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Synthesis and study of antiproliferative, antitopoisomerase II, DNA-intercalating and DNA-damaging activities of arylnaphthalimides

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ABSTRACT

A series of arylnaphthalimides were designed and synthesized to overcome the dose-limiting cytotoxicity of N-acetylated metabolites arising from amonafide, the prototypical antitumour naphthalimide whose biomedical properties have been related to its ability to intercalate the DNA and poison the enzyme Topoisomerase II. Thus, these arylnaphthalimides were first evaluated for their antiproliferative activity against two tumour cell lines and for their antitopoisomerase II in vitro activities, together with their ability to intercalate the DNA in vitro and also through docking modelization. Then, the well-known DNA damage response in *Saccharomyces cerevisiae* was employed to critically evaluate whether these novel compounds can damage the DNA in vivo. By performing all these assays we conclude that the 5-arylsubstituted naphthalimides not only keep but also improve amonafide's biological activities.

1. Introduction

Naphthalimides, a class of compounds that bind to DNA by intercalation have shown high anticancer activity against a variety of murine and human tumor cells.[1–4] Two representative compounds, mitonafide and amonafide (Fig. 1), have reached clinical trials as potential anticancer agents.[5,6] However, amonafide, although effective in its phase 2 clinical trials when administered either alone or in combination, suffered from the dose-limiting bone marrow toxicity caused by the toxic *N*-acetyl-amonafide generated through N-acetylation by the N-acetyltransferase 2 (NAT2).[7,8] Amonafide is a DNA intercalating agent that induces apoptotic signalling by promoting topoisomerase II (Topo II)-mediated DNA cleavage (i.e., it 'poisons' the enzyme).[9] Topo II is a nuclear enzyme that regulates the topological structure of chromatin. It is mainly involved in chromosome condensation and sister chromatid decatenation in mitosis. Topo II poisons are well validated chemotherapy agents.[10] Epipodophyllotoxins (etoposide), aminoacridines (amsacrine) and anthracyclines (doxorubicin, daunorubicin, etc.) are all potent Topo II poisons. Amonafide is distinct from other Topo II poisons in being able to evade Glycoprotein 1 (PgP) and related transporters responsible for multi-drug resistance.[11]

With the aim of accessing to less cytotoxic naphthalimides against bone-marrow while keeping their antitumoral activities, we synthesized several non-amino substituted naphthalimides. In this paper, we describe the preparation and biological evaluation of a series of aromatic substituted naphthalimides. We include the antiproliferative activity against two cancer cell lines of the synthesized naphthalimides together with the antitopoisomerase I and II in vitro activities. Besides, we carried out docking studies in order to further confirm the ability of the arylnaphthalimides to intercalate the DNA and thus complement the results obtained in vitro during the topoisomerase (I and II)-mediated relaxation.

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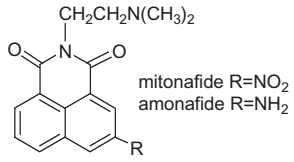


Figure 1. Structures of mitonafide and amonafide.

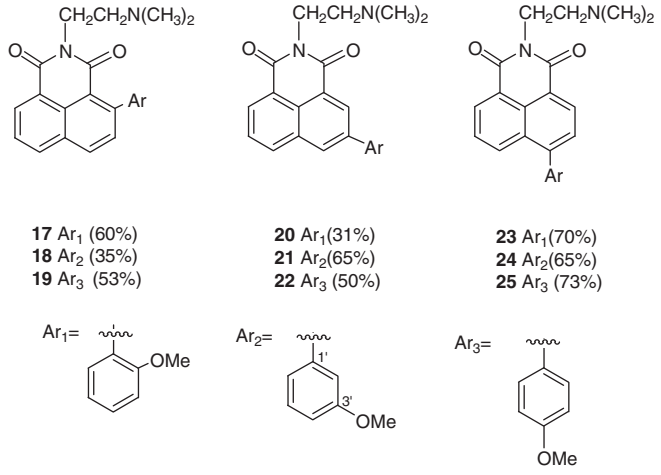
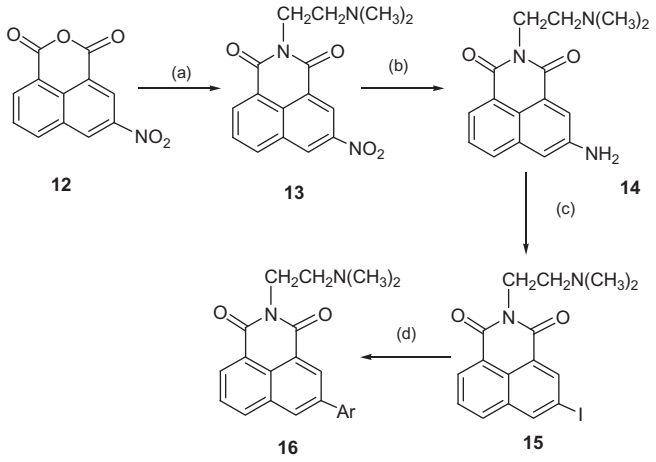


Figure 2. Synthesized arylnaphthalimides.

Finally, we have also evaluated the ability of these compounds to damage the DNA in vivo by studying the well-known DNA damage response in *Saccharomyces cerevisiae*.

2. Results and discussion

With the aim to explore if an extension of the conjugation of the naphthalimide core through an aromatic substituent could led to more active and less toxic derivatives, we decided to prepare 4-, 5-, and 6-aromatic substituted naphthalimides. There are only a few examples of 5-and 6- non fused aromatic arylnaphthalimides[12,13] and only one example of 4-arylnaphthalimide derivative.[14] To obtain the 4- and 6-aromatic substituted naphthalimides we followed the synthetic approach shown in Scheme 1.

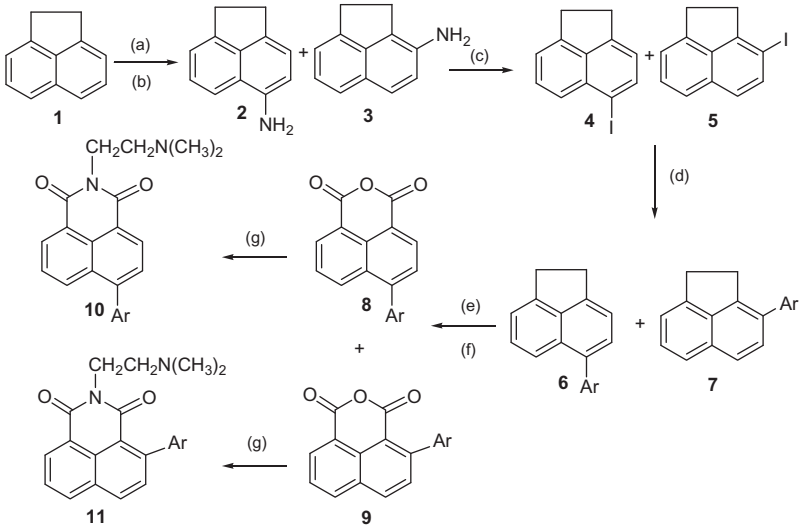


Scheme 2. Preparation of 5-arylnaphthalimides. Reagents and conditions: (a) $NH_2(CH_2)_2N(CH_3)_2$, EtOH, Δ (67%); (b) HCO_2H, TEA, Pd/C (100%); (c) $NaNO_2$, H_2SO_4, KI, 0 °C (44%); (d) $ArB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, EtOH.

Thus, compounds **10** and **11** were synthesized in six steps. The nitration of acenaphthene (**1**)[15] and subsequently hydrogenation[16] led to a mixture of two isomers: 5- and 3-aminoacenaphthene (**2** and **3**). In the following Sandmeyer reaction, the iodo derivatives (**4**) and (**5**) were formed and used for Suzuki coupling with commercial aryl boronic acids.[17,18] The mixture of aryl derivatives (**6**) and (**7**) was then oxidized with $Na_2Cr_2O_7$ in AcOH to yield the corresponding anhydrides (**8**) and (**9**), which were separated by column chromatography. Finally, each anhydride was treated with *N*,*N*-dimethyl-ethane-1,2-diamine under reflux to yield the desired aryl naphthalimides (**10**) and (**11**).

To obtain the 5-aromatic substituted naphthalimides, we employed the commercial 3-nitro-1,8-naphthalic anhydride (**12**) as starting material and we followed the synthetic approach shown in Scheme 2.[19–21]

Figure 2 shows the structures of the synthesized arylnaphthalimides (**17**–**25**) and the obtained yields from the corresponding precursors **8**, **9** or **15**. These arylnaphthalimides were subjected to antiproliferative evaluation against the human breast cancer cell



Scheme 1. Preparation of 4- and 6-arylnaphthalimides. Reagents and conditions: (a) HNO_3, 15 °C, Ac_2O, 3 h (98%); (b) H_2, Pd/C (10%), in THF (98%); (c) $NaNO_2$, H_2SO_4, KI, 0 °C (71%); (d) $ArB(OH)_2$, $Pd(PPh_3)_4$, C_7H_8//EtOH; (e) $Na_2Cr_2O_7$/AcOH; (f) separation by column chromatography; (g) $NH_2(CH_2)_2N(CH_3)_2$, DCM/C_7H_8, Δ, DMAP.

Table 1
Antiproliferative activity[a] of arylnaphthalimides (**17**–**25**) against the Human Breast Cancer MCF-7 and SK-Br-3 cell lines

Compound	MCF-7	SKBr-3	Compound	MCF-7	SKBr-3	Compound	MCF-7	SKBr-3
17	24.0 ± 4.1	24.0 ± 4.1	**20**	25.7 ± 2.9	11.9 ± 3.1	**23**	>30	>30
18	>30	>30	**21**	0.5 ± 0.1	0.3 ± 0.1	**24**	>30	>30
19	>30	>30	**22**	7.6 ± 0.8	1.5 ± 0.3	**25**	>30	27.6 ± 3.1

[a] Expressed as IC_{50} values given in μm and determined as means ± SD ($n = 3$).

