Pergamon

Bioorganic & Medicinal Chemistry 7 (1999) 1067–1074

BIOORGANIC & MEDICINAL CHEMISTRY

Indolocarbazoles: Potent, Selective Inhibitors of Human Cytomegalovirus Replication

Martin J. Slater,* Stuart Cockerill, Robert Baxter, Robert W. Bonser, Kam Gohil, Clare Gowrie, J. Edward Robinson, Edward Littler, Nigel Parry, Roger Randall and Wendy Snowden

GlaxoWellcome Medicines Research Centre, Gunnels Wood Road, Stevenage, SG1 2NY, UK

Received 7 August 1998; accepted 21 October 1998

Abstract—In our search for new, safer anti-HCMV agents, we discovered that the natural product Arcyriaflavin A (**1a**) was a potent inhibitor of HCMV replication in cell culture. A series of analogues (symmetrical indolocarbazoles) was synthesised to investigate structure–activity relationships in this series against a range of herpes viruses (HCMV, VZV, HSV1, and 2). This identified a number of novel, selective and potent inhibitors of HCMV, 12,13-dihydro-2,10-difluoro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-(6*H*)-dione (**1d**) being the best example ($IC_{50} = 40$ nM, therapeutic index > 1450). Compounds described in this series were generally poor inhibitors of protein kinase C βII, and no correlation was found between the ability to inhibit HCMV and the enzyme PKC.

Introduction

Human cytomegalovirus (HCMV) disease is the most common life-threatening opportunistic viral infection in the immunocompromised. It is the primary cause of death in recipients of bone marrow and renal transplants,[1,2] and is the most prevalent serious infection in AIDS patients, frequently giving rise to pneumonitis and retinitis.[3,4] Current treatments are limited to ganciclovir (GCV), foscarnet (PFA) and cidofovir (HPMPC).[5–7] While these drugs have made a significant contribution to the treatment of HCMV disease, each has major drawbacks, and all suffer from poor oral bioavailability. GCV has significant myelosuppressive activity,[5] whilst foscarnet and cidofovir are associated with significant renal toxicity.[8,9] All three anti-HCMV agents exert their primary antiviral effects by inhibition of viral DNA polymerase. GCV and cidofovir may also be incorporated into viral DNA.[10,11] In the search for more potent, selective, novel anti-HCMV drug candidates, we therefore considered targeting alternative viral protein functions.

HCMV does not appear to code for a thymidine kinase. The HCMV gene UL97 encodes for a protein that shares homology with protein kinases, guanylyl kinase and bacterial phosphotransferases, and is capable of phosphorylating ganciclovir.[12,13] We thus screened known kinase inhibitors and discovered the simple, unsubstituted indolocarbazole Arcyriaflavin A (**1a**) to be a potent, selective inhibitor of HCMV replication in vitro. Herein we describe the synthesis and anti-HCMV activities of novel ring substituted Arcyriaflavin analogues.

Protein kinase C (PKC) isoenzymes are a family of phospolipid-dependent serine/threonine protein kinases that play an important role in signal transduction pathways. Consequently, PKC controls a large variety of cellular processes such as proliferation, differentiation and smooth muscle contraction.[14] Since disruption of these processes have severe implications for many physiological functions, it was important to identify compounds that are selective for HCMV and have no effect on PKC. We thus chose to prepare analogues maintaining the imide group, since the staurosporine aglycone (**10**) is known to be a better PKC inhibitor than **1a** (~20-fold).[15]

Results and Discussion

Chemistry

In order to investigate the effect of substitution in the benzene nuclei of **1a**, the preparation of bis-indolylmaleimides[16] and their oxidative cyclisation[17] to indolo-

Key words: Human cytomegalovirus; antiviral; indolocarbazoles; protein kinase C; inhibitors.

*Corresponding author. Tel.: 44 (0) 1438 763416; fax: 44 (0) 1438 763616; e-mail: MJS40312@glaxowellcome.co.uk

0968-0896/99/$ - see front matter
PII: S0968-0896(99)00032-2

carbazoles appeared to be the most versatile method.[18a–h] Additionally, this method provided access to bis-indolylmaleimides, allowing us to contrast antiviral activities of the indolocarbazoles and bis-indolylmaleimides in vitro. Thus, indole magnesium bromides were readily prepared (from indoles **3**) and reacted with 2,3-dichloro-*N*-methylmaleimide (**2**) to afford the deep red bis-indolyl-*N*-methylmaleimides (**4a–f**) along with the yellow mono substitution adducts. These were readily separated by flash chromatography over silica (Scheme 1). Conversion to the corresponding maleic anhydrides (**5a–f**) with aqueous hydroxide and then to the parent bis-indolylmaleimides (**6a–f**) using hot ammonium acetate was performed in good yields over two steps.[17] Aromatisation to the planar, yellow, fluorescent, indolocarbazole targets (**1a–f**) was achieved using 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) and catalytic *p*-toluene sulphonic acid (PTSA) in a mixture of toluene and dioxan under reflux. The DDQ/PTSA method failed with the 4,8-difluoro-bis-indolylmaleimide (**6f**), resulting in considerable decomposition from which the product could not be isolated. A possible explanation is that aromatisation forces the fluorine substituents into close proximity to the imide carbonyl atoms. We therefore sought an alternative procedure. Photocyclisation in isopropanol in the presence of iodine and air[19] also gave a complex mixture of products. Fortunately, clean conversion to the required product (**1f**) was accomplished photochemically in isopropanol using an excess of iodine in an inert atmosphere, removing oxidative side reactions.[20] The 2,10-dihydroxy (**1g**) and 3,9-dihydroxy (**1h**) derivatives were prepared from the corresponding dimethoxy indolocarbazoles using neat pyridine hydrochloride in a sealed tube.[18a]

