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Antiviral activity of natural and semi-synthetic chromone alkaloids

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Abstract

The activity against human immunodeficiency virus (HIV) and herpes simplex virus (HSV), of the non-polar fraction of a methanolic extract of the rootbark of *Schumanniophyton magnificum* was found to be present in a fraction containing the chromone secondary amine schumannificine **1**. Other chromone alkaloids present in the plant were isolated and tested for inhibition of HIV and HSV infections in C8166 and Vero cells, respectively. Acyl and methyl derivatives were prepared and tested. Of all the compounds tested, schumannificine **1** displayed the greatest activity against HIV, whereas potent anti-HSV activity was observed for a number of its derivatives. The presence of a piperidine ring and unsubstituted hydroxy groups on the molecules seems to favour the anti-HIV activity. The anti-HIV activity is considered to be due to irreversible binding to gp120 rather than inhibition of reverse transcriptase or protease.

Keywords: Chromone alkaloid; HIV; HSV; gp120 inhibition

1. Introduction

The search for natural products active against HIV has resulted in a number of compounds which have shown activity in vitro and some of these have reached the stage of clinical trials (Lednicer and Snader, 1991). A variety of alkaloids are included in this group and the isolation of the chromone alkaloids prompted us to investigate their

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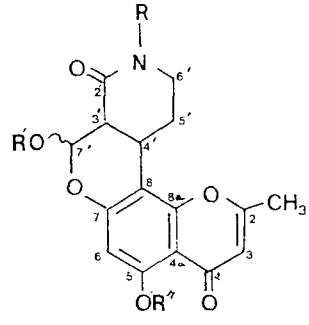
activity against HIV since a crude extract of the alkaloids from *S. magnificum* showed anti-HIV activity in preliminary tests. The chromone alkaloids are a novel type of alkaloid, most of which have been isolated from *Schumanniophyton magnificum* Harms. (Rubiaceae) in the pharmacognosy research laboratories at King's College (Houghton and Yang, 1985; Houghton and Yang, 1987, Houghton, 1987; Houghton, 1988). The structure of some of these alkaloids has recently been revised in the light of ^{13}C-NMR evidence (Houghton et al., 1994). The chromone alkaloids bear structural similarities to the flavonoids, some of which have recently been shown to have activity against human immunodeficiency virus (HIV) and herpes simplex virus (HSV) (Mahmood et al., 1993a). Of particular interest is the anti-HIV activity shown by the flavonoidal alkaloid *O*-demethylbuechanavine (Beutler et al., 1992).



	R	R'	R''
1	H	H	H
2	CH_3	H	H
11	H	BrBz	H
12	H	BrBz	BrBz
13	CH_3CO	CH_3CO	H
14	CH_3CO	CH_3CO	CH_3CO
15	H	$CH_3(CH_2)_2CO$	H
16	H	CH_3	CH_3
17	H	CH_3	H
18	$(CH_3)_2$	H	H

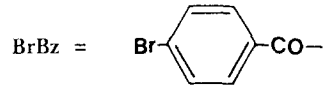
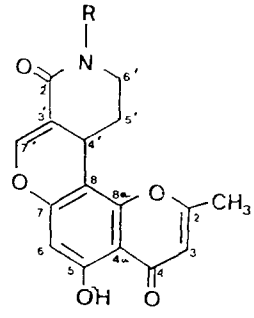
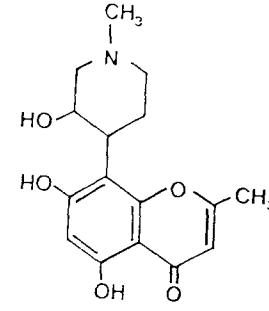


Fig. 1. Structures of compounds tested. **1** Schumannificine; **2** *N*-methylschumannificine; **3** Anhydroschumannificine; **4** *N*-methylanhydroschumannificine; **5** Rohitukine; **6** *N*-demethylrohitukine acetate; **7** Schumanniophytine; **8** Isoschumanniophytine; **9** *N*-methylschumanniophytine; **10** Noreugenin; **11** 7′-(4-bromobenzoyl)schumannificine; **12** 7′,5-di(4-bromobenzoyl)schumannificine; **13** *N*,7′-diacetylschumannificine; **14** *N*,7′,5-triacetylschumannificine; **15** 7′-butylschumannificine; **16** 7′,5-dimethoxyschumannificine; **17** 7′-methoxyschumannificine; **18** *N*,*N*-dimethylschumannificine.



