthalimide in quantitative yield.

Step II: to a solution of 1,8-naphthalimide (1 mmol) in dry acetonitrile (25 mL), anhydrous potassium carbonate (8 mmol) and the corresponding dibromoalkane (1.5 mmol) were added and the mixture was refluxed for 8 h. After this time, potassium carbonate was removed by filtration and the solvent was evaporated under reduced pressure. The resulting solid was washed several times with hexane and filtered under vacuum to yield the crude of the corresponding *N*-bromoalkylnaphthalimide. This product was employed in the next step without further purification.

Step III: to a solution of the previous *N*-bromoalkylnaphthalimide in dry acetonitrile (25 mL) was added the corresponding *N*-Boc-monoprotected cycloalkanediamine (0.6 mmol) and anhydrous potassium carbonate (6 mmol) and the mixture refluxed for 8 h. Then, potassium carbonate was removed by filtration and the solvent evaporated under reduced pressure. The residue was subjected to flash chromatography (ethyl acetate–methanol mixtures) to obtain the corresponding Boc-protected naphthalimidecycloalkanediamine conjugate.

Step IV: the previous compound was refluxed in a mixture of aqueous HCl (20 mL, 3 M) and ethanol (2 mL) during 5 h. The resulting solution was washed twice with *tert*-butyl methyl ether (2 × 15 mL) and the aqueous phase concentrated to dryness to give a white solid. Further crystallization from methanol: *tert*-butyl methyl ether provided pure compounds as dihydrochloride salts (**6**–**17**).

*4.1.1.1. 2-{3-{[(1R,2R)-2-Aminocyclopentyl]amino}propyl}-1H-benzo[de]isoquinoline-1,3(2H)-dione dihydrochloride (**6**).* Global yield: 50%; $[\alpha]^{20}_D$ –15.0 (*c* 0.10, MeOH) > 99% *ee*; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.74–1.90 (m, 4H), 2.05–2.12 (m, 4H). 3.06 (m, 4H), 3.57 (m, 1H), 3.76 (br s, 1H), 4.11 (t, 2H, ^3J = 6.6 Hz), 7.83 (t, 2H, ^3J = 7.8 Hz), 8.40–8.45 (m, 4H), 8.66 (br s, NH), 9.68 (br s, NH); ^{13}C NMR (75.5 MHz) δ 22.3 (CH_2), 25.3 (CH_2), 28.2 (CH_2), 30.1 (CH_2), 37.6 (CH_2), 44.5 (CH_2), 54.1 (CH), 62.0 (CH), 122.4 (2 × C), 127.6 (2 × CH), 127.8 (C), 131.2 (2 × CH_2), 131.7 (C), 134.8 (2 × CH), 164.0 (2 × C═O); MS (APCI) *m/z* (rel. intensity): 338.1 [(M + H)$^+$, 100]; HRMS (ESI^+, *m/z*) calcd. for $(C_{20}H_{24}N_3O_2)^+$ [(M + H)$^+$] 338.1863; found 338.1877

*4.1.1.2. 2-{3-{[(1S,2S)-2-Aminocyclopentyl]amino}propyl}-1H-benzo[de]isoquinoline-1,3(2H)-dione dihydrochloride (**7**).* Global yield: 50%; $[\alpha]^{20}_D$ + 19.0 (*c* 0.10, MeOH) > 99% *ee*; MS (APCI) *m/z* (rel. intensity): 338.1 [(M + H)$^+$, 100]; HRMS (ESI^+, *m/z*) calcd. for $(C_{20}H_{24}N_3O_2)^+$ [(M + H)$^+$] 338.1863; found 338.1886

*4.1.1.3. 2-{3-{[(1R,2R)-2-Aminocyclohexyl]amino}propyl}-1H-benzo[de]isoquinoline-1,3(2H)-dione dihydrochloride (**8**).* Global yield: 60%; $[\alpha]^{20}_D$ –8.5 (*c* 0.10, MeOH) > 99% *ee*; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.05–1.30 (m, 2H), 1.40–1.80 (m, 4H). 2.00–2.30 (m, 4H), 3.01 (m, 1H), 3.10–3.40 (br s, 3H), 4.10 (br s, 2H), 7.82 (t, 2H, ^3J = 7.8 Hz), 8.40–8.44 (m, 4H), 8.79 (br s, NH), 9.46 (br s, NH); ^{13}C NMR (75.5 MHz) δ 23.0 (CH_2), 23.2 (CH_2), 25.2 (CH_2), 26.4 (CH_2), 29.6 (CH_2), 37.6 (CH_2), 43.2 (CH_2), 50.8 (CH), 58.3 (CH), 122.4 (2 × C), 127.6 (2 × CH), 127.8 (C), 131.2 (2 × CH_2), 131.6 (C), 134.8 (2 × CH), 164.1 (2 × C═O); MS (APCI) *m/z* (rel. intensity): 352.1 [(M + H)$^+$, 100]; HRMS (ESI^+, *m/z*) calcd. for $(C_{21}H_{26}N_3O_2)^+$ [(M + H)$^+$] 352.2020; found 352.2012