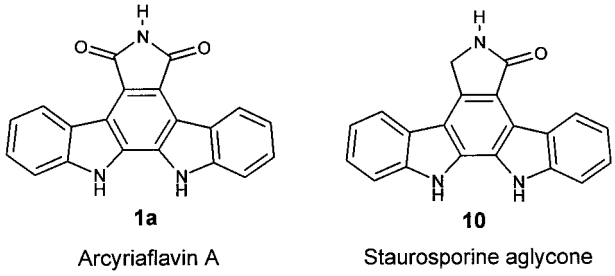
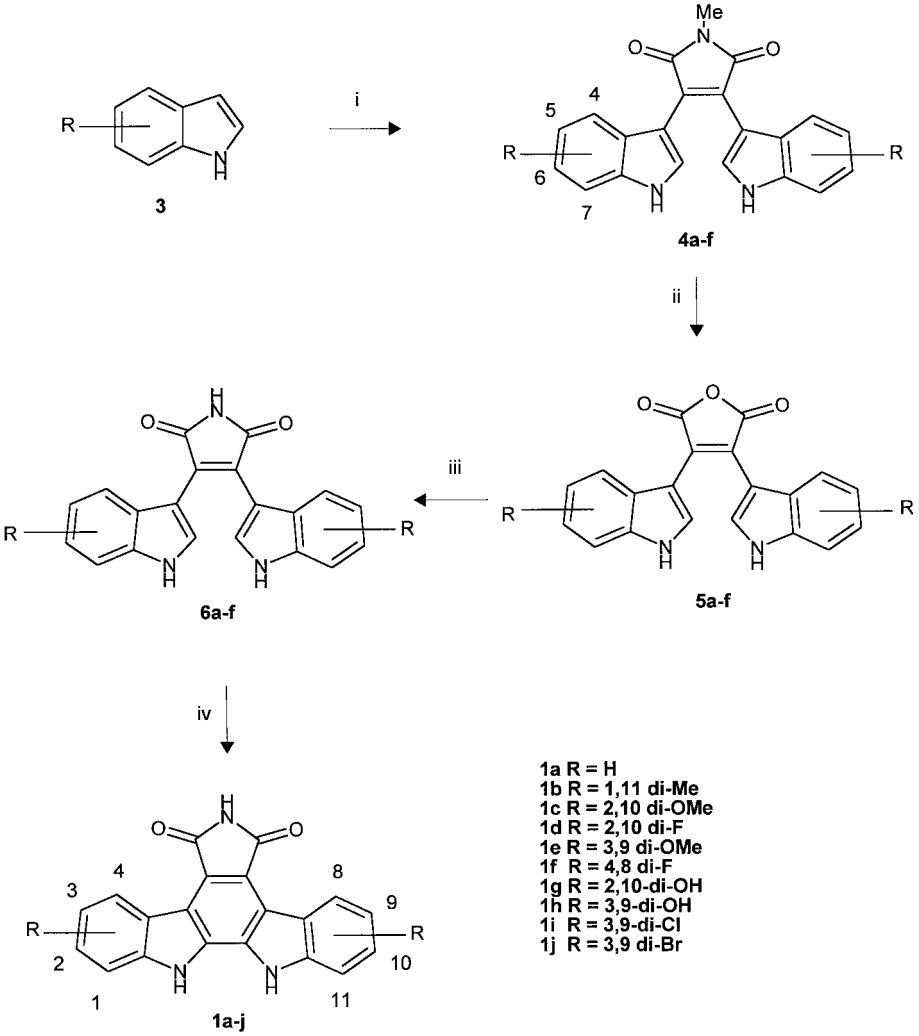


Figure 1. Structures of Arcyriaflavin A and Staurisporine aglycone.



Scheme 1. Synthesis of indolocarbazoles. *Reagents*: (i) a. EtMgBr, THF, benzene b. **2**, THF, benzene; (ii) KOH, MeOH or dioxan; (iii) NH_4OAc, 140 °C; (iv) DDQ, PTSA, toluene, 100 °C for examples **1a–1e**, hv, iodine, isopropanol for example **1f**. For conversions **1c** into **1g** and **1e** into **1h**, pyridine hydrochloride, 120 °C. For conversion **1a** into **1i**, *N*-chlorosuccinimide, MeCN. For conversion **1a** into **1j**, *N*-bromosuccinimide, MeCN.

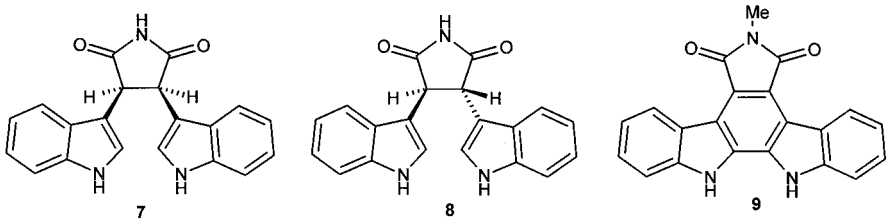


Figure 2.

It was reasoned that the 3,9-dichloro (**1i**) and 3,9-dibromo (**1j**) analogues could be synthesised from **1a** using the appropriate *N*-halo succinimide in acetonitrile, since these sites are favoured for electrophilic and radical substitution in carbazoles. This was indeed the case, the products being less soluble than **1a** and the monohalogenated intermediates, allowing purification by recrystallisation. To investigate the effect of the conformation of the indole groups on antiviral activity, the *cis* (**7**) and *trans* (**8**) bis-indolylsuccinimides were prepared from **6a** by literature routes.[21]

Biology

Antiviral efficacy was measured by plaque assay against a panel of herpes viruses (HCMV, HSV-1, HSV-2, VZV), initially at 10 and 100 μM. GCV was included for comparison. The bis-indolylmaleimides (**6a–f**) were generally toxic in the range 10–100 μM under the assay conditions, and inactive below 10 μM. Antiviral activity is restricted to indolocarbazoles, and these are selective for the inhibition of HCMV (Table 1, only HCMV antiviral data shown). The imide NH was found to be essential for anti-HCMV activity, as replacement of the proton by a methyl group as in **9** completely abolished activity.[18d] Interestingly, the imide proton is also essential for inhibition of protein kinase enzymes. Good anti-HCMV activity is maintained with symmetrical substituents in the 2, 10 or 3, 9 sites (see examples **1c**, **1d**, **1i**, **1j**). Indeed, the 2,10-difluoroindolocarbazole (**1d**) is the most potent in the series with an IC_{50} of 40 nM and a therapeutic index of 1450 based on the $CCID_{50}$ in Vero cells. 1,11 (**1b**) and 4,8 (**1f**) disubstitution resulted in loss of anti-HCMV activity.

