Synthesis of various substituted 3-aryl-4-hydroxycoumarins as new possible drugs of the tenacibaculosis disease

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Abstract

Bacterial diseases of freshwater fish epithelia have been extensively studied; however in marine fishes relatively few bacterial skin and gill diseases have been characterized. *Tenacibaculum Maritimum*, a Gram-negative and filamentous bacterium, has been described as the etiological agent of tenacibaculosis in marine fish. Since 1990, as the farming of fish became a steadily growing industry, this disease has been widely distributed in several countries and is considered a limiting factor for the culture of many species of commercial value in the world. Among the drugs used in the past few years enrofloxacin proved to be the most useful compound in the control of tenacibaculosis outbreaks, although the rapid appearance of resistant strains has already been described. Therefore, it is important to discover new classes of selective antimicrobial drugs.

Coumarin derivatives are a class of compounds that present a wide range of pharmacological activities. Among others, recent studies pay special attention to the antibiotic properties of the coumarin moiety. In order to contribute to the development of new agents, in the present communication we have synthesized new 3-arylcoumarin derivatives, structurally related with well know antibiotic novobiocin.

On this scaffold we introduced substituents with different electronic, steric and/or lipophilic properties in different positions of the aromatic rings.

The compounds have been prepared via a palladium-catalyzed coupling type Suzuki reaction of phenyliodonium zwitterion species previously synthesized by us. Further, were evaluated for antibacterial activity against bacterial strains. The preliminary results of biological activity and/or selectivity against Tenacibaculum Maritimum are presented.

Key words: coumarin, *Tenacibaculum Maritimum*, Suzuki reaction.

Introduction

The rapid expansion of the aquaculture industry in the last decade has increased the losses caused by systemic bacterial infections in marine fish farming throughout the world. Bacterial diseases of freshwater fish epithelia have been extensively studied; however in marine fishes relatively few bacterial skin and gill diseases have been characterized. Marine tenacibaculosis, which is caused by *Tenacibaculum Maritimum* (formerly *Flexibacter maritimus*), is an economically important disease in a great variety of European cultured fish.[1] This pathogen, a Gram-negative and filamentous bacterium, primarily attacks skin, mouth, fins and tail of fish, causing severe necrotic and ulcerative lesions on the body surface. The manifestations of this infection depend on the species and age of the fish.

Up to now, most treatments were proposed for the tenacibaculosis outbreaks. Among the drugs used in the past few years the antibiotic enrofloxacin proved to be the most useful compound in the control of tenacibaculosis, although the rapid appearance of resistant strains has already been described.[2] Therefore, it is important to discover new classes of selective antimicrobial drugs.

Coumarins are a large family of compounds, of natural and/or synthetic origin, that presents different pharmacological activities.[3-7] Due to their structurally variability, they occupy an important place in the realm of natural products and synthetic organic chemistry. Among others, recent studies pay special attention to the antibiotic properties of the coumarin moiety.[8-10]

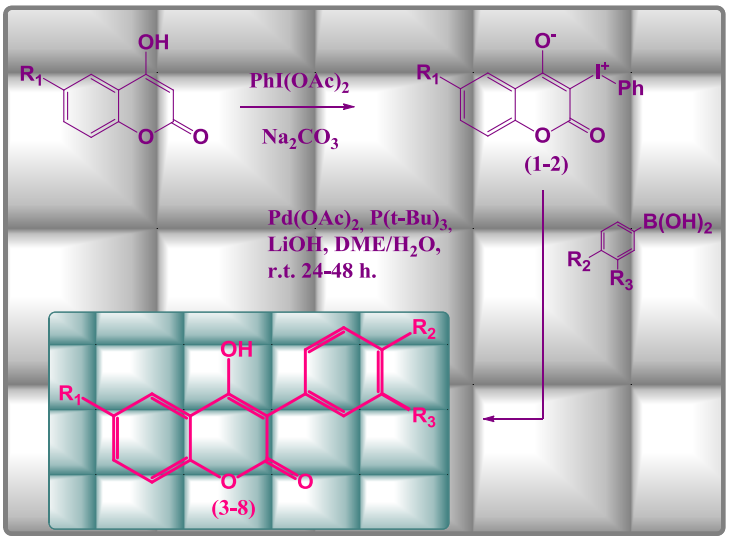
Whit the aim to contribute to the development of new agents, in the present communication we report the synthesis of new 3-arylcoumarin derivatives, via a palladium-catalyzed coupling type Suzuki reaction.

Results and discussion

In the present work we have designed and evaluated a series of 3-phenylcoumarin derivatives with different number of chloro substituent in both the 3-aryl ring and coumarin nucleus. The compounds were synthesized according to Scheme 1 and details are given in the Experimental section.[11]

The key step for the synthesis of the 3-arylcoumarin skeleton was achieved by a palladium-catalyzed coupling Suzuki reaction between phenyliodonium zwitterions species previously synthesized by us and aryl conveniently substituted boronic acids.

