Full Paper

Synthesis and *In Vitro* Cytotoxicity Evaluation of Novel Naphthindolizinedione Derivatives

Andrea Defant, Graziano Guella and Ines Mancini

Laboratorio di Chimica Bioorganica, Dipartimento di Fisica, Università degli studi di Trento, Trento, Italy

Novel 6,11-dioxo-6,11-dihydro-benzo[*f*]pyrido[1,2-*a*]indole-12-carboxamide derivatives and the corresponding 7,10-dihydroxy analogues were designed in accordance with Moore's and Pindur's theory and synthesized based on the structural similarity with known antitumour agents such as ellipticine, daunorubicin, mitoxantrone and 9-aminoacridine-4 carboxamide derivatives. These compounds, including structural variations of the amide side chain, were evaluated in the NCI panel of human tumour cell lines, from which 6,11-dioxo-6,11-dihydro-benzo[*f*]pyrido[1,2-*a*]indole-(2-dimethylamino-ethyl)-12-carboxamide **11a** was found to be the most potent agent within the series. It showed good selectivity towards leukaemia, colon and renal cancer cell lines, with significant GI_{50} values, from lower than 10 nM to 0.2 μM. Moreover, its cytotoxicity against the adriamicine-resistant breast tumour cell line at a concentration lower than 1 μM turned out to be higher than the values using the clinical anticancer agents, daunorubicin and mitoxantrone.

Keywords: Antitumor activity / Cytotoxic activity / DNA-topoisomerases / Polycyclic amines / Synthesis

Received: September 29, 2006; accepted: December 19, 2006

DOI 10.1002/ardp.200600160

Introduction

Current cancer drug research ideally seeks to define candidate compounds that interact with molecular targets that are unique to tumour cells, forming the basis for current cancer drugs with fewer side effects. Much of the success in cancer chemotherapy has involved the use of drugs that damage the DNA of tumour cells, simultaneously showing the highest non-toxicity for the normal cells. In this context, molecules of natural origin such as ellipticine **1** [1] and daunorubicin **2** [2] or of synthetic derivation, such as mitoxantrone **3** [3] and *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide (termed DACA) series, including the 9-amino-5-methyl derivative **4** [4] (Fig. 1) have been shown to interact with DNA intimately by intercalation. However, their potent antitumour effect, which is due to interaction with the drug-DNA *in vivo*, is still partly unknown. In accordance with Moore's and Pindur's theory [5, 6], the structural properties required to maximise the activity of DNA intercalators include a planar tricyclic or tetracyclic ring system with a length of 3 – 4 Å, a width of 6 – 8 Å and a surface area of 28 $Å^2$, and a π-conjugated quinone moiety. The redox activity of quinones has also been found to play a role in the radical cleavage of DNA, as shown in significant examples including doxorubicin, daunorubicin **2** and mitoxantrone **3** [6, 7]. The presence of two hydroxyl groups in *para* positions on the aromatic nucleus (as seen in mitoxantrone) substantially increases the activity against both leukaemia and some solid tumours in mice [8]. In addition, the presence of a cationic side chain at a physiological pH, positioned at a fixed distance (about 8 Å) on a DACA structure (N-[2-(dimethylamino)ethyl]acridine-4-carboxamide) (as in compound **4**), has been reported to be essential for bioactivity, playing an additional binding contact with guanosine-cytosine base pairs in the DNA [9].

To the best of our knowledge, only one example of a naphthindolizinedione derivative has been studied for

Correspondence: Ines Mancini, Laboratorio di Chimica Bioorganica, Dipartimento di Fisica, Università degli studi di Trento, via Sommarive 14, I-38050 Povo Trento, Italy
E-mail: mancini@science.unitn.it
Fax: +39 0461 882-009

