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Synthesis and anti-picornavirus activity of homo-isoflavonoids

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Summary

Substituted homo-isoflavonoids were synthesized in order to study their *in vitro* anti-picornavirus activity. The maximum non-toxic concentration of the compounds for susceptible cells (HeLa) was determined, and the ability of non-cytotoxic concentrations to interfere with plaque formation by human rhinovirus (HRV) 1B and 14 and poliovirus (PV) 2 was examined. All the tested compounds were weakly effective against PV-2, while they exhibited a variable degree of activity against HRV-1B and -14 infection. Serotype 1B was much more sensitive than 14 to the action of the compounds, and the presence of one or more chlorine atoms increased the antiviral effect in all homo-isoflavonoids tested, confirming the positive influence of this substituent on activity.

Keywords: Anti-rhinovirus activity; anti-poliovirus activity; homo-isoflavonoids.

Introduction

Homo-isoflavonoids constitute a small class of natural products whose first member was isolated in 1967 from bulbs of *Eucomis bicolour* Bak (Bohler & Tamm, 1967). Subsequently, a number of homo-isoflavonoids were found to be prevalent in several genera of *Liliaceae* and *Caesalpinioideae* (Leguminosae) (Namikoshi *et al.*, 1987). These compounds belong to three structural types: (*E*)- and (*Z*)-3-benzylidenechroman-4-ones (**1**), 3-benzyl-4-chromones (**2**) and 3-benzylchroman-4-ones (**3**) (Fig. 1). Although exclusively (*Z*)-3-benzylidenechroman-4-ones occur in living plants, (*Z*),(*E*)-isomerization takes place very readily and therefore only the (*E*)-isomers are usually detected. During the last few decades, homo-isoflavonoids have been studied for their chemical and biochemical synthesis and medicinal application. Natural and synthetic homo-isoflavonoids have been recognized to possess various biological properties such as anti-fungal (Ravisé & Kirkiacharian, 1978; Al Nakib *et al.*, 1990), anti-mutagenic (Wall *et al.*, 1989), anti-inflammatory, analgesic (Darmanaden *et al.*, 1984; Della Loggia *et al.*, 1989), angioprotective, anti-allergic, anti-histaminic (Kirkiacharian *et al.*, 1989) and phosphodiesterase inhibitor (Amschler *et al.*, 1996) activities. However, no data are available on the antiviral activity of this group of flavonoids, although their structure is closely related to known anti-picornavirus compounds such as chalcone Ro 09-0410 and flavone Ro 09-0179 (De Meyer *et al.*, 1991; Ishitsuka *et al.*, 1982). (Fig. 2).

Therefore, because of their structural similarity to the flavanoids previously studied by us (Burali *et al.*, 1987; Conti *et al.*, 1988, 1990a, b, 1992; Desideri *et al.*, 1990, 1992, 1995; Quaglia *et al.*, 1991, 1992, 1993; Superti *et al.*, 1989; Genovese *et al.*, 1995), we became interested in synthesizing a series of substituted homo-isoflavones (**1**, **2**, **3a–g**) (Fig. 3) and evaluating them for anti-picornavirus activity. The antiviral potency of these synthetic compounds was evaluated against human rhinovirus (HRV) 1B and 14 and poliovirus (PV) 2 by a plaque reduction assay in HeLa cell cultures.

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Figure 1. Structures of natural homo-isoflavonoids

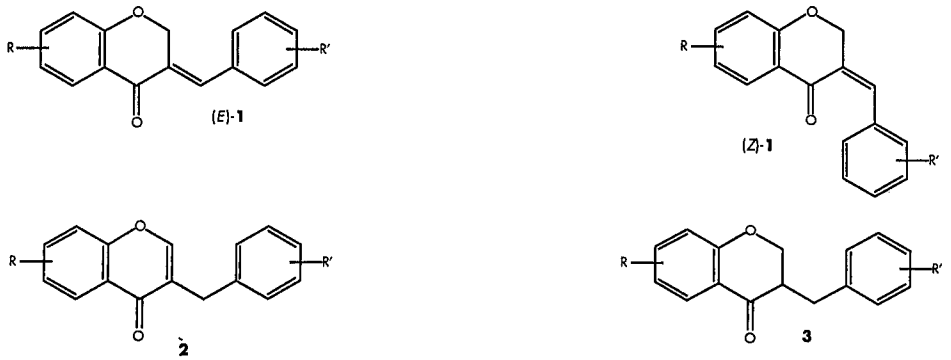


Figure 2. Antipicornavirus compounds Ro 09-0410 and Ro 09-0179

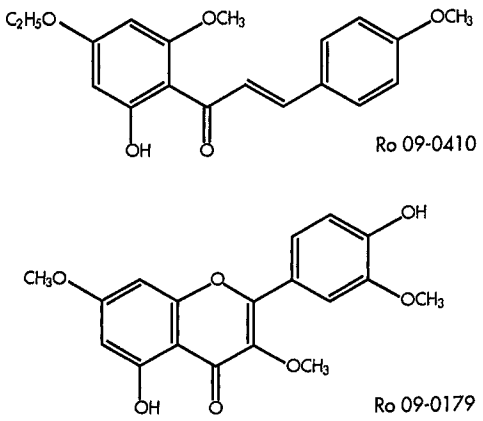
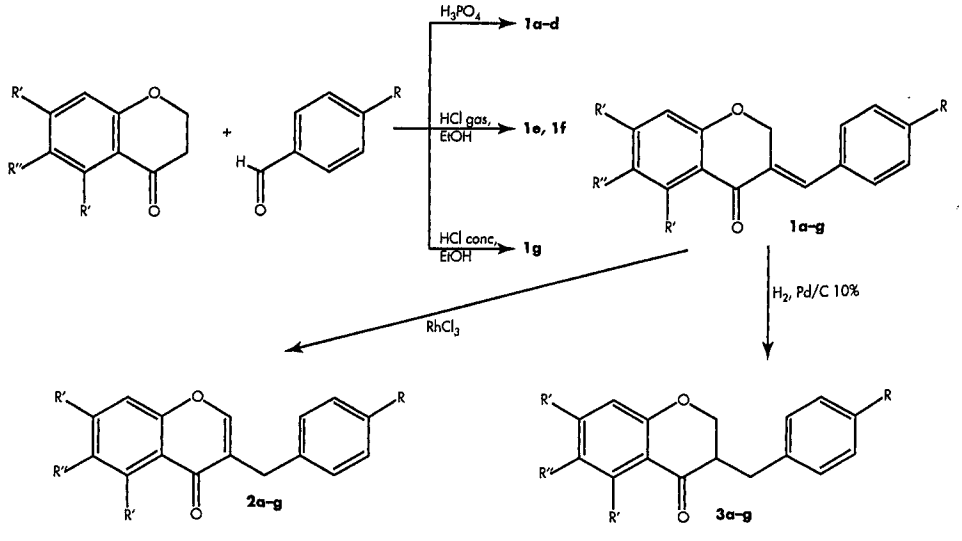