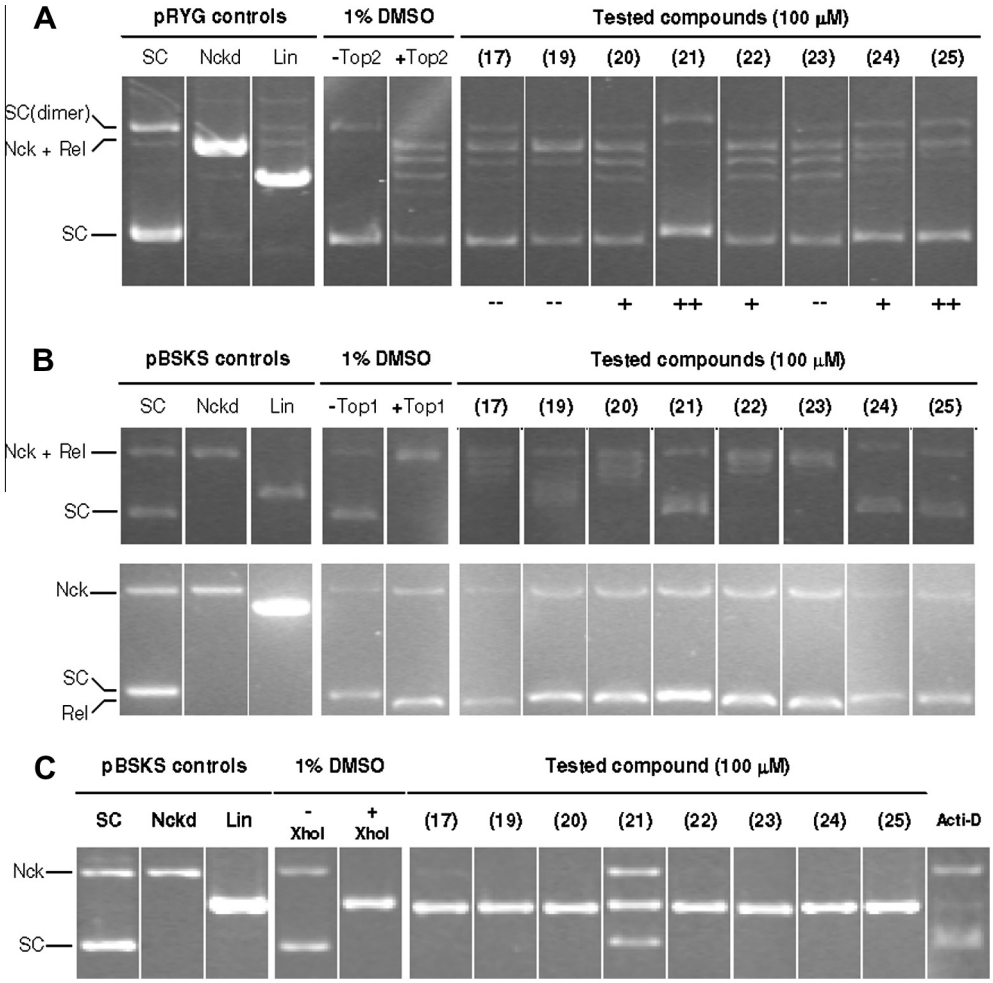
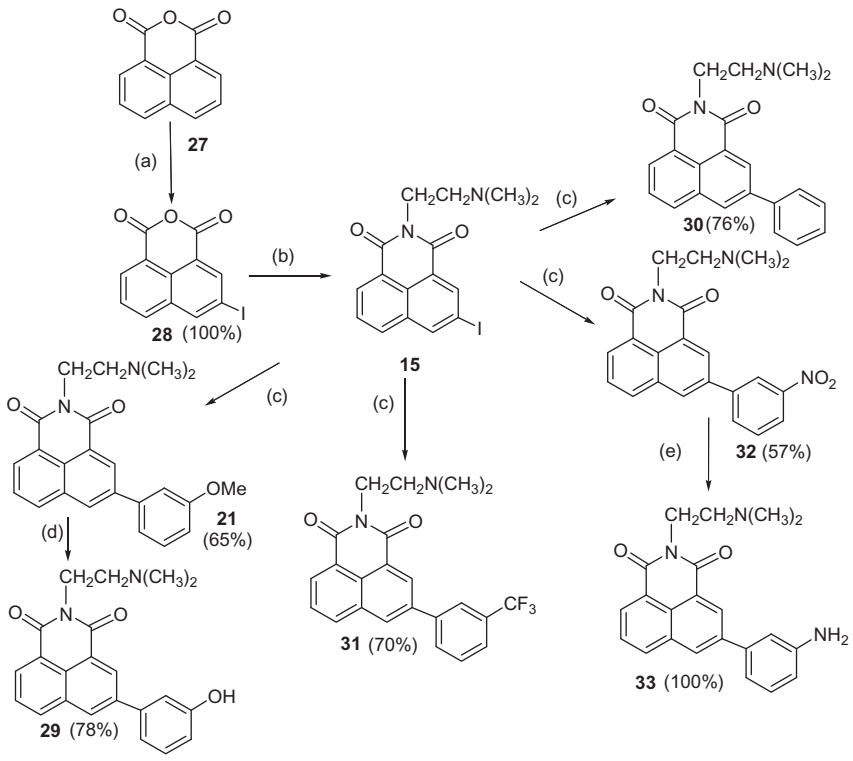


Figure 3. Inhibition of Topo I- and Topo II-mediated supercoiled DNA relaxation by arylnaphthalimides (**17**, **19**–**25**). (A) Agarose gel electrophoresis (without ethidium bromide) to separate substrates (SC form) and products (relaxed topoisomers) of the hTopo IIα reaction. Covalently closed negative supercoiled pRYG plasmid (SC form) was treated with hTopo IIα in the presence of either just 1% (v/v) DMSO, or 100 μM of a set of arylnaphthalimides in 1% DMSO. Concentration below 100 μM were also used and the inhibition profile is indicated underneath (––, no inhibition at 100 μM; +, inhibition at 100 μM; ++, inhibition at 60 μM; +++, inhibition at 30 μM). (B) Agarose gel electrophoresis (upper gel run without ethidium bromide and lower gel run with it) to separate substrates (SC form) and products (relaxed topoisomers) of the wTopoI reaction. For this assay, covalently closed negative supercoiled pBSKS plasmid (SC form) was treated with wTopoI in the presence of either just 1% (v/v) DMSO, or 100 μM of arylnaphthalimides in 1% DMSO. (C) Inhibition of endonucleolytic cleavage of DNA by restriction enzymes. The assay was carried out like above using the restriction enzyme XhoI and the plasmid pBSKS as substrate. The linear form of the plasmid is the final product, being the nicked form a reaction intermediate. Actinomycin D (Acti-D) was used as a control of a DNA intercalating agent capable of inhibiting the endonucleolytic reaction. Note how the presence of the 5-arylnaphthalimide **21** greatly inhibit all hTopo IIα, wTopo I and XhoI reactions (as compared to controls without enzyme). Nck (nicked plasmids), Rel (fully relaxed plasmids), Lin (linearized plasmids), SC (supercoiled plasmids).

lines MCF-7 and SKBr-3 (Table 1) and antitopoisomerase I and II in vitro activities (Fig. 3).

From the preliminary antiproliferative assay, we concluded that only the 5-arylsubstituted derivatives show good activities against MCF-7 and SKBr-3 cell lines. Within the 5-arylsubstituted naphthalimides series, the best results were obtained with the compound having a methoxy group at C-3′ (compare **21** vs **20** and **22**).

We next tested whether the synthesized arylnaphthalimides were good at inhibiting Topoisomerase II since this enzyme seems the relevant molecular target for the antiproliferative actions in tumour cells.[9,22] Thus, we observed that compound **21** was the best at inhibiting Topo II (Fig. 3A). Interestingly, the supercoiled form of the DNA substrate appeared shifted after being incubated with compound **21** and Topo II. This might indicate that this compound is also a potent DNA intercalator. To further explore this possibility we included an anti-Topo I assay (Fig. 3B) and also evaluated the cutting efficiency of a restriction endonuclease in the presence of the compounds (Fig. 3C). Anti-Topo I assays can measure both ac-



Scheme 3. Preparation of arylnaphthalimides **15**, **21**, **29**–**33**. Reagents and conditions: (a) H_2SO_4, Ag_2SO_4, I_2, 65 °C; (b) $NH_2CH_2CH_2N(CH_3)_2$, EtOH, Δ; (c) $ArB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, EtOH; (d) BBr_3, DCM, −78 °C; (e) H_2/Pd/C.

tual inhibition of Topo I and DNA intercalation since the latter non-specifically affects the relaxation activity.[23] Compounds **21**, **24** and **25** inhibited DNA relaxation by Topo I (Fig. 3B, upper panel). However, none seemed to poison the enzyme since the nicked form of DNA (Nckd) was not enriched in this assay when we forced the relaxed form (Rel) to become supercoiled again under the presence of another intercalating agent (i.e., ethidium bromide) (Fig. 3B, lower panel). Intercalating agents are known to inhibit DNA cleavage by restriction enzymes.[24] We also used this approach and found that compound **21** prevented full digestion of a supercoiled plasmid which carries a single recognition site for the restriction enzyme XhoI (Fig. 3C).

With these results in our hands, we decided to prepare other 5-aryl derivatives and evaluate the influence in the activity of other groups such as, –OH, $–NH_2$, $–NO_2$, CF_3, etc. Since the synthetic approach followed in Scheme 2 led to 5-arylsubstituted naphthalimides after four steps in moderated global yield, we planned an alternative synthetic route. Our objective was to obtain 3-iodo-1,8-naphthalic anhydride from direct iodination of the corresponding anhydride and to avoid the Sandmeyer reaction following a modified methodology described by Xie et al.[13] After using several iodination conditions, we obtained quantitatively the desired iodide derivative (**28**) by using of iodine in the presence of Ag_2SO_4/H_2SO_4.[25] This compound was converted into the imide (**15**) by treating with *N*,*N*-dimethyl-ethane-1,2-diamine. The desired 5-aryl derivatives were obtained under Suzuki coupling conditions with the corresponding aryl boronic acid in good yields (Scheme 3). Thus, this new approach permits the access to 5-arylnaphthalimides in three steps and in higher global yield than the synthetic route shown in Scheme 2. The hydroxyl derivative **29** was obtained by demethylation of **21** using BBr_3/DCM and the hydrogenation of **32** yielded the derivative **33**.

This new set of derivatives was again evaluated for antiproliferative (Table 2) and antitopoisomerase I and II activities (Fig. 4). Noteworthy, we also included at this point amonafide in order to directly test if our compounds globally keep (or even improve) amonafide's biological activities.

From these results, several structure–activity relationships can be outlined. The presence of hydrogen bond donor groups in the aromatic ring at C-5 (compounds **29** and **33**) led to the lower antiproliferative activities. Compounds **31** and **32** having electron withdrawing groups (NO_2 and CF_3) showed the higher values for the HEL cell lines while compounds **21** with a OMe group or compound **30** resulted to be the most active compounds against the SK-Br-3 cell line. Importantly, most of the 5-aryl derivatives exhibited higher antiproliferative activities than amonafide.