*4.1.1.4. 2-{3-{[(1S,2S)-2-Aminocyclohexyl]amino}propyl}-1H-benzo[de]isoquinoline-1,3(2H)-dione dihydrochloride (**9**).* Global yield: 55%; $[\alpha]^{20}_D$ + 9.5 (*c* 0.10, MeOH) > 99% *ee*; MS (APCI) *m/z* (rel. intensity): 352.1 [(M + H)$^+$, 90]; HRMS (ESI^+, *m/z*) calcd. for $(C_{21}H_{26}N_3O_2)^+$ [(M + H)$^+$] 352.2020; found 352.2036

*4.1.1.5. 2-{3-{[(1S,2R)-2-Aminocyclopentyl]amino}propyl}-1H-benzo[de]isoquinoline-1,3(2H)-dione dihydrochloride (**10**).* Global yield: 55%; $[\alpha]^{20}_D$ –9.0 (*c* 0.10, MeOH) > 99% *ee*; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.56 (m, 1H), 1.70–2.30 (m, 7H). 3.13 (m, 2H), 3.60 (m, 1H), 3.77 (m, 1H), 4.11 (br s, 2H), 7.83 (t, 2H, ^3J = 7.5 Hz), 8.40–8.44 (m, 4H), 8.50–10.05 (br s, NH); ^{13}C NMR (75.5 MHz) δ 19.7 (CH_2), 25.3 (CH_2), 25.6 (CH_2), 28.5 (CH_2), 37.6 (CH_2), 45.2 (CH_2), 51.7 (CH), 59.7 (CH), 122.4 (2 × C), 127.6 (2 × CH), 127.8 (C), 131.2 (2 × CH_2), 131.7 (C), 134.8 (2 × CH), 164.1 (2 × C═O); MS (APCI) *m/z* (rel. intensity): 338.1 [(M + H)$^+$, 100]; HRMS (ESI^+, *m/z*) calcd. for $(C_{20}H_{24}N_3O_2)^+$ [(M + H)$^+$] 338.1863; found 338.1850

*4.1.1.6. 2-{3-{[(1R,2S)-2-Aminocyclopentyl]amino}propyl}-1H-benzo[de]isoquinoline-1,3(2H)-dione dihydrochloride (**11**).* Global yield: 57%; $[\alpha]^{20}_D$ + 11.3 (*c* 0.10, MeOH) > 99% *ee*; MS (APCI) *m/z* (rel. intensity): 338.1 [(M + H)$^+$, 100]; HRMS (ESI$^+$, *m/z*) calcd. for $(C_{20}H_{24}N_3O_2)^+$ [(M + H)$^+$] 338.1863; found 338.1859

*4.1.1.7. 2-{3-{[(1S,2R)-2-Aminocyclohexyl]amino}propyl}-1H-benzo[de]isoquinoline-1,3(2H)-dione dihydrochloride (**12**).* Global yield: 50%; $[\alpha]^{20}_D$ –7.7 (*c* 0.10, MeOH) > 99% *ee*; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.20–1.40 (m, 2H). 1.50–2.05 (m, 6H), 2.16 (t, 2H, ^3J = 6.9 Hz), 3.12 (m, 2H), 3.40 (m, 1H, overlapped by solvent), 3.80 (br s, 1H), 4.11 (t, 2H, ^3J = 6.0 Hz), 7.83 (t, 2H, ^3J = 7.5 Hz), 8.40–8.45 (m, 4H), 8.70 (br s, NH), 9.40 (br s, NH); ^{13}C NMR (75.5 MHz) δ 18.8 (CH_2), 23.2 (2 × CH_2), 25.1 (CH_2), 27.4 (CH_2), 37.6 (CH_2), 44.1 (CH_2), 48.0 (CH), 57.9 (CH), 122.4 (2 × C), 127.7 (2 × CH), 127.8 (C), 131.3 (2 × CH_2), 131.7 (C), 134.9 (2 × CH), 164.1 (2 × C═O); MS (APCI) *m/z* (rel. intensity): 352.1 [(M + H)$^+$, 100]; HRMS (ESI$^+$, *m/z*) calcd. for $(C_{21}H_{26}N_3O_2)^+$ [(M + H)$^+$] 352.2020; found 352.2044

