Filipa Sameiro de Oliveira

Prenylated xanthone derivatives: Synthesis of structural analogues of α-mangostin and antitumor activity evaluation

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Faculty of Pharmacy, University of Coimbra

Author: Filipa Sameiro de Oliveira

filipasameiro@gmail.com

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INDEX

INDEX OF FIGURES

FIGURE

INDEX OF TABLES

TABLE

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ABSTRACT

Xanthones or xanthen-*9*-ones (dibenzo-γ-pirone) comprise an important class of oxygenated heterocycles whose role is well-known in Medicinal Chemistry. The biological activities of this class of compounds are associated with their tricyclic scaffold but vary depending on the nature and/or position of the different substituents. To obtain more structural diversity and quantities for biological assays, suitable and efficient synthetic processes are necessary.

Different synthetic methodologies, "classical" and "non-classical", namely microwave assisted organic synthesis, heterogeneous catalysis and a combination of heterogeneous catalysis with microwave irradiation, were applied to obtain new xanthone derivatives.

Thus, this thesis reports the synthesis and structure elucidation of seven new compounds: 1,3-dihydroxy-5-methoxyxanthone (**X1**), 1-hydroxy-3-mesyloxy-5-methoxyxanthone (**X2**), 1-hydroxy-5-methoxy-3-(3-methylbut-2-enyloxy)xanthone (**P1**), 1-hydroxy-5-methoxy-4-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (**P2**), 1-hydroxy-5-methoxy-4-(3-methylbut-2-enyl)-6',6'-dimethyl-4',5'-dihydropyran(2',3':3,2) xanthone (**P3**), 1-hydroxy-5-methoxy-6',6'-dimethyl-4',5'-dihydropyran(2',3':3,2)xanthone (**P4**) and 1-hydroxy-5-methoxy-6',6'-dimethyl-4',5'-dihydropyran(2',3':3,4)xanthone (**P5**), as well as the evaluation of their antitumor activity.

The synthesized xanthones and respective derivatives with prenyl units were structurally elucidated by spectroscopic methods including IR, NMR (^{1}H, ^{13}C, HSQC and HMBC) and HRMS. Their biological activity was evaluated by a screening assay for inhibition of growth of human tumor cell lines.

Keywords: Prenylated xanthones, synthesis, microwave assisted organic synthesis (MAOS), heterogeneous catalysis, growth inhibitory activity of tumor cell lines.

RESUMO

As xantonas ou xanten-9-onas (dibenzo-γ-pirona) constituem uma importante classe de heterociclos oxigenados com um papel bem conhecido na Química Medicinal. As atividades biológicas desta classe de compostos estão relacionadas com o seu esqueleto tricíclico e variam consoante a natureza e/ou posição dos diferentes substituintes. De forma a obter maior diversidade estrutural e quantidades para ensaios biológicos, são necessários processos sintéticos adequados e eficientes.

Diferentes metodologias sintéticas, "clássicas" e "não-clássicas", nomeadamente síntese orgânica assistida por micro-ondas, catálise heterogénea e a combinação de catálise heterogénea com radiação micro-ondas, foram aplicadas para obter novos derivados xantónicos.

Assim, esta tese descreve a síntese e elucidação estrutural de sete novos compostos: 1,3-di-hidroxi-5-metoxixantona (**X1**), 1-hidroxi-3-mesiloxi-5-metoxixantona (**X2**), 1-hidroxi-5-metoxi-3-(3-metilbut-2-eniloxi)xantona (**P1**), 1-hidroxi-5-metoxi-4-(3-metilbut-2-enil)-3-(3-metilbut-2-eniloxi)xantona (**P2**), 1-hidroxi-5-metoxi-4-(3-metilbut-2-enil)-6',6'-dimetil-4',5'di-hidropirano(2',3':3,2) xantona (**P3**), 1-hidroxi-5metoxi-6',6'-dimetil-4',5'-di-hidropirano(2',3':3,2)xantona (**P4**) e 1-hidroxi-5-metoxi-6',6'-dimetil-4',5'-di-hidropirano(2',3':3,4)xantona (**P5**), assim como a avaliação da sua atividade antitumoral.

As xantonas e os respetivos derivados prenilados sintetizados foram caraterizados estruturalmente por métodos espetroscópicos, nomeadamente IV, RMN (^{1}H, ^{13}C, HSQC e HMBC) e EM de alta resolução. A sua atividade biológica foi avaliada através de um *screening* para a inibição de crescimento de linhas celulares tumorais humanas.

Palavras-chave: Xantonas preniladas, síntese, síntese assistida por micro-ondas, catálise heterogénea, atividade inibidora do crescimento de linhas celulares tumorais.

ABBREVIATIONS AND SYMBOLS

AChE	Acetylcholinesterase
A375-C5	Malignant melanoma cell line
^{13}C NMR	Carbon nuclear magnetic resonance
^{1}H NMR	Proton nuclear magnetic resonance
CEQUIMED-UP	Centro de Química Medicinal – Universidade do Porto
d	Doublet
DCX	Dicamphanoyl-dihydropyranoxanthone
dd	Double doublet
DMSO	Dimethyl sulfoxide
DMXAA	5,6-dimethylxanthenone-4-acetic acid
GI_{50}	Concentration of compound that causes 50% inhibition of the growth of tumor cell lines
HIV	Human immunodeficiency virus
HMBC	Heteronuclear Multiple Bond Correlation
HRMS	High Resolution Mass Spectrometry
HSQC	Heteronuclear Single Quantum Correlation
IBX	2-Iodoxybenzoic acid
IR	Infrared spectroscopy
IUPAC	International Union of Pure and Applied Chemistry
J	Coupling constant
LDL	Low density lipoprotein
m	Multiplet
MAO	Monoamine oxidase
MAOS	Microwave assisted organic synthesis
MCF-7	Breast adenocarcinoma cell line
mp	Melting point

MRSA	Methicillin-resistant *Staphylococcus aureus*
MW	Microwave
NCI	National Cancer Institute
NCI-H460	Non-small cell lung cancer cell line
NMR	Nuclear magnetic resonance
N,N-DMA	*N,N*-dimethylaniline
NO	Nitric oxide
NSCLC	Non-small cell lung cancer
PAF	Platelet activating factorP-gpP-glycoprotein
q	Quadruplet
s	Singlet
SRB	Sulforhodamine B
S_NAr	Aromatic nucleophilic substitution
t	Triplet
tan δ	Loss tangent
THF	Tetrahydrofuran
TLC	Thin layer chromatography
VEGF	Vascular endothelial growth factor
VRE	Vancomycin-resistant *enterococci*
WHO	World Health Organization

OUTLINE OF THE THESIS

The present thesis consists of five chapters:

I. INTRODUCTION

The state-of-the-art concerning the major classes of xanthone derivatives – prenylated and caged xanthones are presented. The biological activities of xanthones, namely antitumor are highlighted. The synthesis through “classical” and “non-classical” methodologies like MAOS and heterogeneous catalysis are described.

II. AIMS

Herein the main objectives of the present thesis are described.

III. RESULTS AND DISCUSSION

This section is divided in three parts: synthesis, structural elucidation and biological assays of the obtained compounds, concerning inhibitory activity of growth of human tumor cell lines.

IV. EXPERIMENTAL PART

In this chapter, the experimental procedures for the synthesis, structure characterization and biological evaluation of the synthesized compounds are detailed.

V. CONCLUSIONS

This chapter includes the general conclusions of the developed work.

REFERENCES/ATTACHMENTS

I.INTRODUCTION

INTRODUCTION

Throughout the ages, Nature has catered to the basic needs of humans, not the least of which is the provision of medicines for the treatment of a wide spectrum of diseases. Plants, in particular, have given a significant contribution to the development of therapeutic systems. The continuing and essential role played by plant-based systems in the healthcare of many different cultures has been extensively documented and the World Health Organization (WHO) has estimated that in some Asian and African countries, 80% of the population depend on traditional medicine for primary health care (Cragg, G.M. *et al.*, 2009; http://www.who.int/en/).

Besides directly isolated natural products can be the actual drugs used for the treatment of a given disease, these natural molecules can serve as lead compounds for the development of analogues, with optimized pharmacological properties. They have been evolutionarily selected to bind to biological macromolecules representing some of them "privileged structures", which are excellent templates for the synthesis of novel, biologically active, natural product-like molecules.

Some problems concerning natural products are the lack of druglike properties, namely associated with pharmacokinetic properties and/or toxicity, so they need to be fine-tuned to possess the properties desired in a clinically useful drug. Optimization frequently entails modification, removal, or introduction of functional groups and stereocenters or more drastic remodelling of the basic scaffold to improve physicochemical and pharmacokinetic properties. Also the structural diversity is limited by the available biosynthetic pathways of the host organism, but the power of synthetic chemistry can be harnessed to access a greater extent of possible modifications and structural diversity (Cragg, G.M. *et al.*,2009; Kinghorn, A.D. *et al*., 2011).

Xanthones are one of the main compounds of the exotic fruit of *Garcinia Mangostana* L., mangosteen, which have showed to posses diverse health benefits. In Southeast Asia, this fruit has been appreciated for centuries and strongly used as folk medicine due to their bioactive compounds. These allowed the scientists to notice its potential as a source of new therapeutic agents (Adnan, N. and Othman, N., 2012; Saslis-Lagoudakis, C.H. *et al*., 2011; Wittenauer, J. *et al*., 2012).

As in CEQUIMED-UP the synthesis of xanthone derivatives and their investigation as potential antitumor agents has been a research area in increasing development (Palmeira, A. *et al*., 2010; Pinto, M. and Castanheiro, R., 2009a and 2009b), this class of compounds was chosen to be studied in this thesis.

1. XANTHONES

Xanthones or xanthen-9-ones are heterocyclic compounds with the dibenzo-γ-pyrone scaffold (Pinto, M. and Castanheiro, R., 2009a and 2009b; Sousa, M.E. and Pinto, M., 2005) (**Figure 1**). Xanthone derivatives are secondary metabolites produced by plants and microorganisms that have a structural relationship with other γ-pyrone moieties: flavonoids (**2**) and chromones (**3**) (**Figure 2**). The xanthones from higher plants appear to be associated mainly with the families *Clusiaceae* and *Gentianaceae* (Nualkaew, N. *et al.*, 2012; Pinto, M. and Castanheiro, R., 2009a and 2009b; Vieira, L. and Kijjoa, A., 2005). Because of their diverse pharmacological properties, xanthone derivatives have an important and well-known role in Medicinal Chemist.

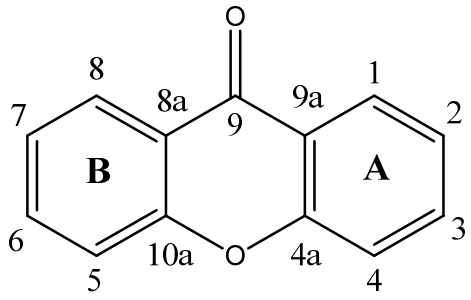

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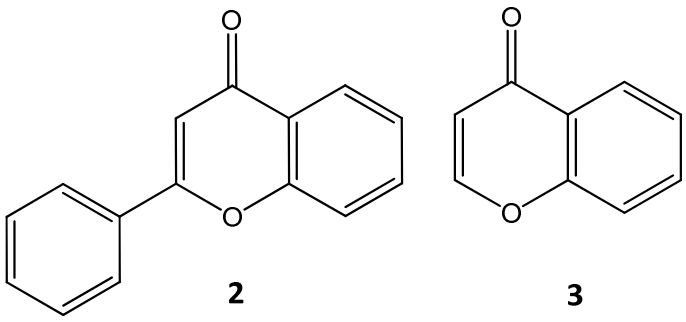
Figure 1. Xanthonic scaffold and numbering (according to IUPAC, http://www.iupac.org;http://www.chem.qmul.ac.uk/iupac/)



2 **3**

Figure 2. Scaffolds containing a γ-pyrone moiety (present in bioactive secondary metabolites)

Concerning the biosynthetic metabolic route in higher plants, xanthones are generated through two pathways: acetate (**Figure 1 -** ring A, numbered from 1 to 4) and shikimate (**Figure 1 -** ring B, numbered from 5 to 8).

Xanthones can present different substituents on their scaffold, allowing great structural diversity (Na, Y., 2009; Sousa, M.E. and Pinto, M., 2005). According to this, xanthones can be classified into the major groups: simple oxygenated xanthones, xanthones glycosides, prenylated and related xanthones, xanthonic dymers, caged xanthones, xanthonolignoids and miscellaneous xanthones. The simple oxygenated xanthones can further be subdivided into sub-groups according to the degree of oxygenation in mono-, di-, tri-, tetra-, penta- and hexaoxygenated xanthones (Fotie, J. and Bohle, D.S., 2006).

