Synthesis of a series of different hydroxycoumarins and their cytotoxic activity

Silvia Serra[1,3]*, Andrea Chicca[2], Jürg Gertsch[2], Lourdes Santana[3], Eugenio Uriarte[3], Giovanna Delogu[1].

[1]*Dipartimento di Scienze della Vita e dell'Ambiente, sezione di Scienze del Farmaco, Università degli Studi di Cagliari, Via Ospedale 72, 09124 Cagliari, Italy*
[2]*Departamento de Química Orgánica, Facultad de Farmacia, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain*
[3]*Institute of Biochemistry and Molecular Medicine, NCCR TransCure, University of Bern, Switzerland*

Email: silvserra@tiscali.it

Abstract: Cancer is at present one of the most leading causes of death in the developed countries, and many efforts have been made to discover agents endowed with cytotoxic action. Natural products, with their ability to interact with more than one target, represent in medicinal chemistry a significant source of inspiration for the design of structural analogues with an improved pharmacological profile. Coumarins are an important family of natural and synthetic origin that has been attracting an intense interest in recent years for their remarkable array of biological activities, usually associated to low toxicity. It has been shown that 4-hydroxycoumarins derivatives bearing an aryl group in the 3 position of the coumarin skeleton, inhibit cell proliferation. Furthermore, a recent study shows that the most simple 4-hydroxycoumarin leads to a selective cytoskeleton disorganization in melanoma cells without affecting a non-tumoral fibroblastic cell line. Taking into account previous data about anti-tumor effects of coumarin derivatives we synthesized a new series of compounds structurally related to these, but exhibiting a new pattern of substitution that does not occur in nature and is not present in previously tested compounds.
In particular in this communication we investigated the impact of different substituents at C-6 and the introduction of a hydroxyl group in diverse positions on the 3-aryl ring of the 4-hydroxycoumarin moiety on their antiproliferative activity by using two cancer cell lines.

Keywords: 4-hydroxycoumarins, cytotoxic activity.

Introduction

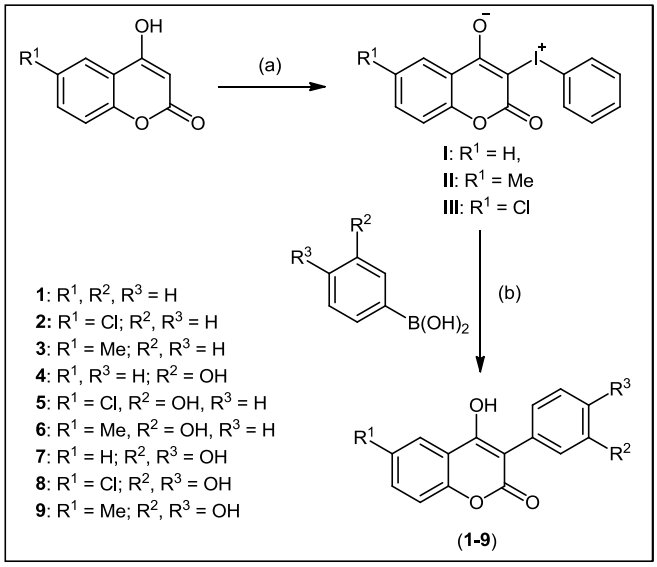
Coumarin is an important class of benzopyrones that is found in nature and acts as a structural subunit of more complex natural products. During the last decades, coumarins have been extensively studied for their pharmacological applications [l-7].
Among their various biological properties, antitumor activities and antiproliferative effects have aroused considerable attention [8-10]. In this regard, it has been found that several 4-hydroxycoumarins bearing an aryl group in 3 position of the coumarin nucleus, inhibit cell proliferation [11]. Furthermore, it has been reported that the most simple 4-hydroxycoumarin leads to a selective cytoskeleton disorganization in melanoma cells without affecting a non-tumoral fibroblastic cell line [12].
In this context, a new series of these derivatives has been synthesized and evaluated. In particular we studied the impact of the presence of different substituents at C-6 and the introduction or no of a hydroxyl group in various positions on the 3-aryl ring of the 4-hydroxycoumarin skeleton on their antiproliferative activity. We have analyzed the cytotoxic properties on breast adenocarcinoma (MCF-7) and human promyelocytic leukemia cells (HL-60).

Methods and experimental part

Chemistry

The synthesis of the compounds **1-9** was achieved by the preparation of different phenyliodonium coumarinate species (**I-III**) starting from the corresponding 3-unsubstituted 4-hydroxycoumarin.

Then we carried out the palladium catalyzed Suzuki coupling reaction between phenyliodonium zwitterions and the conveniently substituted phenyl boronic acids to afford the final compounds (Scheme 1 and Table 1) [13-14].



