Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 3413–3421

BIOORGANIC & MEDICINAL CHEMISTRY

# Synthesis and Cytotoxic Activity of Different Open Indolocarbazole Alkaloid Analogues

Esther Caballero,[a,*] Marta Adeva,[a] Suzanne Calderón,[a] Heidi Sahagún,[a] Fernando Tomé,[a] Manuel Medarde,[a] José Luis Fernández,[b] Miguel López-Lázaro[c] and Maria Jesús Ayuso[c]

[a]*Laboratorio de Química Orgánica y Farmacéutica, Facultad de Farmacia, 37007 Salamanca, Spain*
[b]*Instituto Biomar SA. 24231 Onzonilla, León, Spain*
[c]*Departamento de Farmacología, Facultad de Farmacia, Sevilla, Spain*

Received 4 March 2003; accepted 8 May 2003

**Abstract**—An array of 4-(aryl or indolyl)pyrrolo[3,4-*c*]carbazole-1,3-diones (open analogues of indolocarbazole alkaloids), 10-(aryl or indolyl)pyrrolo[3,4-*b*]carbazole-1,3-diones, and different derivatives have been prepared using a Diels–Alder plus Fischer indolization approach and tested as cytotoxic agents. Some representative compounds display interesting cytotoxic profiles.


Indolocarbazole alkaloids are a class of natural products isolated from microorganisms, slime molds, and marine organisms that show an array of interesting biological activities.[1]

Three representative members of this family are Arcyriaflavin-A,[2] an inhibitor of human cytomegalovirus replication,[3] which was originally isolated from the slime mold *Arcyria denudata* (myxomycetes), rebeccamycin[4] and staurosporine[5] (Fig. 1), both displaying antitumour activity, which were respectively isolated from *Nocardia aerocoligenes* and *Streptomyces staurosporeus* (both actinomycetes).

The considerable research interest in these compounds lies in the potent antitumour properties of many of them. This has given rise to a huge amount of research aimed at their synthesis, the preparation of analogues, and an understanding of their mechanism of action. Compounds whose basic structure is the pyrroloindolocarbazole system with one *N*-glycosidic bond, such as rebeccamycin, act by DNA topoisomerase I inhibition, whereas those with two *N*-glycosidic bonds, such as staurosporine, are mainly protein kinase C (PKC) inhibitors.[6] The mechanism by which Arcyriaflavin-A and simple related compounds inhibit human cytomegalovirus (HCMV) replication seems to be through blockade of the activity of protein kinase pUL97.[7] Currently, several indolocarbazole alkaloids or analogues,

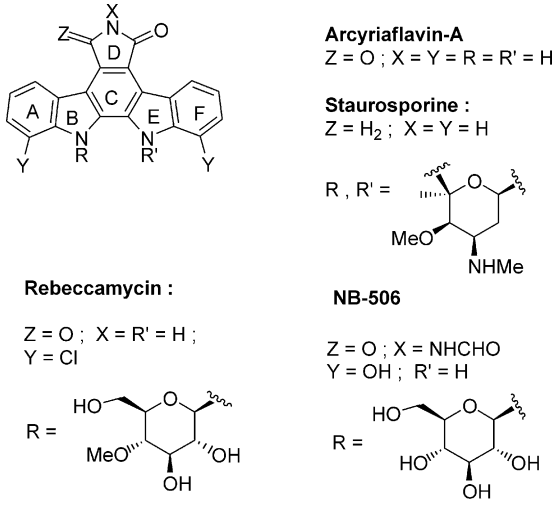



**Figure 1.**

*Corresponding author. Tel.: +34-923-214528; fax: +34-923-294515; e-mail: escab@usal.es

0968-0896/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0968-0896(03)00308-0

such as the topoisomerase I poison NB-506, are in clinical trials for their potential use in cancer therapy.[8]

Attracted by the novel structural features and biological relevance of this family of alkaloids, together with our interest in applying novel aryl-trialkylsiloxy-dienes to the synthesis of natural products and analogues, some time ago we set up a research line aimed at the preparation of analogues of indolocarbazole alkaloids and other natural products, following a Diels–Alder methodology in all cases. The results of this research can be broken down into four different parts: (a) Synthesis and reactivity of novel aryl/aryloxy/heteroaryl dienes,[9] (b) Synthesis of anthracycline analogues,[10] (c) New synthesis of Arcyriaflavin-A[11] and its analogues,[12] (d) Cytotoxic evaluation.[10a,12a]

We now wish to report the cytotoxic activity of the broad number of *open* analogues that we have synthesized to date, together with some hitherto unpublished synthetic procedures for obtaining those compounds. All compounds prepared are 4-(aryl or indolyl)-pyrrolo[3,4-*c*] or 10-(aryl or indolyl)pyrrolo[3,4-*b*] carbazole-1,3-diones and derivatives, and can be divided into three families (Fig. 2).

**Family I** was designed taking into account the presence of the trimethoxyphenyl unit in a variety of cytotoxic agents such as colchicine and podophyllotoxin[13] (tubulin polymerisation inhibitors), etoposide (a topoisomerase II inhibitor, with 4-hydroxy-3, 5-dimethoxyphenyl unit) and duocarmycin SA[14] (a DNA alkylating and minor groove binder). We also focused on the tubulin polymerisation inhibitor 1069C,[15] in which the best antitumour profile is provided by the 3,4,5-trimethoxy- and 2,5-dimethoxyphenyl groupings. Thus, we attempted to modulate the possible cytotoxic activity by introducing these characteristic structural features.

**Family II** was chosen because the nitro group also confers cytotoxic activity in many antitumour agents and in order to use the corresponding analogues as intermediates in the synthesis of the natural product Arcyriaflavin A and its unsymmetrical analogues.

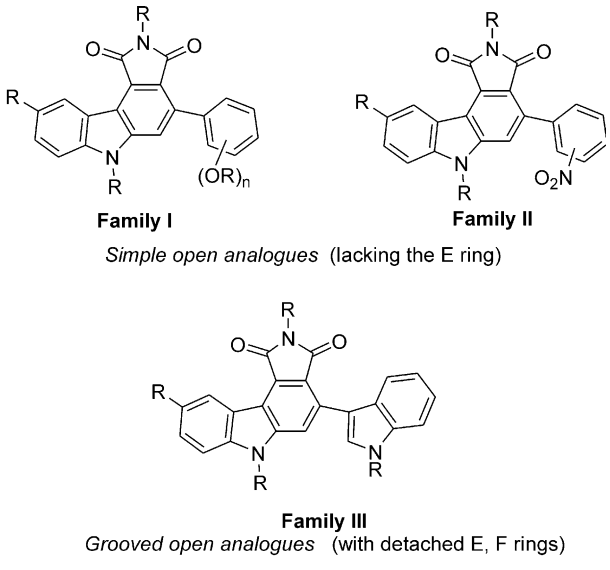



**Figure 2.**

Finally, we considered the preparation of **Family III**, due to the importance of the indolyl ring in many bioactive compounds. Moreover, in this case we took some natural marine products such as wakayin[16] and eudistomin U[17] as models (Fig. 3), all of them displaying cytotoxic activity (topoisomerase I/II inhibitors). We expected that the combination of a planar system and a unit (in this case indolyl) able to interact with DNA through hydrophobic or H-bond forces, as postulated for topoisomerase inhibitors,[18] would lead to compounds having some interesting bioactivity.