Again, we carried out anti-Topo II assays for this new set of compounds. We found that compounds **30**–**32** strongly inhibited Topo II (Fig. 4A). Compound **32** was the best one and inhibition was seen even at concentrations as low as 30 μM, a concentration where both amonafide and **21** cannot completely inhibit the relaxation activity (Fig. 4A). Once again, the supercoiled form of the DNA substrate appeared shifted after being incubated with compounds **30**–**32** and Topo II. Compound **32** (Fig. 4A) was the one where that shift was maximum. This result points out that at least some of these derivatives are also potent DNA intercalators. At this point, we also performed an assay to check whether these compounds stabilized the cleavage complex during the Topo II reaction (i.e., poison the enzyme) since it is believed that amonafide hinders the religation step of topoisomerase II action and thus stabilizes the cleavable complex.[9] We found that all tested 5-arylnaphthalimides increased the visibility of this reaction intermediate (linearized form) compared to a control with the vehicle (Fig. 4B). This intermediate was equally present for amonafide and all the other naphthalimides although slightly less abundant than when treated with the well-known Topo II poison etoposide. Overall, we hypothesize that our arylnaphthalimides, at least those that show the best antiprofilerative activities, act by intercalating the DNA and then poisoning Topo II in a similar manner as amonafide does.[9]

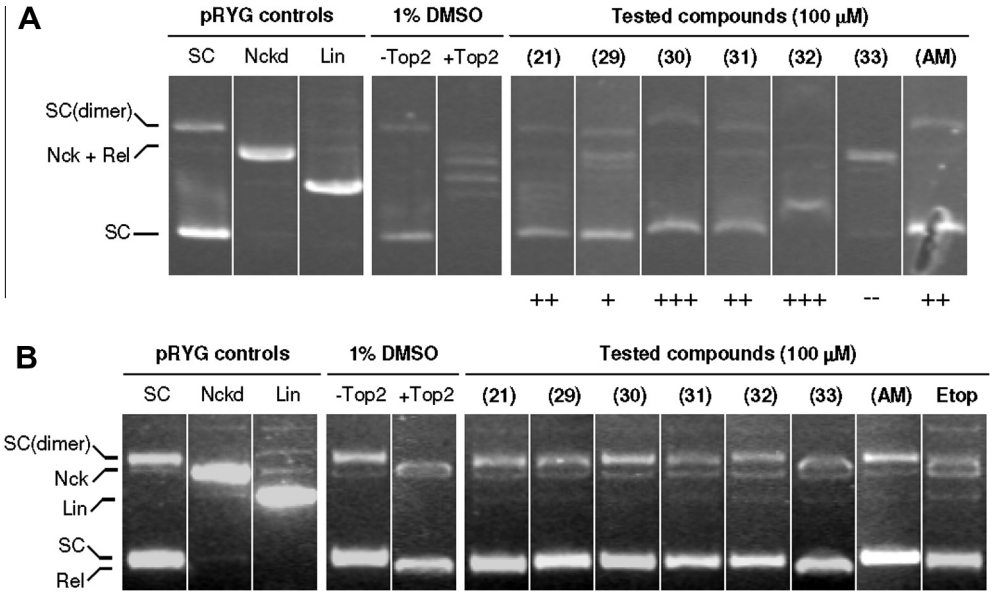


Figure 4. Topo II-mediated supercoiled DNA relaxation and cleavage under the presence of 5-arylnaphthalimides related to **21**. (A) Agarose electrophoresis run under the absence of ethidium bromide to separate substrates (SC form) and products (relaxed topoisomers) of the hTopo IIα reaction. Covalently closed negative supercoiled pRYG plasmid (SC form) was treated with hTopo IIα in the presence of 100 μM of 5-arylnaphthalimides in 1% (v/v) DMSO. Amonafide (AM) was also included for cross-comparisons. Concentration below 100 μM were also used and the inhibition profile is indicated underneath (––, no inhibition at 100 μM; +, inhibition at 100 μM; ++, inhibition at 60 μM; +++, inhibition at 30 μM). (B) Agarose electrophoresis run under the presence of ethidium bromide to separate substrates (SC form) and intermediate products (linearized plasmid) of the hTopo IIα cleavage assay. Covalently closed negative supercoiled pRYG plasmid (SC form) was treated with high concentrations of hTopo IIα in the presence of 100 μM of the same set of 5-aryl-naphthalimides in 1% (v/v) DMSO. Etoposide (Etop) was used as a positive control for this assay. Note how most of these arylnaphthalimide inhibit hTopo IIα. They further poison hTopo IIα (as compared to controls without enzyme). The shift of the SC form in the Topo II inhibition assay for compounds **30**–**32** further indicate that they intercalate into the DNA even better that amonafide and **21**. Nck (nicked plasmids), Rel (fully relaxed plasmids), Lin (linearized plasmids), SC (supercoiled plasmids).

Table 2
Antiproliferative activity[a] of amonafide and 5-aryl-naphthalimides (**21**, **29**–**33**) against HEL (Human Erythroleukemia) and SK-Br-3 (Human Breast Cancer) cell lines

Compound	HEL	SKBr-3
21	2.7 ± 0.3	0.3 ± 0.1
29	7.0 ± 0.9	4.4 ± 0.8
30	6.0 ± 0.9	0.8 ± 0.1
31	1.6 ± 0.2	1.2 ± 0.3
32	0.6 ± 4.1	1.2 ± 0.4
33	13.5 ± 2.2	14.2 ± 2.4
Amonafide	3.4 ± 0.5	7.4 ± 0.9

[a] Expressed as IC_{50} values given in μm and determined as means of ± SD ($n = 3$).

With the aim to explore the structural determinants responsible for the better intercalating properties of some of these 5-arylnaphthalimides, we performed docking calculations by using the Glide software.[26,27] The self-complementary oligodeoxy nucleotide d(ATGCAT)$_2$ was used as a model.[28–30] The best docking scores were found in the range from −6.13 to −8.21 kcal mol^{-1} (see Table S1 in Supplementary data). The analysis of docking results reveals that all ligands share a similar binding mode. The common structural portion, constituted by a polycyclic system, is fundamental for the lineup of the complex with the biological target, through a base pairs intercalation. In this sense, for example, the planar portions of the structure of the most active derivative **32** establishes π–π interactions with DNA aromatic rings, through intercalated naphthalimides and these interactions are extended thanks to the presence of the new aromatic portion where the nitro group is present (Fig. 5). The aminoalkyl chain establishes Van der Waals contacts and the amino groups interact with N7 of G3 through hydrogen bond with nucleic acids.

Drugs that poison Topo II are known to cause DNA damage in vivo, especially in the form of highly cytotoxic DNA double strand breaks (DSB).[10] It is this type of damage which makes cancer

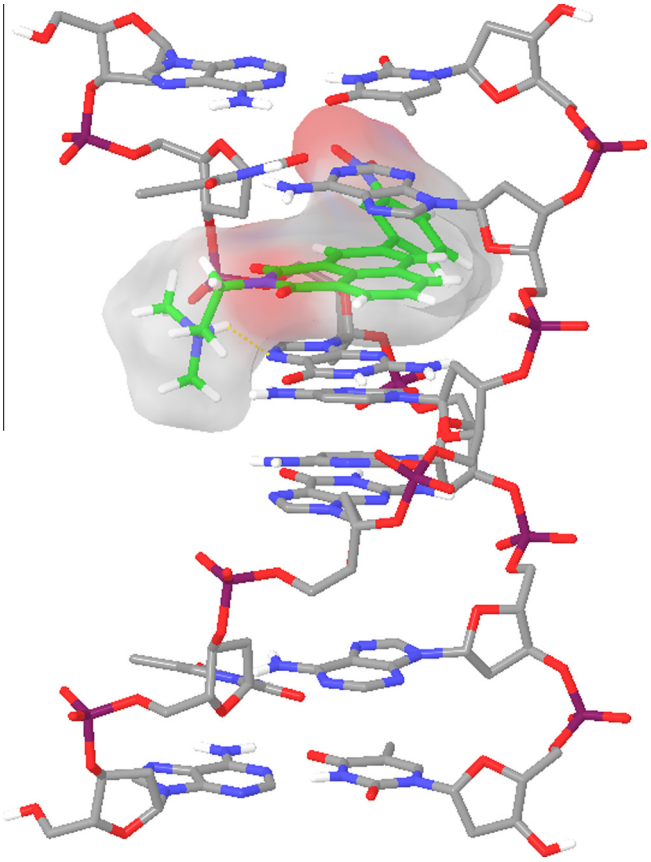


Figure 5. Compound **32**, into the DNA binding site. The figure shows the interaction of the amino groups of **32** with N7 of G3 through hydrogen bond. The DNA is represented by tube (coloured by atom type: C, grey; polar H, white; N, dark blue; O, red). Compound **32** is depicted in green.