Although natural products have always played an important role in drug discovery, providing bioactive compounds of great interest in Medicinal Chemistry (Kinghorn, A.D. *et al*., 2011), xanthones from natural origin are relatively limited in type and position of the substituents imposed by the biosynthetic pathways (Sousa, M.E. and Pinto, M., 2005). Due to their interesting structural scaffold and vast pharmacological properties, a lot of compounds was isolated from natural resources and/or many new xanthone-natural mimics were synthesized as novel drug candidates (Jun, K.Y. *et al.*, 2011). Therefore, natural products can also be an important source of novel molecular architecture.

The biological activities of this class of compounds are associated with their tricyclic scaffold but vary depending on the nature and/or position of the different substituents. The bioactivities of natural and synthetic analogues have been extensively reported and reviewed in the literature (El-Seedi, H.R. *et al.*, 2010; Pinto, M. and Castanheiro, R., 2009a and 2009b; Pinto, M. *et al.*, 2005). In this thesis, diverse activities will be discussed later, particularly antitumor (Giri, R. *et al*., 2010; Jun, K.Y. *et al.*, 2011; Krajarng, A. *et al*., 2012; Na, Y., 2009; Woo, S. *et al.*, 2007).

The major group of naturally occurring xanthones are prenylated xanthones, a group that have very important and diverse pharmacological activities. As a consequence of this, using the prenylated xanthone α-mangostin as a model, synthetic strategies leading to new prenylated derivatives have been explored.

1.1. PRENYLATED XANTHONES

Prenylated xanthones are characterized by the presence of a prenyl group (C_5 unit) in the xanthonic scaffold and represent the major group of naturally occurring xanthones (Pinto, M. and Castanheiro, R., 2009a and 2009b). The study of xanthone derivatives with important bioactivities has shown a relationship between biological activity and the presence of prenyl groups in key-positions on the xanthone nucleus. Therefore, this feature becomes an important structural point for the interaction of xanthones with some targets, allowing an increase of selectivity and potency.

Although the *C*-prenyl derivatives are much more represented in Nature, oxyprenylated compounds are also found, and among them, only a small number are both *C* and *O*-prenylated.

The main substituents (C_5 group) found in prenylated xanthones included the common 3-methylbut-2-enyl or isoprenyl group (**A**), the less frequent 2-hydroxy-3-methylbut-3-enyl group (**B**) and also the 1,1-dimethylprop-2-enyl or 1,1-dimethylallyl group (**C**). Compounds containing the 2,2-dimethyldihydropyran (**D**), the 2,2-dimethylpyran (**E**) and the 2,3,3-trimethyldihydrofuran (**F**) groups, which are the result of cyclization of the substituents **A** and **C** respectively, with the *ortho* hydroxyl group, could also be found (**Figure 3**). Modifications of these side chains by hydroxylation, hydrogenation, cyclization, and Claisen rearrangement reactions can also occur (Pinto, M. and Castanheiro, R., 2009a).

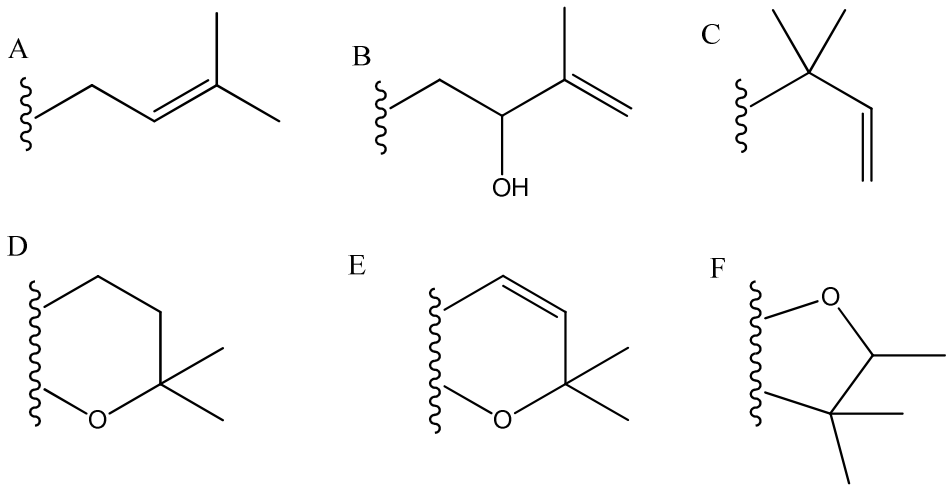


Figure 3. Main substituents found in prenylated xanthones (Pinto, M., Castanheiro, R., 2009a)

The prenyl substituents can occur in any position of the xanthonic scaffold, but there are some preferential positions. For example, substituents **A** and **C** usually appear in C_2, C_4 and C_8,

while cyclic substituents like **D** and **E** frequently appear in C_2-C_3, C_3-C_4 (and C_5-C_6, C_7-C_8 for **D**) of the xanthonic scaffold (Pinto, M. and Castanheiro, R., 2009a).

The biological activities of prenylated xanthones are vast, standing out the antitumor activity. Pinto and Castanheiro carefully review the activities of these xanthones and highlighted their importance as chemopreventive agents against chemical induced carcinogenesis and also as antitumor agents (Pinto, M. and Castanheiro, R., 2009b). In Obolskiy study (Obolskiy, D. *et al*., 2009), several xanthone derivatives, extracted from the steam and root bark of mangosteen, particularly α-mangostin, β-mangostin and γ-mangostin (**Figure 4**), showed strong antitumor and antioxidant activity. These and other studies suggest that prenylated xanthones could be useful as chemotherapeutic agents for the treatment of certain cancers (Castanheiro, R. *et al*., 2009a; Giri, R. *et al.*, 2010; Jun, K.Y. *et al.*, 2011; Krajarng, A. *et al.*, 2012; Na, Y., 2009; Woo, S. *et al.*, 2007). It is important to note that prenylated xanthones exhibit a wide range of other activities in addition to this one, as will be discussed later in this thesis.

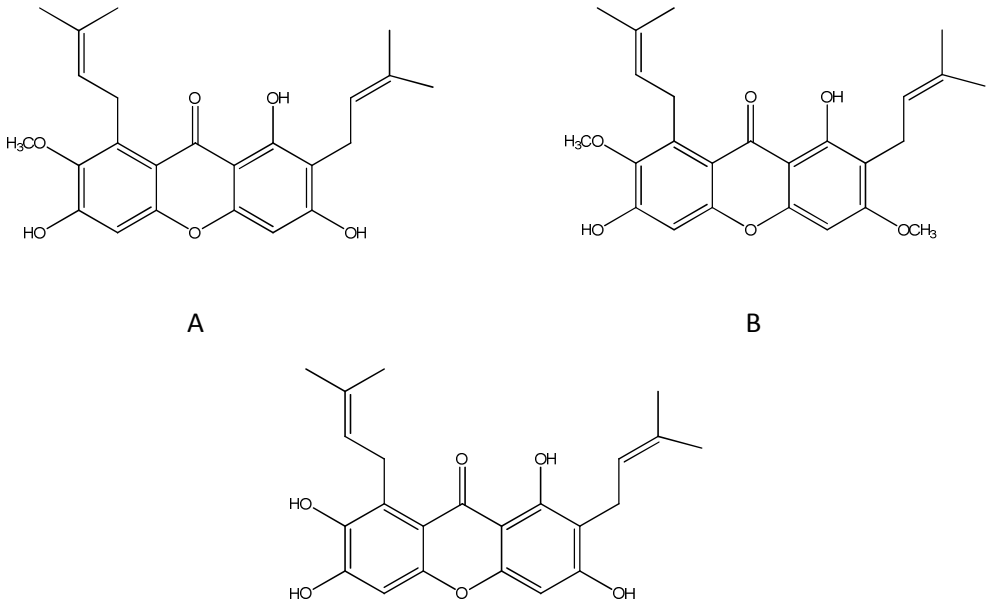


Figure 4. α-mangostin (**A**), β-mangostin (**B**) and γ-mangostin (**C**)

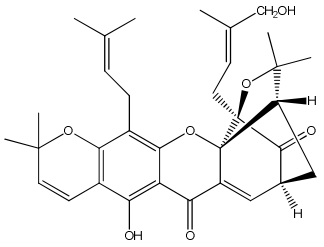
1.2. CAGED XANTHONES

Caged xanthones are a group of polyprenylated xanthones mainly extracted from the *Garcinia* genus (*Guttiferae* family). They are characterized by a unique 4-oxa-tricyclo[4.3.1.0]dec-2-one scaffold, in which a highly substituted tetrahydrofuran core with three quaternary carbon centers is featured (**Figure 5**).

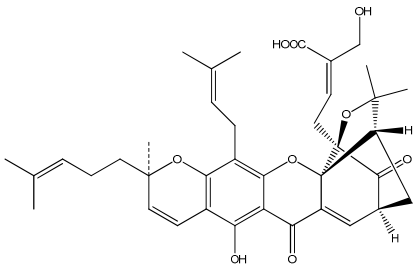
Most of the *Garcinia* genus contain prenylated xanthones, but caged xanthones mainly occur in five species: *G. morella, G. hanburyi, G.bracteata, G. gaudichaudii, and G. scortechinii*, widely distributed in Southeast Asia. From the biosynthetic point of view, caged xanthones are thought to be derived from a common benzophenone intermediate of a mixed shikimate-acetate pathway that has undergone plant-specific prenylations, rearrangements, and/or oxidation reactions (Han, Q-B. and Xu, H-X, 2009).

As suggested by structure-activity relationship studies, the caged core is responsible for the bioactivities of this class of compounds. Among other activities like anti-viral, antibacterial and neurotrophic, they have been reported to have potent antitumor activity, with gambogic acid being the more notorious (Reutrakul, V. *et al.*, 2007; Yen, C. *et al*., 2012).

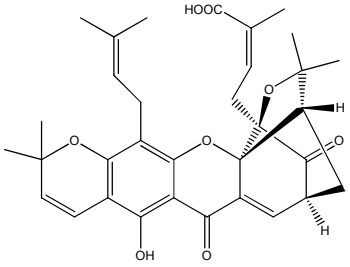
With an unusual caged skeleton and notable bioactivity, this promising class of xanthones attracts increasing attention among scientist from diverse areas.



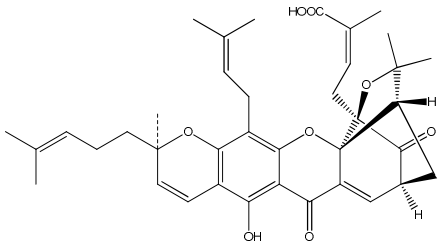
Isomorellinol



30-Hydroxygambogic acid



Morellic acid



Gambogic acid

Figure 5. Examples of caged xanthones with antitumor activity

2. ACTIVITY OF XANTHONES

Xanthone scaffold is considered a “privileged structure” since this class of compounds can interact with diverse drug targets and exhibit multiple pharmacological effects. In the field of Medicinal Chemistry, groups of compounds that can bind to different classes of receptors have attracted much attention as potential drug candidates (Kappe, C.O. and Dallinger, D., 2005; Vieira, L. and Kijjoa, A., 2005).

Many naturally occurring xanthones and their prenylated derivatives are found to exhibit significant biological and pharmacological properties, such as antibacterial, antifungal and antitumor activities (El-Seedi, H.R. *et al.*, 2010) and it can be inferred that the presence of prenyl groups can be associated with an improvement of potency and selectivity for some of these properties. Therefore, there is interest in obtaining this type of compounds to evaluate their antitumor activity. For this purpose, in this thesis, molecular modifications of the 1,3-dihydroxy-5-methoxyxanthone (**X1**) were carried out to obtain xanthone derivatives with prenyl substituents, either in a cyclic or as an open-chain form (Castanheiro, R. *et al.*, 2009b).

Once xanthones posses a wide range of biological properties, it will be described the main activities reported in the last ten years (**Figure 6**), with special emphasis on antitumor activity.