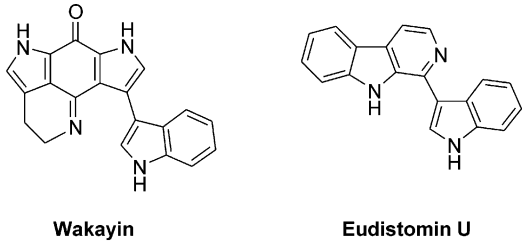


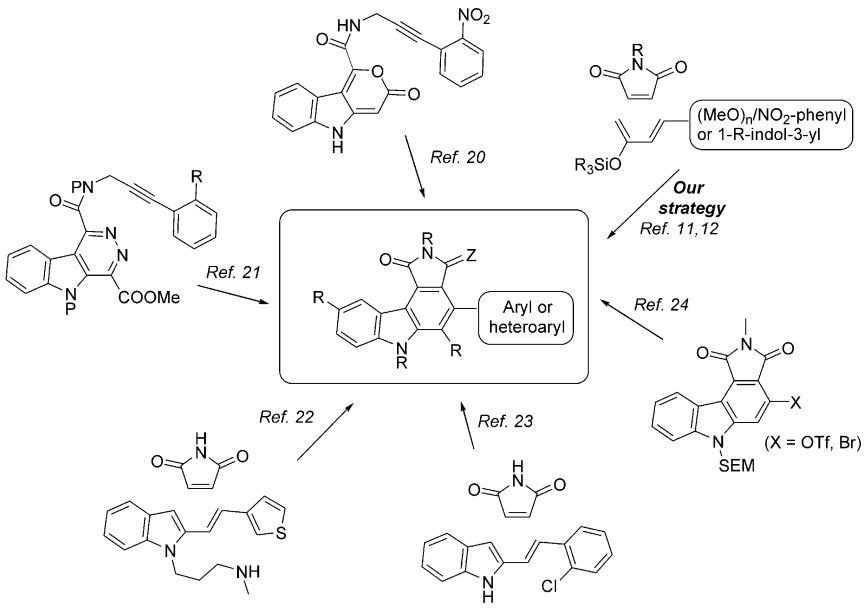

**Figure 3.**

Many approaches to the synthesis of indolocarbazole alkaloids and analogues have been described and are summarised in different reviews and publications[19] (Scheme 1). By contrast, there are few references to the synthesis of analogues with structures similar to those described in this paper, and most of them use a Diels–Alder methodology.

Moody's group reported[20] an intramolecular cycloaddition between an alkyne and a pyranoindolone, yielding an *open* 4-(2-nitrophenyl)pyrrolo[3,4-*c*]carbazole analogue, which was transformed into the staurosporine aglycone via nitrene insertion. Starting from a C-4 ester of a pyridazino[4,5-*b*]indole, a similar approach to *open* analogues has also been reported.[21] The Hoffman-La Roche and Goedecke groups developed a strategy based on the use of maleimides as dienophiles and 2-aryl/heteroarylvinyl-substituted indoles as dienes, leading to 4-aryl/heteroarylpyrrolo[3,4-*c*]carbazoles[22,23] (which show cytotoxicity through PKC inhibition). McCort et al. have recently synthesised a series of other 4-aryl/heteroarylpyrrolo[3,4-*c*]carbazoles, introducing the aryl/heteroaryl moiety by means of a palladium catalysed cross-coupling, after a Diels–Alder reaction similar to that of the two latter references.[24]

Further examples of the synthesis of this kind of analogues are a series of 4-[2-(*N,N*-dimethylaminoethoxy)-phenyl]pyrrolo[3,4-*c*]carbazoles (some of them with thrombopoietic activity)[25] and the formation of 4-(1-aminophenyl)pyrrolo[3,4-*c*]carbazoles from bisindolylmaleimides.[26]

We followed an original strategy that was also based on the Diels–Alder reaction, in this case between novel aryl/heteroaryl-dienes and maleimides, followed by a




**Scheme 1.** Diels–Alder synthetic approaches to 4-aryl/heteroarylpyrrolo[3,4-*c*]carbazoles.

Fischer indolization with *para*-substituted phenylhydrazines. In addition to its utility for obtaining Arcyriaflavin-A and unsymmetrical derivatives,[11] this methodology is useful for the synthesis of new families of open analogues,[12] as we describe below.

## Chemistry

The methodology used for the synthesis of these compounds is similar to that established by us for the synthesis of Arcyriaflavin-A and other grooved analogues. The first step in the synthesis (Scheme 2) is the Diels–Alder reaction of dienes **IVa** and **IVb** with *N*-substituted maleimides, which is quantitative after 2–4 days, using benzene (toluene) as a solvent for **IVa** and $CH_2Cl_2$/acetone for **IVb**. As reported in our previous studies *endo* products were always obtained and different conformations were observed, depending on the type of substitution of the aromatic moiety.[12]

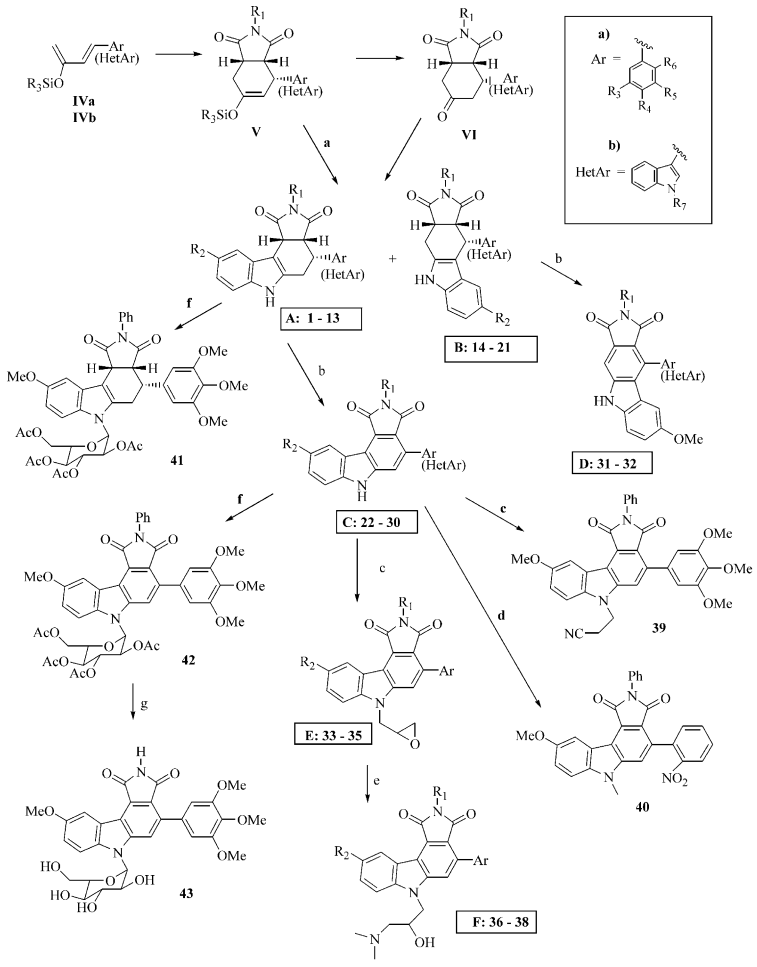
Fischer indolization is usually carried out in acidic medium on ketones **VI**, obtained from the enolcycloadduct **V** by HCl hydrolysis. Since the indolization reaction is carried out under acidic conditions, which could also produce the hydrolysis of the enolether to the ketone, we have now checked the direct indolization reaction on the silylenolether **V** by modifying the Fischer conditions. To the glacial acetic acid/EtOH 1:1 solution some drops of HCl 37% followed by hydrazine were added. For example, from the corresponding enolcycloadduct **Vb** and *p*-methoxyphenylhydrazine, regiosomers **13** and **21** were obtained in good yields. The regioisomeric ratio in this reaction changed, depending on whether compound **V** or **VI** was used, but in all the cases pyrrolo[3,4-*c*]carbazoles **A** were the major products and pyrrolo[3,4-*b*]carbazoles **B** the minor ones.

