Bioorganic & Medicinal Chemistry Letters 22 (2012) 5791–5794

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and cytotoxic activity of non-naturally substituted 4-oxycoumarin derivatives

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ARTICLE INFO

Article history:
Received 5 June 2012
Revised 26 July 2012
Accepted 30 July 2012
Available online 5 August 2012

Keywords:
4-Oxycoumarins
MCF-7
HL-60
U937
Neuro2a
Cytotoxic activity

ABSTRACT

Coumarins are a large family of natural and synthetic compounds exerting different pharmacological effects, including cytotoxic, anti-inflammatory or antimicrobial. In the present communication we report the synthesis of a series of 12 diversely substituted 4-oxycoumarin derivatives including methoxy substituted 4-hydroxycoumarins, methyl, methoxy or unsubstituted 3-aryl-4-hydroxycoumarins and 4-benzyloxycoumarins and their anti-proliferative effects on breast adenocarcinoma cells (MCF-7), human promyelocytic leukemia cells (HL-60), human histiocytic lymphoma cells (U937) and mouse neuroblastoma cells (Neuro2a). The most potent bioactive molecule was the 4-hydroxy-5,7-dimethoxycoumarin (compound **1**) which showed similar potency (IC_{50} 0.2–2 μM) in all cancer cell lines tested. This non-natural product reveals a simple bioactive scaffold which may be exploited in further studies.

Natural products, with their inherent ability to interact with biological targets, represent a significant source of inspiration for drug discovery and development.[1] A primary aim in medicinal chemistry is the design of structural analogues of bioactive natural products with an improved pharmacological profile. Such examples have been provided with anticancer agents derived from natural products, such as microtubule targeting drugs.[2]

In the current report, we present the synthesis and biological activity of a series of 4-oxycoumarin derivatives, which show substitution patterns that are not found in nature. Coumarins are a large class of natural and/or synthetic products that have attracted interest because of their remarkable array of biological activities, usually associated to a low toxicity.[3–9] The pharmacological and biochemical properties of coumarins depend upon the pattern of substitution of the naturally occurring scaffold. Potential anti-tumour effects of coumarin derivatives have been previously examined.[10–12]

The naturally occurring 7-hydroxycoumarin derivatives have been investigated as potential lead structures for cancer drug development and the related compounds scopoletin (7-hydroxy-6-methoxycoumarin) and esculetin (6,7-dihydroxycoumarin) (Fig. 1) show anti-proliferative effects in several tumor cell lines and thus have been proposed as potential anticancer agents.[12–16]

4-Hydroxycoumarins constitute another important class of coumarin derivatives. Many of them display pharmacological activities including anticoagulant,[14] antibacterial[17] or anti-inflammatory.[18] It has been shown that 4-hydroxycoumarins such as compound (A) (Fig. 1) bearing an aryl group in the 3 position, inhibit cell proliferation.[19] Furthermore, a recent study shows that the

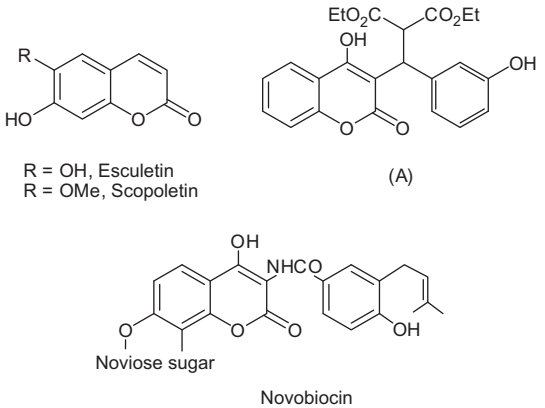

Figure 1. Chemical structures of esculetin, scopoletin, compound (A) and novobiocin.

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http://dx.doi.org/10.1016/j.bmcl.2012.07.099

most simple 4-hydroxycoumarin leads to a selective cytoskeleton disorganization in melanoma cells without affecting a non-tumoral fibroblastic cell line.[20] This effect is correlated with the reduction in cell adhesion and inhibition of random motility. Because adhesion of tumor cells to extracellular matrix is required during the metastatic process, the 4-hydroxycoumarin has been suggested as useful treatment in the adjuvant therapy for melanoma.[20]

Novobiocin (Fig. 1), a member of the amino-4-hydroxycoumarin family, has been shown to possess anticancer activity, and several derivatives were prepared based on structural modifications in the amide side chain, the coumarin ring, and/or the sugar moiety.[21]

However, the most simple 4-oxycoumarin scaffold comprising a bicyclic ring system has not been explored in sufficient detail. Therefore, taking into account previous data, in the present paper we report the synthesis of a series of 12 different 4-oxycoumarin derivatives, structurally related to the mentioned compounds, but exhibiting a new pattern of substitution that does not occur in nature and is not present in previously tested compounds: two simple 4-hydroxycoumarins dimethoxy substituted in 5 and 7 or in 7 and 8 positions (compounds **1–2**); 4-hydroxycoumarins in which the group present in 3 position in compound (A) or in novobiocin is substituted by a phenyl ring (compounds **3–8**) and finally different substituted 4-(benzyloxy)coumarins (compounds **9–12**).

