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Naphthoindole-based analogues of tryptophan and tryptamine: Synthesis and cytotoxic properties

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Abstract—The efficacy of anthracycline based anticancer drugs is limited by pleiotropic drug resistance of tumor cells. Aiming at the design of anthracyclinone congeners capable of circumventing drug resistance, we synthesized naphthoindole containing derivatives of tryptophan and tryptamine. In doing so we adapted the traditional, gramine based approach for tryptophan and tryptamine synthesis. The most potent new compound, 3-(2-aminoethyl)-4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-dione (**16**), was equally cytotoxic (IC_{50} within low micromolar concentrations) for human K562 leukemia and HCT116 colon carcinoma cell lines and their isogenic sublines with genetically defined determinants of altered drug response, that is, the expression of the multidrug transporter P-glycoprotein and loss of pro-apoptotic p53. Each of these mechanisms conferred resistance to the reference drug adriamycin. In contrast, naphthotryptamine **16**, although less potent than adriamycin, was equally toxic for wild type cell lines and drug resistant counterparts. Moreover, at 3–5 μM **16** inhibited topoisomerase I in vitro. Thus, our novel naphthoindole based derivative of tryptamine gained new activities important for anticancer therapy, namely, suppression of topoisomerase I and the ability to overcome resistance mediated by P-glycoprotein expression and p53 dysfunction.

1. Introduction

The anthracycline antibiotics (e.g., adriamycin (**1**)) belong to the most valuable anticancer drugs.[1] However, their clinical use is frequently limited by organ toxicities (mostly dose-limiting heart and bone marrow toxicity), and by emergence of drug resistance in tumor cells. Numerous synthetic analogues of anthracycline have been developed aiming at drugs with high anticancer potency and attenuated general toxicity, such as the derivatives of anthracene-9,10-dione (mitoxantrone and ametantrone),[2] their aza-analogues (pixantrone),[3] and anthrapyrazoles (loxanthazol).[4] Still, tumor cells that possess the molecular determinants of altered chemotherapeutic drug response are resistant to anthracycline derivatives, thereby seriously limiting their therapeutic potential.[5] A critical prerequisite for an efficient anthracycline analogue is the ability to circumvent at least two factors involved in the resistance to these agents: (1) the transmembrane efflux pump P-glycoprotein (Pgp), a mechanism whereby the intracellular drug concentration is decreased in multidrug resistant (MDR) cells, and (2) dysfunction of p53, a major sensor of DNA damage by numerous stimuli including anthracyclines.[6] Our approach to design novel type synthetic analogues of anthracyclines with improved activity against drug resistant cells is based on modifications of 4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-dione (**2**), which can be considered as a heterocyclic analogue of the aglycon of anthracycline antibiotics in which the pyrrole cycle replaces the cyclohexene (A) ring. Importantly, several derivatives of naphtho[2,3-*f*]indole-5,10-dione demonstrated the ability to kill cultured human tumor cells and their either Pgp expressing or p53-null counterparts at low micromolar concentrations.[6,7]

Keywords: Derivatives of 4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-dione; Tryptophan; Tryptamine; Cytotoxic; Inhibitor of topoisomerase I; P-glycoprotein; p53; Circumvention of drug resistance; Human tumor cells.
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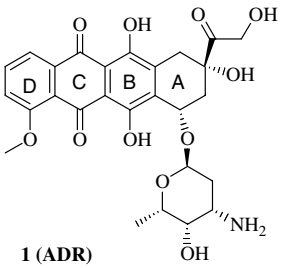
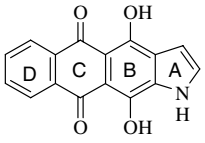
Natural and synthetic derivatives of tryptamine and tryptophan have long been an active pursuit of the pharmaceutical chemistry.[8] These data prompted us to synthesize 4,11-dihydroxy-1*H*-naphtho[2,3-*f*]indole-5,10-dione derivatives of tryptamine and tryptophan, and study their cytotoxicity and tentative intracellular targets important for cell death.



1 (ADR)



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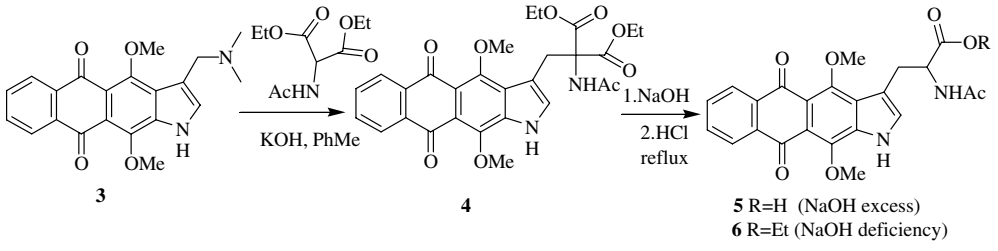
2. Results and discussion

2.1. Chemistry

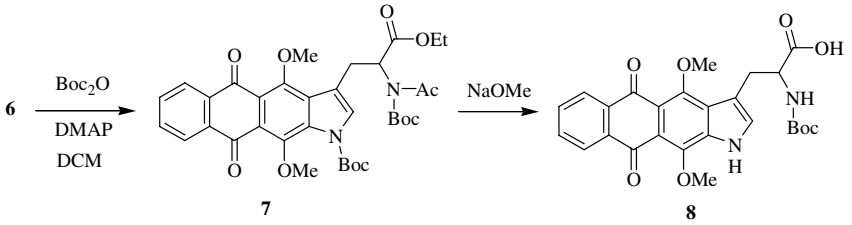
Our approach to synthesize the naphthoindole based derivatives of tryptophan and tryptamine was based on the consideration that naphthoindole chromophores can be readily reduced or hydrolyzed. Condensation of dimethylaminomethyl derivative of naphthoindole **3**[9] with diethyl acetamidomalonate under basic conditions[10] yielded compound **4** (Scheme 1). The acetylaminomalonic ester **4** was hydrolyzed by alkali. By varying the amount of alkali, one or both ester groups in **4** can be selectively hydrolyzed. Decarboxylation of the intermediate malonic acid derivatives led to *N*-acylnaphthotryptophan **5** or its ethyl ester **6** depending on the amount of alkali used for hydrolysis.