3 R = H
4 R = CH_3



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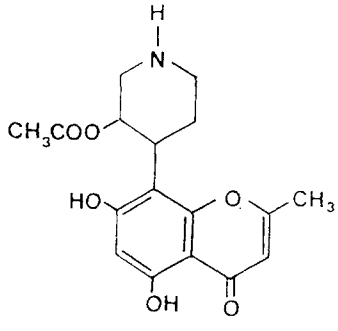

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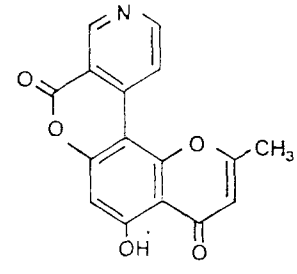

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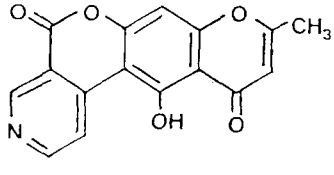

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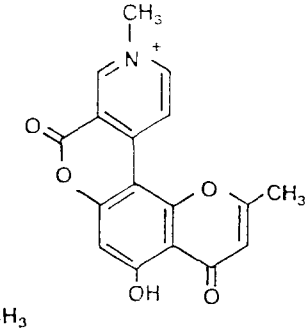

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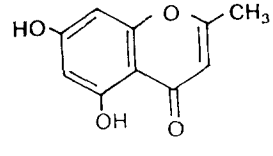

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Fig. 1 (continued).

2. Materials and methods

2.1. Compounds

The naturally occurring alkaloids **1–9** and the chromone noreugenin **10** were isolated from the rootbark of *Schumanniophyton magnificum* as previously described (Houghton and Yang, 1985; Houghton and Yang, 1987). The identity of each alkaloid was checked by chromatography and NMR spectroscopy against the authentic alkaloids previously extracted.

Compounds **11,12** were obtained by dissolving 60 mg of **1** in 1 ml pyridine and adding 60 mg dimethylaminopyridine (DMAP) and 120 mg 4-bromo benzoyl chloride (4BrBzCl) dissolved in 1 ml dichloromethane. The solution was made up to 6 ml with dichloromethane and kept at 25° for 72 h. The reaction mixture was poured into 20 ml 1 M $NaHCO_3$ and extracted with 3 × 10 ml chloroform. The chloroform was washed with water and evaporated off under reduced pressure. The residue was examined on TLC and two products **11,12** isolated by preparative TLC (silica-gel; chloroform/methanol, 12:1). The mass spectrum and ^{1}H-NMR spectrum of each product was obtained (Fig. 1).

11 Mono (4-bromobenzoyl) schumannificine R_f value 0.64.

Mass spectrum: 500 (40) M^+ $C_{23}H_{18}O_7NBr$ calculated 500.0118, measured 500.0116, 301(100).

^{1}H-NMR spectrum ($CDCl_3$) δ ppm from TMS.

12.7 (1H,s,5-OH), 7.81 (2H, d, J = 8.6, Hz 3′5′-H), 7.54 (2H, d, J = 8.6 Hz, 2′6′-H), 7.14 (1H, d, J = 3.7 Hz, 7′-H), 6.38 (1H, s, 6-H), 6.12 (1H, s, 3-H), 4.82 (1H, bs, N-H) 3.82 (1H, m, 4′-H), 3.32 (1H, m, 6′-H), 3.18 (1H, m, 3′-H), 3.07 (1H, m, 6′-H), 2.80 (1H, m, 5′-H), 2.43 (3H, s, 2-CH_3), 2.19 (1H, m, 5′-H).

12 Di (4-bromobenzoyl) schumannificine R_f value 0.73.

Mass spectrum: 682 (15) M^+ $C_{30}H_{21}O_7NBr_2$ calculated 682.9341, measured 682.9340, 301(100).