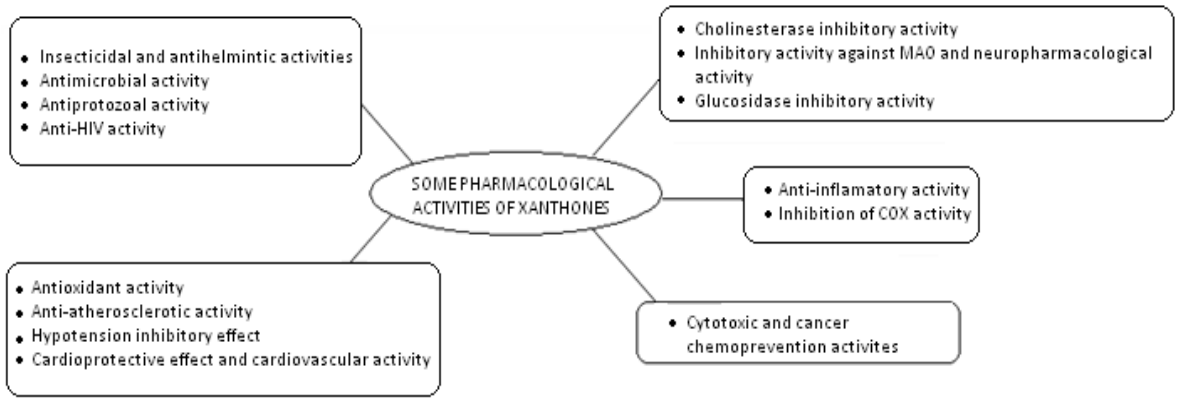
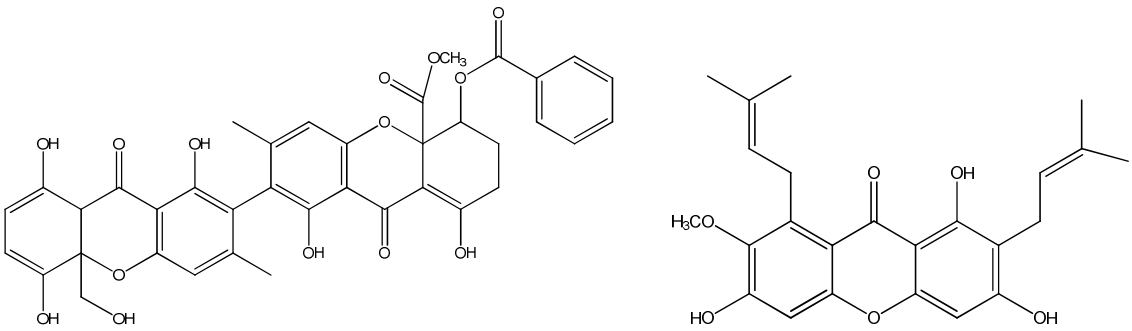


Figure 6. Some pharmacological activities of xanthones (El-Seedi, H.R. *et al.*, 2010)

- Insecticidal and Antihelmintic Activities

Although the significant number of helminth infections, few anthelminthic drugs are available for human use. The activities of α-mangostin (**Figure 7**), a major bioactive xanthone isolated from *Garcinia mangostana* and of the synthetic derivative mangostin diacetate were tested by Keiser, J. α-Mangostin has showed promising activities against the trematodes *Schistosoma mansoni, Echinostoma caproni,* and *Fasciola hepatica in vitro*, but not *in vivo* (Keiser, J. *et al.*, 2012).

Xanthonol (**Figure 7**) has been shown to have moderate insecticidal and antihelmintic activities, being considered a promising lead for safe, efficacious systemic antiparasitic drugs, with new modes of action. This result is very important because an effective treatment for human and animal infections is urgently needed, since the efficacy of current insecticides and anthelmintics is limited by low therapeutic indices, environmental hazards and development of resistance. (Ondeyka, J.G. *et al*., 2006).



Xanthonol α-Mangostin

Figure 7. Xanthonol and α-mangostin structures

- Antimicrobial Activity

Bacterial and fungal resistance to antibiotics has become a serious problem in the treatment of infectious diseases. The discovery of new antimicrobial agents is very important because of the increased incidence of infections by opportunistic fungi, especially in patients whose immune system has been compromised by AIDS, cancer, diabetes, age or other causes. Several studies have shown that diverse xanthones (α-, β-, γ-mangostin, among others) exhibit moderate to high inhibitory activities against diverse human pathogenic microorganisms,

particularly multi drug-resistant organisms such as the methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *enterococci* (VRE). These compounds warrant further attention as possible antibiotics with activities against various human pathogenic microorganisms (El-Seedi, H.R. *et al*., 2010).

- Antiprotozoal Activity

Protozoa such as trypanosomes, leishmania and the malaria parasites (*Plasmodium* spp.) are responsable for serious diseases. Malaria remains the most important parasitic disease, causing 2–3 million deaths every year, and the emergence and rapid spread of chloroquine-resistant strains of *Plasmodium falciparum* threaten to increase the annual death toll (Azebaze, A.G.B. *et al*., 2007). Being in the past considered a disease of underdeveloped countries, today, due to climate changes, it is estimated that it can reach developed countries like United States, and therefore the attention and the investment applied in the discovery of new therapeutic drugs against malaria are increasing. Xanthones have already been evaluated by their antimalarial activity and had proven to be potential antimalarial agents (Dua, V.K. *et al*., 2004; Portela, C. *et al.*, 2007).

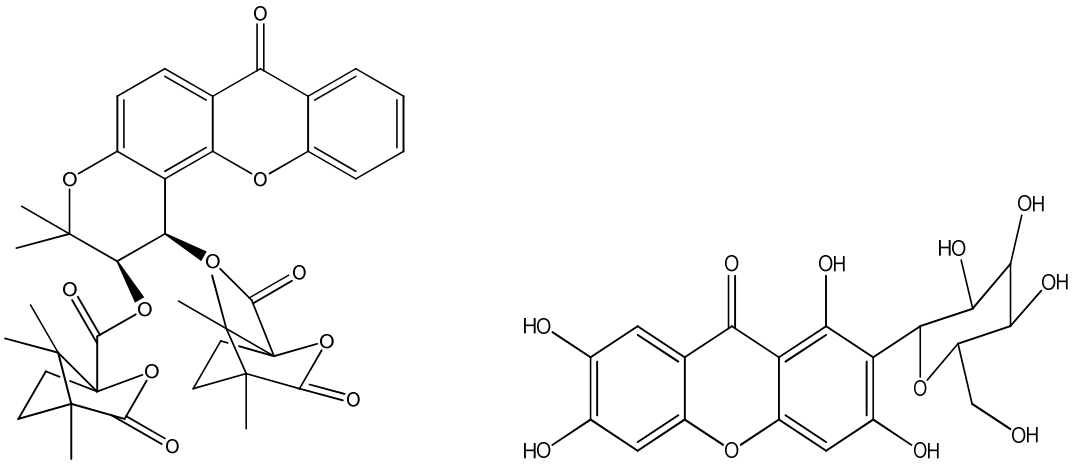
Prenylated xanthones from *Garcinia subelliptica* have shown trypanocidal activity against *Trypanosoma cruzi*, the etiologic agent for Chagas' disease (Abe, F. *et al.*, 2003).

- Antiviral and Anti-HIV-1 Activities

Xanthones are being evaluated by their neuraminidase inhibitory activity (Ryu, H.W. *et al*., 2010). The neuraminidase family is a group of exo-acting enzymes that are important in a varied array of cell–cell interactions and cell–molecule recognition processes. Neuraminidase has a well known role in infectivity of the influenza virus and recent studies have also shown that it can enable several bacterial pathogens to evade the host immune system. Thus development of inhibitors of neuraminidase may provide a new weapon for the treatment of several pathogenic diseases.

Concerning to anti-HIV activity, mangiferin (**Figure 8**) showed good activities, being now a novel anti-HIV agent effective against resistant HIV-strains (Wang, R.R. *et al*., 2011a). Gambogic

acid (**Figure 5**) have also shown moderate HIV-1 inhibitory activities in the reverse transcriptase assay (Reutrakul, V. *et al*., 2007). More recently, dicamphanoyl-dihydropyranoxanthone (DCX-**Figure 8**) derivatives, previously discovered as novel anti-HIV agent, were evaluated for their potential to reverse multi-drug resistance in a cancer cell line over-expressing P-glycoprotein (P-gp). DCX can act as dual inhibitor of HIV replication and chemoresistance mediated by P-gp. As such, they may be useful in combination therapy to overcome P-gp-associated drug resistance for AIDS treatment (Zhou, T. *et al.*, 2012a and 2012b).



DCX Mangiferin

Figure 8. DCX and Mangiferin structures

- Antioxidant Activity

Xanthones with phenolic groups have been described for their remarkable antioxidant activities, namely scavenging ones (Chin, Y-W. *et al*., 2008; Mahabusarakam, W. *et al*., 2006; Rana, V.S. and Rawat, M.S.M., 2005). Beside free radical scavengers, they can act as metal chelators as well as inhibitors of lipid peroxidation. These properties have been implicated with their hepatoprotective, anti-inflammatory and cancer chemopreventive actions (Pinto, M. *et al.*, 2005).

One example of xanthone with this activity is the mangiferin (**Figure 8**), a "super antioxidant" more potent than vitamin C and E (Wu, Z. *et al.*, 2010).

- Cardioprotective effect and cardiovascular activity

Many xanthones with catechol systems are considered anti-atherosclerotic since they have anti-low density lipoprotein (LDL) oxidation and acyl CoA:cholesterol acyltransferase (ACAT) inhibition activities, both of which are potentially useful for treating and/or preventing atherosclerosis and hypercholesterolemia (Park, K.H. *et al.*, 2006). They showed to have potential as cardiovascular protective agents (Jiang, D.J. *et al.*, 2004; Marona, H. *et al.*, 2009; Wang, Y. *et al*., 2008).

Xanthones can be useful in several pathologies like asthma, allergies, inflammation and thrombosis, once they are PAF-antagonists. PAF – exogenous platelet activating factor, initiates anaphylactic hypotension being an important mediator of those pathologies. Xanthones isolated from various plants showed inhibitory effects on PAF-induced hypotension, some of them with an activity higher than that of gingkolide-B, a recognized natural PAF-antagonist from *Gingko biloba* (Oku, H., *et al*., 2005).

- Anti-allergic and Anti-inflammatory Activity

Mast cells are known to participate in allergic and anaphylactic reactions related to immune responses. Various stimuli can activate mast cells, causing the release a number of biologically active molecules like histamine, serotonin, Interleukin-6 (IL-6), leukotriene C_4 (LTC_4), and prostaglandin D_2 (PGD_2), leading to allergic and inflammatory diseases, especially skin inflammation and allergic asthma. The diprenylated xanthones isolated from *Garcinia Mangostana* α-and γ-mangostin were investigated for their inhibitory effect on the allergy mechanism (Chae, H., *et al*., 2012). α-and γ-mangostin were found to modulate the production of IL-6, LTC_4, PGD_2 and the release of histamine, and also repress cyclooxygenase-2 expression, suggesting that α-and γ-mangostin may be useful as anti-allergic and anti-inflammatory molecules in preventing or treating various diseases (Nakatani, K. *et al.*, 2002).

- Neuropharmacological Activity

Alzheimer's disease is the most common age-related degenerative brain disorder, which causes gradual, irreversible losses of memory and other mental abilities. Once it is associated with low brain neurotransmitters levels, it is fundamental to find new cholinesterase inhibitors as new drug candidates to treat Alzheimer's disease and related dementias. Most currently known natural inhibitors of acetylcholinesterase (AChE) are alkaloids, which have the disadvantages of short half-lives and undesirable side effects (Lopez, S. et al., 2002). Thus, xanthones can play an important role as non-alkaloid cholinesterase inhibitors, exhibiting potent inhibitory activities against AChE (Urban, A. *et al*., 2004). Since multiple pathogenic factors, including aggregated amyloid-β peptide and tau protein, excessive levels of transition metals, oxidative stress and reduced acetylcholine levels, are implicated in Alzheimer's disease, multipotent agents with diverse targets are expected to be more effective for treating Alzheimer's disease than single-target counterparts (Mattson, M.P., 2004). Once xanthone are "privileged structures" that can act in different receptors, multiple pharmacological effects can be combined into one xanthone molecule.

In this way, is also important to note that xanthones are also capable to inhibit monoamine oxidase (MAO), an enzyme that exists as two isoenzymes – MAO-A and MAO-B, with an important role in the metabolism of neurotransmitters, including serotonin and dopamine (Harkcom, W.T. and Bevan, D.R., 2007; Nunez, M.B. *et al.*, 2004; Ohishi, N, *et al.*, 2000; Thull, U., *et al*., 1993). It was proved that the dysfunction of this enzyme is associated with neurological disorders like depression, drugs abuse and attention deficit, revealing the importance of finding new and effective MAO-inhibitors and therefore the importance of the xanthones (El-Seedi *et al.*, 2010).

Xanthones are multipotent agents, found to be efficient radical scavengers, MAO (isoenzymes A and B) inhibitors and potential AChE inhibitors (El-Seedi *et al.*, 2010; Mattson, M.P., 2004), thereby fundamental tools in the treatment of dementias.

- α-Glucosidase Inhibitory Activity

Nowadays, several diseases like obesity, heart disease and diabetes are rising. In the case of diabetes, glycosidases, a large family of enzymes that are involved in the processing of complex carbohydrates have been consider as an important therapeutic target. α-Glucosidase inhibitors

also show promising therapeutic potential in the treatment of disorders such as human immunodeficiency virus (HIV), as referred above, metastatic cancer, and lysosomal storage diseases (Melo, E.B. *et al*., 2006). In the specific case of diabetes mellitus type II (Floris, A.L. *et al*., 2005), α-glucosidase is a key enzyme required for cleavage of maltose for absorption of glucose into blood in the small intestine. Thus, abnormally high levels of plasma glucose after a carbohydrate meal may be regulated by α -glucosidase inhibitors.

