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Bioorganic & Medicinal Chemistry 13 (2005) 2285–2291
Bioorganic & Medicinal Chemistry

3-Aminomethyl derivatives of 4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-dione for circumvention of anticancer drug resistance

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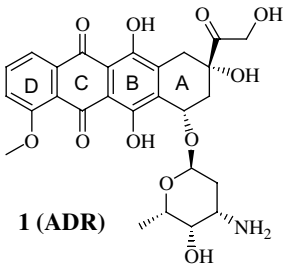
Received 27 August 2004; revised 21 December 2004; accepted 22 December 2004
Available online 18 January 2005

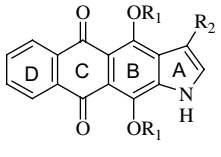
Abstract—A series of 3-aminomethyl derivatives of 4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-dione was synthesized by Mannich reaction or by the transamination of 3-dimethylaminomethyl 4,11-dihydroxy- or 4,11-dimethoxynaphtho[2,3-*f*]indole-5,10-dione. The potency of novel derivatives was tested on a National Cancer Institute panel of 60 human tumor cell lines as well as in cells with genetically defined determinants of cytotoxic drug resistance, P-glycoprotein (Pgp) expression, and p53 inactivation. Mannich derivatives of 4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-dione with an additional amino function in their side chain, demonstrated equal cytotoxicity against the parental K562 leukemia cells and their Pgp-positive subline, whereas the latter showed ~7-fold resistance to adriamycin, a Pgp transported drug. 3-(1-Piperazinyl)methyl and 3-(quinuclidin-3-yl)aminomethyl derivatives of 4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-dione killed HCT116 colon carcinoma cells (carrying wild type p53) and their p53-null variant within the similar range of concentrations. We conclude that Mannich modification of 4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-dione, especially when cyclic diamine (e.g., piperazine, quinuclidine) is used, confers an important feature to the resulting compounds, namely, the potency for tumor cells otherwise resistant to a variety of anticancer drugs.

1. Introduction

The continuing therapeutic success of the anthracycline adriamycin (**ADR**, doxorubicin, **1**) continues to interest investigations of anthracyclines and their analogs as anticancer agents. Unfortunately, the blood and heart toxicity as well as emergence of multidrug resistance in tumor cells can limit the efficacy of treatment.[1] Many efforts have been made to the design of semi- or entirely synthetic analogs of anthracycline antibiotics that retain the antitumor activity and improve the efficacy of the drug. Of particular importance are the compounds capable of circumventing multidrug resistance in cancer cells. In our previous paper we have described the preparation and cytotoxic properties of 4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-dione (**2**) derivatives carrying *N*-(ω-aminoalkyl) substituents.[2] Compound **2** can be considered as a heterocyclic analog of an anthracycline antibiotic aglycon in which the pyrrole cycle substitutes the cyclohexane (A) ring. The *N*-(4-aminobutyl)naphthoindolediones demonstrated high antiproliferative activity against human cancer cell lines and had higher potency than **ADR** or mitoxantrone for **ADR** selected, multidrug resistant breast cancer cell line NCI/ADR. In these compounds the distance between the terminal amino group



1 (ADR)



2 R_1=R_2=H
3 R_1=Me; R_2=H
4 R_1=H; R_2=CH_2NMe_2
5 R_1=Me; R_2=CH_2NMe_2

Keywords: Naphthoindolediones; Mannich reaction; Circumvention of anticancer drug resistance.
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doi:10.1016/j.bmc.2004.12.044

and chromophore is equivalent to five C–C bonds. On the other hand, 3-dimethylaminomethyl derivatives (**4** and **5**) of 4,11-dihydroxy- or 4,11-dimethoxynaphtho[2,3-*f*]indole-5,10-dione (**2** and **3**) synthesized by us earlier[3] also showed high antiproliferative properties though lower than those of **1**.

The aims of this study were to synthesize 3-aminomethyl derivatives of naphtho[2,3-*f*]indole-5,10-dione that contain, in their side chain, an additional amino function separated from the chromophore by five C–C and C–N bonds and to test the antiproliferative activity of novel derivatives in a broad panel of tumor cell lines. In particular, we sought to determine whether the expression of transmembrane transporter P-glycoprotein (Pgp) and inactivation of p53 mediated death pathway alter the response of cells to our novel compounds. These mechanisms are critically important as the determinants of altered response to chemotherapy in many malignancies.[4] Transmembrane ATP dependent efflux pumps represent the first cellular barrier encountered by the drug, and Pgp-mediated outward transport of xenobiotics is implicated in decreased intracellular uptake and long-term cell survival.[4,5] The p53 pathway largely mediates apoptosis triggered by DNA damaging stimuli including anthracyclines; 'loss-of-function' of p53, the most common genetic alteration in malignant tumors, can thereby limit drug induced cell death.[6]

