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Original article

Synthesis and biological activity of 6*H*-isoindolo[2,1-*a*] indol-6-ones, analogues of batracylin, and related compounds

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Abstract

Closely related to batracylin, 6*H*-isoindolo[2,1-*a*]indol-6-ones including 2-nitro- **13a**, 2-amino- **14**, and 2-diethylaminopropionamide derivative **16** as well as D-ring substituted **13b**, **13c** or A-ring substituted **13d** and **20** analogues, were synthesised and evaluated against L1210 leukaemia. Subsequent treatment of **13b** and **13c** with *N*,*N*-diethylethylenediamine at 180 °C, led to compounds **17a** and **17b** arising from an unexpected opening of the pyrrolidinone ring and amidification of the keto group. Under the same conditions, the dichloro derivative **13d** led to the monoalkyl compound **20** which was the most cytotoxic of the series.

Keywords: Batracylin; Isoindolo[2,1-*a*]indole; Indole; Amino-basic chain; Intramolecular Wittig reaction

1. Introduction

Batracylin **1** [8-amino-isoindolo[1,2-*b*]quinazolin-12(10H)-one] is a water-insoluble compound synthesised by Kabbe [1], acting as an ATP-insensitive topoisomerase II poison [2]. Originally investigated in vivo against murine tumours, this drug displays an original spectrum of activity. Although inactive against L1210 leukaemia, B16 melanoma and marginally active against P388 leukaemia, it inhibits tumour growth of the subcutaneous implanted colon 38 adenocarcinoma [3]. In addition, it possesses collateral sensitivity against, both adriamycin and cisplatin-resistant leukaemia P-388 [4]. Preclinical toxicities studies revealed that batracylin was much more toxic when administered orally to rats than to mice. The responsible metabolite was identified to be *N*-acetylbatracylin **2** [5] which is a potent inducer of unscheduled DNA synthesis [6], and cytochrome P450 3A was probably involved into this transformation [7]. The systemic toxicity, which occurs especially in rats, but also in human liver [8] and the high dose required for antineoplastic activity were the principal adverse effects of batracylin.

Taking these data into account, several studies have been undertaken to find compounds endowed with decreased toxicity. The structural modifications, applied to batracylin, were undertaken in order to examine the influence of additional nitrogen atom in "A" or "D" ring (**3** [9], **4** [6]), or increasing the size of the polycyclic system (**5**) [9]. Contraction of the "C" ring from a six-membered into a five-membered ring providing the benzimidazol analogues of batracylin (**6a–b**) was also reported [6] (Fig. 1). It must be noticed than none of these compounds was found to act as a topoisomerase inhibitor.

For our part, in a general program within discovering new potential antitumour heterocycles [10], we designed and synthesised new isoindolo[2,1-*a*]indole-6-ones, of type **A** (Fig. 2), which can be considered as C-pyrrole-containing analogues of batracylin. The influence of nitrogen introduction on rings A or D, as a free amino group, a diethylamino ethylamide or a diethylamino ethylamine side-chain, such as in **14**, **16** and **20**, respectively, was studied. This is the subject of the present report.

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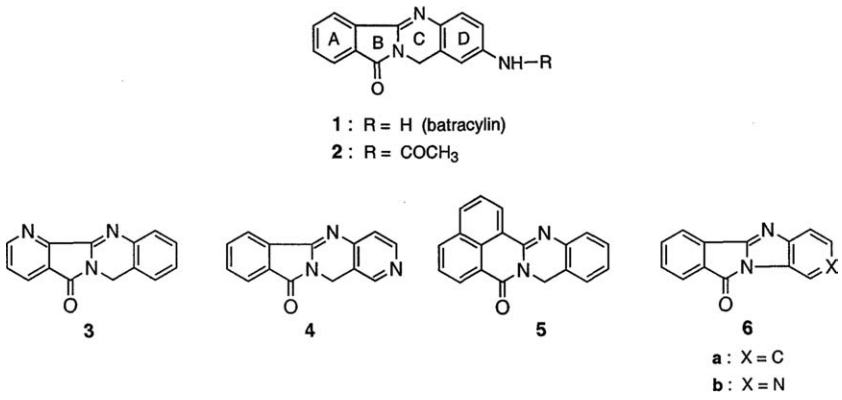

Fig. 1. Chemical structures of batracylin **1** and related compounds.

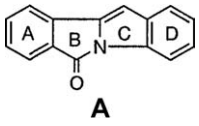

Fig. 2. Structure of type A.

2. Chemistry

The synthesis of the tetracyclic system was carried out via an intramolecular Wittig reaction, using the protocol that we had already reported [10]. Diversely substituted phthalimides **9** were obtained by reaction of the corresponding phthalic anhydrides **7** with various aniline derivatives **8** in acetic acid. Next, the derivatives **10** were prepared by bromination of **9** with *N*-bromosuccinimide (NBS) under radical conditions. This led to the inseparable mixtures of the expected monobromo compounds **10**, along with the dibromo compounds **11** and the recovered starting materials **9** (Table 1).

Therefore, the crude mixtures were engaged in the following Wittig step and the resulting phosphonium salts **12**, when treated with a base in toluene under reflux, provided the expected isoindolo[2,1-*a*]indol-6-one derivatives **13**. Catalytic hydrogenation of the 2-nitro-6*H*-isoindolo[2,1-*a*]indol-6-one **13a** furnished **14** along with **15** resulting from reduction of the double-bond of the indole ring (ratio of 55:45, as estimated by ^{1}H NMR). After these compounds had been separated by column chromatography, condensation of **14** with 3-(diethylamino)propionic acid hydrochloride, in the presence of DCC and DMAP afforded **16** (46% yield) (Fig. 3).