Compounds	Yield	Mp
1	63 %	237-239 °C
2	68 %	263-265 °C
3	57 %	243-245 ° C
4	58 %	265-267 °C
5	69 %	283-285°C
6	68 %	244-246 °C
7	60 %	265-267 °C
8	65 %	299-300 °C
9	54 %	264-266 °C

Scheme 1: Reagents and conditions: (a) $PhI(OAc)_2$, Na_2CO_3, H_2O, r.t., 14 h; (b) $Pd(OAc)_2$, P(*t*-Bu)$_3$, LiOH, DME/H_20, r.t., 24-48 h.
Table 1: Yields and Mp obtained for compound **1**-**9**.

General procedure for the preparation of 3-phenyliodonium coumarinates **I**, **II** *and* **III**: iodobenzene diacetate (10 mmol) was suspended in a solution of Na_2CO_3 (10 mmol) in water (100 mL) and was stirred for 30 min at room temperature. To this solution was added a mixture of the corresponding 4-hydroxycoumarin (10 mmol) and Na_2CO_3 (10 mmol) in water (100 mL). After the mixture was stirred at room temperature for 14 h, the precipitate was collected by filtration, washed with water (5 x 20 mL) and dried under vacuum. The resulting white solid was used without further purification.
General procedure for the preparation of 3-aryl-4-hydroxycoumarins **1-9**: a degassed solution of appropriated phenyl boronic acid (1.21 mmol) and P(*t*-But)$_3$ (0.109 mmol) in DME and H_2O (4:1, 12.5 mL) was added to a mixture of iodonium ylide (0.55 mmol), LiOH/H_2O (1.65 mmol) and $Pd(OAc)_2$ (0.027 mmol) under argon at room temperature. After being stirred at the same temperature for 24-48 h. The resulting mixture was purified by FC (hexane/ethyl acetate, 7:3)to give the desired compound.

HL-60 and MCF-7 antiproliferative assays

The inhibition of cell proliferation by these diversely substituted coumarins **1-9** was evaluated *in vitro* using the human breast MCF-7 cells and the human promyelocytic leukemia HL-60 cells. Cell proliferation assay was carried out by using the Cell Proliferation Reagent WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulphonate) (Roche, Mannheim, Germany) based on the mitochondrial enzymatic cleavage of the WST-1 to formazan salt, whose formation has been monitored by measuring the absorbance at 450 nm, as previously described [15].
The HL60 and the MCF-7 cells line were purchased from the American Type Culture Collection. HL60 cells were grown in Iscove's modified Dulbecco's medium with 2 mM $_{L}$-glutamine supplemented with 20% fetal bovine serum, 1 µg/ml amphotericin B, 100 units/ml penicillin, and 100 µg/ml streptomycin (all from Life Technologies Invitrogen, Basel, Switzerland). MCF-7 cells were cultured in RPMI 1640 medium containing penicillin (100 U/ml), streptomycin (100 µg/ml), amphotericin B (1 µg/ml), 2 mM $_{L}$-glutamine and 10% fetal bovine serum (all from Life Technologies Invitrogen, Basel, Switzerland). All cells were grown in a humidified incubator at 37°C and 5% CO_2. Cells were seeded into 96-well plates at a density of $2x10^6$ per well and incubated at 37 °C with 5% CO_2. After 24 h incubation to allow cell attachment, cells were treated for 72 h with the acetylenic compounds in the range 0.1–100 µM. For the HL-60 cells it is not necessary to wait the time of incubation to allow cell attachment because the cells are in suspension. At the end of the

exposure time, WST-1 was added at 1/10 of the total volume and after 60 min of incubation at 37 °C, the absorbance was measured at 450 nm with a microplate reader (Wallac; Perkin Elmer,Wellesley, MA, USA).

Results and Discussion

The antiproliferative effects obtained at 10 μM with compounds **1-9** are reported in Table 2. The inhibition of cell proliferation was expressed as percentage of viable cells in treated samples as compared to vehicle-treated cells. Each value was obtained from two/three independent experiments carried out in triplicate.

Table 2. Percentage of cell viability ± SEM (Standard error of the mean) for the synthesized compounds **1-9**.

Compounds	Percentage cell viability ± SEM	
	MCF-7	HL-60
1	96.7±5.6	73.4±4.6
2	86.9±14.8	85.1±1.7
3	72.6±0.6	73.9±1.4
4	55.4±2.1	85.7±3.2
5	73.4±12.6	96.5±1.3
6	75.9±2.2	100.1±5.1
7	124.5±4.6	74.7±5.9
8	95.2±8.8	71.2±7.1
9	65.1±3.9	65.4±7.4

The results found that the compound **4** is the most potent molecule in the series. So we calculated by nonlinear least squares curve fitting (GraphPad Software, San Diego, CA, USA) its 50% inhibitory concentration on MCF-7 proliferation (IC_{50}) (Table 3).

Table 3. IC50 ± SEM values of antiproliferative activity of compounds **4** and reference compounds.