*4.1.1.8. 2-{3-{[(1R,2S)-2-Aminocyclohexyl]amino}propyl}-1H-benzo[de]isoquinoline-1,3(2H)-dione dihydrochloride (**13**)..* Global yield: 52%; $[\alpha]^{20}_D$ + 11.0 (*c* 0.10, MeOH) > 99% *ee*; MS (APCI) *m/z* (rel. intensity): 352.1 [(M + H)$^+$, 100]; HRMS (ESI$^+$, *m/z*) calcd. for $(C_{21}H_{26}N_3O_2)^+$ [(M + H)$^+$] 352.2020; found 352.2036

*4.1.1.9. 2-{4-{[(1R,2R)-2-Aminocyclopentyl]amino}butyl}-1H-benzo[de]isoquinoline-1,3(2H)-dione dihydrochloride (**14**).* Global yield: 56%; $[\alpha]^{20}_D$ –20.7 (*c* 0.10, MeOH) > 99% *ee*; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.65–1.90 (m, 8H), 2.05–2.20 (m, 2H). 3.02 (m, 2H), 3.60 (m, 1H), 3.75 (m, 1H), 4.05 (br s, 2H), 7.85 (t, 2H, ^3J = 7.8 Hz), 8.30–8.45 (m, 4H), 8.65 (br s, NH), 9.70 (br s, NH); ^{13}C NMR (75.5 MHz) δ 22.4 (CH_2), 24.0 (CH_2), 25.3 (CH_2), 28.3 (CH_2), 30.2 (CH_2), 39.5 (CH_2), 46.1 (CH_2), 54.0 (CH), 62.0 (CH), 122.3 (2 × C), 127.6 (2 × CH), 127.6 (C), 131.2 (2 × CH_2), 131.7 (C), 134.8 (2 × CH), 163.8 (2 × C═O); MS (APCI) *m/z* (rel. intensity): 352.2 [(M + H)$^+$, 100]; HRMS (ESI$^+$, *m/z*) calcd. for $(C_{21}H_{26}N_3O_2)^+$ [(M + H)$^+$] 352.2020; found 352.2028

*4.1.1.10. 2-{4-{[(1S,2S)-2-Aminocyclopentyl]amino}butyl}-1H-benzo[de]isoquinoline-1,3(2H)-dione dihydrochloride (**15**).* Global yield: 60%; $[\alpha]^{20}_D$ + 24.7 (*c* 0.10, MeOH) > 99% *ee*; MS (APCI) *m/z* (rel. intensity): 352.1 [(M + H)$^+$, 100]; HRMS (ESI$^+$, *m/z*) calcd. for $(C_{21}H_{26}N_3O_2)^+$ [(M + H)$^+$] 352.2020; found 352.2041

*4.1.1.11. 2-{3-{[(1R,2R)-2-Aminocyclopentyl]amino}hexyl}-1H-benzo[de]isoquinoline-1,3(2H)-dione dihydrochloride (**16**).* Global yield: 48%; $[\alpha]^{20}_D$ –8.5 (*c* 0.10, MeOH) > 99% *ee*; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.50–1.90 (m, 12H), 2.05–2.15 (m, 2H). 2.95 (m, 2H), 3.65 (m, 1H), 3.75 (m, 1H), 4.10 (br s, 2H), 7.70 (t, 2H, ^3J = 7.6 Hz), 8.35–8.45 (m, 4H), 8.70 (br s, NH), 9.50 (br s, NH); ^{13}C NMR (75.5 MHz) δ 19.5 (CH_2), 21.0 (CH_2), 22.4 (CH_2), 24.0 (CH_2), 25.3 (CH_2), 28.3 (CH_2), 30.2 (CH_2), 39.5 (CH_2), 46.1 (CH_2), 53.7 (CH), 61.5 (CH), 122.3 (2 × C), 127.6 (2 × CH), 127.6 (C), 131.2 (2 × CH_2), 131.7 (C), 134.8 (2 × CH), 163.8 (2 × C═O); MS (APCI) *m/z* (rel. intensity): 380.2 [(M + H)$^+$, 35]; HRMS (ESI$^+$, *m/z*) calcd. for $(C_{23}H_{30}N_3O_2)^+$ [(M + H)$^+$] 380.2333; found 380.2350