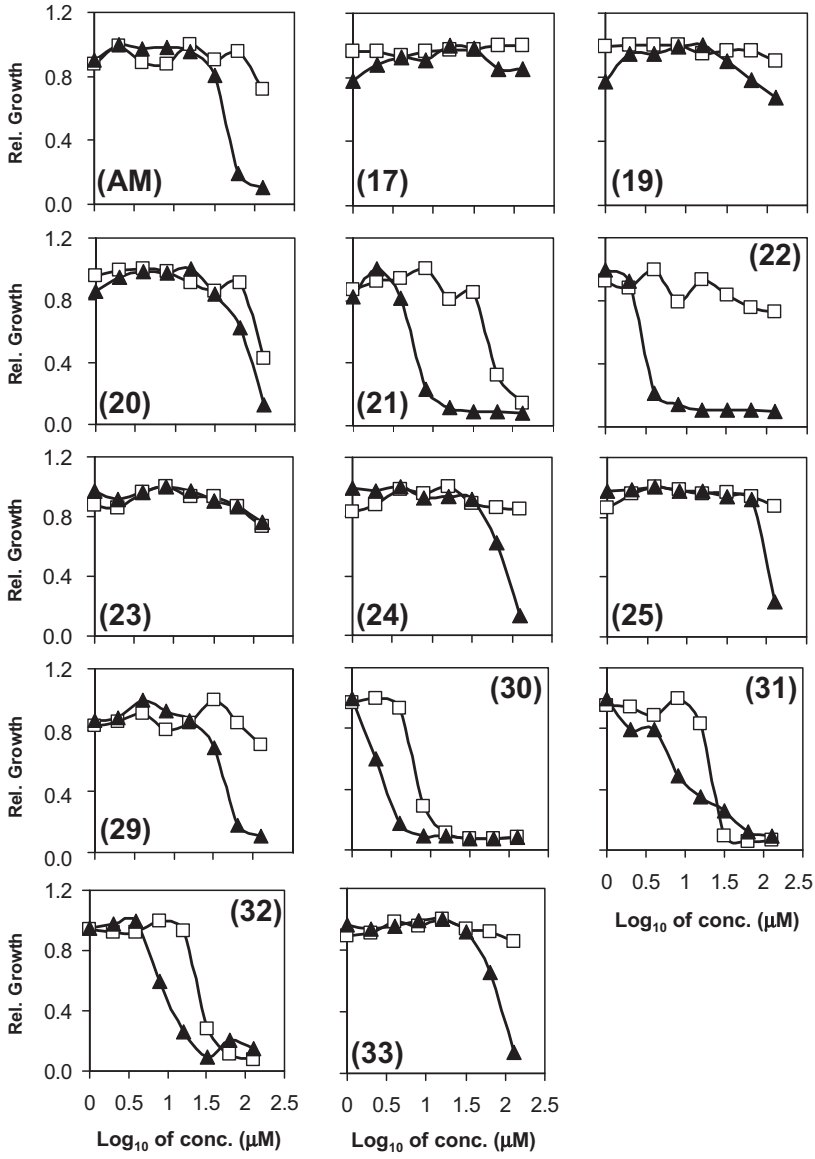


Figure 6. Sensitivity to the set of arylnaphthalimides (**17**, **19**–**25**, **29**–**33**) in wild type and homologous recombination deficient yeast cells. Strains proficient (*RAD52*) and deficient (*rad52Δ*) for the homologous recombination pathway were grown for 24 h under increasing concentrations of the tested naphthalimides. Relative growth to a control with DMSO 1% (v/v) was plotted against the drug concentrations. Note how: (i) compounds **21**, **30**–**32** are stronger than amonafide (AM) against yeast; and (ii) the *rad52Δ* mutant was always hypersensitive. In the graph, open squares depict growth of the *RAD52* strain and filled triangles depict the *rad52Δ* strain.

cells hypersensitive to these agents. In order to explore whether our compounds were making DSBs in vivo, we decided to carry out assays with the model organism *Saccharomyces cerevisiae*. This yeast has been extensively used for studies on DSB repair since it strongly depends on simple pathways that can be knocked out without compromising cell viability under non-toxic conditions. The central DBS repair pathway in yeast is homologous recombination (HR). This repair mechanism fully depends on the *RAD52* gene.[31] Thus, any yeast-permeable drug that causes DSBs would make yeast cells deficient in Rad52 hypersensitive to that drug. Furthermore, if that protein is labelled (e.g., Rad52-YFP) and we cytologically followed it we would see how it concentrates onto nuclear factories while DSBs occur.[32] Finally, DSBs also trigger checkpoints that arrest the cell cycle to allow time for repair. This arrest mainly takes place in G2 unless the number of DSBs is very high.[33] We made used of these three expected phenotypes to determine if the arylnaphthalimides (**17**, **19**–**25**, **29**–**33**) generate DSBs. First, we performed dose–response growth curves for each naphthalimide (including amonafide), and compared a wild-type *RAD52* yeast strain to the isogenic single *rad52Δ* mutant strain (Fig. 6).

We observed that amonafide did not inhibit the growth of the *RAD52* strain in the conditions we employed (1–100 μM concentration range with an initial yeast inoculum of $\sim 10^5$ cells/mL). Nevertheless, many of our arylnaphthalimides did inhibit the growth (compounds **21**, **30**–**32**). Importantly, those that did were the ones that gave the best antiproliferative profiles against tumor cell lines (Tables 1 and 2), strongly inhibited Topo II in vitro (Figs. 3 and 4), and likely are the best DNA intercalating agents (Figs. 3–5). Remarkably, the *rad52Δ* mutant was hypersensitive to most tested naphthalimides, including those moderately cytotoxic against tumor cell lines but not wild type yeast (e.g., compound **22**, **29**, **33** and amonafide itself). Arylnaphthalimides which were non-cytotoxic against tumor cell lines did not cause growth inhibition in the *rad52Δ* mutant either (e.g., **19** and **23**).

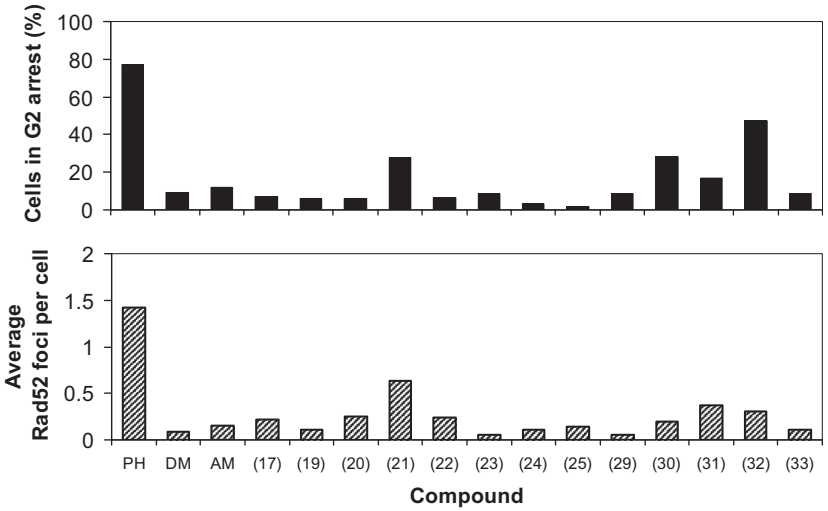


Figure 7. Cell cycle G2 arrests and homologous recombination nuclear factories in yeast cells treated with the set of naphthalimides (**17**, **19**–**25**, **29**–**33**). A yeast strain which expresses Rad52 fused to YFP was treated with 100 μM of the novel set of arylnaphthalimides or amonafide (AM) in DMSO 1% (v/v). The vehicle alone (DM) or phleomycin (PH) were used as the negative and positive control, respectively. Note how compounds **21**, **30**–**32** increased both the percentage of cells arrested in G2 and the average number of Rad52 foci per cell.

Besides, we used a *RAD52-YFP* strain to follow up the cell cycle arrest and formation of nuclear Rad52 repair factories after a short treatment (3 h) with the corresponding naphthalimides (Fig. 7). We observed that naphthalimides to which *RAD52* yeast cells were hypersensitive (i.e., **21**, **30**–**32**) partly triggered a G2 checkpoint as well (Fig. 7, upper panel). These same compounds led to the formation of more Rad52 foci than amonafide or other less-cytotoxic derivatives (Fig. 7, lower panel).

3. Conclusions

In short, we have synthesized a set of 4-, 5- and 6-aryl naphthalimides. The antiproliferative assay against the Human Breast Cancer MCF-7 and SK-Br-3 cell lines showed that only the 5-arylsubstituted derivatives have good activities. We prepared new 5-aryl derivatives and evaluated the influence of other groups such as, –OH, $–NH_2$, $–NO_2$, CF_3, etc., in the antiproliferative, and antitopoisomerase I and II activities. We found that all synthesized 5-arylnaphthalimides but those carrying H-bond donor groups, strongly inhibited Topo II and that the DNA substrate appeared electrophoretically shifted after being incubated with these compounds and Topo II, which points out that these derivatives are also potent DNA intercalators. These compounds also stabilized the cleavage complex during the Topo II reaction. Docking studies using a self-complementary oligodeoxynucleotide $d(ATGCAT)_2$ as a model corroborated that 5-arylnaphthalimides can act as DNA intercalating agents. In order to explore whether our compounds were making DSBs in vivo, assays with the model organism *Saccharomyces cerevisiae* were carried out. Remarkably, the *rad52Δ* mutant was hypersensitive to most tested naphthalimides, including those moderately cytotoxic against tumor cell lines (e.g., compound **22**, **29**, **33** and amonafide itself). However, some 5-arylnaphthalimides (i.e., **21**, **30**–**32**) were the most cytotoxic derivatives in yeast as well as in tumor cell lines. They were also able to arrest yeast cells in G2 and promote the formation of Rad52 foci, which fit well with DSBs being generated by these compounds in vivo.

4. Experimental

4.1. Chemistry

All solvents and reagents were purified by standard techniques reported in Ref. 34 or used as supplied from commercial sources as appropriate. Reactions were monitored by TLC (on silica gel POLYGRAM SIL G/UV_{254} foils). Purification by column flash-chromatography used Merk Kiesel 60-H (0.063–0.2 mm) as adsorbent and different mixtures of hexanes–EtOAc as eluent. Pre-coated TLC plates SIL G-100 UV_{254} (Machery-Nagel) were used for preparative-TLC purification. ^1H NMR spectra were recorded in $CDCl_3$ or C_6D_6 at 300 or 400 MHz, using a Bruker AMX300 or Bruker AMX400 instruments. For ^1H spectra, chemical shifts are given in parts per million (ppm) and are referenced to the residual solvent peak. Coupling constants (*J*) are given in Hertz (Hz) to the nearest 0.5 Hz. ^{13}C NMR spectra were recorded at 75 and 100 MHz using a Bruker AMX300 or Bruker AMX400 instruments. Carbon spectra assignments are supported by DEPT-135 spectra, $^{13}C–^1H$ (HMQC) and $^{13}C–^1H$ (HMBC) correlations where necessary. Chemical shifts are quoted in ppm and are referenced to the appropriate residual solvent peak. MS and HRMS were recorded on a VG Micromass ZAB-2F. IR spectra were taken on a Bruker IFS28/55 spectrophotometer. The preparation of 3-nitro-acenaphthene, 5-nitro-acenaphthene, 5-amino-acenaphthene (**2**), 3-amino-acenaphthene (**3**), 5-iodo-acenaphthene (**4**), 3-iodo-acenaphthene (**5**), 5-aryl-acenaphthenes (**6a**–**6c**), 3-aryl-acenaphthenes (**7a**–**7c**), 5-aryl-1,8-naphthalic anhydrides (**8a**–**8c**), 3-aryl-1,8-naphthalic anhydride (**9a**–**9c**), 2-(2-dimethylamino-ethyl)-5-nitro-benzo[*de*] isoquinoline-1,3-dione (**13**), 2-(2-dimethylamino-ethyl)-5-amino-benzo[*de*]isoquinoline-1,3-dione (**14**), 2-(2-dimethylamino-ethyl)-5-iodo-benzo[*de*]isoquinoline-1,3-dione (**15**) was carried out following the procedure described in Refs. 15–21 and the experimental procedures and spectroscopic characterization are included in Supplementary data.