Xanthones are now being study as α-glucosidase inhibitors because of the high level of natural abundance and antioxidant potential (Ryu, H.W., *et al*., 2011). They proved to have antihyperglycemic activity, helping to low postprandial glucose absorption by retarding the cleavage of complex carbohydrates (Seo, E.J. *et al.*, 2007). One good example of this inhibitory activity is γ-mangostin (Ryu, H.W., *et al*., 2011).

- Cytotoxicity and Cancer Chemoprevention Activities

Despite the advances in anticancer drug development, cancer is still a major cause of human mortality and morbidity. Today there are powerful tools for the development of new cancer treatments, but small molecules like xanthones possessing potent anticancer activities are still attractive as novel anticancer drug candidates (Woo, S. *et al.*, 2010).

Among a myriad of biological activities described for xanthones, the *in vitro* growth inhibitory activity on tumor cell lines appeared to be quite significant, since they exert their effect on a wide range of different tumor cell lines. Apart from the antitumor effect, some extracts containing xanthones have been described for their antimutagenic properties and for their cancer chemopreventive effect, acting as inhibitors of tumoral promoters. The xanthones reported to date as antitumor agents include the xanthone molecule itself (**Figure 1**), as well as other natural and synthetic derivatives (Palmeira, A. *et al*., 2010; Pinto, M. *et al.*, 2005).

Mangosteen, *Garcinia mangostana*, is a fruit found in South East Asia that has been used as traditional medicine. Its therapeutic properties are related with xanthones, its principal secondary metabolites (Peres, V. *et al*., 2000). Prenylated xanthones isolated from young fruits of *Garcinia mangostana*, namely α, β, and γ-mangostin (**Figure 4**) showed cytotoxic activity against several human tumor cell lines (El-Seedi, H.R. *et al*., 2010; Suksamrarn, S. *et al.*, 2006). α-Mangostin (**Figure 7**), one of major xanthones isolated from mangosteen, has been reported for its antiproliferative, antitumor growth, metastasis suppression and apoptotic effects against

human breast cancer, prostate cancer , colon cancer and leukaemia (Han, A.-R. *et al.*, 2009; Lee, Y.-B. *et al*., 2010; Na, Y., 2009; Woo, S. *et al*., 2007).

Another impressive natural xanthone is psorospermin (**Figure 9**), a dihydrofuranxanthone of the African plant *Psorospermum febrifugum*. This molecule showed excellent anticancer activity against human and murine cancer cell lines (Na, Y., 2009). Psorospermin advanced to clinical trials but further development for the commercial market suffered from limited resources. Meanwhile stereoselective total synthesis for psorospermin was reported (Schwaebe, M.K., *et al.*, 2005), giving a future opportunity to clinical trials with this compound. Psorospermin has shown biological activities via intercalation of the xanthone group with DNA base pairs (Shen, R. *et al*., 2010) and alkylation of epoxide by N7-guanine in the presence of topoisomerase II (Na, Y., 2009; Woo, S. *et al.*, 2007).

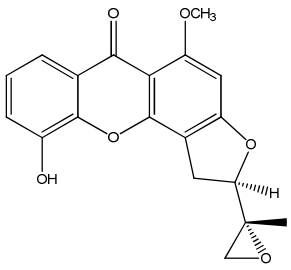
Topoisomerases are critical cellular enzymes necessary for cell proliferation, being involved in the DNA replication. Because of the importance of these enzymes in the cell proliferative process, topoisomerases are one of the major targets in anti-cancer drug development. Also, compounds that can act as DNA cross-linking agents, inhibiting replication and transcription and therefore causing cell death, are an important class of anti-tumor drugs targeting DNA. In Sangwook Woo study, these two mechanisms were tested and the synthesized xanthones exhibited strong inhibitory activity against different cancer cell lines (Woo, S. *et al*., 2010).

Also among these interesting xanthones is the polyprenylated xanthone gambogic acid (**Figure 5**), the most abundant caged *Garcinia* xanthones, isolated from the resin of the *Garcinia hurbury* tree. In finished phase II clinical trials in China, this compound was identified as a potent anticancer agent during highthroughput screening to determine if this agent could be used as a novel anticancer agent. A variety of mechanisms have been proposed by which gambogic acid inhibits the proliferation of cancer cells and induces apoptosis. These include inhibition of antiapoptotic proteins, induction of apoptosis-associated proteins, inhibition of topoisomerase II, among others. Other reported pathway is the inhibition of angiogenesis by suppressing vascular endothelial growth factor (VEGF) signalling, a factor that is highly expressed in human cancer tissues. It was also found that the apoptotic effect of gambogic acid was not related to cell cycle arrest, a common pathway for many current natural anticancer drugs, including paclitaxel and that gambogic acid reversed docetaxel resistance in gastric cancer cells, further supporting the potential of gambogic acid as a prospective anticancer drug candidate (Sun, H. *et al*., 2012; Wang, X. *et al*., 2011b).

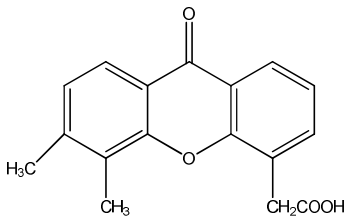
One the best-known synthetic xanthone candidate as anticancer drug is 5,6-dimethylxanthenone-4-acetic acid (DMXAA), also known as Vadimezan (**Figure 9**). Once angiosenesis is a fundamental process in tumor growth and progression, considerable efforts have been directed to antiangiogenic therapy as a new treatment for human cancers. DMXAA, a simple carboxylated xanthone, was discovered in a structure-activity relationship study involving a series of xanthone-4-acetic acids related to the parent drug flavone acetic acid. DMXAA was the most effective analog and was then selected for detailed evaluation (Palmeira, A. *et al*., 2010).

DMXAA is a vascular disrupting agent that leads to the collapse of tumor vasculature and subsequent tumor cell death (Na, Y., 2009) by immunomodulation and cytokines induction. It also possesses inductive effects in 5-hydroxytryptamine and nitric oxide (NO). In this way, DMXAA may be applied in synergy not only with conventional cytotoxic agents and other antivascular agents, but also with immunomodulatory agents that increase host-mediated responses such as cytokines and NO (Pinto, M. *et al*., 2005). This compound has attracted scientific interest because of its excellent pharmacological profile since its discovery. Its multiple actions can be used as a basis to improve antivascular therapy.

Until recently, DMXAA was the most advanced in clinical development among investigational vascular disrupting agents, being tested mainly for lung cancer. However, two phase III trials of this small molecule in the first- and second-line settings in non-small cell lung cancer (NSCLC) were terminated early after the observation of no overall survival benefit (Reckamp, K.L., 2012; Rogosin, S. and Sandler, A.B., 2012).



Psorospermin



DMXAA

Figure 9. Psorospermin and DMXAA structures

Once xanthones can exhibit their antitumor activity through different mechanisms of action, in **table 1** are summarized xanthone derivatives/substituents and their mechanisms.

Table 1. Mechanisms of action for xanthone derivates with antitumor activity (Pinto, M. *et al*., 2005 and references cited therein)

MECHANISMS	**XANTHONE DERIVATIVES/SUBSTITUENTS**
Apoptosis via caspase 3	Prenyl; Glycosyl; Methylenecarboxy
Aromatase inhibition	Imidazolyl; Triazolyl
DNA binding	Pyrano; Amino; Oxygenated
DNA breaks and DNA-proteins cross-links	Furano; Epoxy
DNA synthesis suppression	Epoxy; Polycyclic; Prenyl; Dialkylamine; Oxygenated; Furano
11-β-Hydroxylase inhibition	Xanthone-anthraquinone
17-α-Hydroxylase/C17,20-lyase inhibition	Imidazolyl; Triazolyl
Immunomodulation, cytokines induction	Methylenecarboxyl
Kinases modulation	Dihydroxy; Dihydroxy/nitro; Methylenecarboxy; Prenyl; Glycosyl; Formyl; Hydroxyl; Xanthonolignoids; Oxygenated
Phospholipase C inhibition	Xanthone-chromone, Tetra-oxygenated
Prostaglandin (PG) E2 receptors blocking	Prenyl; Carboxy
Protein synthesis suppression and RNA synthesis suppression	Epoxy
Sphingomyelinases inhibition	Prenyl
Topoisomerases I and II inhibition	Furano; Prenyl; Oxygenated; Pyrano; Xanthonolignoids; Carboxamide; Epoxy; Acyl; Thioxanthones

Transforming growth factor-β (TGF-β) gene expression increasing	Glycosyl
Vasculogenic mimicry inhibition	Methylenecarboxyl

With all these biological activities and their promising value is easily understandable that xanthones, either from synthesis or natural resources, are considered a class of compounds with great interest.

3. SYNTHESIS OF XANTHONES

Once the biosynthetic routes are a limiting factor for the structural variation of naturally-occurring xanthones, the chemical synthesis can be a good alternative to create new derivatives as well as to rationalize the structure-activity relationship.

One of the first synthesis of xanthones was made by Michael and Kostanecki, involving the distillation of a mixture of a phenol, an *o*-hydroxybenzoic acid and acetic anhydride (Sousa, M.E. and Pinto, M., 2005). Since then, different methods have been developed, with higher yields and less drastic experimental conditions (Sousa, M.E. and Pinto, M., 2005).

Three classical methods can be used to synthesize xanthones:

- the Grover, Shah, and Shah (GSS) reaction;
- the synthesis *via* benzophenone;
- the synthesis *via* diaryl ethers intermediates.

The GSS reaction (**Figure 10**) is a one-pot process for preparing hydroxyxanthones and still popular due to usually accessible materials. The xanthone is obtained by adding a salicylic acid derivative (**1**) and a suitable phenol (**2**), both heated together with zinc chloride in phosphoryl chloride as solvent. It can afford the xanthone skeleton (**4**), directly only if the benzophenone intermediate (**3**) carries another hydroxyl group at 6 or 6' position, which means if an alternative site for cyclization is available (*****).

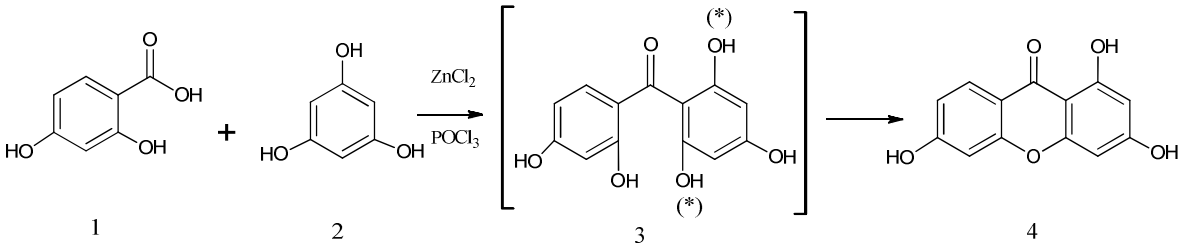


Figure 10. Synthesis of xanthones *via* GSS reaction

The xanthone synthesis via benzophenone intermediate (**Figure 11, I**) begins with condensation, by Friedel-Crafts acylation, of a substituted benzoyl chloride with a phenolic derivative (a) in the presence of aluminium chloride and anhydrous diethyl ether as solvent, followed by a intramolecular cyclization (b).

The xanthone synthesis *via* diaryl ether (**Figure 11, II**) is an Ullman condensation of sodium phenolates with *ortho*-halogenated benzoic acids (c), followed by ring formation by electrophilic cycloacylation of the 2-aryloxybenzoic acids (d).

Once intermolecular acylations give generally higher yields than Ullmann ether syntheses, the chosen route for xanthone synthesis is usually the acylation, followed by cyclization to form the heterocyclic ring (Sousa, M.E. and Pinto, M., 2005).