Figure 3. Synthesis of *(E)*-3-benzylidenchroman-4-ones (**1 a–g**), 3-benzyl-4-chromones (**2 a–g**) and 3-benzylchroman-4-ones (**3 a–g**)



1, 2, 3	R	R′	R″
a	H	H	H
b	OH	H	H
c	OCH_3	H	H
d	Cl	H	H
e	Cl	H	Cl
f	Cl	Cl	H
g	OCH_3	OCH_3	H

Table 1. IR and ^1H NMR data of *(E)*-3-benzylidenechroman-4-ones (**1a–g**).

Comp.	IR (KBr) cm^{-1}	^1H NMR (solvent) δ
1a	1660	($CDCl_3$) 8.04 (dd, 1H, H_5, J_{5-6} 7.8 Hz); 7.87 (bs, 1H, H_9); 7.50–7.39 (m, 1H, H_7, J_{5-7} 1.7 Hz); 7.40–6.96 (m, 7H, H_6, H_8, $H_{2'-6'}$); 5.33 (d, 2H, H_2, J_{all} 1.7 Hz).
1b	3100–3300, 1650	(acetone d_6) 9.00 (bs, 1H, OH); 7.93 (dd, 1H, H_5, J_{5-6} 7.9 Hz, J_{5-7} 1.6 Hz); 7.75 (bs, 1H, H_9); 7.61–7.50 (m, 1H, H_7, J_{6-7} 7.2 Hz, J_{5-7} 1.6 Hz, J_{7-8} 8.4 Hz); 7.37 (d, 2H, $H_{2'}$, $H_{6'}$, $J_{2'-3'}$ 8.6 Hz); 7.15–6.94 (m, 4H, H_6, H_8, $H_{3'}$, $H_{5'}$); 5.46 (d, 2H, H_2, J_{all} 1.7 Hz).
1c	1660	($CDCl_3$) 8.01(dd, 1H, H_5, J_{5-6} 7.8 Hz, J_{5-7} 1.7 Hz); 7.83 (bs, 1H, H_9); 7.52–7.40 (m, 1H, H_7); 7.28 (d, 2H, $H_{2'}$, $H_{6'}$, $J_{2'-3'}$ 8.7 Hz); 7.11–6.90 (m, 4H, H_6, H_8, $H_{3'}$, $H_{5'}$); 5.37 (d, 2H, H_2, J_{all} 1.9 Hz); 3.85 (s, 3H, OCH_3).
1d	1660	($CDCl_3$) 8.02 (dd, 1H, H_5, J_{5-6} 7.8 Hz, J_{5-7} 1.4 Hz); 7.80 (bs, 1H, H_9); 7.56–7.38 (m, 3H, H_7, $H_{2'}$, $H_{6'}$); 7.24 (d, 2H, $H_{3'}$, $H_{5'}$, $J_{2'-3'}$ 8.5 Hz); 7.12–6.93 (m, 2H, H_6, H_8); 5.31 (d, 2H, $2H_2$, J_{all} 1.5 Hz).
1e	1660	($CDCl_3$) 7.97 (d, 1H, H_5, J_{5-7} 2.6 Hz); 7.82 (bs, 1H, H_9); 7.48–7.39 (m, 3H, H_7, $H_{2'}$, $H_{6'}$); 7.24(d, 2H, $H_{3'}$, $H_{5'}$, $J_{2'-3'}$ 8.5 Hz); 6.93 (d, 1H, H_8, J_{7-8} 8.8 Hz); 5.31 (d, 2H, $2H_2$, J_{all} 1.9 Hz).
1f	1660	($CDCl_3$) 7.82 (bs, 1H, H_9); 7.42 (d, 2H, $H_{2'}$, $H_{6'}$, $J_{2'-3'}$ 8.5 Hz); 7.21 (d, 2H, $H_{3'}$, $H_{5'}$, $J_{2'-3'}$ 8.5 Hz); 7.12 (d, H_6, J_{6-8} 2.0 Hz); 6.95 (d, H_8, J_{6-8} 2.0 Hz); 5.25 (d, 2H, $2H_2$, J_{all} 1.8 Hz).
1g	1665	($CDCl_3$) 7.77 (bs, 1H, H_9); 7.24 (d, 2H, $H_{2'}$, $H_{6'}$, $J_{2'-3'}$ 8.8Hz); 6.95 (d, 2H, $H_{3'}$, $H_{5'}$, $J_{2'-3'}$ 8.8 Hz); 6.12 (d, 1H, H_6, J_{6-8} 2.3 Hz); 6.07 (d, 1H, H_8, J_{6-8} 2.3 Hz); 5.24 (d, 2H, H_2, J_{all} 1.8 Hz); 3.91 (s, 3H, OCH_3); 3.85 (s, 3H, OCH_3); 3.83 (s, 3H, OCH_3).