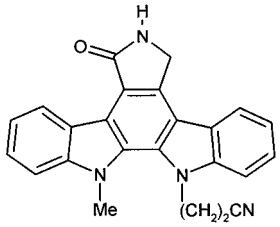
Table 1. Anti-HCMV activities, PKC inhibition data and cell cytotoxicities of indolocarbazoles and selected compounds

	HCMV (IC_{50}/μM)	$CCID_{50}$/μM	PKC: % inhibition at 100 μM
1a	0.2	> 125	< 50
1b	> 10	> 31	32
1c	0.5	< 8	43
1d	0.04	58	59
1e	10	> 60	IC_{50} = 60 μM
1f	> 10	> 125	1
1g	4.6	84	IC_{50} = 50 μM
1h	> 10	> 31	IC_{50} = 0.5 μM
1i	0.16	> 125	18
1j	0.6	> 125	0
6a	T100	34	IC_{50} = 5 μM
7	> 10	143	−6
8	> 10	101	−11
9	> 100	~100	−2
GCV	3	> 500	Not tested

T100 = unable to measure an antiviral effect due to cell toxicity at 100 μM.

As **1a** was found to have a similar level of PKC inhibition against PKC from rat brain and recombinant PKCβII, we chose to routinely use the latter as this was experimentally more convenient. Thus, we could readily prepare one large batch of protein with consistent properties and providing consistent inhibition data. Generally, the indolocarbazoles (**1a–j**) are poor inhibitors of PKCβ, and there is no clear correlation between anti-HCMV activity and inhibition of PKCβ, suggesting that this is not the mode of action for these compounds. Indeed, the introduction of 2,10 or 3,9 dihydroxy groups (in **1g** and **1h**) appears to promote inhibition of PKC and reduce activity against HCMV. The ability of the 3,9-dihydroxy derivative (**1h**) to inhibit PKC (IC_{50} = 0.5 μM) is particularly noteworthy. Staurosporine itself was rather toxic in MDCK cells and it was not possible to measure any antiviral activities, whilst the bis-indolylsuccinimides (**7** and **8**) were inactive, confirming the importance of planarity for anti-HCMV activity.

Arcyriaflavin A analogues represent a novel class of antiviral compounds, selective for the inhibition of HCMV. The mode of action is not known, but does not appear to be related to the inhibition of PKC. To the best of our knowledge, this is the first report of indolocarbazoles with selective antiviral activity unrelated to PKC inhibition. The closest literature example we are aware of is the reported anti-HIV-1 activity of the indolocarbazole lactam Go 6976 (Fig. 3). This antiviral effect is due to the prevention of PKC-mediated induction of viral replication in latently infected cells, and is specific for the U1 cell line.[22] We thought it would be interesting nevertheless to examine whether indolocarbazoles **1a–j** were able to inhibit the replication of



Go 6976

Figure 3. Structure of Go 6976.

HIV-1 in MT4 cells.[23] No activity was observed up to a concentration of 50 μM.

The UL97 protein was conceptually the target that lead to the discovery of these inibitors, and has recently been shown to be a kinase capable of autophosphorylation on serine and threonine.[24] The mode of action of indolocarbazoles could be inhibition of UL97, an alternative viral kinase, or inhibition of an induced or elevated host kinase.[25,26] Further experiments to identify the mode of action of these inhibitors are under way in our laboratories.

In combination experiments using constant drug ratios of **1a** and GCV, the combination was found to have an additive antiviral effect. Thus, it should be possible to use indolocarbazole inhibitors in combination with GCV to treat HCMV disease.

Conclusion

We have discovered Arcyriaflavin A (**1a**) to be a potent, selective inhibitor of HCMV replication in cell culture. A series of novel symmetrical analogues have been prepared to investigate the SAR, affording a number of very active compounds, such as the 2,10-difluoro derivative (**1d**), which is approximately 100 times more potent than GCV in vitro. Indolocarbazoles can be used in conjunction with GCV, and warrant further investigation as potential candidates for the treatment of HCMV disease. Anti-HCMV activity does not appear to be related to the inhibition of protein kinase C. The synthesis and evaluation of further analogues are underway in our laboratories and will be reported in due course.

Experimental

PKC assay

Production of recombinant PKCβII (kindly donated by Dr Christy King, GlaxoWellcome, RTP, North Carolina) was carried out by the infection of Sf9 cells with recombinant baculovirus stocks in spinner flasks and culturing for 5 days at room temperature. Cells were harvested, washed with PBS, and suspended in 0.002 volumes of lysis buffer (20 mM Tris.HCl pH 7.5, 2 mM EDTA, 5 mM EGTA, 1% Triton X-100, 1 mM PMSF). Cells were lysed by homogenisation in a Potter homogenizer and the soluble fraction obtained after centrifugation at 100,000 *g* for 60 min at 4 °C. This fraction was then loaded onto a DE52 anion exchange column equilibrated with buffer A (25 mM Tris.HCl pH7.5, 10 mM 2-mercaptoethanol, 10% glycerol). Proteins were eluted in a linear gradient from buffer A to buffer B (buffer A containing 500 mM NaCl). Fractions were assayed for phorbol ester dependent PKC activity, and the active fractions pooled and stored at −70 °C.

PKC activity was measured essentially as in Ref. 27. Briefly, mixed micelles containing 20 mol% phosphatidylserine and 80 mol% Triton X-100 ± 200 μM PDBu were prepared by sonication in 250 mM Tris.HCl pH 7.5. Samples of diluted enzyme (8.8 μg protein/10 μL) were added to microtubes containing 30 μL reaction buffer (25 mM Tris.HCl pH 7.5, 8.35 mM EGTA, 8.8 mM CaCl2, 16.7 mM MgCl2, 160 μM glycogen synthase peptide substrate (PLSRTLSVAAKK), 5 μCi/mL [g32P]-ATP), 10 μL prepared micelles and 1 μL inhibitor or vehicle (DMSO). This mixture gives a buffered free calcium level of 100 μM. After 15 min at room temp., 40 μL samples were spotted onto squares of P81 paper and washed three times with 75 mM orthophosphoric acid. The amount of [^{32}P]-labelled peptide bound to the P81 paper was quantitated by liquid scintillation counting in 10 mL Picofluor scintillant.