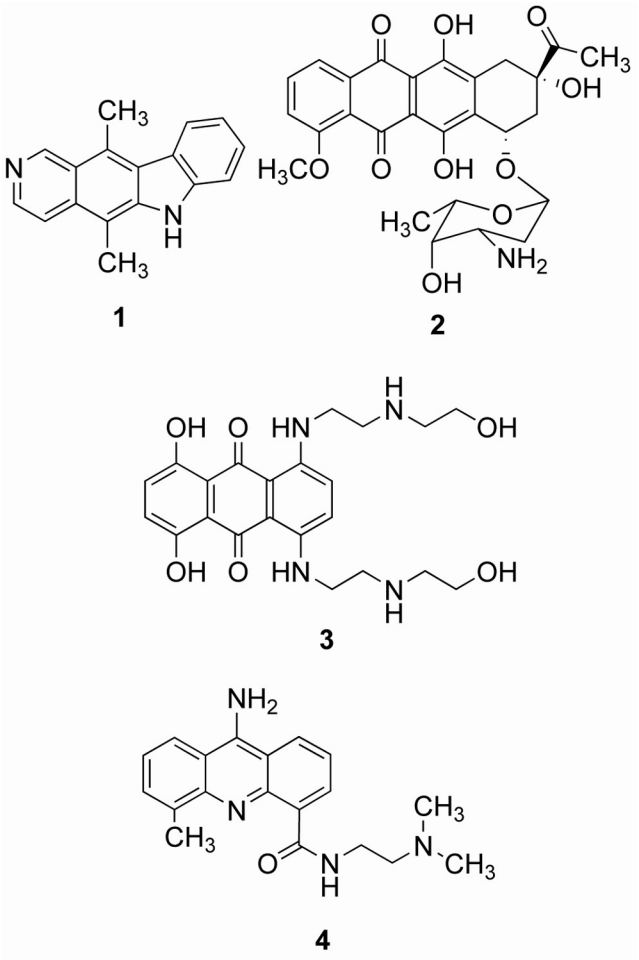
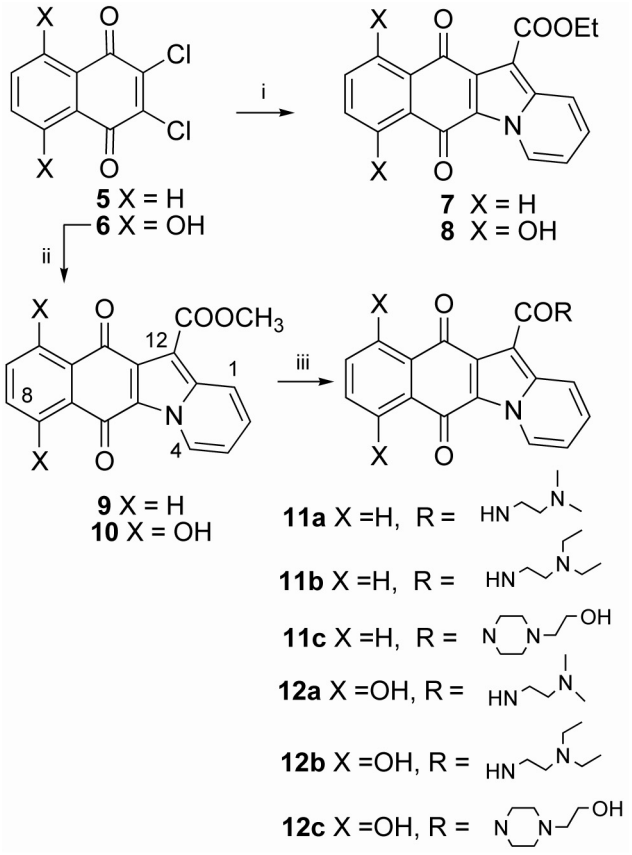


Figure 1. Chemical structures of known DNA intercalator molecules: ellipticine **1**, daunorubicin **2**, mitoxantrone **3** and 9-amino-5-methyl DACA derivative **4**.



Reagents and conditions: (i) ethyl acetoacetate, pyridine, 2-propanol reflux, 4 h; 75% for **7**, 54% for **8**; (ii) methyl acetoacetate, pyridine, 2-propanol reflux, 4 h; 75% for **9**, 54% for **10**; (iii) amine in excess, 80°C, 3 h, 98%.

Scheme 1. Synthesis of naphthindolizinedione derivatives.

antitumour activity, the ester **7**. It was evaluated both for topo I and topo II inhibition and for activity as a cytotoxic agent against A549, col2 and SNU-638 cell lines (IC_{50} values of 16.3, 15.4 and 0.2 μg/mL, respectively) [10]. Although selective, the latter data indicated a moderate cytotoxic activity in cultured stomach cancer cells.

This study was aimed at the synthesis and the investigation of the cytotoxicity against a large panel of human tumour cell lines for a new series of tetracyclic compounds with structural variations both on the heterocyclic moiety and by the presence of an amide chain, or by hydroxyl groups on the aromatic system. In our preliminary design based on Moore's and Pindur's theory [5–7], the AM1 semi-empirical calculations carried on the target compounds **11a–c** and **12a–c** showed minimised structures with values of 2.954 and 8.437 Å relating to the distances C1–C4 and C3–C8, respectively. In addition, the presence of an amide side chain in this common tetracyclic planar system was thought to increase the potential bioactivity of these molecules.

Results and discussion

Chemistry

Target compounds **11a–c** and **12a–c** were obtained according to the reactions depicted in Scheme 1. Precursors methyl 6,11-dioxo-6,11-dihydro-benzo[*f*]pyrido [1,2-*a*]indole-12-carboxylate **9** and methyl 7,10-dihydroxy-6,11-dioxo-6,11-dihydro-benzo[*f*]pyrido[1,2-*a*]indole 12-carboxylate **10** were obtained through a one-pot cyclisation starting from the commercially available 2,3-dichloro-1,4-naphtoquinone **5** or 2,3-dichloro-5,8-dihydroxy-1,4-naphtoquinone **6**, respectively, by refluxing them in ethanol with pyridine and methyl acetoacetate [11]. Higher yields were obtained by using 2-propanol as opposed to the better-known procedure starting with the corresponding ethyl ester substrate and using the pro-

Table 1. *In vitro* cytotoxic activity of the compounds *8, 11a – 11c* and *12a – 12c* in the NCI three cell line / one dose pre-screening.

Compound	Growth percentage (at conc. = 100 μM)			Activity
	Lung NCI-H460	Breast MCF7	CNS SF-268	
8	100	121	109	Inactive
11a	0	0	0	Active
11b	0	0	44	Active
11c	50	66	77	Inactive
12a	−1	−1	−2	Active
12b	31	40	84	Active
12c	0	0	82	Active

cess of cyclization in ethanol [11, 12]. The choice of methyl esters **9** and **10** as intermediates for the preparation of compounds **11a – c** and **12a – c**, respectively, was required by the successive formation of the corresponding amides. In fact, the amide preparation by direct conversion of methyl ester was demonstrated by us to be a better procedure than the alternative route relying on amide formation *via* the acyl chloride prepared from the carboxylic acid, which, in turn, can be obtained by the hydrolysis of the esters **7** and **8** [13]. Thus, compounds **11a – c** and **12a – c** were obtained by a two-step sequence from the commercial naphtoquinones **5** and **6**, with an overall yield of 74% and 53%, respectively. The molecular composition of the products was supported by HR-EIMS measurements and ESI-MS tandem fragmentation experiments, whereas their structures were fully characterised by extensive NMR analysis, including NOESY, 1D- and 2D-one bond and long range ^{1}H-^{13}C hetero-correlated experiments (see Experimental, section 4).