Table 2. IR and ^1H NMR data of 3-benzyl-4-chromones (**2a–g**)

Comp.	IR (KBr) cm^{-1}	^1H NMR (solvent) δ
2a	1640	($CDCl_3$) 8.24 (dd, 1H, H_5, J_{5-6} 8.0 Hz, J_{5-7} 1.8 Hz); 7.70–7.58 (m, 2H, H_2, H_7); 7.50–7.20 (m, 7H, H_6, H_8, $H_{2'-6'}$); 3.82 (s, 2H, CH_2).
2b	3100–3400, 1630	(acetone d_6) 8.88 (bs, 1H, OH); 8.12 (dd, 1H, H_5, J_{5-6} 8.0 Hz, J_{5-7} 1.5 Hz); 8.01(bs, 1H, H_2); 7.80–7.69 (m, 1H, H_7, J_{6-7} 7.1 Hz, J_{7-8} 8.4 Hz, J_{5-7} 1.5 Hz); 7.56–7.47 (m, 2H, H_6, H_8); 7.18 (d, 2H, $H_{2'}$, $H_{6'}$, $J_{2'-3'}$ 8.4 Hz); 6.72 (d, 2H, $H_{3'}$, $H_{5'}$, $J_{2'-3'}$ 8.4 Hz); 3.68 (s, 2H, CH_2).
2c	1635	($CDCl_3$) 8.23 (dd, 1H, H_5, J_{5-6} 8.0 Hz, J_{5-7} 1.8 Hz); 7.68–7.55 (m, 2H, H_2, H_7); 7.43–7.31 (m, 2H, H_6, H_8); 7.22 (d, 2H, $H_{2'}$, $H_{6'}$, $J_{2'-3'}$ 8.7 Hz); 6.85 (d, 2H, $H_{3'}$, $H_{5'}$, $J_{2'-3'}$ 8.7 Hz); 3.79 (s, 3H, OCH_3); 3.76 (s, 2H, CH_2).
2d	1630	($CDCl_3$) 8.21 (dd, 1H, H_5, J_{5-6} 8.0 Hz; J_{5-7} 1.6 Hz); 7.70–7.58 (m, 2H, H_2, H7); 7.46–7.31 (m, 2H, H_6, H_8); 7.30–7.19 (m, 4H, $H_{2'}$, $H_{3'}$, $H_{5'}$, $H_{6'}$); 3.77 (s, 2H, CH_2).
2e	1630	($CDCl_3$) 8.17 (d, 1H, H_5, J_{5-7} 2.6 Hz); 7.64 (bs, 1H, H_2); 7.60 (dd, 1H, H_7, J_{7-8} 8.9 Hz, J_{5-7} 0.6 Hz); 7.38 (d, 1H, H_8, J_{7-8} 8.9 Hz); 7.29–7.18 (m, 4H, $H_{2'}$, $H_{3'}$, $H_{5'}$, $H_{6'}$); 3.76 (s, 2H, CH_2).
2f	1630	($CDCl_3$) 7.50 (bs, 1H, H_2); 7.35 (d, 1H, H_6, J_{6-8} 2.0 Hz); 7.32 (d, 1H, H_8, J_{6-8} 2.0 Hz); 7.27 (d, 2H, $H_{2'}$, $H_{6'}$, $J_{2'-3'}$ 8.5 Hz); 7.20 (d, 2H, $H_3H_{5'}$, $J_{2'-3'}$ 8.5 Hz); 3.71 (s, 2H, CH_2).
2g	1640	($CDCl_3$) 7.35 (bs, 1H, H_2); 7.21 (d, 2H, $H_{2'}$, $H_{6'}$, $J_{2'-3'}$ 8.4 Hz); 6.83 (d, 2H, $H_{3'}$, $H_{5'}$, $J_{2'-3'}$ 8.4 Hz); 6.37 (d, 1H, H_6, J_{6-8} 2.2 Hz); 6.33 (d, 1H, H_8, J_{6-8} 2.2 Hz); 3.92 (s, 3H, OCH_3); 3.85 (s, 3H, OCH_3); 3.78 (s, 3H, OCH_3); 3.68 (s, 2H, CH_2).

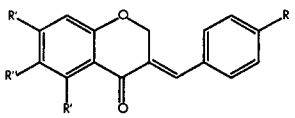
Table 3. IR and ^{1}H NMR data of 3-benzylchroman-4-ones (**3a–g**)