The hydrolysis of *N*-acetyl derivatives in tryptophan synthesis is traditionally performed in an alkaline solution.[11] However, no identifiable products were obtained after prolonged heating of naphthotryptophan **5** under alkaline or acidic conditions. The attempts to hydrolyze the acetyl group in naphthotryptophan **5** with acylase from *Aspergillus melleus*[12] were unsuccessful, probably because bulky chromophore prevents **5** from fitting well into the catalytic site of the enzyme. In some cases the hydrolysis of amides was accomplished after their carbamoylation.[13] Using this methodology, di-Boc-derivative **7** was synthesized by acylation of ethyl ester of naphthotryptophan **6** with Boc_2O in the presence of DMAP (Scheme 2). Hydrolysis of **7** by treatment with sodium methoxide[14] afforded the Boc-derivative of naphthotryptophan **8** in high yield.

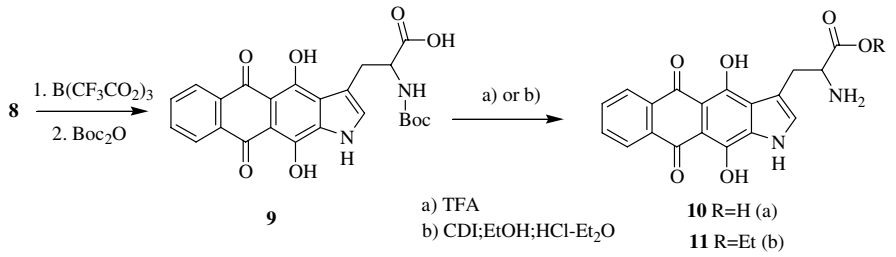
Earlier we have shown that naphthoindolediones containing the hydroxy groups in the chromophore moiety were more cytotoxic than their methoxy analogues.[6] *O*-Demethylation of methoxynaphthoindoles was carried out using a variety of Lewis acids, and BBr_3 gave good results.[6,7] However, demethylation of compound **8** with BBr_3 in DCM proceeded with low yield (<30%) probably due to the formation of the insoluble complex of **8** with BBr_3. To overcome this obstacle, demethylation of **8** was performed with Tris(trifluoroacetoxy)boron, a deprotecting reagent used in peptide chemistry[15] (Scheme 3). However, the obtained naphthotryptophan was poorly soluble in major organic solvents, which hampered the isolation and purification of the product. Therefore, by acylation with Boc_2O the demethylated amino acid was converted into N-Boc-derivative **9** isolated in 76% yield. Treatment of **9** with TFA gave pure naphthotryptophan **10**. Activation of the acid **9** with CDI followed by esterification and deblocking of the amino group produced ethyl ester of naphthotryptophan **11** in a reasonable yield.



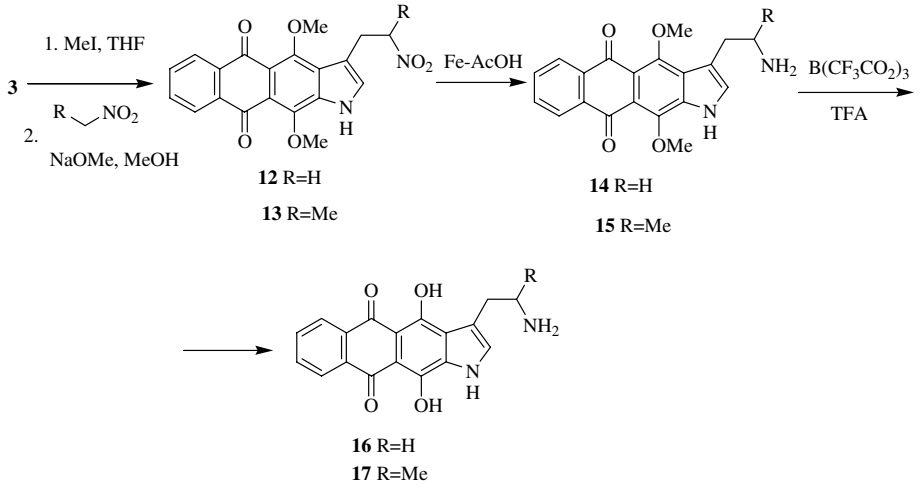
Scheme 1.



Scheme 2.



Scheme 3.



Scheme 4.

To prepare the naphthoindole analogues of tryptamine we used the conventional route of tryptamine synthesis, starting with gramines and proceeding via 3-nitroethylindoles. As in the case of gramine,[16] the dimethylamino derivative **3** reacted with nitromethane in the presence of the base, resulting in the nitroethyl derivative **12** in poor yield (15–20%). Quarternization[17] of the Mannich base **3** with iodomethane and subsequent treatment with sodium salts of nitromethane or nitroethane gave the nitroethane derivatives **12** and **13**, respectively, in 65–75% yields (Scheme 4).

The routine methods of nitroethylindole reduction[16,18–21] were not applicable for the reduction of nitro groups in **12** and **13** due to catalytic hydrogenation of the quinone moiety or reduction with $LiAlH_4$. The reduction of nitroalkanes by metals under acidic conditions, a classical method of alkylamine synthesis, has been recently proposed for preparing tryptamine.[22,23] Accordingly, treatment of **12** or **13** with iron powder in acetic acid resulted in the selective reduction of nitro groups without a significant reduction of the chromophore moiety, affording tryptamine and α-methyltryptamine in 50–60% yields. Demethylation of methoxy derivatives **14** and **15** by $B(CF_3CO_2)_3$ yielded final hydroxytryptamines **16** and **17**.

