Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 3095–3097

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Flavonoids: Structural Requirements for Antiproliferative Activity on Breast Cancer Cells

Christelle Pouget, Fabienne Lauthier, Alain Simon, Catherine Fagnere, Jean-Philippe Basly, Christiane Delage and Albert-José Chulia*

UPRES EA 1085, 'Biomolécules et cibles cellulaires tumorales', Faculté de Pharmacie, 2 rue du Docteur Marcland, 87025 Limoges Cedex, France

Received 6 April 2001; revised 24 June 2001; accepted 3 September 2001

Abstract—Several classes of flavonoids (flavones, flavanones, 2′-hydroxychalcones and flavan-4-ols) having a variety of substituents on A ring were investigated for their antiproliferative activity against MCF-7 human breast cancer cells. Structure–activity relationships of these compounds were discussed. 2′-hydroxychalcones and methoxylated flavanones were found to be potent inhibitors of MCF-7 cells growth whereas flavones and flavan-4-ols appeared to be weak inhibitory agents except 7,8-dihydroxyflavone.

Introduction

Many clinically successful anticancer drugs are themselves either natural products or have been developed from naturally occurring lead compounds. Great interest is currently being paid to flavonoids—one of the major classes of natural products with widespread distribution in fruits, vegetables, spices, tea and soy-based foodstuff[1]—for their interesting pharmacological activities.[2,3] The antiproliferative effects against breast cancer cells,[4] anti-aromatase activity[5,6] and binding affinities for the estrogen receptor[7] of these compounds have drawn attention due to their role as potential anti-breast cancer agents.

In our attempt to design flavonoid-related compounds having a balance between the above activities, we undertook a screening relating to several classes of flavonoids and we recently described the aromatase inhibitory effects of some flavones, flavanones[8] and chalcones.[9] The aim of the present study was to explore thoroughly the structural requirements on A-ring of chalcones, flavanones and flavones for inhibition of MCF-7 breast cancer cells growth, both to identify an optimal candidate among currently available compounds, as well as to ascertain potential directions for synthetic lead-optimization studies.

Finally, a previous study about anticancer activity of *cis*- and *trans*-4′,7-dihydroxyisoflavan-4-ols,[10] two proposed metabolites of daidzein (4′,7-dihydroxyisoflavone) prompted us to investigate for the first time the antiproliferative activity against MCF-7 cells of synthetic flavan-4-ols,[11] which are of a rare occurrence in nature.

Chemistry

For this study, we synthesized a set of flavonoid derivatives, increased by commercially available compounds (flavanones **1a** and **1d**–**1i**, chalcones **2a** and **2c** and flavones **3a**–**3e**), all of which bearing either hydrogen, hydroxyl and/or methoxy substituents on the A-ring (Table 1).

2′,4′-dihydroxychalcone **2b** was synthesized as previously described[12] (yield 36%). Flavanones **1d**–**1h** were treated by a methanolic KOH solution to afford 2′-hydroxychalcones **2d**–**2h** respectively (yield 50–60%) while flavanones **1b** and **1c** were obtained, also in moderate yields, by cyclization of the corresponding 2′-hydroxychalcones **2b** and **2c** in a methanolic H_2SO_4 solution. Inspection of the ^1H NMR spectra of both commercial and synthesized chalcones clearly indicated that they were configured *trans* ($J_{H\alpha,\ H\beta} = 15$–$16$ Hz).

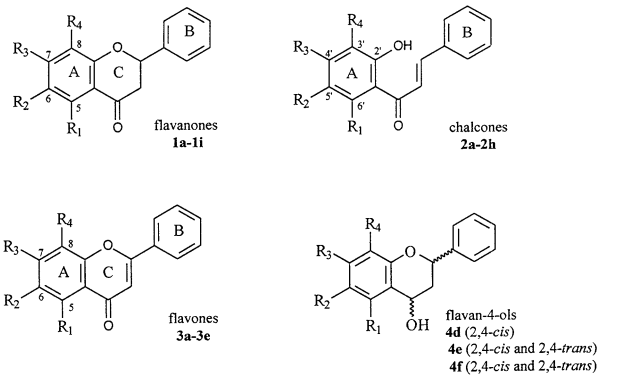
Reduction of the flavanones **1d**–**1f** was performed with sodium borohydride to give the corresponding flavan-4-ols

*Corresponding author. Tel.: +33-555-435-834; fax: +33-555-435-910; e-mail: jose.chulia@unilim.fr

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00617-5

4d–**4f** for which the assignment of stereochemistry was readily made on the basis of the ^{1}H NMR coupling constants of the heterocyclic ring protons.[11] $NaBH_4$ reduction of 7-methoxyflavanone **1d** was stereoselective leading to the 2,4-*cis*-7-methoxyflavan-4-ol while reduction of the 4-keto group of the 5-methoxyflavanone **1e** and of the 5,7-dimethoxyflavanone **1f** afforded a mixture of 2,4-*cis*- and 2,4-*trans*-flavan-4-ols which were separated by TLC.