Biological activity

Compounds **8**, **11a – c** and **12a – c** were evaluated for their *in vitro* activity against cancer cell lines by the National Cancer Institute (NCI), following its anticancer drug development programme based on automated sulphorhodamine blue (SRB) cytotoxicity assay [14]. Compounds **8** and **11c** showed low growth inhibition (GI_{50} values $\geq 10^{-4}$ M) in the pre-screened panel including three highly sensitive human tumour lines (MCF-7 breast, NCI-H640 lung and SF-268 glioma), as summarised in Table 1. Therefore, they were not selected for the advanced testing in the full 60 cell lines screening, whereas **11a – b** and **12a – c** underwent the latter advanced bioassays (Table 2).

In Table 2 their most significant GI_{50} values are reported compared with the corresponding data for the antitumour agents **1 – 4**. These data indicate a decisive role of the amide chain when compared to the corresponding ethyl esters. To look at this in more detail, whereas the known ester **7** exhibited value of GI_{50} = 51 μM [10] for the tested A549 cell line, all the amides **11a**, **11b** and **12a – 12c** resulted as being more potent against the same lung tumour cell line, with amide **11a** showing a more than 100-fold better value.

Moreover, a drastic enhancement of cytotoxicity and a good selectivity within the series of the substituted amides under investigation were observed by changing the side chain. Indeed, the GI_{50} values change from $\geq 10^{-4}$ M for the amide **11c** to $\geq 10^{-6}$ M in the case of **11b**, and become even better for **11a**, showing a $< 10^{-8}$ M value in the case of leukaemia HL60 (TB) cell line. The difference in bioactivity of *N,N*-dimethylaminoethyl as far as the *N,N*-diethylaminoethyl chain is concerned, is similar to the result of modified anthracene-9,10-diones, where the structural features of the side chain and the antileukaemic activity were correlated. In particular, this study exhibited that both the steric variation on the distal nitrogen and the basic characteristics of the amino group significantly affected the antitumour bioactivity [15]. As far as the difference in the substituents on the quinone moiety are concerned, the data for compound **12b** are quite similar to those obtained for **11b**, indicating that the additional presence of the hydroxyl groups does not play an important role. Instead, a striking outcome is the effect of the hydroxyl aromatic groups in **12a** in comparison with the most active compound **11a** where such groups are missing. A strong reduction in cytotoxicity observed for **12a** was unpredictable on the base of the known data for ametantrone and the dihydroxyl analogue mitoxantrone, the latter showing higher activity against both leukaemia and some solid tumours [8].

Among the series of tetracyclic compounds which were investigated, **11a** turned out to be the most powerful cytotoxic molecule, with remarkable values against some specific leukaemia, colon and renal cancer cell lines. Moreover, its sub-micromolar cytotoxicity against the breast cell line resistant to adriamicine (NCI/ADR-RES) was comparable to ellipticine **1** and DACA derivative **4**, or also higher than the values of the reference compounds daunorubicin **2** and mitoxantrone **3** (Table 2).

Conclusions

This study focused on the design and synthesis of novel naphthindolizinedione derivatives **11a – c** and **12a – c**, the structures of which summarise some peculiarities present in antitumour molecules such as ellipticine, daunorubicin, mitoxantrone and DACA derivatives. Contrary to the case of daunorubicin and mitoxantrone, the presence of two hydroxyl groups in *para* positions on the aromatic nucleus does not improve the cytotoxicity, whereas

Table 2. Inhibition of *in vitro* human cancer cell lines by compounds **11a–b** and **12a–c**, in comparison with the antitumour agents **1–4.**

