Synthesis of novel amino/nitro substituted 3-arylcoumarins as antibacterial agents

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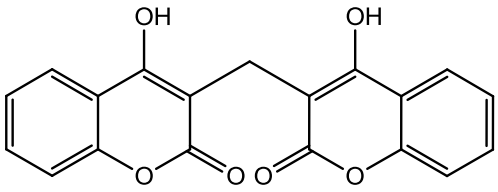
With the aim of exploring the antibacterial interest of the 3-arylcoumarinic skeleton, a new series of amino/nitro substituted 3-arylcoumarins was synthesized and evaluated for their antibacterial activity against clinical isolates *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). This series was selected thinking on finding out the structural features for their antibacterial activity and selectivity. Therefore, in the present manuscript different positions of nitro, methyl, methoxy, amino and bromo substituents, under the 3-arylcoumarin scaffold, were reported. Some of these molecules exhibited antibacterial activity against *S. aureus* comparable to the standards – oxolinic acid and ampicillin. The best derivative of the studied series is compound **6**, active and selective against *S. aureus*, with a MIC = 8 µg/mL. The preliminary structure-activity relationship (SAR) study showed that the antibacterial activity against *S. aureus* depends on the position of the substitution pattern on the 3-arylcoumarin moiety. Compound **6** will be the inspiration for the search of new active and selective antibacterial compounds.

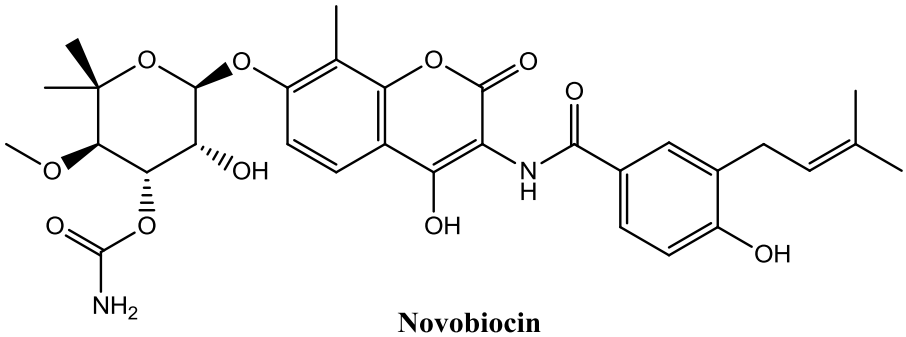
Keywords: Amino/nitro substituted 3-arylcoumarins; Perkin reaction; Antibacterial assays; *S. aureus*; *E. coli*.

Introduction

Drug discovery is considered a complex and slow activity. However, new approaches and methods have been developed by the medicinal chemistry researchers with the intention of discovering new chemical entities in a new and more efficient way. Some of the new products are based on a diversity of molecules found and extracted from natural sources. Coumarins are a large family of compounds of natural or synthetic origin, associated with remarkable pharmacological activities (Borges et al. 2005; Borges et al. 2009). They occur naturally in plants and microorganisms and approximately 1000 coumarin derivatives have been isolated from over 800 species (Borges et al. 2009). The fused heterocyclic framework of coumarins has been used as a prototype scaffold for the synthesis of a wide variety of analogues in order to study and improve their biological properties. Its structural variety is responsible for the important place that they occupy in the natural products and synthetic organic chemistry realm (Borges et al. 2009; Chilin et al. 2008). Some coumarins have been studied for their antimicrobial (Hopper et al. 1982; Ostrov et al. 2007; Chimenti et al. 2010), cardioprotective (Vilar et al. 2006), anticancer (Riveiro et al. 2008; Belluti et al. 2010), antioxidative (Roussaki et al. 2010) and enzymatic inhibition properties (Fais et al. 2009; Matos et al. 2010; Matos et al. 2011; Delogu et al. 2011). The antibacterial properties of coumarins were described for the first time in 1945 by Goth when they studied the dicoumarol (Figure 1). Some other coumarin derivatives have proved to display antibacterial and antifungal activities (Hoettecke et al. 2008; Basanagouda et al. 2010; Shi et al. 2011; Patil et al. 2011; Sabry et al. 2011). A study of coumarin derivatives substituted on the pyrone ring indicated that 3-carboxyl derivatives show significant activities (Kawase et al. 2001). Different 4-substituted coumarins, such as 4-chlorocoumarins, exhibit also an interesting antimicrobial profile (Patel et al. 2010). Novobiocin (**Figure 1**), chlorobiocin and coumermycin A_1 are important natural occurring antibiotics in which coumarin nucleus is present in their skeleton (Laurin et al. 1999).