Antiviral assay

HCMV activity was determined by plaque reduction assay as previously described, using strain AD169 in MRC 5 cells.[28] For IC_{50} determination, a dose–response curve was obtained from which the 50% inhibitory concentration (IC_{50}) was calculated.

Cytotoxicity assay

Cytotoxicity determinations were performed in Vero cells as previously reported.[28] The concentration required for a 50% inhibition of cell growth compared to control cells grown in the absence of compound is termed the $CCID_{50}$. The yellow-orange color of many of the compounds in this study interfered with the tetrazolum dye measurement (MTT). We therefore introduced a modification in which the compound was washed from the cells prior to visualisation with the dye. In many cases, it was only possible to define a lower limit for toxicity due to the poor solubility of the compounds in the assay conditions.

Chemistry

General

2,3-Dichloromaleic anydride was purchased from Aldrich. Thin-layer chromatography (TLC) was performed on E. Merck 60F254 No. 5719 glass backed plates. Flash column chromatography was conducted using E. Merck silica gel 60 (230–400 mesh). Solvents employed were A (hexane/ethyl acetate, 4/1), B (hexane/ethyl acetate, 2/1), C (hexane/ethyl acetate, 1/1), D (ethyl acetate), E (cyclohexane, ethyl acetate, 4/1). All ^{1}H NMR spectra were recorded on a Bruker 200 MHz spectrometer in DMSO-d_6 unless stated otherwise, with tetramethylsilane as internal standard. Chemical shifts are reported in δ ppm. Elemental analyses were determined by the Physical Sciences Department of Wellcome Research Labs, Beckenham. Electron impact mass spectra (EIMS) were recorded on a Kratos Concept 1S spectrometer. Fast atomic bombardment mass spectra (FABMS) were recorded on Kratos MS50 or MS80 spectrometers using 3-nitrobenzyl alcohol or thioglycerol matrices. Melting points were obtained using a Gallenkamp apparatus and are uncorrected. Photochemical reactions were performed on a Rayonet Type

RS Preparative Photochemical Reactor, employing 8 lamps with wavelengths of 254, 300 and 350nM (Southern N. E. Ultraviolet Co., Middleton, Connecticut, USA).

Preparation of 3,4-dichloro-*N*-methylmaleimide (2). To a suspension of 2,3-dichloromaleic anhydride (13.36 g, 80 mmol) in glacial acetic acid (25 mL) was added aqueous methylamine (10.7 mL of an approx 25% solution in water, ~84 mmol) with stirring and cooling. The solution was heated under reflux for 330 min, cooled, and water (30 mL) added. The mixture was placed at 4 °C overnight, affording the product (**2**) as yellow crystals (7.75 g, 54%) after filtration, washing with water and drying in vacuo. mp 82–83 °C; ^{1}H NMR 3.12 (s, N-Me); Anal. $C_5H_3Cl_2NO_2$ requires C, 33.36%; H, 1.68%; N, 7.78%; found C, 33.04%; H, 1.65%; N,7.62%.

General procedure for the preparation of bis-indolyl-*N*-methylmaleimides (4a–4f). To a solution of ethyl magnesium bromide (57 mmol) in THF (25 mL) under nitrogen at 0 °C was added a solution of the appropriate indole **3** (57 mmol) in benzene (35 mL) over 40 min with stirring. After heating the solution for 30 min at 50 °C, a solution of **2** (14 mmol) in benzene was added dropwise over 30 min at room temperature. The dark violet solution was then heated under reflux for 1–4 h. After cooling, the mixture was quenched with 20% w/v citric acid solution or ammonium chloride solution, ethyl acetate added, the organic layer separated, dried ($MgSO_4$) and evaporated. The dark-red residue was chromatographed over flash silica using the eluants described for the individual compounds below. The excess indole (**3**) was eluted first, followed by the yellow mono-indole substituted maleimides, and finally the red bis-indolylmaleimides (**4a–f**).

2,3-Bis-(1*H*-indol-3-yl)-*N*-methylmaleimide (4a). Data consistent with that reported in Ref. 16.

2,3-Bis-(7-methyl-1*H*-indol-3-yl)-*N*-methylmaleimide (4b). Chromatography solvent: A; Yield 30%; Recrystallised from EtOAc (dark-red solid); mp >320 °C; ^{1}H NMR 11.6 (2H, brs, NH), 7.7 (2H, s), 6.8 (2H, d, J=7.5 Hz), 6.7 (2H, d, J=7.5 Hz), 6.6 (2H, pseudo t, J=7.5 Hz), 3.05 (3H, s, N-Me), 2.4 (6H, s); Anal. $C_{23}H_{19}N_3O_2.0.2CH_3CO_2Et$ requires C, 73.86%; H, 5.36%; N, 10.86%; found C, 73.96%; H, 5.22% N, 11.00%; EIMS m/z 369 (M^+).

2,3-Bis-(6-methoxy-1*H*-indol-3-yl)-*N*-methylmaleimide (4c). Chromatography solvent C; Yield 62%; Recrystallised from EtOAc (dark-red solid); mp >320 °C; ^{1}H NMR 11.45 (2H, brs, NH), 7.6 (2H, s), 6.85 (2H, d, J=1.5 Hz), 6.7 (2H, d, J=7.5 Hz), 6.3 (2H, dd, J=7.5 Hz, 1.5 Hz), 3.7 (3H, s, OMe), 3.0 (3H, s, NMe); Anal. $C_{23}H_{19}N_3O_4$ requires C, 68.82%.; H, 4.77%; N, 10.47%; found C, 68.71%; H, 4.66%; N, 10.37%; FABMS m/z 402 (MH^+).

2,3-Bis-(6-fluoro-1*H*-indol-3-yl)-*N*-methylmaleimide (4d). Chromatography solvent C; Yield 43%; Recrystallised from EtOAc (red solid); mp 274–276 °C; ^{1}H NMR 11.75 (2H, brs, NH) 7.75 (2H, s), 7.25 (2H, dd, J=10 Hz, 1.5 Hz), 6.8 (2H, dd, J=10 Hz, 5 Hz), 6.5 (2H, ddd, J=10 Hz, 5 Hz, 1.5 Hz), 3.05 (3H, s, NMe); Anal. $C_{21}H_{13}F_2N_3O_4$. 1.13 CH_3CO_2Et requires C, 66.48%; H, 3.64%; N, 10.81%; found C, 66.35%; H, 3.53%; N, 10.78%; EIMS m/z 377 (M^+).

