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Antiproliferative activity of various flavonoids and related compounds: additive effect of interferon-α2b

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Abstract—The antiproliferative activity of several natural and synthetic flavonoids and some related compounds was evaluated in vitro against a cell line derived from a human cervical carcinoma (WISH cells). According to their activities, the most potent derivatives were 2′-nitroflavone (**14**), 2′,6-dinitroflavone (**15**) and the *n*-buthyl ester of caffeic acid (**29**). When these compounds were tested in the presence of recombinant human interferon-α2b (rhIFN-α2b), a cytokine exhibiting an antimitogenic action on WISH cells, an additive effect on cell growth inhibition was observed. Time course studies of the antiproliferative action exerted by the active derivatives or the rhIFN-α2b suggested that these compounds induced cell death.

Flavonoids are a group of natural products present in a wide variety of plants. They are found in seeds, citrus fruits, olive oil, tea and red wine and are commonly consumed with the human diet.[1,2] Flavonoids exhibit a broad range of biological activities, including antiviral, antiinflammatory, antioxidant and antitumoral actions.[1,3] Many of the pharmacological properties of these compounds have been related to their ability to inhibit enzymes involved in cell activation, such as phosphodiesterases, kinases, topoisomerases and other regulatory enzymes.[3]

It has been previously established that some natural flavonoids as well as certain synthetic flavone derivatives obtained in our laboratories behave as ligands for the central benzodiazepine-binding site.[4] These compounds exhibit medium-high affinity in vitro and anxiolytic activity in vivo. Although the biological actions of various synthetic compounds on the central nervous system have been extensively characterized,[4] their potential as antitumoral drugs has not yet been explored.

The alpha interferons (IFNα) represent a family of structurally related proteins that exert antiviral, antiproliferative and immunoregulatory actions.[5,6] These effects are triggered after IFN binding to cell membrane receptors located in different cell types.[5,6] In a previous work, we characterized the antimitogenic activity of the recombinant human IFN-α2b (rhIFN-α2b) in a cellular line (WISH) derived from a human cervical carcinoma.[7] In the present study, the effect of several natural and synthetic flavonoids and a series of related derivatives on the growth of WISH cells was examined. In addition, the antiproliferative activity of the most active compounds was evaluated in the presence of rhIFN-α2b.

The synthesis of flavone derivatives was performed as described previously.[8,9] Briefly, a hydroxyacetophenone and a benzoyl chloride derivative were first converted into a benzoyl ester and this species was then treated with base, forming a 1,3-diketone. Treatment of the diketone with acid leads to the generation of the desired flavone.[8] The products obtained were recrystalized from ethanol/water and their identification were achieved on the basis of ^{1}H NMR, ^{13}C NMR and mass spectra.[8,9] Flavanone (**24**) was obtained from Extrasynthese, Genay, France. Flavone (**1**), chrysin (**21**), apigenin (**22**), quercetin (**23**), α-naphthoflavone (**25**), β-naphthoflavone (**26**), cinnamic acid (**27**) and caffeic acid (**28**) were from Sigma Chem. Co, St. Louis, MO, USA. Flavonoids were dissolved in dimethyl sulfoxide (DMSO) as 10 mM stock solutions and stored at −70 °C. Prior to use, the compounds were diluted 1:10 in ethanol and

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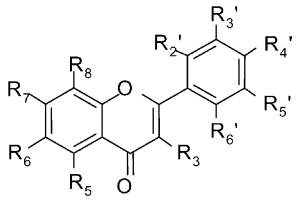
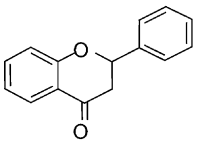
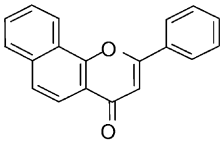
Table 1. Molecular structures of the compounds tested