2.2. Biological testing

Study of antiproliferative activity of newly synthesized compounds revealed that the naphthoindole derivative of tryptophan **10** was inactive at concentrations >50 μM (Table 1). In contrast, compound **11**, the ethyl ester of **10**, inhibited the growth of HCT116 colon carcinoma cells, although at concentrations one order of magnitude higher than **ADR**. Among the tryptamine derivatives, 3-(2-aminoethyl)-4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-dione (**16**) was the most potent for both HCT116 and K562 cell lines. This compound caused cell death at low micromolar concentrations (Table 1). Most importantly, **16** was toxic for the wild type cell lines and their isogenic drug resistant counterparts. Indeed, K562i/S9 cells express functional Pgp and are resistant to Pgp transported agents.[24] In line with these observations the K562i/S9 subline was resistant to the reference drug **ADR** (RI = 14.3; Table 1). In striking contrast, the activity of **16** for K562i/S9 cells was even higher than for K562 cells (RI = 0.6). Furthermore, **16** caused similar toxicity in HCT116 cells ($p53^{+/+}$) and $p53^{-/-}$ variant HCT116p53KO (RI = 1.1), whereas HCT116p53KO cells were less sensitive to **ADR** than HCT116 cells (RI = 3.1; Table 1). Also, α-methyltryptamine analogue **17** showed similar potency for wild type and drug resistant cell lines; however, this compound was less active than **16** against each of the tested models.

These results indicate that modifications of 4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-dione can yield the compounds with improved characteristics as potential anticancer drugs. Indeed, the water soluble tryptamine derivatives **16** and **17** are advantageous for circumventing

Table 1. Cytotoxicity (IC_{50} by MTT test) of 4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-dione derivatives and **ADR** (**1**) for wild type and drug resistant cell lines

Compound	IC_{50} (μM)		RI[a]	IC_{50} (μM)		RI[a]
	K562	K562i/S9		HCT116	HCT116p53KO	
1	0.14 ± 0.04	2.0 ± 0.1	14.3	1.4 ± 0.1	4.4 ± 0.4	3.1
10	>50	nt[b]	—	>50	nt[b]	—
11	>50	nt[b]	—	14.9 ± 0.9	nt[b]	—
16	4.0 ± 0.5	2.2 ± 0.3	0.6	1.1 ± 0.1	1.2 ± 0.1	1.1
17	12.0 ± 0.5	13.4 ± 0.5	1.1	4.8 ± 0.4	5.0 ± 0.5	1.0

Mean ± S.E. of 3 experiments.
[a] RI, resistance index = IC_{50}(K562i/S9)/IC_{50}(K562) or IC_{50}(HCT116p53KO)/IC_{50}(HCT116), respectively.
[b] Not tested.

drug resistance mediated by Pgp expression or p53 dysfunction, the two major determinants of resistance to anthracycline antibiotics including **ADR**.

To identify tentative intracellular target important for cytotoxicity of 4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-dione derivatives, we tested the most potent compound **16** for the ability to modulate topoisomerase I (topo I) activity in vitro. As shown in Figure 1a, addition of purified topo I caused complete relaxation of supercoiled DNA and formation of topoisomers (relaxed DNA; compare lanes 1 and 2). These effects were attenuated by compound **16** in a dose dependent manner. At 2.5 μM of **16** an increased amount of nicked DNA was detectable (lanes 3 and 4). At 5 μM **16** almost completely prevented the relaxation of supercoiled DNA (lanes 5 and 6), and no relaxation occurred at 10 μM (lanes 7 and 8). Figure 1b shows that already at 3 μM of **16** topo I activity was inhibited. This conclusion was substantiated by the analysis of DNA topoisomers (generated in the same reaction as in Fig. 1b) resolved in ethidium bromide-containing gel (Fig. 1c). Similar results were obtained in DNA relaxation assays performed with HCT116 nuclear extracts instead of purified topo I (data not shown). Thus, compound **16** inhibited topo I in vitro at low micromolar concentrations, with submaximal inhibition at 3–5 μM. In striking contrast, camptothecin, a classical topo I inhibitor,[25] attenuated topo I mediated DNA relaxation only at ≫10 μM.

Earlier we have demonstrated that, among *N*-aminoalkyl derivatives of 4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-

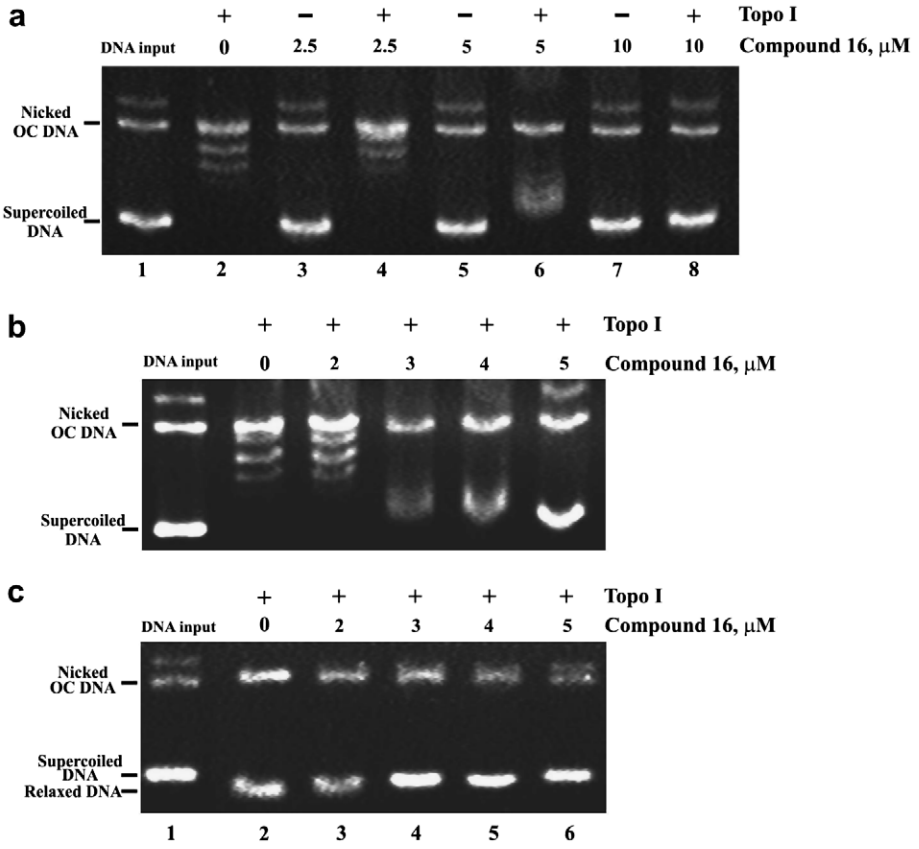


Figure 1. Inhibition of topo I activity by compound **16**. (a) DNA topoisomers were resolved by electrophoresis in 1% agarose gel and stained with ethidium bromide. (b and c) Products of the same decatenation reaction. (b) DNA topoisomers were analyzed as in a; c, electrophoresis was performed with ethidium bromide in the gel and buffer. OC, open circular DNA. See text for details.