4.1.1. Preparation of 2-(2-dimethylamino-ethyl)-4-(2-methoxyphenyl)-benzo[*de*]isoquinoline-1,3-dione (17)

15 μL (0.13 mmol) of *N*,*N*-dimethyl-ethane-1,2-diamine and catalytic amount of DMAP were added to 15 mg (0.05 mmol) of 3-(2-methoxyphenyl)-1,8-naphthalic anhydride in 5 mL of DCM/C_7H_8 (1:1). The reaction mixture was refluxed under argon for 2 h. Then, the solvent was removed under reduced pressure and the resulting residue was dissolved in 10 mL of EtOAc and treated with a 5% solution of HCl and with brine. The aqueous phase was separated and the organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated to obtain 20 mg of a residue, which was purified by preparative-TLC using DCM:MeOH (9:1) to yield

11.4 mg (60%) of compound **17** as an amorphous yellow solid. ^{1}H NMR ($CDCl_3$) δ: 2.41 (6H, s, H-3″, H-4″), 2.73 (2H, t, J = 7.0 Hz, H-2″), 3.71 (3H, s, OCH_3), 4.39 (2H, t, J = 7.1 Hz, H-1″), 7.09 (1H, d, J = 8.2, H-3′), 7.14 (1H, t, J = 8.2 Hz, H-5′), 7.30 (1H, m, H-6′), 7.51 (1H, t, J = 8.2 Hz, H-4′), 7.65 (1H, d, J = 8.4 Hz, H-6), 7.67 (1H, d, J = 7.6 Hz, H-3), 7.97 (1H, d, J = 8.4 Hz, H-5), 8.61 (1H, d, J = 7.6 Hz, H-4), 8.66 (1H, d, J = 8.4 Hz, H-7). ^{13}C NMR ($CDCl_3$) δ: 37.8 (t, C-1″), 45.4 (q, C-3″, C-4″), 55.4 (q, OCH_3), 56.7 (t, –C-2″), 111.1 (d, C-3′), 120.8 (d, C-5′), 121.7 (s, C-1a), 122.6 (s, C-7a), 126.4 (d, C-6), 127.5 (s, C-1′), 128.4 (d, C-6′), 130.1 (d, C-4′), 130.2 (s, C-4b), 130.6 (s, C-4a), 130.8 (d, C-3), 131.0 (d, C-7), 131.4 (d, C-5), 133.1 (d, C-4), 144.1 (s, C-2), 156.7 (s, C-2′), 164.2 (s, C-1), 164.5 (s, C-8). EIMS m/z (%): 374 (9), 316 (24), 303 (91), 245 (10) 189 (17). HREIMS: 374.1647 (calcd for $C_{23}H_{22}N_2O_3$ $[M]^+$ 374.1630). IR ν_{max}: 826, 1236, 1671, 1699, 2783, 2866, 2989 cm^{-1}.

4.1.2. Preparation of 2-(2-dimethylamino-ethyl)-4-(3-methoxyphenyl)-benzo[*de*]isoquinoline-1,3-dione (18)

15 μL (0.13 mmol) of *N*,*N*-dimethyl-ethane-1,2-diamine and 2 mg of DMAP were added to 10 mg (0.05 mmol) of 3-(3-methoxyphenyl)-1,8-naphthalic anhydride in 5 mL of DCM/C_7H_8 (1:1). The reaction mixture was refluxed under argon for 6 h. After carrying out the same procedure described above, the resulting residue was purified by preparative-TLC using DCM:MeOH (9:1) to yield 4 mg (35%) of compound **18** as an amorphous yellow solid. ^{1}H NMR ($CDCl_3$) δ: 2.41 (6H, s, H-3″, H-4″), 2.73 (2H, t, J = 7.0 Hz, H-2″), 3.84 (3H, s, OCH_3), 4.28 (2H, t, J = 6.5 Hz, H-1″), 6.95 (3H, m, H-4′, H-6′, H-2′), 7.38 (1H, t, J = 8.0 Hz, H-5′), 7.55 (1H, d, J = 8.4 Hz, H-4), 7.78 (1H, t, J = 7.6 Hz, H-6), 8.15 (1H, d, J = 8.4 Hz, H-3), 8.21 (1H, d, J = 8.0 Hz, H-5), 8.66 (1H, d, J = 6.4 Hz, H-7). ^{13}C NMR ($CDCl_3$) δ: 32.1 (t, C-1″), 45.2 (q, C-3″, C-4″), 55.4 (q, OCH_3), 55.6 (t, –C-2″), 113.0 (d, C-4′), 113.8 (d, C-2′), 119.3 (s, C-7a), 120.5 (d, C-6′), 123.1 (s, C-1a), 126.9 (d, C-5′), 129.0 (s, C-4b), 129.3 (d, C-3), 131.3 (s, C-4a), 131.6 (d, C-6), 131.9 (d, C-4), 132.9 (d, C-5), 134.2 (d, C-7), 143.8 (s, C-1′), 147.9 (s, C-2), 159.5 (s, C-3′), 163.4 (s, C-1), 164.3 (s, C-8). ESMS m/z (%): 352 (43), 397 (100), 413 (88). HRESMS: 397.1523 (calcd for $C_{23}H_{22}N_2O_3Na$ $[M+Na]^+$ 397.1528); IR ν_{max}: 816, 1246, 1671, 1698, 2763, 2865, 2990 cm^{-1}.

4.1.3. Preparation of 2-(2-dimethylamino-ethyl)-4-(4-methoxyphenyl)-benzo[*de*]isoquinoline-1,3-dione (19)

10 μL (0.09 mmol) of *N*,*N*-dimethyl-ethane-1,2-diamine and catalytic amount of DMAP were added to 10 mg (0.03 mmol) of 3-(4-methoxyphenyl)-1,8-naphthalic anhydride in 5 mL of DCM/C_7H_8 (1:1), and the reaction mixture was refluxed under argon for 6 h. After carrying out the same procedure described for compound **17**, the resulting residue was purified by preparative-TLC to yield 6.0 mg (53%) of compound **19** as an amorphous yellow solid. ^{1}H NMR ($CDCl_3$) δ: 2.39 (6H, s, H-3″, H-4″), 2.70 (2H, t, J = 6.8 Hz, H-1″), 3.91 (3H, s, OCH_3), 4.29 (2H, t, J = 6.8 Hz, H-2″), 7.03 (2H, d, J = 8.5 Hz, H-3′, H-5′), 7.37 (2H, d, J = 8.5 Hz, H-2′, H-6′), 7.58 (1H, d, J = 8.4 Hz, H-3), 7.77 (1H, t, J = 8.0 Hz , H-6), 8.16 (1H, d, J = 8.4 Hz, H-4), 8.22 (1H, d, J = 8.0 Hz, H-5), 8.68 (1H, d, J = 8.0 Hz, H-7). ^{13}C NMR ($CDCl_3$) δ: 37.5 (t, C-1″), 45.2 (c, C-3″, C-4″), 55.2 (c, OCH_3), 56.5 (t, C-2″), 113.6 (d, C-3′, C-5′), 119.0 (s, C-1a), 122.8 (s, C-7a), 126.5 (d, C-3), 129.1 (s, C-4b), 129.5 (d, C-6′, C-2′), 130.9 (s, C-4a), 131.6 (d, C-6), 132.0 (d, C-5), 132.7 (d, C-4), 133.9 (d, C-7), 134.3 (s, C-1′), 147.9 (s, C-2), 159.1 (s, C-4′), 163.6 (s, C-1), 164.1 (s, C-8); EIMS m/z (%): 374 (5), 316 (21), 303 (60), 243 (8) 189 (17); HREIMS: 374.2136 (calcd for $C_{23}H_{22}N_2O_3$ $[M]^+$ 374.1630); IR ν_{max}: 819, 1244, 1659, 1698, 2773, 2851, 2924 cm^{-1}.

4.1.4. Preparation of 2-(2-dimethylamino-ethyl)-5-(2-methoxyphenyl)-benzo[*de*]isoquinoline-1,3-dione (20)

110 mg (0.28 mmol) of compound **15** in 3 mL of dry toluene, tetrakis(triphenylphosphine)palladium (7 mol %) and 0.3 mL (0.56 mmol) of a 2 M Na_2CO_3 solution were treated with 64 mg (0.42 mmol) of 2-methoxyphenyl boronic in 3 mL of EtOH. The reaction mixture was refluxed under argon for 16 h, next 0.1 mL of H_2O_2 (30%) was added and the reaction mixture was stirred for one additional hour. The aqueous phase was extracted several times with AcOEt, the combined organic layers were dried over anhydrous magnesium sulfate, filtered and concentrated. The resulting residue was purified by flash chromatography using DCM/MeOH 9:1 to yield 33 mg of compound **20** as a yellow amorphous solid (31%). ^{1}H NMR ($CDCl_3$) δ: 2.38 (6H, s, H-3″, H-4″), 2.69 (2H, t, J = 7.2 Hz, H-2″), 3.84 (3H, s, OCH_3), 4.35 (2H, t, J = 7.2 Hz, H-1″), 7.03 (2H, m, H-5′, H-3′), 7.41 (2H, m, H-4′, H-6′), 7.71 (1H, t, J = 7.2 Hz, H-6), 8.20 (1H, d, J = 7.2 Hz, H-5), 8.29 (1H, s, H-4), 8.54 (1H, d, J = 7.2 Hz, H-7), 8.79 (1H, s, H-2). ^{13}C NMR ($CDCl_3$) δ: 38.1 (t, C-1″), 45.7 (q, C-3″, C-4″), 55.7 (q, OCH_3), 57.0 (t, C-2″), 111.4 (d, C-3′), 121.2 (d, C-5′), 122.1 (s, C-1a), 122.5 (s, C-7a), 127.0 (d, C-6′) 127.2 (s, C-4b), 128.7 (s, C-1′), 129.8 (d, C-4′), 130.9 (d, C-6), 131.0 (d, C-5), 131.8 (s, C-4a), 133.6 (d, C-7), 134.0 (d, C-2), 134.1 (d, C-4), 137.8 (s, C-3), 156.6 (s, C-2′), 164.4 (s, C-8, C-1). EIMS m/z (%): 374 (17), 316 (13), 303 (8), 189 (15), 71 (96), 58 (100). HREIMS 374.1638 (calcd for $C_{23}H_{22}N_2O_3$ $[M]^+$ 374.1630). IR ν_{max}: 757, 1029, 1664, 1703, 2774, 2837, 2944 cm^{-1}.