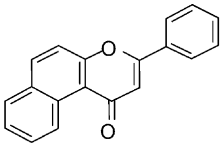
Compd	R_3	R_5	R_6	R_7	R_8	$R_{2'}$	$R_{3'}$	$R_{4'}$
Flavone (**1**)	H	H	H	H	H	H	H	H
6-Fluoroflavone (**2**)	H	H	F	H	H	H	H	H
6-Chloroflavone (**3**)	H	H	Cl	H	H	H	H	H
6-Bromoflavone (**4**)	H	H	Br	H	H	H	H	H
6-Nitroflavone (**5**)	H	H	NO_2	H	H	H	H	H
6-Methylflavone (**6**)	H	H	CH_3	H	H	H	H	H
3′-Bromo-6-methylflavone (**7**)	H	H	CH_3	H	H	H	Br	H
3′-Methyl-6-bromoflavone (**8**)	H	H	Br	H	H	H	CH_3	H
3′-Bromoflavone (**9**)	H	H	H	H	H	H	Br	H
3′-Nitroflavone (**10**)	H	H	H	H	H	H	NO_2	H
4′-Bromoflavone (**11**)	H	H	H	H	H	H	H	Br
3-Bromoflavone (**12**)	Br	H	H	H	H	H	H	H
3,6-Dibromoflavone (**13**)	Br	H	Br	H	H	H	H	H
2′-Nitroflavone (**14**)	H	H	H	H	H	NO_2	H	H
2′,6-Dinitroflavone (**15**)	H	H	NO_2	H	H	NO_2	H	H
2′,6-Dinitro-3-bromoflavone (**16**)	H	H	NO_2	H	H	NO_2	Br	H
2′,6-Difluoroflavone (**17**)	H	H	F	H	H	F	H	H
2′-Fluoro-6-chloroflavone (**18**)	H	H	Cl	H	H	F	H	H
2′-Fluoro-6-bromoflavone (**19**)	H	H	Br	H	H	F	H	H
5,7-Dimethoxyflavone (**20**)	H	CH_3O	H	CH_3O	H	H	H	H
Chrysin (**21**)	H	OH	H	OH	H	H	H	H
Apigenin (**22**)	H	OH	H	OH	H	H	H	OH
Quercetin (**23**)	OH	OH	H	OH	H	H	OH	OH



Flavanone (**24**)

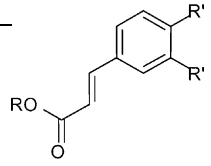


α-Naphthoflavone (**25**)



β-Naphthoflavone (**26**)

Compd	R	R′
Cinnamic acid (**27**)	H	H
Caffeic acid (**28**)	H	OH
Caffeic acid *n*-buthyl ester (**29**)	1-Buthyl	OH
Caffeic acid ethyl ester (**30**)	Ethyl	OH



added at the indicated concentrations to the culture medium.

Recombinant human IFN-α2b with an specific activity of 2×10^8 U/mg protein was supplied by Bio Sidus S.A., Buenos Aires, Argentina.

WISH cell line (ATCC CCL 25) was grown in Minimum Essential Medium (MEM, Gibco BRL, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (FBS, Gibco BRL, Gaithersburg, MD, USA), 2 mM L-glutamine, 50 U/mL penicillin and 50 μg/mL streptomycin. Although this line was originally thought to be derived from human normal amnion, WISH cells were derived via HeLa contamination (human cervix adenocarcinoma), as indicated by American Type Culture Collection.

A cell proliferation assay was performed as described previously.[7] Briefly, 0.1 mL of WISH cells (10,000 cells) were incubated for 72 h at 37 °C in 96-well culture microplates in the presence or absence of rhIFN-α2b and/or different natural or synthetic derivatives in a total volume of 0.2 mL of culture medium. Since solutions of flavonoids diluted in DMSO/ethanol (1:10) were usually employed, controls were treated under similar conditions and a maximum concentration of 20 μL of solvent/mL of culture medium was used in all experiments. Total cell number was evaluated by colorimetric determination of hexosaminidase levels.[10] IC_{50}

values, that is, the molar drug concentrations required to cause 50% growth inhibition, were determined from dose–response curves. Viable cells, identified by trypan blue exclusion, were counted with a hemocytometer.

The effect of a series of natural and synthetic flavonoids and some other related compounds (see Table 1) on the proliferation of WISH cells is shown in Figure 1. When tested at 20 μM, the majority of compounds caused a reduction $\leq$20% in cell growth; the caffeic acid ethyl ester (**30**) inhibited almost 50%, whereas 2′-nitroflavone (**14**), 2′,6-dinitroflavone (**15**) and caffeic acid *n*-buthyl ester (**29**) were the most active cytotoxic agents ($\sim$70% inhibitory effect). Results obtained from dose–response curves revealed that 50% of cell growth inhibition was achieved with similar concentrations of compounds **14**, **15**, and **29** (IC_{50} $\sim$3 μM).