Dicoumarol



Novobiocin

Figure 1. Chemical structures of dicoumarol and novobiocin.

The most active of them is novobiocin, isolated from *Streptomyces niveus*, which is mainly active against Gram-positive bacteria. Although they antagonize the B subunit of the essential *E. coli* DNA gyrase supertwisting activity *in vitro* and the bacterial multiplication, they are not used in clinic. This is due their relatively weak activity against the Gram-negative bacteria, side effects and poor water solubility (Hopper et al. 1982; Laurin et al. 1999). On the other hand, these three examples are antibiotics which present activity against methicillin-resistant *S. aureus* (MRSA) (Laurin et al. 1999). The number of multidrug-resistant (MDR) bacteria is increasing and of particular importance is Gram-positive MRSA. The ability of *S. aureus* species to develop resistance to virtually all antibiotics is a major concern and the discovery of new antimicrobial agents is essential to combat this problem (Nathan 2004). MRSA are often an important problem for severe infections in patients, especially immunesuppressed persons, during long stays in hospitals (Nathan 2004). The dramatic world-wide increase of dangerous infections by resistant and multi-resistant microbes makes, therefore, the search of new molecules and new chemical entities an important topic in medicinal chemistry (Rachakonda et al. 2004; Walsh 2003). As the ideal drug candidate has not been attained, an intensive search for new and innovative antimicrobials is still needed. Due to economic reasons, unfortunately, pharmaceutical companies have considerably decreased this effort in recent years (Von Nussbaum et al. 2006).

Experimental

Chemistry

The entire chemicals used in the synthesis were supplied by Aldrich and Merck. Melting points were determined using a Reichert Kofler thermopan or in capillary tubes on a Büchi 510 apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1640FT spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX spectrometer at 300 and 75.47 MHz, respectively, using TMS as internal standard (chemical shifts in δ values, *J* in Hz). Mass spectra were obtained using a Hewlett Packard 5988A spectrometer. Elemental analyses were performed using a Perkin-Elmer 240B microanalyser and were within ± 0.4% of calculated values in all cases. Silica gel (Merck 60, 230–00 mesh) was used for flash chromatography (FC). Analytical thin layer chromatography (TLC) was performed on plates precoated with silica gel (Merck 60 F254, 0.25 mm). The purity of compounds **1-11** was assessed by HPLC and was found to be higher than 95%.

General Procedure for the preparation of 3-arylcoumarins (1-10): In a 20 mL dry Schlenk tube, to a solution of the conveniently substituted salicylaldehyde (2.46 mmol) and the arylacetic acid (2.46 mmol) in acetic anhydride (6 mL), NaH (2.46 mmol) was added, in small portions, and the reaction mixture was stirred for 3 hours, at room temperature. The obtained crude was filtered and washed with diethyl ether. The solid was then purified by flash chromatography (hexane/ethyl acetate 9:1) to give the desired coumarins (**1**–**10**).

3-(4'-Nitrophenyl)-6-nitrocoumarin (1) Yield 71%; m.p. 252-253 ºC (biblio. Girouard et al. 2005, m.p. 250 ºC)

3-(4'-Methoxyphenyl)-6-nitrocoumarin (2) Yield 75%; m.p. 62-63 ^{o}C; ^{1}H NMR ($CDCl_3$-300 MHz) *δ*: 3.80 (s, 3H, OCH_3), 7.05 (d, 2H, H-3', H-5', J=9.45), 7.62-7.69 (m, 3H, H-2', H-6', H-8), 8.36 (s, 1H, H-4), 8.73 (d, 2H, H-5, H-7, J=2.93). Mass (*m/z*): 297 (M^+, 100).

3-(3´-Methylphenyl)-6-nitrocoumarin (3) Yield 77%; m.p. 209-210 ºC (biblio. Piazzi et al. 2007, m.p. 210-211 ºC)

3-(3'-Nitrophenyl)-6-nitrocoumarin (4) Yield 69%; m.p. 87-88 ^{o}C; ^{1}H NMR ($CDCl_3$-300 MHz) *δ*: 7.57-7.63 (m, 2H, H-8, H-6'), 7.71-7.77 (m, 1H, H-5'), 8.15 (s, 1H, H-4), 8.42-8.63 (m, 4H, H-4', H-2, H-5, H-7). Mass (*m/z*): 312 (M^+, 100).