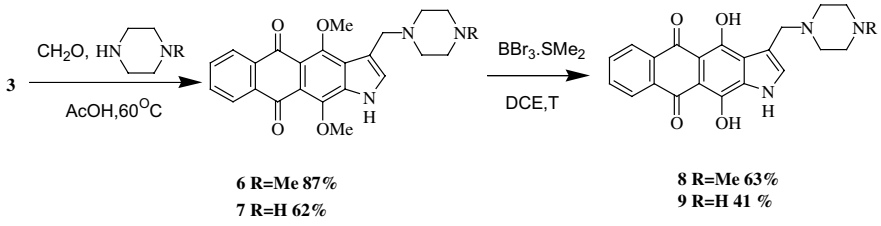
2. Results and discussion

2.1. Chemistry

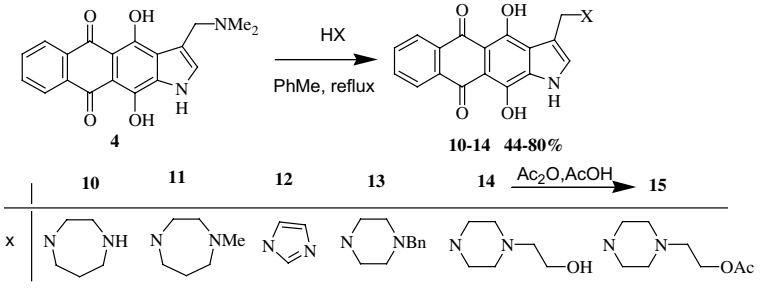
Earlier we have shown that 4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-dione (**2**) is less reactive in Mannich reaction than its di-*O*-methylderivative **3**.[3] Compound **2** does not react with formaldehyde and a secondary amine, however, it can be aminomethylated by Eschenmoser's reagent to give **4**. Another route to compound **4** is the demethylation of compound **5**. This approach was used for the preparation of piperazine analogs of compound **4**. Naphtho[2,3-*f*]indole **3** was aminomethylated by formaldehyde and piperazine or 1-methylpiperazine in acetic acid to produce compounds **6** or **7**, respectively (Scheme 1). Demethylation of **6** and **7** by $BBr_3{\cdot}SMe_2$ yielded compounds **8** and **9**. However, our attempt to use this method for the preparation of homopiperazine or imidazole derivatives failed as naphtoindole **3** did not produce Mannich compounds when the corresponding diamines were used.

We have developed another approach to preparation of various aminomethyl derivatives of 4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-dione (**2**) based on the transamination of gramine derivative **4**.[7,8] The interaction of compound **4** with various secondary amines in boiling toluene led to Mannich bases isolated as mono- (**12**) or dihydrochlorides **10**, **11**, **13**, **14** in 44–80% yields (Scheme 2). 1-(2-Hydroxyethyl)piperazine derivative **14** yielded the corresponding *O*-acetyl derivative **15** after acetylation by Ac_2O in acetic acid in 82% yield.

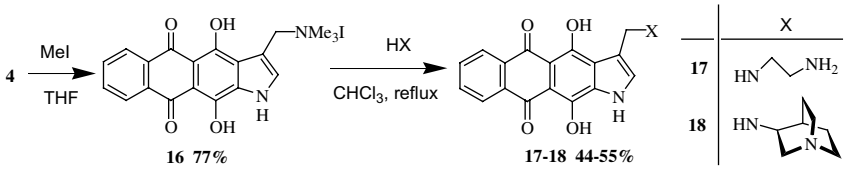
However, we could not use primary diamines in the transamination reaction because multicomponent mixtures were formed which were difficult to separate. To overcome this problem, we used quaternary salt **16** obtained by the interaction of **4** with MeI (77% yield). Compound **16** reacted with primary amines more easily than compound **4**. Transamination proceeded in milder conditions,[9] and the formation of side products was limited. The use of ethylenediamine or 3-aminoquinuclidine in this reaction provided compounds **17** and **18**, respectively, in moderate yields (Scheme 3).



Scheme 1.



Scheme 2.



Scheme 3.

2.2. Biology

The 4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-dione derivatives as hydrochlorides **4**, **8**, **9**, **14**, **18** were studied using a panel of 60 human cancer cell lines in National Cancer Institute Drug Screen Program.[10] The following parameters were determined for every cell line: GI_{50} (concentration inhibiting 50% net cell growth), TGI (concentration that totally inhibited net cell growth) and IC_{50} (concentration leading to 50% net cell death). For each of these parameters the averaged values of mean graph midpoint (MG_MID) were calculated.[11] The GI_{50} values for selected cell lines, along with MG_MID values, are shown in Table 1.