Type of cancer	Cell line	Cytotoxicity GI_{50} (μM)[a]								
		11a[b]	**11b**	**12a**	**12b**	**12c**	**1**	**2**	**3**	**4**
Leukemia	CCRF-CEM	1.64 ± 0.94	4.07	2.55	3.75	37.4	0.398	0.0158	0.00631	0.0100
	HL60(TB)	<0.0100	n.d.	2.30	n.d.	n.d.	0.794	0.0158	0.0100	0.0100
	K562	0.992 ± 0.01	n.d.	0.738	n.d.	16.0	0.501	0.0631	0.126	0.0158
	MOLT-4	0.268 ± 0.025	10.3	0.771	2.41	0.702	0.251	0.00316	0.00501	0.0100
	RPMI-8226	0.853 ± 0.48	3.03	4.97	3.12	29.8	0.631	0.0199	0.200	0.0200
	SR	0.318 ± 0.11	2.00	0.293	2.17	0.365	n.d.	0.0158	0.00794	0.0100
Non-Small	A549/ATCC	0.395 ± 0.097	4.25	0.806	5.92	48.6	0.501	0.125	0.0126	0.0158
Cell Lung	HOP-92	1.28 ± 0.70	2.08	3.62	4.46	30.4	0.501	0.0117	0.0158	0.0631
	NCI-H226	0.412 ± 0.37	4.344	1.82	8.71	48.7	0.158	0.0398	0.0158	0.0501
	NCI-H322M	6.92 ± 6.08	4.42	0.825	7.61	43.9	0.316	0.316	0.251	0.0398
	NCI-H460	3.91 ± 0.5	2.16	0.361	2.86	38.0	0.158	0.00501	0.00631	0.0100
Colon	COLO 205	0.31 ± 0.21	10.0	1.84	12.5	36.3	0.501	0.251	0.100	0.0316
	HCT-116	0.194 ± 0.184	3.65	n.d.	2.90	18.0	0.251	0.0631	0.0501	0.0316
	HCT-15	0.277 ± 0.23	3.49	1.58	4.41	46.3	0.398	1.58	0.251	0.0158
	HT29	0.146 ± 0.094	5.47	2.44	6.03	48.1	0.794	0.0794	0.251	0.0251
	KM12	6.55 ± 6.05	4.87	1.48	7.05	28.3	0.501	0.316	0.501	0.0501
	SW-620	1.68 ± 1.27	4.82	0.996	3.43	55.8	0.398	0.0199	0.0501	0.0251
CNS	SF-295	5.61 ± 4.79	3.95	1.75	7.00	36.7	0.794	n.d.	0.0200	0.0316
	SNB-19	0.574 ± 0.22	15.6	3.52	25.5	>100	0.794	0.0398	0.0158	0.0501
Melanoma	LOX IMVI	0.275 ± 0.25	3.64	1.12	3.32	36.6	0.501	0.0501	0.0200	0.0316
	SK-MEL-5	1.39 ± 0.77	14.3	2.86	12.4	18.5	1.00	n.d.	0.0501	0.126
Ovarian	IGROV-1	2.65 ± 2.0	7.88	1.97	10.6	33.5	0.794	0.100	0.200	0.0316
	OVCAR-8	0.444 ± 0.084	4.72	1.89	5.54	66.7	0.398	0.0794	0.0398	0.0631
Renal	ACHN	0.169 ± 0.159	2.25	0.693	3.60	49.3	n.d.	0.0251	0.0126	0.0158
	CAKI-1	5.66 ± 5.14	3.60	3.53	4.71	20.3	1.26	0.0501	0.0100	0.0158
	SN12C	0.278 ± 0.25	2.27	2.10	5.32	57.7	1.00	0.0794	0.00794	0.0158
	UO-31	3.51 ± 3.10	11.6	2.41	14.8	29.8	1.99	0.251	0.316	0.100
Breast	NCI/ADR-RES	0.638 ± 0.59	5.44	1.95	5.56	38.6	0.630	1.25	3.98	0.398
	MDA-MB-231/ATCC	0.573 ± 0.49	3.37	6.04	10.4	34.5	n.d.	0.316	0.199	0.050
	MGM #	0.944	6.446	2.75	8.51	36.3	0.749	0.0443	0.0591	0.0477

[a] GI_{50} values, defined as the concentration that inhibits growth by 50% from NCI screening. About the 60 cell lines panel investigated for the compounds **11a–b** and **12a–c**, only the data for cell lines giving values $=10^{-6}$ M are reported. n.d. = not determined, for compounds **1-4**, values accessible by NCI database.

[b] Average values from re-testing of the compound showing a good MGM value (Mean graph medium).

MGM as average GI_{50} (μM) over all cell lines investigated.

changes in the amide side chain play a decisive role. In particular, the *N,N*-dimethylethylamino chain has been found to be the most relevant structural modification for obtaining the highest activity and selectivity towards leukaemia, colon and renal cancer cell lines, with GI_{50} values from lower than 10 nM to 0.2 μM. Moreover, the cytotoxicity of compound **11a** against the sub-panel breast cancer cell line, resistant to adriamicine (NCI/ADR-RES), resulted in comparable or higher values than those of all the reference compounds **1–4**.

Following these results, further efforts will be addressed to the study of this most active compound as a potential DNA intercalator, as well as to investigate the effect of structural modifications on the naphthindolizinedione skeleton.

We would like to thank the National Cancer Institute (NCI) at Bethesda, USA for the antitumour screening tests of our compounds and we are grateful to Mr. A. Sterni and Mr. M. Rossi for their technical support with mass and UV spectra, respectively.

Experimental

Chemistry

General

All evaporations were carried out at room temperature at reduced pressure. Yields are given for reacted compounds. All reagents and solvents were purchased from Sigma Aldrich Europe (Milan, Italy) and used without purification. TLC was carried on Kieselgel 60 PF_{254} and RP-18 F_{254} (Merck, Darmstadt, Germany) and flash-chromatography (FC) was carried out on Merck silica gel 60, 20-50 μm; HPLC analysis for compounds **11a–c** and **12 a–c** was realized on 25 × 1 cm columns packed with Merck-LiChrospher RP-18 (7 μm) under UV monitoring at λ 254 nm and solvent flux 1 mL/min. M.p.: Reichert Thermovapor microscope (C.

Reichert, Vienna, Austria). UV spectra (λ_{max} in nm) were taken with a Perkin-Elmer Lambda-3 spectrophotometer, (Perkin Elmer, Norwalk, CT, USA). NMR spectra were taken with an Avance 400 Bruker spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany); ^{1}H at 400 MHz and ^{13}C at 100 MHz in $CDCl_3$, δ values in ppm rel. to $SiMe_4$ (0 ppm), J values in Hz. Structural assignments are from ^{1}H,^{1}H-COSY, heteronuclear single quantum correlation (HSQC), Heteronuclear Multiple Bond Correlation (HMBC) and 2D NOESY experiments; multiplicities from Attached Proton Test (APT) experiment. ^{13}C-NMR data were deduced by HMBC correlations for compounds **8**, **11a**, **12b** and **12c**. Electron-impact (EI) mass spectra (m/z; rel.%) and HR-EI data were taken with a Kratos-MS80 mass spectrometer (Kratos, Ltd., Manchester, UK) with home-built computerised acquisition software. ESI-MS data, and tandem fragmentation spectra (MS^n), were taken with a Bruker Esquire-LC™ spectrometer (Bruker Daltonik GmbH, Bremen, Germany), equipped with an electrospray ionization ion source used in positive ion mode by injection of the sample into the source from a methanol solution. Semi-empirical calculations were achieved using the free MOPAC 6.0 software.