3-(3'-Methoxyphenyl)-6-nitrocoumarin (5) Yield 71%; m.p. 53-54 ^{o}C; ^{1}H NMR ($CDCl_3$-300 MHz) *δ*: 3.71 (s, 3H, OCH_3), 6.80 (s, 1H, H-2'), 7.17-7.22 (m, 3H, H-4', H-5', H-6'), 7.57 (d, 1H, H-8, J=6.5), 7.77 (s, 1H, H-5), 8.19 (d, 1H, H-7 J=6.5), 8.39 (s, 1H, H-4). Mass (*m/z*): 297 (M^+, 100).

3-(3'-Methylphenyl)-6-nitrocoumarin (6) Yield 69%; m.p. 55-56 ^{o}C; ^{1}H NMR ($CDCl_3$-300 MHz) δ: 2.25 (s, 3H, CH_3), 6.90-7.30 (m, 5H, H-2', H-4', H-5', H-6', H-8), 7.75 (s, 1H, H-4), 8.44 (d, 1H, H-7, J=2.8), 8.62 (d, 1H, H-5, J=2.8). Mass (*m/z*): 281 (M^+, 100).

3-(3'-Bromophenyl)-6-nitrocoumarin (7) Yield 60%; m.p. 240-241 ^{o}C; ^{1}H NMR ($CDCl_3$-300 MHz) δ: 7.12-7.26 (m, 2H, H-4', H-5'), 7.46-7.71 (m, 5H, H-2', H-6', H-5, H-7, H-8), 7.78 (s, 1H, H-4). Mass (*m/z*): 345 (M^+, 100).

3-(4´-Nitrophenyl)-6-methoxycoumarin (8) Yield 72%; m.p. 275-276 ºC (biblio. Rao et al. 1972, m.p. 278 ºC)

6-Methoxy-3-(3'-nitrophenyl)coumarin (9) Yield 73%; m.p. 84-85 ^{o}C; ^{1}H NMR ($CDCl_3$-300 MHz) δ: 3.77 (s, 3H, OCH_3), 7.54-7.59 (m, 2H, H-5', H-6'), 7.71 (d, 1H, H-8, J=7.6), 8.06-8.09 (m, 2H, H-2', H-4'), 8.15 (s, 1H, H-4). Mass (*m/z*): 297 (M^+, 100).

6-Methyl-3-(4'-nitrophenyl)coumarin (10) Yield 75%; m.p. 100-101 ^{o}C; ^{1}H NMR ($CDCl_3$-300 MHz) δ: 2.36 (s, 3H, CH_3), 7.21 (d, 2H, H-7, H-8, J=8.2), 7.38 (s, 1H, H-5), 7.63-7.70 (m, 2H, H-2', H-6'), 7.99-8.20 (m, 2H, H-3', H-5'), 8.38 (s, 1H, H-4). Mass (*m/z*): 281 (M^+, 98).

Procedure for the preparation of 3-(3'-Aminophenyl)-6-aminocoumarin (11) The previously prepared nitro-3-(arylnitro)coumarin **4** (2.46 mmol) was dissolved in ethanol (5 mL) and a calatytic amount of Pd/C was added to the mixture. The solution was stirred, at room temperature, under H_2 atmosphere, for 3 hours. After the completion of the reaction, the mixture was filtered to eliminate the catalyst. The obtained crude was then purified by flash chromatography (hexane/ethyl acetate 9:1) to give the desired coumarin **11**. Yield 80%; m.p. 105-106 ^{o}C; ^{1}H NMR ($CDCl_3$-300 MHz) δ: 4.02 (s, 2H, NH_2), 6.50-6.55 (m, 1H, H-4') 6.56 (s, 1H, H-2'), 6.69-6.73 (m, 1H, H-6') 6.80 (dd, 2H, H-5, H-7, J=7.8, J=2.4), 7.30-7.38 (m, 2H, H-5', H-8), 8.4 (s, 1H, H-4). Mass (*m/z*): 252 (M^+, 100).