In our case, the separation of both regioisomers (**A**, **B**) by CC or crystallisation followed by aromatization with DDQ gave representative planar compounds in the **C** and **D** series. Since we were more interested in type **C** analogues, we prepared different *N*-substituted derivatives in order to test their cytotoxicities against representative cancer cell lines. According to structure–activity relationship studies described in the literature for staurosporine and rebeccamycin analogues, a planar system is required, whereas substitution on the carbazole nitrogen is variable, and sugar, aminoalkyl or cyanoalkyl residues have been reported—among others—as substituents that confer good activity.[27] On the basis of this, we proposed the modifications indicated for the families **I**, **II** and **III**.

The synthesis of these compounds is shown in Scheme 2 and the compounds synthesized are summarised in Table 1 (for experimental details, see this and previous papers). We decided to synthesize epoxides **E** (**33**–**35**), aminoalcohols **F** (**36**–**38**), the cyano derivative **39**, and the glycoside **43** to obtain a representation of most common substituents on the carbazole nitrogen encountered among the indolocarbazole derivatives. Since compound **1** proved to be the most potent of the non-planar compounds of type **A** or **B**, we also transformed it into the 4′-hydroxy derivative **7** in order to know the influence of this moiety on the activity of non-planar systems.

## Biological Assays

All the products synthesized were subjected to cytotoxicity assays against neoplastic cell lines representative of solid tumours and leukaemia in order to know their potential as antineoplastic agents. The results obtained for representative compounds are presented in



**Scheme 2.** Synthesis of **1–43**: (a) $R_2$–MeO–$C_6H_4$–NH–$NH_2$, HOAc/EtOH 1:1, drops HCl (37%), reflux; (b) DDQ, $C_6H_6$, rt or reflux; (c) NaOH, $Bu_4NSO_4H$, RX, $C_6H_6$, rt; (d) NaH, DMF, RX; rt; (e) $Me_2NH$, EtOH, rt; (f) tetraacetyl-α-D-glucopyranosyl bromide, $Ag_2O$, $C_6H_6$, reflux; (g) $NH_4OH$/MeOH, rt

Table 2, from which several conclusions can be deduced. The compounds not included in Table 2 were inactive.

The initially synthesized compound, **1**, is among the most active compounds in the series, with several new derivatives with the same order of cytotoxicity, although no important increase in their activity was observed. The most cytotoxic derivatives ($IC_{50}$ = 0.3–5.0 μM in at least two of the cell lines tested) are those with a non-aromatic C ring (**1, 41**) or aromatic derivatives with different substitutions: imido-*N*-phenyl (**33**, **36**) or-*N*-methyl (**27**, **37**, **38**), and carbazole- *N*–H (**25**, **27**, **28**) or carbazole-*N*-substituted (**36**), although in general the C-aromatised derivatives (series **C**–**F**) are more cytotoxic than their non-aromatic analogues (series **A**–**B**). Among the C-aromatised carbazole with free N–H (series **C**), the most active are those presenting *ortho*-substituents on the phenyl group (**25** $R_6$ = OMe; **28** $R_6 = NO_2$).

**Table 1.** Structure of compounds **1**–**38.** See Scheme 2 for general structures **A**–**F**

| Compounds | | | | | | Substitution | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | B | C | D | E | F | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ | $R_7$ | Ar/HetAr |
| **1** | **14** | **22** | | **33** | **36** | Ph | OMe | OMe | OMe | OMe | H | | Ar |
| **2** | **15** | **23** | **31** | | | Me | OMe | OMe | OMe | OMe | H | | Ar |
| **3** | **16** | **24** | | | | H | OMe | OMe | OMe | OMe | H | | Ar |
| **4** | | **25** | | | | Ph | OMe | OMe | H | H | OMe | | Ar |
| **5** | **17** | **26** | **32** | **34** | **37** | Me | OMe | OMe | H | H | OMe | | Ar |
| **6** | **18** | **27** | | **35** | **38** | Me | OMe | OMe | OMe | H | H | | Ar |
| **7** | | | | | | Ph | OMe | OMe | OH | OMe | H | | Ar |
| **8** | | | | | | Ph | Me | OMe | OMe | OMe | H | | Ar |
| **9** | | | | | | Me | Br | OMe | OMe | OMe | H | | Ar |
| **10** | **19** | **28** | | | | Ph | OMe | H | H | H | $NO_2$ | | Ar |
| **11** | **20** | | | | | Ph | OMe | $NO_2$ | H | H | | | Ar |
| **12** | | **29** | | | | Me | OMe | | | | | Me | HetAr |
| **13** | **21** | | | | | Me | OMe | | | | | $SO_2Ph$ | HetAr |
| | | **30** | | | | Me | OMe | | | | | H | HetAr |

Table 2, from which several conclusions can be deduced. The compounds not included in Table 2 were inactive.

The initially synthesized compound, **1**, is among the most active compounds in the series, with several new derivatives with the same order of cytotoxicity, although no important increase in their activity was observed. The most cytotoxic derivatives ($IC_{50}$ = 0.3–5.0 μM in at least two of the cell lines tested) are those with a non-aromatic C ring (**1, 41**) or aromatic derivatives with different substitutions: imido-*N*-phenyl (**33**, **36**) or-*N*-methyl (**27**, **37**, **38**), and carbazole- *N*–H (**25**, **27**, **28**) or carbazole-*N*-substituted (**36**), although in general the C-aromatised derivatives (series **C**–**F**) are more cytotoxic than their non-aromatic analogues (series **A**–**B**). Among the C-aromatised carbazole with free N–H (series **C**), the most active are those presenting *ortho*-substituents on the phenyl group (**25** $R_6$ = OMe; **28** $R_6 = NO_2$).

This variability in structure and cytotoxicity allowed us to propose two general observations: the presence of the 3,4,5-trimethoxyphenyl group as the aryl (Ar) moiety is associated with the active (**1**, **33**, **36**, **41**, **42**) or moderately active (**14**, **15**, **22**, **31**) compounds, and the introduction of a 3-(*N,N*-dimethylamino)-2-hydroxypropyl moiety on the carbazole nitrogen (series **F**) is accompanied by a noticeable increase in cytotoxicity in comparison with the corresponding carbazole free N–H analogues (series **C**). In the light of the lower cytotoxicity of these derivatives (**12**, **13**, **21**, **29**, **30**), the replacement of the phenyl-substituted moiety (Ar) by the