Table 1. Structure of flavanone, chalcone, flavone and flavan-4-ols derivatives



Compd	R_1	R_2	R_3	R_4
a	H	H	H	H
b	H	H	OH	H
c	H	H	OH	OH
d	H	H	OMe	H
e	OMe	H	H	H
f	OMe	H	OMe	H
g	OH	H	OMe	H
h	H	OMe	H	H
i	H	OH	H	H

Proliferation assay

The growth inhibitory activity of the compounds was determined in the MCF-7 human breast cancer cell line using the MTT assay as described by Mosman et al.[13] Results were represented as % of inhibition compared to control absorbance. The IC_{50} concentration was calculated graphically and represents the concentration which results in a 50% decrease in cell growth after 6 days incubation. The given values are mean values of three experiments. The deviations were within $\pm 5\%$.

Results and Discussion

The antiproliferative effects of flavanones and flavones on MCF-7 cells are reported in Figure 1 while results from testing 2′-hydroxychalcones are summarized in Table 2.

Some structure–activity relationships for cytotoxicity are apparent from these results. First, the unsubstituted flavanone **1a** appeared to be a weak inhibitor of MCF-7 cells growth as well as flavanones with hydroxyl groups like compounds **1b**, **1c**, **1g** and **1i**. In contrast, the substitution by a methoxy group at position 7 and/or 5 increased the antiproliferative activity (the IC_{50} of flavanones **1d**, **1e** and **1f** were, respectively, 35.7, 35.7 and 36.0 μM) while a 6-methoxy group (compound **1h**) did not enhance the growth inhibition.

Flavone **3a** and 7-hydroxyflavone **3b** were shown to have only a weak antiproliferative activity as previously described for the corresponding flavanones. Then, the presence of a 7-methoxy group on the A-ring of flavone did not enhance the inhibitory effect as shown by the low activity of the 7-methoxyflavone **3d** while the substitution by a methoxy group at position 5 (flavone **3e**) increased the antiproliferative activity. Among the

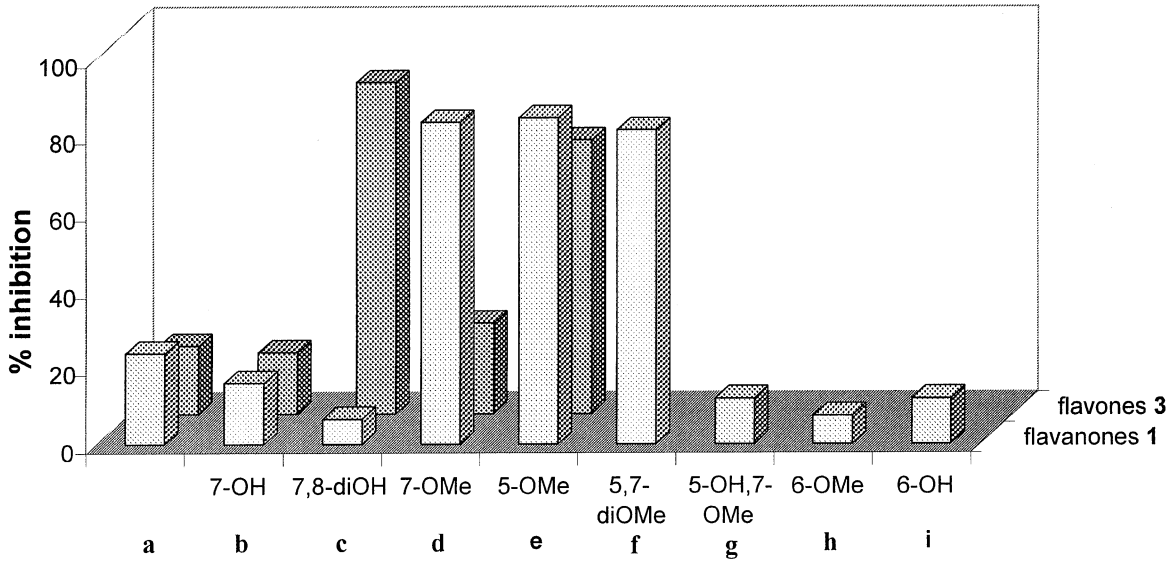


Figure 1. Antiproliferative activity of flavanones and flavones: MCF-7 cells were incubated with flavanones at 60 μM for 6 days. Cell viability was determined by MTT assay. Results were expressed as percentage of control (untreated cells); data points represent the mean values of three independent experiments.

Table 2. Cytotoxicity of 2′-hydroxychalcones

Compd	IC_{50} (μM)
2a	28.4
2b	16.3
2c	22.2
2d	26.8
2e	18.3
2f	20.0
2g	>60
2h	29.2

flavones tested, 7,8-dihydroxyflavone **3c** was found to be the most potent ($IC_{50}=27.5$ μM) whereas the corresponding flavanone **1c** was inactive.