2,3-Bis-(5-methoxy-1*H*-indol-3-yl)-*N*-methylmaleimide (4e). Chromatography solvent C; Yield 36%; Recrystallised from EtOAc (dark-red solid); mp >320 °C; ^{1}H NMR 11.55 (2H, brs, NH), 7.8 (2H, d, J=2.5 Hz), 7.25 (2H, d, J=7.5 Hz), 6.6 (2H, dd, J=7.5 Hz, 2.5 Hz), 6.2 (2H, d, J=2.5 Hz), 3.1 (6H,s, OMe), 3.05 (3H, s, N-Me); Anal. $C_{23}H_{19}N_3O_4.0.36CH_3CO_2Et$ requires C, 67.77%; H, 5.09%; N, 9.70%; found C, 67.56%; H, 4.88%; N, 9.80%; EIMS m/z 401 (M^+).

2,3-Bis-(4-fluoro-1*H*-indol-3-yl)-*N*-methylmaleimide (4f). Chromatography solvent C; Yield 26% (dark-red solid); mp >320 °C; ^{1}H NMR 11.75 (2H, brs, NH), 7.5 (2H, s), 7.2 (2H, d, J=7.5 Hz), 7.0 (2H, ddd, J=10 Hz, 7.5 Hz, 5 Hz), 6.6 (2H, dd, J=10 Hz, 7.5 Hz), 3.2 (3H, s, NMe); Anal. $C_{21}H_{13}F_2N_3O_2.0.2H_2O$ requires C, 66.22%; H, 3.55%; N, 11.03%; found C, 66.13%; H, 3.47%; N, 10.80%; EIMS m/z 377 (M^+).

General procedure for the preparation of 2,3-bis-(1*H*-indol-3-yl)-maleic anhydrides (5a–5f). A solution of the appropiate bis-indolyl-*N*-methylmaleimide **4** (1 mmol) in 10% aqueous potassium hydroxide (20 mL) and dioxane (10 mL) was heated under reflux for 4–6 h (until starting material was absent as measured by TLC analysis on silica). The mixture was cooled, and acidified to pH 1 with 2 N HCl. If the product precipitated at this stage, it was isolated by filtration. Otherwise, the product was extracted into ethyl acetate and purified by crystallisation or column chromatography over silica.

2,3-Bis-(1*H*-indol-3-yl)-maleic anhydride (5a). Data consistent with that reported in Ref. 16.

2,3-Bis-(7-methyl-1*H*-indol-3-yl)-maleic anhydride (5b). Chromatography solvent E; Yield 54%; mp 310 °C; TLC (EtOAc/Hexane, 1/1) R_f 0.37; ^{1}H NMR 11.9 (2H, brs, NH), 7.8 (2H, s), 6.85 (2H, d, J=7.5 Hz), 6.75 (2H, d, J=7.5 Hz), 6.65 (2H, t, J=7.5 Hz), 2.5 (6H, s); Anal. $C_{22}H_{16}N_2O_3.0.2CH_3CO_2Et.0.3H_2O$ requires C, 72.18%; H, 4.84%; N, 7.38%; found C, 72.08%; H, 4.71%; N, 7.21%; EIMS m/z 356 (M^+).

2,3-Bis-(6-methoxy-1*H*-indol-3-yl)-maleic anhydride (5c). This product was not obtained pure. The crude material following the ethyl acetate extraction was taken directly into the ammonium acetate reaction below (**5c** into **6c**).

2,3-Bis-(6-fluoro-1*H*-indol-3-yl)-maleic anhydride (5d). Chromatography solvent B; Yield 31%; mp 291–292 °C; ^{1}H NMR 11.95 (2H, brs, NH), 7.9 (2H, s), 7.2 (2H, dd, J=10 Hz, 1.5 Hz), 6.8 (2H, dd, J=10 Hz, 5 Hz), 6.6 (2H, ddd, J=10 Hz, 5 Hz, 1.5 Hz); Anal. $C_{20}H_{10}F_2N_2O_3.0.55CH_3CO_2Et$ requires C, 64.60%; H, 3.52%; N, 6.79%; found C, 64.79%; H, 3.71%; N, 6.64%; EIMS m/z 364 (M^+).

2,3-Bis-(5-methoxy-1*H*-indol-3-yl)-maleic anhydride (5e). Yield ~100% following extraction from EtOAc,and used directly in the next step. TLC (Hex/EtOAc, 1/1) R_f 0.27; ^{1}H NMR 11.8 (2H, brs, NH), 7.9 (2H, s), 7.3 (2H, d, J=7.5 Hz), 6.6 (2H, dd, J=7.5 Hz, 2.5 Hz), 6.25 (2H, d, J=2.5 Hz), 3.1 (6H, s, OMe); Anal. $C_{22}H_{16}N_2O_5.0.6H_2O$ requires C, 66.20%; H, 4.34%; N, 7.08%; found C, 66.18%; H, 4.15%; N, 6.97%; EI-MS m/z 388 (M^+).

2,3-Bis-(4-fluoro-1*H*-indol-3-yl)-maleic anhydride (5f). Yield ~100% (red solid) following extraction from EtOAc. mp 161–162 °C; ^{1}H NMR 12.0 (2H, brs, NH), 7.65 (2H, s), 7.25 (2H, d, J=7.5 Hz), 7.1 (2H, m), 6.65 (2H, dd, J=10 Hz, 7.5 Hz); Anal. $C_{20}H_{10}F_2N_2O_3.H_2O$ requires C, 62.74%; H, 3.18%; N, 7.32%; found C, 62.95%; H, 3.24%; N, 7.08%; FABMS m/z 365 (MH^+).