As described by Krapcho et al. [11,12] in anthracene-9,10-dione series, we were also planning to obtain the [[(diethylamino)ethyl]amino]isoindolo[2,1-*a*]indol analogues, by substitution of either the fluorine or chlorine group of **13b** or **13c** by *N*,*N*-diethylethylenediamine. In order to be efficient, the reaction must be performed at 180 °C for 4 days under pressure in steel vessel, and without solvent [13–15]. Under these drastic conditions, instead of the expected isoindoloindole derivatives, this reaction led to the opened derivatives, the fluoro and chloro derivatives of *N*-[2-(diethylamino)ethyl]-2-(indol-2-yl) benzenecarboxamide **17a**, **17b** (Fig. 4). These structures were assigned on the basis of mass spectrometry and X-ray spectra of **17b** (Fig. 5). The relative fragility of the lactam bond was also observed during the reduction of the 2-methoxyisoindolo [2,1-*a*]indol-6-one **18** with $LiAlH_4$ which provided the alcohol **19** (Fig. 6).

In contrast, treatment of the dichloro derivative **13d** by *N*,*N*-diethylethylenediamine, under the afore-mentioned conditions, readily afforded the mono-substituted product **20**. It can be hypothesised that the halogen displacement (ipso substitution) is favoured in the latter case at C-7 by the presence of the adjacent carbonyl group in peri position [11]. This was not the case for **13b** and **13c** or for the chlorine present at C-10 in **13d** (Fig. 7).

Table 1
Bromination of 2-(2-methylphenyl)-1*H*-isoindole-1,3(2*H*) dione **9**

Compounds	NBS equivalent[a]	Ratio[b]		
		Starting Material	CH_2Br **10**	$CHBr_2$ **11**
10a/11a	1.1	38	48	14
	1.25	31	53	16
	1.5	25	54	21
10b/11b	1.1	12	64	24
10c/11c	1.1	10	66	24
10d/11d	1.1	11	66	23

[a] All experiments were conducted in CCl_4 at 76 °C for 8 h except in the case of **10d/11d**, 1 h.

[b] The ratio were calculated from an average of ^{1}H NMR signals integrations of each component.

3. Biological results and discussion

Compounds **14**, **15**, **16**, **17a**, **17b** and **20** were tested in vitro against the murine L1210 leukaemia and the human HT-29 colon carcinoma cell lines (Table 3). Although these compounds did not show properties comparable to that of the reference drugs (adriamycin and camptothecin), it should be noted that the most active were the basic chain-containing derivatives **16** and **20**, followed by the parent compound **14**.

Despite the reported [4] inhibition of adenocarcinoma 38 growth in mice (but at 400 mg kg^{-1}) by batracylin, the relative lack of in vitro activity against L1210 and HT-29 was not surprising, since the same relative inactivity in vitro was already demonstrated against the leukaemia cell line [3] but also the

