Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1993–1995

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis of *N*-Alkyl Substituted Indolocarbazoles as Potent Inhibitors of Human Cytomegalovirus Replication

Martin J. Slater,* Robert Baxter, Robert W. Bonser, Stuart Cockerill, Kam Gohil, Nigel Parry, Ed Robinson, Roger Randall, Clive Yeates, Wendy Snowden and Adeline Walters

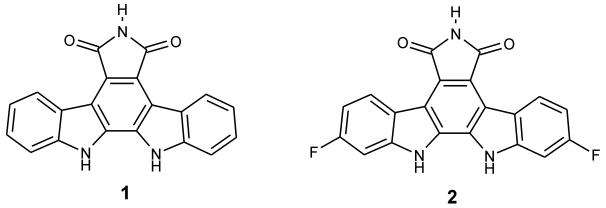
GlaxoSmithKline Medicines Research Centre, Gunnels Wood Road, Stevenage SG1 2NY, UK

Received 26 March 2001; accepted 16 May 2001

Abstract—The synthesis and antiviral evaluation of unsymmetrical indolocarbazole derivatives of Arcyriaflavin A, substituted with a range of alkyl groups at the indole nitrogen, is described. Structure–activity relationships in this series against human cytomegalovirus (HCMV) replication in cell culture are reported. Compound **4b** was identified as potent inhibitor of HCMV ($IC_{50} = 19$ nM), which retained activity against a range of HCMV strains including ganciclovir resistant isolates.

Human cytomegalovirus (HCMV) disease is a common life-threatening opportunistic viral infection in the immunocompromised. It is a primary cause of death in recipients of bone marrow and renal transplants, and is one of the most prevalent serious infections in AIDS patients, frequently giving rise to pneumonitis and retinitis.[1–6] The introduction of highly active antiretroviral therapy (HAART) has had a major impact on the treatment of HCMV disease in HIV-infected individuals, with the successful suppression of HIV RNA levels and immune reconstitution permitting discontinuation of HCMV maintenance therapy without relapse.[7–9] How long anti-HCMV therapy can be interrupted and when treatment should be initiated, is likely to be related to the emergence of HIV resistance and the potential progression of HIV disease and AIDS. Current treatment options for HCMV disease are limited to ganciclovir (GCV), foscarnet (PFA), cidofovir (HPMPC) and formivirsen.[10–15] While these drugs have made significant contributions to the treatment of HCMV disease, all possess significant side effects and have poor oral bioavailability, requiring intravenous or intraocular routes of administration. Here, we report on a group of compounds identified during our search for an agent with potent anti-HCMV activity and an improved profile of safety and bioavailability. We recently reported the discovery that symmetrical indolocarbazoles such as **1** (Arcyriaflavin A) are a new class of potent, selective inhibitors of HCMV replication (Fig. 1).[16] We explored one aspect of the structure–activity relationships (SARs) and discovered that symmetrical substitution into the phenyl indole groups with small lipophilic moieties (e.g., 2,10-difluoro substitution, **2**) was well tolerated.[16] We now report the syntheses and SARs of unsymmetrical *N*-alkyl substituted indolocarbazoles.

Our objective was to investigate the effect on antiviral activity of substitution at the indole nitrogen, removing the symmetry in the process. We chose to introduce simple alkyl groups at the indole nitrogen atoms, since indolocarbazoles bearing more complex functionalities, particularly furan and pyran derivatives, are known to be potent inhibitors of protein kinase C (PKC) and/or topoisomerase I,[17,18] properties which must be absent in



1 Arcyriaflavin A **2**

Figure 1.