*Preparation of esters **7–10***

To a mixture of commercially available 2,3-dichloro-1,4-naphtoquinone **5** (0.284 g, 1.25 mmol) or 2,3-dichloro-5,7-dihydroxy-1,4-naphtoquinone **6** (0.324 g, 1.25 mmol) and ethyl acetoacetate (0.24 mL, 1.88 mmol) or methyl acetoacetate (0.20 mL, 1.88 mmol), dissolved in 2-propanol (13 mL), pyridine (0.60 mL, 7.5 mmol) was added and the resultant solution was refluxed for 4 h. After cooling, the solvent was removed *in vacuo* and the residue was partitioned between water and dichloromethane, the organic phases were separated (× 3), combined and washed in sequence with 0.1 M aq. HCl, and water, dried on anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was subjected to flash-chromatography on silica gel with hexane / EtOAc gradient elution, to give the respective esters **7**, **9** (each 75%) and **8**, **10** (each 54%). The purity of ester **8**, later subjected to bioassays, was tested to be higher than 99.9% by RP-18 HPLC analysis with 99.5 : 0.5 MeOH/TFA (t_R 4.9 min).

*Ethyl 6,11-dioxo-6,11-dihydro-benzo[f]pyrido[1,2-a]indole 12-carboxylate **7***

Orange-brown solid; m. p. 157°C (ethanol) [lit. [11]:157-158°C]; ^{1}H-NMR (400 MHz, $CDCl_3$) δ : 1.50 (t, 3H, J = 7.00 Hz, OCH_2CH_3), 4.51 (q, 2H, J = 7.20 Hz, OCH_2CH_3), 7.16 (t, 1H, J = 7.00 Hz, H-3) 7.43 (m, 1H, H-2), 7.71 (m, 2H, H-8 and H-9), 8.22 (br d, 2H, J = 7.70 Hz, H-7 and H-10), 8.31(d, 1H, J = 9.20 Hz , H-1), 9.85 (br d, 1H, J = 6.80Hz, H-4); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 14.33 (q, CH_3), 61.03 (t, OCH_2), 106.10 (s, C-12), 117.32 (d, C-3), 123.62 (s, C-10a), 120.94 (d, C-1), 126.04 (d, C-2), 127.34, 127.78 (two d, C-7 and C-10), 128.53 (d, C-4), 128.70 (s, C-5a), 133.16, 133.34 (two d, C-8 and C-9), 133.77 (s, C-6a), 134.24 (s, C-10a), 139.59 (s, C-12a), 163.64 (s, *C*OOEt), 175.24 (s, C-6), 180.31 (s, C-11). EI-MS m/z (%): 319 [M^+] (85), 274 (87), 247 (100); HR(EI)MS: 319.0840 ± 0.003 ($C_{19}H_{13}NO_4$, calc. 319.0844).

*Ethyl-7,10-dihydroxy-6,11-dioxo-6,11-dihydro-benzo[f]pyrido[1,2-a]indole 12-carboxylate **8***

Orange-brown solid; m. p. 179 – 181°C (ethanol); UV/Vis (EtOH) λ_{max} nm (ε) = 528 (4430), 500 (4690), 343(25890), 248 (16320), 202 (13290); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.48 (t, 3H, J = 7.20 Hz, $-OCH_2CH_3$), 4.50 (q, 2H, J = 7.20 Hz, OCH_2CH_3), 7.16 (m, 1H, H-3) 7.17 (s, 2H, H-8 and H-9), 7.43 (m, 1H, H-2), 8.26 (br d, 1H, J = 9.1 Hz, H-1), 9.75 (br d, 1H, J = 6.90 Hz, H-4), 12.88 (s, 1H, OH), 13.05 (s, 1H, OH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 14.25 (q, CH_3), 61.60 (t, OCH_2), 107.24 (s, C-12), 117.40 (d, C-3), 129.28 (two d, C-8 and C-9), 121.13 (d, C-1), 128.01 (d, C-2), 128.53 (d, C-4), 128.10 (s, C-5a), 139.98 (s, C-12a), 157.37, 158.46 (two s, C-7 and C-10), 163.29 (s, *C*OOEt), 178.37 (s, C-6), 184.72 (s, C-11); EI-MS m/z (%): 351 [$M^{+\bullet}$] (51), 305 (100), 277 (39); HR(EI)MS: 351.0728 ±0.003 ($C_{19}H_{13}NO_6^{+\bullet}$, calc. 351,0743).

*Methyl-6,11-dioxo-6,11-dihydro-benzo[f]pyrido[1,2-a]indole 12-carboxylate **9***

Orange solid; m. p. 191°C (acetic acid) [lit. [11]: 190 – 191°C]; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.07 (s, 3H, OCH_3), 7.16 (t, 1H, J = 7.00 Hz, H-3), 7.43 (m, 1H, H-2), 7.71 (m, 2H, H-8 and H-9), 8.22 (br d, 2H, J = 7.70 Hz, H-7 and H-10), 8.31(d, 1H, J = 9.20 Hz , H-1), 9.85 (br d, 1H, J = 6.80 Hz, H-4); EI-MS m/z (%): 305 [$M^{+\bullet}$] (62), 274 (100), 247 (23); HR(EI)MS: 305.0693 ± 0.005 ($C_{18}H_{11}NO_4$, calc. 305.0688).