Microbiology

Disk Diffusion Test (method A)

The antimicrobial activity of the compounds was assayed by the disk diffusion method, following the procedures of the Clinical and Laboratory Standards Institute (CLSI, 2006). The inoculum was prepared by making a saline suspension of colonies from a 24 h Mueller Hinton Agar (MHA) (Cultimed, Spain) plate culture of the microorganisms, containing approximately $1x10^8$ colony forming units (CFU)/mL (OD_{620} of 0,09). Colony counts on inoculum suspension were verified by the plate dilution method using MHA plates and counting the bacterial colonies produced. The entire surface of the MHA plate was inoculated by streaking with the swab containing the inoculum. Antimicrobial solution (concentration of 10000 µg/mL) was

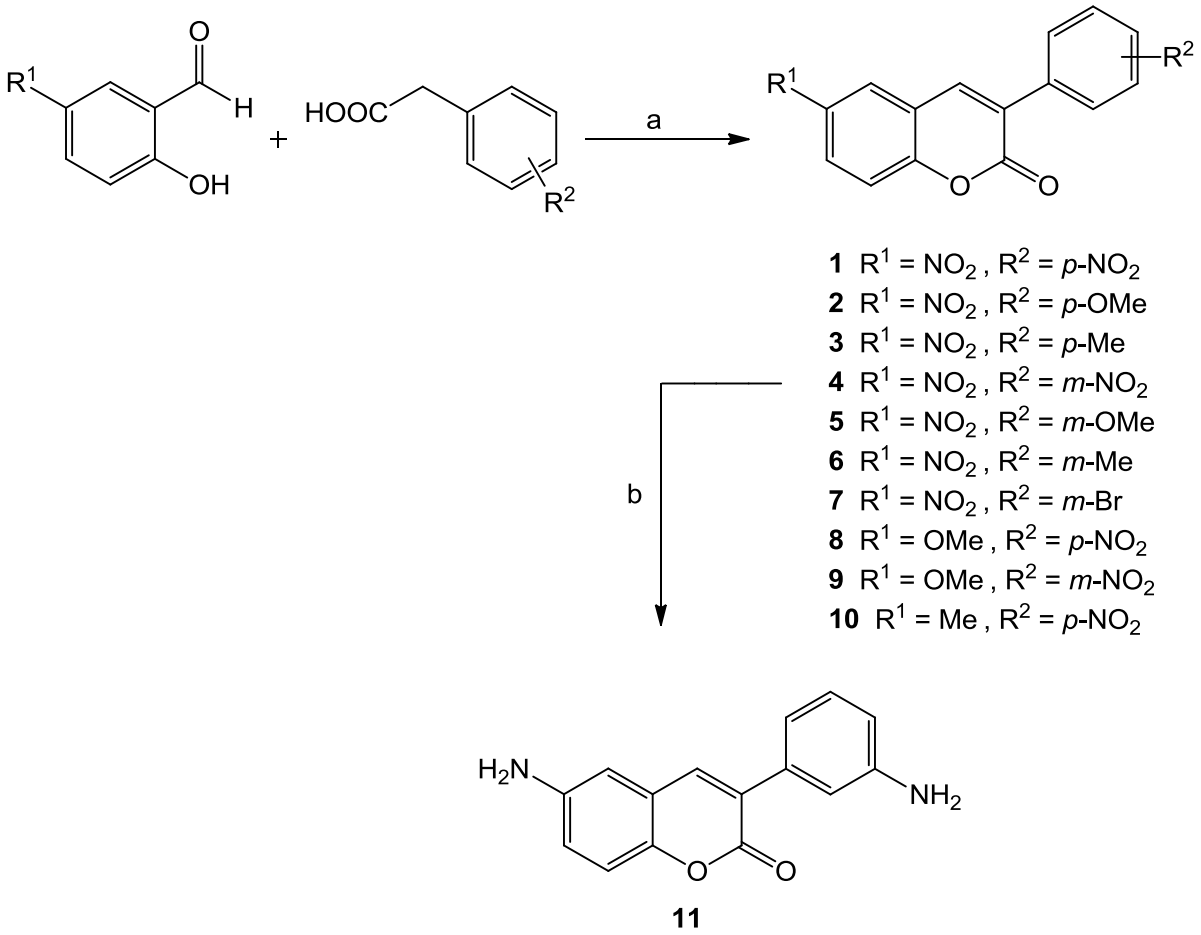
prepared by using DMSO as solvent. Sterile disks of 6 mm diameter (Liofilchem, Italy) embedded in the drug at a final concentration of 100 µg/disk were kept on agar surface. Sterile disks embedded with DMSO were used as a negative control. The plates were incubated at 37 ºC for 24 h. Zones of inhibition were measured in millimeter.