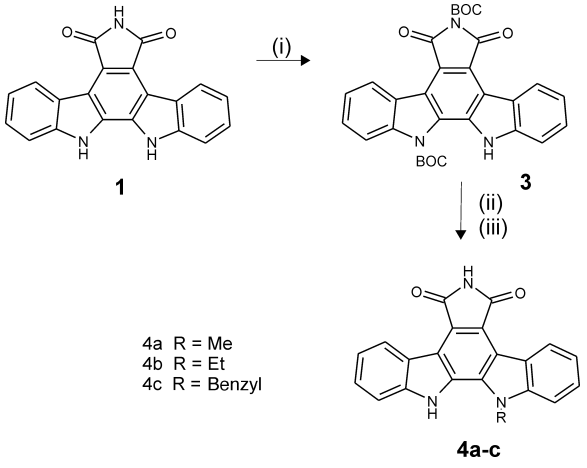
*Corresponding author. Tel.: +44-1438-763416; fax: +44-1438-763616; e-mail: mjs40312@glaxowellcome.co.uk

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00352-3

an anti-HCMV agent. We have already established that Arcyriaflavin A is essentially inactive against PKC and does not intercalate into DNA.[16,19]

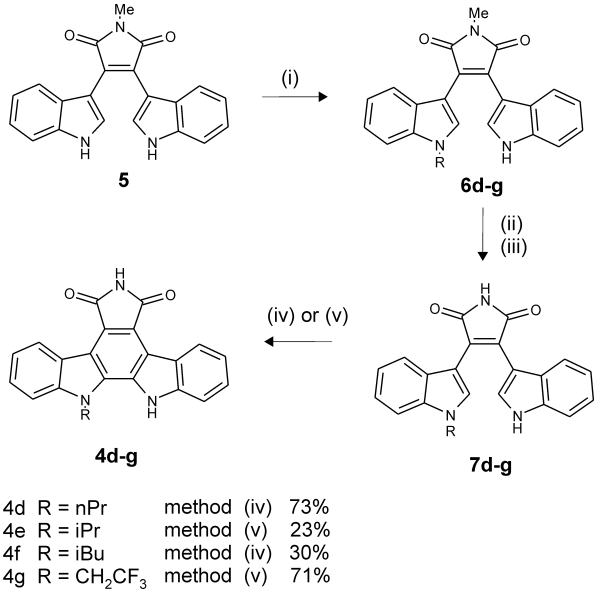
In order to synthesise the mono-alkylated indolocarbazole targets, we initially prepared the previously unreported bis-BOC protected intermediate of **1**. Thus, treatment of **1** with $(BOC)_2O$ and sodium hydride in DMF and subsequent purification by column chromatography afforded **3** in good yield. This could be readily derivatised with the more reactive alkylating agents (methyl iodide, ethyl iodide, benzyl iodide) and deprotected in a separate step to afford the targets **4a–c** (Scheme 1). For less reactive alkyl halides, such as *n*-propyl iodide and isopropyl iodide, several highly-fluorescent impurities formed during the alkylation stage, from which it was very difficult to isolate the pure products. It transpires that the BOC groups are not entirely stable during the reaction conditions and a mixture of mono- and di-alkylated compounds are formed over prolonged reaction times. We therefore sought an alternative approach.

Published approaches to the regiocontrolled introduction of substituents at the indolocarbazole nitrogens either take advantage of their differing electronic character on the staurosporine lactam aglycone, or utilise an orthogonally protected bis-indole maleimide strategy.[17,20–22] With a large stock of the bis-indolyl-*N*-methyl maleimide **5** available,[16] we chose to simply alkylate this intermediate since the mono- and di-substituted products were readily separated by chromatography over silica. Subsequent conversion of the mono-alkyl derivatives (**6d–g**) to the corresponding maleic anhydrides, and then to the parent bis-indolyl maleimides (**7d–g**) was accomplished in good yields as previously described.[16] The final cyclisation step was achieved either with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) and catalytic *p*-toluenesulphonic acid (PTSA) in toluene and dioxan solvent mixtures under reflux, or photochemically in the presence of iodine in isopropanol (Scheme 2).[16] Generally, we have found the photochemical method to be preferred, giving higher yields and cleaner reaction profiles, particularly in the case of β-branched substituents. In the case of the isopropyl compound **4e** for example, no product could be isolated using DDQ/PTSA, whereas a modest 23% yield was attainable using light and iodine.



