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Short communication

Antiviral activity of substituted homoisoflavonoids on enteroviruses

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Abstract

The antiviral activity of homoisoflavonoids, a class of flavonoids, was determined *in vitro* against a large panel of enteroviruses. The inhibition of viral replication was monitored on BGM (Buffalo Green Monkey) cells, and the concentration required for 50% inhibition (IC_{50}), as well as the selectivity index (SI) were determined. None of the substances were effective against Sabin type 1 poliovirus (PV1), but most of them showed a low cytotoxicity and a marked antiviral activity against Coxsackie virus B1, B3, B4, A9 and echovirus 30.

Keywords: Antivirals; Homoisoflavonoids; Enteroviruses

Picornaviruses, in particular enteroviruses (EVs) and rhinoviruses (HRVs), are responsible for several human viral diseases, ranging from mild upper respiratory diseases to fatal neurological or cardiac-based illnesses. Enteroviruses cause aseptic meningitis, encephalitis, febrile illness, foot and mouth diseases, myocarditis, and pancreatitis, whereas, rhinoviruses are estimated to cause approximately one-third of all upper respiratory tract viral infections (Pallansch and Roos, 2001).

Due to the widespread nature of the diseases associated with picornaviruses and the difficulty of vaccine development for the majority of these viruses, extensive efforts have been made in the search for effective anti-picornavirus agents. However, despite the in vitro activity of several specific compounds, to date only few drugs have shown efficacy in humans and none have been approved for clinical use (Shih et al., 2004; Pevear et al., 2005; Rawlinson, 2001).

Natural and synthetic flavanoids and flavonoids interfere with picornavirus replication preventing the decapsidation of viral particles and RNA release within cells (Tisdale and Selway, 1984; Castrillo et al., 1986; Conti et al., 1990a; González et al., 1990; Genovese et al., 1995; Salvati et al., 2004) or blocking viral RNA synthesis (Robin et al., 2001).

Several substituted flavanoids (flavans, isoflavans and 3(2H)-isoflavenes) have been reported to have a broad antiviral spectrum of activity, efficiently inhibiting HRV 1B, Sabin type 2 poliovirus, hepatitis A virus, coxsackievirus B4, echovirus 6, and enterovirus 71 infections in vitro (Burali et al., 1987; Conti et al., 1990a,b; Desideri et al., 1990, 1992; Quaglia et al., 1993; Genovese et al., 1995).

Among flavonoids, both natural and synthetic flavanones and flavones presented a large spectrum of activity, although they are less potent than flavanoids. In particular flavones, generally poorly active compounds, achieved good anti-picornavirus potency through the introduction of substituents in position 3 (Van Hoof et al., 1984; De Meyer et al., 1991; Desideri et al., 1995).

Homoisoflavonoids constitute a small class of natural products structurally related to other known anti-picornavirus flavonoids. Synthetic analogues (3-benzylidenechroman-4-ones, 3-benzyl-4-chromones, and 3-benzylchroman-4-ones) (Fig. 1) were prepared and tested for their antiviral activity against picornaviruses. The homoisoflavonoids **1**–**3**(**a**–**g**) were reported to be weakly effective against poliovirus type 2, while they exhibited a variable degree of activity against HRV 1B and 14, selected as representative serotypes for groups B and A of HRVs, respectively (Desideri et al., 1997; Quaglia et al., 1999).

The aim of the present study was to evaluate the antiviral activity of this latter class of flavonoids against a larger panel of pathogenic enteroviruses, including Coxsackie virus B3 (CVB3), one of the major causes of virus-induced acute or chronic heart diseases (Maier et al., 2004), Coxsackie virus B4

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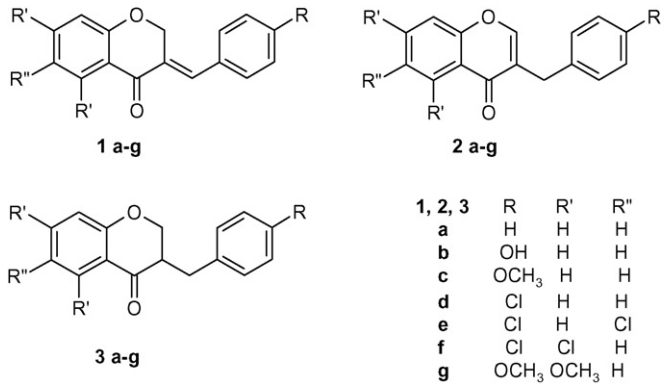

Fig. 1. Chemical structures of homoisoflavonoids: (E)-3-benzylidenechroman-4-ones (**1a**–**g**), 3-benzyl-4-chromones (**2a**–**g**) and 3-benzylchroman-4-ones (**3a**–**g**).

(CVB4) and A9 (CAV9), correlated with pancreatitis (Roivainen et al., 2000; Huber and Ramsingh, 2004) and echovirus 30 (Echo30) associated with meningitis (Savolainen et al., 2001). Among the three series of homoisoflavonoids described above, 3-benzyl-4-chromones (**2a**–**g**) (Fig. 1) were selected since they proved less cytotoxic in HeLa cell cultures (Desideri et al., 1997).