Minimum inhibitory concentrations (MICs) (method B)

The MICs were evaluated using the broth microdilution method, following the procedures of the Clinical and Laboratory Standards Institute (CLSI, 2006). Broth microdilution tests were performed with 96 sterile flat-bottom microtiter plates (Becton Dickinson Labware Europe). Antimicrobials were serially diluted (512 to 1 µg/mL) in Mueller-Hinton Broth (MHB) (Cultimed, Spain) and then one hundred microliters of each dilution were deposited into each well. The inoculum was prepared by making a MHB suspension of colonies from a 24 h MHA (Cultimed, Spain) plate culture of the microorganisms, containing approximately $1x10^8$ colony forming units (CFU)/mL (OD_{620} of 0,09). The inoculum was diluted 1:100 in MHB to yield $1x10^6$ CFU/mL. Ten microliters of this suspension was inoculated into the each well. Colony counts on inoculum suspension were verified by the plate dilution method using MHA plates and counting the bacterial colonies produced. MHB with and without inoculum was used as growth-control and negative control, respectively. The plates were incubated at 37 ºC for 24 h. The MIC was established comparing the amount of growth in the wells containing the antimicrobial agents with the amount of growth in the growth-control wells both with the unaided eye and by using a photometric device (OD_{620}).

Results and Discussion

Based on previous findings in the area (Patel et al. 2010), and in our experience with substituted 3-arylcoumarins, (Matos et al. 2010; Matos et al. 2011) in the present work we proposed the synthesis and antibacterial evaluation of a series of different substituted amino/nitro 3-arylcoumarins (**Scheme 1**). With the aim of finding out structural features for the antibacterial activity and selectivity, we decided to explore the importance of the nature and position of small groups (methoxy, bromo, nitro, amino and methyl substituents) into both coumarin nucleus and 3-aryl ring (Scheme 1).



Scheme 1. *Reagents and conditions:* (a) NaH, acetic anhydride, r.t., 3 h; (b) H_2, Pd/C, ethanol, r.t., 3 h.

The coumarin derivatives **1-11** (Krishnaswamy et al. 1969; Rao et al. 1972; Kremky 1979; Piazzi et al. 2007) were efficiently synthesized according to the synthetic protocol outlined in scheme 1. 3-Arylcoumarins **1-10** were synthesized in a dry Schlenk tube, with acetic anhydride as solvent, in the presence of sodium hydride, at room temperature, for three hours. The reaction mixture was purified by flash chromatography, using hexane/ethyl acetate, in a proportion of 9:1, as eluent. Starting from different substituted commercially available salicylaldehydes and the respectively substituted phenylacetic acids, we obtained ten derivates in good yields (60-77%). The derivative **11** was obtained from **4**, in ethanol, with palladium/carbon as catalytic and under hydrogen atmosphere, for three hours. The reaction mixture was purified by flash chromatography, using hexane/ethyl acetate, in a proportion of 9:1, as eluent. We obtained the desired derivate in good yield (80%). Then, two different methodologies were employed in the antibacterial evaluation of these compounds: disk diffusion test and microdilution method. Clinical antimicrobial drugs –oxolinic acid and ampicillin– were used as positive controls. The inhibition zones for growth inhibitory effects of

the new compounds and controls were measured in millimetres (Table 1 – method A). The minimum inhibitory concentrations (MICs) of the new compounds and controls were measured in microgram/microlitter (Table 1 – method B).

Table 1. *In vitro* antibacterial activity[a] of amino/nitro substituted 3-arylcoumarins **1-11** (A) expressed as inhibition zones (mm)[b] and (B) expressed as MICs (µg/mL).

Compounds	Method A (mm)		Method B (µg/mL)	
	S. aureus	***E. coli***	***S. aureus***	***E. coli***
1	19	NA	128	-
2	25	NA	128	-
3	22	NA	32	-
4	22	NA	32	-
5	25	NA	64	-
6	32	NA	8	-
7	14	NA	256	-
8	NA	NA	> 512	-
9	NA	NA	> 512	-
10	NA	NA	> 512	-
11	18	NA	128	-
Oxolinic acid	26	31	2	< 1
Ampicillin	32	23	2	8

[a] This results are average results of three experiments.