Scheme 1. (i) $2(BOC)_2O$, 2NaH, DMF, 70%; (ii) methyl iodide or ethyl iodide or benzyl bromide, NaH, DMF; (iii) HCl, EtOAc or 90% aq TFA.

The new indolocarbazoles (**4a–g**) were assayed for antiviral activity in standard plaque reduction assays against HSV-1, HSV-2, HCMV, VZV, influenza A and HIV-1 (Table 1, HCMV data shown only),[23] and for



Scheme 2. (i) R-X, NaH, DMF; (ii) KOH, MeOH or dioxan; (iii) NH_4OAc, 140 °C; (iv) DDQ, PTSA, toluene, dioxan; (v) I_2, hν, isopropanol.

Table 1. Anti-HCMV activity, PKC inhibition data and cell cytotoxicity of indolocarbazoles

Compound	R	HCMV IC_{50}/μM	$CCID_{50}$ (Vero cells)/μM	PKC % Inhibition at 100 μM
1	H	0.2	>125	<50
4a	Me	0.06	>31	47
4b	Et	0.019	>125	IC_{50} = 10 μM
4c	Benzyl	T10	<31	23
4d	*n*Pr	0.15	<8	IC_{50} = 50 μM
4e	*i*Pr	0.1	28	IC_{50} = 50 μM
4f	*i*Bu	0.03	<8	71
4g	CH_2CF_3	0.07	<8	IC_{50} = 25 μM
GCV	—	3	>500	Not tested

T10 = unable to measure an antiviral effect due to cell toxicity at 10 μM.

Table 2. Activity of **4b** against various strains of HCMV in MRC5 cells

Compound	HCMV strain IC_{50}/(μM)					
	AD169	Towne	Davis	Xba F[a]	C8805[b]	2599R
4b	0.019	0.09	0.03	0.02	0.06	0.13
GCV	3	1.5	1.4	>10	ND	1.6

[a]GCV resistant due to 4 amino acid deletion in UL97 gene.
[b]GCV resistant due to mutation in DNA polymerase.

their ability to inhibit protein kinase C (PKC).[24] The pattern of activity mirrored that observed with the aryl ring substituted series, in that antiviral activity was specific for HCMV. All new *N*-alkyl indolocarbazoles possessed significant anti-HCMV activity, with the exception of the *N*-benzyl compound **4c**. The ethyl derivative **4b** was the most potent example, with an IC_{50} of 19 nM, being two orders of magnitude more active than ganciclovir. A clear trend is apparent in the cell cytotoxicity data, with side chains bigger than ethyl conferring significant cytotoxicity. The ethyl compound (**4b**) itself was not toxic, and has an apparent therapeutic index >6500. There is a good separation between the concentration required to inhibit HCMV replication in cell culture and that required to inhibit PKC. No correlation was noted in this series between HCMV activity, cell cytotoxicity and PKC inhibition.

The activity of the ethyl derivative **4b** was maintained against several strains of HCMV, including two which are resistant to GCV (Table 2), indicating that the mechanism of action of **4b** is different from that of GCV, and that GCV-resistance does not confer cross-resistance to **4b**.

The specificity of the antiviral activity of **4b** was further verified by the generation of resistant viruses by in vitro passage in MRC5 cells of the laboratory strain AD169 in the presence of increasing concentration of **4b**. After approximately 3 months passage in a maximum concentration of 200 nM **4b** (10 times the IC_{50} of the parental wild-type virus AD169), a virus was isolated (2599R) which possessed approximately 6-fold reduction in susceptibility to **4b**, but retained normal susceptibility to GCV. These data (Table 2) confirm the specificity of antiviral activity of **4b** and the likely different mechanism of action of **4b** from GCV. Marker rescue experiments to identify the location of any mutation(s) which may be responsible for conferring resistance to **4b** are underway, and should help determine the mechanism of action of these agents.