General procedure for the preparation of 2,3-bis-(1*H*-indol-3-yl)-maleimides (6a–6f). A mixture of the 2,3 bis-(1*H*-indol-3-yl)-maleic anhydride (**5**) and ammonium acetate (100–250 equiv) were heated at 140 °C (bath temperature) until TLC (silica) indicated complete reaction (15–200 min). The mixture was cooled, partitioned between water and ethyl acetate, the organic layer washed with 5% sodium bicarbonate solution, separated, dried ($MgSO_4$) and evaporated. The product was purified by flash chromatography over silica unless specified otherwise.

2,3-Bis-(1*H*-indol-3-yl)-maleimide (6a). Data consistent with that reported in Ref. 16.

2,3-Bis-(7-methyl-1*H*-indol-3-yl)-maleimide (6b). Chromatography solvent E; Yield 86%; mp >320 °C; ^{1}H NMR 11.69 (2H, brs, NH), 10.85 (1H, brs, NH), 7.7 (2H, s), 6.8 (2H, d, J=7.5 Hz), 6.7 (2H, d, J=7.5 Hz), 6.55 (2H, t, J=7.5 Hz), 2.45 (6H, s); Anal. $C_{22}H_{17}N_3O_2.0.1CH_3CO_2Et$ requires C, 73.87%; H, 4.93%; N, 11.54%; found C, 73.94%; H, 5.01%; N, 11.42%; EIMS m/z 355 (M^+).

2,3-Bis-(6-methoxy-1*H*-indol-3-yl)-maleimide (6c). Chromatography solvent C; Recrystallised from EtOAc; Yield 50% from **4c**; mp 280 °C; ^{1}H NMR 11.49 (2H, brs, J=2.5 Hz, NH), 10.8 (1H, brs, NH), 7.6 (2H, d, J=2.5 Hz), 6.85 (2H, d, J=2.5 Hz), 6.7 (2H, d, J=10 Hz), 6.3 (2H, dd, J=10 Hz, 2.5 Hz), 3.7 (6H, s); Anal. $C_{22}H_{17}N_3O_4.0.9CH_3CO_2Et$ requires C, 65.88%; H, 5.22%; N, 9.00%; found C, 66.04%; H, 5.22%; N, 8.89%; EIMS m/z 387 (M^+).

2,3-Bis-(6-fluoro-1*H*-indol-3-yl)-maleimide (6d). Crude product recrystallised from EtOAc; Yield 28% from **4d**; mp 281–282 °C; ^{1}H NMR 11.7 (2H, brs, NH), 10.9 (1H, brs, NH), 7.75 (2H, s), 7.15 (2H, dd, J=10 Hz, 1.5 Hz), 6.7 (2H, dd, J=10 Hz, 5 Hz), 6.5 (2H, ddd, J=10 Hz, 5 Hz, 1.5 Hz); Anal. $C_{20}H_{11}F_2N_3O_2.1.2CH_3CO_2Et$ requires C, 63.45%; H, 4.45%; N, 8.91%; found C, 63.18%; H, 4.19%; N, 9.90%; FABMS m/z 364 (MH^+).

2,3-Bis-(5-methoxy-1*H*-indol-3-yl)-maleimide (6e). Chromatography solvent F; Yield 95%; mp ~280 °C; ^{1}H NMR 11.55 (2H, brs, NH), 10.8 (1H, brs, NH), 7.75 (2H, s), 7.2 (2H, d, J=10 Hz), 6.55 (2H, dd, J=10 Hz, 2.5 Hz), 6.2 (2H, d, J=2.5 Hz), 3.1 (6H, s); Anal. $C_{22}H_{17}N_3O_4.0.2CH_3CO_2Et$ requires C, 67.61%; H, 4.63%; N, 10.38%; found C, 67.52%; H, 4.50%; N, 10.22%; EIMS m/z 387 (M^+).

2,3-Bis-(4-fluoro-1*H*-indol-3-yl)-maleimide (6f). Crude product recrystallised from EtOAc; Yield 95%; mp 300–302 °C; ^{1}H NMR 11.75 (2H, brs, NH), 10.95 (1H, brs, NH), 7.55 (2H, s), 7.25 (2H, m), 6.65 (2H, dd, J=10 Hz, 7.5 Hz); Anal. $C_{20}H_{11}$ $F_2N_3O_2.0.2_6H_2O$ requires C, 65.28%; H, 3.16%; N, 11.42%.; found C, 65.20%; H, 3.08%; N, 11.44%; FABMS m/z 364 (MH^+).

General procedure for the preparation of 12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-(6*H*)-diones (1a–1e). To a solution of the 2,3-bis-(1*H*-indol-3-yl)-maleimide **6a–e** (0.5 mmol)) in dioxan (25 mL) and toluene (75 mL) at 100 °C was added DDQ (125 mg, 0.55 mmol) and PTSA (4.75 mg, 0.025 mmol), and the mixture maintained at 100 °C for 15 min. After cooling, the solvent was evaporated in vacuo, and the products purified as described for the individual examples.

12,13-Dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-(6*H*)-diones (1a). Data consistent with that reported in Ref. 18a.

12,13-Dihydro-1,11-dimethyl-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-(6*H*)-dione (1b). The crude product was dissolved in EtOAc, washed with 5% sodium bicarbonate solution, dried ($MgSO_4$), and evaporated. Pure product was obtained by successive trituration with EtOAC, acetone, and THF (25 mL each). Yield 20% (yellow solid); mp >300 °C; ^{1}H NMR 11.45 (2H, brs, NH), 10.4 (1H, brs, NH), 8.8 (2H, d, J=7.5 Hz), 7.35 (2H, d, J=7.5 Hz), 7.25 (2H, pseudo t, J=7.5 Hz), 2.75 (6H, s); Anal. $C_{22}H_{15}N_3O_2.2.33H_2O$ requires C, 66.83%; H, 5.01%; N, 10.63%; found C, 66.97%; H, 4.75%; N, 10.68%; FABMS m/z 35 (MH^+).

12,13-Dihydro-2,10-dimethoxy-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-(6*H*)-dione (1c). The crude product was purified directly by flash chromatography over silica, eluting with hexane/EtOAc (3/7). Yield 46% (yellow solid); mp >330 °C; ^{1}H NMR 11.45 (2H, brs, NH), 10.8 (1H, brs, NH), 8.8 (2H, d, J=10 Hz), 7.3 (2H, d, J=2.5 Hz), 6.95 (2H, dd, J=10 Hz, 2.5 Hz), 3.9 (6H, s); Anal. $C_{22}H_{15}N_3O_4.0.4CH_3CO_2Et$ requires C, 66.83%; H, 4.42%; N, 9.91%; found C, 66.83%; H, 4.22%; N, 9.70%; EIMS m/z 385 (M^+).