The comparison of data for compounds **4** and **8**, **9**, **14**, **18** demonstrated that the introduction of an additional amino group in the side chain increased the cytotoxicity (see GI_{50} MG_MID values, Table 1). All tested compounds (**4**, **8**, **9**, **14** and **18**) were less potent than **1**, however, **ADR** selected, multidrug resistant breast cancer cells were more sensitive to each of these compounds than to **1**. The ratio of **ADR** GI_{50} for NCI/ADR versus MCF-7 cells was equal to 1000; in striking contrast, the corresponding ratios for **4**, **9**, and **18** were 1.34, 0.05, and 1.25, respectively (Table 1). These compounds inhibited the growth of NCI/ADR cells at low micromolar concentrations (GI_{50} 1.5–4.3 mkM), whereas GI_{50} of **1** was 20 mkM (Table 1). Besides 3-aminomethyl derivatives 4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-dione had selective cytotoxicity for colon and renal cancer cells. 3-(1-Piperazinylmethyl)- and 3-[(quinuclidin-3-ylamino)methyl]- derivatives of 4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-dione **9** and **18** demonstrated the most potent cytocidal effects and were almost as active as **1** (LC_{50} MG_MID 25.1, 31.6, and 14.5 mkM, respectively).

We next tested compounds **4**, **5**, **8**–**18** for cytotoxicity against cells that express Pgp, the transporter responsible for the most well documented phenotype of multidrug resistance.[4,5] Each of these agents was less toxic than **1** for human leukemia cell line K562 (Table 2). However, the compounds containing piperazine moiety (**8**, **9**, **13**–**15**) were equally potent for these cells and their Pgp-positive subline at submicromolar or low micromolar concentrations. Mean IC_{50} ratios of these compounds for

Table 2. Cytotoxicity (IC_{50}) of 3-aminomethyl-4,11-dihydroxynaphtho[2,3-f]indole-5,10-dione derivatives **4**, **5**, **8**–**18** for K562 and K562i/S9 cells

Compd	IC_{50} (mkM)		IC_{50} ratios
	K562	K562i/S9	K562i/S9 to K562
1	0.2 ± 0.1	1.5 ± 0.1	7.5
4	1.2 ± 0.1	12.5 ± 0.3	10.4
5	6.5 ± 0.2	14.1 ± 0.3	2.2
8	1.2 ± 0.2	1.2 ± 0.2	1.0
9	1.2 ± 0.2	1.2 ± 0.2	1.0
10	1.8 ± 0.2	1.9 ± 0.2	1.1
11	2.7 ± 0.2	3.0 ± 0.3	1.1
12	1.0 ± 0.2	1.8 ± 0.2	1.8
13	1.0 ± 0.1	1.1 ± 0.2	1.1
14	1.7 ± 0.2	1.7 ± 0.2	1.0
15	1.0 ± 0.1	2.1 ± 0.2	2.1
16	20 ± 0.5	20 ± 0.5	1.0
17	4.8 ± 0.3	6.2 ± 0.3	1.3
18	3.2 ± 0.2	2.2 ± 0.2	0.7

Table 1. Activity (GI_{50}, mkM) of naphthoindolediones **4**, **8**, **9**, **14**, and **18** in the NCI in vitro 60-cell line Drug Screen Program

Compd	Molt-4[a]	NCI-H226[b]	NCI-H460[b]	HCT-15[c]	KM-12[c]	M-14[d]	OVCAR-8[e]	TK-10[f]	MCF-7[g]	NCI/ADR[h]	MG_MID[i]
1	0.01	0.05	0.5	1.6	0.3	0.2	0.15	0.1	0.02	20.0	0.13
4	3.4	17.0	3.5	5.0	4.7	17.2	8.6	5.1	3.2	4.3	6.9
8	4.0	16.8	5.4	3.5	3.5	1.8	6.3	1.5	5.2	11.7	8.7
9	3.3	12.2	20.1	1.3	10.2	1.3	1.5	10.2	33.0	1.5	2.3
14	1.4	10.5	2.8	3.1	1.6	1.7	9.8	2.3	3.7	10.2	4.2
18	0.02	16.5	1.0	1.2	0.7	4.0	18.1	0.5	1.2	1.5	1.6

Origin of cell lines:
[a] Leukemia.
[b] Non-small cell lung cancer.
[c] Colon cancer.
[d] Melanoma.
[e] Ovarian cancer.
[f] Renal cancer.
[g] Breast cancer.
[h] **ADR** selected multidrug resistant breast cancer cell line.
[i] Mean graph midpoint over the NCI 60-cell panel.

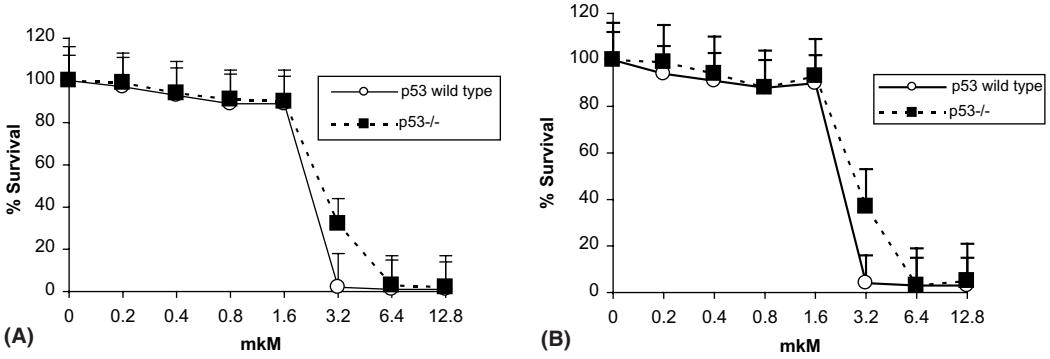


Figure 1. Role of p53 in cytotoxicity of naphthoindolediones **9** (**A**) and **18** (**B**) to HCT116 cells.