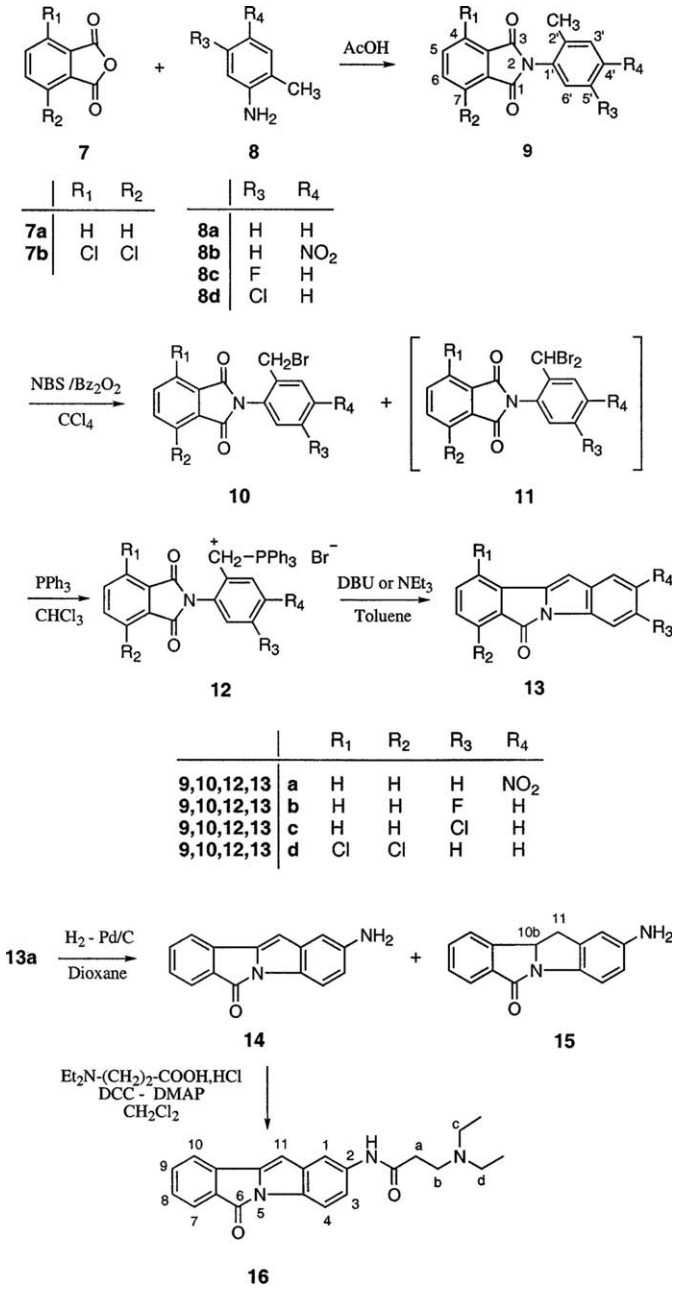

Fig. 3. Synthesis of 2-amino-6*H*-isoindolo[2,1-*a*]indol-6-one **(14)** and related derivatives.

Fig. 4. Synthesis of *N*-[2-(diethylamino)ethyl]-2-(indol-2-yl)benzenecarboxamides **(17)**.

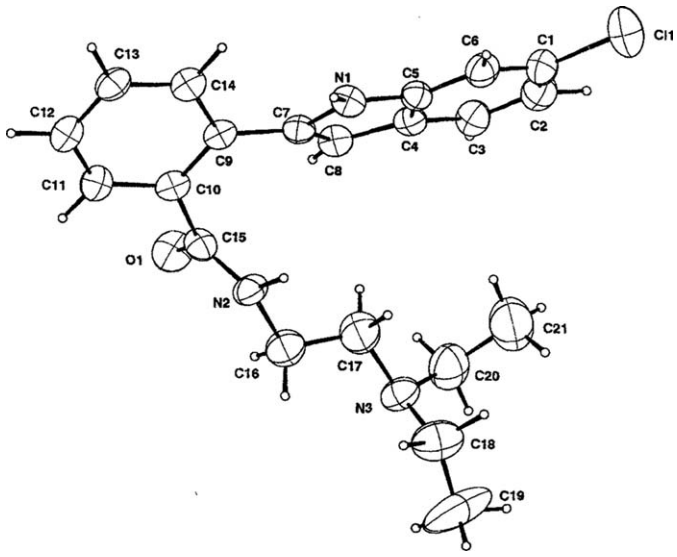

Fig. 5. X-ray crystallography of **17b**.

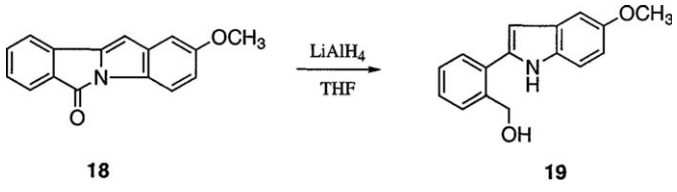

Fig. 6. Reduction of 2-methoxyisoindolo[2,1-*a*]indol-6-one **(18)**.

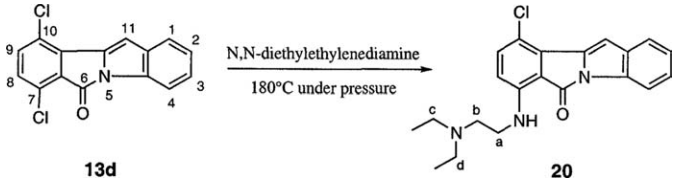

Fig. 7. Synthesis of 10-chloro-7-[[(diethylamino)ethyl]amino]-6*H*-isoindol[2,1-*a*]indol-6-one **(20)**.

cancer lung (H-125), human colon (CX1) and human intestinal (HCT-8) adenocarcinoma [19].

A tetracyclic aromatic scaffold is a critical feature to retain antiproliferative activity as demonstrated by the dramatic decrease in activity when partial reduction occurred (see **15**) or when the ring B was cleaved (see **17a** and **17b**).

Blockade of the cellular cycle in G2 + M was observed in the case of **14** and **20,** the active compound **16** being too toxic to observe a specific effect.

These amino compounds were also tested for topoisomerases I and II inhibition. None of the tested compounds was a specific topoisomerase I inhibitor; only a non-specific effect was observed with compounds **14**, **16** and **20**. Indeed these three compounds bind to DNA and interfere non-specifically with the topoisomerase I catalytic cycle. Noteworthy, these compounds are planar aromatic molecules and when a double-bond is lost likely in compound **15,** the molecule was devoid of the DNA-binding properties. The conformation of

Table 2
Crystal data of compound **17b** for $C_{21}H_{24}ClN_3O$, CH_3OH

Empirical formula	$C_{22}H_{28}ClN_3O_2$
Crystal class	Triclinic
Shape	Parallelepiped
Colour	Colourless
M (g mol^{-1})	401.94
Space group	P–1
Temperature (K)	295
Wavelength (Å)	0.710690
a (Å)	11.837 (3)
b (Å)	13.505 (4)
c (Å)	13.717 (2)
α (°)	89.74 (2)
β (°)	86.42 (1)
γ (°)	84.08 (2)
Volume (A^3)	2177 (5)
Z	4
Density	1.23
Crystal size (mm)	0.5 × 0.4 × 0.3
Scan type	2 θ/ω
θ_{min}, θ_{max}	1–25
μ (mm^{-1})	0.197
Radiation type	MoKα
Reflections measured	7993
Independent reflections	7641
Reflections used	3184
Number of parameters	507
Goodness of fit	1.09
$R = \Sigma \, IIF_oI–IF_cII/\Sigma \, IF_oI$	
$R_w = [\Sigma w \, (IIF_oI–IF_cII)^2/\Sigma \, IF_oI^2]^{1/2}$	

compounds **17a** and **17b** allowed even less DNA binding. Furthermore, the presence of the amino side-chains in **16** and **20** increased DNA affinity as compared to **14**.