**Table 2.** Cytotoxicity of representative compounds against cancer cell lines. $IC_{50}$ (μM)

| Compd | Structural type | P-388 | A-549 | HT-29 | MEL-28 | UACC-62 | TK-10 | MCF-7 |
|---|---|---|---|---|---|---|---|---|
| **1** | **A** | 0.5 | 1.9 | 1.9 | 1.9 | | | |
| **7** | **A** | 10 | 10 | 10 | 10 | | | |
| **12** | **A** | 7.4 | 7.4 | 7.4 | 7.4 | | | |
| **14** | **B** | 9.8 | 9.8 | 9.8 | 9.8 | | | |
| **15** | **B** | 11.1 | 22.2 | 11.1 | 22.2 | | | |
| **20** | **B** | 12 | 12 | 1.2 | 12 | | | |
| **22** | **C** | 9.8 | 9.8 | 9.8 | 9.8 | >10 | >10 | >10 |
| **25** | **C** | 2.1 | 2.1 | 10.5 | 2.1 | | | |
| **27** | **C** | | | | | 2.3 | 2.7 | 8.9 |
| **28** | **C** | 2.8 | 0.3 | 2.2 | 2.2 | 6.6 | >10 | 11.5 |
| **29** | **C** | >5 | >5 | >5 | >5 | | | |
| **31** | **D** | 11.2 | 11.2 | 11.2 | 11.2 | | | |
| **33** | **D** | 2.2 | 2.2 | 17.7 | 2.2 | | | |
| **35** | **E** | 8.2 | 8.2 | 8.2 | 8.2 | | | |
| **36** | **F** | 0.8 | 4.1 | 4.1 | 4.1 | >10 | >10 | >10 |
| **37** | **F** | 4.8 | 4.8 | 4.8 | 4.8 | 11.3 | 2.3 | 10.9 |
| **38** | **F** | 1.0 | 2.4 | 2.4 | 2.4 | | | |
| **41** | | 3.0 | >10 | >10 | 3.0 | | | |
| **42** | | 5.9 | 5.9 | 5.9 | 5.9 | | | |
| **43** | | >5 | >5 | >5 | >5 | 5.2 | 9.9 | 6.4 |

P-388: mouse lymphoma. A-549 human lung carcinoma. HT-29: human colon adenocarcinoma.
MEL-28 and UACC-62: human melanoma. TK-10: human renal adenocarcinoma. MCF-7: human breast adenocarcinoma.

3-indolyl residue (HetAr) is not a worthwhile modification.

Thus, there is a certain degree of cytotoxicity associated with some of the compounds with the pyrrolo[3,4-*c*]-carbazole skeleton, and this cytotoxicity is mainly displayed by compounds having a highly hydrophobic residue (3,4,5-trimethoxyphenyl) and/or a solubilising moiety (3-(*N*,*N*-dimethylamino)-2-hydroxypropyl) on the carbazole nitrogen. The planarity of the indolo-carbazole system is not directly associated with cytotoxic compounds, but the non-planar non-aromatic C-ring derivatives are in general less active.

The activity of our first model compound **1** was not improved but did persist in some of the derivatives described in this paper. This suggests that compound **1** is a particular case of a folded molecule whose cytotoxicity may be due to a mechanism of action other than those of planar molecules: that already described for indolocarbazoles (DNA topoisomerase I inhibition or PKC inhibition) or that one to be investigated for our open analogues of the natural alkaloids.

In conclusion, the methodology applied in this work is highly suitable for the synthesis of open indolocarbazole analogues. Although not highly potent, many of these derivatives displayed cytotoxicity against the cell lines assayed, thereby stimulating the preparation of new related compounds.

## Experimental

### General methods

Melting points were determined on a Büchi 510 apparatus and are uncorrected. IR spectra were recorded on Nicolet Impact 410 spectrophotometer and the values are expressed in $cm^{-1}$. NMR spectra were recorded on Bruker WP 200 SY spectrometer in $CDCl_3$ or the reported solvent. Mass spectra were obtained by EI or FAB methods on a VGTS-250 mass spectrometer. Microanalyses were carried out on Perkin–Elmer 2400 CHN. Flash chromatography was performed with Merck 60 silica gel (0.063–0.2 or 0.040–0.063 mm). Solvents of analytical grade were used as purchased and, when necessary, dried using standard procedures.

**Fischer indolization. Preparation of 13 and 21.** To a solution of 2.18 g (3.7 mmol) of cycloadduct **V** (R = *i*Pr, $R_1$ = Me, $R_7 = SO_2Ph$) in 300 mL of AcOH/EtOH (1:1 vol/vol) and 3.3 mL of HCl (37%), 7.4 mmol of *p*-methoxyphenylhydrazine were added. The reaction mixture was refluxed for 6 h and then rendered basic with solid $Na_2CO_3$ and extracted with EtOAc, washed with brine, and dried. Evaporation of the solvent, followed by flash chromatography (silica gel, hexane/AcOEt 1:1) and crystallization gave **13** (1.18 g, 59%) and **21** (0.55g, 27%).

**(±)-(3a*S*,4*S*,10c*S*)-4-(*N*-phenylsulphonylindol-3-yl)-2-methyl-9-methoxy-3a,4,5,10*c*-tetrahydro-6*H*-pyrrolo[3,4-*c*]carbazole-1,3-dione (13).** Yellow solid; $R_f$ (hexane/AcOEt 1:1) 0.53; mp 280 °C (ether/MeOH). IR (KBr): 3426, 1758, 1706, 1175 $cm^{-1}$; $^1H$ NMR (DMSO) δ 10.94 (s, 1H), 7.94 (d, *J* = 7.8 Hz, 2H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.75 (d, *J* = 7.6 Hz, 1H), 7.67 (d, *J* = 7.8 Hz, 1H), 7.65 (s, 1H), 7.57 (dd, *J* = 7.8, 7.8 Hz, 2H), 7.34 (dd, *J* = 8.0, 7.6 Hz, 1H), 7.31 (d, *J* = 2.4 Hz, 1H), 7.27 (dd, *J* = 7.6, 7.6 Hz, 1H), 7.22 (d, *J* = 8.8 Hz, 1H), 6.73 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.43 (d, *J* = 7.6 Hz 1H), 3.95 (dd, *J* = 7.6, 4.3 Hz, 1H), 3.77 (s, 3H), 3.68 (m,1H), 3.11 (m, 2H), 2.58 (s, 3H), $^{13}C$ NMR (DMSO) δ 177.1, 176.7, 153.3, 137.2, 135.6, 134.5, 134.2, 131.1, 130.2, 129.8 (2C), 126.9, 126.7 (2C), 124.9, 124.4, 123.3 (2C), 120.2, 113.2, 111.6, 110.5, 102.9, 101.9, 55.4, 43.2, 40.8, 30.7, 25.4, 24.1; HRMS (FAB) calcd for $C_{30}H_{25}N_3O_5S$

539.1515, found *m*/*z* 540.1593. Anal. calcd for $C_{30}H_{25}N_3O_5S$: C, 66.78, H, 4.67, N, 7.79, S, 5.94, found: C, 66.83, H, 4.80, N, 7.96, S, 5.77.

**(±)-(3a*R*,10*R*,10a*S*)-10-(*N*-phenylsulphonylindol-3-yl)-2-methyl-8-methoxy-3a,4,10,10a-tetrahydro-5*H*-pyrrolo[3,4-*b*]carbazole-1,3-dione (21).** $R_f$ (hexane/AcOEt 1:1) 0.24; mp 265 °C (ether/MeOH). IR (KBr): 3421, 1745, 1697, 1173 $cm^{-1}$; $^1H$ NMR (DMSO) δ 8.32 (brs, 1H), 6.8–7.9 (m, 11H), 6.76 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.56 (d, *J* = 2.2 Hz, 1H), 5.07 (d, *J* = 6.8 Hz, 1H), 3.65 (dd, *J* = 17.8, 3.0 Hz, 1H), 3.60 (s, 3H), 3.53 (dd, *J* = 9.8, 6.8 Hz, 1H), 3.38 (td, *J* = 9.8, 3.0 Hz, 1H), 3.12 (dd, *J* = 17.8, 9.8 Hz, 1H), 1.93 (s, 3H). $^{13}C$ NMR (DMSO) δ 178.9, 177.5, 154.1, 137.6, 135.1, 133.8, 132.6, 131.4, 130.6, 129.1 (2C), 127.1 (2C), 126.1, 125.7, 125.1, 123.1, 122.2, 121.1, 114.1, 113.2, 111.7, 110.6, 100.1, 55.8, 46.1, 38.9, 29.8, 23.7, 18.8. HRMS (FAB) calcd for $C_{30}H_{25}N_3O_5S$ 539.1515, found *m*/*z* 540.1291. Anal. calcd for $C_{30}H_{25}N_3O_5S$: C, 66.78; H, 4.67, N: 7.79; S, 5.94, found: C, 66.36; H, 4.33; N, 7.36; S, 5.41.