12,13-Dihydro-2,10-difluoro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-(6*H*)-dione (1d). The crude product was dissolved in EtOAc, washed with 5% sodium bicarbonate solution, dried ($MgSO_4$), and evaporated. Pure product was obtained by successive trituration with EtOAC and acetone. Yield 55% (yellow solid); mp >300 °C; ^{1}H NMR 11.8 (2H, brs, NH), 11.1 (1H, brs, NH), 8.95 (2H, dd, J=7.5 Hz, 5 Hz), 7.63 (2H, dd, J=7.5 Hz, 1.5 Hz), 7.15 (2H, m); Anal. $C_{20}H_9F_2N_3O_2.0.74CH_3CO_2Et$

requires C, 64.66%; H, 3.53%; N, 9.85%; found C, 64.48%; H, 3.34%; N, 9.93%; EI-MS m/z 361 (M^+).

12,13-Dihydro-3,9-dimethoxy-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-(6*H*)-dione (1e). The crude product was purified directly by flash chromatography over silica, eluting with solvent C. Yield 38% (red solid); mp >320 °C; ^{1}H NMR 11.6 (2H, brs, NH), 10.9 (1H, brs, NH), 8.55 (2H, d, J=2.5 Hz), 7.7 (2H, d, J=10 Hz), 7.2 (2H, dd, J=10 Hz 2.5 Hz), 3.9 (6H, s); Anal. $C_{22}H_{15}N_3O_4 0.8H_2O$ requires C, 66.09%; H, 3.78%; N,10.51%; found C, 66.28%; H, 3.72%; N, 10.49%; EIMS m/z 385 (M^+).

12,13-Dihydro-4,8-difluoro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-(6*H*)-dione (1f). A solution of **6f** (28 mg, 77 μmol) and iodine (7 mg) in propan-2-ol (40 mL) was sonicated under a flow of nitrogen for 10 min. The solution was then irradiated at wavelengths of 254 nm, 300 nm, 350 nm for 48 h. The solvent was evaporated in vacuo, and the residue triturated with acetone (2 mL) to afford **1f** (20 mg, 72%) as a yellow solid. mp >300 °C; ^{1}H NMR 12.1 (2H, s, NH), 10.75 (1H, s, NH), 7.65 (2H, d, J=7.5 Hz), 7.55 (2H, m), 7.1 (2H, dd, J=10 Hz, 7.5 Hz); EIMS m/z 361 (M^+).

12,13-Dihydro-2,10-dihydroxy-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-(6*H*)-dione. A mixture of **1c** (76 mg, 0.2 mmol) and pyridine hydrochloride (800 mg, 6.9 mmol) was heated at 180 °C in a sealed tube for 60 min. After cooling, ethyl acetate and water were added, the organic fraction washed successively with 2 N hydrochloric acid and brine, then dried over $MgSO_4$. Following evaporation in vacuo to approximately 20 mL, the mixture was applied to a flash silica column, and eluted with hexane, then hexane/ethyl acetate (4/1). Pooling and evaporation of the appropriate fractions afforded the product (**1g**) as a yellow solid (62 mg, approx 90% pure). A pure sample was obtained following crystallisation from acetone (2 mL), yield 22 mg (31%); mp 145–147 °C; TLC (EtOAc) R_f 0.35; ^{1}H NMR 11.25 (2H, brs, NH), 10.75 (1H, brs, NH), 9.7 (2H, brs, OH), 8.7 (2H, d, J=10 Hz), 7.1 (2H, d, J=2.5 Hz), 6.8 (2H, dd, J=10 Hz, 2.5 Hz); FABMS m/z 358 (MH^+).

12,13-Dihydro-3,9-dihydroxy-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-(6*H*)-dione (1h). A mixture of **1e** (66 mg, 0.17 mmol) and pyridine hydrochloride (800 mg, 6.9 mmol) was heated at 180 °C in a sealed tube for 60 min. After cooling, ethyl acetate and water were added, the organic fraction washed successively with 2 N hydrochloric acid and brine, then dried over $MgSO_4$. Following evaporation in vacuo to approximately 20 mL, the red mixture was applied to a flash silica column, and eluted with hexane, then hexane/ethyl acetate (4/1). Pooling and evaporation of the appropriate fractions afforded the product (**1h**) as a red solid (31 mg, 51%). This product was found to be unstable unless maintained in the freezer. mp >300 °C; TLC (EtOAc) R_f 0.59; ^{1}H NMR 10.85 (2H, brs, NH), 9.65 (1H, brs, NH), 8.55 (2H, d, J=2.5 Hz), 7.55 (2H, d, J=10 Hz), 7.1 (2H, dd, J=10 Hz, 2.5 Hz); EIMS m/z 357 (M^+).

12,13-Dihydro-3,9-dichloro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-(6*H*)-dione (1i). To a warm solution of **1a** (320 mg, 0.98 mmol) in acetonitrile (120 mL) was added *N*-chlorosuccinimide (262 mg, 2.2 mmol), and the mixture heated under reflux for 2 h. Cooling provided the crude product, which was isolated by filtration, and purified by repeated crystallisation from hot THF (3×). Yield 12 mg, 3.1%. mp >330 °C; ^{1}H NMR 11.9 (2H, brs, NH), 11.1 (1H, brs, NH), 8.9 (2H, d, J=1.5 Hz), 7.85 (2H, d, J=7.5 Hz), 7.55 (2H, dd, J=7.5, 1.5 Hz); EIMS m/z 397, 395, 393 (M^+).