K562i/S9 versus K562 cells were 0.7–2.1 compared to 7.5 for **1** and 10.4 for starting gramine derivative **4** (Table 2). Ethylene diamine derivative **17**, albeit 3- to 4-fold less active than the compounds containing cyclic diamine moieties **8**–**15**, **18**, was still capable of killing Pgp-expressing cells at doses similar to those for wild type cells (Table 2). The quaternary salt **16** was the least cytotoxic for wild type K562 cells; however, Pgp did not confer survival to this compound (Table 2).

Finally, we addressed the role of p53 in cytotoxicity of novel derivatives of 4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-dione. Compounds **9** and **18** were selected for these experiments because of their high potency for NCI/ADR multidrug resistant cells. One can expect that the activity for p53-deficient cells would provide an additional advantage to these compounds as potential drugs. Figure 1 shows that both **9** and **18** were toxic for cells carrying wild type p53 (HCT116 cell line) and their isogenic p53-null subline at low micromolar concentrations. However, at 3.2 mkM naphthoindoles **9** or **18** the viability of $p53^{-/-}$ cells was higher than that of $p53^{+/+}$ counterparts, and difference was statistically significant (Fig. 1A,B; $p < 0.05$). Concentrations >3.2 mkM were equally cytotoxic regardless of the p53 status of tested cells. IC_{50} of adriamycin (**1**) to HCT116 cell line ($p53^{+/+}$) and the isogenic MDR line $p53^{-/-}$ are 1.4 and 4.4 mkM, respectively.

Design of agents potent for cells otherwise resistant to conventional chemotherapeutics should presume the escape from the efflux pumps as a critical requirement for drug efficacy. Equal sensitivity of the wild type and Pgp-expressing cells to our novel compounds strongly suggests that these derivatives are capable of overcoming the transmembrane barrier, a prerequisite for delivery of the drug into the cell. Once the drug is in the cell, its efficacy as a toxin (and therefore, cell death or survival) would depend on the balance between pro- and anti-apoptotic signals. Given that tumor cells accumulate diverse survival mechanisms (such as constitutive activation of anti-apoptotic Bcr-Abl tyrosine kinase in K562 cells or lack of caspase 3 expression in MCF-7 cells[12–14]), the functionality of p53 as a key regulator of apoptosis is important for drug induced cell death. Similar toxicity of naphthoindolediones **9** and **18** for cells carrying wild type p53 and their p53-null counterparts implies that indole-containing analogs of anthracyclines, although their potency is attenuated, possess an important advantage over the prototypic drug **1**. Indeed, these derivatives were active for cells with clinically relevant mechanisms of drug resistance such as Pgp and loss of p53 function. However, p53-null cells were found somewhat less sensitive to a certain concentration of **9** and **18** in a 72 h viability assay (Fig. 1). Further studies should demonstrate whether the loss of functional p53 does confer long-term cell survival or the demise of cells treated with our novel compounds is merely delayed but inevitable. If the former hypothesis holds true and the doses of our agents below a certain threshold may be insufficient for the complete elimination of tumor cells, strategies to restore p53 death pathway[15,16] should be considered for sensitization of cells to naphthoindoles.

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 these solvents as internal standards ($CDCl_3$: δ ^{1}H (residual) 7.25 ppm, CD_3OD: δ ^{1}H (residual) 3.32 ppm, DMSO-d_6: δ ^{1}H (residual) 2.50 ppm). 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. HRMS mass spectra were registered on a MAT-8430 Finnigan instrument with data operating system SS-300 (EI, 70 eV, direct introduction, temperature of ion source 250 °C). UV spectra were recorded on Hitachi-U2000 spectrophotometer. All solutions were dried over sodium sulfate and evaporated at reduced pressure on a Buchi-211 rotary evaporator at the temperature below 45 °C.

3.2. Cell lines, drugs, 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 gene transfer and immunoflow cytometry-based sorting of Pgp-positive cells,[17] HCT116 colon carcinoma cell line

(ATCC) with wild type p53 and HCT116p53KO subline (in which both alleles of p53 were deleted by homologous recombination[18]) were cultured in RPMI-1640 supplemented with 5% fetal calf serum (BioWhittaker, Belgium), 2 mM L-glutamine, 100 U/mL penicillin, 100 mkg/mL streptomycin at 37 °C, 5% CO_2 in humidified atmosphere. Cells in logarithmic phase of growth were used in all experiments. Novel compounds were dissolved in 10% aqueous DMSO as 10 mM stock solutions followed by serial dilutions in water immediately before experiments.