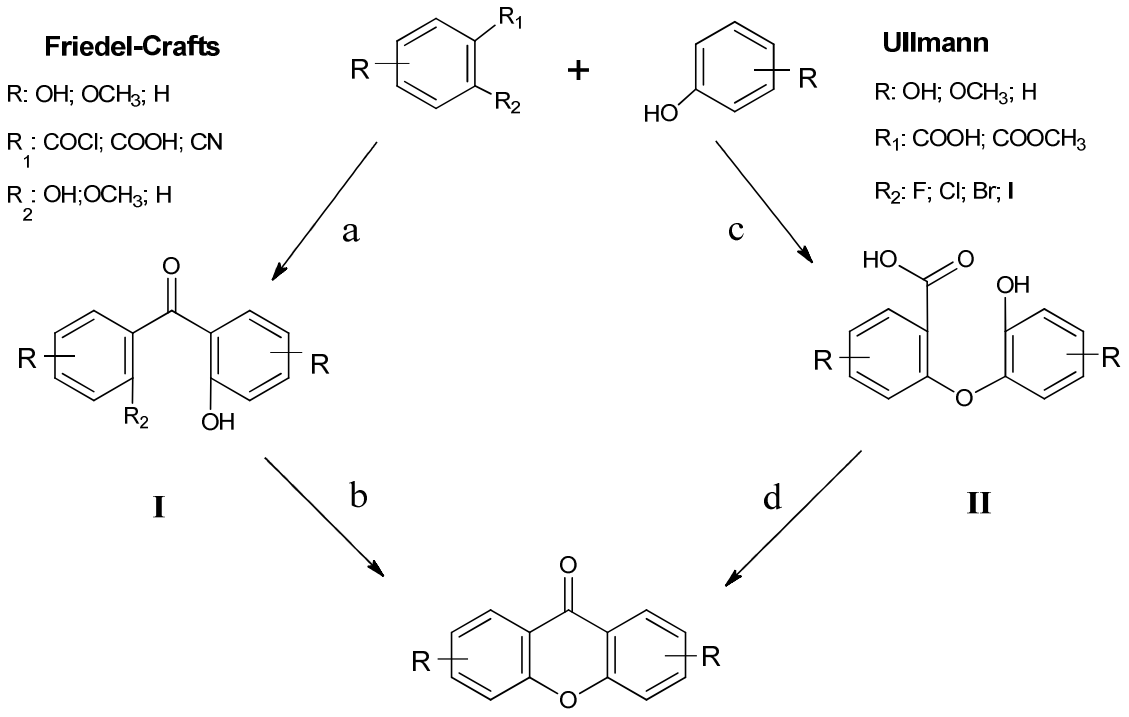


Figure 11. Synthesis of xanthones *via* benzophenone and *via* diaryl ethers intermediate

Different methods of construction of xanthone core have been reported in the last years. Modifications to the GSS reaction (**Figure 10**) have emerged. For example, in some cases, better results can be obtained using as catalyst the Eaton's reagent (phosphorus pentoxide and methanesulfonic acid) instead of the traditional mixture of phosphorus oxychloride and zinc chloride (Davies, J.S.H. *et al.*, 1958; Grover, P.K. *et al.*, 1955; Pillai, R.K.M. *et al.*, 1986; Sousa, M.E. and Pinto, M., 2005). Later, this alternative method will be discussed in this thesis applied to the xanthone synthesis.

Very recently, Verbanac (Verbanac, D. *et al*., 2012) described an efficient microwave-assisted chemical synthesis of (thio)xanthones. According to them, from a mixture of phenolic acids and phenol derivatives with Lewis acid (**Figure 12**), heated under microwave irradiation, xanthones can be obtained with the desired regioselectivity in a shorter reaction time (50 seconds) and with very good yields (upper than 80%).

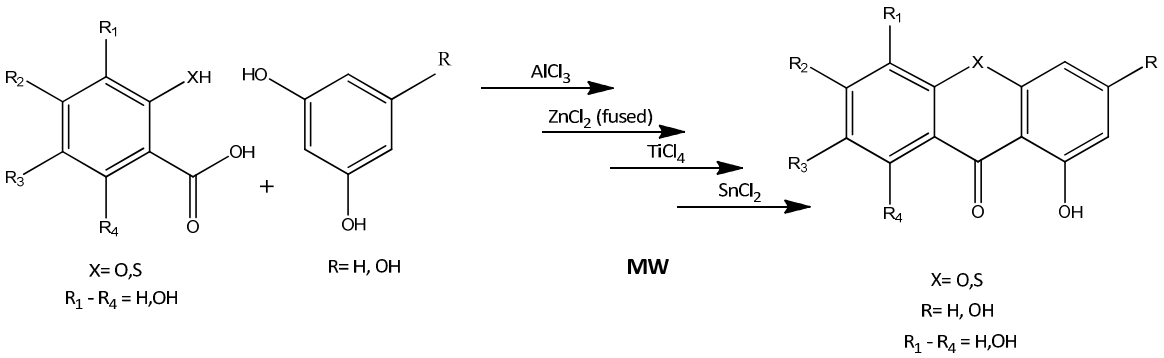


Figure 12. Xanthone synthesis by Verbanac, D. *et al.*, 2012

Others methodologies for the synthesis of xanthones can be found in a very recent review by Key-Simeon Masters and Stefan Bräse (Masters, K-S. and Bräse, S., 2012).

4. SYNTHESIS OF PRENYLATED XANTHONES

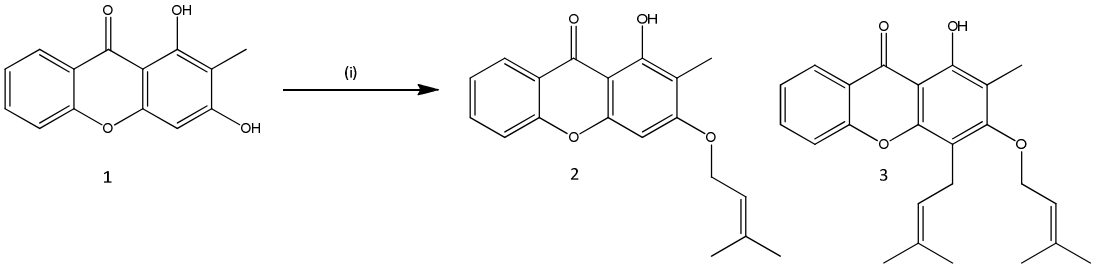
4.1. "CLASSICAL" SYNTHETIC METHODOLOGIES

The methodologies to obtain synthetic prenylated xanthones and respective cyclic derivatives are based on different approaches of molecular modification (Pinto, M. and Castanheiro, R., 2009a) namely:

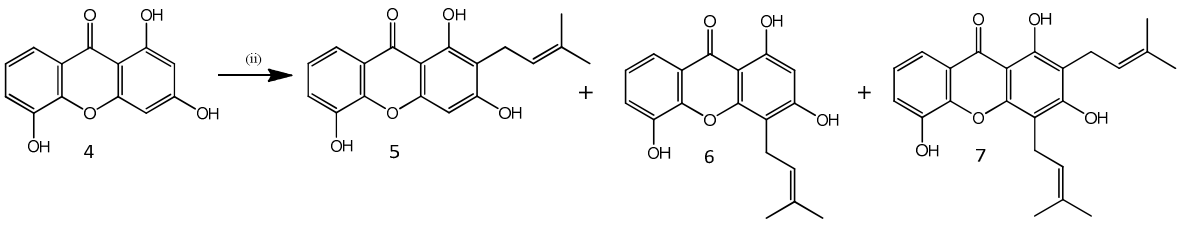
- Molecular extension through prenylation of xanthonic scaffold (**Figure 13**);
- Molecular rigidification by Claisen rearrangement and/or cyclization of prenylated precursors (**Figures 14 and 15**).

4.1.1. Molecular extension through prenylation of xanthonic scaffold

The xanthonic scaffold can be prenylated by a nucleophilic substitution reaction with prenyl bromide, in alkaline medium. The products of this reaction are usually prenyloxy xanthones, however, in some cases, diprenylated derivatives with the prenyl group on the carbon adjacent to the prenyloxy substituent can also be obtained (**Figure 13 (i)**). If the reaction is performed in an aqueous medium, for example a KOH solution, *C*-prenylation can also occur (**Figure 13 (ii)**) (Pinto, M. and Castanheiro, R., 2009a).



(i) Prenyl bromide, K_2CO_3, Acetone, reflux, 8h (2, 48%; 3, 3%)



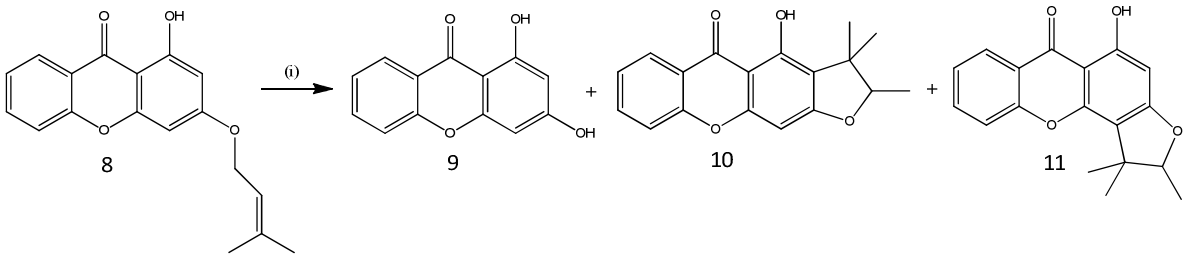
(ii) Prenyl bromide, aq. KOH 10%, room temperature, overnight (5, 11%; 6, 13%; 7, 10%).

Figure 13. Prenylation of the xanthonic scaffold with different experimental conditions (i, ii).

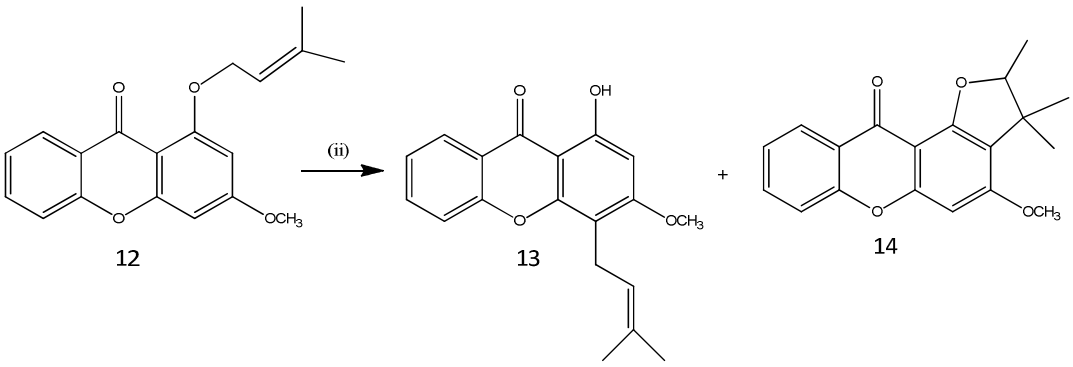
4.1.2. Molecular rigidification by Claisen rearrangement and/or cyclization of prenylated precursors

- Claisen rearrangement of prenylated precursors

The molecular rigidification can be the result of Claisen rearrangement. In **figure 14 (i)**, the 1-hydroxy-3-(3-methylbut-2-enyloxy)-9*H*-xanthen-9-one (**8**) were heated in vacuum at 200-210°C to obtain rearranged prenyl xanthones and/or cyclic derivatives. Three products were formed: 1,3-dihydroxy-9*H*-xanthen-9-one (**9**), and two products with 4,4,5-trimethyl-4,5-dihydrofuran-ring condensed in either linear (**10**) or angular ways (**11**). These dihydrofuran derivatives results of Claisen rearrangement in both the available *ortho* positions of the xanthone scaffold to give (1,1-dimethylallyl) derivatives (**Figure 3, C**) followed by spontaneous cyclization involving the 3-hydroxy group (Pinto, M. and Castanheiro, R., 2009a).



(i) Vacuum, 200-210°C (9, 32%; 10, 18%; 11, 9%)



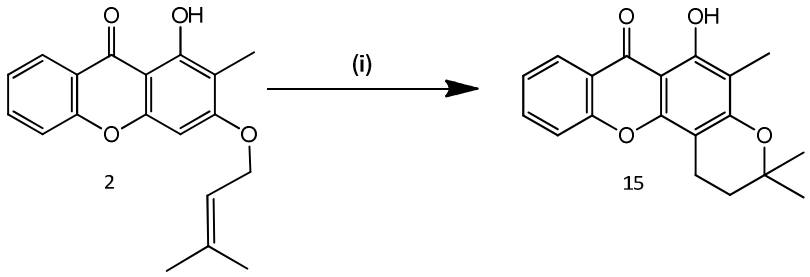
(ii) *N,N*-DMA, 200°C (13, 60%; 14, 30%)

Figure 14. Claisen rearrangement of prenylated precursors

Other example of the Claisen rearrangement is the reaction of 3-methoxy-1-(3-methylbut-2-enyloxy)-9*H*-xanthen-9-one (**12**) performed in *N,N*-dimethylaniline (*N,N*-DMA) (**Figure 14 (ii)**). A mixture of 1-hydroxy-3-methoxy-4-(3-methylbut-2-enyl)-9*H*-xanthen-9-one (**13**) and the dihydrofuranoxanthone (**14**) were obtained, along with a small amount of starting material (**12**)**.** The xanthone **13** resulted of a *para* Claisen rearrangement of xanthone **12**, while dihydrofuranoxanthone **14** was obtained from an *ortho* Claisen rearrangement followed by a spontaneous cyclization with the 1-hydroxy group (Pinto, M. and Castanheiro, R., 2009a).