**Aromatisation and deprotection of the *N*-sulphonyl group.** 250 mg (0.46 mmol) of tetrahydropyrrolocarbazole **13** dissolved in 20 mL of toluene was reacted with 1 mmol of DDQ for 3 h at reflux. The crude reaction mixture was diluted in EtOAc, washed with $NaHCO_3$ and brine, dried, and evaporated. By precipitation in $CHCl_3$, 176 mg (70%) of dehydrogenated product were obtained. This product was treated, in THF, with 4 equiv of TBAF and maintained under reflux for 24 h. By evaporation of the solvent and chromatography (silica gel, hexane/AcOEt 1:1), **30** (68 mg, 61%) was isolated. Mp 210 °C (ether/MeOH). IR (KBr): 3311, 1750, 1691, 1026 $cm^{-1}$; $^1H$ NMR (DMSO) δ 8.48 (d, *J* = 2.0, 1H), 7.85 (s, 1H), 7.84 (s, 1H), 7.66 (m, 2H), 7.66 (d, *J* = 7.6 Hz, 1H), 7.49 (d, *J* = 8.1 Hz, 1H), 7.48 (d, *J* = 7.6 Hz, 1H), 7.17 (dd, *J* = 8.1, 2.0 Hz, 1H), 7.16 (t, *J* = 7.1 Hz, 1H), 7.07 (t, *J* = 6.8 Hz, 1H), 3.87 (s, 3H), 3.06 (s, 3H). $^{13}C$ NMR (DMSO) δ 168.9, 168.8, 153.6, 144.8, 136.8, 136.1, 130.8, 127.5, 127.2, 126.1, 121.4, 120.6, 119.6, 118.8, 118.2, 117.3, 116.9, 115.6, 112.2, 111.9, 110.9, 107.1, 55.4, 23.1; HRMS (FAB) calcd for $C_{24}H_{17}N_3O_3$ 395.1270, found *m*/*z* 396.1348.

**General procedures for *N*-alkylation of pyrrolo[3,4-*c*]carbazole: (A) Phase transfer conditions.** To a suspension of the corresponding pyrrolo[3,4-*c*]carbazole (1 mmol) in benzene (1 mL) were added a solution of NaOH (50%) (0.5 mL), tetra-*N*-butyl ammonium hydrogen sulphate (0.5 mmol), and the alkylating agent. The reaction mixture was allowed to react for 14 h at room temperature, after which it was diluted in $CHCl_3$, washed with brine, and dried over $Na_2SO_4$. Solvent evaporation followed by column chromatography afforded the *N*-alkylated product.

**(B) NaH in DMF.** To a solution of the corresponding pyrrolo[3,4-*c*]carbazole (1 mmol) in dry DMF (3.3 mL), under Ar, was added NaH (80%) (1.5 mmol). The reaction mixture was stirred for 50 min and then the alkylating agent (2 mmol) was added. The mixture was allowed to react for 14 h at room temperature, diluted in $CHCl_3$, washed with brine, and dried over $Na_2SO_4$. Solvent evaporation and purification by diethyl ether precipitation or column chromatography yielded the corresponding *N*-alkylated product.

**6-(2,3-epoxypropyl)-9-methoxy-2-phenyl-4-(3,4,5-trimethoxyphenyl)-6*H*-pyrrolo[3,4-*c*]carbazole-1,3-dione (33).** Using procedure **A** and epichlorohydrin (2 mmol), after CC (hexane/EtOAc 4:6) product **33** was obtained in 23% yield. Using procedure **B**, 33% of **33** was obtained; yellow solid; IR (KBr): 1761, 1710, 1587 $cm^{-1}$; $^1H$ NMR δ 8.73 (d, *J* = 2.6 Hz, 1H), 7.66 (s, 1H), 7.40–7.60 (m, 5H), 7.45 (d, *J* = 8.8 Hz, 1H), 7.27 (dd, *J* = 8.8, 2.6 Hz, 1H), 6,89 (s, 2H), 4.81 (dd *J* = 15.9, 2.6 Hz, 1H), 4.36 (dd *J* = 15.9, 5.5 Hz, 1H), 3.98 (s, 3H), 3.93 (s, 9H), 3.36 (m, 1H), 2.85 (t, *J* = 4.6 Hz, 1H), 2.55 (dd, *J* = 4.6, 2.6 Hz, 1H); $^{13}C$ NMR δ 168.1, 167.9, 155.3, 152.8 (2C), 145.2, 138.4 (2C), 137.0, 133.2 (3C), 129.2 (2C), 128.0, 127.2 (2C), 121.2 (2C), 119.3, 119.0, 115.3, 110.0, 107.7, 107.4 (2C), 61.1, 56.4 (2C), 56.1, 50.6, 45.0, 44.9; MS (FAB) *m*/*z*: 564 ($M^+$, 32).

**4-(2,5-dimethoxyphenyl)-6-(2,3-epoxypropyl)-2-methyl-9-methoxy-6*H*-pyrrolo[3,4-*c*]carbazole-1,3-dione (34).** Using procedure **A** and epichlorohydrin (2 mmol), by CC (hexane/EtOAc 4:6) 42% of **34** was obtained; yellow solid; IR (KBr): 1703, 1593, 1503, 1126 $cm^{-1}$; $^1H$ NMR δ 8.67 (d, *J* = 2.6 Hz, 1H), 7.43 (s, 1H), 7.41 (d, *J* = 8.8 Hz, 1H); 7.24 (dd, *J* = 8.8, 2.6 Hz, 1H), 6.95 (m, 3H), 4.68 (dd, *J* = 15.7, 4.6 Hz, 1H), 4.32 (dd, *J* = 15.7, 5.1 Hz, 1H), 4.01 (s, 3H), 3.81 (s, 3H), 3.71 (s, 3H), 3.34 (m, 1H), 3.18 (s, 3H), 2.81 (t, *J* = 4.6 Hz, 1H), 2.54 (dd, *J* = 4.6, 2.6 Hz, 1H) ; $^{13}C$ NMR δ 170.9, 170.1, 155.0, 153.4, 151.2, 144.9, 138.6, 135.2, 133.6 (2C), 121.8 (3C), 118.6, 117.2, 114.9 (2C), 111.9, 110.0, 107.5, 56.3, 56.0 (2C), 50.4, 45.1 (2C), 23.8.