Comp.	IR (KBr) cm^{-1}	^{1}H NMR (solvent) δ
3a	1680	($CDCl_3$): 7.95 (dd, 1H, H_5, J_{5-6} 7.8 Hz, J_{5-7} 1.7 Hz); 7.54–7.41 (m, 1H, H_7, J_{6-7} 7.3 Hz, J_{7-8} 8.3 Hz, J_{5-7} 1.7 Hz); 7.40–7.20 (m, 5H, $H_{2'-6'}$); 7.09–6.96 (m, 2H, H_6, H_8); 4.38 (dd, 1H, H_2, J_{gem} 11.5 Hz, J_{2-3} 4.4 Hz); 4.18 (dd, 1H, H_2, J_{gem} 11.5 Hz, J_{2-3} 8.2 Hz); 3.30 (dd, 1H, H_9, J_{gem} 13.6 Hz, J_{3-9} 4.1 Hz); 3.10–2.85 (m, 1H, H_3); 2.71 (dd, 1H, H_9, J_{gem} 13.6 Hz, J_{3-9} 10.4 Hz).
3b	3100–3400, 1665	(acetone d_6): 8.20 (bs, 1H, OH); 7.85 (dd, 1H, H_5, J_{5-6} 7.8 Hz, J_{5-7} 1.7 Hz); 7.60–7.48 (m, 1H, H_7, J_{6-7} 7.2 Hz, J_{7-8} 8.4 Hz, J_{5-7} 1.7 Hz); 7.17–6.93 (m, 4H, $H_{2'}$, $H_{6'}$, H_6, H_8); 6.80 (d, 2H, $H_{3'}$, $H_{5'}$, $J_{2'-3'}$ 8.5 Hz); 4,43 (dd, 1H, H_2, J_{gem} 11.5 Hz, J_{2-3} 4.6 Hz); 4.21 (dd, 1H, H_2, J_{gem} 11.5 Hz, J_{2-3} 9.1 Hz); 3.16 (dd, 1H, H_9, J_{gem} 13.7 Hz, J_{3-9} 4.6 Hz); 3.05–2.83 (m, 1H, H_3); 2.05 (dd, 1H, H_9, J_{gem} 13.7 Hz, J_{3-9} 9.6 Hz).
3c	1675	($CDCl_3$): 7.92 (dd, 1H, H_5, J_{5-6} 7.8 Hz, J_{5-7} 1.7 Hz); 7.52–7.42 (m, 1H, H_7, J_{6-7} 7.3 Hz, J_{7-8} 8.3 Hz); 7.16 (d, 2H, $H_{2'}$, $H_{6'}$, $J_{2'-3'}$ 8.5 Hz); 7.07–6.91 (m, 2H, H_6, H_8); 6.86 (d, 2H, $H_{3'}$, $H_{5'}$, $J_{2'-3'}$ 8.5 Hz); 4.37 (dd, 1H, H_2, J_{gem} 11.5 Hz, J_{2-3} 4.3 Hz); 4.17 (dd, 1H, H_2, J_{gem} 11.5 Hz, J_{2-3} 7.9 Hz); 3.79 (s, 3H, OCH_3); 3.20 (dd, 1H, H_9, J_{gem} 13.6 Hz, J_{3-9} 4.2 Hz); 2.96–2.78 (m, 1H, H_3); 2.68 (dd, 1H, H_9, J_{gem} 13.6 Hz, J_{3-9} 10.2 Hz).
3d	1670	($CDCl_3$): 7.91 (dd, 1H, H_5, J_{5-6} 7.9 Hz, J_{5-7} 1.8 Hz); 7.55–7.43 (m, 1H, H_7, J_{6-7} 7.2 Hz, J_{7-8} 8.4 Hz); 7.29 (d, 2H, $H_{2'}$, $H_{6'}$, $J_{2'-3'}$ 8.4 Hz); 7.17 (d, 2H, $H_{3'}$, $H_{5'}$, $J_{2'-3'}$ 8.4 Hz); 7.09–6.92 (m, 2H, H_6, H_8); 4.37 (dd, 1H, H_2, J_{gem} 11.5 Hz, J_{2-3} 4.3 Hz); 4.15 (dd, 1H, H_2, J_{gem} 11.5 Hz, J_{2-3} 8.1 Hz); 3.23 (dd, 1H, H_9, J_{gem} 13.5 Hz, J_{3-9} 4.3 Hz); 2.98–2.81 (m, 1H, H_3); 2.71 (dd, 1H, H_9, J_{gem} 13.5 Hz, J_{3-9} 9.9 Hz).
3e	1675	($CDCl_3$): 7.86 (d, 1H, H_5, J_{5-7} 2.7 Hz); 7.40 (dd, 1H, H_7, J_{5-7} 2.7 Hz, J_{7-8} 8.8 Hz); 7.28 (d, 2H, $H_{2'}$, $H_{6'}$, $J_{2'-3'}$ 8.5 Hz); 7.14 (d, 2H, $H_{3'}$, $H_{5'}$, $J_{2'-3'}$ 8.5 Hz); 4.37 (dd, 1H, H_2, J_{gem} 11.6 Hz, J_{2-3} 4.4 Hz); 4.24 (dd, 1H, H_2, J_{gem} 11.6 Hz, J_{2-3} 4.4 Hz); 3.22 (dd, 1H, H_9, J_{gem} 13.7 Hz, J_{3-9} 9.6 Hz); 2.97–2.81 (m, 1H, H_3); 2.70 (dd, 1H, H_9, J_{gem} 13.7 Hz, J_{3-9} 9.6 Hz).
3f	1680	($CDCl_3$): 7.29 (d, 2H, $H_{2'}$, $H_{6'}$, $J_{2'-3'}$ 8.5 Hz); 7.15 (d, 2H, $H_{3'}$, $H_{5'}$, $J_{2'-3'}$ 8.5 Hz); 7.06 (d, 1H, H_6, J_{6-8} 2.0 Hz); 6.92 (d, 1H, H_8, J_{6-8} 2.0 Hz); 4.37 (dd, 1H, H_2, J_{gem} 11.6 Hz, J_{2-3} 4.5 Hz); 4.15 (dd, 1H, H_2, J_{gem} 11.6 Hz, J_{2-3} 8.7 Hz); 3.24 (dd, 1H, H_9, J_{gem} 13.8 Hz, J_{3-9} 4.6 Hz); 3.00–2.84 (m, 1H, H_3); 2.65 (dd, 1H, H_9, J_{gem} 13.8 Hz, J_{3-9} 9.8 Hz).
3g	1660	($CDCl_3$): 7.16 (d, 2H, $H_{2'}$, $H_{6'}$, $J_{2'-3'}$ 8.5 Hz); 6.86 (d, 2H, $H_{3'}$, $H_{5'}$, $J_{2'-3'}$ 8.5 Hz); 6.10–6.02 (m, 2H, H_6, H_8, J_{6-8} 2.3 Hz); 6.06 (d, 1H, H_8, J_{6-8} 2.3 Hz); 4.27 (dd, 1H, H_2, J_{gem} 11.5 Hz, J_{2-3} 3.9 Hz); 4.08 (dd, 1H, H_2, J_{gem} 11.5 Hz, J_{2-3} 7.0Hz); 3.89 (s, 3H, OCH_3); 3.82 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 3.20 (dd, 1H, H_9, J_{gem} 12.4 Hz, J_{3-9} 3.7 Hz); 2.81–2.55 (m, 2H, H_3, H_9).