dione (**2**), *N*-aminobutyl derivatives were the most potent, whereas *N*-aminoethyl congeners had only marginal activity.[7] Studying the cytotoxicity of 4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-diones with the pharmacophore groups in position 3, we found surprisingly high cytotoxicity of the derivatives with short spacers, for example, tryptamine and gramine analogues [this article and Ref. 6]. We hypothesize that the terminal position of amino group in 3-aminoethyl derivative of naphtho[2,3-*f*]indole-5,10-dione **16** could be a reason for poor recognition of this compound by Pgp and kill of MDR cells.

Importantly, our naphthoindole based derivative of tryptamine gained an activity that has not been commonly attributed to anthracyclines, that is, inhibition of topo I. Indeed, **ADR** is a topo II blocker,[26] and the cellular content of topo II is a more important prerequisite for cytotoxicity of anthracyclines than topo I content.[27] Mono- (idarubicin) and disaccharide anthracyclines have been shown to trap topo I-DNA complexes, although the inhibition of topo II by these compounds was more pronounced.[27] On the other hand, aclarubicin, a trisaccharide containing anthracycline derivative, displays a dual activity against topo I and II.[28,29] Whereas inhibition of topo II catalytic activity can be secondary due to drug binding to DNA, aclarubicin has been reported to exert a concomitant poisoning effect on topo I, in a fashion similar to that of camptothecin.[27] In our experiments **ADR** blocked topo I mediated DNA relaxation at >50 μM, whereas the naphthoindole derivative of tryptamine (compound **16**) was active at 3 μM. Altogether, one may expect that chemical modifications of the anthracycline moiety would yield new agents with activities against single cellular target and/or a combination of targets. Studies are in progress by our group to reveal whether compound **16** binds DNA and blocks topo II. Should any or both activities be identified, this will expand the list of intracellular targets for naphthoindole based derivatives of tryptamine or tryptophan. We conclude that tryptamine- or tryptophan-containing naphthoindole analogues are perspective anticancer agents potent for cells with Pgp expression and p53 deletion.

3. Experimental

3.1. General

NMR spectra were registered on a Varian VXR-400 instrument operated at 400 MHz (^{1}H NMR). Chemical shifts were measured in CD_3OD, DMSO-d_6 or $CDCl_3$ using tetramethylsilane or residual protic solvents as internal standards. Analytical TLC was performed on Silica Gel F_{254} plates (Merck) and column chromatography on Silica Gel Merck 60. Melting points were determined on a Buchi SMP-20 apparatus and are uncorrected. Mass-spectra were obtained on an SSQ 710 Finnigan instrument. UV spectra were recorded on Hitachi-U2000 spectrophotometer. All solutions were dried over Na_2SO_4 and evaporated at reduced pressure on a Buchi-R200 rotary evaporator at the temperature below 45 °C. All products were vacuum dried at room temperature.

3.2. Cell lines, drug treatment, and viability assay

The K562 human leukemia cell line (American Type Culture Collection; ATCC, Manassas, VA) and its variant K562i/S9 that expresses Pgp after *MDR*1/Pgp cDNA transfer and immunoflow cytometry-based sorting of Pgp-positive cells,[30] HCT116 colon carcinoma cell line (ATCC) with wild type p53 ($p53^{+/+}$) and HCT116p53KO subline (both alleles of p53 deleted by homologous recombination[31]) ($p53^{-/-}$) were cultured in RPMI-1640 supplemented with 5% fetal calf serum (HyClone, Logan, UT), 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C, 5% CO_2 in humidified atmosphere. Cells in logarithmic phase of growth were used in all experiments. All reagents were from Sigma Chemical Co., St. Louis, MO unless specified otherwise. Novel compounds were dissolved in 10% aqueous DMSO as 10 mM stock solutions followed by serial dilutions in water immediately before experiments. The cytotoxicity was determined in a formazan conversion assay (MTT test).[32] Briefly, cells (5×10^3 in 190 μL of culture medium) were plated into a 96-well plate (Becton Dickinson, Franklin Lakes, NJ) and treated with 0.1% dimethylsulfoxide (DMSO; vehicle control) or with increasing concentrations of tested compounds (each dose in duplicate) for 72 h. After the completion of drug exposure, 50 μg of 3-(4,5-dimethylthiazol-2)-2,5-diphenyl tetrazolium bromide was added into each well for an additional 1–3 h. Formazan was dissolved in dimethylsulfoxide, and the absorbance at $\lambda = 540$ nm was measured. Cell viability at given drug concentration was calculated as percentage of absorbance in wells with drug treated cells to that of vehicle control cells (100%).