On the other hand, compounds **14** and **16** showed an inhibitory potency of topoisomerase II comparable to that of etoposide (50% of linear DNA in the presence of 20 μM of drug). However, at high concentrations, compound **16** proved to be highly toxic. Despite the fact that the chloro-derivative **20** was less active for inhibiting topoisomerase II, this compound displayed the most relevant cytotoxicity against HT-29 and L1210 cell lines.

4. Conclusion

The synthesis of isoindolo analogues of batracylin was undertaken. Contrary to what was observed with stricto sensu five-membered ring analogues, some of these new compounds displayed a topoisomerase II inhibition comparable to that of etoposide. They also displayed modest but slightly superior antiproliferative effect against HT-29 and L1210 cell lines versus batracylin itself. Dihydro derivatives and B-ring opened analogues were completely devoid of activity underlining the importance of the planar tetracyclic aromatic system in this class of compounds.

5. Experimental protocols

5.1. Chemistry

5.1.1. General remarks

Uncorrected melting points were determined on a Kofler hot stage or an electrothermal capillary melting apparatus. The ^1H NMR spectra were recorded at 90 MHz using a Varian EM 390, and at 300 MHz on a Brucker AM-300 spectrometer with TMS as internal standard (s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublet, m = multiplet); coupling constants (*J*) were given in hertz (Hz). Electronic impact (EI, 70 eV) and chemical ionisation (CI) mass spectra (MS) were recorded on a Nermag R10-10-C spectrometer. Elemental analyses were performed by the "Service de Microanalyses du CNRS" (Vernaison-Lyon, France); all compounds had analytical results within ± 0.4% of their theoretical values. The thin-layer chromatographic analyses were performed using precoated silica gel (Merck, $60F_{254}$). Column chromatographies were carried out on Merck silica gel 60 (70–230 mesh).

Table 3
Cytotoxicity, effect on the cellular cycle and activity against topoisomerase I and II of **14, 15, 16, 17a, 17b, 20**

Compounds	HT-29	L1210	% of L1210 in G2 + M[a]		TOPO I	TOPO II
	IC_{50} (μM)	IC_{50} (μM)	%	Conc. (μM)	MIC [b] (μM)	linDNA (%) [c] (at 20 μM)
Adriamycin	0.068	0.022	80%	0.1		
Camptothecin	0.008	0.025	78%	0.25	10	
Batracylin	> 50	> 50				
Etoposide	0.83		76%	2.5		50
14	15.1	10.2	71%	25	20	50
15	80	> 50	NE		> 100	0
16	NE	6.7	NS [d]	20	50	50
17a	NE	42.9			> 100	0
17b	NE	14.6			> 100	0
20	3.2	4.4	52%	10	50	30

NE: not evaluated; NS: non-specific.
[a] Distribution of control cells in the cycle: 37% (G1), 39% (S), 24% (G2 + M).
[b] MIC: minimal concentration of drug for which an inhibition of relaxation in the presence of topoisomerase I is detected.
[c] linDNA (%): percentage of linear DNA measured in a DNA relaxation assay in the presence of topoisomerase II reported at 20 μM of drug.
[d] Toxic at 25 μM and inactive at lower concentrations.

5.1.2. Synthesis of 2-(2-methylphenyl)-1H-isoindole-1,3(2H)-diones ***9***

General procedure: the 3,6-dichlorophthalic anhydride was prepared from tetrachlorophthalic anhydride [13,15].

A mixture of phthalic anhydride **7** (10 mmol) and aniline **8** (10.5 mmol) was refluxed for 6 h in 20 ml of acetic acid. The mixture was poured into water, the resulting precipitate was filtered off, washed with water, dried and recrystallised.

5.1.2.1. 2-(2-Methyl-4-nitrophenyl)-1H-isoindole-1,3(2H)-dione ***(9a)***. This compound was synthesised from **7a** and **8b**: yield 91%; m.p. 200–202 °C (EtOH); ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.33 (s, 3H, CH_3), 7.40 (d, 1H, $H_{6'}$, $J = 9$ Hz), 7.76–8.06 (m, 4H arom.), 8.16–8.33 (m, $H_{3'}$, $H_{5'}$). Anal. $C_{15}H_{10}N_2O_4$ (C, H, N).

5.1.2.2. 2-(5-Fluoro-2-methylphenyl)-1H-isoindole-1,3(2H)-dione ***(9b)***. This compound was synthesised from **7a** and **8c**: yield 95%; m.p. 158–160 °C (EtOH); ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.16 (s, 3H, CH_3), 6.86–7.46 (m, $H_{3'}$, $H_{4'}$, $H_{6'}$), 7.73–8.00 (m, 4H arom.). Anal. $C_{15}H_{10}FNO_2$ (C, H, N, F).