The inhibitory effects of some of the flavanones were compared to those of corresponding 2′-hydroxychalcones. We noted that in all cases, 2′-hydroxychalcones were more potent against MCF-7 cells growth than corresponding flavanones. Surprisingly, the most active was the 2′,4′-dihydroxychalcone **2b** corresponding to the 7-hydroxyflavanone **1b** which is inactive against MCF-7 cells proliferation. The 2′,3′,4′-trihydroxychalcone **2c** was also active whereas the corresponding 7,8-dihydroxyflavanone **1c** had no effect on MCF-7 cells growth. Therefore, unlike flavanones, hydroxyl substituents on A ring of chalcones are not critical for antiproliferative effect except hydroxylation at position 6′ since 2′,6′-dihydroxy-4′-methoxychalcone **2g** is much less active than 2′-hydroxy-4′-methoxychalcone **2d**.

All the flavan-4-ols synthesized showed no inhibition of MCF-7 cells proliferation (data not shown) while the corresponding flavanones had significant activity. So, the 4-keto functionality appeared to be essential for antiproliferative activity against this human breast cancer cell line. This result provides further support to previous studies which underlined the importance of this structural feature for interaction with different cellular mechanisms involved in cancer growth. Thus, Constantinou et al.[14] have reported that some flavonoids were DNA topoisomerase inhibitors and that the C-4 carbonyl group of these compounds was essential for the inhibition of topoisomerase activity. Edwards et al.[15] have also shown that chalcones were effective antimitotic agents by binding to tubulin and they noticed that reduction of the carbonyl group of chalcones diminished this activity. Interestingly, the 4-keto group of the flavonoids is also essential for aromatase inhibitory effect since the flavan-4-ols were found to be weak aromatase inhibitors (unpublished results).

In conclusion, on the basis of the above findings, the 7-methoxyflavanone **1d** and the 7,8-dihydroxyflavone **3c** scaffolds were selected as skeleton for the development of flavonoid structurally-related compounds having a balance between different anti-breast cancer activities since these compounds were found to possess not only antiproliferative activity against MCF-7 cells but also aromatase inhibitory effect.[8] In spite of their great antiproliferative activity, chalcones could not be selected in our synthetic lead-optimization study because they were shown to be only weak inhibitors of aromatase activity.[9] However, a further evaluation of chalcones will be undertaken, concerning the structural requirements on B-ring for inhibition of hormone-dependent human breast cancer cell line growth.

Acknowledgements

The authors are grateful to the Region Limousin for its financial support and Y. Champavier for running the NMR spectra.

References and Notes

1. Peterson, J.; Dwyer, J. *Nutrition Res.* **1998**, *18*, 1995.
2. Di Carlo, G.; Mascolo, N.; Izzo, A. A.; Capasso, F. *Life Sci.* **1999**, *65*, 337.
3. Dimmock, J. R.; Elias, D. W.; Beazely, M. A.; Kandepu, N. M. *Curr. Med. Chem.* **1999**, *6*, 1125.
4. So, F. V.; Guthrie, N.; Chambers, A. F.; Carroll, K. K. *Cancer Lett.* **1997**, *112*, 127.
5. Ibrahim, A. R.; Abul-Hajj, Y. J. *J. Steroid Biochem. Mol. Biol.* **1990**, *37*, 257.
6. Jeong, H. J.; Shin, Y. G.; Kim, I. H.; Pezzuto, J. M. *Arch. Pharm. Res.* **1999**, *22*, 309.
7. Miksicek, R. J. *Proc. Soc. Exp. Biol. Med.* **1995**, *208*, 44.
8. Le Bail, J. C.; Laroche, T.; Marre-Fournier, F.; Habrioux, G. *Cancer Lett.* **1998**, *133*, 101.
9. Le Bail, J. C.; Pouget, C.; Fagnere, C.; Basly, J. P.; Chulia, A. J.; Habrioux, G. *Life Sci.* **2001**, *68*, 751.
10. Wähälä, K.; Koskimies, J. K.; Mesilaakso, M.; Salakka, A. K.; Leino, T. K.; Adlercreutz, H. *J. Org. Chem.* **1997**, *62*, 7690.
11. Pouget, C.; Fagnere, C.; Basly, J. P.; Levêque, H.; Chulia, A. J. *Tetrahedron* **2000**, *56*, 6047.
12. Hsieh, H. K.; Lee, T. H.; Wang, J. P.; Wang, J. J.; Lin, C. N. *Pharm. Res.* **1998**, *15*, 39.
13. Mosman, T. *J. Immunol. Meth.* **1983**, *65*, 5563.
14. Constantinou, A.; Mehta, R.; Runyan, C. *J. Nat. Prod.* **1995**, *58*, 217.
15. Edwards, M. L.; Stemerick, D. M.; Sunkara, P. S. *J. Med. Chem.* **1990**, *33*, 1948.