Materials and Experimental Procedures: Chemistry

Melting points were determined on a Büchi SMP-390 apparatus and are uncorrected. All compounds were routinely checked by thin-layer chromatography (TLC) and ^{1}H NMR. TLC was performed using 0.25 mm silica gel or aluminium oxide fluorescent coated plates (Kieselgel or aluminium oxide 60 F254; Merck). Compounds were visualized by UV light. Column chromatography was performed using silica gel (0.05–0.20 mm; Carlo Erba) or aluminium oxide (70–230 mesh; Merck). Elemental analyses of the new compounds were performed by the Microanalytical Laboratory (University of Padova, Italy) and were within ±0.4% of theoretical values. Syntheses of (*E*)-3-benzylidenechroman-4-ones **1a–d** and **1g**, 3-benzyl-4-chromones **2a–d** and **2g** and 3-benzylchroman-4-ones **3a**, **3c**, **3d** and **3g** have been reported previously (Al Nakib *et al.*, 1990; Bohler & Tamm, 1967; Chatterjea *et al.*, 1974; Farkas *et al.*, 1971; Kirkiacharian & Aloulou, 1984; Lévai & Schag, 1979; Mulvagh *et al.*, 1979; Pfeiffer *et al.*, 1949). ^{1}H NMR spectra were detected with a Varian EM-390 or a Bruker M200 instrument using Me_4Si as the internal standard. IR

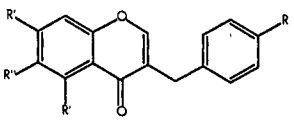
Table 4. Physical and chemical data of substituted *(E)*-3-benzylidenechroman-4-ones (**1a–g**)



Compound	R	R′	R″	Chromatog. system[a]	Crystn. solvent	M.p. (°C)	Yield (%)	Formula[b]
1a	H	H	H	A	EtOH	110–112[c]	66	$C_{16}H_{12}O_2$
1b	OH	H	H	–	EtOH	224–225[d]	48	$C_{16}H_{12}O_3$
1c	OCH_3	H	H	B	EtOH	131–133[e]	69	$C_{17}H_{14}O_3$
1d	Cl	H	H	–	EtOH	170–173[f]	54	$C_{16}H_{11}O_2Cl$
1e	Cl	H	Cl	C	AcOEt	202–205	40	$C_{16}H_{10}O_2Cl_2$
1f	Cl	Cl	H	–	AcOEt	162–165	41	$C_{16}H_9O_2Cl_3$
1g	OCH_3	OCH_3	H	D	EtOH	142–144[g]	38	$C_{19}H_{18}O_5$

[a] A, silica gel/AcOEt:petroleum ether 1:10; B, silica gel/AcOEt:petroleum ether 1:5; C, silica gel/AcOEt:petroleum ether 1:6; D, silica gel/AcOEt:petroleum ether 3:1.
[b] Elemental analyses were within ±0.4% of the theoretical values.
[c] 111–112°C, Lévai & Schag (1979).
[d] 224–226°C, Lévai & Schag (1979).
[e] 131–132°C, Lévai & Schag (1979).
[f] 172–173°C, Lévai & Schag (1979).
[g] 141–144°C, Bohler & Tamm (1967).

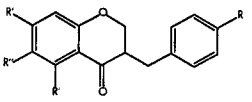
Table 5. Physical and chemical data of substituted 3-benzyl-4-chromones (**2a–g**)



Compound	R	R′	R″	Heating time (h)	Chromatog. system[a]	Crystn. solvent	M.p. (°C)	Yield (%)	Formula[b]
2a	H	H	H	52	A	EtOH	111–112[c]	55	$C_{16}H_{12}O_2$
2b	OH	H	H	11	B	EtOH	194–195[d]	47	$C_{16}H_{12}O_3$
2c	OCH_3	H	H	12	C	EtOH	103–105[e]	35	$C_{17}H_{14}O_3$
2d	Cl	H	H	7.5	A	EtOH	139–141[f]	22	$C_{16}H_{11}O_2Cl$
2e	Cl	H	Cl	24	D	EtOH	158–160	60	$C_{16}H_{10}O_2Cl_2$
2f	Cl	Cl	H	18	E	EtOH	107–108	38	$C_{16}H_9O_2Cl_3$
2g	OCH_3	OCH_3	H	11	–	MeOH	114–115[g]	30	$C_{19}H_{18}O_5$

[a] A, silica gel/AcOEt:petroleum ether 1:15; B, aluminium oxide/EtOH:diethyl ether 1:15; C, silica gel/CH_2Cl_2; D, silica gel/AcOEt:petroleum ether 1:6; E, silica gel/AcOEt:petroleum ether 1:10.
[b] Elemental analyses were within ±0.4% of theoretical values.
[c] 112–113°C, Chatterjea *et al.* (1974).
[d] 194–196°C, Mulvagh *et al.* (1979).
[e] 94–95°C, Chatterjea *et al.* (1974).
[f] 140°C, Al Nakib *et al.* (1990).
[g] 115–117°C, Farkas *et al.* (1971).

Table 6. Physical and chemical data of substituted 3-benzylchroman-4-ones (**3a–g**)



Compound	R	R′	R″	Chromatog. system[a]	Crystn. solvent	M.p. (°C)	Yield (%)	Formula[b]
3a	H	H	H	A	n-Hexane	63–65[c]	50	$C_{16}H_{14}O_2$
3b	OH	H	H	B	Benzene	137–138	48	$C_{16}H_{14}O_3$
3c	OCH_3	H	H	C	n-Hexane	96–99[d]	60	$C_{17}H_{16}O_3$
3d	Cl	H	H	C	n-Hexane	89–92[e]	77	$C_{16}H_{13}O_2Cl$
3e	Cl	H	Cl	–	n-Hexane	102–103	77	$C_{16}H_{12}O_2Cl_2$
3f	Cl	Cl	H	–	n-Hexane	101–102	80	$C_{16}H_{11}O_2Cl_3$
3g	OCH_3	OCH_3	H	–	AcOEt/ petroleum ether	83–85[f]	75	$C_{19}H_{20}O_5$

[a] A, silica gel/AcOEt:petroleum ether 1:10; B, silica gel/AcOEt:petroleum ether 1:2; C, silica gel/CH_2Cl_2.
[b] Elemental analyses were within ±0.4% of theoretical values.
[c] 58°C, Pfeiffer *et al.* (1949).
[d] 99°C, Pfeiffer *et al.* (1949).
[e] 84°C, Kirkiacharian & Aloulou (1984).
[f] 82°C, Farkas *et al.* (1971).

spectra were recorded on a Perkin-Elmer 1310 spectrophotometer. ^1H NMR and IR data of all synthesized compounds are shown in Tables 1–3.