^{1}H-NMR spectrum ($CDCl_3$) δ ppm from TMS.

8.04 (2H, d, J = 8.6, 3″,5″H), 7.85 (2H, d, J = 8.6 Hz, 3′5′-H), 7.85 (2H, d, J = 8.6, 2″6″-H), 7.60 (2H, d, J = 8.6 Hz, 2′6′-H), 7.17 (1H, d, J = 3.7 Hz, 7′-H), 6.76 (1H, s, 6-H), 6.01 (1H, s, 3-H), 4.82 (1H, bs, N-H) 3.89 (IH, m, 4′-H), 3.35 (1H, m, 6′-H), 3.24 (1H, m, 3′-H), 3.15 (1H, m, 6′-H), 2.80 (1H, m, 5′-H), 2.43 (3H, s, 2-CH_3), 2.25 (1H, m, 5′-H).

Compounds **13,14** were obtained by acetylation of **1** as carried out previously (Houghton and Yang, 1985).

Compound **15** was made by treating 50 mg schumannificine **1** with 1 ml butyric anhydride and 1 ml pyridine. The mixture was kept at room temperature for 72 h and the solvents then evaporated under reduced pressure. The residue was acidifled with 20 ml 1 M HCl and extracted with chloroform. The chloroform was washed with water and then evaporated to small volume and the major product purified by preparative TLC (silica-gel; chloroform/methanol, 12:1).

15 7′-butyrylschumannificine.

Mass spectrum: 387 (40) M^+, $C_{20}H_{21}O_7N$ calculated 387.1312, measured 387.1314, 301(100).

^{1}H-NMR spectrum ($CDCl_3$) δ ppm from TMS.

12.7 (1H,s,5-OH), 7.11 (1H, d, J = 3.7 Hz, 7′-H), 6.36 (1H, s, 6-H), 6.11 (1H, s, 3-H), 4.82 (1H, bs, N-H) 3.82 (1H, m, 4′-H), 3.32 (1H, m, 6′-H), 3.18 (1H, m, 3′-H), 3.07 (1H, m, 6′-H), 2.80 (1H, m, 5′-H), 2.43 (3H, s, 2-CH_3), 2.28 (2H, t, 2″-CH_2), 2.19 (1H, m, 5′-H), 1.58 (2H, quintet, 3″-CH_2), 0.91 (3H, t, 4″-CH_3).

Compounds **16,17** were obtained by dissolving 30 mg **1** in 1 ml 0.2 m trimethylanilium hydroxide in methanol and refluxing for 1 h. The solution was evaporated to dryness and acidified with 1 M HCl and extracted with 3 × 15 ml chloroform.

Two products **16,17** were isolated using preparative TLC (silica-gel; chloroform/methanol, 12:1) and the mass spectrum and ^{1}H-NMR spectrum of each product was obtained.

16 5,7′-dimethoxyschumannificine R_f 0.62.

Mass spectrum: 345 (100) M^+ $C_{18}H_{19}O_6N$ calculated 345.1207, measured 345.1207.

^{1}H-NMR spectrum ($CDCl_3$) δ ppm from TMS.

7.16 (1H, bs, NH), 6.38 (1H, s, 6-H), 6.09 (1H, s, 3-H), 5.57 (1H, d, J = 4.0, 7′-H), 3.81 (1H, m, 4′-H), 3.78 (3H, s, 5-OCH_3), 3.76 (3H, s, 7′-OCH_3), 3.24-3.08 (3H, m, 3′-H, 6-CH_2), 2.81 (1H, m, 5′-H), 2.41 (3H, s, 2-CH_3), 2.22 (1H, m, 5′-H).

17 7′-methoxyschumannificine R_f 0.46.

Mass spectrum: 331 (100) M^+ $C_{17}H_{17}O_6N$ calculated 331.1051, measured 331.1050.

^{1}H-NMR spectrum ($CDCl_3$) δ ppm from TMS.