*4.1.1.12. 2-{3-{[(1S,2S)-2-Aminocyclopentyl]amino}hexyl}-1H-benzo[de]isoquinoline-1,3(2H)-dione dihydrochloride (**17**)..* Global yield: 50%; $[\alpha]^{20}_D$ + 13.6 (*c* 0.10, MeOH) > 99% *ee*; MS (APCI) *m/z* (rel. intensity): 380.2 [(M + H)$^+$, 40]; HRMS (ESI$^+$, *m/z*) calcd. for $(C_{23}H_{30}N_3O_2)^+$ [(M + H)$^+$] 380.2333; found 380.2338

*4.1.2. General procedure for the synthesis of **18**–**19***

To a solution of (*R*,*R*)- or (*S*,*S*)-*trans*-2-aminocyclopentanol (1 mmol) in dry acetonitrile (25 mL), anhydrous potassium carbonate (8 mmol) and *N*-(3-bromopropylnaphthalimide (1.5 mmol) were added and the mixture was refluxed for 8 h. After this time, potassium carbonate was removed by filtration and the solvent was evaporated under reduced pressure. Next, the residue was subjected to flash chromatography (ethyl acetate–methanol mixtures). The purified compound was stirred with aqueous HCl (10 mL, 3 M) during 30 min and concentrated to dryness to yield a white solid. Further crystallization from methanol: *tert*-butyl methyl ether provided hydrochloride salts **18** and **19**, respectively.

*4.1.2.1. 2-{3-{[(1R,2R)-2-Hydroxycyclopentyl]amino}propyl}-1H-benzo[de]isoquinoline-1,3(2H)-dione hydrochloride (**18**).* Global yield: 30%; $[\alpha]^{20}_D$ –10.7 (*c* 0.10, MeOH) > 99%; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.70–1.90 (m, 4H), 2.05–2.10 (m, 4H). 3.00 (m, 4H), 3.60 (m, 1H), 3.90 (br s, 1H), 4.15 (t, 2H, ^3J = 6.8 Hz), 4.40 (br s, 1H, OH), 7.80 (t, 2H, ^3J = 7.5 Hz), 8.40–8.55 (m, 4H), 9.60 (br s, NH); ^{13}C NMR (75.5 MHz) δ 21.5 (CH_2), 25.0 (CH_2), 28.2 (CH_2), 30.5 (CH_2), 37.7 (CH_2), 44.5 (CH_2), 54.3 (CH), 73.1 (CH), 122.4 (2 × C), 127.5 (2 × CH), 127.8 (C), 131.0 (2 × CH_2), 131.6 (C), 134.8 (2 × CH), 164.0 (2 × C═O); MS (APCI) *m/z* (rel. intensity): 339.1 [(M + H)$^+$, 100]; HRMS (ESI$^+$, *m/z*) calcd. for $(C_{23}H_{30}N_3O_2)^+$ [(M + H)$^+$] 339.1703; found 339.1725

*4.1.2.2. 2-{3-{[(1S,2S)-2-Hydroxycyclopentyl]amino}propyl}-1H-benzo[de]isoquinoline-1,3(2H)-dione hydrochloride (**19**).* Global yield: 32%; $[\alpha]^{20}_D$ + 8.7 (*c* 0.10, MeOH) > 99%; MS (APCI) *m/z* (rel. intensity): 339.1 [(M + H)$^+$, 100]; HRMS (ESI$^+$, *m/z*) calcd. for $(C_{23}H_{30}N_3O_2)^+$ [(M + H)$^+$] 339.1703; found 339.1740

*4.1.3. Procedure for the synthesis of **20**–**21***

To a solution of **6** or **7** (1 mmol) in dry acetonitrile (25 mL), anhydrous potassium carbonate (8 mmol) and *N*-(3-bromopropylnaphthalimide (1.5 mmol) were added and the mixture was refluxed for 8 h. After this time, potassium carbonate was removed by filtration and the solvent was evaporated under reduced pressure. Next, the residue was subjected to flash chromatography (ethyl acetate–methanol mixtures). The purified compound was stirred with aqueous HCl (10 mL, 3 M) during 30 min and concentrated to dryness to yield a white solid. Further crystallization from methanol: *tert*-butyl methyl ether provided dihydrochloride salts **20** and **21** respectively.