**4-(3,4-dimethoxyphenyl)-6-(2,3-epoxypropyl)-2-methyl-9-methoxy-6*H*-pyrrolo[3,4-*c*]carbazole-1,3-dione (35).** Using procedure **A** and epichlorohydrin (2 mmol), by CC (hexane/EtOAc 4:6), 41% of **35** was obtained); yellow solid; IR (KBr): 1751, 1603, 1519, 1120 $cm^{-1}$; $^1H$ NMR δ 8.71 (d, *J* = 2.2 Hz, 1H), 7.54 (s, 1H), 7.42 (d, *J* = 8.8 Hz, 1H), 7.16–7.28 (m, 3H), 7.00 (d, *J* = 8.4 Hz, 1H), 4.75 (dd, *J* = 16.1, 2.6 Hz, 1H), 4.32 (dd, *J* = 16.1, 5.1 Hz, 1H), 4.03 (s, 3H), 3.97 (s, 6H), 3.35 (m, 1H), 3.22 (s, 3H), 2.84 (t, *J* = 4.7 Hz, 1H), 2.54 (dd, *J* = 4.7, 2.6 Hz, 1H); $^{13}C$ NMR δ 169.2, 169.1, 155.1, 149.3, 149.0, 145.5, 137.9, 137.7, 130.4, 128.4, 122.3, 121.3, 120.0, 119.0, 118.7 114.5, 113.5, 110.8, 109.9, 107.7, 56.2 (3C), 50.6, 45.0 (2C), 23.9.

**6-(2-cyanoethyl)-2-phenyl-9-methoxy-4-(3,4,5-trimethoxyphenyl)-6*H*-pyrrolo[3,4-*c*] carbazole-1,3-dione (39).** Using procedure **A** and 1,1 mmol of 3-bromopropionitrile, by CC (hexane/EtOAc 3:7) 23% of **39** was obtained; yellow solid; IR (KBr): 2260, 1709, 1586 $cm^{-1}$; $^1H$ NMR δ 8.76 (d, *J* = 2.5 Hz, 1H), 7.68 (s, 1H), 7.40–7.55 (m, 5H), 7.37 (d, *J* = 8.9 Hz, 1H), 7.29 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.90 (s, 2H), 4.73 (t, *J* = 6.5 Hz, 2H), 3.98 (s, 3H), 3.92 (s, 3H), 3.91 (s, 6H), 2.93 (t, *J* = 6.5 Hz, 2H); $^{13}C$ NMR δ 167.8, 167.4, 155.5, 152.8

(2C), 144.1, 138.6, 138.2, 136.2, 133.7, 132.7, 131.9, 129.1 (2C), 128.0, 127.0 (2C), 121.7, 119.5, 119.4, 119.0, 117.1 (2C), 114.7, 109.1, 108.1, 107.3 (2C), 60.9, 56.3 (2C), 56.0, 39.3, 17.7; HRMS (FAB) calcd for $C_{33}H_{27}N_3O_6$ 561.1900, found *m/z* 562.1978.

**6-methyl-9-methoxy-4-(2-nitrophenyl)-2-phenyl-6*H*-pyrrolo[3,4-*c*]carbazole-1,3-dione (40).** Using procedure **B,** and purification by precipitation in diethyl ether 70% of **40** was obtained, yellow powder; IR (KBr): 1708, 1608, 1520 $cm^{-1}$; $^1H$ NMR (DMSO) δ 8.46 (d, *J* = 2.7, 1.5 Hz, 1H), 8.23 (dd, *J* = 8.0, 1.5 Hz, 1H), 8.00 (s, 1H), 7.87 (td, *J* = 8.0, 1.5 Hz, 1H), 7.75 (td, *J* = 8.0, 1.5 Hz, 1H), 7.30–7.70 (m, 8H), 3.98 (s, 3H), 3.86 (s, 3H); $^{13}C$ NMR (DMSO) δ 167.5, 167.3, 154.3, 148.2, 145.0, 137.9, 133.8, 133.2, 132.8 (4C), 129.7, 128.8 (2C), 127.9, 127.3 (2C), 125.6, 124.2, 119.9 (2C), 118.0, 115.0, 111.2, 106.8, 55.5, 29.9; MS (FAB) *m/z*: 478 ($M^+ + H$, 4).

**General procedure for the preparation of *N*-(3-dimethylamino-2-hydroxypropyl)-pyrrolo[3,4-*c*]carbazoles.** A solution of the corresponding epoxide (1 mmol) in EtOH and another solution of dimethylamine in EtOH (30%) (3.8 mL) was stirred at room temperature for 3 h. Solvent evaporation and—sometimes—purification by flash chromatography afforded the amino-alcohol.

**6-(3-dimethylamino-2-hydroxypropyl)-9-methoxy-2-phenyl-4-(3,4,5-trimethoxyphenyl)-6*H*-pyrrolo[3,4-*c*]carbazole-1,3-dione (36).** By CC (EtOAc/MeOH 1:1) 76% of **36** was obtained; yellow solid; IR (KBr): 3446, 1709, 1586 $cm^{-1}$; $^1H$ NMR δ 8.79 (d, *J* = 2.2 Hz, 1H), 7.72 (s, 1H), 7.20–7.65 (m, 7H), 6.61 (s, 2H), 4.45 (m, 2H), 4.23 (m, 1H), 4.01 (s, 3H), 3.95 (s, 3H), 3.94 (s, 6H), 2.52 (t, *J* = 11.1, 1H), 2.41 (dd, *J* = 11.1, 2.2 Hz, 1H), 2.31 (s, 6H); $^{13}C$ NMR δ 168.1, 167.6, 155.0, 152.7 (2C), 145.5, 138.1 (2C), 138.0, 133.2 (2C), 132.1, 129.0 (2C), 127.8, 127.0 (2C), 121.1, 119.1, 118.7, 118.6, 115.7, 110.2, 107.6, 107.4 (2C), 66.9, 62.7, 60.9, 56.1 (2C), 55.9, 47.7, 45.7 (2C); HRMS (FAB) calcd for $C_{35}H_{35}N_3O_7$ 609.2475, found *m/z* 610.2552.

**6-(3-dimethylamino-2-hydroxypropyl)-4-(2,5-dimethoxyphenyl)-9-methoxy-2-methyl-6*H*-pyrrolo[3,4-*c*]carbazole-1,3-dione (37).** (95%); yellow solid; IR (KBr): 3377, 1699, 1605, 1518 $cm^{-1}$; $^1H$ NMR δ 8.68 (d, *J* = 2.6 Hz, 1H), 7.59 (s, 1H), 7.44 (d, *J* = 8.8 Hz), 7.23 (dd, *J* = 8.8 Hz, *J* = 2.6 Hz, 1H), 6.95 (m, 3H), 4.35 (m, 2H), 4.15 (m, 1H), 4.02 (s, 3H), 3.81 (s, 3H), 3.71 (s, 3H), 3.18 (s, 3H), 2.42 (t, *J* = 10.9 Hz, 1H), 2.32 (dd, *J* = 10.9, 6.9 Hz, 1H), 2.23 (s, 6H); $^{13}C$ NMR δ 170.0, 169.0, 154.8, 153.3, 151.5, 145.3, 138.1 (2C), 133.3, 133.2, 121.3 (2C), 119.0, 118.4, 117.2, 115.4, 113.9, 111.9, 110.4, 107.3, 66.9, 62.8, 56.3, 55.9 (2C), 47.6, 45.6 (2C), 23.7; HRMS (FAB) calcd for $C_{29}H_{31}N_3O_6$ 517.2213, found *m/z* 518.2263.