*Methyl-7,10-dihydroxy-6,11-dioxo-6,11-dihydro-benzo[f]pyrido[1,2-a]indole 12-carboxylate **10***

Orange-brown powder, m. p. 214°C (hexane/ethyl acetate); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.01 (s, 3H, OCH_3), 7.19 (t, 1H, *J* = 6.90 Hz, H-3), 7.20 (s, 2H, H-8 and H-9), 7.46 (m, 1H, H-2), 8.30 (br d, 1H, J = 9.1 Hz, H-1), 9.80 (br d, 1H, J = 6.90 Hz, H-4), 12.90 (s, 1H, OH), 13.10 (s, 1H, OH); EIMS m/z (%): 337 [$M^{+\bullet}$] (40), 305 (100), 277 (28).

General procedure for the conversion to amides

The methyl ester (0.020 g, 0.066 mmol of **9** or 0.060 mmol of **10**) was stirred in the presence of a molar excess of amine (0.5 mL) at 80°C for 3 h. The crude mixture was evaporated *in vacuo*, water (5 mL) was added to the residue or treated with a phosphate buffer solution at pH 6 (5 mL) for the preparation of the amides **12a – c**, and extracted with dichloromethane (× 3), to give a combined organic phase, which was washed with brine. After drying over anhydrous Na_2SO_4 and evaporation, the residue was subjected to preparative TLC eluted with CH_2Cl_2 / MeOH (99.5 : 0.5) to give pure amides in 98% yields.

The purity of each amide was tested to be higher than 99.8% by RP-18 HPLC analysis with MeOH/TFA (99.5 : 0.5): **11a** (t_R 15.1 min), **11b** (t_R 13.3 min), **11c** (t_R 3.7 min), **12a** (t_R 3.4 min), **12b** (t_R 2.4 min), **12c** (t_R 4.0 min).

*6,11-Dioxo-6,11-dihydro-benzo[f]pyrido[1,2-a]indole-(2-dimethylamino-ethyl)-12-carboxamide **11a***

Deep red solid; m. p. 142 – 143°C (chloroform/methanol); UV/Vis (EtOH) λ_{max} nm (ε) = 488 (5150), 347 (7230), 326 (12380), 254 (38300), 202 (31230); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.35 (s, 6H, $N(CH_3)_2$), 2.67 (t, 2H, J = 6.7 Hz, $CH_2N(CH_3)_2$), 3.66 (pseudo q, 2H, J = 6.7 Hz, $CONHCH_2$), 7.18 (td, 1H, J = 6.9 Hz, 1.3 Hz, H-2), 7.42 (br t, 1H, J = 6.9 Hz, H-3), 7.70 (td, 1H, J = 7.5, 1.4 Hz, H-8), 7.76 (td, 1H, J = 7.5, 1.4 Hz, H-9), 8.22 (br t, 1H, J = 7.5 Hz, H-7), 8.25 (br t, 1H, J = 7.5 Hz, H-10), 9.12 (br d, 1H, J = 9.2 Hz, H-1), 9.90 (br d, 1H, J = 6.9 Hz, H-4), 10.48 (br s, 1H, CONH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 37.28 (t, $CONHCH_2$), 45.30 (q, $N(CH_3)_2$), 58.23 (t, $CH_2N(CH_3)_2$), 109.98 (s, C-12), 118.30 (d, C-3), 123.41 (d, C-1), 121.69 and 125.34 (s, C-5a, C-11a), 126.21 (d, C-7), 127.69 (d, C-10), 127.62 (s, C-5a), 127.70 (d, C-4), 127.79 (d, C-2), 133.06 (d, C-8), 133.35 (s, C-10a), 133.97 (s, C-6a), 134.42 (d, C-9), 140.58 (s, C-12a), 140.79 (s, C-5a),

163.35 (s, CONH), 174.55 (s, C-6), 184.22 (s, C-11); EI-MS m/z (%): 361 [$M^{+\bullet}$] (1), 291 (2), 274 (6), 58 (100); HR(EI)MS: 361.1417 ± 0.003 ($C_{21}H_{19}N_3O_3^{+\bullet}$, calc. 361.1426). ESI (+)-MS/MS m/z: 362 ([M+H]$^+$) → 317.

6,11-Dioxo-6,11-dihydro-benzo[f]pyrido[1,2-a]indole-(2-diethylamino-ethyl)-12- carboxamide ***11b***

Deep red solid; m. p. 200°C (chloroform/methanol); UV/Vis (EtOH) λ_{max} nm (ε) = 490 (5830), 349 (8560), 319 (14200), 250 (46300), 205 (147860); ^{1}H-NMR (400 Mz, $CDCl_3$) δ: 1.10 (t, 6H, J = 6.4 Hz, N(CH_2CH_3)$_2$), 2.64 (q, 4H, J = 6.4 Hz, N(CH_2CH_3)$_2$), 2.78 (t, 2H, J = 6.7 Hz, CH_2NEt_2), 3.59 (pseudo q, 2H, J = 6.7 Hz, CONHCH_2),7.22 (td, 1H, J = 6.9, 1.3 Hz, H-2), 7.45 (br t, 1H, J = 6.9 Hz, H-3), 7.73 (td, 1H, J = 7.5, 1.4 Hz , H-8), 7.78 (td, 1H, J = 7.5, 1.4 Hz, H-9), 8.22 (m, 2H, H-7, H-10), 9.10 (br d, 1H, J = 9.2 Hz, H-1), 9.94 (br d, 1H, J = 6.9 Hz, H-4), 10.50 (br s, 1H, CONH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 11.80 (q, N(CH_2CH_3)$_2$), 32.60 (t, CONHCH_2), 42.24 (two t, N(CH_2CH_3)$_2$), 51.95 (t, CONHCH$_2$CH$_2$), 110.08 (s, C-12), 118.24 (d, C-3), 123.47 (d, C-1), 121.58, 125.24 (two s, C-11a and C-5a), 126.14 (d, C-7), 127.54, 127.63, 127.66 (three d, C-2, C-4, and C-10), 132.99 (d, C-8), 133.94 and 133.34 (s, C-6a and C-10a), 134.30 (d, C-9), 140.75 (s, C-12a), 163.16 (s, CONH), 174.33 (s, C-6), 183.93 (s, C-11); EI-MS m/z (%): 389 [M^+] (4), 274 (13), 86 (100); HR(EI)MS: 389.1731 ± 0.003 ($C_{23}H_{23}N_3O_3^{+\bullet}$, calc. 389.1739).