In conclusion, we have have identified 12-ethyl-12,13-dihydro-5*H*-indolo[2,3a]pyrrolo[3,4c]carbazole-5,7(6*H*)-dione (**4b**) as a potent, selective inhibitor of HCMV replication in cell culture. Compound **4b** is a promising lead for the development of new anti-HCMV agents. The poor solubility of **4b** has precluded further development of this particular compound. Our approaches to apply the SARs described herein in order to design potent analogues with improved solubility will be reported in due course.

Acknowledgements

The authors wish to thank Alan Emerson and Chris Roberts for excellent technical assistance with cytotoxicity assays, Dr. Karen Biron for the provision of virus strains Xba F and C8805, Dr. Margaret Tisdale for influenza and HIV assays and Drs. Peter Collins, Eddy Littler and George Hardy for advice.

References and Notes

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*, 12 S.
5. DeJong, M. D.; Galasso, G. J.; Gazzard, B.; Griffiths, P. D.; Jabs, D. A.; Kern, E. R.; Spector, S. A. *Antiviral Res.* **1998**, *39*, 141.
6. Hoffman, V. F.; Skiest, D. J. *Expert Opin. Invest. Drugs* **2000**, *9*, 107.
7. Cooper, D. A. *Antiviral Ther.* **1998**, *3* (Suppl. 4), 19.
8. Komanduri, K. V.; Viswanathan, M. N.; Wieder, E. D.; Schmidt, D. K.; Bredt, B. M.; Jacobsen, M. A.; McCune, J. M. *Nat. Med. (N. Y.)* **1998**, *4*, 953.
9. Garrett, N. W.; Boeckh, M. *J. Clin. Virol.* **2000**, *16*, 25.
10. Temsamani, J.; Pari, G. S.; Guinot, P. *Expert Opin. Invest. Drugs* **1997**, *6*, 1157.
11. Faulds, D.; Heel, R. C. *Drugs* **1990**, *39*, 597.
12. Chrisp, P.; Clissold, S. P. *Drugs* **1991**, *41*, 104.
13. Kirsch, L. S.; Arevalo, J. F.; De Clerg, E.; Chavez de la Paz, E.; Munguia, D.; Garcia, R.; Freeman, W. R. *Am. J. Ophthalmol* **1995**, *119*, 466.
14. 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.
15. Polis, M. A.; Spooner, K. M.; Baird, B. F.; Manischewitz, J. F.; Jaffe, H. S.; Fisher, P. E.; Falloon, J.; Davey, R. T., Jr.; Kovacs, J. A.; Walker, R. E.; Whitcup, S. M.; Nussenblatt, R. B.; Lane, H. C.; Masur, H. *Antimicrob. Agents Chemother.* **1995**, *39*, 882.
16. 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.
17. Pindur, U.; Kim, Y.-S.; Mehrabani, F. *Curr. Med. Chem.* **1999**, *6*, 29.
18. Qu, X.; Chaires, J. B.; Ohkubo, M.; Yoshinari, T.; Nishimura, S.; Bailly, C. *Anti-Cancer Drug Des.* **1999**, *14*, 433.
19. Cory, M. GlaxoSmithKline, personal communication.
20. Eils, S.; Winterfeldt, E. *Synthesis* **1998**, *275*.
21. Wood, J. L.; Stoltz, B. M.; Dietrich, H.-J. *J. Am. Chem. Soc.* **1995**, *117*, 10413.
22. Link, J. T.; Raghavan, S.; Gallant, M.; Danishefsky, S. J.; Chou, T. C.; Ballas, L. M. *J. Am. Chem. Soc.* **1996**, *118*, 2825.
23. 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.
24. Bonser, R. W.; Thompson, N. T.; Hodson, H. F.; Beams, R. M.; Garland, L. G. *FEBS Lett.* **1988**, *234*, 341.