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Potential of synthetic chalcone derivatives to prevent marine biofouling

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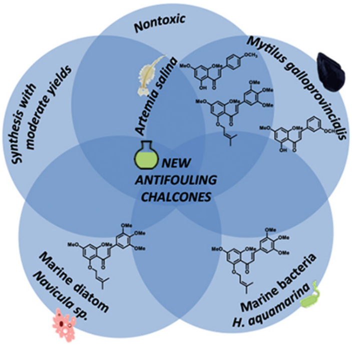
HIGHLIGHTS

- Marine biofouling impacts atmosphere, hydrosphere, and biosphere negatively.
- Antifouling paints in use release toxic and persistent organic pollutants.
- Synthesis of new nontoxic antifoulants was achieved in this work.
- These compounds are able to be obtained in short time and in suitable amounts.

GRAPHICAL ABSTRACT



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ABSTRACT

Biofouling represents a major economic, environmental and health concern for which new eco-friendly solutions are needed. International legislation has restricted the use of biocidal-based antifouling coatings, and increasing efforts have been applied in the search for environmentally friendly antifouling agents. This research work deals with the assessment of the interest of a series of synthetic chalcone derivatives for antifouling applications. Sixteen chalcone derivatives were synthesized with moderate yields (38–85%). Antifouling bioactivity of these compounds was assessed at different levels of biological organization using both anti-macrofouling and anti-microfouling bioassays, namely an anti-settlement assay using mussel (*Mytilus galloprovincialis*) larvae, as well as marine bacteria and microalgal biofilms growth inhibition bioassays. Results showed that three compounds (**11**, **12**, and **16**) were particularly active against the settlement of mussel larvae (EC_{50} 7.24–34.63 μM), being compounds **12** and **16** also able to inhibit the growth of microfouling species (EC_{50} 4.09–20.31 μM). Moreover, the most potent compounds **12** and **16** were found to be non-toxic to the non-target species *Artemia salina* (<10% mortality at 25 μM). A quantitative structure-activity relationship model predicted that descriptors describing the ability of molecules to form hydrogen bonds and encoding the shape, branching ratio and constitutional diversity of the molecule were implied in the antifouling activity against the settlement of mussel larvae. This work elucidates for the first time the relevance of synthesizing chalcone derivatives to generate new nontoxic products to prevent marine biofouling.

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1. Introduction

Both macro and microorganisms are part of the community responsible for the natural process known as biofouling, which occurs when marine species attach to natural or artificial underwater surfaces (Callow and Callow, 2002). Marine biofouling causes not only material and economic loss for the marine sector operations, but also creates a series of environmental and health problems affecting atmosphere, hydrosphere, biosphere due to over-consumption of fuel and carbon dioxide emissions, and also the spread of pathogenic bacteria and indigenous species which contributes to health problems and biodiversity reduction (Qian et al., 2010; Schultz et al., 2011).

Antifouling (AF) paints have been and remain the primary strategy for biofouling control. Biocidal paints based on tributyltin (TBT) have been effective AF agents. Nevertheless, these coatings were banned in several countries since 2008 (IMO, 2008), given its detrimental effect to non-target organisms and whole marine environment (Antizar-Ladislao, 2008). In fact, environmental studies demonstrated that organotin compounds do not readily degrade in the environment, affecting marine organisms and possibly biomagnifying through the food chains (Konstantinou and Albanis, 2004). Booster biocides based on copper, zinc, and several organic compounds were more recently introduced as AF agents, but have also been found to be harmful to many non-target organisms (Thomas and Brooks, 2010). Therefore, there is a high demand for environmentally benign, non-toxic AF agents as an alternative to the biocide-based coatings currently in use (Almeida and Vasconcelos, 2015).

A wide range of diverse natural AF compounds have been identified lately (Qian et al., 2015; Satheesh et al., 2016; Wang et al., 2017). These compounds have been reported as acting against micro and macrofouling species with low toxicity, and were recently considered as models to the synthesis of nature-inspired AF agents namely, synoxazolidinone A (Trepos et al., 2014), 2,5-diketopiperazine (Liao et al., 2015), zosteric acid (Almeida et al., 2017; Catto et al., 2015), batatasin III (Moodie et al., 2018; Moodie et al., 2017a), polygodial (Moodie et al., 2017b).

Chalcones represent one of the major subclasses of flavonoids and have long been recognised for their myriad of biological activities (Singh et al., 2014). Regarding AF properties, the evaluation of their effect is limited to studies using marine bacterial biofilms such as *Vibrio natriegens*, *Bacillus flexus*, and *Pseudomonas fluorescens* (Sivakumar et al., 2010a; Sivakumar et al., 2010b). Moreover, chalcones have been used as anticorrosive agents (Bouklah et al., 2006), alone and combined with iodide ions to synergize the activity of the latter in acid-mediated corrosion of steel (Bouklah et al., 2003; Elayyoubi et al., 2002). Considering the antibacterial and slimicidal activities together with anticorrosive properties, chalcones were proposed as ideal candidates to be used as AF agents in anticorrosive coatings (Sivakumar et al., 2010a). At the best of our knowledge, no studies concerning their effects on other fouling organisms have been conducted.