12.68 (1H, s, 5-OH), 7.16 (1H, bs, NH), 6.38 (1H, s, 6-H), 6.09 (1H, s, 3-H), 5.57 (1H, d, J = 4.0, 7′-H), 3.81 (1H, m, 4′-H), 3.76 (3H, s, 7′-OCH_3), 3.24-3.08 (3H, m, 3′-H, 6-CH_2), 2.81 (1H, m, 5′-H), 2.41 (3H, s, 2-CH_3), 2.22 (1H, m, 5′-H).

Compound **18** was obtained by refluxing 20 mg *N*-methylschumannificine **2** with 5 ml methyl iodide for 30 min. After evaporation under reduced pressure the residue was dissolved in methanol and the major product isolated after preparative TLC (silica-gel; ethyl acetate/propan-2-ol/10% ammonia, 65:35:10) to yield 16 mg **18**.

18 *N,N* dimethylschumannificine.

Mass spectrum: 346 (100) M^+ $C_{18}H_{20}O_6N$ calculated 346.1285, measured 346.1284.

^{1}H-NMR spectrum ($CDCl_3$) δ ppm from TMS.

12.60 (1H, s, 5-OH), 6.78 (1H, bs, 7′-OH), 6.33 (1H, s, 6-H), 6.11 (1H, s, 3-H), 5.58 (1H, d, J = 4.0, 7′-H), 4.35 (6H, s, N-CH_3), 3.71 (1H, m, 4′-H), 3.31-3.10 (3H, m, 3′-H, 6′-CH_2), 2.65 (1H, m, 5′-H), 2.3 (3H, s, 2-CH_3), 2.22 (1H, m, 5′-H).

2.2. Antiviral assays

The antiviral activities and toxicities of compounds were assessed in C8166 cells infected with HIV-1 strain$_{(IIIB)}$ and Vero cells infected with HSV-1 (strain 17-I).

The methods are outlined below but are described in detail in earlier publications (Mahmood et al. 1993a, Mahmood et al. 1993b). Six dilutions of the compounds were used for each test and different ranges of concentrations for each compound. Tests were repeated three times.

The anti-HIV activity was measured in microtitre plate wells by mixing 4×10^4 C8166 cells with HIV-1$_{(IIIB)}$. Cells were grown in RPMI 1640 with 10% foetal calf serum. 4×10^4 cells per microtitre plate well were mixed with 5-fold dilutions of

compound prior to addition of 10 $CCID_{50}$ (50% cell culture infectious dose) units of virus and incubated for 5 days.

Formation of syncytia was examined from 2 days post-infection. Viral gp120 antigen produced after 5 days was measured by ELISA following the method described by Mahmood and Hay (1992). The EC_{50} was determined as the concentration of compound (μM) which reduced the production of gp120 by 50%. The viability of virus-infected and uninfected control cells was measured by the MTT-Formazan method (Pauwels et al., 1988), and the TC_{50} was determined as the concentration of compound which reduced the viability of uninfected cells by 50%.

Antiviral activity against HSV-1 was determined by measuring viral antigen produced in infected Vero cells as described previously (Mahmood et al., 1993b). Five-fold dilutions of compounds were added to duplicate wells just before adding virus at a multiplicity of infection of 0.01 plaque-forming units per cell. The cells were incubated 16–18 h at 37° and then fixed with 3% formalin for 1–2 h. Antigen was detected by ELISA using rabbit anti-HSV-1 antibodies obtained from Dakopatts, Denmark. The cytotoxicity was assessed using the MTT-Formazan assay on growing Vero cells and also on human embryonic lung cells.

2.3. Enzyme assays

In vitro tests for the inhibitory effect of schumannificine **1** on the HIV-1 enzymes reverse transcriptase were carried out as described previously (Mahmood et al., 1993a; Mahmood et al., 1993b). Briefly, the test consisted of incubating reverse transcriptase (RT) 1.4 μg/ml (0.05 U/ml) with 25 μl of a mixture of 50 mM Tris buffer (pH 8.0),