*4.1.3.1. 2,2′-{[(1R,2R)-2-Cyclopentane-1,2-diylbis(azanediyl)]bis(propane-3,1-diyl)]}-bis(1H-benzo[de]isoquinoline-1,3(2H)-dione dihydrochloride (**20**).* Global yield: 40%; $[\alpha]^{20}_D$ + 31.0 (*c* 0.10, MeOH) > 99% *ee*; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 1.80 (m, 2H), 1.95 (m, 2H), 2.05–2.15 (m, 6H). 3.04 (m, 2H), 3.12 (m, 2H), 3.78 (br s, 2H), 4.15 (t, 4H, ^3J = 6.6 Hz), 7.83 (t, 4H, ^3J = 7.2 Hz), 8.44 (d, 4H, ^3J = 7.2 Hz), 8.51 (d, 4H, ^3J = 7.2 Hz), 9.70 (br s, NH), 10.02 (br s, NH); ^{13}C NMR (150 MHz) δ 22.7 (CH_2), 25.4 (2 × CH_2), 28.1 (2 × CH_2), 37.8 (2 × CH_2), 44.5 (2 × CH_2), 61.3 (2 × CH), 122.6 (4 × C), 127.6 (4 × CH), 128.0 (2 × C), 131.2 (4 × CH_2), 131.8 (2 × C), 134.8 (4 × CH), 164.1 (4 × C═O); MS (APCI) *m/z* (rel. intensity): 575.2 [(M + H)$^+$, 70]; HRMS (ESI$^+$, *m/z*) calcd. for $(C_{35}H_{35}N_4O_4)^+$ [(M + H)$^+$] 575.2653; found 575.2675

4.1.3.2. 2,2′-{[(1S,2S)-2-Cyclopentane-1,2-diylbis(azanediyl)]bis(propane-3,1-diyl)]}-bis(1H-benzo[de]isoquinoline-1,3(2H)-dione dihydrochloride (21). Global yield: 35%; $[\alpha]^{20}_D$ –26.7 (*c* 0.10, MeOH) > 99% *ee*; MS (APCI) *m/z* (rel. intensity): 575.2 [(M + H)$^+$, 40]; HRMS (ESI$^+$, *m/z*) calcd. for $(C_{35}H_{35}N_4O_4)^+$ [(M + H)$^+$] 575.2653; found 575.2659

*4.1.4. Procedure for the synthesis of **22**–**23***

The synthesis of compounds **22** and **23** was accomplished following an identical procedure to that described in the section 4.1.1 for compounds **6**–**17,** but using 3-nitro-1,8-naphthalic anhydride as precursor. Thus, the pair of enantiomers **22**–**23** was isolated as dihydrochloride salts.

*4.1.4.1. 2-{4-{[(1R,2R)-2-Aminocyclopentyl]amino}butyl}-5-nitro-1H-benzo[de]isoquinoline-1,3(2H)-dione dihydrochloride (**22**).* Global yield: 42%; $[\alpha]^{20}_D$ –19.3 (*c* 0.10, MeOH) > 99% *ee*; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.60–1.90 (m, 8H), 2.05–2.20 (m, 2H). 3.00 (m, 2H), 3.55 (m, 1H), 3.70 (m, 1H), 4.10 (br s, 2H), 8.03 (t, 1H, $^3J = 7.6$ Hz), 8.41 (d, 1H, $^3J = 7.5$ Hz), 8.70 (d, 1H, $^3J = 7.5$ Hz), 8.85 (d, 1H, $^4J = 1.6$ Hz), 9.41 (d, 1H, $^4J = 1.5$ Hz), 9.65 (br s, NH), 9.80 (br s, NH); ^{13}C NMR (75.5 MHz) δ 21.8 (CH_2), 23.6 (CH_2), 25.0 (CH_2), 28.1 (CH_2), 29.6 (CH_2), 40.0 (CH_2), 46.2 (CH_2), 54.9 (CH), 62.0 (CH), 122.1 (C), 123.2 (CH), 124.3 (C), 129.7 (CH), 129.9 (C), 130.1 (CH), 131.2 (C), 134.4 (CH), 136.8 (CH), 146.1 (C), 162.7 (C═O), 163.2 (C═O); MS (APCI) *m/z* (rel. intensity): 397.2 [$(M + H)^+$, 100]; HRMS (ESI$^+$, *m/z*) calcd. for $(C_{21}H_{25}N_4O_4)^+$ [$(M + H)^+$] 397.1870; found 397.1855