3.3. Topo I assay

The ability of **16** to modulate the activity of topo I in vitro was determined in a DNA relaxation assay using the Topo I kit (TopoGen, Port Oranges, FL) as recommended by the manufacturer. Briefly, 4 U of purified topo I (Amersham Biosci., UK) was incubated with 0.25 μg of supercoiled pHOT1 plasmid DNA in the buffer (10 mM Tris–HCl, pH 7.9, 1 mM ethylenediaminetetraacetic acid disodium salt, 0.15 M NaCl, 0.1% bovine serum albumin, 0.1 mM spermidine, and 5% glycerol) in the presence of 0.1% DMSO (vehicle control) or compound **16** (2–10 μM) at 37 °C for 30 min. The reaction was terminated by the addition of sarcosyl (1% final concentration). DNA topoisomers were resolved by electrophoresis in 1% agarose gel in the buffer containing 40 mM Tris-acetate, pH 7.6, 1 mM ethylenediaminetetraacetic acid disodium salt (3 h, 50 V) in the presence or absence of 0.5 μg/mL ethidium bromide. In some experiments topo I inhibition assays were performed using extracts prepared from the nuclei of HCT116 cells essentially as described.[33]

3.4. Diethyl 2-(acetylamino)-2-[(4,11-dimethoxy-1*H*-naphtho[2,3-*f*]indole-5,10-dione-3-yl)methyl]malonate (4)

To a well-stirring solution of gramine derivative **3**[9] (800 mg, 2.2 mmol) in toluene (100 mL) diethyl acetamidomalonate (750 mg, 3.5 mmol) and powdered KOH

(40 mg, 0.8 mmol) were added. The mixture was stirred with reflux for 6 h. The resulting solution was cooled, washed with water, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography with chloroform–methanol (10:1 → 3:1) to give **4** (940 mg, 80%); 182–184 °C (toluene–*n*-heptane (1:1)); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.32 (br s, 1H, N-H), 8.23 (m, 2H, 6-H, 9-H), 7.73 (m, 2H, 7-H, 8-H), 7.16 (d, 1H, J = 2.5 Hz 2-H), 6.62 (br s, 1H, N-H), 4.27 (m, 4H, –CH_2CH$_3$), 4.09 (s, 3H, –OCH$_3$), 3.97 (s, 3H, –OCH$_3$), 3.47 (dd, 1H, J = 4.5 Hz –CH$_2$–), 3.33 (m, 1H, –CH$_2$–), 2.02 (s, 3H, –COCH$_3$), 1.28 (t, 6H, –CH$_2$CH_3); MS *m*/*z* 536 (18), 477 (13), 320 (100), 302 (28). Anal. Calcd for $C_{28}H_{28}N_2O_9$: C, 62.68; H, 5.26; N, 5.22. Found: C, 62.04; H. 5.61; N. 5.33.

3.5. *N*-Acetyl-3-(4,11-dimethoxy-1*H*-naphtho[2,3-*f*]indole-5,10-dione-3-yl)alanine (5)

To a stirring solution of diethyl malonate derivative **4** (200 mg, 0.36 mmol) in mixture of THF (30 mL) and methanol (20 mL) was added solution NaOH (100 mg, 2.5 mmol) in water (2 mL). The mixture was heated at 50 °C for 2 h. After the complete conversion of **4** (as determined by TLC) the reaction mixture was evaporated, quenched with water (30 mL), and acidified by 0.1 N HCl to pH 4.0. The resulting solution was refluxed for 1.5 h and cooled. The obtained solid was filtered, dried, and crystallized from toluene–1,4-dioxane mixture (1:1) to afford 120 mg (74%) title compound as an yellow solid, mp 294–296 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.18 (d, 1H, J = 2.5 Hz, NH), 8.22 (d, 1H, J = 7.9 Hz, N-H), 8.13 (m, 2H, 6-H, 9-H), 7.83 (m, 2H, 7-H, 8-H), 7.40 (d, 1H, J = 2.5 Hz, 2-H), 4.57 (m, 1H, –CH–), 3.96 (s, 3H, –OCH$_3$), 3.94 (s, 3H, –OCH$_3$), 3.44 (dd, 1H, J = 4.5 Hz, –CH$_2$–), 3.05 (dd, 1H, J = 10.1 Hz, –CH$_2$–), 1.79 (s, 3H, –COCH$_3$); ^{13}C NMR (400 MHz, DMSO-d_6) δ 182.27 (C═O), 181.95 (C═O), 173.67 (CO_2H), 169.41 (CONH), 154.06 (C), 145.12 (C), 134.62 (C), 134.52 (C), 134.21 (C), 126.03 (C), 118.48 (C), 116.42 (C), 114.88 (C), 133.58 (CH), 133.38 (CH), 129.21 (CH), 126.20 (CH), 125.95 (CH), 53.07 (CH), 27.97 (CH_2), 62.18 (CH_3), 61.72 (CH_3), 22.44 (CH_3); MS *m*/*z* 436 (21), 377 (16), 320 (100), 302 (31). Anal. Calcd for $C_{23}H_{20}N_2O_7$: C, 63.30; H, 4.62; N, 6.42. Found: C, 63.12; H. 4.65; N. 6.49.

3.6. *N*-Acetyl-3-(4,11-dimethoxy-1*H*-naphtho[2,3-*f*]indole-5,10-dione-3-yl)alanine ethyl ester (6)

To a stirring solution of diethyl malonate derivative **4** (0.6 g, 1.1 mmol) in the mixture of THF (40 mL) and ethanol (50 mL) solution of NaOH (90.0 mg, 2.2 mmol) in water (1 mL) was added. The mixture was heated at 50 °C for 3 h, quenched with water (100 mL), and acidified by 0.05 N HCl to pH 6.0. The resulting solution was refluxed for 3 h and cooled. The product was extracted with warm AcOEt (3× 70 mL), the organic layers were combined, washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by chromatography on silica gel with chloroform–methanol (10:1 → 3:1) to give 310 mg (62%) of alanine derivative **6** as an yellow solid, mp 168–170 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.72 (br s, 1H, N-H), 8.24 (m, 2H, 6-H, 9-H), 7.74 (m, 2H, 7-H, 8-H), 7.23 (s, 1H, 2-H), 6.89 (d, 1H, J = 7.9 Hz, N-H), 4.84 (m, 1H, –CH–), 4.21 (m, 2H, –CH_2CH$_3$), 4.08 (s, 3H, –OCH$_3$), 4.06 (s, 3H, –OCH$_3$), 3.47 (dd, 1H, J = 4.5 Hz, –CH$_2$–), 3.33 (m, 1H, –CH$_2$–), 1.91 (s, 3H, –COCH$_3$), 1.26 (t, 3H, –CH$_2$CH_3); MS *m*/*z* 464 (27), 405 (31), 320 (100), 302 (34). Anal. Calcd for $C_{25}H_{24}N_2O_7$: C, 64.65; H, 5.21; N, 6.03. Found: C, 64.77; H, 5.40; N, 6.12.