[b] The compounds were used at the concentration of 100 µg/disk and the inhibition zones are stated in mm.

NA = not active; diameter of inhibition zone ≤ 5 mm.

The obtained inhibition zones revealed that all the 6-nitro derivatives (compounds **1**-**7**) presented antibacterial activity against *S. aureus*, showing inhibition zones between 14 and 32 mm. The amino derivative **11** presented activity against the same bacteria strain. The 3-arylcoumarins nitro substituted only in the 3-aryl ring (compounds **8**-**10**) were inactive against *S. aureus* in the disk diffusion test *in vitro* assays, independently of the relative position of this nitro group (*para* or *meta* positions). From the data, it is shown that compound **6** and ampicillin presented the same inhibition zone against *S. aureus* (32 mm) and a higher inhibition zone than the oxolinic acid (26 mm). So, the preliminary experimental data revealed that some of the

tested compounds showed very interesting activity profile against *S. aureus* when compared with the standards ampicillin and oxolinic acid. Also, the different nature and position of the substituent in the coumarin scaffold seems to influence the antibacterial activity.

In order to deeply study the antibacterial activities and quantify the previously mentioned information, MICs of compounds **1**-**11** against *S. aureus* were also determined. This study was performed against the same bacteria strain, by a microdilution assay with serial dilutions (from 512 to 1 μg/mL) of the synthesized and the reference compounds. MICs of compounds **1**-**11** against *E. coli* were not evaluated, taking into account the lack of inhibition zones in the previous disk diffusion test (inhibition zones ≤ 5 mm). Analyzing the MICs results for *S. aureus*, it is clear that the presence of a nitro substituent at six position of coumarin moiety (compounds **1**-**7**) is significantly better to the activity than its presence only at *meta* or *para* positions of the 3-aryl ring (compounds **8**-**10**).

Compounds with nitro substitution either in the coumarin moiety and in the aryl group (compounds **1** and **4**) are not better than compounds where the nitro in the aryl ring is substituted for a methyl or a methoxy group (compounds **2** and **3** compared to compound **1**, and compounds **5** and **6** compared to compound **4**). However, compound **4** (MIC = 32 μg/mL), with the nitro substituent in *meta* position of the 3-aryl ring, is better than compound **1** (MIC = 128 μg/mL), with the same substituent in *para* position. When there is a nitro substituent in the six position of the coumarin moiety, the presence of a methyl group in position *meta* is the best substitution (compound **6**, MIC = 8 μg/mL). In general, substitutions in position *meta* are more favorable than the *para*, being the most active the *meta*-methyl substituent, followed by *meta*-nitro and finally *meta*-methoxy. A *para*-methyl substituent in the 3-aryl ring (compound **3**, MIC = 32 μg/mL) is also the better substitution in this *para* position. Like the *meta* substitutions, *para*-methyl substitution is better than *para*-nitro and *para*-methoxy substitutions. The introduction of a bromo atom at *meta* position was the worst substitution of the studied 3-aryl-6-nitrocoumarins (compound **7**, MIC = 256 μg/mL). Therefore, the substitutions with bromines were discarded. Comparing the two positions in the 3-aryl ring, the substitutions by methyl groups are more favorable for the desired activity. The substitution of nitro by amino groups, under the 3-arylcoumarins decreases significantly the activity of the described compounds (comparing compounds **4** and **11**).

Conclusions

In conclusion, in the present study it was shown that eight of the eleven synthesized amino/nitro substituted 3-arylcoumarins have inhibitory activity against *S. aureus*. MICs

determination proved that the tested compounds presented different profiles against *S. aureus* due to their substituents, being the 3-(3'-methylphenyl)-6-nitrocoumarin (compound **6**) the best one. A nitro substituent at six position of the coumarin moiety seems to be essential for the antibacterial activity of this kind of compounds. The substitution of nitro by amino groups seems to decrease the described activity. The pharmacological potential of these amino/nitro substituted 3-arylcoumarins confirms that this scaffold could be effectively optimized into a candidate for the treatment of some bacterial infectious diseases.