12-[4-(2-Hydroxy-ethyl)-piperazine-1-carbonyl]-benzo[f]pyrido[1,2-a]indole-6,11-dione ***11c***

Orange solid; m. p. 249–251°C (chloroform/methanol); UV/Vis (EtOH) λ_{max} nm (ε) = 477 (2950), 320 (6670), 280 (30230), 254 (29150); ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 2.29, 2.59,2.83, 3.39,3.46, 3.61, 3.91, 4.05 (series of m, 12H), 7.14 (t, 1H, J = 7.10 Hz, H-3), 4.46 (br s, 1H, OH), 7.33 (br t, 1H, J = 7.10 Hz , H-2), 7.69 and 7.75 (two t, 1H each, J = 7.10 Hz, H-8 and H-9), 7.73 (d, J = 9.00 Hz, H-1), 8.14 (d, 1H, J = 7.10 Hz, H-10), 8.28 (d, 1H, J = 7.10 Hz, H-7), 9.68 (d, 1H, J = 7.10 Hz, H-4); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 41.61 and 46.43 (two t, (CH_2)$_2$NCO), 52.58, 53.04 (two t, (CH_2)$_2$NCH$_2$), 57.56 (t, CH_2CH_2OH), 59.53 (t, CH_2OH), 109.92 (s, C-12), 117.74, 119.73 (two d, C-2 and C-3), 120.68 (s, C-11a), 126.43 (d, C-1 and C-7), 126.85 (d, C-10), 128.04 (d, C-4), 132.97 (d, C-9), 133.98 (d, C-8), 134.80 (s, C-10a), 133.98 (s, C-6a), 134.77 (d, C-9), 136.97 (s, C-12a), 125.97 (s, C-5a), 163.34 (s, CONH), 174.25 (s, C-6), 181.59 (s, C-11); EI-MS m/z (%): 403 [M^+] (2), 385 (6), 372 (11), 316 (15), 274 (100); HR(EI)MS: 403.1519 ± 0.003 ($C_{23}H_{21}N_3O_4^{+\bullet}$, calc. 403.1532). ESI (+)-MS/MS m/z: 404 ([M+H]$^+$) → 274.

7,10-Dihydroxy-6,11-dioxo-6,11-dihydro-benzo[f]pyrido[1,2-a]indole-(2-dimethylamino -ethyl)-12-carboxamide ***12a***

Violet solid; m. p. 174–175°C (chloroform/methanol); UV/Vis (EtOH) λ_{max} nm (ε) = 557 (14370), 520 (13020), 350 (6260), 275 (33300), 246 (42750); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.41 (s, 6H, N(CH_3)$_2$), 2.69 (t, 2H, J = 6.7 Hz , NCH$_2CH_2$), 3.68 (pseudo q, 2H, J = 6.7 Hz, CONHCH_2), 7.22 (m, 3H, H-3, H-8 and H-9), 7.49 (br t, 1H, J = 6.9 Hz, H-2), 9.15 (d, 1H, J = 8.9 Hz, H-1), 9.88 (d, 1H, J = 6.8 Hz, H-4), 10.28 (br.s, 1H, CONH), 12.85 and 13.20 (two br s, 2H, 2 OH);^{13}C-NMR(100 MHz, $CDCl_3$) δ: 37.35 (t, CONHCH_2), 45.52 (q, N(CH_3)$_2$), 58.34 (t, CH_2NMe_2), 110.73 (s, C-12), 112.57, 112.99 (two s, C-6a and C-10a), 118.33 (d, C-3), 123.59 (d, C-1), 125.38 (s, C-5a), 127.96 (d, C-2), 128.14 (d, C-4), 128.63, 130.66 (two d, C-8 and C-9), 139.80 (s, C-12a), 157.90, 159.28 (two s, C-7 and C-10), 162.81 (s, CONH), 177.52 (s, C-6), 186.88 (s, C-11); EI-MS m/z (%): 393 [M^+] (3), 323 (3), 306 (10), 58 (100); HR(EI)MS: 393.1315 ±0.003 ($C_{21}H_{19}N_3O_5^{+\bullet}$, calc. 393.1325).