In this research work, a series of chalcone derivatives was synthesized (Fig. 1, **1**–**16**) and evaluated for their AF activity against both micro and macrofouling species, namely five biofilm-forming marine bacteria (*Cobetia marina*, *Vibrio harveyi*, *Pseudoalteromonas atlantica*, *Halomonas aquamarina and Roseobacter litoralis*), four marine diatom strains (*Cylindrotheca* sp., *Halamphora* sp., *Nitzschia* sp. and *Navicula* sp.) and the adhesive larvae of the macrofouling mussel *Mytilus galloprovincialis*. A quantitative structure-activity relationship (QSAR) model to predict the AF activity against larvae of *Mytilus galloprovincialis* was also developed.

Compounds showing promising AF bioactivity were submitted to complementary assays to evaluate the viability of the selected compounds as AF agents, including the assessment of general ecotoxicity using *Artemia salina* standard ecotoxicity assay, and the evaluation of possible mechanisms of action related with adhesion and neurotransmission pathways.

2. Material and methods

2.1. Synthesis and structure elucidation

Microwave (MW) reactions were performed using a glassware setup for atmospheric pressure reactions and a 100 mL Teflon reactor (internal reaction temperature measurements with a fiber-optic probe sensor), and were carried out in an Ethos MicroSYNTH 1600 Microwave Labstation from Milestone. The reactions were monitored by thin-layer chromatography (TLC). Compounds purification was performed by flash column chromatography using Macherey-Nagel silica gel 60 (0.04–0.063 mm), and preparative thin-layer chromatography (TLC) using Macherey-Nagel silica gel 60 (GF254) plates. Melting points were obtained in a Köfler microscope and are uncorrected. ^{1}H and ^{13}C NMR spectra were taken in $CDCl_3$ at room temperature, on Bruker Avance 300 instrument (300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C). Chemical shifts are expressed in δ (ppm) values relative to tetramethylsilane (TMS) as an internal reference; ^{13}C NMR assignments were made by 2D (HSQC and HMBC) NMR experiments (long-range C, H coupling constants were optimized to 7 Hz). HRMS mass spectra were recorded at C.A.C.T.I.-University of Vigo, Spain. Experiments were performed on an APEXQe FT-ICR MS (Bruker Daltonics, Billerica, MA), equipped with a 7 T actively shielded magnet. Ions were generated using a Combi MALDI-electrospray ionization (ESI) source. Ionization was achieved by electrospray, using a voltage of 4500 V applied to the needle, and a counter voltage of 300 V applied to the capillary. Samples were prepared by adding a spray solution of 70:29.9:0.1 (v/v/v) CH_3OH/water/formic acid or 70:29.9:0.1 (v/v/v) CH_3CN/water/formic acid to a solution of the sample at a v/v ratio of 1 to 5% to give the best signal-to-noise ratio. Data acquisition was performed using the ApexControl software version 3.0.0, and data processing was performed using the DataAnalysis software, version 4.0 both from Bruker Daltonics. 2-Hydroxy-4,6-dimethoxyacetophenone and benzaldehydes were purchased from Sigma Aldrich. Chalcone derivatives **1**–**6** (33–42%) (Pereira et al., 2016), **8** (46%) (Detsi et al., 2009), **9** (60%) (Kadival et al., 1962), **10** (47%) (Boeck et al., 2006), **11** (40%) (Boeck et al., 2006), **12** (77%) (Thieury et al., 2017), **13** (39%) (Detsi et al., 2009), **14** (38%) (Alvim et al., 2010), **15** (71%) (Mateeva et al., 2002), and **16** (73%) (Neves et al., 2012) were synthesized as described elsewhere. The NMR data of compound **9** was described for the first time, as indicated below. The new chalcone derivative **7** was synthesized and purified by the following procedures.

2.1.1. Synthesis of chalcone 7

2-Hydroxy-4-methoxy-3-propylacetophenone was synthesized (quantitative yield) and characterized according to a previous described procedure (Pereira et al., 2016). Then, to a solution of 2-hydroxy-4-methoxy-3-propylacetophenone (1.000 mmol, 0.208 g) in methanol was added an aqueous solution of 40% sodium hydroxide until pH 13–14. Then, a solution of 2 mmol of 4-chlorobenzaldehyde in methanol was slowly added to the reaction mixture. The reaction was submitted to successive 15 min periods of MW irradiation at 180 W. Total irradiation time was 30 min and the final temperature was 75 °C. The solution was extracted with chloroform (3 × 50 mL). The combined organic layers were rinsed with brine and water, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The obtained residue was purified by flash column chromatography (SiO_2; n-hexane/ethyl acetate 8:2) affording compound **7** as orange crystals in 57% yield.

(*E*)-1-(2-hydroxy-4-methoxy-3-propylphenyl)-3-(4-Clorophenyl) prop-2-en-1-one (7). mp (ethyl acetate): 109–112 °C; ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 13.30 (1H, s, 2′-OH), 7.82 (1H, d, $J = 15.0$ Hz, H-β), 7.78 (1H, d, $J = 9.2$ Hz, H-6′), 7.58 (1H, d, $J = 15.0$ Hz, H-α), 7.57 (2H, d, $J = 8.5$ Hz, H-2,6), 7.40 (2H, d, $J = 8.5$ Hz, H-3,5), 6.50 (1H, d, $J = 9.2$ Hz, H-5′), 3.90 (3H, s, 4′-OCH_3), 2.66 (2H, t, $J = 7.7$ Hz, H-1″), 1.59–1.51 (2H, m, H-2″), 0.96 (3H, t, $J = 7.4$ Hz, H-3″); ^{13}C NMR ($CDCl_3$,