12,13-Dihydro-3,9-dibromo-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7-(6*H*)-dione (1j). To a suspension of **1a** (300 mg, 0.92 mmol) in acetonitrile (25 mL) at room temperature was added *N*-bromosuccinimide (414 mg, 2.5 mmol) and the suspension stirred for 90 min. The dark green precipitate was filtered off, and recrystallised from hot THF (3×), to afford the product (**1j**) as an orange solid (60 mg, 13.5%). mp >320 °C; ^{1}H NMR 11.8 (2H, brs, NH), 11.1 (1H, brs, NH), 9.1 (2H, d, J=1.5 Hz), 7.85 (2H, d, J=7.5 Hz), 7.65 (2H, dd, J=7.5, 1.5 Hz); EIMS m/z 485, 484, 483, 482, 481 (M^+).

Acknowledgements

The authors wish to thank Alan Emerson and Chris Roberts for excellent technical assistance with cytotoxicity assays, and Dr. Margaret Tisdale for HIV data.

References

1. Myers, J. D.; Flournoy, N.; Thomas, E. D. *J. Infect. Dis.* **1986**, *153*, 478.
2. Glenn J. *Rev. Infect Dis.* **1981**, *3*, 1151.
3. Jacobsen, M. A.; Mills J. *Ann. Intern Med.* **1988**, *108*, 585.
4. Heinemann, M. H. *J. Am. Med.* **1992**, *92*, 12S.
5. Faulds, D.; Heel, R. C. *Drugs* **1990**, *39*, 597.
6. Chrisp, P.; Clissold, S. P. *Drugs* **1991**, *41*, 104.
7. Kirsch, L. S.; Arevalo, J. F.; De Clerq E.; Chavez de la Paz, E.; Munguia, D.; Garcia, R.; Freeman, W. R. *Am. J. Ophthalmol.* **1995**, *119*, 466.
8. Bacigalupo, A.; van Lint, M. T.; Tedone, E.; Moro, F.; Sanna, M. A.; Longren, M.; Trespi, G.; Frassoni, F.; Occhini, D.; Gualandi, F.; Lamparelli, T.; Marmont, A.; M. *Bone Marrow Transplant* **1994**, *13*, 753.
9. Polis, M. A.; Spooner, K. M.; Baird, B. F.; Manischewitz, J. F.; Jaffe, H. S.; Fisher, P. E.; Falloon, J.; Davey Jr, R. T.; Kovacs, J. A.; Walker, R. E.; Whitcup, S. M.; Nussenblatt, R. B.; Lane, H. C.; Masur, H. *Antimicrob. Agents Chemotherapy* **1995**, *39*, 882.
10. Tyms, A. S; Taylor, D. L.; Parkin, J. M. *Antimicrob. Agents Chemotherapy* **1989**, *23(A)*, 89.
11. Xiong, X.; Smith, J. L.; Chen, M. S. *Antimicrob. Agents Chemotherapy* **1997**, *41*, 594.
12. Littler, E.; Stuart, A. D.; Chee, M. S. *Nature* **1992**, *358*, 160.
13. Sullivan, V.; Talarico, C. L.; Stanat, S. C.; Davis, M.; Biron, K. K. *Nature* **1992**, *358*, 162.
14. Prudhomme, M. *Curr. Pharm Des.* **1997**, *3*, 1381.
15. Fabre, S.; Prudhomme, M. *Bioorg. Med. Chem.* **1993**, *1*, 193.
16. Brenner, M.; Rexhausen, H.; Steffan B., Steglich, W. *Tetrahedron* **1988**, *44*, 10.

17. Joyce, P.; Gainor J. A.; Weinreb, S. M. *J. Org. Chem.* **1987**, *52*, 7.
18. For alternative syntheses see: (a) Hughes, I.; Nolan, W. P.; Raphael, R. A. *J. Chem. Soc. Perkin Trans. 1* **1990**, 2475. (b) Bergman, J.; Pelcman, B. *J. Org. Chem.* **1989**, *54*, 824. (c) Saulnier, M. G.; Frennesson, D. B; Deshpande, M. S.; Vyas, D. M. *Tetrahedron Letts.* **1995**, *36*, 7841. (d) Gribble, G. W.; Berthel, S. J. *Tetrahedron* **1992**, *48*, 8869. (e) Somei, M.; Kodama, A. *Heterocycles* **1992**, *34*, 1285. (f) Bergman, J; Pelcman, B. *Tetrahedron Letts.* **1987**, *38*, 4441. (g) Kaneko, T.; Wong, H. *Tetrahedron Letts.* **1985**, *34*, 4015. (h) Wood, J. L.; Stoltz, B. M.; Dietrich, H.-J.; Petsch, D. T. *J. Am. Chem. Soc.* **1997**, *119*, 9641.
19. Gallant, M.; Link, J. T.; Danishefsky, S. J. *J. Org. Chem.* **1993**, *58*, 343.
20. Liu, L.; Yang, B.; Katz, T. J.; Poindexter, M. K. *J. Org. Chem.* **1991**, *56*, 3769.
21. Davis, P. D.; Hill, C. H.; Lawton, G.; Nixon, J. S.; Wilkinson, S. E.; Hurst, S. A.; Keech, E.; Turner, S. E. *J. Med Chem.* **1992**, *35*, 177.
22. Patzold, S.; Schneider, J.; Rudolph, C.; Marmie, D.; Schachtele, C. *Antiviral Research* **1993**, *22*, 273.
23. Tisdale, M.; Kemp, S. D.; Parry, N. P.; Larder, B. A. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 5653.
24. He, Z. W.; He, Y. S.; Kim, Y.; Chu, L. L.; Ohmstede, C.; Biron, K. K.; Coen, D. M. *J. Virol.* **1997**, *71*, 405.
25. Michelson, S.; Tardy-Panit, M.; Barzu, O. *Virology* **1984**, *134*, 259.
26. Muganda, P. M.; Fischer, A.; Bernal, R. A. *Biochem. Biophys. Res. Commun.* **1995**, *207*, 740.
27. Bonser, R. W.; Thompson, N. T.; Hodson, H. F.; Beams, R. M.; Garland, L. G. *FEBS Letters* **1988**, *234*, 341.
28. Rahim, S. G.; Trivedi, N. T.; Bogunovic-Batchelor, M. V.; Hardy, G. W.; Mills, G.; Selway, J. W. T.; Snowden, W.; Littler, E.; Coe, P. L.; Basnak, I.; Whale, R. F.; Walker, R. T. *J. Med Chem.* **1996**, *39*, 789.