**6-(3-dimethylamino-2-hydroxypropyl)-4-(3,4-dimethoxyphenyl)-9-methoxy-2-methyl-6*H*-pyrrolo[3,4-*c*]carbazole-1,3-dione (38).** (95%); yellow solid; IR (KBr): 3338, 1702, 1633 $cm^{-1}$; $^1H$ NMR δ 8.70 (d, *J* = 2.7 Hz, 1H), 7.61 (s, 1H), 7.42 (d, *J* = 9.1 Hz, 1H), 7.24 (dd, *J* = 9.1, 2.7 Hz, 1H), 7.21 (d, *J* = 2.6 Hz, 1H), 7.20 (dd, *J* = 8.5, 2.6 Hz, 1H), 7.00 (d, *J* = 8.5 Hz, 1H), 4.37 (m, 2H), 4.17 (m, 1H), 4.01 (s, 3H), 3.97 (s, 6H), 3.20 (s, 3H), 2.45 (dd, *J* = 11.1, 9.9 Hz, 1H); 2.32 (dd, *J* = 11.1, 4.0 Hz, 1H), 2.25 (s, 6H); $^{13}C$ NMR δ 169.4, 169.0, 155.0, 149.3, 148.4, 145.5, 137.9, 137.7, 131.0, 128.9, 122.3, 121.3 (2C), 118.7, 118.5, 114.9, 113.7, 110.8, 110.2, 107.6, 66.9, 62.8, 56.2, 56.0 (2C), 47.8, 45.4 (2C), 23.8; HRMS (FAB) calcd for $C_{29}H_{31}N_3O_6$ 517.2213, found *m/z* 518.2284.

**General procedure for the preparation of α-tetra-*O*-acetylglucosides.** A mitxture of the corresponding pyrrolo[3,4-*c*]carbazole (1 mmol) in benzene (375 mL), 2,3,4,6-tetraacetyl-α-D-glucopyranosyl bromide (5 mmol) and $Ag_2O$ (10 mmol) was refluxed for 3 h and then filtered off. The filtrate was evaporated and purified by flash chromatography to give the *N*-α-tetra acetylglucoside.

**(±)-(3a*S*,4*S*,10c*S*)-9-methoxy-2-phenyl-6-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-4-(3,4,5-trimethoxyphenyl)-3a,4,5,10c-tetrahydro-6*H*-pyrrolo[3,4-*c*]carbazole-1,3-dione (41).** CC (hexane/EtOAc 3:7) 27%; yellow solid; IR (KBr): 1748, 1592, 1586, 1502 $cm^{-1}$; $^1H$ NMR δ 7.81 (d, *J* = 9.1 Hz, 1H), 7.46 (d, *J* = 2.2 Hz, 1H), 7.40 (m, 3H), 6.88 (dd, *J* = 9.1, 2.2 Hz, 1H), 6.71 (m, 2H), 6.48 (s, 2H), 5.75 (d, *J* = 5.9 Hz, 1H), 5.27 (m, 1H), 4.96 (bd, *J* = 9.9 Hz, 1H), 4.42 (d, *J* = 7.3 Hz, 1H), 4.40–4.15 (m, 3H), 4.00–3.40 (m, 5H), 3.92 (s, 3H), 3.79 (s, 3H), 3.61 (s, 6H), 2.00–2.15 (s, 12H); $^{13}C$ NMR δ 176.4, 175.5, 171.5, 170.8, 170.2, 169.7, 154.9, 153.1 (2C), 136.9, 136.0, 134.6, 131.9, 131.0, 129.1 (2C), 128.6, 127.3, 126.4 (2C), 112.5, 111.4, 105.7 (2C), 104.0, 102.1, 96.9, 73.2, 69.9, 68.3, 67.4, 66.7, 60.9, 56.0 (2C), 55.7, 46.2, 40.7, 40.3, 27.2, 24.6, 20.8 (3C); MS (EI) *m/z*: 842 ($M^+$, 12).

**9-methoxy-2-phenyl-6-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-4-(3,4,5-trimethoxyphenyl)-6*H*-pyrrolo[3,4-*c*]-carbazole-1,3-dione (42).** CC (hexane/EtOAc 4:6) 50%; yellow solid; IR (KBr): 1750, 1712, 1586 $cm^{-1}$; $^1H$ NMR δ 8.80 (d, *J* = 2.8 Hz, 1H), 8.31 (s, 1H), 8.03 (d, *J* = 9.3 Hz, 1H), 7.35–7.60 (m, 5H), 7.21 (dd, *J* = 9.3, 2.8 Hz, 1H), 6.91 (s, 2H), 5.81 (d, *J* = 5.5 Hz, 1H), 5.34 (m, 1H), 4.99 (bd, *J* = 9.5 Hz, 1H), 4.25–4.30 (m, 1H), 4.15–4.25 (m, 3H), 3.94 (s, 3H), 3.93 (s, 3H), 3.92 (s, 6H), 2.05–2.20 (s, 12H); $^{13}C$ NMR δ 170.7, 169.7 (2C), 169.0, 167.9, 167.4, 155.4, 152.8 (2C), 143.5, 138.7, 138.3, 136.0, 133.2 (2C), 132.0, 129.1 (2C), 128.0, 127.1 (2C), 122.7, 121.7, 120.0, 119.1, 118.6, 113.8, 107.5 (3C), 97.1, 73.1, 69.7, 68.4, 67.1, 63.0, 61.0, 56.4 (2C), 55.9, 22.6, 20.9, 20.7 (2C); MS (FAB) *m/z*: 839 ($M^+ + H$, 12); $[\alpha]_D^{25}$ 1.50 (c 0.07 in $CH_2Cl_2$).

**Preparation of 9-methoxy-6-α-glucopyranosyl-4-(3,4,5-trimethoxyphenyl)-6*H*-pyrrolo[3,4-*c*]carbazole-1,3-dione (43).** To a solution of **42** (1 mmol) in MeOH (220 mL) was added $NH_4OH$ (33% in water) (100 mL). The reaction mixture was stirred for 4 h at room temperature. The solvent was removed and the residue dissolved in EtOAc and washed with brine. The organic phase was dried over $Na_2SO_4$ and the solvent was evaporated. Purification by column chromatography (EtOAc/MeOH 97:3, 39%) led to **43** as a yellow powder; IR (KBr): 3350, 1713, 1588 $cm^{-1}$; $^1H$ NMR ($CD_3OD$) δ

8.39 (d, $J=2.7$ Hz, 1H), 8.23 (s, 1H), 7.97 (d, $J=9.1$ Hz, 1H), 7.10 (dd, $J=9.1$, 2.7 Hz, 1H), 6.91 (s, 2H), 5.81 (d, $J=5.2$ Hz, 1H), 3.20–4.20 (m, 5H), 3.76 (s, 9H), 3.73 (s, 3H); $^{13}$C NMR ($CD_3OD$) δ 169.8, 169.6, 156.1, 153.9 (2C), 140.9, 139.2, 139.0 (2C), 137.4, 135.2, 123.7, 121.8, 119.5, 119.0, 118.9, 114.9, 108.4 (3C), 99.5, 77.8, 75.0, 73.9, 70.5, 61.2 (2C), 56.8 (2C), 56.0; MS (FAB) *m*/*z*: 594 ($M^+$, 10); $[\alpha]_D^{25}$ 1.20 (c 0.06 in $CH_2Cl_2$).

## Biological Assays

### Antitumoral assays

Cells were seeded into 16 mm wells (multidishes, NUNC 42001) at concentrations of $1\times10^4$ (P-388), $2\times10^4$ (A-549, HT-29 and MEL-28) cells well$^{-1}$, respectively, in 1 mL aliquots of MEM 10FCS medium. The day after the inoculum, media were replaced by 1 mL aliquots of MEM 10FCS containing the different concentrations of sample. In both cases a separate set of cultures without sample was counted daily to ensure that the cells remained in exponential growth. Cells were incubated at 37 °C in a 10% $CO_2$ humid atmosphere. All determinations were carried out in duplicate. After three days of incubation, cells were counted and the $IC_{50}$ for each sample was determined.