Table 1
Anti-HIV activity of naturally occurring chromone alkaloids

Compound	Syncytium formation *	EC_{50} (μM)	TC_{50} (μM)	Selectivity index TC_{50}/EC_{50}
AZT	0.016	0.016	> 1000	62500
Piperidino-alkaloids				
Schumannificine **1**	8	1.6	100	62.5
N-methylschumannificine **2**	40	5	100	20
Anhydroschumannificine **3**	2	0.4	4	10
N-methylanhydroschumannificine **4**	200	20	100	5
Rohitukine **5**	200	30	400	13.3
N-demethylrohitukine acetate **6**	500	inactive	500	-
Pyridino-alkaloids				
Schumanniophytine **7**	20	8	100	12.5
Isoschumanniophytine **8**	80	80	100	1.25
N-methylschumanniophytine **9**	200	80	500	6.25
Chromone				
Noreugenin **10**	200	40	500	12.5

* Maximum concentration at which syncytia were just appearing.
EC_{50} = the concentration of compound (μM) which reduced the production of gp120 by 50%.
TC_{50} = the concentration of compound which reduced the viability of uninfected cells by 50% measured by the MTT-Formazan method (Pauwels et al., 1988).

Table 2
Anti-HIV activity of chromone alkaloid derivatives

Compound	Syncytium formation *	EC_{50} (μM)	TC_{50} (μM)	Selectivity index TC_{50}/EC_{50}
AZT	0.016	0.016	> 1000	62500
7′-(4-bromobenzoyl)schumannificine **11**	20	10	40	4
7′,5-di(4-bromobenzoyl)schumannificine **12**	20	4	40	10
N,7′-diacetylschumannificine **13**	40	8	40	5
N,7′,5-triacetylschumannificine **14**	20	4	100	25
7′-butylschumannificine **15**	8	2	40	20
7′,5-dimethoxyschumannificine **16**	200	40	400	10
7′-methoxyschumannificine **17**	50	5	250	50
N,*N*-dimethylschumannificine **18**	20	5	100	20

* Maximum concentration at which syncytia were observed.
EC_{50} = the concentration of compound (μM) which reduced the production of gp120 by 50%.
TC_{50} = the concentration of compound which reduced the viability of uninfected cells by 50% measured by the MTT-Formazan method (Pauwels et al., 1988).

100 mM KCl, 6 mM $MgCl_2$, 5 mM DTT, 10 μM [^{3}H] dTTP (4Ci mmol^{-1}) and 1.6 μg ml^{-1} template primer (p(rA.$(dT)_{12-18}$). Enzyme activity was linear with time for 30 min. The tests were conducted without compound **1** and with **1**.

The test for proteinase activity was carried out according to the method described by Shearer (1992).

2.4. Test for effect of compound on virus infectivity

HIV-$1_{(IIIB)}$ (10^5–10^6 $TCID_{50}$) was incubated with the compound at 37° for 2 h. The virus was serially diluted, mixed with C8166 cells and incubated at 37° for 5 days. The infectivity endpoint was determined by visual examination for syncytium formation and by the MTT-Formazan assay. The virus titre ($TCID_{50}$) was expressed as the reciprocal of the dilution which gave a 50% end-point. In all cases the compound was diluted to well below the EC_{50} such that residual compound did not interfere with the virus titration. Results are shown in Table 5.

3. Results and discussion

The results for the anti-HIV tests of the naturally-occurring compounds are shown in Table 1 and of schumannificine the derivatives in Table 2. The results for the anti-HSV tests are similarly shown in Tables 3 and 4.

The greatest activity against HIV-1 was shown by the piperidone schumannificine **1**, whilst its *N*-methyl analogue **2** showed less activity. Tests for the inhibitory effect of schumannificine on the activity of reverse-transcriptase and proteinase were negative but the considerable reduction in viral titre in cells exposed to HIV previously incubated with schumannificine **1** (Table 5) indicates that **1** binds irreversibly to gp120 and thus prevents the virus binding to the cells and subsequent infection.