4.1.5. Preparation of 2-(2-dimethylamino-ethyl)-5-(3-methoxyphenyl)-benzo[*de*]isoquinoline-1,3-dione (21)

110 mg (0.28 mmol) of compound **15** in 3 mL of dry toluene, tetrakis(triphenylphosphine)palladium (7 mol %) and 0.3 mL (0.56 mmol) of a 2 M Na_2CO_3 solution were treated with 64 mg (0.42 mmol) of 3-methoxyphenyl boronic acid in 3 mL of EtOH. The reaction mixture was refluxed under argon for 16 h. Then 0.1 mL of 30% H_2O_2 solution was added and the reaction mixture was stirred for one additional hour. After carrying out the same procedure described for compound **20**, the resulting residue was purified by flash chromatography using DCM/MeOH 9:1 to yield 67 mg of compound **21** as a yellow amorphous solid (65%). ^{1}H NMR($CDCl_3$) δ: 2.38 (6H, s, H-3″, H-4″), 2.69 (2H, t, J = 7.2 Hz, H-2″), 3.91 (3H, s, OCH_3), 4.35 (2H, t, J = 7.2 Hz, H-1″), 6.98 (1H, d, J = 7.6 Hz, H-4′), 7.25 (1H, s, H2′), 7.32 (1H, d, J = 7.6 Hz, H-6′), 7.42 (1H, d, J = 7.6 Hz, H-5′), 7.73 (1H, t, J = 7.3 Hz, H-6), 8.22 (1H, d, J = 7.4 Hz, H-5), 8.33 (1H, s, H-4), 8.54 (1H, d, J = 7.3 Hz, H-7), 8.81 (1H, s, H-2). ^{13}C NMR ($CDCl_3$) δ: 38.0 (t, C-1″), 45.6 (q, C-3″, C-4″), 55.4 (q, OCH_3), 56.9 (t, C-2″), 112.9 (d, C-2′), 113.7 (d, C-4′), 119.7 (d, C-6′), 122.4 (s, C-1a), 122.9 (s, C-7a), 127.2 (d, C-6), 130.1 (d, C-5′), 130.6 (d, C-2), 130.9 (d, C-7), 131.2 (d, C-4), 131.9 (s, C-1′, C-4b), 134.0 (d, C-5), 139.7 (s, C-3), 140.5 (s, C-4a), 160.2 (s, C-3′), 164.1 (s, C-8, C-1). EIMS m/z (%): 374 (16), 316 (12), 303 (8), 189 (15) 71 (96), 58 (100). HREIMS: 374.1638 (calcd for $C_{23}H_{22}N_2O_3$ $[M]^+$ 374.1630). IR ν_{max}: 783, 1015, 1734, 1775, 2849, 2923, 3067 cm^{-1}.

4.1.6. Preparation of 2-(2-dimethylamino-ethyl)-5-(4-methoxyphenyl)-benzo[*de*]isoquinoline-1,3-dione (22)

110 mg (0.28 mmol) of compound **15** in 3 mL of dry toluene, tetrakis(triphenylphosphine)palladium (7 mol %) and 0.56 mmol of a 2 M sodium carbonate solution were treated with 64 mg (0.42 mmol) of 3-methoxyphenyl boronic acid in 3 mL of EtOH. The reaction mixture was refluxed for 16 h under argon. Then 0.1 mL of 30% H_2O_2 was added and the reaction mixture was stirred for one additional hour. After carrying out the same procedure described for compound **20**, the resulting residue was purified by flash chromatography using DCM/MeOH 9:1 to yield 50 mg of compound **22** as a yellow amorphous solid (50%). ^{1}H NMR ($CDCl_3$) δ: 2.37 (6H, s, H-3″, H-4″), 2.66 (2H, t, J = 7.4 Hz, H-2″), 3.88 (3H, s, OCH_3), 4.32 (2H, t, J = 7.4 Hz, H-1″), 7.02 (2H, d, J = 8.4 Hz, H-5′, H-3′), 7.65 (2H, d, J = 8.4 Hz, H-4′, H-6′), 7.68 (1H, m, H-6), 8.17 (1H, d, J = 8.1 Hz, H-5), 8.24 (1H, s, H-4), 8.45 (1H, d, J = 7.3 Hz, H-7), 8.74

(1H, s, H-2). ^{13}C NMR ($CDCl_3$) δ: 38.1 (t, C-1″), 45.7 (q, C-3″, C-4″), 55.3 (q, OCH_3), 56.9 (t, C-2″), 114.5 (d, C-3′ C-5′), 122.4 (s, C-1a), 122.8 (s, C-7a), 126.8 (s, C-4b) 127.1 (d, C-6), 128.3 (d, C-6′ C-2′), 130.1 (d, C-2), 130.2 (d, C-7), 130.5 (d, C-4), 131.3 (s, C-1′), 133.7 (d, C-5), 139.0 (s, C-4b), 139.4 (s, C-3), 141.8 (s, C-4a), 159.9 (s, C-4′), 164.1 (s, C-8 or C-1), 164.2 (s, C-8 or C-1). EIMS *m*/*z* (%): 374 (5), 316 (26), 303 (100), 189 (14) 69 (17), 58 (5). HREIMS: 374.1638 (calcd for $C_{23}H_{22}N_2O_3$ $[M]^+$ 374.1630). IR ν_{max}: 787, 1034, 1662, 1698, 2850, 2923, 3065 cm^{-1}.

4.1.7. Preparation of 2-(2-dimethylamino-ethyl)-6-(2-methoxyphenyl)-benzo[*de*]isoquinoline-1,3-dione (23)

50 mg (0.16 mmol) of 5-(2-methoxyphenyl)-1,8-naphthalic anhydride in 5 mL of DCM/C_7H_8 (1:1), were treated with 26 μL (0.24 mmol) of *N*,*N*-dimethyl-ethane-1,2-diamine and 2.0 mg of DMAP. After carrying out the same procedure described for compound **17**, the resulting residue was purified by flash chromatography using DCM:MeOH (9:1) to yield 42 mg (70%) of compound **23** as an amorphous yellow solid. ^{1}H NMR ($CDCl_3$) δ: 2.41 (6H, s, H-3″, H-4″), 2.73 (2H, t, *J* = 6.8 Hz, H-2″), 3.71 (3H, s, OCH_3), 4.39 (2H, t, *J* = 6.8 Hz, H-1″), 7.09 (1H, m, H-3′), 7.10 (1H, t, *J* = 8.3 Hz, H-5′), 7.29 (1H, m, H-6′), 7.51 (1H, td, *J* = 1.6 Hz, *J* = 8.3, H-4′), 7.65 (1H, d, *J* = 8.3 Hz, H-6), 7.67 (1H, d, *J* = 7.6 Hz, H-2), 7.69 (1H, d, *J* = 7.6 Hz, H-3). 7.97 (1H, d, *J* = 7.8 Hz, H-5). 8.61 (1H, d, *J* = 7.8 Hz, H-7). ^{13}C NMR ($CDCl_3$) δ: 37.8 (t, C-1″), 45.4 (q, C-3″, C-4″), 55.4 (q, OCH_3), 56.7 (t, C-2″), 111.1 (d, C-3′), 120.8 (d, C-5′), 121.7 (s, C-1a), 122.6 (s, C-7a), 126.4 (d, C-6),127.5 (s, C-4a), 128.4 (d, C-6′), 130.1 (d, C-4′), 130.6 (s, C-4b), 130.8 (d, C-3), 131.0 (d, C-7), 131.4 (d, C-2), 133.1 (d, C-5) 144.1 (s, C-4), 156.7 (s, C-2′), 164.2 (s, C-8 or C-1), 164.5 (s, C-1 or C-8). EIMS *m*/*z* (%): 374 (5), 316 (7), 303 (15), 189 (10.7), 58 (100); HREIMS: 374.1614 (calcd for $C_{23}H_{22}N_2O_3$ $[M]^+$ 374.1630); IR ν_{max}: 758, 768, 1658, 1698, 2852, 2926 cm^{-1}.

4.1.8. Preparation of 2-(2-dimethylamino-ethyl)-6-(3-methoxyphenyl)-benzo[*de*]isoquinoline-1,3-dione (24)

45 mg (0.15 mmol) of 6-(3-methoxyphenyl)-1,8-naphthalic anhydride in 5 mL of DCM/C_7H_8 (1:1), were treated with 25 μL (0.22 mmol) of *N*,*N*-dimethyl-ethane-1,2-diamine and 2.0 mg of DMAP. After carrying out the same procedure described for compound **17**, the resulting residue was purified by flash chromatography using DCM:MeOH (9:1) to yield 36 mg (65%) of compound **24** as an amorphous yellow solid. ^{1}H NMR ($CDCl_3$) δ: 2.38 (6H, s, H-3″, H-4″), 2.69 (2H, t, *J* = 7.0 Hz, H-2″), 3.89 (3H, s, OCH_3), 4.37 (2H, t, *J* = 6.8 Hz, H-1″), 7.06 (3H, m, H-4′, H-2′, H-6′), 7.47 (1H, t, *J* = 7.3 Hz, H-5′), 7.71 (2H, m, H-6, H-3), 8.30 (1H, d, *J* = 8.3 Hz, H-5), 8.63 (1H, d, *J* = 6.5 Hz, H-7), 8.64 (1H, d, *J* = 7.4 Hz, H-2); ^{13}C NMR ($CDCl_3$) δ: 38.1 (t, C-1″), 45.7 (c, C-3″, C-4″), 55.3 (q, OCH_3), 56.9 (t, C-2″), 113.7 (d, C-2′), 115.6 (d, C-4′), 121.7 (s, C-1a), 122.3 (d, C-6′), 122.8 (s, C-7a) 126.8 (d, C-3), 127.6 (d, C-6), 128.6 (s, C-4b), 129.6 (d, C-5′), 130.0 (s, C-4a), 130.8 (d, C-7), 131.2 (d, C-2), 132.6 (d, C-5), 140.1 (s, C-1′), 146.7 (s, C-4), 159.6 (s, C-3′), 164.1 (s, C-8 or C-1), 164.2 (s, C-1 or C-8). EIMS *m*/*z* (%): 374 (4), 316 (7), 3.42 (15), 303 (100), 217 (17) 189 (28), 58 (100). HREIMS 374.1623 (calcd for $C_{23}H_{22}N_2O_3$ $[M]^+$ 374.1630); IR ν_{max}: 745, 766, 1645, 1698, 2882, 2986 cm^{-1}.