*4.1.4.2. 2-{4-{[(1S,2S)-2-Aminocyclopentyl]amino}butyl}-5-nitro-1H-benzo[de]isoquinoline-1,3(2H)-dione dihydrochloride (**23**).* Global yield: 40%; $[\alpha]^{20}_D$ + 15.5 (*c* 0.10, MeOH) > 99% *ee*; MS (APCI) *m/z* (rel. intensity): 397.2 [$(M + H)^+$, 100]; HRMS (ESI$^+$, *m/z*) calcd. for $(C_{21}H_{25}N_4O_4)^+$ [$(M + H)^+$] 397.1870; found 397.1847

4.2. Cell culture

Human cell lines HL-60 and LoVo were cultured in RPMI 1640 medium (Sigma). HeLa and A-549 were cultured in Dulbecco's Modified Eagle's Medium (Sigma). All cell lines were supplemented with 10% fetal bovine serum (Invitrogen), 100 U/ml penicillin, 100 μg/ml streptomycin and glutamine (Sigma) at 37 °C in a humidified atmosphere with 5% CO_2.

4.3. Assesment of cytotoxicity

For cytotoxicity assays in 96-well microculture plates, 2500–5000 cells were seed per well depending on the doubling time. Seven parallel wells were performed for each treatment and, each experiment was replicated three times. Initially each new compound was tested for one single dose at 10 μM. Analogs with best activity values were also evaluated in an 8-dose screen from 1 nM to 10 μM. After incubation for 48 h with the compounds, WST-8 assay was carried out according to de manufactureŕs instruction (Cell Counting Kit − 8, Sigma-Aldrich, St. Louis, MO, USA). The absorbances were read at a wavelength of 450 nm. The IC_{50} values were calculated using GraphPad Prism (GraphPad Software; La Jolla, CA).

4.4. Cell cycle analysis

HL-60, HeLa, Lovo and, A549 cell lines were incubated with the different compounds at 10 μM for 48 h. After this time, the cells were collected, washed with PBS and cell cycle was analyzed following the method described by Vindelov et al. [21] Stained DNA was detected with Cytomis FC500 from Beckman Coulter and ModFit LT program (Verity Software House) was used to calculate the different cell cycle phases.

4.5. Annexin V-FITC staining

Apoptosis was evaluated after 48 h of treatment through Annexin V-FITC apoptosis detection kit from Immunostep; Spain. Briefly, 500.000 cells were collected and washed with PBS, and resuspended in 500 μl of Binding Buffer (10 μM Hepes/NaOH pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$). After adding 5 μl of Annexin V-FITC and 2.5 μl of propidium iodide, the samples were incubated for 15 min in the dark and analyzed by flow cytometry with Cytomis FC500 from Beckman Coulter.

4.6. Apoptosis study for normal cells

Buffy-coats from healthy donors (n = 4) were provided by Centro Comunitario de Sangre y Tejidos de Asturias (Oviedo, Spain). Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll gradient centrifugation and cultured in RPMI 1640 (Lonza) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Sigma-Aldrich), 1 mM sodium pyruvate, 2 mM L-glutamine, 100 U/mL penicillin and 10 μg/mL streptomycin at 37 °C and 5% CO_2. For apoptosis assays, PBMCs were treated with **2** and **22** (1, 5 and 10 μM) for 48 h and DMSO was used as control. Afterwards, samples were double-stained with Annexin V-FITC and PI as described above and analyzed with Cytomis FC500 from Beckman Coulter

4.7. DNA photocleavage

Plasmid DNA photocleavage experiments were performed using close pUC18 DNA. DNA (0.1 μg/μL) was incubated in Tris-HCl (pH 7.5) with corresponding compounds under photo-irradiation (366 nm) for 2 h. Non irradiated reactions were run in parallel. All reactions were quenched by loading buffer and agarose gel electrophoresis was carried out. The resolved bands were visualized with a UV transilluminator.

4.8. Maximum tolerated dose

Healthy CD-1 female mice, provided by the University of Oviedo Animal Facility, were used to determine the acute maximum tolerated dose of compound 23. Single IP injection of increasing doses was administered to groups of 4 mice. Body weight, deaths, changes in behavior, mobility, eating and drinking habits, and any other sign of local or systemic toxicity were recorded daily.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2021.104859.