Some structure–activity relationships of the flavone derivatives, useful to define certain molecular requirements leading to the generation of a more active antiproliferative compound, were established. Thus, although the flavone nucleus itself (**1**) behaved as an inactive compound, the flavanone (**24**), its C2–C3 dihydro derivative, slightly reduced WISH cell proliferation. The introduction of fluoro, chloro, bromo, nitro or methyl group at C6 of the flavone nucleus (**2**, **3**, **4**, **5**, and **6**) did not modify the biological activity, while substitutions at C3′, C4′ or C3 (**7**, **8**, **9**, **10**, **11**, **12**, and **13**) appeared to be more favorable. The addition of nitro groups at positions 2′ and/or 2′ and 6 of the flavone nucleus (**14** and **15**) induced a striking decrease in cell proliferation, although other groups at the same positions did not improve the biological potency. Thus, a moderate activity was observed for compounds bearing either fluoro groups at C2′ and C6 (**17**) or a fluoro at C2′ and a chloro or a bromo at C6 (**18** and **19**). In addition, the introduction of a bromo at C3′ of compound **15** (**16**) led to a less active derivative.

Several natural flavonoids, such as compounds **20**, **21**, **22**, **23**, **25**, and **26**, together with cinnamic acid (**27**) showed slight antiproliferative effects. The examination of other related compounds revealed that although caffeic acid (**28**) behaved as an inactive derivative, its esterification resulted to improve the biological activity (**29** and **30**), being the caffeic acid *n*-buthyl ester (**30**) among the most active compounds.

The growth inhibitory activity of synthetic derivatives **14**, **15**, and **29** was determined after 24, 48, and 72 h of incubation. Furthermore, the time course of the antiproliferative effect exhibited by the rhIFN-α2b, an antimitogenic agent previously characterized on WISH cells,[7] was also studied. As shown in Figure 2, compounds **14**, **15**, and **29** as well as the rhIFN-α2b caused a significant decrease in the number of viable cells, indicating that they induced a cytotoxic effect. On the other hand, similar cell number values were reached after incubating cells for 72 h in the absence of flavonoids (control) or in the presence of an inactive derivative (**5**) (Fig. 2).

When the highly potent derivatives **14**, **15**, and **29** were assayed in the presence of a suboptimal concentration of rhIFN-α2b, a higher growth inhibition, equivalent to

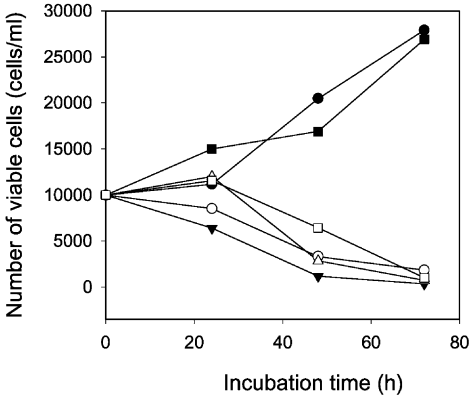


Figure 2. Effect of rhIFN-α2b and some synthetic derivatives on the number of viable cells at different incubation times. Cells (10,000 per well) were incubated for 24, 48, and 72 h at 37 °C with 5×10^{-2} μM rhIFN-α2b (○) or 20 μM of different synthetic compounds. The number of viable cells was determined with a hemocytometer by trypan blue exclusion. Control (●); 6-nitroflavone (**5**) (■); caffeic acid *n*-buthyl ester (**29**) (□); 2′,6-dinitroflavone (**15**) (△); 2′-nitroflavone (**14**) (▼).

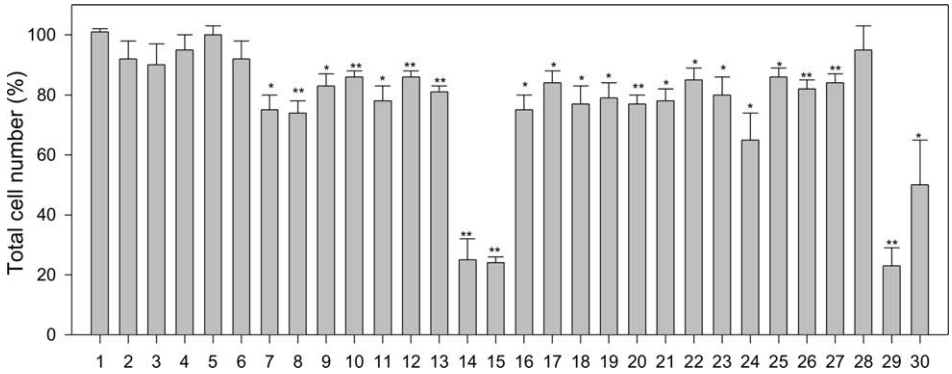


Figure 1. Effect of flavonoids and synthetic derivatives on WISH cell proliferation. Cells (10,000 per well) were incubated in the presence or absence of 20 μM of different compounds for 72 h at 37 °C. Cell growth was determined by colorimetric determination of hexosaminidase levels.[10] Results are expressed as the percentage of total cell number obtained in the absence of flavonoids (control) and represent the mean ±S.E. of three different experiments. Statistical significance in comparison with the control value was calculated by Student's *t*-test and is indicated by * ($p < 0.01$) or ** ($p < 0.005$).