5.1.2.3. 2-(5-Chloro-2-methylphenyl)-1H-isoindole-1,3(2H)-dione ***(9c)***. This compound was synthesised from **7a** and **8d**: yield 75%; m.p. 170–172 °C (EtOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.17 (s, 3H, CH_3), 7.22–7.34 (m, 3H, $H_{3'}$, $H_{4'}$, $H_{6'}$), 7.81 (m, 2H, H_5, H_6), 7.96 (m, 2H, H_4, H_7); IR ($CDCl_3$) 1709 cm^{-1} (C=O). Anal. $C_{15}H_{10}ClNO_2$ (C, H, N, Cl).

5.1.2.4. 4,7-Dichloro-2-(2-methylphenyl)-1H-isoindole-1,3(2H)-dione ***(9d)***. This compound was synthesised from **7b** and **8a**: yield 91%; m.p. 201–203 °C (EtOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.16 (s, 3H, CH_3), 7.17 (d, 1H, $H_{3'}$, $J = 7.64$), 7.30–7.39 (m, 3H, $H_{4'}$, $H_{5'}$, $H_{6'}$), 7.64 (s, 2H, H_5, H_6). Anal. $C_{15}H_9Cl_2NO_2$ (C, H, N, Cl).

5.1.3. Synthesis of bromo compounds ***10***

General procedure for bromination:

A suspension of methylated derivatives **9** (10 mmol), NBS (according to the quantity indicated on Table 1) and benzoyl peroxide (50 mg) in dry carbon tetrachloride (80 ml) was refluxed under stirring, under exposure of a 150 W tungsten lamp, for the indicated time. The reaction mixture was poured into water, extracted with dichloromethane, and the solvent was evaporated.

The dosage of each component, monobromo **10** and dibromo compound **11** of the crude reaction mixture was analysed by ^{1}H NMR spectroscopy.

5.1.4. Synthesis of phosphonium salts ***12***

General procedure:

To a solution of crude mixture of bromo derivatives (3 g) in $CHCl_3$ (25 ml) was added triphenylphosphine (1.05 equivalent with regard to the theoretical quantity of mono bromo compound present in the mixture). After heating at reflux for 18 h, the solvent was evaporated. Under agitation in dimethoxyethane (100 ml), the gummy residue crystallised. The precipitate was filtered, carefully washed with dimethoxyethane to eliminate the impurities, and dried at 80 °C in vacuo.

5.1.4.1. [[2-(1,3-Dihydro-1,3-dioxo-2H-isoindol-2-yl)-5-nitrophenyl]methyl] triphenyl phosphonium bromide ***(12a)***. Yield (with regard to **9a**) 35%, m.p. 272–274 °C (dec.); ^{1}H NMR (90 MHz, $CDCl_3$): δ 5.55 (d, 2H, CH_2–P, $J = 15$ Hz), 7.26–8.00 (m, 20H arom), 8.16–8.26 (m, $H_{3'}$), 8.56 (m, 1H, $H_{5'}$). Anal. $C_{33}H_{24}BrN_2O_4P$ (C, H, N, Br).

5.1.4.2. [[2-(1,3-Dihydro-1,3-dioxo-2H-isoindol-2-yl)-4-fluorophenyl]methyl] triphenyl phosphonium bromide ***(12b)***. Yield (with regard to **9b**) 67%, m.p. 218–220 °C (dec.); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.03 (d, 2H, CH_2–P, $J = 13.8$ Hz), 6.82 (d, 2H, $H_{3'}$, $H_{4'}$, $J = 8.4$ Hz), 7.33–7.48 (m, 15 H arom.), 7.56 (m, 2H, H_5, H_6), 7.68 (m, 1H, $H_{6'}$), 7.78 (m, 2H, H_4, H_7). Anal. $C_{33}H_{24}BrFNO_2P$, $C_4H_{10}O_2$, dimethoxyethane (C, H, Br, F, N).

5.1.4.3. [[2-(1,3-Dihydro-1,3-dioxo-2H-isoindol-2-yl)-4-chlorophenyl]methyl] triphenyl phosphonium bromide ***(12c)***. Yield (with regard to **9c**) 66%, m.p. 204–206 °C (dec.); ^{1}H NMR (90 MHz, $CDCl_3$): δ 5.26 (d, 2H, CH_2–P, $J = 15$ Hz), 7.10 (s, 1H, $H_{3'}$), 7.20–8.06 (m, 21 H arom.). Anal. $C_{33}H_{24}BrClNO_2P$ (C, H, Br, Cl, N).

5.1.4.4. [[2-(4,7-Dichloro-1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)phenyl]methyl] triphenyl phosphonium bromide ***(12d)***. Yield (with regard to **9d**) 51%, m.p. 220–222 °C (dec.); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.19 (d, 2H, CH_2–P, $J = 13.9$ Hz), 7.10 (d, 1H, $H_{3'}$, $J = 7.8$ Hz), 7.22 (m, 2H, $H_{4'}$, $H_{5'}$), 7.35–7.67 (m, 15 H arom.), 7.74 (s, 2H, H_5, H_6), 7.87 (d, 1H, $H_{6'}$, $J = 8.2$ Hz). Anal. $C_{33}H_{23}BrCl_2NO_2P$ (C, H, Br, Cl, N).

5.1.5. Synthesis of 6H-isoindol[2,1-a]indol-6-one derivatives ***13****, by intramolecular Wittig reaction*

General procedure:

To a suspension of phosphonium salts **12** (10 mmol) in toluene (125 ml) was added the under-mentioned quantity of triethylamine (NEt_3) or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The mixture was refluxed for the indicated time. After cooling, the insoluble was filtered and the mother liquor was directly chromatographed on the silica column using toluene as eluent.