Different methods have been reported for the synthesis of the simple 4-hydroxycoumarin moiety: condensation of *o*-hydroxyacetophenones with diethyl carbonate in the presence of an alkali metal[22] or cyclocondensation of malonates with phenols in presence of anhydrous aluminum chloride at about 180 °C.[23]

For the sake of an easily reproducible methodology, we have synthesized in good yields the compounds **1–2** using appropriately disubstituted phenols, malonic acid, $ZnCl_2$ as Lewis agent and phosphorus oxychloride ($POCl_3$), as condensing agent (Scheme 1).[23]

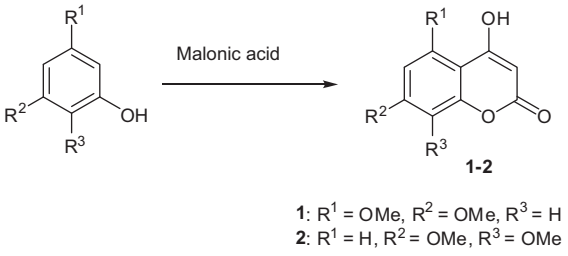
First, the synthesis of the compounds **3–8**[24–29] was achieved by the preparation of different phenyliodonium coumarinate species (**I–II**) starting from the corresponding 3-unsubstituted 4-hydroxycoumarin. Then we carried out the palladium-catalyzed Suzuki coupling reaction between phenyliodonium zwitterions and the conveniently substituted phenyl boronic acids to afford the final compounds (Scheme 2).

The preparation of compounds **9–12** is outlined in Scheme 3. For compounds **9** and **10**, the synthesis has been executed by reaction of commercially available 4-hydroxycoumarin and 4-hydroxy-6-methylcoumarin with benzyl chloride in EtOH in the presence of anhydrous potassium carbonate.[30,31] Compounds **11** and **12** were obtained by reaction of the previously synthesized compounds **3** and **4** with benzyl bromide in acetone.[32,33]

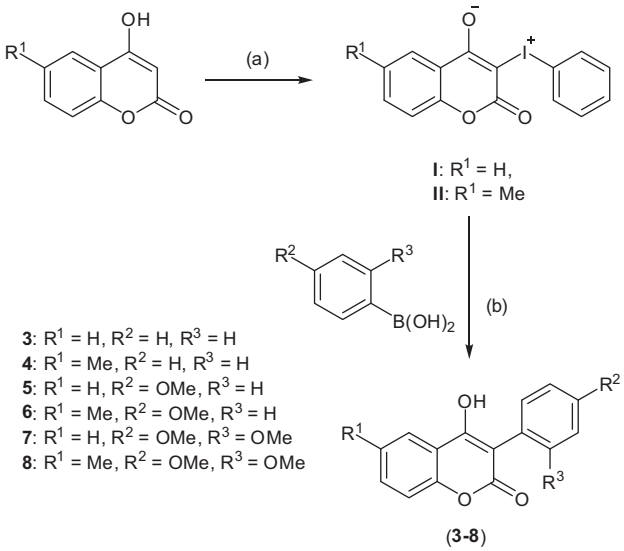
The inhibition of cell proliferation by these diversely substituted coumarins **1**–**12** was then evaluated in vitro using the human breast MCF-7 cells and the human promyelocytic leukemia HL-60 cells.

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.[34,35]

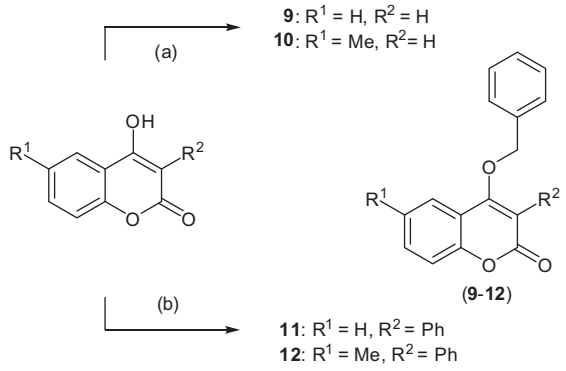
Inhibition of cell proliferation was expressed as percentage of viable cells in treated samples as compared to vehicle-treated cells and the 50% inhibitory concentration of cell proliferation (IC_{50}) was calculated by nonlinear least squares curve fitting (GraphPad Software, San Diego, CA, USA). Each value was obtained from two/three



Scheme 1. Reagents and conditions: $ZnCl_2$, $POCl_3$, reflux, 48 h.



Scheme 2. 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.



Scheme 3. Reagents and conditions: (a) $PhCH_2Cl$, K_2CO_3, EtOH, reflux, 6 h; (b) $PhCH_2Br$, K_2CO_3, acetone, reflux, 6 h.

Table 1
Percentage of cell viability ± SEM for the synthesized compounds **1**–**12** at 10 μM

Compounds	Percentage cell viability ± SEM	
	MCF-7	HL-60
1	17.1 ± 0.9	23.2 ± 0.1
2	114.2 ± 3.9	28.9 ± 0.6
3	96.7 ± 5.6	73.4 ± 4.6
4	72.6 ± 0.6	73.9 ± 1.4
5	114.9 ± 8.4	86.6 ± 3.1
6	106.2 ± 3.7	83.9 ± 2.0
7	91.9 ± 5.3	100.4 ± 3.1
8	69.0 ± 6.6	74.4 ± 11.4
9	95.0 ± 2.1	72.7 ± 5.5
10	104.3 ± 2.2	76.0 ± 1.3
11	113.0 ± 10.2	94.7 ± 2.0
12	53.5 ± 0.1	92.3 ± 3.7