(E)-3-benzylidenechroman-4-ones (1a–d)

A mixture of chroman-4-one (0.01 mol), substituted benzaldehyde (0.01 mol) and 85% phosphoric acid (20.0 mL) was heated at 80°C for 6 h. After cooling the mixture was diluted with water and the precipitate was filtered off, washed with water and chromatographed (**1a, 1c**) or crystallized (**1b, 1d**). Physical and chemical data are reported in Tables 1 and 4.

(E)-3-benzylidenechroman-4-ones (1e, 1f)

A mixture of substituted chroman-4-one (Canalini *et al.*, 1967; Merchant & Upasani, 1980) (0.01 mol) and 4-chlorobenzaldehyde (0.011 mol) in dry ethanol saturated with hydrochloric acid (40.0 mL) was stirred at room temperature for 24 h. The mixture was diluted with ice-water and the precipitate was filtered off, washed with water and chromatographed (**1e**) or crystallized (**1f**). Physical and chemical data are reported in Tables 1 and 4.

(E)-5,7-dimethoxy-3-(4-methoxybenzylidene) chroman-4-one (1g)

A solution of 5,7-dimethoxychroman-4-one (Loudon & Razdan, 1954) (0.01 mol) and 4-methoxybenzaldehyde (0.013 mol) in dry ethanol (80.0 mL) and concentrated HCl (1.6 mL) was heated at 80°C for 12 h while stirring. After cooling, ethanol was evaporated under reduced pressure. The residue was taken up with ethyl acetate and the solution was washed with water and dried over Na_2SO_4. After evaporation of the solvent, the crude compound was chromatographed. Physical and chemical data are reported in Tables 1 and 4.

3-Benzyl-4-chromones (2a–g)

To a solution of (*E*)-3-benzylidenechroman-4-ones (**1a–g**) (0.01 mol) in chloroform (24 mL for **1a–e, 1g** and 70 mL for **1f**) and ethanol (24 mL for **1a–e, 1g** and 70 mL for **1f**), $RhCl_3$ (0.5 mmol) and water (1.8 mL for **1a–e** and 5.4 mL for **1f**) were added. The mixture was refluxed under stirring for the time reported in Table 5. After cooling, the solution was poured into water and extracted with chloroform. The organic layer was dried over Na_2SO_4, the solvent was evaporated under reduced pressure and the crude compound was chromatographed (**2a–e**) or crystallized (**2f, 2g**). Physical and chemical data are reported in Tables 2 and 5.

3-Benzylchroman-4-ones (3a–g)

A solution of (*E*)-3-benzylidenechroman-4-ones (**1a–g**) (0.001 mol) in ethyl acetate (33 mL for **1a–d, 1g**) or THF (36 mL for **1e**, 14 mL for **1f**) was hydrogenated at room temperature over 10% Pd/C (170 mg), until absorption of the theoretical hydrogen amount. After filtration of the catalyst and evaporation of the solvent, the residue was chromatographed (**3a–d**) or crystallized (**3e–g**). Physical and chemical data are reported in Tables 3 and 6.

Materials and Experimental Procedures: Virology

Cells

HeLa (Ohio) cells were routinely grown at 37°C in 72 cm^2 tissue culture flasks using Eagle's MEM supplemented with 100 $\mu g\ mL^{-1}$ of streptomycin and 100 U mL^{-1} of penicillin G. Eight percent heat-inactivated fetal calf serum (FCS) was added for cell growth (growth medium); the concentration was reduced to 2% for cell maintenance (maintenance medium).

Compounds

Compounds were initially dissolved in ethanol (1 or 0.1 mg mL^{-1}) and further diluted in tissue culture medium shortly before use.

Virus

HRV-1B and -14, and PV-2 (P712) were grown in HeLa (Ohio) cells at 33°C and 37°C, respectively. Virus stocks were prepared by inoculating cell monolayers at low m.o.i. When the CPE involved most of the cell monolayer, the cultures were freeze–thawed three times and the clarified supernatants titrated by plaque assay, essentially as described by Fiala & Kenny (1966). The virus was stored in aliquots at −80°C until use.

Compound cytotoxicity

Exponentially growing HeLa cells in 24-well plates were cultured for 3 days at 37°C in growth medium in the presence or absence of the two-fold serially diluted compounds to be tested. Three wells were utilized for each drug concentration. On the third day of incubation, treated and mock-treated cells were trypsinized and counted in a haemocytometer and the viability of cells was checked by the trypan blue exclusion method. Cytotoxicity was also scored microscopically as morphological alterations (i.e. swelling, granularity, rounding up, shrinking and detachment). In antiviral tests, compounds were utilized starting from the highest concentration which did not produce any modification of morphology and viability in 100% of the cells in all the wells examined (maximum non-toxic concentration).

Determination of the 50% inhibitory concentration (IC_{50})

The IC_{50} was determined as described previously (Desideri *et al.*, 1992). Briefly, confluent monolayers of HeLa cells in 6-well plates were infected with a virus suspension producing approximately 100 plaques $well^{-1}$. After 1 h of incubation at 37°C (PV) or 33°C (HRV), the virus suspension was removed and the cells were overlaid with medium for plaques, in the presence or absence of fourfold dilutions of drugs. After 2 days of incubation at 37°C (PV) or 3 days at 33°C (HRV), the cells were stained with a neutral red solution at 0.2 mg mL^{-1} in PBS pH 7.4 and the plaques were counted. The IC_{50} was expressed as the concentration of drug reducing the plaque number by 50% as compared to mock-treated controls. It was calculated from a dose–response line obtained by plotting the percentage of plaque reduction, with respect to the control plaque count, versus the $\log_{10}$ of compound dose. Triplicate wells were utilized for each drug concentration tested.