The cytotoxicity of novel agents was determined in a formazan conversion assay (MTT test).[19,20] Briefly, cells (5×10^3 in 190 mkL of culture medium) were plated into a 96-well plate (Becton Dickinson, Franklin Lakes, NJ) and then treated with 0.1% DMSO (vehicle control) or increasing concentrations of tested compounds (in 10 mkL; each dose in duplicate) for 72 h. After the completion of drug exposure, 20 mkL of 5 mg/mL aqueous solution of 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazane (Sigma Chemical Co., St. Louis, MO) were added into each well for an additional 3 h. Formazan was dissolved in acidified DMSO, and the absorbance at λ 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 mock-treated cells (100%).

3.3. 4,11-Dimethoxy-3-[(4-methyl-1-piperazinyl)methyl]-1*H*-naphtho[2,3-*f*]indole-5,10-dione (6)

To the stirring solution of 4,11-dimethoxynaphtho[2,3-f]indole-5,10-dione (**3**; 0.10 g, 0.3 mmol) in acetic acid (20.0 mL) methylpiperazine (0.1 mL, 1.0 mmol) and an aqueous solution (40%) of formaldehyde (0.5 mL, 7.9 mmol) were added, and the mixture was heated at 50 °C for 3 h and followed by evaporation. The residue was quenched with water (50 mL), and 10% Na_2CO_3 solution was added until pH 11.0 was reached. The aqueous solution was extracted with ethyl acetate (4×30 mL). The organic layers were combined, washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel in chloroform–methanol–concd NH_4OH (10:2:0 → 10:3:1) to give crude **6** as a yellow solid. Crude **6** was recrystallized from toluene–*n*-heptane mixture (1:5) to afford **6** (0.12 g, 87%) as a yellow powder; mp 163–165 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.50 (br s, 1H, N–H), 8.23 (m, 2H, 6-H, 9-H), 7.71 (m, 2H, 7-H, 8-H), 7.43 (br s, 1H, 2-H), 4.09 (s, 3H, OCH_3), 4.06 (s, 3H, OCH_3), 3.93 (s, 2H, $-CH_2N$), 2.60 (br m, 8H, NCH_2), 2.41 (s, 3H, NCH_3); MS *m*/*z* 419 (M^+, 11), 404 (15), 385 (12), 321 (18), 307 (64), 292 (100), 114 (85); HRMS calcd for $C_{24}H_{25}N_3O_4$ 419.1845, found 419.1849.

3.4. 4,11-Dimethoxy-3-(1-piperazinylmethyl)-1*H*-naphtho[2,3-*f*]indole-5,10-dione (7)

This was similarly prepared from **3** and piperazine in 41% yield; mp 169–171 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.51 (br s, 1H, N–H), 8.21 (m, 2H, 6-H, 9-H), 7.70 (m, 2H, 7-H, 8-H), 7.58 (br s, 1H, 2-H), 4.08 (s, 3H, OCH_3), 4.06 (s, 3H, OCH_3), 3.89 (s, 2H, $-CH_2N$), 2.60 (br m, 8H, NCH_2); MS *m*/*z* 404 (M^+-1, 12), 390 (15), 321 (20), 307 (61), 292 (33), 114 (100); Anal. Calcd for $C_{23}H_{23}N_3O_4$: C, 68.13; H, 5.72; N, 10.36. Found: C, 68.35; H, 5.99; N, 10.48.

3.5. 4,11-Dihydroxy-3-[(4-methyl-1-piperazinyl)methyl]-1*H*-naphtho[2,3-*f*]indole-5,10-dione dihydrochloride (8)

The stirring mixture of **6** (80 mg, 0.19 mmol) and 1 M solution of $BBr_3{\cdot}SMe_2$ in dichloromethane (1.2 mL, 1.2 mmol) was mixed in dry dichloroethane (20.0 mL) and refluxed for 1 h, cooled, quenched by the dropwise addition of methanol (2.0 mL), and then evaporated. The residue was partitioned between *n*-butanol (50 mL) and aqueous solution of $NaHCO_3$ (2%, 20 mL). The extract was washed with brine, water, dried, and evaporated. The residue was purified by column chromatography on silica gel using chloroform–methanol–concd NH_4OH (10:2:0 → 10:3:1), the red oil obtained after evaporation was dissolved in methanol (0.3 mL), and a solution of HCl in ether (0.1 N, 3 mL) was added. The mixture was frozen overnight and the red crystal precipitates were filtered, washed with ether and, after drying, yielded dihydrochloride **8** (56 mg, 63%), mp 231–233 °C (dec); ^{1}H NMR (400 MHz, CD_3OD) δ 8.19 (m, 2H, 6-H, 9-H), 7.74 (m, 2H, 7-H, 8-H), 7.56 (br s, 1H, 2-H), 4.62 (s, 2H, $-CH_2N$), 3.60 (br m, 8H, NCH_2), 2.95 (s, 3H, NCH_3); UV (ethanol) λ_{max} 240, 262, (295), (450), 477, 511 nm; MS *m*/*z* 391 (M^+, 12), 293 (100), 279 (64), 114 (31); Anal. Calcd for $C_{22}H_{21}N_3O_4{\cdot}2HCl$: C, 56.91; H, 4.99; Cl, 15.27; N, 9.05. Found: C, 56.54; H, 4.76; Cl, 15.11; N, 8.92.