5.1.5.1. 2-Nitro-6H-isoindolo[2,1-a]indol-6-one ***(13a)***. This compound was prepared, from phosphonium salt **12a** with 1.1 equivalent of DBU for 24 h: yield 89%, m.p. 268–270 °C (chlorobenzene); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.18 (s, 1H, H_{11}), 7.53 (m, 1H, H_8 or H_9), 7.74 (m, 1H, H_8 or H_9), 7.84 (d, 1H, H_7, $J = 7.5$ Hz), 7.92 (m, 2H, H_4 and H_{10}, $J = 9$ Hz), 8.21 (dd, 1H, H_3, $J = 2.3$ Hz, $J = 8.8$ Hz), 8.56 (d, 1H, H_1, $J = 2.2$ Hz). Anal. $C_{15}H_8N_2O_3$ (C, H, N).

5.1.5.2. 3-Fluoro-6H-isoindolo[2,1-a]indol-6-one ***(13b)***. This compound was prepared, from phosphonium salt **12b** with 2 equivalents of NEt_3 for 5 h: yield 86%, m.p. 193–195 °C (toluene/cyclohexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.58 (s, 1H, H_{11}), 6.89 (m, 1H, H_2, $J = 2.3$ Hz, $J = 9.2$ Hz), 7.32–7.39 (m, 2H, H_1, H_8), 7.51–7.53 (m, 2H, H_9, H_{10}), 7.61 (dd, 1H, H_4, $J = 2.3$ Hz, $J = 8.8$ Hz), 7.77 (d, 1H, H_7, $J = 7.9$ Hz). Anal. $C_{15}H_8FNO$ (C, H, F, N).

5.1.5.3. 3-Chloro-6H-isoindolo[2,1-a]indol-6-one ***(13c)***. This compound was prepared, from phosphonium salt **12c** with 1.5 equivalents of NEt_3 for 4 h: yield 92%, m.p. 203–205 °C (toluene/cyclohexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.58 (s, 1H, H_{11}), 7.12 (dd, 1H, H_2, $J = 1.9$ Hz, $J = 8.2$ Hz), 7.35 (d, 1H, H_1, $J = 8.3$ Hz), 7.37 (m, 1H, H_8), 7.53 (m, 2H, H_9, H_{10}), 7.77 (d, 1H, H_7, $J = 6.8$ Hz), 7.90 (d, 1H, H_4). MS (EI) *m/z* 253–255 (M^+, 100%). Anal. $C_{15}H_8ClNO$ (C, H, Cl, N).

5.1.5.4. 7,10-Dichloro-6H-isoindolo[2,1-a]indol-6-one ***(13d)***. This compound was prepared, from phosphonium salt **12d** with 2 equivalents of NEt_3 for 6 h: yield 82%, m.p. 206–208 °C (toluene); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.89 (s, 1H, H_{11}), 7.18 (m, 2H, H_2, H_3), 7.34 (m, 2H, H_1, H_8), 7.48 (d, 1H, H_9, $J = 7.3$ Hz), 7.89 (d, 1H, H_4, $J = 8.1$ Hz). Anal. $C_{15}H_7Cl_2NO$ (C, H, Cl, N).

5.1.6. Reduction of 2-nitro-6H-isoindolo[2,1-a]indol-6-one ***(13a)***

Hydrogenation was accomplished at air-pressure using 1.5 g (5.68 mmol) of nitro compound and 10% Pd–C (335 mg) in dioxane, for 5 h. The reaction mixture was filtered off over celite and the solvent was evaporated. The residue, dissolved in dichloromethane, was chromatographed on silica (increasing the eluent polarity from CH_2Cl_2 to MeOH: 99.5–0.5 to 98–2) to give the following:

5.1.6.1. 2-Amino-6H-isoindolo[2,1-a]indol-6-one ***(14)***. 0.62 g (yield 46%), m.p. 189–191 °C (toluene); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.65 (bs, 2H exchangeable, NH_2), 6.46 (s, 1H, H_{11}), 6.66 (dd, 1H, H_3, $J = 2.2$ Hz, $J = 8.2$ Hz), 6.75 (d, 1H, H_1, $J = 2.2$ Hz), 7.31 (m, 1H, H_9), 7.47 (m, 2H, H_8, H_{10}), 7.67 (d, 1H, H_4, $J = 8.3$ Hz), 7.72 (d, 1H, 1H, H_7, $J = 7.9$ Hz); MS (EI) *m/z* 234 (M^+). Anal. $C_{15}H_{10}N_2O$ (C, H, N).

5.1.6.2. Hydrochloride. The free base was dissolved in dry dichloromethane and dry HCl gas was bubbled through the solution. The hydrochloride was collected by filtration, m.p. > 260 °C. Anal. $C_{15}H_{10}N_2O$, HCl (C, H, Cl, N).

5.1.6.3. 2-Amino-6H-isoindolo[2,1-a]indolin-6-one ***(15)***. 0.47 g (yield 35%), m.p. 165–167 °C (toluene)**;** ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.97 and 3.35 (2 m, 2H, H_{11a}, H_{11b}), 3.43 (bs, 2H exchangeable, NH_2), 5.56 (t, 2H, H_{10b}), 6.60 (m, 2H, H_1, H_3), 7.45–7.52 (m, 3H, H_4, H_8, H_{10}), 7.58 (t, 1H, H_9), 7.90 (m, 1H, H_7). MS (EI) *m/z* 236 (M^+, 100%). Anal. $C_{15}H_{12}N_2O$ (C, H, N).