Results

Chemistry

(*E*)-3-Benzylidenechroman-4-ones (**1a–g**), 3-benzyl-4-chromones (**2a–g**) and 3-benzylchroman-4-ones (**3a–g**) utilized in the present study were prepared using previously described methods. In general, (*E*)-3-benzylidenechroman-4-ones were obtained either by acid- or base-catalysed condensation of chroman-4-ones with aromatic aldehydes (Blaskò & Cordell 1988; Chatterjea *et al.*, 1979; Lévai *et al.*, 1981; Lévai & Szabò, 1992; Namikoshi *et al.*, 1987). We easily prepared (*E*)-3-benzylidenechroman-4-ones (**1a–d**) using a phosphoric acid catalyst; however, this method could not be conveniently used for the synthesis of polychloro derivatives (**1e, 1f**) and for (*E*)-5,7-dimethoxy-3-(4-methoxybenzylidene)chroman-4-one (**1g**). Therefore these latter compounds were obtained using concentrated or dry hydrogen chloride in ethyl alcohol (Fig. 3). The ^1H NMR spectra show that only the (*E*)-isomer was obtained in each case. Owing to the anisotropic effect of the carbonyl group, the vinyl C-9 proton appears downfield near 7.8 p.p.m., whereas for the (*Z*)-isomer this signal would appear around 6.9 p.p.m. Also, the C-2 protons of the (*Z*)-isomer would be shielded with respect to those of the *trans*-isomer, owing to the greater distance from the side-chain phenyl group.

The synthesis of 3-benzyl-4-chromones (**2a–g**) was achieved by exocyclic–endocyclic migration of the double bond of *(E)*-3-benzylidenechroman-4-ones (**1a–g**) using rhodium trichloride as a catalyst (Andrieux *et al.*, 1977) (Fig. 3). The products of isomerization were identified by their IR and ^1H NMR spectra. The characteristic C=O band appeared between 1665 and 1650 cm^{-1} for *(E)*-3-benzylidenechroman-4-ones (**1a–g**) and between 1640 and 1630 cm^{-1} for 3-benzyl-4-chromones (**2a–g**). ^1H NMR data showed the upfield shift the of CH_2 signal of 3-benzyl-4-chromones (**2a–g**) if compared to that of isomers **1a–g** and the disappearance of the allyl coupling (J 1.5–1.9 Hz) between CH_2 and vinyl protons.

Reduction of the exocyclic double bond was obtained by hydrogenation at room temperature using palladium as the catalyst, until absorption of the theoretical hydrogen amount, to avoid the reduction of carbonyl group (Fig. 3) (Chatterjea *et al.*, 1979). The endo isomer was observed as

Table 7. Cytotoxicity and anti-picornavirus activity of 3-benzylidenechroman-4-ones (**1a–g**), 3-benzyl-4-chromones (**2a–g**) and 3-benzylchroman-4-ones (**3a–g**).

Compound	R	R′	R″	Maximum non-toxic concentration (μM)		IC_{50} (μM)[a]		
				Morphology	Growth	HRV-1B	HRV-14	PV-2
1a	H	H	H	6.25	6.25	>6.25 (32)	Inactive	>6.25 (26)
1b	OH	H	H	6.25	6.25	Inactive	Inactive	>6.25 (12)
1c	OCH_3	H	H	6.25	6.25	>6.25 (21)	Inactive	>6.25 (19)
1d	Cl	H	H	6.25	6.25	3.40	Inactive	>6.25 (22)
1e	Cl	H	Cl	6.25	0.39	0.28	>6.25 (29)	>6.25 (27)
1f	Cl	Cl	H	6.25	0.39	0.36	>6.25 (12)	>6.25 (15)
1g	OCH_3	OCH_3	H	6.25	1.56	>6.25 (41)	Inactive	4.29
2a	H	H	H	25.00	50.00	4.64	6.20	>25 (25)
2b	OH	H	H	25.00	50.00	>25.00 (22)	16.20	>25 (21)
2c	OCH_3	H	H	25.00	50.00	7.86[b]	15.37	>25 (29)
2d	Cl	H	H	25.00	50.00	0.20	>25.00 (46)	>25.00 (8)
2e	Cl	H	Cl	25.00	25.00	3.24	>25.00 (26)	>25.00 (7)
2f	Cl	Cl	H	25.00	25.00	3.90[b]	4.27	16.89
2g	OCH_3	OCH_3	H	6.25	1.56	5.93[b]	Inactive	>6.25 (38)
3a	H	H	H	>50.00	6.25	9.38	>50 (10)	18.75
3b	OH	H	H	6.25	1.56	Inactive	Inactive	>6.25 (12)
3c	OCH_3	H	H	6.25	6.25	5.98	Inactive	>6.25 (25)
3d	Cl	H	H	>50.00	6.25	1.23	32.64	9.21
3e	Cl	H	Cl	25.00	25.00	2.75[b]	13.54	18.56
3f	Cl	Cl	H	25.00	< 6.25	3.70	14.22	20.03
3g	OCH_3	OCH_3	H	6.25	25.00	>6.25 (38)[b]	>6.25 (15)	>6.25 (41)[b]
BW683C				>50.00	25.00	0.025	Inactive	Inactive

[a] The IC_{50} value was the concentration reducing the plaque number by 50% and was calculated by plotting the $\log_{10}$ of drug concentration versus the percentage plaque reduction. When the IC_{50} value was higher than the maximum non-toxic dose the percentage inhibition of plaque number, obtained at this concentration, is shown in parentheses.

[b] Compound producing a reduction (about 50%) in the mean viral plaque size at the highest dose tested, beside an effect on plaque number.

a byproduct of the reaction.

Antiviral activity

In the first set of experiments, the maximum non-toxic concentration of compounds, in 100% of the cells, was determined. Serial twofold dilutions of compounds in growth medium were applied to HeLa cells and, after 3 days of incubation at 37°C, cell morphology and growth were examined. Data are reported in Table 7. In general, *(E)*-3-benzylidenechroman-4-ones (**1a–g**) appeared more toxic than 3-benzyl-4-chromones (**2a–g**), while the results for 3-benzylchroman-4-ones (**3a–g**) were found to be variable. For compounds **3a** and **3d**, the maximum non-toxic concentration was found to be higher than the saturation concentration in cell culture medium (50 μM).