Table 3
Anti-HSV activity of naturally occurring chromone alkaloids

Compound	EC_{50} (μM)	TC_{50} (μM) Vero	TC_{50} (μM) MRC5	Selectivity index TC_{50}/EC_{50} [+]
Piperidino-alkaloids				
Schumannificine **1**	0.5	500	500	1000
N-methylschumannificine **2**	0.5	> 500	1000	> 1000
Anhydroschumannificine **3**	0.06	250	> 500	> 4000
N-methylanhydroschumannificine **4**	0.5	400	> 250	800
Rohitukine **5**	1.6	200	> 100	12.5
N-demethylrohitukine acetate **6**	100	> 500	> 1000	> 10
Pyridino-alkaloids				
Schumanniophytine **7**	40	> 500	500	> 12.5
Isoschumanniophytine **8**	50	> 500	1000	> 10
N-methylschumanniophytine **9**	50	> 500	> 1000	> 10
Chromone				
Noreugenin **10**	50	> 500	1000	10

[+] EC_{50} values for Vero cells.
EC_{50} = the concentration of compound (μM) which reduced the production of viral antigen by 50%.
TC_{50} = the concentration of compound which reduced the viability of uninfected cells by 50% measured by the MTT-Formazan method (Pauwels et al., 1988).

Schumannificine exists as two isomers at the 7′ position and attempts were made to separate these to investigate the activity of each of the two isomers (Houghton and Woldemariam, 1994). Although separation was effected by use of HPLC using a porous graphitic carbon stationary phase, the two isomers underwent isomerisation so rapidly that in the test system a racemic mixture would be formed even if the pure isomer were introduced. The tertiary amines **2** and **4** gave an activity less than their secondary

Table 4
Anti-HSV activity of chromone alkaloid derivatives

	EC_{50} (μM)	TC_{50} (μM) Vero	TC_{50} (μM) MRC5	Selectivity index TC_{50}/EC_{50} [+]
Compound				
7′-(4-bromobenzoyl)schumannificine **11**	0.4	400	500	1000
7′,5-di(4-bromobenzoyl)schumannificine **12**	0.4	> 500	1000	1250
N,7′-diacetylschumannificine **13**	0.1	> 50	> 100	> 500
N,7′,5-triacetylschumannificine **14**	0.5	400	300	800
7′-butylschumannificine **15**	0.4	100	ND	250
7′,5-dimethoxyschumannificine **16**	0.2	250	1000	1250
7′-methoxyschumannificine **17**	15	400	500	26.7
N,*N*-dimethylschumannificine **18**	15	300	> 50	20

[+] EC_{50} values for Vero cells.
EC_{50} = the concentration of compound (μM) which reduced the production of viral antigen by 50%.
TC_{50} = the concentration of compound which reduced the viability of uninfected cells by 50% measured by the MTT-Formazan method (Pauwels et al., 1988).

Table 5
Effect of compounds on virus infectivity

Compound	Concentration (μM)	Virustitre ($TCID_{50} \times 10^{-3}$)
Schumannificine **1**	500	5
DS_{500}	100	128
Heparin	25	128
Control	0	128

HIV-$1_{(IIIB)}$ (10^5–10^6 $TCID_{50}$) was incubated with the compound at 37° for 2 h. The virus was serially diluted, mixed with C8166 cells and incubated at 37° for 5 days. The infectivity end-point was determined by visual examination for syncytium formation and by the MTT-Formazan assay. The virus titre ($TCID_{50}$) was expressed as the reciprocal of the dilution which gave a 50% end-point. In all cases the compound was diluted to well below the EC_{50} such that residual compound did not interfere with the virus titration.

analogues **1** and **3**. The piperidones were generally more active than the pyridino-alkaloids. The activity of noreugenin, the part of the molecule which does not contain nitrogen, is low and it therefore seems that an alkaloidal moiety is necessary for significant activity.

Derivatives of the most active compound, schumannificine **1**, were made which would have greater lipophilicity. Such compounds have been found to increase cell penetration, and therefore activity, for other anti-HIV agents such as 1-deoxynojirimycin (Fellows, 1992). It can be seen that most of the compounds made show less cytotoxicity but also reduced anti-HIV activity.

The quaternary analogues of both schumannificine and schumanniophytine, compounds **18** and **9**, respectively, show fairly good activity. These are more water-soluble than the corresponding tertiary compounds and thus may be more attractive for formulation purposes.