3.7. *N*-Acetyl-*N*-(*tert*-butoxycarbonyl)-3-[1-(*tert*-butoxycarbonyl)-4,11-dimethoxy-1*H*-naphtho[2,3-*f*]indole-5,10-dione-3-yl]alanine ethyl ester (7)

A solution of alanine derivative **6** (260 mg, 0.56 mmol), 4-(*N*,*N*-dimethylamino)pyridine (240 mg, 2 mmol), and Boc_2O (400 mg, 1.9 mmol) in dichloromethane (20.0 mL) was refluxed for 4 h. The resulting reaction mixture was washed with 0.5 N HCl, washed with brine, dried, and evaporated. The residue was purified by flash chromatography on silica gel pad using chloroform–methanol (10:0 → 7:1) to give 310 mg (84%) di-Boc-derivative **7** as an yellow foam, mp 158–160 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (m, 2H, 6-H, 9-H), 7.74 (m, 2H, 7-H, 8-H), 7.33 (s, 1H, 2-H), 5.58 (m, 1H, –CH–), 4.25 (m, 2H, –CH_2CH$_3$), 4.07 (s, 3H, –OCH$_3$), 3.96 (s, 3H, –OCH$_3$), 3.81 (m, 1H, –CH$_2$–), 3.38 (m, 1H, –CH$_2$–), 2.42 (s, 3H, –COCH$_3$), 1.67 (s, 9H, *tert*-Bu), 1.30 (t, 3H, –CH$_2$CH_3), 1.22 (s, 9H, *tert*-Bu); MS *m*/*z* 664 (M^+, 41), 564 (17), 464 (100), 405 (81), 320 (87), 57 (81). Anal. Calcd for $C_{35}H_{40}N_2O_{11}$: C, 63.24; H, 6.07; N, 4.21. Found: C, 63.12; H, 6.01; N, 4.32.

3.8. *N*-(*tert*-Butoxycarbonyl)-3-(4,11-dimethoxy-1*H*-naphtho[2,3-*f*]indole-5,10-dione-3-yl)alanine (8)

To a stirring solution of di-Boc-derivative **7** (260 mg, 0.4 mmol) in methanol (20 mL) solution MeONa (0.1 N, 15 mL) was added, and the mixture was stirred for 2 h. The resulting solution was evaporated, quenched with water (20 mL), and acidified by the addition of aqueous HCl (0.05 N) to pH 5.0. The product was twice extracted with BuOH, the organic layers were combined, washed with brine, dried over Na_2SO_4, and evaporated. The residue was recrystallized from toluene–1,4-dioxane mixture (3:1) to afford N-Boc-alanine derivative **8** (180 mg, 92%) as an yellow solid, mp 235–238 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.14 (br s, 1H, N-H), 8.13 (m, 2H, 6-H, 9-H), 7.83 (m, 2H, 7-H, 8-H), 7.41 (s, 1H, 2-H), 7.06 (d, 1H, N-H), 4.30 (m, 1H, –CH–), 3.97 (s, 3H, –OCH$_3$), 3.96 (s, 3H, –OCH$_3$), 3.01 (m, 2H, –CH$_2$–), 1.31 (s, 9H, *tert*-Bu); MS *m*/*z* 494 (M^+, 22), 437 (19), 421 (62), 362 (100), 334 (85), 320 (82), 306 (38), 56 (85). Anal. Calcd for $C_{26}H_{26}N_2O_8$: C, 63.15; H, 5.30; N, 5.67. Found: C, 63.10; H, 5.22; N, 5.55.

3.9. *N*-(*tert*-Butoxycarbonyl)-3-(4,11-dihydroxy-1*H*-naphtho[2,3-*f*]indole-5,10-dione-3-yl)alanine (9)

A solution of BBr_3 (10 mL, 1 M in DCM) was added at 0 °C to a solution of TFA (4.0 mL, 3.5 mmol) in DCM (40 mL). The resulting solution was evaporated,

the residue dissolved in TFA (15 mL), and a solution of **8** (150 mg, 0.3 mmol) in TFA (5.0 mL) was added. After 15 min the violet solution was concentrated and quenched by the addition of water (50 mL). To the stirred mixture 1 N NaOH was added dropwise until pH 11.0, the resulting solution was heated at reflux for 15 min and then cooled down to room temperature. The solution was quenched with isopropanol (50 mL) followed by the addition of Boc_2O (180 mg, 0.4 mmol). The mixture was stirred overnight, concentrated to 40–50 mL volume, cooled to 0 °C, and acidified by careful addition of aqueous HCl (0.05 N) to pH 3.0. The products were extracted with *n*-butanol (3× 40 mL), washed with brine, dried, and evaporated. The residue was purified by chromatography on silica gel with chloroform–methanol (10:1 → 2:1) to give 105 mg (76%) Boc-derivative **9** as a red solid, mp 264–266 (dec) °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 15.28 (br s, 1H, OH), 14.77 (br s, 1H, OH), 12.88 (br s, 1H, NH), 8.20 (m, 2H, 6-H, 9-H), 7.77 (m, 2H, 7-H, 8-H), 7.19 (s, 1H, 2-H), 7.02 (d, 1H, N-H), 4.26 (m, 1H, –CH–), 3.36 (m, 1H, $-CH_2-$), 3.02 (m, 1H, $-CH_2-$), 1.30 (s, 9H, -*tert*-Bu); MS *m*/*z* 466 (M^+, 27), 410 (57), 349 (100), 321 (54), 304 (36), 292 (60), 56 (78). Anal. Calcd for $C_{24}H_{22}N_2O_8$: C, 61.80; H, 4.75; N, 6.01. Found: C, 61.96; H, 4.81; N, 6.15.