5.1.7. N-(6-oxo-6H-isoindolo[2,1-a]indol-2-yl)-3-diethylaminopropionamide ***(16)***

To a solution of the amino compound **14** (117 mg, 0.5 mmol) in CH_2Cl_2 were added 3-(diethyamino)propionic acid hydrochloride (181.5 mg, 1 mmol), dicyclohexyl carbodiimide (DCC, 1 M in solution in CH_2Cl_2, 1.05 ml, 1 mmol), and dimethylamino-pyridine (DMAP, 244 mg, 2 mmol). The mixture was allowed to stir for 16 h at room temperature under argon atmosphere. The resulting white precipitate of dicyclohexylurea was removed by filtration. The solvent was evaporated in vacuo and the residue was purified on silica gel (eluent CH_2Cl_2/MeOH 98:2). Compound **16** was obtained in 46% yield, m.p. 92–94 °C (toluene-C_6H_{12}); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.16 (t, 6H, $2CH_3$), 2.53 (t, 2H, H_b), 2.69 (q, 4H, H_c, H_d), 2.80 (t, 2H, H_a), 6.59 (d, 1H, H_{11}, $J = 1.1$ Hz), 7.12 (d, 1H, H_1, $J = 8.7$ Hz), 7.33 (m, 1H, H_3), 7.51 (m, 2H, H_8, H_9), 7.75 (m, 2H, H_7, H_{10}), 8.00 (s, 1H, H_4), 11.37 (s, 1H, NH); MS (CI–NH_3) *m/z* 362 $(M + H)^+$. Anal. $C_{22}H_{23}N_3O_2$,H_2O (C, H, N).

5.1.8. Synthesis of ***17*** *and* ***20***

General procedure:

A mixture of halogenoisoindolo[2,1-*a*]indol-6-one **13** (1 g) and freshly distilled *N*,*N*-diethylethylenediamine (20 ml) was heated in a steel vessel at 180 °C for 4 days. Excess of diamine was removed under reduced pressure and the residue was directly chromatographed on a silica column (eluent: CH_2Cl_2/MeOH 99:1).

5.1.8.1. N-[2-(diethylamino)ethyl]-2-(6-fluoroindol-2-yl)benzenecarboxamide ***17a***. This compound was synthesised from **13b**: yield 88%, m.p. 93–95 °C (hexane): ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85 (t, 6H, 2 CH_3), 2.38 (q, 4H, H_c, H_d), 2.47 (t, 2H, H_b), 3.42 (q, 2H, H_a), 6.58 (bs, 1H exchangeable, NH–CO), 6.72 (s, 1H, H_3), 6.86 (m, 1H, H_5, $J = 2.1$ Hz, $J = 9.2$ Hz), 7.11 (dd, 1H, H_7, $J = 1.9$ Hz, $J = 9.7$ Hz), 7.36 (m, 1H, $H_{4'}$, $J = 1.3$ Hz, $J = 7.8$ Hz), 7.47–7.54 (m, 3H, H_4, $H_{3'}$, $H_{5'}$), 7.75 (d, 1H, $H_{6'}$, $J = 1.4$ Hz, $J = 7.7$ Hz), 10.20 (bs, NH-indole). MS (CI–NH_3) *m/z* 354 $(M + H)^+$. Anal. $C_{21}H_{24}FN_3O$ (C, H, F, N).

5.1.8.2. N-[2-(diethylamino)ethyl]-2-(6-chloroindol-2-yl)benzenecarboxamide ***17b***. This compound was synthesised from **13c**: yield 70%, m.p. 107–109 °C (hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85 (t, 6H, 2 CH_3), 2.38 (q, 4H, H_c, H_d), 2.47 (t, 2H, H_b), 3.41 (q, 2H, H_a), 6.50 (bs, 1H exchangeable, NH–C=O), 6.71 (d, 1H, H_3, $J = 0.9$ Hz), 7.06 (dd, 1H, H_5, $J = 1.9$ Hz, $J = 8.5$ Hz), 7.36 (m, 1H, $H_{4'}$), 7.42 (m, 1H, H_7), 7.47–7.52 (m, 3H, H_4, $H_{3'}$, $H_{5'}$), 7.75 (d, 1H, $H_{6'}$, $J = 7.9$ Hz), 10.25 (bs, NH-indole). MS (CI–NH_3) *m/z* 370–372 $(M + H)^+$. Anal. $C_{21}H_{24}ClN_3O$ (C, H, N, Cl).

5.1.8.3. 10-Chloro-7-[[(diethylamino)ethyl]amino]-6H-isoindol[2,1-a]indol-6-one ***20***. This compound was synthesised from **13d**: yield 64%, m.p. 118–120 °C (hexane); ^{1}H NMR (90 MHz, $CDCl_3$): δ 1.13 (t, 6H, 2 CH_3), 2.60–2.86 (m, 6H,

CH_2–$N(CH_2)_2$), 3.33 (q, 2H, NH–CH_2), 6.46 (d, 1H, H_8, $J = 9$ Hz), 6.80 (s, 1H, H_{11}), 7.00–7.60 (m, 4H, H_1, H_2, H_3, H_9), 7.83 (d, 1H, H_4, $J = 8.7$ Hz); MS (CI–NH_3) *m/z* 368–370 $(M + H)^+$. Anal. $C_{21}H_{22}ClN_3O$ (C, H, N).