References

[1] (a) M.F. Braña, A. Ramos, Curr. Med. Chem.: Anti-Cancer Agents 1 (2001) 237–255;
(b) S. Banerjee, E.B. Veale, C.M. Phelan, S.A. Murphy, G.M. Tocci, L.J. Gillespie, D. O. Frimannsson, J.M. Kelly, T. Gunnlaugsson, Chem. Soc. Rev. 42 (2013) 1601–1618;
(c) A. Kamal, N.R. Bolla, P.S. Srikanth, A.K. Srivastava, Expert. Opin. Ther. Patents 23 (2013) 299–317;
(d)Skubic, K., Adams, M., Horn, J., Priddy, A., Oelrich, R., Lewis, Znosko, B., Arnatt, C. The FASEB Journal 2018, 31, lb535.(e) H.-Q. Dong, T.-B. Wei, X.-Q. Ma, Q.-Y. Yang, Y.-F. Zhang, Y.-J. Sun, B.-B. Shi, H. Yao, Y.-M. Zhang, Q.J. Lin, Mater. Chem. C. 8 (2020) 13501–13529.
[2] (a)Braña, M. F., Cacho, M., García, M. A., de Pascual de Teresa, B., Ramos, A., Domínguez, M. T., Pozuelo, J. M., Abradelo, C., Rey-Stolle, M. F., Yuste, M., Bañez-Coronel, M., Lacal, J. M. J. Med. Chem. 2004, 47, 1391-1399.(b) L. Ingrassia, F. Lefranc, R. Kiss, T. Mijatovic, S.A. Unibioscreen, Curr. Med. Chem. 16 (2009) 1192–1213.
[3] E. Van Quaquebeke, T. Mahieu, P. Dumont, J. Dewelle, F. Ribaucour, G. Simon, S. Sauvage, J.-F. Gaussin, J. Tuti, M. El Yazidi, F. Van Vynckt, T. Mijatovic, F. Lefranc, F. Darro, R. Kiss, J. Med. Chem. 50 (2007) 4122–4134.
[4] Z.-Y. Tian, S.-Q. Xie, Y.-W. Du, Y.-F. Ma, J. Zhao, W.-Y. Gao, C.-J. Wang, Eur. J. Med. Chem. 44 (2009) 393–399.
[5] J. Ma, Y. Li, L. Li, K. Yue, H. Liu, J. Wang, Z. Xi, M. Shi, S. Zhao, Q. Ma, S. Liu, S. Guo, J. Liu, L. Hou, C. Wang, P.G. Wang, Z. Tian, S. Xie, Front. Chem. 8 (2020) 166.
[6] (a) A. Wu, Y. Xu, X. Qian, Bioorg. Med. Chem. 17 (2009) 592–599;
(b) A. Balachandra, T. Govindaraju, J. Org. Chem. 85 (2020) 1525–1536.(c)Li, X.; Lin, Y., Yuan, Y., Liu, K., Qian, X. Tetrahedron 2011, 67, 2299-2304.

(d)(d) Shalini, J. L., Adebayo, A. A., Kisten, P., Rosenthal, P. J., Singh, P., Kumar, V. ACS Med. Chem. Lett. 2020, 11, 154-161.
(e)Nekvinda, J., Rozycka, D., Rykowski, S., Wyszko, E., Fedoruk-Wyszomirska, A., Gurda, D., Orlicka-Plocka, M., Giel-Pietraszuk, M., Kiliszek, A., Rypniewski, W., Bachorz, R., Wojcieszak, J., Gruner, B., Olejniczak, A. B. Bioorg. Chem. 2020, 94, 103432.
(f)Rizzo, C., Cancemi, P., Mattiello, L., Marullo, S., D'Anna, F. ACS Appl.Mater. Interfaces 2020 (in press); DOI: 10.1021/acsami.0c17149.

[7] (a) K.J. Kilpin, C.M. Clavel, F. Edafe, P.J. Dyson, Organometallics 31 (2012) 7031–7039;
(b) K.G. Leslie, D. Jacquemin, E.J. New, K.A. Jolliffe, Chem. Eur. J. 24 (2018) 5569–5573;
(c)Shalini, Johansen, M. D., Kremer, L., Kumar, V. Bioorg. Chem. 2019, 92, 103241.(d) C.D. Wight, Q. Xiao, H.R. Wagner, E.A. Hernandez, V.M. Lynch, B.L. J. Iverson, Am Chem. Soc. 142 (2020) 17630–17643.