Acknowledgements

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References

Borges F, Roleira F, Milhazes N, Santana L, Uriarte E (2005) Simple coumarins and analogues in medicinal chemistry: occurrence, synthesis and biological activity. Curr Med Chem 12:887-916.

Borges F, Roleira F, Milhazes N, Uriarte E, Santana L (2009) Simple coumarins: privileged scaffolds in medicinal chemistry. Front Med Chem 4:23-85.

Chilin A, Battistutta R, Bortolato A, Cozza G, Zanatta S, Poletto G, Mazzorana M, Zagotto G, Uriarte E, Guiotto A, Pinna L, Meggio F, Moro S (2008) Coumarin as attractive casein kinase 2 (CK2) inhibitor scaffold: an integrate approach to elucidate the putative binding motif and explain structure-activity relationships. J Med Chem 51:752-759.

Hooper DC, Wolfson JS, McHugh GL, Winters MB, Swartz MN (1982) Effects of novobiocin, coumermycin A1, clorobiocin, and their analogs on Escherichia coli DNA gyrase and bacterial growth. Antimicrob Agents Chemother 22:662-671.

Ostrov DA, Hernández Prada JA, Corsino PE, Finton KA, Le N, Rowe TC (2007) Discovery of novel DNA gyrase inhibitors by high-throughput virtual screening. Antimicrob Agents Chemother 51:3688-3698.

Chimenti F, Bizzarri B, Bolasco A, Secci D, Chimenti P, Granese A, Carradori S, Rivanera D, Zicari A, Scaltrito MM, Sisto F (2010) Synthesis, selective anti-Helicobacter pylori activity, and cytotoxicity of novel N-substituted-2-oxo-2H-1-benzopyran-3-carboxamides. Bioorg

Med Chem Lett 20:4922-4926.

Vilar S, Quezada E, Santana L, Uriarte E, Yanez M, Fraiz N, Alcaide C, Cano E, Orallo F (2006) Design, synthesis and vasorelaxant and platelet antiaggregatory activities of coumarin-resveratrol hybrids. Bioorg Med Chem Lett 16:257-261.

Riveiro ME, Moglioni A, Vazquez R, Gomez N, Facorro G, Piehl L, de Celis ER, Shayo C, Davio C (2008) Structural insights into hydroxycoumarin-induced apoptosis in U-937 cells. Bioorg Med Chem 16:2665-2675.

Belluti F, Fontana G, Bo L, Carenini N, Giommarelli C, Zunino F (2010) Design, synthesis and anticancer activities of stilbene-coumarin hybrid compounds: identification of novel proapoptotic agents. Bioorg Med Chem 18:3543-3550.

Roussaki M, Kontogiorgis C, Hadjipavlou-Litina DJ, Hamilakis S, Detsi A (2010) A novel synthesis of 3-aryl coumarins and evaluation of their antioxidant and lipoxygenase inhibitory activity. Bioorg Med Chem Lett 20:3889-3892.

Fais A, Corda M, Era B, Fadda MB, Matos MJ, Quezada E, Santana L, Picciau C, Podda G, Delogu G (2009) Tyrosinase inhibitor activity of coumarin-resveratrol hybrids. Molecules 14:2514-2520.

Matos MJ, Viña D, Quezada E, Picciau C, Delogu G, Orallo F, Santana L, Uriarte E (2009) A new series of 3-phenylcoumarins as potent and selective MAO-B inhibitors. Bioorg Med Chem Lett 19:3268-3270.

Matos MJ, Viña D, Janeiro P, Borges F, Santana L, Uriarte E (2010) New halogenated 3-phenylcoumarins as potent and selective MAO-B inhibitors. Bioorg Med Chem Lett 20:5157-5160.

Matos MJ, Terán C, Pérez-Castillo Y, Uriarte E, Santana L, Viña D (2011) Synthesis and study of a series of 3-arylcoumarins as potent and selective monoamine oxidase B inhibitors. J Med Chem 54:7127–7137.

Delogu G, Picciau C, Ferino G, Quezada E, Podda G, Uriarte E, Viña D (2011) Synthesis, human monoamine oxidase inhibitory activity and molecular docking studies of 3-heteroarylcoumarin derivatives. Eur J Med Chem 49:1147-1152.

Goth A (1945) The antibacterial properties of dicumarol. Science 101:383.