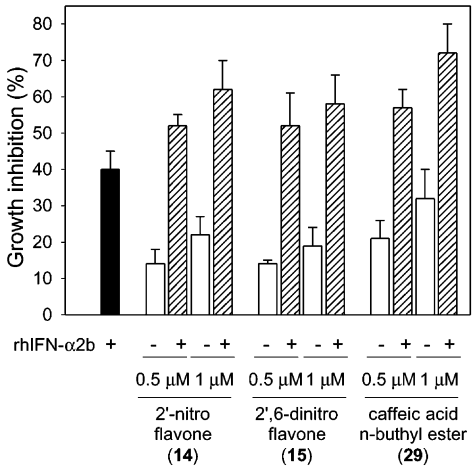


Figure 3. Combined effect of rhIFN-α2b and some synthetic derivatives on WISH cell proliferation. Cells (10,000 per well) were incubated for 72 h at 37 °C with different concentrations of compounds **14**, **15**, and **29** in the presence (+) or absence (−) of rhIFN-α2b (3×10^{-4} μM). Results are expressed as percentage of growth inhibition with respect to the control value (cell growth obtained in the absence of rhIFN-α2b and synthetic compounds). The solid bar shows the inhibitory effect produced after incubating cells only with rhIFN-α2b. Each bar represents the mean ±SD of three determinations.

the addition of the effect of either individual compound, was observed (Fig. 3).

The molecular mechanisms responsible for the antitumoral action produced by the rhIFN-α2b are not clearly understood. It has been reported that, depending on the cell line studied, cell growth inhibition could or not be related to the induction of an apoptotic response.[11,12] Although the cellular pathways specifically involved in WISH cell death remains to be elucidated, IFN actions are certainly triggered after its binding to previously characterized cell membrane receptors.[7] On the contrary, a different cellular target site would probably be required for synthetic derivatives to exert their antigrowth effects. Remarkably, the structural requirements to inhibit WISH cell proliferation did not correlate with the reported ability of natural and synthetic flavonoids to bind to central-type benzodiazepine receptors.[4] Thus, whatever the mechanism of antitumoral action is, distinct signaling pathways would contribute to the additive effect exhibited by the flavonoids/interferon associations on the inhibition of WISH cell proliferation. Further experiments would be required to evaluate the possible therapeutic efficacy of these combinations in vivo.

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References and notes

1. Harborne, J. B., Ed. *The Flavonoids. Advances in Research since 1986*; Chapman and Hall: London, 1999.
2. Middleton, E., Jr.; Kandaswami, C.; Theoharides, T. C. *Pharmacol. Rev.* **2000**, *52*, 673.
3. Manthey, J. A.; Grohmann, K.; Guthrie, N. *Curr. Med. Chem.* **2001**, *8*, 135.
4. Marder, M.; Paladini, A. C. *Curr. Top. Med. Chem.* **2002**, *2*, 853.
5. Pestka, S.; Langer, J. A.; Zoon, K. C.; Samuel, C. E. *Annu. Rev. Biochem.* **1987**, *56*, 727.
6. Pestka, S. *Biopolymers* **2000**, *55*, 254.
7. Blank, V. C.; Sterin-Prync, A.; Retegui, L.; Vidal, A.; Criscuolo, M.; Roguin, L. P. *Eur. J. Biochem.* **1999**, *265*, 11.
8. Marder, M.; Zinczuk, J.; Colombo, M. I.; Wasowski, C.; Viola, H.; Wolfman, C.; Medina, J. H.; Rúveda, E. A.; Paladini, A. C. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2003.
9. Marder, M.; Viola, H.; Bacigaluppo, J. A.; Colombo, M. I.; Wasowski, C.; Wolfman, C.; Medina, J. H.; Ruveda, E. A.; Paladini, A. C. *Biochem. Biophys. Res. Commun.* **1998**, *249*, 481.
10. Landegren, U. *J. Immunol. Methods* **1984**, *67*, 379.
11. Sangfelt, O.; Erickson, S.; Castro, J.; Heiden, T.; Einhorn, S.; Grander, D. *Cell Growth Differ.* **1997**, *8*, 343.
12. Thyrell, L.; Erickson, S.; Zhivotovsky, B.; Pokrovskaja, K.; Sangfelt, O.; Castro, J.; Einhorn, S.; Grander, D. *Oncogene* **2002**, *21*, 1251.