4.1.9. Preparation of 2-(2-dimethylamino-ethyl)-6-(4-methoxyphenyl)-benzo[*de*]-isoquinoline-1,3-dione (25)

70 mg (0.22 mmol) of 5-(4-methoxyphenyl)-1,8-naphthalic anhydride in 5 mL of DCM/C_7H_8 (1:1), were treated with 26 μL (0.09 mmol) of *N*,*N*-dimethyl-ethane-1,2-diamine and catalytic amount of DMAP. After carrying out the same procedure described for compound **17**, the resulting residue was purified by flash chromatography using DCM:MeOH (9:1) to yield 63 mg (73%) of compound **25** as an amorphous yellow solid. ^{1}H NMR ($CDCl_3$) δ: 2.39 (6H, s, H-3″, H-4″), 2.71 (2H, t, *J* = 6.8 Hz, H-2″), 3.93 (3H, s, OCH_3), 4.38 (2H, t, *J* = 6.9 Hz, H-1″), 7.10 (2H, d, *J* = 8.5 Hz, H-3′, H-5′), 7.45 (2H, d, *J* = 8.5 Hz, H-2′, H-6′), 7.69 (1H, dd, *J* = 7.4 Hz, *J* = 8.5 Hz, H-6), 7.71 (1H, d, *J* = 7.5 Hz , H-3), 8.33 (1H, dd, *J* = 1.6 Hz, *J* = 8.5 Hz, H-5), 8.65 (2H, d, *J* = 7.4 Hz, H-7, H-2). ^{13}C NMR ($CDCl_3$) δ: 38.0 (t, C-1″), 45.6 (q, C-3″, C-4″), 55.4 (q, OCH_3), 56.8 (t, C-2″), 114.1 (d, C-3′, C-5′), 120.9 (s, C-1a), 122.8 (s, C-7a), 126.6 (d, C-3), 127.7 (d, C-6), 128.8 (s, C-4b), 130.1 (s, C-4a), 130.9 (d, C-7), 131.0 (d, C-2), 131.1 (d, C-2′, C-6′), 132.7 (d, C-5), 146.7 (s, C-4), 158.4 (s, C-1′), 159.9 (s, C-4′), 164.2 (s, C-8 or C-1), 164.4 (s, C-1 or C-8). EIMS *m*/*z* (%): 374 (5), 316 (7), 303 (15), 189 (11), 58 (100). HREIMS: 374.1614 (calcd for $C_{23}H_{22}N_2O_3$ $[M]^+$374.1630). IR ν_{max}: 673, 788, 1023, 1736, 1777, 2853, 2922, 2962 cm^{-1}.

4.1.10. Preparation of 3-iodo-1,8-naphthalic anhydride (28)

3.0 g (15 mmol) of 1,8-naphthalic anhydride in 60 mL of H_2SO_4 were treated with 3.4 g of Ag_2SO_4 (11 mmol) and 6.9 g of I_2 (27.5 mmol) pulverized in a mortar. The reaction mixture was stirred at 65 °C for 24 h, then it was poured onto ice, and the resulting yellow precipitate was filtered through a büchner funnel. The precipitate was washed with cold water, saturated solution of sodium carbonate, and saturated solution of sodium bisulphite. The obtained solid was dried in an oven at 80 °C to yield quantitatively compound (**28**) (5.9 g). ^{1}H NMR (DMSO-d_6) δ: 7.91 (1H, t, *J* = 7.9 Hz H-6), 8.45 (1H, d, *J* = 7.9 Hz, H-5), 8.51 (1H, d, *J* = 7.8 Hz, H-7), 8.60 (1H, d, *J* = 1.4 Hz, H-2), 8.99 (1H, d, *J* = 1.4 Hz, H-4). ^{13}C NMR (DMSO-d_6) δ: 93.3 (s, C-3), 119.7 (s, C-1a), 121.1 (s, C-7a), 128.8 (d, C-6) y (c, C-4b), 133.1 (d, C-7), 1333.3 (c, C-4a), 134.5 (d, C-5), 139.6 (d, C-2), 143.4 (d, C-4), 160.0 (c, C-1), 160.6 (c, C-8). EIMS *m*/*z* (%): 325.9 (2), 324.9 (13), 323.9 (100), 279.9 (46), 153 (41). HREIMS: 323.9282 (calcd for $C_{12}H_5IO_3$ $[M]^+$ 323.9282. IR ν_{max}: 782, 1012, 1290, 1771, 3056 cm^{-1}.

4.1.11. Preparation of 2-(2-dimethylamino-ethyl)-5-iodo benzo[*de*]isoquinoline-1,3-dione (15) from 28

To a solution of 3-iodo-1,8-naphthalic anhydride (1.0 g, 3.08 mmol) of **28** in 10 mL of absolute EtOH were added 0.99 mL of *N*,*N*-dimethylethylendiamine and catalytic amounts of DMAP. The reaction mixture was refluxed under argon for 45 min, then it was filtered hot, the filtrate was cooled and the resulting orange precipitate was filtered through a buchner funnel, and washed with cold EtOH (100 mL) and two portions of hexane (20 mL). 850 mg of compound **15** were obtained without furthermore purification (70%).

4.1.12. Preparation of 2-(2-dimethylamino-ethyl)-5-(3-hydroxy-phenyl)-benzo[*de*]isoquinoline-1,3-dione (29)

35 mg (0.09 mmol) of compound **21** in dry DCM at −78 °C were treated with 0.55 mL of a 1 M BBr_3 solution in DCM. The reaction mixture was stirred at 0 °C under argon until disappearance of the starting compound, then was treated with $NaHCO_3$ and stirred for 30 min. The aqueous phase was extracted several times with DCM, the organic layers were dried over anhydrous magnesium sulfate, filtered and concentrated. The resulting residue was purified by flash chromatography using DCM/MeOH (85:15) to yield 23 mg (60%) of compound **29** as a yellow amorphous solid. ^{1}H NMR ($CDCl_3$) δ: 2.51 (6H, s, H-3″, H-4″), 2.98 (2H, t, *J* = 7.2 Hz, H-2″), 4.47 (2H, t, *J* = 7.2 Hz, H-1″), 6.34 (1H, s, H-2′), 6.40 (1H, d, *J* = 6.6 Hz, H-6′), 6.85 (1H, d, *J* = 7.4 Hz, H-4′), 6.92 (1H, t, *J* = 7.4 Hz, H-5′), 7.72 (1H, t, *J* = 7.8 Hz, H-6), 8.06 (1H, s, H-4), 8.17 (1H, d, *J* = 7.8 Hz, H-5), 8.48 (1H, s, H-2), 8.52 (1H, d *J* = 6.5 Hz, H-7). ^{13}C NMR ($CDCl_3$) δ: 37.5 (t, C-1″), 45.4 (q, C-3″, C-4″), 57.3 (t, C-2″), 113.7 (d, C-2′), 115.2 (d, C-6′), 117.6 (d, C-4′), 122.6 (s, C-7a), 122.8 (s, C-1a), 127.1 (d, C-6), 129.6 (d, C-5′, C-2), 130.4 (d, C-4), 130.7 (d, C-7), 131.9 (s, C-4a), 134.1 (d+s, C-5, C-

4b), 139.0 (s, C-3), 139.4 (s, C-1′), 157.5 (s, C-3′), 164.8 (s, C-8, C-1). EIMS *m*/*z* (%): 361 (25), 360 (89), 189 (15), 71 (54), 58 (100). HREIMS: 361.1538 (calcd for $C_{23}H_{22}N_2O_3$ $[M+1]^+$ 361.1530). IR ν_{max}: 783, 1015, 1734, 1775, 2849, 2923, 3067 cm^{-1}.

4.1.13. 2-(2-Dimethylamino-ethyl)-5-(phenyl)-benzo[*de*]isoquinoline-1,3-dione (30)

50.0 mg (0.125 mmol) of compound **15** in 2 mL of dry toluene, tetrakis(triphenylphosphine) palladium (10 mol %) and 0.14 mL of a 2 M solution of Na_2CO_3 were treated with 62.6 mg (0.42 mmol) of phenyl boronic acid dissolved in 1 mL of absolute ethanol. The reaction mixture was refluxed under argon for 6 h. Then 0.05 mL of 30% H_2O_2 was added, and the reaction mixture was stirred for one additional hour. The aqueous phase was extracted several times with AcOEt, the combined organic layers were dried over anhydrous magnesium sulfate, filtered and concentrated. The residue was purified by flash chromatography using DCM/MeOH 9:1 to yield 33.0 mg of compound **30** as an orange amorphous solid (76%). Compound **30** showed identical spectroscopic data to those previously reported.[12,13]

4.1.14. Preparation of 2-(2-dimethylamino-ethyl)-5-(3-trifluormethyl-phenyl)-benzo[*de*]isoquinoline-1,3-dione (31)

100 mg (0.25 mmol) of compound **15** in 4 mL of dry toluene were treated with calatytic amounts of tetrakis(triphenylphosphine) palladium (10 mol %), 0.28 mL of a 2 M solution of sodium carbonate and 64 mg (0.42 mmol) of 3-trifluormethyl-phenyl boronic acid in 3 mL of absolute ethanol. The reaction mixture was refluxed under argon for 14 h. Then 0.1 mL of 30% H_2O_2 was added, and the reaction mixture was stirred for one additional hour. The aqueous phase was extracted several times with AcOEt, the combined organic layers were dried over anhydrous magnesium sulfate, filtered and concentrated. The residue was purified by flash chromatography using DCM/MeOH 9:1 to yield 70 mg of compound **31** as an orange amorphous solid (70%). ^1H NMR ($CDCl_3$) δ: 2.35 (6H, s, H-3″, H-4″), 2.68 (2H, t, J = 6.9 Hz, H-2″), 4.33 (2H, t, J = 6.9 Hz, H-1″), 7.65 (2H, m, H-4′, H-6′), 7.74 (1H, t, J = 7.5 Hz H 6), 7.89 (1H, d, J = 7.6 Hz, H-5′), 7.93 (1H, s, H- 2′), 8.20 (1H, d, J = 8.2 Hz, H-5), 8.30 (1H, d, J = 1.6 Hz, H-4), 8.51 (1H, d, J = 7.2 Hz, H-7), 8.73 (1H, d, H-2, J = 1.6 Hz).^{13}C NMR ($CDCl_3$) δ: 38.1 (t, C-1″), 45.6 (q, C-3″, C-4″), 56.9 (t, C-2″), 122.6 (s, C-7a), 123.4 (s, C-1a), 123.1 (d, C-2′), 124.1 (d, C-6′),127.3 (s, C-4a) 127.5 (d, C-6), 129.7 (d, C-4′), 130.1 (d, C-2), 130.6 (d, C-5′), 131.3 (d, C-4, C-7), 132.0 (s, C-1′, C-4b), 134.0 (d, C-5), 138.3 (s, C-3), 163.9 (s, C-3′), 164.0 (s, C-8, C-1). EIMS *m*/*z* (%): 412 (19), 340 (51), 270 (70), 202 (98), 71 (15), 58 (100); HREIMS: 412.1389 (calcd for $C_{23}H_{19}F_3N_2O_2$ $[M]^+$ 412.1399); IR ν_{max}: 770, 1075, 1744, 1775, 2850, 2925, 3097 cm^{-1}.