We have used 2 differents commercial coumarins to produce two coumarin-based phenyliodonium zwitterions (**1-2**). Then we have carried on the palladium-catalyzed coupling reaction to introduce the aryl group at the 3 position of coumarin scaffold (Compounds **3-8,** Table 1).



Scheme 1

Compounds	R1	R2	R3	Yield %	M.P.
3	H	H	H	63	237-239 °C
4	Cl	H	H	68	263-265 °C
5	H	Cl	H	52	267-269 °C
6	Cl	Cl	H	66	314-316 °C
7	H	Cl	Cl	51	321-323 °C
8	Cl	Cl	Cl	43	347-349 °C

Table 1

All the compounds were screened for their in vitro antibacterial activity by disk diffusion method. Most of them were inactive against different species of Gram-pos. and Gram-neg. organisms, but surprisingly all tested compounds have be found to exhibit antibacterial activity against *Tenacibaculum Maritimum*, showing significant zone of inhibition of the growth of the bacteria in the agar plates (Table 2). The general procedure[12] and details are given in the Experimental section.

The compound **6** shows the best activity with a zone of inhibition of 38 mm. (Photo 1)

Compounds	Diameter of the zone of inhibition of the growth of ***T. Maritimum***
3	27 mm
4	18 mm
5	20 mm
6	38 mm
7	25 mm
8	21 mm

Table 2: Evaluation of diameter of the zone of inhibition of *Tenacibaculum Maritimum* inhibitory of compounds **3**–**8.**

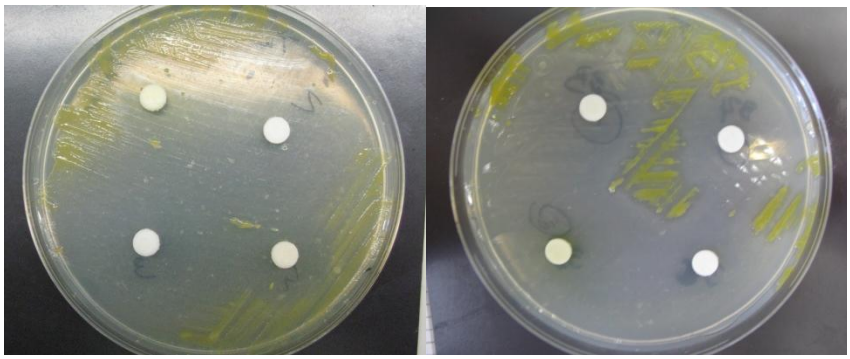


Photo1: zone of inhibition of compound **3** and **6** respectively.

Conclusions

In summary, we report a versatile methodology for the synthesis of a new library of coumarin derivatives in a short number of steps and satisfactory yields. The compounds were then evaluated for antibacterial activity against different bacterial and fungal strains. The preliminary results indicate that most of the compounds showed remarkable antibacterial activity and interesting selectivity against *Tenacibaculum Maritimum*, especially compound 6 with an inhibition zone of 38 mm. These results suggest that this new scaffold can be further optimized for building potent and selective antibacterials used in Tenacibaculosis disease.

Experimental section

Chemistry

All reactions were carried out under dry and deoxygenated argon atmosphere. Identification of the compounds and course of the reactions were visualized using TLC plates (Merck, silica gel 60F254) under UV light (254-366 nm).

Melting points were determined using a Reichert Kofler thermopan or in capillary tubeson a Buchi 510 apparatus and are uncorrected.

General procedure for the preparation of 3-phenyliodonium coumarinates (**1-2**).

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). 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 in the next reaction without further purification.

General procedure for the preparation of 3-aryl-4-hidroxycoumarins (**3-8**).

A degassed solution of appropriated phenyl boronic acid (2.2 equiv.) and P(*t*-But)$_3$ (27 mL) in DME and H_2O (4:1, 12.5 mL) was added to a mixture of iodonium ylide (0.55 mmol), LiOH/H_2O (3 equiv.) and

$Pd(OAc)_2$ (6.2 mg) under argon at room temperature and stirred for 24-48 h. The mixture was then purified by FC (hexane:ethyl acetate, 7:3) to give the desired compound. Yields 43-68 %.

Biological assay

The Antibacterial activity was assessed by disk diffusion method, following the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) (2006). [12] To this aim, were recoated sterile discs (Liofilchem Bacteriology Products, Italy) with the synthesized compounds at final concentrations of 10 and 100 μg/disc and were applied on the surface of agar plates Mueller Hinton Agar (Difco) previously inoculated with the bacterial strains. The plates were incubated at 37 ºC during 24h and it was determined the diameter of the growth zone of inhibition as an index of their sensitivity to the antimicrobials tested.

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