3.6. 4,11-Dihydroxy-3-(1-piperazinylmethyl)-1*H*-naphtho[2,3-*f*]indole-5,10-dione dihydrochloride (9)

This was similarly prepared from **7** in 41% yield; mp 238–241 °C (dec); ^{1}H NMR (400 MHz, CD_3OD) δ 8.19 (m, 2H, 6-H, 9-H), 7.74 (m, 2H, 7-H, 8-H), 7.56 (br s, 1H, 2-H), 4.51 (s, 2H, $-CH_2N$), 3.52 (br m, 8H, NCH_2); MS *m*/*z* 377 (M^+, 3), 339 (3), 293 (80), 279 (100); Anal. Calcd for $C_{21}H_{19}N_3O_4{\cdot}2HCl$: C, 56.01; H, 4.70; Cl, 15.75; N, 9.33. Found: C, 55.88; H, 4.87; Cl, 15.56; N, 9.21.

3.7. 4,11-Dihydroxy-3-[(1-homopiperazinyl)methyl]-1*H*-naphtho[2,3-*f*]indole-5,10-dione dihydrochloride (10)

The stirring solution of gramine **4** (0.10 g, 0.3 mmol) and homopiperazine (0.30 mL, 3.0 mmol) in dry toluene (20 mL) was refluxed for 2–3 h under dry argon flow. After complete conversion of **4** (as determined by TLC) the reaction mixture was evaporated, the residue was partitioned between *n*-butanol (50 mL) and aqueous solution of $NaHCO_3$ (2%, 20 mL). The extract was washed with brine, water, dried, and evaporated. The residue was purified by column chromatography on silica gel by chloroform–methanol–concd NH_4OH (10:2:0 → 10:3:2), the red solid obtained after evaporation was dissolved in hot methanol, and a solution of HCl in ether (0.1 N, 3 mL) was added. The mixture

was frozen overnight and the precipitated red crystals were filtered, washed with ether and, after drying, yielded dihydrochloride **10** (60 mg, 44%), mp 237–239 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6), δ 15.03 (br s, 1H, OH), 14.55 (br s, 1H, OH), 9.55 (br s, 1H, NH), 8.40 (m, 2H, 6-H, 9-H), 7.90 (m, 2H, 7-H, 8-H), 7.82 (br s, 1H, 2-H), 4.63 (s, 2H, –CH_2N), 3.63 (br m, 8H, NCH_2), 2.15 (br m, 2H, $CH_2CH_2CH_2$); MS *m*/*z* 391 (M^+, 3), 293 (100), 279 (23); Anal. Calcd for $C_{22}H_{21}N_3O_4$·2HCl: C, 56.91; H, 4.99; Cl, 15.27; N, 9.05. Found: C, 56.81; H, 4.78; Cl, 15.12; N, 9.01.

3.8. 4,11-Dihydroxy-3-[(4-methyl-1-homopiperazinyl)-methyl]-1*H*-naphtho[2,3-*f*]indole-5,10-dione dihydrochloride (11)

This was similarly prepared from gramine **4** and *N*-methylhomopiperazine in 60% yield; mp 228–229 °C (dec); ^{1}H NMR (400 MHz, D_2O) δ 7.76 (d, 1H, J = 6.8 Hz, 9-H), 7.65 (d, 1H, J = 6.8 Hz, 6-H), 7.57 (t, 1H, J = 6.8 Hz, 8-H), 7.51 (t, 1H, J = 7.8 Hz, 7-H), 7.31 (s, 1H, 2-H), 4.47 (s, 2H, –CH_2N), 3.58 (br s, 4H, NCH_2), 3.52 (br m, 4H, NCH_2), 3.09 (s, 3H, NCH_3), 2.40 (br s, 2H, CH_2); MS *m*/*z* 405 (M^+, 6), 339 (3), 293 (100), 279 (21), 114 (35); Anal. Calcd for $C_{23}H_{23}N_3O_4$·2HCl: C, 57.75; H, 5.27; N, 8.78. Found: C, 57.99; H, 5.29; N, 8.56.