The piperidones are therefore seen to be the most active compounds of this group so far tested and represent a novel class of compound active against HIV. It should, however, be pointed out that the toxicity:activity ratio is not very high. It was decided to assay this novel class of compound against herpes simplex virus which also causes undesirable infections in AIDS patients.

It was interesting to see that piperidones **1–4** were highly active against HSV with selectivity indices ranging from 1000–4000 (Table 1). Removing the lactol ring, as in **5**, reduced the anti-herpes activity and increased the cytotoxicity, whereas minimal activity and cytotoxicity were noted when the 3′ OH of such a compound with no lactol ring was acetylated (as in compound **6**).

As observed for HIV, noreugenin and the pyridino compounds exhibited much reduced activity against HSV compared with the piperidino type.

The synthetic derivatives of schumannificine **1** retained inhibitory activity with EC_{50} values ranging from 0.1–15.0 μM. There was an increase in cytotoxicity, especially with the introduction of a butyl group which made **15** quite toxic. Pronounced differences in activity were noted between **16** and **17**, where 7′,5-dimethoxyschumannificine **16** being 50–100 times more active than the corresponding 7′-methoxy compound **17**.

These unusual novel compounds, though less active against HIV, are promising candidates as anti-HSV agents, showing low toxicities against Vero and also against MRC5 cells of human origin in vitro.

Acknowledgments

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References

Beutler, J.A., Cardellina II, J.H., McMahon, J.B., Boyd, M.R. and Cragg, G.M. (1992) Anti-HIV and cytotoxic alkaloids from *Buchenavia capitata*. J. Nat. Prod. 55, 207.

Fellows, L. (1992) Pharmaceuticals from traditional medicinal plants and others: future prospects, In: J.D. Coombes (Ed), New Drugs from Natural Sources, pp. 93–100, IBC Technical Services Ltd, London.

Houghton, P.J. and Yang, H. (1985) Novel chromone alkaloids from *Schumanniophyton magnificum*. Planta Med. 51, 23.

Houghton, P.J. and Yang, H. (1987) Further chromone alkaloids from *Schumanniophyton magnificum*. Planta Med. 53, 262.

Houghton, P.J. (1987) Revision of structures of some *Schumanniophyton* alkaloids. Planta Med. 53, 264.

Houghton, P.J. (1988) New chromone alkaloids from the stem bark of *Schumanniophyton magnificum*. Planta Med. 54, 239.

Houghton, P.J. and Woldemariam, T.Z. (1994) Use of a porous graphitic carbon column to separate isomers of piperidino-type chromone alkaloids. Phytochem. Anal., in press.

Houghton, P.J., Osibogun, I.M., Woldemariam, T.Z. and Jones, K. (1994) Heteronuclear NMR studies of the chromone alkaloids and revision of the structure of some piperidino-chromone alkaloids. Planta Med., in press.

Lednicer, D. and Snader, K.M. (1991) Plants and Other Organisms as a Source of Anti-Human Immunodeficieny Virus (HIV) Drugs, In: H. Wagner and N.R. Farnsworth (Eds), Economic and Medicinal Plant Research **5**, pp. 1–20, Academic Press, London.

Mahmood, N., Pizza, C., Aquino, R., De Tommasi, N., Piacente, S., Colman, S., Burke, A. and Hay, A.J. (1993a) Inhibition of HIV infection by flavanoids. Antiviral Res. 22, 189–199.

Mahmood, N., Moore, P.S., De Tommasi, N., De Simone, F., Colman, S., Hay, A.J. and Pizza, C. (1993b) Inhibition of HIV infection by caffeoylquinic acid derivatives. Antiviral Chem. Chemother. 4, 235–240.

Mahmood, N. and Hay, A.J. (1992) An ELISA utilising immobilised snowdrop lectin GNA for the detection of envelope glycoproteins of HIV and SIV J. Immunol. Methods 151, 9–13.

Pauwels, R., Balzarini, J., Baba, M., Snoeck, R., Schols, D., Herdewijn, P., Desmyter, J. and De Clercq, E. (1988) Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. J. Virol. Methods 20, 309–321.

Shearer, A. (1992) Spectrophotometric assay of HIV proteinase using a chromophoric synthetic peptide. Medical Research Council ADP Reagents Catalogue, p. 94.