[8] (a) S.-Q. Xie, Y.-H. Zhang, Q. Li, F.-H. Xu, J.-W. Miao, J. Zhao, C.-J. Wang, Apoptosis 17 (2012) 725–734;
(b) D. Mahadevan, D.W. Northfelt, P. Chalasani, D. Rensvold, S. Kurtin, D.D. Von Hoff, M.J. Borad, R. Tibes, Int. J. Clin. Oncol. 18 (2013) 934–941;
(c) Y. Ye, S. Huang, Y. Wu, Cancer Manag. Res. 11 (2019) 2339–2348.

[9] Q. Yang, P. Yang, X. Qian, L. Tong, Bioorg. Med. Chem. Lett. 18 (2008) 6210–6213.

[10] (a) Y.L. Bennani, S. Hanessian, Chem. Rev. 97 (1997) 3161–3195;
(b) C. Peña, J. González-Sabín, F. Rebolledo, V. Gotor, Tetrahedron Asymmetry 18 (2007) 1981–1985;
(c) C. Peña, J. González-Sabín, F. Rebolledo, V. Gotor, Tetrahedron 64 (2008) 7709–7717;
(d) J. González-Sabín, F. Rebolledo, V. Gotor, Chem. Soc. Rev. 38 (2009) 1916–1925;
(e) I. Alfonso, Curr. Org. Synth. 7 (2010) 1–23.

[11] M.F. Braña, J.M. Castellano, C.M. Roldán, A. Santos, D. Vázquez, A. Jiménez, Cancer Chemother. Pharmacol. 4 (1980) 61–66.

[12] M.K. Hadden, B.S.J. Blagg, Anticancer Agents Med. Chem. 8 (2008) 807–816.

[13] K. Suzuki, H. Nagasawa, Y. Uto, Y. Sugimoto, K. Noguchi, M. Wakida, K. Wierzba, T. Terada, T. Asao, Y. Yamada, K. Kitazato, H. Hori, Bioorg. Med. Chem. 13 (2005) 4014–4021.

[14] (a) A. Wu, Y. Xu, X. Qian, J. Wang, J. Liu, Eur. J. Med. Chem. 44 (2009) 4674–4680;
(b) V. Tumiatti, A. Milelli, A. Minarini, M. Micco, A.G. Campani, L. Roncuzzi, D. Baiocchi, J. Marinello, G. Capranico, M. Zini, C. Stefanelli, C. Melchiorre, J. Med. Chem. 52 (2009) 7873–7877.

[15] (a) H.C. Becker, B. Norden, J. Am. Chem. Soc. 122 (2000) 8344–8349;
(b) X. Qu, J.O. Trent, I. Fokt, W.J. Priebe, B. Chaires, PNAS 97 (2000) 12032–12037;
(c) F. Eker, K. Griebenow, R. Schweitzer-Stenner, J. Am. Chem. Soc. 125 (2003) 8178–8185.

[16] H. Zhu, M. Huang, F. Yang, Y. Chen, Z.H. Miao, X.H. Qian, Y.F. Xu, Y.X. Qin, H. B. Luo, X. Shen, M.Y. Geng, Y.J. Cai, J. Ding, Mol. Cancer Ther. 6 (2007) 484–495.

[17] (a) H. Zhu, Z.-H. Miao, M. Huang, J.-M. Feng, Z.-X. Zhang, J.-J. Lu, Y.-J. Cai, L.-J. Tong, Y.-F. Xu, X.-H. Qian, J. Ding, Neoplasia 11 (2009) 1226–1234;
(b) Y.V. Suseela, S. Das, S.K. Pati, T. Govindaraju, ChemBioChem 17 (2016) 2162–2171.

[18] J.A. Hickman, Cancer Metastasis Rev. 11 (1992) 121–139.

[19] (a) Q. Yang, P. Yang, X. Qian, L. Tong, Bioorg. Med. Chem. Lett. 18 (2008) 6210–6213;
(b) X. Li, Y. Lin, Q. Wang, Y. Yuan, H. Zhang, X. Qian, Eur. J. Med. Chem. 46 (2011) 1274–1279.

[20] E. Van Quaquebeke, T. Mahieu, P. Dumont, J. Dewelle, F. Ribaucour, G. Simon, S. Sauvage, J.F. Gaussin, J. Tuti, M. El Yazidi, F. Van Vynckt, T. Mijatovic, F. Lefranc, F. Darro, R. Kiss, J. Med. Chem. 50 (2007) 4122–4134.

[21] L.L. Vindelov, I.J. Christensen, J.I. Nissen, Cytometry 3 (1983) 323–327.