3.10. 3-(4,11-Dihydroxy-1*H*-naphtho[2,3-*f*]indole-5,10-dione-3-yl)alanine (10)

Boc-derivative **9** (50 mg, 0.1 mmol) was dissolved in TFA (5.0 mL) and after 1 h the solution was evaporated. The residue was dissolved in 0.1 N NaOH (10 mL) and acidified by the addition of aqueous HCl (0.1 N) to pH 5.0. The precipitated product was filtered, washed with water, and dried to give the tryptophan derivative **10** (37 mg 94%) as a red solid, mp 290–295 (dec) °C; ^{1}H NMR (400 MHz, DMSO-d_6 CD_3OD, 2:1, 35 °C) δ 8.33 (m, 2H, 6-H, 9-H), 7.87 (m, 2H, 7-H, 8-H), 7.33 (s, 1H, 2-H), 4.29 (dd, 1H, J = 6.1 Hz, –CH–), 3.50 (dd, 1H, J = 6.1 Hz, $-CH_2-$), 3.50 (dd, 1H, J = 8.5 Hz, $-CH_2-$); UV (ethanol) λ_{max} ($\log\varepsilon$) 237 (4.5), 272 (4.6), 451 sh (4.2), 475 (4.4), 510 (4.3) nm; MS *m*/*z* 322 (M^+-CO_2, 18), 304 (29), 293 (100), 292 (81). Anal. Calcd for $C_{19}H_{14}N_2O_6$: C, 62.30; H, 3.85; N, 7.65. Found: C, 62.44; H, 3.90; N, 7.66.

3.11. 3-(4,11-Dihydroxy-1*H*-naphtho[2,3-*f*]indole-5,10-dione-3-yl)alanine ethyl ester hydrochloride (11)

Boc-derivative **9** (40 mg, 0.09 mmol) was dissolved with heating in stirred DMF (10 mL) and then carbonyldiimidazole (17 mg, 0.1 mmol) was added. The resulting solution was stirred at 35 °C for 30 min followed by the addition of absolute ethanol (10 mL). The mixture was refluxed for 20 min and concentrated. The residue was diluted with $CHCl_3$ and washed with water. The aqueous layer was again extracted with $CHCl_3$, the organic extracts were pooled, washed with water, dried, and evaporated. The product was treated with 0.5 N HCl in ethanol (10 mL), stirred overnight, and quenched with Et_2O (20 mL). The precipitated product was filtered, washed with Et_2O, and dried to afford hydrochloride of ethyl ester **11** (31 mg, 85%) as a red solid, mp 242–245 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.32 (m, 2H, 6-H, 9-H), 7.79 (m, 2H, 7-H, 8-H), 7.15 (s, 1H, 2-H), 4.52 (dd, 1H, J = 6.1 Hz, –CH–), 4.28 (m, 2H, $-CH_2CH_3$), 3.54 (dd, 1H, J = 6.1 Hz, $-CH_2-$), 3.24 (dd, 1H, J = 7.8 Hz, $-CH_2-$), 1.28 (t, 3H, $-CH_2CH_3$); MS *m*/*z* 394 (M^+, 41), 376 (11), 321 (100), 304 (79), 293 (79), 292 (87). Anal. Calcd for $C_{21}H_{19}ClN_2O_6$: C, 58.54; H, 4.45; N, 6.50. Found: C, 58.40; H, 4.54; N, 6.60.

3.12. 4,11-Dimethoxy-3-(2-nitroethyl)-1*H*-naphtho[2,3-*f*]indole-5,10-dione (12)

To a stirring solution of gramine **3**[9] (400 mg, 0.55 mmol) in tetrahydrofurane (30 mL) CH_3I (1.4 mL, 5.0 mmol) was added, and the mixture was stirred overnight. The precipitate was filtered and washed with ether. After drying crude iodomethylate of gramine **3** (470 mg, 85%) was afforded as yellow solid. To a stirring solution MeONa (1 N, 12 mL) in MeOH were added nitomethane (1.0 mL, 16.4 mmol) and, 10 min later, quaternary salts (220 mg, 0.4 mmol). The mixture was stirred for 2 h and concentrated. The residue was diluted with water and acidified by the addition of 1 N HCl to pH 3.0. The product was extracted with AcOEt (3× 50 mL), the organic layers were collected, washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by chromatography on silica gel with chloroform–methanol (10:0 → 5:1) to give 106 mg (64%) of nitroethane derivative **12** as an yellow solid, mp 178–179 °C (benzene); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.17 (br s, 1H, NH), 8.25 (m, 2H, 6-H, 9-H), 7.73 (m, 2H, 7-H, 8-H), 7.23 (d, 1H, J = 2.7 Hz, 2-H), 4.79 (t, 2H, J = 9.1 Hz, $-CH_2NO_2$), 4.08 (s, 3H, $-OCH_3$), 4.07 (s, 3H, $-OCH_3$), 3.54 (t, 2H, J = 9.1 Hz, $-CH_2-$); MS *m*/*z* 380 (M^+, 74), 334 (32), 318 (58), 304 (88), 301 (100), 290 (42), 276 (62). Anal. Calcd for $C_{20}H_{16}N_2O_6$: C, 63.16; H, 4.24; N, 7.37. Found: C, 63.24; H, 4.11; N, 7.43.

3.13. 4,11-Dimethoxy-3-(2-nitropropyl)-1*H*-naphtho[2,3-*f*]indole-5,10-dione (13)

This compound was prepared from naphthoindole **3** and nitroethane as described for **12**. Yield 73%, mp 154–156 °C (benzene); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.08 (br s, 1 H, NH), 8.27 (m, 2H, 6-H, 9-H), 7.75 (m, 2H, 7-H, 8-H), 7.23 (d, 1H, J = 2.7 Hz, 2-H), 5.14 (m, 1H, $-CHNO_2$), 4.10 (s, 3H, $-OCH_3$), 4.09 (s, 3H, $-OCH_3$), 3.54 (dd, 1H, J = 9.0 Hz, $-CH_2-$), 3.54 (dd, 1H, J = 4.9 Hz, $-CH_2-$), 1.64 (d, 3H, J = 6.7 Hz, $-CH_3$); MS *m*/*z* 394 (M^+, 100), 348 (41), 332 (38), 314 (62), 304 (32), 290 (35). Anal. Calcd for $C_{21}H_{18}N_2O_6$: C, 63.96; H, 4.60; N, 7.10. Found: C, 63.84; H, 4.72; N, 7.22.