7,10-Dihydroxy-6,11-dioxo-6,11-dihydro-benzo[f]pyrido[1,2-a]indole-(2-diethylamino-ethyl)- 12-carboxamide ***12b***

Purple solid; m. p. 225°C (chloroform/methanol); UV/Vis (EtOH) λ_{max} nm (ε) = 550 (4330), 346 (2530), 250 (14080), 205 (19130); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.17 (t, 6H, J = 6.7 Hz, N(CH_2CH_3)$_2$), 2.84 (q, J = 6.7 Hz, 4H, N(CH_2CH_3)$_2$), 2.70 (t, 2H, J = 6.7 Hz, CH_2NEt_2), 3.68 (pseudo q, 2H, J = 6.7 Hz, CONHCH_2), 7.23 (m, 3H, H-3, H-8 and H-9),7.49 (br t, 1H, J = 6.9 Hz, H-2), 8.99 (d, 1H, J = 8.9 Hz, H-1), 9.87 (d, 1H, J = 6.8 Hz, H-4), 10.54 (br s, 1H, CONH), 12.70 and 13.17 (two br s, 1H each, 2OH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 11.93 (q, N(CH_2CH_3)$_2$), 32.58 (t, CONHCH_2), 42.21 (two t, N(CH_2CH_3)$_2$), 51.83 (t, CONHCH$_2$CH$_2$), 110.69 (s, C-12), 112.53, 112.81 (two s, C-6a and C-10a), 118.18 (d, C-3), 123.34 (d, C-1), 125.42 (s, C-5a), 127.88 (d, C-2), 128.02 (d, C-4), 128.71, 130.79 (two d, C-8 and C-9), 139.65 (s, C-12a), 157.54, 159.04 (two s, C-7 and C-10), 162.78 (s, CONH), 177.47 (s, C-6), 185.99 (s, C-11); EI-MS m/z (%): 421 [$M^{+\bullet}$] (4), 306 (10), 86 (100); HR(EI)MS: 421.1631 ± 0.003 ($C_{23}H_{23}N_3O_5^{+\bullet}$, calc. 421.1638).

7,10-Dihydroxy-12-[4-(2-hydroxy-ethyl)-piperazine-1-carbonyl]-benzo[f]pyrido[1,2-a]indole-6,11-dione ***12c***

Deep red solid; m. p. 235–236°C (chloroform/methanol); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.29, 2.59,2.83, 3.39,3.46, 3.61, 3.91, 4.05 (series of m, 12H), 7.21 (m, 3H, H-3,H-8 and H-9), 7.37 (br t., 1H, J = 6.9 Hz, H-2), 7.75 (br d., 1H, J = 9.0 Hz, H-1), 9.62 (br d, 1H, J = 6.9 Hz, H-4), 12.85 (s, 1H, OH), 13.04 (s, 1H, OH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 41.59, 46.33 (two t, (CH_2)$_2$NCO), 52.38, 53.11 (two t, (CH_2)$_2$NCH$_2$), 57.24 (t, CH_2CH_2OH), 58.93 (t, CH_2OH), 109.99 (s, C-12), 112.84, 113.12 (two s, C-6a and C-10a), 117.53, 119.21 (two d, C-2 and C-3), 125.88 (s, C-5a), 126.12 (d, C-1), 128.21 (d, C-4), 128.40, 129.12 (two d, C-8 and C-9), 138.35 (s, C-12a), 158.67, 158.71 (two s, C-7 and C-10), 162.99 (s, CONH), 176.50 (s, C-6), 184.31 (s, C-11); ESI(+)-MS m/z: 458 [M+Na]$^+$, 436 [M+H]$^+$, MS/MS (458) → 328; ESI(-)-MS: 434 [M-H]$^-$, MS/MS (434) → 404, 304, 278.

References

[1] K. M. Tewey, G. L. Chen, E. M. Nelson, L. F. Liu, *J. Biol. Chem.* **1984**, *259*, 9182–9187.

[2] R. B. Weiss, *Semin. Oncol.* **1992**, *19*, 670–686.

[3] H. Malonne, G. Atassi, *Anti-Cancer Drugs* **1997**, *8*, 811–822.

[4] M. R. McCrystal, B. D. Evans, V. J. Harvey, P. I. Thompson, *et al.*, *Cancer Chemother. Pharmacol.* **1999**, *44*, 39–44.

[5] M. H. Moore, W. H. Hunyer, B. L. d'Estaintot, O. Kennard, *J. Mol. Biol.* **1989**, *206*, 693–705.

[6] U. Pindur, M. Harber, K. Sattler, *J. Chem. Educ.* **1993**, *70*, 263–272.

[7] A. Bolognese, G. Correale, M. Manfra, A. La Vecchia, *et al.*, *J. Med. Chem.* **2004**, *47*, 849–858.

[8] K. C. Murdock, R. G. Child, P. F. Fabio, R. B. Angier, *et al.*, *J. Med. Chem.* **1979**, *22,* 1024–1030.

[9] G. W. Rewcastle, G. J. Atwell, D. Chambers, B. C. Baguley, W. A. Denny, *J. Med. Chem*. **1986**, *29*, 472 – 477.

[10] H. J. Park, H-J. Lee, E-J. Lee, H. J. Hwang, *et al*., *Biosci. Biotechnol. Biochem.* **2003**, *67*, 1944 – 1949.

[11] E. F. Pratt, R. W. Luckenbaugh, R. L. Erickson, *J. Org. Chem.* **1954**, *19*, 176 – 182.

[12] M. S. Mathur, B. D. Tilak, *J. Scient. & Ind. Res.* **1958**, *17B,* 33 – 40 [Chem. Abstr. **1958**, *52*, 72342].

[13] J. M. Khurana, A. Sehgal, *Org. Prep. Proced. Int.* **1994**, *26*, 580 – 583.

[14] http://dtp.nci.nih.gov/branches/btb/ivclsp.html

[15] A. P. Krapcho, M. E. Petry, Z. Getahun, J. J. Landi, *et al*., *J. Med. Chem*. **1994**, *37*, 828 – 837.