Compounds	IC_{50} (μM) ± SEM	
	MCF-7	HL-60
4	35.4 ± 0.1	-
Tamoxifen	8.6 ± 1.6	14.3 ± 2.6
Coumarin	> 50	> 50

Unfortunately, between all synthesized coumarins only compound **4**, that presents a *m*-hydroxy group in the 3-aryl ring, showed a significant antiproliferative activity on MCF-7 cells. It was more potent than simple coumarin, but not that tamoxifen, used as reference compounds. As regard the citotoxic activity on HL-60 cells, the compound **9** turns out to be the most potent of evaluated series even if the values obtained (65.4 % of cell viability) are over the border-line range.
Although the results obtained are not as expected, they are important to better understand what molecular fragments are essential to obtain and or improve the activity. It appears that the presence of a substituent at 6 position in 4-hydroxycoumarin nucleus is not really fundamental. Whereas the introduction of a hydroxyl group on the 3-aryl ring changes the activity. In particular the *meta* position seems to be the good goal in the antiproliferative activity of MCF-7 cells. When the hydroxyl group is included in *para* position the antiproliferative activity is lost.

Conclusion

As conclusion, we synthesized a series of nine compounds using an efficient and versatile synthetic route. We investigated and evaluated their antiproliferative activity on MCF-7 and HL-60 cells. We

found that the most potent of these molecules has a *m*-hydroxyl group in the 3 aryl ring. These preliminary results allows us to make structural improvements in the 4-hydroxycoumarin nucleus and encourage us to continue the efforts towards the optimization of its pharmacological profile.

Acknowledgements

This work was partially supported by Spanish Ministry (PS09/00501), Xunta de Galicia (09CSA030203PR), Italian Ministry (PRIN 2008, F21J10000010001) and by Fondazione Banco di Sardegna 2012.

References

1. Borges, F.; Roleira, F.; Milhazes, N.; Santana, L.; Uriarte, E. *Curr. Med. Chem.* **2005**, *12*, 887.
2. Santana, L.; Uriarte, E.; González-Díaz, H.; Zagotto, G.; Soto-Otero, R.; Méndez-Álvarez, E. *J. Med. Chem.* **2006**, *49*, 1149.
3. Riveiro, M. E.; Vazquez, R.; Moglioni, A.; Gomez, N.; Baldi, A.; Davio, C.; Shayo, C. *Biochem. Pharmacol*. **2008**, *75*, 725.
4. Borges, F.; Roleira, F.; Milhazes, N.; Uriarte, E.; Santana, L. *Front. Med. Chem.* **2009**, *4*, 23.
5. Matos, M. J.; Terán, C.; Pérez-Castillo, Y.; Uriarte, E.;Santana, L.; Viña, D. *J. Med. Chem.* **2011**, *54*, 7127.
6. Viña, D.; Matos, M. J.; Yáñez, M.; Santana, L.; Uriarte, E. *Med. Chem. Comm.* **2011**, *3*, 213.
7. Serra, S.; Ferino, G.; Matos, M. J.; Vázquez-Rodríguez, S.; Delogu, G.; Viña, D.; Cadoni, E.; Santana, L.; Uriarte, E. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 258.
8. Alvarez-Delgado, C.; Reyes-Chilpa, R.; Estrada-Muñiz, E.; Mendoza-Rodriguez, C. A.; Quintero-Ruiz, A.; Solano, J.; Cerbon, M. A. *J. Biochem. Mol. Toxicol.* **2009**, *23*, 263.
9. Omarbasha, B.; Fair, W. R.; Heston, W. D. W. *Cancer Res*. **1989**, *49*, 3045.
10. Kim, E. K.; Kwon, K. B.; Shin, B. C.; Seo, E. A.; Lee, Y. R.; Kim, J. S.; Park,
11. Stanchev, S.; Momekov, G.; Jensen, F.; Manolov, I. *Eur. J. Med. Chem.* **2008**, *43*, 694.
12. Velasco-Velazquez, M. A.; Agramonte-Hevia, J.; Barrera, D.; Jimenez-Orozco, A.; Garcia-Mondragon, M. J.; Mendoza-Patino, N. .*Cancer Lett.* **2003**, *5*, 179.
13. Zhu, Q.; Wu, J.; Fathi, R.; Yang, Z. *Org. Lett.* **2002**, *4*, 3333.
14. Pérez-Cruz, F.; Serra, S.; Delogu, G.; Lapier, M.; Maya, J. D.; Olea-Azar, C.; Santana, L.; Uriarte, E. *Bioorg. Med. Chem. Lett*, **2012**, *22*, 5569.
15. Chicca, A.; Pellati, F.; Adinolfi, B.; Matthias, A.; Massarelli, I.; Benvenuti, S.; Martinotti, E.; Bianucci, A. M.; Bone, K.; Lehmann, R.; Nieri, P. *Br. J. Pharmacol.* **2008**, *153*, 879.