The antiviral potency of *(E)*-3-benzylidenechroman-4-ones (**1a–g**), 3-benzyl-4-chromones (**2a–g**) and 3-benzylchroman-4-ones (**3a–g**) was evaluated in HeLa cell cultures infected with HRV-1B and -14 and PV-2, selected as representative members of the rhinovirus and enterovirus genera. 4′,6-Dichloroflavan (BW683C) (Bauer *et al.*, 1981) was included as a control. The inhibitory effect of homo-isoflavonoids on virus replication was monitored by the reduction of plaque number and size. The 50% effective concentrations on plaque formation (IC_{50}), obtained from dose–response plots, are shown in Table 7. When the IC_{50} value was higher than the maximum non-toxic concentration, the percentage inhibition obtained at this dose is reported in parentheses.

Compounds **2c**, **2f**, **2g**, **3e** and **3g** caused a reduction (about 50%) in viral plaque size of HRV-1B at the highest dose tested, in addition to an effect on plaque number, suggesting a slowing down in the kinetics of viral replication; none of the other compounds altered the mean viral plaque size.

Substituted homo-isoflavonoids were generally poorly active towards PV-2 infection and the few active analogues reduced the viral plaque number by 50% only at very high doses.*(E)*-5,7-dimethoxy-3-(4-methoxybenzylidene) chroman-4-one (**1g**) was the most effective compound

against this virus even though its therapeutic index was very low.

The rhinovirus serotypes tested exhibited a different sensitivity to the compounds. HRV-1B, belonging to the minor group of rhinoviruses according to membrane receptor recognition, was much more susceptible than HRV-14, a representative of the major group. With the exception of 3-(4-hydroxybenzyl)-4-chromone (**2b**), which was effective at high concentration on HRV-14 only, all the homo-isoflavonoids active on HRV-14 (**2a, 2c, 2f, 3d–f**) were also effective against HRV-1B and not vice versa.

Among the (*E*)-3-benzylidenechroman-4-ones synthesized, the chloro-substituted compounds (**1d–f**) were highly active only against HRV-1B infection, while all the 3-benzyl-4-chromones tested were efficacious towards serotype 1B, with the exception of 3-(4-hydroxybenzyl)-4-chromone (**2b**). 3-(4-Chlorobenzyl)-4-chromone (**2d**) was the most effective analogue in this study towards HRV-1B, with an IC_{50} of 0.20 μM and a high therapeutic index.

Also, the majority of 3-benzylchroman-4-ones interfered with HRV-1B infection of HeLa cells (**3a, 3c–f**) and chlorinated compounds (**3c–f**) exhibited the highest potency. The activity of chloro-substituted compounds of this series extended to all viruses tested.

Owing to the correlation between anti-HRV-1B activity and the presence of chlorine substituents, it was interesting to evaluate the effects of the addition of further chloro atoms on 4′-chloro-homo-isoflavonoids (**1d, 2d** and **3d**). Polychloro substituted 3-benzylidenechroman-4-ones (**1e** and **1f**) resulted in more potent inhibitors than 3-(4-chlorobenzylidene)chroman-4-one (**1d**). In contrast, the introduction of a chlorine in the 6 position or of two chlorine atoms in the 5 and 7 positions on **2d** or **3d** led to a progressive reduction of activity. For **2f** the lower effect against HRV-1B was accompanied by an inhibitory activity towards the other virus tested.

Discussion

In this study we described the synthesis and anti-picornavirus activity of three related classes of homo-isoflavonoids (**1a–g, 2a–g** and **3a–g**). Besides a well documented effect on several biological activities (Ravisé & Kirkiacharian, 1978; Darmanaden *et al.*, 1984; Della Loggia *et al.*, 1989; Kirkiacharian *et al.*, 1989; Wall *et al.*, 1989; Al Nakib *et al.*, 1990; Amschler *et al.*, 1996), to our knowledge, this is the first report describing the antiviral properties of this group of compounds. Therefore the homo-isoflavonoids can be considered another class of anti-picornavirus flavonoids along with flavans, flavones, isoflavones, isoflavans, calchones, etc.

Homo-isoflavonoids were more effective against HRV than PV. Among HRVs, serotype 1B was more sensitive than serotype 14. Only 3-(4-hydroxybenzyl)-4-chromone (**2b**) exhibited a higher activity against HRV-14. Considerable differences were frequently noted in the sensitivity of different HRV serotypes to each compound tested. Flavonoids were generally more effective against serotype 1B (Batchelor *et al.*, 1979; Fujui *et al.*, 1980). Most of the homo-isoflavonoids tested showed an anti-HRV activity comparable with that of flavonoids previously studied by us (Burali *et al.*, 1987; Conti *et al.*, 1990a; Desideri *et al.*, 1990, 1992, 1995).

The replication of PV-2 was usually weakly sensitive to homo-isoflavonoids. Only compound **1g** showed a higher anti-PV activity. It is interesting to note that PV-2 and other enteroviruses were also generally less susceptible than HRV-1B to the action of substituted flavans, isoflavans and isoflavenes, previously synthesized by us (Burali *et al.*, 1987; Conti *et al.*, 1990a,b; Desideri *et al.*, 1990; Genovese *et al.*, 1995). The different sensitivity of rhinoviruses and enteroviruses to our compounds is not surprising. The structure of rhinoviruses is known to be slightly different from that of enteroviruses (Lonberg-Holm & Butterworth, 1976; Newman *et al.*, 1973). Our compounds may recognize differences in the fine structure or mechanism of replication among picornaviruses.

Some of the synthesized homo-isoflavonoids (**2f, 3d–f**) showed a spectrum of action wider than that of the reference drug (BW683C). Interestingly, all these compounds contain at least one chlorine atom, confirming the positive influence of this substituent on anti-picornavirus activity (Burali *et al.*, 1987; Conti *et al.*, 1990a; Desideri *et al.*, 1990, 1992, 1995; Genovese *et al.*, 1995).

Further evaluations should be carried out to define more accurately the structure–activity relationships and the mechanism of action towards picornaviruses.

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