Initially, we confirmed the low cytotoxicity of these compounds on BGM (Buffalo Green Monkey) cells, widely used in enterovirus isolation because of their susceptibility to most of picornaviruses. The 50% cytotoxic concentration (CC_{50}) of the compounds, defined as the concentration reducing the viability of untreated cell cultures by 50%, was determined (Table 1). Confluent cell monolayers grown in 96-well plates were incubated with 10-fold serial dilutions (from 10 to 100 μM) of compounds for 3 days (37 °C, 5% CO_2) in D-MEM supplemented with 2% FCS. Cells were then fixed and stained with a methanol solution of crystal violet, as previously described (Sugarman et al., 1987; Horn et al., 1990). After dye extraction, the optical density of individual wells was quantified spectrophotometrically at 590 nm with a microplate reader. Cell viability in individual compound-treated wells was determined as the percentage of the mean value of optical density resulting from the average of three replicates with respect to the mock-treated cell control set as 100%.

The CC_{50} values of compounds **2a**–**d**, **2f** and **2g** ranged from 39 to 89 μM, while derivative **2e** was not toxic up to the highest concentration tested (100 μM). These data indicated that all the substances had a low cytotoxicity for BGM cells, comparable or lower than WIN 51711, a broad-spectrum anti-picornavirus compound used as reference compound (Otto et al., 1985). Cytotoxicity was dependent on the nature of substituents, the chloro-substituted derivatives **2d**–**f** being the least toxic among the 3-benzyl-4-chromones tested (Table 1).

The inhibitory activity of the homoisoflavonoids **2a**–**g** was evaluated against Sabin type 1 poliovirus (PV1), Coxsackie virus B1 (CVB1), B3 (CVB3), B4 (CVB4), A9 (CAV9) and echovirus 30 (Echo30) by focus reduction neutralization assay, as previously described (Di Lonardo et al., 2002). Briefly, BGM cell monolayers were grown in 96-well microtiter plates. After

Table 1
Cytotoxicity and antiviral activity of 3-benzyl-4-chromones (**2a**–**g**), 3-(4′-hydroxybenzyliden)chroman-4-one (**1b**) and 3-(4′-hydroxybenzyl) chroman-4-one (**3b**) against Sabin type 1 poliovirus (PV1), coxsackievirus B1 (CVB1), coxsackievirus B3 (CVB3), coxsackievirus B4 (CVB4), coxsackievirus A9 (CAV9) and echovirus 30 (Echo30)

Compound	CC_{50} (μM)	IC_{50} (μM)						SI[a]				
		PV1 (Sab)	CVB3	CVB4	CAV9	CVB1	Echo30	PV1 (Sab)	CVB3	CVB4	CAV9	CVB1	Echo30
2a	53.6	Not active	20.0 ± 1.8	12.0 ± 1.1	23.3 ± 1.5	4.0 ± 0.2	8.0 ± 0.5	–	2.7	4.5	2.3	13.4	6.7
2b	46.8	30.0 ± 1.6	10.0 ± 0.8	14.0 ± 1.3	38.0 ± 1.8	4.0 ± 0.3	13.0 ± 1.1	1.6	4.7	3.3	1.2	11.7	3.6
2c	55.5	>55.5[b]	4.0 ± 0.2	8.0 ± 0.5	20.0 ± 1.3	2.5 ± 0.1	13.0 ± 1.0	–	13.9	6.9	2.8	22.2	4.3
2d	85.0	50.0 ± 2.1	20.0 ± 1.6	15.0 ± 1.2	2.5 ± 0.1	3.0 ± 0.1	2.5 ± 0.1	1.7	4.3	5.7	34.0	28.3	34.0
2e	>100.0	43.0 ± 1.8	30.0 ± 2.1	30.0 ± 1.8	39.3 ± 1.4	7.5 ± 0.4	25.0 ± 1.6	>2.3	>3.3	>3.3	>2.5	>13.3	>4.0
2f	88.6	Not active	20.0 ± 1.6	10.0 ± 0.6	7.5 ± 0.5	20.0 ± 1.4	5.0 ± 0.3	–	4.4	8.9	11.8	4.4	17.7
2g	39.1	>39.0[b]	25.0 ± 1.5	20.0 ± 1.5	36.5 ± 1.7	17.5 ± 1.3	15.0 ± 1.3	–	1.6	2.0	1.1	2.2	2.6
WIN51711	53.2	2.0 ± 0.1	5.0 ± 0.3	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	26.6	10.6	26.6	26.6	26.6	26.6
1b	20.0	15.0 ± 1.3	15.0 ± 1.4	13.0 ± 1.2	8.5 ± 0.4	5.0 ± 0.3	3.5 ± 0.2	1.3	1.3	1.5	2.4	4.0	5.7
3b	91.4	30.0 ± 1.6	26.0 ± 1.7	20.0 ± 1.6	20.0 ± 1.3	14.0 ± 1.3	7.5 ± 0.5	3.0	3.5	4.6	4.6	6.5	12.2

All experiments were performed in triplicate on BGM cells. WIN 51711 was included as control.
[a] SI: ratio CC_{50}/IC_{50}.
[b] $>CC_{50}$: when IC_{50} value was higher than the corresponding CC_{50}.