Efficient synthesis of coumarin-chalcones hybrids as new scaffold with antibacterial interest.

Saleta Vazquez-Rodriguez[a *], Silvia Serra[a] , Ysabel Santos[b] and Lourdes Santana[a]

[a] *Department of Organic Chemistry, Faculty of Pharmacy, University of Santiago de Compostela, 15782, Spain.*

[b] *Departement of Microbiology and parasitology, CIBUS building-Faculty of Biology, Univesrsity of Santiago de Compostela, 15782, Spain.*

* *E-mail: svre77@hotmail.com*

Abstract: Due to the potential antibacterial activity of the chalcone and coumarin moieties, hybrid compounds containing both structures have been synthesized in good yield using the Knoevenagel reaction as the key step.

Keywords: Coumarin, Chalcone, Knoevenagel , Antibacterial

Introduction

Chemotherapy, in its most general sense, is the treatment of diseases by chemicals especially by killing micro-organisms or cancerous cells. Nowadays are known a wide range of different chemotherapeutic agents. In this sense, the emergence of multyidrug-resistance bacteria has made treatment of infectious diseases difficult. This means that it is necessary the discovery of novel antibacterial agents.

One of our aims in the last years has been the development of new tools and methodologies for drug discovery. The molecular manipulation of promising lead compounds is still a major line of approach to develop new and efficient drugs. Following this aim we designed hybrid molecules coming from two natural occurring compounds: coumarin and chalcones. Coumarin derivatives have well known

pharmacological activities such as antibacterial, antitumor, anti-inflammatory, antithrombotic, cardio protectors or enzymatic inhibitors.[1-5]

Chalcones (a,β-unsaturated ketones) are an important group of natural or synthetic flavonoids that are know to exhibit an impressive array of biological properties[6-8]. Particularly, their antimicrobial and antifungal action is attributed to the reactive enone moiety[9]. As Michael acceptor enone, reactions of chalcones are modulated by electron withdrawing/donating character of sustituents at the *p*-positions of the aromatic groups.

Therefore in the present study, the chalcone functionality has been attached in a coumarin nucleus. The new scaffold incorporate two Michel enones in a single molecule and introducing different sustituents in the aromatic ring allow us to modulate the acceptor character for the thiol nucleophilic attack of microbial proteins.

Using this strategy a series of 3-cinnamoylcoumarin derivatives has been synthesized as potential antibacterial compounds.

Results and Discussion

The aim of this work has been to synthesize new coumarin-chalcone hybrids containing different sustituents in the aromatic rings that can potentially be used as new lead compounds in drug discovery, particularly as antimicrobial agents.

Compounds were synthesized using a two steps synthetic strategy that allows us to obtain the desired compounds in good yields (***Figure 1***)

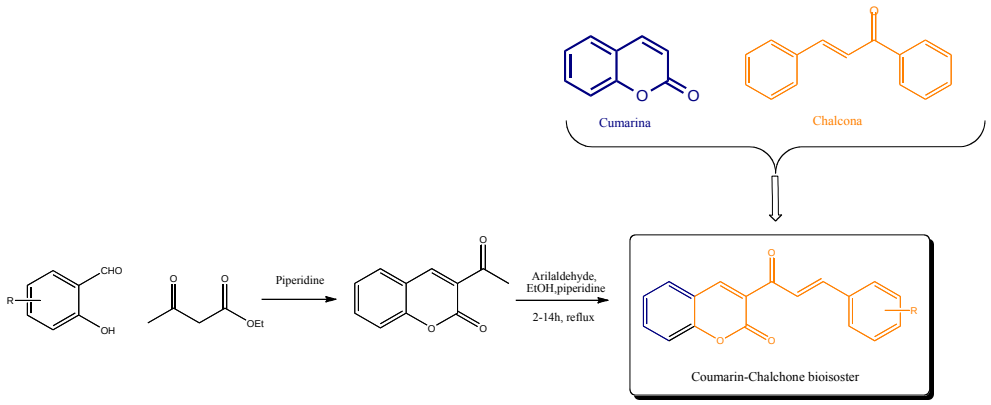
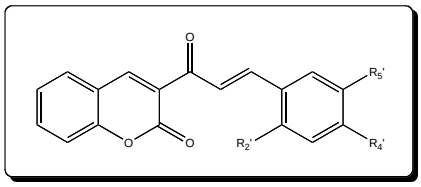


Figure 1*. Synthetic route used to hybrids of coumarin-chalcone.*

The 3-acetylcoumarin precursor was prepared by a Knoevenagel reaction in basic conditions in 89% yield. The final step was a Claisen-Schmidt aldolic condensation in basic conditions that allow us to obtain the final compounds in good yield (***Table 1***)

Table 1. *Synthesized compounds*



Compound	R_2'	R_4'	R_5'	Yield (%)
2	H	H	H	60
3	H	OMe	H	61
4	OMe	OMe	H	80
5	OMe	OMe	OMe	50
6	OMe	NO_2	H	67
7	H	H	NO2	53

The followed methodologies in a parallel synthesis way, bring the opportunity of synthesize structural related compounds with punctual modifications in the aromatic rings depending on the starting materials.