- Cyclization of prenylated precursors

The molecular rigidification can also be the result of cyclization of prenylated precursors. In **figure 15**, the monoprenylated xanthone **2** is used as precursor for the synthesis of dihydropyranoxanthones. By heating **2** with zinc chloride in *o*-xylene, the angular dihydropyranoxanthone **15** is obtained (Pinto, M. and Castanheiro, R., 2009a; Castanheiro, R. *et. al.*, 2009b).



(i) $ZnCl_2$, *o*-xylene, 200°C, 21h (15, 22%) (Castanheiro, R. *et. al*, 2009b)

Figure 15. Synthesis of dihydropyranoxanthones

4.1.3. Different approaches for the synthesis of prenylated xanthones

Prenylated xanthones can also be generated by a condensation and consequent cyclization reaction of prenylated building blocks. For example, using this method the naturally occurring α-mangostin (**Figure 16**, **20**) can be synthesized. The coupling reaction between the building blocks (**16 and 17**) leads to benzophenone intermediate (**19**) that cyclizes, to give the natural xanthone (**20**) (Pinto, M. and Castanheiro, R., 2009a).

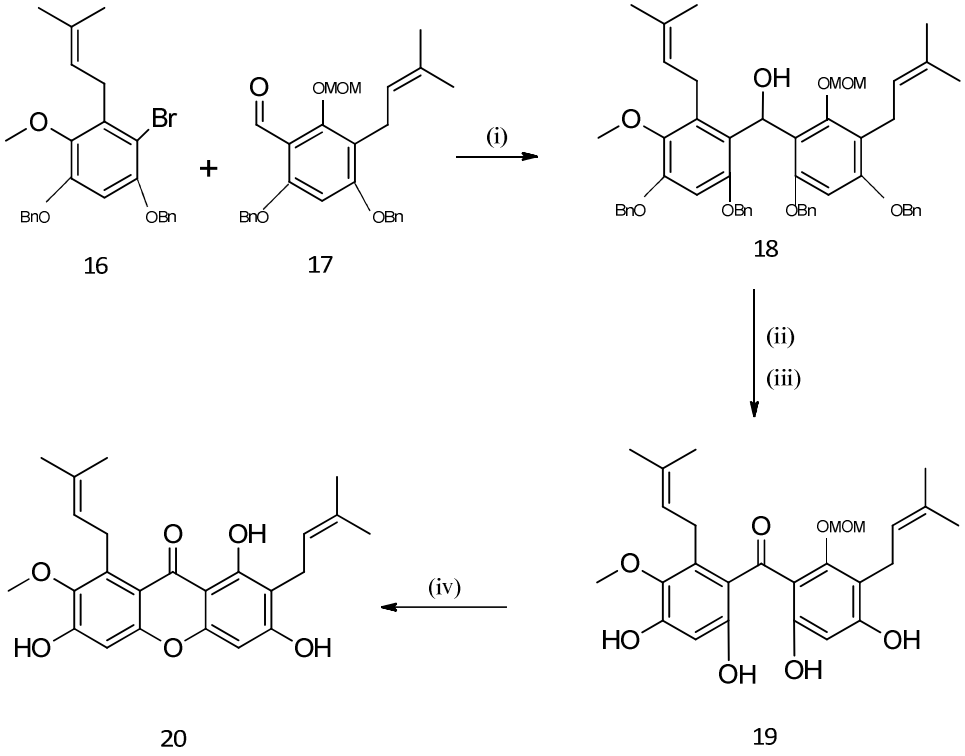


Figure 16. Synthesis of α-mangostin (i): *s*BuLi, THF, -78°C, 49%; (ii) IBX, toluene/DMSO (1/1), room temperature, 76%; (iii) 10% Pd/C, HCO_2NH_4, acetone, room temperature, 63%; (iv) PPh_3, CCl_4, THF, room temperature, silica gel, 43%

4.2. “NON-CLASSICAL” SYNTHETIC METHODOLOGIES

The synthetic procedures can be achieved not only by the application of classical methodologies, such as the use of conventional heating, but more recently following “non-classical” synthetic alternatives like microwave-assisted organic synthesis (MAOS) and/or heterogeneous catalysis. In this thesis, the molecular modifications to obtain prenylated xanthones were attained by the application of these two techniques discussed later on.

To quickly and efficiently synthesize a large number of xanthones derivatives, alternative synthetic methodologies to the classical ones are being increasingly explored. The lack of reproducibility, difficult and expensive scale-up and standardization of synthetic processes are the main hurdles towards the industrial production. Time- and energy-consuming synthetic routes, usually involving the use of volatile and toxic organic solvents, can be apparently cost-viable and environmentally acceptable for the synthesis at a laboratory scale. However, in an industrial scale, due to the product high cost and to the negative impact they cause on the environment, that routes are often not viable. Also, the appearing of a new philosophy that aims to minimize the use of non-renewable resources and organic solvents, the generation of toxic secondary products and the consumption of energy and the emission of gases – the so called green chemistry or sustainable chemistry, a concept that has received enormous attention in recent times - has encouraged the use of “non-classical” synthesis (Sosnik, A. *et al.*, 2011).

To overcome those hurdles and achieved the aims of the green chemistry, synthetic alternatives like microwave-assisted organic synthesis (MAOS) and heterogeneous catalysis are being applied.

- **MAOS - Microwave-assisted organic synthesis**

MAOS, first reported in the late 1980s, relies on the application of microwave (MW) irradiation as the energy source for organic reactions. MWs comprise electromagnetic radiation with a frequency between 0.3 and 300 GigaHertz (GHz), with domestic and synthetic ovens usually opperating between 2 and 8 GHz (**Figure 17**).

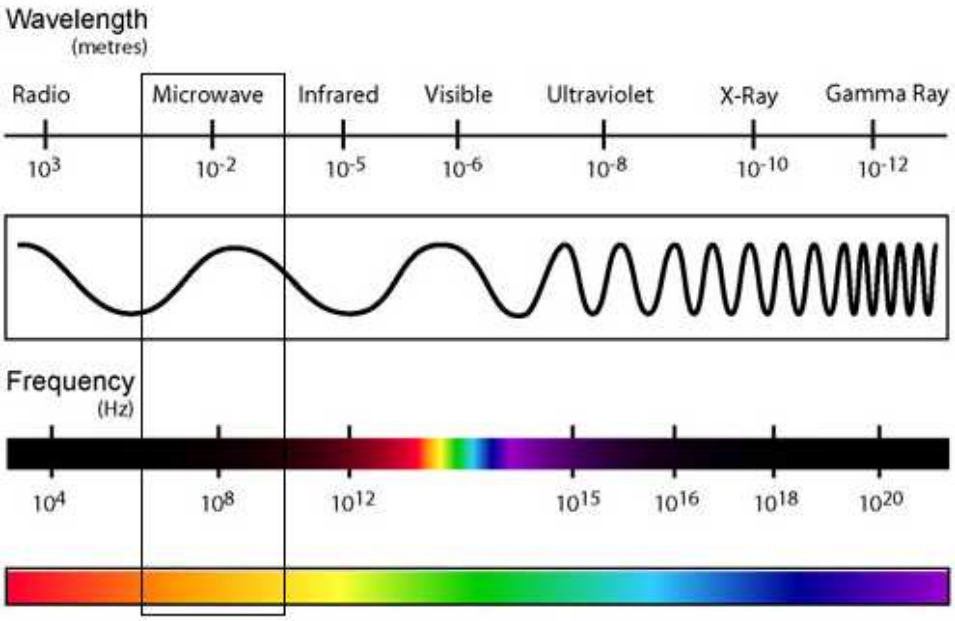


Figure 17. Electromagnetic spectrum (http://labs.ciid.dk/experiments/)

MAOS is mainly based on the efficient heating of materials by microwave dielectric heating effects. Microwave dielectric heating is dependent on the ability of a specific material (solvent or reagent) to absorb microwave energy and convert it to heat. The ability to convert MW energy into heat, at a given frequency and temperature, is determined by the dissipation factor or 'loss tangent', tan δ and in general a reaction medium with a high (>0.5) tan δ value, at the standard operating frequency of a microwave synthesis reactor (2.45 GHz), is required for efficient absorption and rapid heating (Kappe, C.O., 2008).

Microwave irradiation triggers heating by two main mechanisms— dipolar polarization and ionic conduction. Whereas the dipoles in the reaction mixture (for example the polar solvent molecules) are involved in the dipolar polarization effect, the charged particles in a sample (usually ions) are affected by ionic conduction. When irradiated at microwave frequencies, the dipoles or ions of the sample align in the applied electric field. As the applied field oscillates, the dipole or ion field attempts to realign itself with the alternating electric field and, in the process, energy is lost in the form of heat (**Figure 18**).

Although is not the only important factor in the conversion of energy into heat, the polarity of the solvent is an important tool to predict if the solvent will be heated under MW irradiation. Usually, polar solvents absorb well MW irradiation, while less polar or non-polar solvents are low absorbing or do not absorb. If the reaction requires the use of solvents that are

non-polar and therefore MW low absorbing, there are passive heating elements, like *weflon*^{TM} (inert phluoropolymer) magnetic bars, that are made out of strongly microwave absorbing materials and therefore increase the MW absorbance level of the medium, allowing the heating of the mixture reaction (Kappe, C.O., 2008; http://www.milestonesci.com).

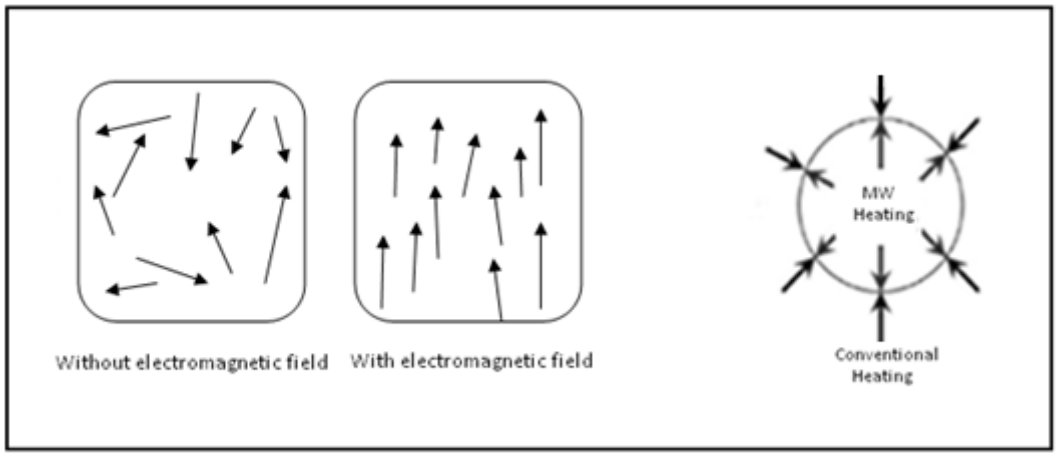


Figure 18. Dipolar polarization; microwave *versus* traditional heating process

Until recently, heating reaction mixtures on a laboratory scale was typically performed using isomantles, oil baths or hot plates applying a reflux set-up where the reaction temperature is controlled by the boiling point of the solvent. This traditional form of heating – surface heating - is a rather slow and inefficient method for transferring energy into a reaction mixture and often results in the temperature of the reaction vessel being higher than that of the reaction mixture. In contrast, microwave irradiation produces efficient internal heating by direct coupling of microwave energy with the molecules present in the reaction mixture (**Figure 18**) (Kappe, C.O., 2008; Salema, A.A. and Ani, F.N., 2011; Sosnik, A. *et al.*, 2011). Since the reaction vessels employed in microwave chemistry are made out of essentially microwave transparent materials such as glass or Teflon only the reaction mixture is heated.

The equipment used in this work was the *MicroSYNTH* from Milestone. There are different adaptable accessories to thus equipment, allowing it to be used widely, in a small or large scale, at different values of pressure and temperatures. The inside temperature of the vessel is measured by an optic fiber sensor and the outside temperature, in the cavity, is controlled through an infrared sensor (**Figure 19**) (Favretto, L., 2003; http://www.milestonesci.com).