4.1.15. Preparation of 2-(2-dimethylamino-ethyl)-5-(3-nitro-phenyl)-benzo[*de*]isoquinoline-1,3-dione (32)

100 mg (0.25 mmol) of compound **15** in 4 mL of dry toluene were treated with catalytic amounts of tetrakis(triphenylphosphine)palladium (10 mol %), 0.28 mL of a 2 M solution of sodium carbonate and 62.6 mg (0.38 mmol) of 3-nitro-phenyl boronic acid dissolved in 2 mL of absolute ethanol. The reaction mixture was refluxed under argon for 12 h, then 0.1 mL of 30% H_2O_2 was added, and the reaction mixture was stirred for one additional hour. The aqueous phase was extracted several times with AcOEt, the combined organic layers were dried over anhydrous magnesium sulfate, filtered and concentrated. The residue was purified by flash chromatography using DCM/MeOH 9:1 to yield 55 mg of compound **32** as an orange amorphous solid (57%). Compound **32** showed identical spectroscopic data to those previously reported.[13]

4.1.16. Preparation of 2-(2-dimethylamino-ethyl)-5-(3-amino-phenyl)-benzo[*de*]isoquinoline-1,3-dione (33)

40 mg (0.10 mmol) of compound **32** in 5 mL of dry THF were hydrogenated in the presence of catalytic amounts of 10% Pd/C for 6 h. Then the reaction mixture was filtered through Celite and the solvent eliminated to yield quantitatively compound **33**. ^1H NMR ($CDCl_3$) δ: 2.37 (6H, s, H-3″, H-4″), 2.68 (2H, t, J = 6.9 Hz, H-2″), 3.95 (2H, s, NH_2), 4.35 (2H, t, J = 7.2 Hz, H-1″), 6.75 (1H, d, J = 7.6 Hz, H-4′), 7.06 (1H, s, H-2′), 7.14 (1H, d, J = 7.6 Hz, H-6′), , 7.29 (1H, t, J = 7.7 Hz, H-5′), 7.74 (1H, t, J = 8.4 Hz, H-6), 8.21 (1H, d, J = 8.2 Hz, H-5), 8.33 (1H, s, H-4), 8.55 (1H, d, J = 7.3 Hz, H-7), 8.82 (1H, s, H-2). ^{13}C NMR ($CDCl_3$) δ: 38.0 (t, C-1″), 45.3 (q, C-3″, C-4″), 57.1 (t, C-2″), 114.4 (d, C-2′), 115.1 (d, C-4′), 119.8 (d, C-6′), 122.7 (s, C-1a), 123.1 (s, C-7a), 127.3 (d, C-6), 130.2 (d, C-5′), 130.9 (d, C-2), 131.0 (d, C-7), 131.2 (d, C-4), 132.2 (s, C-1′, C-4b), 134.1 (d, C-5), 140.3 (s, C-3), 140.4 (s, C-4a), 147.3 (s, C-3′), 164.4 (s, C-8, C-1); EIMS *m*/*z* (%):359 (55), 315 (17) 301 (68), 217 (33), 288 (28), 189 (33), 71 (44), 58 (100); HREIMS: 359.1638 (calcd for $C_{22}H_{21}N_3O_2$ $[M]^+$ 359.1630); IR ν_{max}: 783, 1015, 1734, 1775, 2849, 2923, 3067 cm^{-1}.

4.2. Molecular modeling studies

Docking studies were performed using the Glide 5.7. The PDB structure 1CX3 ($d(ATGCAT)_2$ duplex (D6)) as a template and the starting coordinates were taken from the Protein Data Bank (www.rcsb.org). The receptor was optimized in Maestro 9.2 by using OPLS2005 force field before docking study. A receptor grid was generated using a 1.00 van der Waals (vdW) radius scaling factor and 0.25 partial charge cutoff. The radius of 30 Å from the central atom of DNA was defined as the binding site that covers the entire DNA. Ligands were prepared using Lig-Prep 2.5 as implemented in Maestro 9.2 and were docked using the extra precision mode (XP) without using any constraints and a 0.80 van der Waals (vdW) radius scaling factor and 0.15 partial charge cutoff and using Glidescore for ligand ranking. A modified version of ChemScore,[35] GlideScore implemented in Glide, was used to estimate binding affinity and rank ligands. One poses per ligand were generated and post-docking minimization was carried out.

4.3. Biological assays

The solvent for most stocks of the chemical agents employed was Dimethyl Sulphoxide (DMSO), especial Molecular Biology grade (DNase and RNase-free), from Sigma–Aldrich. Etoposide and Actinomycin D were purchased from Sigma–Aldrich and stored as a 10 mM stock in DMSO at −20 °C. Amonafide and related arylnaphthalimides were also stored in DMSO as a 10 mM stock at −20 °C until their use. Phleomycin was also purchased from Sigma–Aldrich but stored as a 1.5 mg/mL stock in H_2O at −20 °C.

4.3.1. MTT cell viability assay

The human cancer cell lines HL60 (promyelocytic leukemia), MCF-7 (breast adenocarcinoma) and SK-Br3 (breast adenocarcinoma) were purchased from ATCC and cultured in RPMI or DMEN containing 10% FBS, respectively. The MTT assay, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], was used to test cytotoxicity of naphthalimides and cell viability.[36] Briefly, cells were plated in 96-well plates at 10,000 cells/well. Twenty-four hours after plating, vehicle (0.1% DMSO, final concentration) or compound was added to cells at indicated concentrations. Forty eight hours following compound addition, MTT (Sigma–Aldrich, St. Louis, MO) was added to each well (0.5 mg/mL, final concentration) and plates were incubated for an additional 3 h at 37 °C. Medium was then aspirated and the formazan product was solubilized in SDS–HCl (20% SDS; HCl 0.02 M). The

absorbance of each well was measured at 570 nm using a microplate reader. Non linear regression analysis was performed to calculate IC_{50} according to the GraphPad Prism 5 program (GraphPad Software, San Diego, CA). The data are expressed by mean ± SD ($n = 3$).

4.3.2. TopoII-mediated DNA relaxation assay

The hTopoIIα enzyme was purchased from Inspiralis (Norwich, UK). The reaction conditions were as described before[37] with minor modifications. Briefly, the 20 μL reaction mixture contained 250 ng of pRYG plasmid DNA purchased from TopoGEN (Columbus, OH) and 1 mM ATP in the assay buffer [5 mM Tris·HCl (pH 7.5), 12.5 mM NaCl, 1 mM $MgCl_2$, 0.5 mM DTT and 10 μg/mL albumin]. The reaction components were added as follows: assay buffer, DNA, chemical compound from DMSO stock or just DMSO, and finally 1–2 units of hTopoIIα. The reaction mixture was incubated at 37 °C for 30 min, and quenched with 1% (w/v) SDS and 25 mM Na_2-EDTA. The mixture was treated with 0.25 mg/mL proteinase K (Roche) at 55 °C for 30 min to digest the protein. pRYG DNA topoisomers were resolved in 1% (w/v) agarose gel electrophoresis (0.5 V/cm) in 1× TBE buffer (89 mM Tris–borate and 2 mM Na_2-EDTA, pH 8) without ethidium bromide. The photograph was taken after staining the gel with ethidium bromide.

4.3.3. Stabilization of the TopoII cleavage-complex

The same procedure was used as above but this time 10 units of hTopoIIα were added 1–3 min before the compound addition. Electrophoresis was run (0.5 V/cm) in TAE buffer with 0.5 μg/mL ethidium bromide.

4.3.4. TopoI-mediated DNA relaxation assay

The wTopoI enzyme (Promega) was used. The reaction conditions were as follows: assay buffer [50 mM Tris–HCl (pH 7.5 at 25 °C), 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT and 20% glycerol], DNA (250 ng of pBSKS plasmid), chemical compound from DMSO stock or just DMSO, and 5 units of wTopoI enzyme were added in that order. The reaction mixture was incubated at 37 °C for 30 min, and quenched with 1% (w/v) SDS and 25 mM Na_2EDTA. The mixture was treated with 0.25 mg/mL proteinase K (Roche) at 55 °C for 30 min to digest the protein. pBSKS DNA topoisomers were resolved in 1% (w/v) agarose gel electrophoresis (0.5 V/cm) in 1× TBE buffer (89 mM Tris–borate and 2 mM Na_2EDTA, pH 8) without ethidium bromide. Since wTopoI tended to give a nearly fully relaxed form of the plasmid in our conditions, we also run a gel in TAE buffer with 0.5 μg/mL ethidium bromide to rule out that the nicked form was the main product.

4.3.5. Inhibition of DNA endonucleolytic cleavage

One unit of the restriction enzyme XhoI (Promega) was used to digest 250 ng of the pBSKS plasmid for one hour according to manufacturer instructions. This was carried out either with the vehicle alone (DMSO 1% v/v) or 100 μM of the tested compounds.

4.3.6. Dose–response yeast growth curves

Yeast strain FM588 was used as the reference wild type (*RAD52*) during this assay. It is a strain engineered for cell cycle and chromosome segregation studies,[38] which has been previously employed by us for genotoxic analysis.[39] On this strain, we knocked out the *RAD52* gene by PCR methods[40] in order to obtain FM888 (*rad52Δ*). For the assay, both strains were grown overnight at 25 °C to log phase (OD_{620}/mL = 0.4–0.8), then diluted to OD_{620}/mL = 0.01 (~10^5 cells/mL) and aliquoted into 96-well flat bottom plates. Tested compounds were added in eight 1:2 serial dilutions ranging from 1 to 128 μM in DMSO 1% (v/v). The vehicle alone was also used as a control. Growth was measured by OD_{620} readings after 24 h incubation at 25 °C without shaking. Relative growth to just DMSO 1% (v/v) was then calculated and plotted against the logarithm of drug concentrations.

4.3.7. Assay for G2 arrest and DNA repair nuclear Rad52 foci

Strain W3749-14C (*RAD52-YFP*) form S/G2/M-specific nuclear YFP foci under different types of DNA damage; especially those that generate DSBs.[32] For this assay, yeast cells were treated with 100 μM of the tested compounds while asynchronously growing at 25 °C. Chemical treatments with DMSO 1% (v/v) and Phleomycin 25 μg/mL were also included as controls. After 3 h, samples were taken and micrographed. All cells in the field were then classified according to the cell cycle stage they were in. Mononucleated budded cells where the bud size was more than two-thirds that of the mother were considered to be arrested in G2. Rad52 foci were detected and quantified in the YFP channel by using the CellProfiler software.[41] Briefly, whole YFP images were normalized following the procedure: most intense foci in the first photo taken for Phleomycin was set to 1, least intense pixel of the background was set to 0. A lower threshold of 0.1 was set for foci recognition. Then, total number of detected foci were divided by total number of cells to obtain the average foci number per cell.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.08.039.

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