5.1.9. 2-(5-Methoxyindol-2-yl)benzenemethanol **(19)**

To a solution of 2-methoxy-6*H*-isoindol[2,1-*a*]indol-6-one **18** [10] (1 g, 4.01 mmol) in THF (40 ml) stirred at room temperature, $LiAlH_4$ (0.305 g, 8.02 mmol) was added portion-wise and the stirring maintained for 3 h. Then the excess of reagent was quenched carefully with water and the resulting mixture was extracted with CH_2Cl_2.The alcohol was obtained with 87% yield; ^1H NMR (90 MHz, $CDCl_3$): δ 2.23 (bs, 1H exchangeable, OH), 4.73 (s, 2H, $C\underline{H}_2OH$), 6.66 (d, 1H, H_3, $J = 2.1$ Hz), 6.86 (dd, 1H, H_6, $J = 2.4$ Hz, $J = 9$ Hz), 7.10 (d, 1H, H_4, $J = 9$ Hz), 7.23–7.53 (m, 4H, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{6'}$), 7.75 (d, 1H, H_7, $J = 9$ Hz), 10.06 (bs, 1H, NH-indole); MS (CI–NH_3) *m/z* 254 $(M + H)^+$. Anal. $C_{16}H_{15}NO_2$ (C, H, N).

5.2. X-ray diffraction analysis of N-[2-(diethylamino)ethyl]-2-(6-chloroindol-2-yl)benzenecarboxamide **(17b)**

Crystal of **17b**, suitable for X-ray analysis, was obtained by slow recrystallisation from methanol. The carboxamide crystallised with a MeOH molecule.

The data were collected on a Enraf-Nonius CAD-4 diffractometer using graphite-monochromated MoKα radiation (Table 2). The structure was solved by direct methods and subsequent Fourier maps. Refinements were carried out by full-matrix least-squares techniques. Non-hydrogen atoms were anisotropically refined. All hydrogen atoms' positions were found on difference maps, their coordinates were not refined and they were given an overall isotropic thermal parameter. An absorption correction was applied with the DIFABS program [16] from CRYSTALS [17] which was used to carry out all the calculations.

5.3. Biopharmacological methods (bioevaluations)

5.3.1. Cell culture and cytotoxicity

The human HT29 and murine L1210 cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD). Cells were maintained in RPMI 1640 medium supplemented with 10% decomplemented fetal calf serum (FCS), 2 mM L-glutamine, 100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin, and 10 mM HEPES, pH 7.4. Cells were grown at 37 °C in 5% CO_2/95% air. All media and supplements were from Life Technologies (Cergy-Pontoise, France) except FCS which was purchased from Sigma. Cytotoxicity was measured by the microculture tetrazolium assay as described in [18]. Briefly, adherent cells were seeded in 96 well microplates and incubated for 2 days. The tested compounds were then added and the plates were incubated for four doubling times (continuous exposure). The non-adherent L1210 cells were directly incubated for 48 h with the compounds. At the end of this period, 15 μl of 5 mg ml^{-1} 3-(4,5 dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) were added to each well and the plates were incubated for 4 h at 37 °C. The medium was aspirated and the formazan was solubilised by 100 μl of DMSO. The IC_{50} concentration, reducing by 50% the optical density at 540 nm, was calculated by a linear regression performed on the linear zone of the dose–response curve. All the measurements were performed in triplicate.

For the cell cycle analysis, L1210 cells (2.5×10^5 cells ml^{-1}) were incubated for 21 h with various concentrations of the compounds. Cells were then fixed by 70% ethanol (v/v), washed and incubated in PBS containing 100 μg ml^{-1} RNAse and 25 μg ml^{-1} propidium iodide for 30 min at 20 °C. For each sample, 10^4 cells were analysed on an Epics XL/MCL flow cytometer (Beckman Coulter, France). PI fluorescence was collected through a 630 nm band-pass filter. Results are expressed as the percentage of cells in each phase of the cell cycle.

5.3.2. Topoisomerase I-mediated DNA relaxation assay

Supercoiled pBS DNA (0.1 μg) (Stratagene, France) was incubated for 15 min at 30 °C, in a 50 mM Tris–HCl buffer, pH 7.5, containing 1 mM ATP, 60 mM KCl, 10 mM $MgCl_2$, 0.5 mM DTT, 0.1 mM EDTA and 30 mg BSA, in the presence of the drug at the indicated concentration (total reaction volume 10 ml). 2 units of human DNA topoisomerase I (Topo-GEN, Inc., USA) were added to the duplex, preincubated as described, and incubated for 30 min at 30 °C. The DNA–topoisomerase II cleavage complexes were dissociated by addition of SDS (final concentration 0.25%) and of proteinase K (Sigma) to 250 mg ml^{-1}, followed by incubation for 30 min at 55 °C. DNA samples were then added to the electrophoresis dye mixture (5 ml) and electrophoresed (35 V cm^{-1}) for 2 h in a 1% agarose gel in TBEx1 at room temperature. Gels were stained with SYBR® Green I (Molecular Probes), washed and photographed under UV light.

5.3.3. Topoisomerase II-mediated DNA cleavage assay

The assay was performed as described in [10].

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