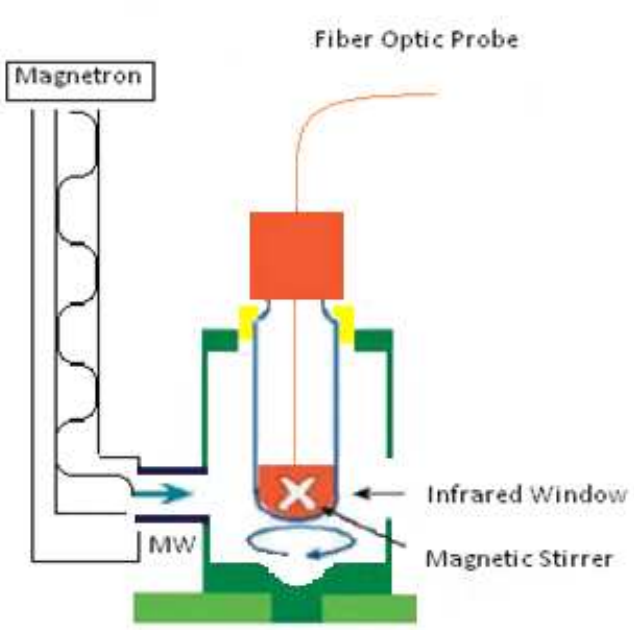


Figure 19. MW equipment system (http://www.milestonesci.com)

Due to unique advantages such as shorter reaction times, higher yields, limited generation of by-products and the relatively easy scale-up without detrimental effects, this technology has steadily become an appealing synthetic tool (Kappe, C.O. and Dallinger, D., 2009; Kappe, C.O., 2008 and 2004; Sosnik, A. *et al.*, 2011).

Comparing the results and reaction conditions with the reaction presented before in **Figure 13 (i)**, it is notably the optimization of the synthetic process when MW irradiation was applied (**Figure 20**). This reaction was faster, with better yields and more selectivity as we can conclude by the analysis of **table 2** (Pinto, M. and Castanheiro, R., 2009a).

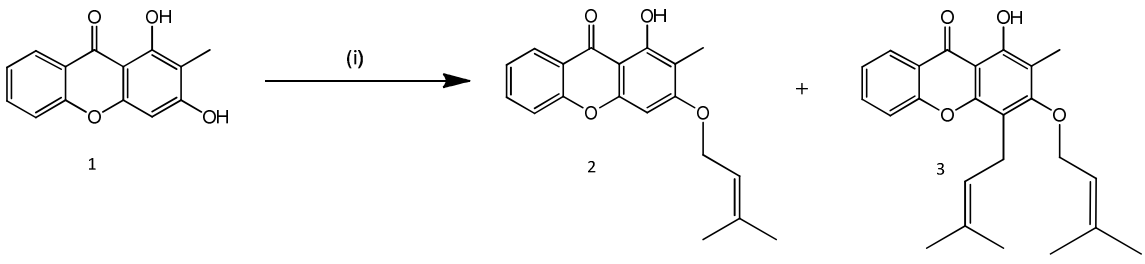


Figure 20. Prenylation using MAOS (i) Prenyl bromide, K_2CO_3, Acetone, MW, 200W, 3x20min, 59°C

Table 2. Prenylation through MW irradiation and conventional heating

	Conventional Heating	MW
Yield	48% (2) 3% (3)	83%(2) 5% (3)
Reaction time	8 h	1h

The microwave heating process, the high temperatures attained and the ability to work under high pressure conditions for relatively short times make reactions faster than under conventional thermal conditions and limit the occurrence of side reactions (Salema, A.A. and Ani, F.N., 2011; Kappe, C.O., 2008 and 2004). Therefore, better yields, more selectivity and reaction times reduced from days and hours to minutes and seconds represent a big gain in areas like Medicinal Chemistry and Drug Discovery, where large libraries of compounds are generated.

In **table 3** are summarized some relevant differences between conventional thermal heating and MW heating (the differences need to be understood as general) (Sosnik, A. *et al.*, 2011).

Table 3. Main general differences between conventional and MW heating (Sosnik, A., *et al.*, 2011).

PROPERTY	CONVENTIONAL HEATING	MW HEATING
Heating rate	Slow	Fast
Maximum reaction temperature	Limited by the bp* of the solvent (reflux)	Overheating above bp* (up to 100°C in a closed-vessel)
Reaction time (rt)	Long	Short
Pressure	High pressure reactions more dangerous (longer reaction time)	High pressure reactions are safer
Homogeneity of heating	Low	High
Yield	Low	High
Amount of secondary products	High	Low
Solvent conditions	Difficult to work without solvent	Easy to work in solvent-free conditions
Reproducibility [a]	Low	High

*bp – boiling point

[a] A higher reproducibility of MW reactions is commonly claimed, but this depends on the sophistication of the equipment, as domestic MW ovens lack optimal reproducibility.

Other advantage of MW technique is, when one of the reactants is liquid, it can act as a solvent and absorb MW irradiation sufficiently to heat the system, so that reactions can be

conducted under solvent-free conditions (Bougrin, K. *et al*., 2005; Kappe, C.O. and Dallinger, D., 2009; Kappe, C.O., 2004). Due to environment and economical advantages, solvent-free reactions are becoming more appealing, optimizing conventional procedures, by turning them cleaner, safer and simpler. Syntheses traditionally with long reaction times can sometimes become faster when MW and solvent-free conditions are associated. Today, more therapeutic targets are being identified and the synthesis and optimization of lead compounds have to be accelerated (Kappe, C.O. and Dallinger, D., 2005). The traditional synthesis is usually slow and can be insufficient to satisfy the growing need of new drugs. In this context, MW and solvent-free methods have high potential in accelerating this discovery. Avoid the use of organic solvents leads to cleaner, cheaper and efficient syntheses (Green Chemistry), with good yields and short reaction times (Verbanac, D. *et al*., 2012).

MW irradiation has shown many advantageous features over the conventional methods and, even if the experimental conditions to expand its applications in industry remain to be optimized, it is obvious that MW represents one the most versatile and promising synthetic and processing technologies available today (Sosnik, A. *et al.*, 2011).

- **Heterogeneous Catalysis**

Nowadays, one of the biggest challenges in chemistry is to develop synthetic routes that are less polluting, designing clean or green chemical transformations that should not cause permanent damage to the environment. Ways to minimize the consumption of energy and raw materials must be deployed so that optimal value of resources could be realized and environmentally friendly products can be obtained at reasonable costs – Green Chemistry. Catalysts can be a great help to achieve many of these goals (Zhou, C.H., 2011).

Catalysts could be synthetic or natural chemicals capable of making an otherwise impracticable reaction to occur under the mildest possible conditions. Recently, an important family of catalysts derived from the soil has raised the interest of the chemists, being the most remarkable ones the clays and zeolites (Nagendrappa, G., 2011 and 2002). The commercially available montmorillonite K10 clay was one of the chosen catalysts to work with.

Clays are widespread, easily available and low-cost chemical substances. Both in their native state and in numerous modified forms, clays are versatile materials that can function as Brönsted and/or Lewis acids, or as bases and are used to catalyze various types of organic reactions, usually allowing higher yields and greater selectivity (Nagendrappa, G., 2011).

Clays are aluminosilicate nanoparticles (<2 mm in diameter) with layered structures and a surface area of about 23000 cm^2 per gram. The layers possess net negative charge that is

neutralized by cations such as Na^+, K^+, Ca^{2+}, which occupy the interlamellar space and can be very easily replaced by other cations or other molecules. Therefore, simple procedures can easily alter clays properties like acidity, pore size, surface area and others, turning clays, in addition to their environmental compatibility and cheapness, in a catalyst of choice.

Several reviews have been written about clays, highlighting the advantages and developments in the area of organic synthesis using clays (Varma, R.S., 1999; Zhou, C.H., 2011). Their chemical composition and crystal structure are the basis on which they are divided into four main groups such as illite, smectite, vermiculite, and kaolinite. In clays groups, the one that is found to be most useful as a catalyst to the synthetic organic chemist is a subgroup of the smectite clay, called montmorillonite. The montmorillonite lattice is composed of a octahedral sheet of $[Al_2(OH)_6]$ between two tetrahedral sheets of $[SiO4]^{4-}$ (**Figure 21**). The three-sheet layer repeats itself, and the interlayer space holds the key to the chemical and the physical properties of the clay, once, like said before, it has cations that can be replaced by other cations or molecules.

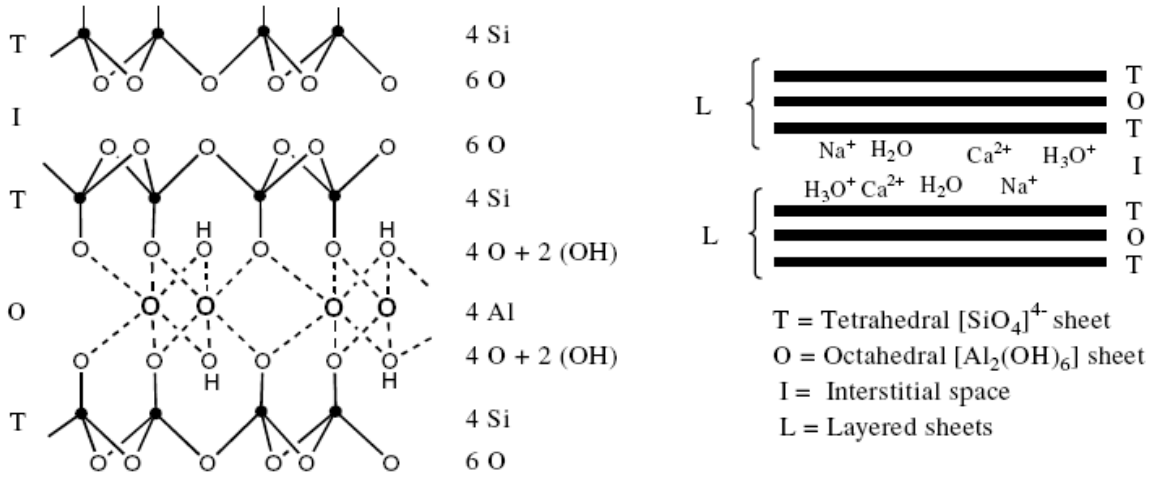
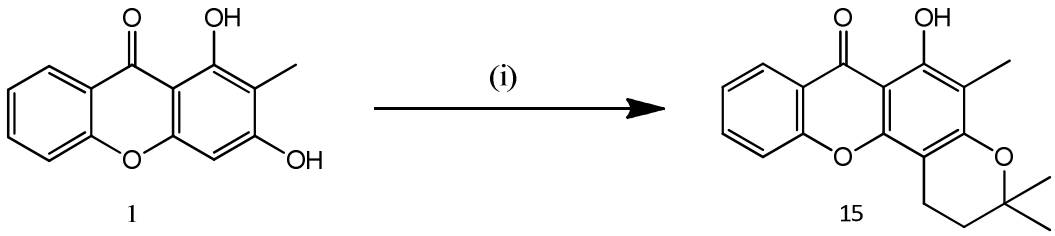


Figure 21. Structure of montmorillonite (Nagendrappa, G., 2002)

When the clay is dry these cations reside in the hexagonal cavities of the silica sheets. When wet, the layers of the clay move apart by the entry of water molecules, the clay swells and the interlayer cations become easily exchangeable by a variety of metallic and non-metallic cations, for example H_3O^+, NH_4^+, Al^{3+}, Fe^{3+}. Therefore the catalytic properties of montmorillonite clay can be manipulated to meet the needs of synthetic organic chemists and a variety of organic reactions can be carried out with great success using such clays as catalysts. With clays, reactions

take place more efficiently, under milder conditions, with better yields, shorter reaction times and greater product selectivity.

Figure 22 shows an example of a prenylation reaction of xanthone **1** that occurred in the presence of K10 clay. Different reaction conditions – room temperature (A), conventional heating at 100 °C (B) and MW irradiation (C) were tested. By analysis of **table 4**, it is clear that the combination of K10 clay-catalysis with MW irradiation was the better method to obtained dihydropyranoxanthone **15** (better yields and shorter reactions times).



1 15

Figure 22. Clay-catalyzed condensation (i) K10 Clay, $CHCl_3$, prenyl bromide, stirring, heating (Castanheiro, R. *et al.*, 2009b)

Table 4. Yields obtained with different reaction conditions (Castanheiro, R. *et al.*, 2009b)

Method	Reaction time	Temperature	Yields with *K10 clay*	Yield with classical synthetic method
A	5 days	Room temperature	51%	10,5%
B	60 minutes	100°C	63%	
C with solvent	20 minutes	110°C	53%	
C without solvent	20 minutes	105°C	86%	

Besides these, clays have others advantages. The work-up and purification are simpler, because the clay is separated easily from the reaction mixture and the catalyst can be reused or

regenerated (Nagendrappa, G., 2002). For all this, the clay-catalyzed synthesis is economical and environmentally favourable, representing a great advantage in a moment where there is urgent to replace the not-so-desirable conventional catalysts by the called green catalysts.

In this thesis, the chemical syntheses catalyzed by montmorillonite K10 clay were performed under microwave irradiation. Associating MW irradiation with inorganic solid supports such as clays, either with solvent or under solvent-free conditions, can bring advantages to the chemical synthesis once it usually allows better reaction rates, high yields, ease of manipulation and selectivity (Castanheiro, R. *et al*., 2009b; Nagendrappa, G., 2011; Varma, R.S., 1999).