Hoettecke N, Rotzoll S, Albrecht U, Lalk M, Fischer C, Langer P (2008) Synthesis and antimicrobial activity of 2-alkenylchroman-4-ones, 2-alkenylthiochroman-4-ones and 2-alkenylquinol-4-ones. Bioorg Med Chem 16:10319-10325.

Basanagouda M, Shivashankar K, Kulkarni MV, Rasal VP, Patel H, Mutha SS, Mohite AA (2010) Synthesis and antimicrobial studies on novel sulfonamides containing 4-azidomethyl

coumarin. Eur J Med Chem 45:1151–1157.

Shi Y, Zhou C (2011) Synthesis and evaluation of a class of new coumarin triazole derivatives as potential antimicrobial agents. Bioorg Med Chem Lett 21:956-960.

Patil SA, Unki SN, Kulkarni AD, Naik VH, Badami PS (2011) Synthesis, characterization, in vitro antimicrobial and DNA cleavage studies of Co(II), Ni(II) and Cu(II) complexes with ONOO donor coumarin Schiff bases. J Mol Struct 985:330-338.

Sabry NM, Mohamed HM, Khattab ES, Motlaq SS, El-Agrody AM (2011) Synthesis of 4H-chromene, coumarin, 12H-chromeno[2,3-d]pyrimidine derivatives and some of their antimicrobial and cytotoxicity activities. Eur J Med Chem 46:765-772.

Kawase M, Varu B, Shah A, Motohashi M, Tani S, Saito S (2001) Antimicrobial activity of new coumarin derivatives. Arzneimittelforschung 51:67-71.

Patel D, Kumari P, Patel N (2010) Synthesis, characterization and biological evaluation of some

thiazolidinone derivatives as antimicrobial agents. J Chem Pharm Res 2:84-91.

Patel D, Kumari P, Patel N (2010) Synthesis and characterization of some new thiazolidinones containing coumarin moiety and their antimicrobial study. Arch Appl Sci Res 2:68-75.

Laurin P, Ferroud D, Klich M, Dupuis-Hamelin C, Mauvais P, Lassaigne P, Bonnefoy A, Musicki B (1999) Synthesis and in vitro evaluation of novel highly potent coumarin inhibitors of gyrase B. Bioorg Med Chem Lett 9:2079-2084.

Nathan C (2004) Antibiotics at the crossroads. Nature 431:899-902.

Rachakonda S, Cartee L (2004) Challenges in antimicrobial drug discovery and the potential of nucleoside antibiotics. Curr Med Chem 11:775-793.

Walsh, C. Antibiotics, actions, origins, resistance; ASM: Washington, DC, 2003.

Von Nussbaum F, Brands M, Hinzen B, Weigand S, Häbich D (2006) Antibacterial natural products in medicinal chemistry–exodus or revival? Angew Chem Int Ed 45:5072-5129.

Matos MJ, Delogu G, Podda G, Santana L, Uriarte E (2010) Regioselective synthesis of different bromo-substituted 3-penhylcoumarins. Synthesis 16:2763-2766.

Matos MJ, Vazquez-Rodriguez S, Borges F, Santana L, Uriarte E (2011) Synthesis of 3-arylcoumarins via Suzuki-cross-coupling reactions of 3-chlorocoumarin. Tetrahedron Lett 72:1225-1227.

Krishnaswamy NR, Seshadri TR, Sharma BR (1969) Nitro derivatives of 3-phenylcoumarins. Ind J Chem 7:49-55.

Rao DV, Sayigh AR, Ulrich H (1972) Aminophenyl alkoxy coumarins. US 3676436 A 19720711.

Kremky E (1979) Chromatographic method for the separation and identification of some coumarin derivatives. Organika 159-163.

Piazzi L, Cavalli A, Belluti F, Bisi A, Gobbi S, Rizzo S, Bartolini M, Andrisano V, Recanatini M, Rampa A (2007) Extensive SAR and computational studies of 3-{4-[(benzylmethylamino)methyl]phenyl}-6,7-dimethoxy-2H-2-chromenone (AP2238) derivatives. J Med Chem 50:4250-4254.

Girouard S, Houle MH, Grandbois A, Keillor JW, Michnick SW (2005) Synthesis and characterization of dimaleimide fluorogens designed for specific labeling of proteins. J Am Chem Soc 127:559-566.