Biological assays as antibacterial agents will be further presented.

General Experimental Procedure

All reactions were carried out under dry and deoxygenated argon atmosphere. Solvents were used as anhydrous by reflux of each solvent over an appropriate dryer agent and further distillate under argon atmosphere.

Qualitative identification of the compounds and course of the reactions were visualized using TLC plates (Merck, silica gel $60F_{254}$) under UV light (254-366 nm). Melting points were determined using a Reichert Kofler thermopan or in capillary tubes on a Büchi 510 apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker WM-250 at 250 MHz using TMS as internal standard (chemical shifts in δ values, *J* in Hz).

Synthesis of 3-acetylcoumarin: A mixture of salicilaldehyde (1 eq.), ethyl acetoacetate (1 eq.) and a few drops of piperidine were mixed for 5 min. at room temperature without

any solvent. Reaction was neutralized with HCl (1M) and finally the product was isolated by filtration. The final compound was then recristalized in EtOH[10].

3-Acetylcoumarin (1): Yield 89%, **Mp.:** 119-121 ºC. **^{1}H NMR** (250 MHz, $CDCl_3$) δ ppm 8.34 (s, 1H), 7.54 – 7.42 (m, 2H), 7.26 – 7.12 (m, 2H), 2.56 (s, 3H).

General procedure for the synthesis of 3-cinnamoylcoumarins (2-7): A mixture of 3-acetylcoumarin (1 eq.) and te corresponding benzaldehyde (1.2 eq.) in EtOH was stired with a few drops of piperidine under reflux during 2-12h. Mixture was cooled and the resulting solid was filtered and purified by recristalization or flash chromatography. Purification of compounds **2-5** was made by recristalization in MeOH, while compounds **6-7** were purified by flash chromatography using a 8:2 mixture of Hexane:AcOEt as eluent.

3-Cinnamoylcoumarin (2): Yield 60% **Mp.:** 202-204 **^{1}H-NMR** (250 MHz, $CDCl_3$) δ ppm 8.60 (s, 1H), 7.92 (d, *J* = 7.9 Hz, 2H), 7.73 – 7.61 (m, 4H,), 7.45 – 7.35 (m, 5H).

3-(4'-Methoxicinnamoyl)cumarin (3): Yield 60% **Mp:** 202-203 ºC. **^{1}H NMR** (250 MHz, $CDCl_3$) δ ppm 8.40 (s, 1H), 7.71 (d, *J* = 15.8 Hz, 1H), 7.62 (d, *J* = 15.8 Hz, 1H) 7.48 (m, 4H), 7.29 – 7.11 (m, 2H), 6.76 (d, *J* = 8.8 Hz, 2H), 3.69 (s, 3H).

3-(2',4'-Dimethoxicinnamoyl)cumarin (4): Yield 80% **Mp.:**192-194ºC. **^{1}H NMR** (250 MHz, $CDCl_3$) δ ppm 8.37 (s, 1H), 8.00 (d, *J* = 15.8 Hz, 1H), 7.72 (d, *J* = 15.8 Hz, 1H), 7.56 – 7.36 (m, 3H), 7.29 – 7.11 (m, 2H), 6.36 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.29 (d, *J* = 2.2 Hz, 1H), 3.73 (s, 3H), 3.69 (s, 3H).

3-(2',4',5'-Trimethoxicinnamoyl)cumarin (5): Yield 50%. **Mp:** 190-192 ºC. **^{1}H NMR** (250 MHz, $CDCl_3$) δ ppm 8.38 (s, 1H), 8.05 (d, *J* = 15.8 Hz, 1H), 7.65 (d, *J* = 15.8 Hz, 1H), 7.55-7.40 (m, 2H), 7.28 – 7.12 (m, 2H), 7.01 (s, 1H), 6.33 (s, 1H), 3.78 (s, 3H), 3.74 (s, 3H), 3.73 (s, 3H).

3-(2'-Methoxy-4'-nitrocinnamoyl)coumarin (6): Yield 57% **Mp:** 227-229 ºC. **^{1}H NMR** (250 MHz, $CDCl_3$) δ ppm 8.57 (s, 1H), 7.96 (d, *J* = 12.6 Hz 1H), 7.84-53 (m, 5H), 7.42 (d, *J* = 8.8 Hz, 2H) 7. 32 (d, *J* = 12.6 *Hz*, 1H), 3.86 (s, 3H).

3-(3'-Nitrocinnamoyl)coumarin (7): Yield 49% **Mp:** 205-207 ºC. . **^{1}H NMR** (250 MHz, $CDCl_3$) δ ppm 7,98 (d, *J* = 7.68 Hz 1H), 7.90 (d, *J* = 1.8 Hz), 7.85 (s,1H) 7.45 (t, *J = 6.57 Hz*, 2H), 7.40 – 7.30 (m, 1H), 7.28-7.17 (m, 5H).

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