3.9. 4,11-Dihydroxy-3-(1*H*-imidazol-1-ylmethyl)-1*H*-naphtho[2,3-*f*]indole-5,10-dione hydrochloride (12)

This was similarly prepared from gramine **4** and imidazole. After complete conversion of **4**, the reaction mixture was washed with water, brine, then dried, and evaporated. The residue was purified by column chromatography on silica gel in chloroform–methanol(10:0 → 10:3), the red solid obtained after evaporation was dissolved in tetrahydrofurane, and a solution of HCl in ether (0.1 N, 3 mL) was added. The mixture was frozen overnight and the red crystal precipitates were filtered off, washed with ether and, after drying, yielded hydrochloride **12** (98 mg, 84%); mp 192–196 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6,) δ 15.01 (br s, 1H, OH), 14.61 (br s, 1H, OH), 13.32 (br s, 1H, NH), 9.21 (s, 1H, 2′-H), 8.26 (m, 2H, 6-H, 9-H), 7.86 (m, 2H, 7-H, 8-H), 7.77 (br s, 1H, 5′-H), 7.68 (br s, 1H, 2-H), 7.65 (br s, 1H, 4′-H), 5.63 (s, 2H, –CH_2N); MS *m*/*z* 359 (M^+, 83), 291 (100), 68 (65); Anal. Calcd for $C_{20}H_{13}N_3O_4$·HCl: C, 60.69; H, 3.57; N, 10.62; Cl, 8.96. Found: C, 60.15; H, 3.42; N, 10.47; Cl, 8.67.

3.10. 3-[(4-Benzyl-1-piperazinyl)methyl]-4,11-dihydroxy-1*H*-naphtho[2,3-*f*]indole-5,10-dione dihydrochloride (13)

This was similarly prepared from gramine **4** and *N*-benzylpiperazine in 78% yield as orange-red powder; mp 165–166 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6,) δ 15.01 (br s, 1H, OH), 14.72 (br s, 1H, OH), 13.27 (br s, 1H, NH), 8.38 (m, 2H, 6-H, 9-H), 7.92 (m, 2H, 7-H, 8-H), 7.74 (br s, 1H, 2-H), 7.50 (m, 2H, 3′-H, 5′-H), 7.41 (m, 2H, 2′-H, 4′-H, 6′-H), 4.55 (s, 2H, –CH_2N), 4.18 (s, 2H, –CH_2N), 3.56 (br m, 8H, NCH_2); MS *m*/*z* 467 (M^+, 9), 293 (35), 176 (29), 134 (81), 91 (100); Anal. Calcd for $C_{28}H_{25}N_3O_4$·2HCl: C, 62.23; H, 5.04; N, 7.78; Cl, 13.12. Found: C, 61.95; H, 5.02; N, 7.45; Cl, 12.89.

3.11. 4,11-Dihydroxy-3-{[4-(2-hydroxyethyl)-1-piperazinyl]methyl}-1*H*-naphtho[2,3-*f*]indole-5,10-dione dihydrochloride (14)

This was synthesized from derivative **4** and 1-(2-hydroxyethyl)pyperazine as described for compound **10** in 72% yield; mp 212–214 °C (dec); ^{1}H NMR (400 MHz, D_2O) δ 7.62 (br s, 1H, 9-H), 7.43 (br m, 3H, 6-H, 8-H, 9-H), 7.23 (s, 1H, 2-H), 4.41 (s, 2H, –CH_2N), 3.98 (br t, 2H, -CH_2O), 3.72 (br m, 8H, NCH_2), 3.46 (br t, 2H, NCH_2); MS *m*/*z* 421 (M^+, 3), 293 (100), 279 (39); Anal. Calcd for $C_{23}H_{23}N_3O_5$·2HCl: C, 55.88; H, 5.10; N, 8.50; Cl, 14.34. Found: C, C, 55.72; H, 5.12; N, 8.34; Cl, 14.12.

3.12. 3-{[4-(2-Acetoxyethyl)-1-piperazinyl]methyl}-4,11-dihydroxy-1*H*-naphtho[2,3-*f*]indole-5,10-dione dihydrochloride (15)

To the stirring solution of **14** (60 mg, 0.3 mmol) acetic acid (10.0 mL) was added in acetic anhydride (0.2 mL, 2.0 mmol) and the mixture was refluxed for 30 min, and then evaporated. The resulting red solid was twice reprecipitated from methanol with ether. The precipitate was filtered, washed with ether and, after drying, yielded dyhydrochloride **15** (53 mg, 82%); mp 202–204 °C (dec); ^{1}H NMR (400 MHz, D_2O) δ 7.62 (br s, 1H, 9-H), 7.42 (br m, 3H, 6-H, 8-H, 9-H), 7.22 (s, 1H, 2-H), 4.50 (br s, 2H, –CH_2O), 4.36 (s, 2H, –CH_2N), 3.70 (br m, 10H, NCH_2), 2.23 (s, 3H, CH_3), MS *m*/*z* 463 (M^+, 7), 403 (12), 293 (58), 279 (22), 112 (37), 99 (100); Anal. Calcd for $C_{25}H_{25}N_3O_6$·2HCl: C, 55.98; H, 5.07; N, 7.83. Found: C, 55.74; H, 4.92; N, 7.53.