For the human cancer cell lines, UACC-62, TK-10, MCF-7 their cytotoxicities were determined following protocols established by the National Cancer Institute, National Institute of Health.[28] The sulphorhodamine B (SRB) assay was used in this study to assess growth inhibition. This colorimetric assay estimates cell number indirectly by staining total cellular protein with the dye SRB. The cancer cells were seeded in 96-well microtiter plates and incubated to allow for cell attachment. After 24 h the cells were treated for 48 h with serial concentrations of compounds (final concentrations of $10^{-4}$, $10^{-5}$, $10^{-6}$, $10^{-7}$ and $10^{-8}$ M). They were dissolved in DMSO whose concentration for the tested dilutions was not greater than 0.25% (vol/vol), the same as in solvent control wells.

## Acknowledgements

Financial support came from the Spanish MCYT and the European FEDER Funds for the Project PPQ2000-1179, and from Junta de Castilla y León (Consejería de Educación y Cultura) for the Project SA048/03. S. Calderón thanks the Spanish MECyD for a predoctoral grant.

## References and Notes

1. Pindur, U.; Kim, Y. S.; Mehrabani, F. *Curr. Med. Chem.* **1999**, *6*, 29.
2. Steglich, W.; Steffan, B.; Kopanski, L.; Eckhardt, G. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 459.
3. Slater, M. J.; Cockerill, S.; Baxter, R.; Bonser, R. W.; Gohil, K.; Gowrie, C.; Robinson, J. E.; Littler, E.; Parry, N.; Randall, R.; Snowden, W. *Bioorg. Med. Chem.* **1999**, *7*, 1067.
4. Nettleton, D. E.; Doyle, T. W.; Krishnan, B.; Matsumoto, G. K.; Clardy, J. *Tetrahedron Lett.* **1985**, *26*, 4011.
5. Omura, S.; Iwai, Y.; Hirano, A.; Nakagawa, A.; Awaya, J.; Tsuchiya, H.; Takahashi, Y.; Masuma, R. *J. Antibiot.* **1977**, *30*, 275.
6. Marminon, C.; Anizon, F.; Moreau, P.; Léonce, S.; Pierré, A.; Pfeiffer, B.; Renard, P.; Prudhomme, M. *J. Med. Chem.* **2002**, *45*, 1330.
7. Marschall, M.; Stein-Gerlach, M.; Freitag, M.; Kupfer, R.; van der Bogaard, M.; Stamminger, T. *J. General Virology* **2002**, *83*, 1013.
8. Akinaga, S.; Sugiyama, K.; Akiyama, T. *Anticancer Drug Des.* **2000**, *15*, 43.
9. (a) Caballero, E.; Guilhot, F.; López, J. L.; Medarde, M.; Sahagún, H.; Tomé, F. *Tetrahedron Lett.* **1996**, *37*, 6951. (b) Adeva, M.; Caballero, E.; García, F.; Medarde, M.; Sahagún, H.; Tomé, F. *Tetrahedron Lett.* **1996**, *38*, 6893. (c) Caballero, E.; Longieras, N.; Zausa, E.; Del Rey, B.; Medarde, M.; Tomé, F. *Tetrahedron Lett.* **2001**, *42*, 7233.
10. (a) Acosta, J. C.; Caballero, E.; Grávalos, D. G.; Medarde, M.; Sahagún, H.; Stoodley, R. J.; Tomé, F. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2955. (b) Caballero, E.; Medarde, M.; Pelaez-Lamamie de Clairac, R.; Sahagún, H.; Tomé, F. *Tetrahedron* **1998**, *54*, 6111.
11. Adeva, M.; Buono, F.; Caballero, E.; Medarde, M.; Tomé, F. *Synlett* **2000**, 832.
12. (a) Caballero, E.; García, F.; Grávalos, D. G.; Medarde, M.; Sahagún, H.; Tomé, F. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2459. (b) Adeva, M.; Sahagún, H.; Caballero, E.; Pelaez-Lamamie de Clairac, R.; Medarde, M.; Tomé, F. *J. Org. Chem.* **2000**, *65*, 3387. (c) Ref 9c.
13. (a) Shi, Q.; Chen, K.; Morris-Natschke, S. L.; Lee, K.-H. *Current Pharmaceutical Design* **1998**, *4*, 219. (b) Sengupta, S. K. Topoisomerase II Inhibitors. Inhibitors of DNA Topoisomerases, In *Cancer Chemotherapeutic Agents*, Foye W. O., Ed.; ACS: Washington, 1995; pp 205–217.
14. Cory, M. DNA Minor-Groove Binding Compounds as Antitumor-Agents, In *Cancer Chemotherapeutic Agents*, Foye W. O., Ed.; ACS: Washington, 1995; pp 311–344.
15. Hodgson, S. T.; Jenkins, D. C.; Knick, V.; Rapson, E.; Watts, S. D. M. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1257.
16. Copp, B. R.; Ireland, C. M.; Barrows, L. R. *J. Org. Chem.* **1991**, *56*, 4596.
17. Badre, A.; Boulanger, A.; Abou-Mansour, E.; Banaigs, B.; Combaut, G.; Francisco, C. *J. Nat. Prod.* **1994**, *57*, 528.
18. MacDonald, T. L.; Lehnert, E. K.; Loper, J. T.; Chow, K.; Ross, W. E. In *DNA Topoisomerase in Cancer*: Potmesil, M.; Kohn, K. W. Eds.: Oxford University Press: New York, 1991; pp 119–214.
19. (a) Bergman, J. In *Studies in Natural Products Chemistry*, Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 1988; Vol. 1, pp 3–30 (b) Gribble, G. W.; Berthel, S. J. In *Studies in Natural Products Chemistry*, Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 1993; Vol. 12, pp 365–409. (c) Ref 1. (d) Merlic, C. A.; Ying, Y.; McInnes, D. M.; Zechman, A. L.; Miller, M. M.; Deng, Q. *Tetrahedron* **2001**, *57*, 5199 and references cited therein. (e) Knölker, H.-J.; Reddy, K. R. *Chem. Rev.* **2002**, *102*, 4303.
20. Moody, C. J.; Rahimtoola, K. F. *J. Org. Chem.* **1992**, *57*, 2105.
21. Nomak, R.; Snyder, J. K. 216th ACS National Meeting, ORGN Abstract 543, Boston, 1998.
22. Broka, C. A. European Patent 695755, 1996; *Chem. Abstr.* **1996**, *124*, 289516.
23. Kleinschroth, J.; Hartenstein, J.; Schaechtele, C.; Rudolph, C.; Dooley, D. J.; Weinheimer, G. U.S. Patent 4912107, 1990; *Chem. Abstr.* **1990**, *113*, 191154.

24. McCort, G.; Duclos, O.; Cadilhac, C.; Guilpain, E. *Tetrahedron Lett*. **1999**, *40*, 6211.
25. Ikuina, Y.; Kanai, F.; Shiotsu, Y.; Iida, T.; Yamashita, K.; Saitoh, Y.; Akinaga, S.; Tamaoki, T.; Murakata, C. 217th ACS National Meeting, MEDI Abstract 089, Anaheim, 1999.
26. Steglich, W. *Pure & Appl. Chem*. **1989**, *61*, 281.
27. (a) Kleinschroth, J.; Hartestein, J.; Rudolph, C.; Schächtele, C. *Bioorg. Med. Chem. Lett*. **1995**, *5*, 55. (b) Facompré, M.; Baldeyrou, B.; Bailly, C.; Anizon, F.; Marminon, C.; Prudhomme, M.; Colson, P.; Houssier, C. *Eur. J. Med. Chem*. **2002**, *37*, 925 and references cited therein.
28. Monks, A.; Scudeiro, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst*. **1991**, *83*, 757.