3.14. 3-(2-Aminoethyl)-4,11-dimethoxy-1*H*-naphtho[2,3-*f*]indole-5,10-dione (14)

To a warm solution of nitroethane derivative **12** (95 mg, 0.25 mmol) in mixture of acetic acid (15.0 mL) and EtOH (15.0 mL) activated iron (500 mg, 6.1 mmol) was added, the mixture was refluxed for 30 min and cooled down. The filtered precipitate was washed with warm EtOH and filtrate was evaporated. The residue

was diluted with water, the aqueous 1 N NaOH was added to pH 9.0, and the product was extracted with BuOH (3× 30 mL). The organic layers were pooled, washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by chromatography on silica gel with chloroform–methanol-concd NH_4OH (10:1:0 → 10:4:1) to give 45 mg (51%) of tryptamine **14** as an yellow solid, mp 155–157 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (m, 2H, 6-H, 9-H), 7.71 (m, 2H, 7-H, 8-H), 7.23 (s, 1H, 2-H), 4.05 (s, 3H, –OCH_3), 4.04 (s, 3H, –OCH_3), 3.31 (m, 4H, –$(CH_2)_2$–); MS *m*/*z* 350 (M^+, 14), 333 (22), 318 (58), 306 (100), 290 (46). Anal. Calcd for $C_{20}H_{18}N_2O_4$: C, 68.56; H, 5.18; N, 8.00. Found: C, 68.46; H, 5.01; N, 8.31.

3.15. 3-(2-Aminopropyl)-4,11-dimethoxy-1*H*-naphtho[2,3-*f*]indole-5,10-dione (15)

This compound was synthesized from naphthoindole **13** as described for **14**. Yield 58%, mp 122–124 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (m, 2H, 6-H, 9-H), 7.70 (m, 2H, 7-H, 8-H), 7.20 (s, 1H, 2-H), 4.06 (s, 6H, –OCH_3), 3.40 (m, 1H, –CH–), 3.06 (dd, 1H, J = 4.6 Hz, –CH_2–), 2.75 (dd, 1H, J = 8.5 Hz, –CH_2–), 1.21 (d, 3H, J = 6.2 Hz, –CH_3); MS *m*/*z* 364 (M^+, 2), 346 (9), 332 (61), 321 (75), 306 (100), 291 (64). Anal. Calcd for $C_{21}H_{20}N_2O_4$: C, 69.22; H, 5.53; N, 7.69. Found: C, 69.14; H, 5.40; N, 7.98.

3.16. 3-(2-Aminoethyl)-4,11-dihydroxy-1*H*-naphtho[2,3-*f*]indole-5,10-dione (16)

A solution of BBr_3 (2.0 mL, 1 M in DCM) was added to a solution of TFA (1.0 mL, 3.5 mmol) in DCM (40 mL) at 0 °C. The resulting mixture was evaporated, the residue dissolved in TFA (2 mL), and a solution of tryptamine **14** (35 mg, 0.1 mmol) in TFA (5.0 mL) was added. After 15 min the violet solution was concentrated and quenched with water (50 mL). To the stirred mixture 1 N NaOH was added dropwise until pH 11.0 was reached, the resulting solution was heated at reflux for 15 min, then cooled to room temperature and acidified with 0.05 N HCl to pH 7.0. The product was extracted with hot *n*-butanol (3× 20 mL), washed with brine, dried, and evaporated. The residue was purified by chromatography on silica gel using chloroform–methanol-concd NH_4OH (10:1:0 → 10:4:1) to give 23 mg (71%) of tryptamine **14** as a red solid, mp > 240 (dec) °C. Hydrochloride mp > 260 (dec) °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.35 (m, 2H, 6-H, 9-H), 7.84 (m, 2H, 7-H, 8-H), 7.29 (s, 1H, 2-H), 2.95 (m, 4H, –$(CH_2)_2$–); UV (ethanol) λ_{max} (log ε) 239 (4.5), 245 (4.6), 279 (4.6), 458 sh (4.2), 475 (4.4), 510 (4.4) nm; MS *m*/*z* 322 (M^+, 8), 293 (100), 277 (5). Anal. Calcd for $C_{18}H_{14}N_2O_4$: C, 67.07; H, 4.38; N, 8.69. Found: C, 66.87; H, 4.21; N, 8.90.

3.17. 3-(2-Aminopropyl)-4,11-dihydroxy-1*H*-naphtho[2,3-*f*]indole-5,10-dione (17)

This compound was synthesized from naphthoindole **15** as described for compound **16** in 73% yield. Hydrochloride mp > 240 (dec) °C; ^{1}H NMR (400 MHz, D_2O) δ 7.36–7.5 (m, 4H, 6-H, 7-H, 8-H, 9-H), 6.66 (s, 1H, 2-H), 3.40 (m, 1H, –CH–), 2.64 (dd, 1H, J = 5.6 Hz, –CH_2–), 2.52 (dd, 1H, J = 8.3 Hz, –CH_2–), 1.21 (d, 3H, J = 6.4 Hz, –CH_3); UV (ethanol) λ_{max} (log ε) 239 (4.6), 245 (4.6), 279 (4.6), 454 sh (4.1), 476 (4.4), 511 (4.4) nm; MS *m*/*z* 336 (M^+, 4), 318 (61), 303 (72), 293 (100), 277 (18). Anal. Calcd for $C_{19}H_{17}ClN_2O_4$: C, 61.21; H, 4.60; N, 7.51. Found: C, 61.11; H, 4.75; N, 7.43.

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