3.13. 4,11-Dihydroxy-3-[(dimethylamino)methyl]-1*H*-naphtho[2,3-*f*]indole-5,10-dione iodomethylate (16)

To the stirring solution of gramine **4** (0.20 g, 0.6 mmol) in tetrahydrofurane (50.0 mL) CH_3I (0.3 mL, 3.5 mmol) was added and stirred overnight. The precipitate was filtered, washed with ether, and after drying afforded crude iodomethylate **16** (220 mg 77%) as red crystals. For analytical purpose crude **16** was purified by column gel-filtration on LH-20 in tetrahydrofurane–water (1:1); mp 257–259 °C (dec); ^{1}H NMR (400 MHz, D_2O) δ 7.54 (br d, 1H, 9-H), 7.42 (br t, 1H, 8-H), 7.32 (br m, 2H, 6-H, 7-H), 7.22 (s, 1H, 2-H), 4.41 (s, 2H, –CH_2N), 3.03 (s, 9H, N$(CH_3)_3$), MS *m*/*z* 351 (M^+, 4), 293 (100), 279 (37); Anal. Calcd for $C_{20}H_{19}N_2O_4I$: C, 50.22; H, 4.00; N, 5.86. Found: C, 50.54; H, 4.13; N, 5.99.

3.14. 3-{[(2-Aminoethyl)amino]methyl}-4,11-dihydroxy-1*H*-naphtho[2,3-*f*]indole-5,10-dione dihydrochloride (17)

The stirring solution of crude iodomethylate **16** (96 mg, 0.2 mmol) and ethylenediamine (0.30 mL, 5.0 mmol) in chloroform (25 mL) was refluxed for 20 min and the mixture was evaporated. The residue was partitioned between *n*-butanol (50 mL) and aqueous solution of $NaHCO_3$ (2%, 20 mL). The extract was washed with brine

and water, then dried, and evaporated. The residue was purified by column chromatography on silica gel in chloroform–methanol–concd NH_4OH (10:2:0 → 10:3:2), the red solid obtained after evaporation was dissolved in hot methanol, and a solution of HCl in ether (0.1 N, 3 mL) was added. The mixture was frozen overnight and the red crystal were filtered, washed with ether, and after drying yielded dihydrochloride **17** (47 mg, 55%), mp 229–231 °C (dec); ^{1}H NMR (400 MHz, D_2O) δ 7.66 (d, 1H, J = 6.9 Hz, 9-H), 7.53 (d, 1H, J = 6.9 Hz, 6-H), 7.42 (t, 1H, J = 6.9 Hz, 8-H), 7.36 (t, 1H, J = 6.9 Hz, 7-H), 7.06 (s, 1H, 2-H), 4.16 (s, 2H, $-CH_2N$), 3.43 (br s, 4H, NCH_2); MS m/z 293 (M^+–NMe_3, 100), 279 (27); Anal. Calcd for $C_{19}H_{17}N_3O_4 \cdot 2HCl$: C, 53.79; H, 4.51; N, 9.90. Found: C, 53.57; H, 4.28; N, 9.69.

3.15. 4,11-Dihydroxy-3-[(quinuclidin-3-ylamino)methyl]-1*H*-naphtho[2,3-*f*]indole-5,10-dione dihydrochloride (18)

This was synthesized from **16** and 3-aminoquinuclidine as described for compound **17**. After chromatographic purification crude dihydrochloride **18** was purified by column gel-filtration on LH-20 in tetrahydrofurane–water (1:1) and crystallized from methanol–tetrahydrofurane to afford **18** (43 mg, 44%) as red crystals; mp 240–242 °C (dec); ^{1}H NMR (400 MHz, D_2O) δ 7.85 (d, 1H, J = 7.8 Hz, 9-H), 7.72 (d, 1H, J = 7.8 Hz, 6-H), 7.59 (t, 1H, J = 7.8 Hz, 8-H), 7.54 (t, 1H, J = 7.8 Hz, 7-H), 7.21 (s, 1H, 2-H), 4.28 (d, 2H, J = 5.4 Hz, $-CH_2N$), 4.0 (br m, 2H, CH), 3.50 (br m, 4H, NCH_2), 3.48 (br m, 1H, NCH), 2.22 (br m, 4H, $C(CH_2)_2$), 2.15 (br m, 1H, CH); MS m/z 417 (M^+, 4), 293 (100), 279 (44); Anal. Calcd for $C_{24}H_{23}N_3O_4 \cdot 2HCl$: C, 58.78; H, 5.14; Cl, 14.46; N, 8.57. Found: C, 58.67; H, 5.01; Cl, 14.21; N, 8.36.

Acknowledgements

We gratefully acknowledge extensive collaboration with Dr. V. Narayanan, Dr. E. A. Sausville, and staff of the Developmental Therapeutics Program at NCI (Bethesda, USA). We thank Dr. B. Vogelstein (The Johns Hopkins University, Baltimore, USA) and Dr. B. P. Kopnin (N. N. Blokhin Cancer Center, Moscow, Russia) for HCT116p53KO subline. This study was supported by Russian Foundation for Basic Research, Grant No. 03-03-32090.

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