II. AIMS

AIMS

This thesis had the main goal the synthesis of xanthone derivatives with prenyl substituents, either in a cyclic or as an open-chain form, and to evaluate their biological activity, namely antitumor. Different synthetic methodologies were applied, considering the principles of the Green Chemistry. To elucidate the structure of the new obtained compounds, spectroscopic methods were a useful tool.

Based on these, the aims of this thesis were:

- To synthesize the building block 1,3-dihydroxy-5-methoxyxanthone;
- To obtained new prenylated xanthone derivates – cyclic and/or open-chain;
- To apply different methodologies to the synthesis of xanthones derivatives – "classical" and "non-classical" – to attempt better yields, shorter reactions times, more selectivity, lower costs and less environmental danger;
- To elucidate the structures of the new compounds using analytical techniques, namely IR, HRMS and NMR (^{1}H, ^{13}C, HSQC and HMBC);
- To evaluate the biological activity of the synthesized compounds, namely the antitumor activity, through their effect on the *in vitro* growth of three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer) and A375-C5 (malignant melanoma).

III. RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

1. Synthesis of xanthone derivatives

PART I

Synthesis of 1,3-dihydroxy-5-methoxyxanthone

The 2-hydroxy-3-methoxybenzoic acid and phloroglucinol (1,3,5-trihydroxybenzene) were used as starting materials for the synthesis of 1,3-dihydroxy-5-methoxyxanthone (**X1**). This xanthonic building block (**X1**) was synthesized through the Grover, Shah and Shah (GSS), method **A** (**Figure 23**), and also applying Eaton's reagent (P_2O_5/CH_3SO_3H) (**B**) as the condensation agent (**Figure 24**), both with conventional heating (Grover, P.K. *et al.*, 1955; Davies, J.S.H. *et al.*, 1958; Pillai, R.K.M. *et al.*, 1986).

In method A (GSS reaction), the use of $ZnCl_2$ and $POCl_3$ results in better yields, obtaining the 1,3-dihydroxy-5-methoxyxanthone (**X1**) with 39% yield.

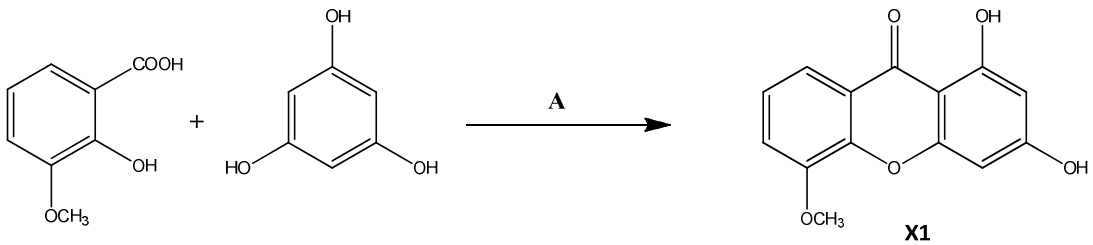


Figure 23. Synthesis of 1,3-dihydroxy-5-methoxyxanthone through method **A** ($ZnCl_2$, $POCl_3$,70°C, 4h30)

In method **B** (Eaton's reaction) (**Figure 24**), due to different reaction conditions, two different xanthones were obtained: 1,3-dihydroxy-5-methoxyxanthone (**X1**) and 1-hydroxy-3-mesyloxy-5-methoxyxanthone (**X2**), with 23% and 15% yield, respectively.

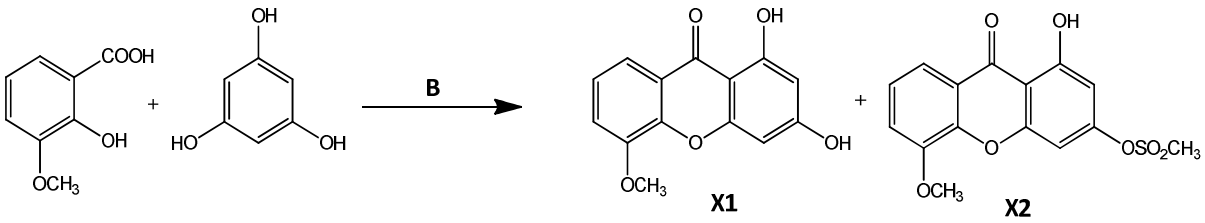


Figure 24. Synthesis of 1,3-dihydroxy-5-methoxyxanthone through method **B** (P_2O_5, CH_3SO_3H, 100°C, 1h)

The purification of **X1** and **X2** was performed by flash column chromatography, using flash silica gel as stationary phase and a gradient of chloroform/acetone as mobile phase. After crystallization from methanol, yellow crystals of both compound **X1** and **X2** were obtained.

PART II

Synthesis of prenylated xanthones

To synthesize prenylated xanthones, the first synthetic approach involves the nucleophilic substitution on the xanthonic building block 1,3-dihydroxy-5-methoxyxanthone (**X1**), with prenyl bromide in alkaline medium (Cs_2CO_3) using acetone as solvent, under 200W microwave irradiation for 1 hour. In this procedure, two main prenylated derivatives were obtained: 1-hydroxy-5-methoxy-3-(3-methylbut-2-enyloxy)xanthone (**P1**) and 1-hydroxy-5-methoxy-4-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (**P2**) in 15% and 5% yield, respectively (**Figure 25**). Other two products were detected but in such small percentage that could not be isolated.

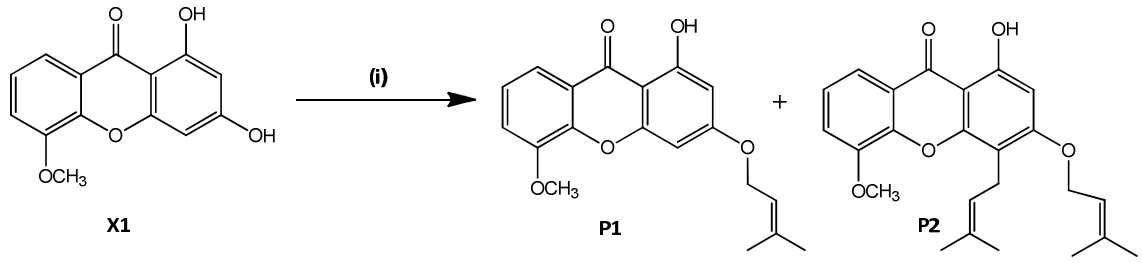


Figure 25. General procedure for the synthesis of **P1** and **P2** by MW irradiation **(i)** Prenyl bromide, Cs_2CO_3, Acetone, MW, 200W, 1h

The second synthetic approach involves a combined methodology using MAOS and montmorillonite K10 clay.

The 1,3-dihydroxy-5-methoxyxanthone (**X1**) was prenylated in the presence of montmorillonite K10 clay, using chloroform as solvent, under 180W microwave irradiation for 45 minutes (**Figure 26**). In this clay-catalyzed synthesis, three main prenylated derivatives were obtained, along with a small amount (13% yield) of starting material: 1-hydroxy-5-methoxy-4-(3-methylbut-2-enyl)-6',6'-dimethyl-4',5'-dihydropyran(2',3':3,2)xanthone (**P3**, 0.3% yield), 1-hydroxy-5-methoxy-6',6'-dimethyl-4',5'-dihydropyran(2',3':3,2)xanthone (**P4**, 3% yield) and 1-hydroxy-5-methoxy-6',6'-dimethyl-4',5'-dihydropyran(2',3':3,4)xanthone (**P5**, 13% yield). Other two products were detected but in such small percentage that could not be isolated.

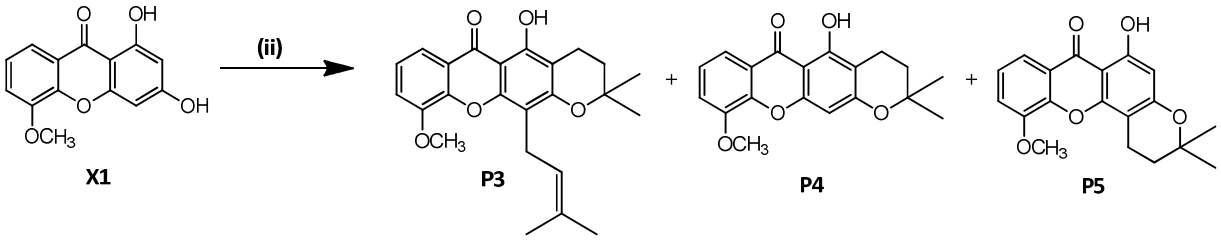


Figure 26. General procedure for the synthesis of **P3, P4** and **P5** by MW irradiation **(ii)** Prenyl bromide, montmorillonite K10 clay, Chloroform, MW, 180W, 45min

The purification of these prenylated xanthone derivatives was performed by flash column chromatography and preparative TLC (SiO_2; hexane/ethyl acetate), and through crystallization.

2. STRUCTURE ELUCIDATION

The structure elucidation of X1, X2, P1, P2, P3, P4 and P5 was established on the basis of melting point, IR, HRMS and NMR (^{1}H, ^{13}C, HSQC and HMBC) techniques.

By the analysis of the IR data, all compounds (**X1, X2, P1, P2, P3, P4 and P5**) show the presence of a band at 3500-3400cm^{-1} corresponding to O-H stretch of hydroxyl groups. It is possible to observe a band corresponding to the C=O, as well as bands corresponding to the C-C aromatic bond typical from the xanthone scaffold. As expected, in **P1** to **P5**, the IR data show the presence of additional bands, corresponding to the C-C and C-H bonds of the prenyl group. Nevertheless, prenylation did not occurred for all hydroxyl groups, once there is still present the band at 3500-3400 cm^{-1}, characteristic of OH group.

The EI-HRMS gave the accurate molecular mass of the compounds and their molecular formula:

X1 -EI-HRMS m⁄z found for $C_{14}H_{10}O_5$: 258.0530;

X2- EI-HRMS m⁄z found for $C_{15}H_{12}O_7S$: 336.0305;

P1 - EI-HRMS m⁄z found for $C_{19}H_{18}O_5$: 326.1157;

P2 - EI-HRMS m⁄z found for $C_{24}H_{26}O_5$: 394.1769;

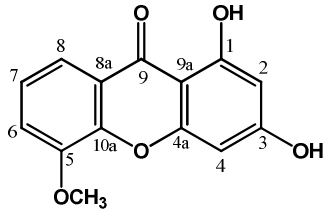
P3 – not determined;

P4 – EI-HRMS m⁄z found for $C_{19}H_{18}O_5$: 326.1153;

P5 - EI-HRMS m⁄z found for $C_{19}H_{18}O_5$: 326.1157.

The ^{1}H NMR and ^{13}C NMR data of X1, X2, P1, P2, P3, P4 and P5 are presented in **tables 5 to 18.**

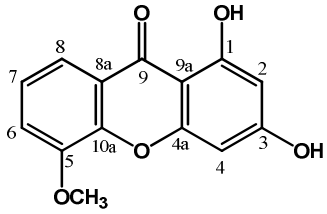
Table 5. ^{1}H NMR data for compound **X1**



X1	**δ_H**
H-2	6.22 (1H, d, *J*= 2.1)
H-4	6.41 (1H, d, *J*= 2.1)
H-6	7.50 (1H, dd, *J*= 8.0 and 1.4)
H-7	7.38 (1H, t, *J*= 8.0)
H-8	7.66 (1H, dd, *J*= 8.0 and 1.4)
O-H_1	12.82 (1H, s)
O-H_3	-
OCH_3	3.97 (3H, s)

¥ Values in ppm (δ_H) relative to Me_4Si as an internal reference. *J* values are in Hz.

Table 6. ^{13}C NMR data for compound **X1**

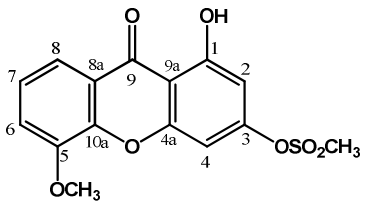


X1	**δ_C**
C-1	162.8
C-2	98.3

C-3	166.1
C-4	94.2
C-4a	157.2
C-5	147.9
C-6	116.7
C-7	124.1
C-8	115.7
C-8a	120.6
C-9	179.8
C-9a	102.1
C-10a	145.4
OCH_3	56.2

¥ Values in ppm (δ_C).

Table 7. ^{1}H NMR data for compound **X2**

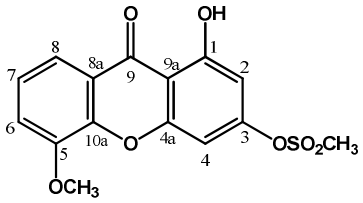


X2	δ_H
H-2	6.83 (1H, d, *J*= 2.1)
H-4	7.18 (1H, d, *J*= 2.1)
H-6	7.58 (1H, dd, *J*= 8 and 1.5)

H-7	7.45 (1H, t, *J*= 8.0)
H-8	7.71 (1H, dd, *J*= 8.0 and 1.5)
O-H_1	12.79 (1H, s)
OCH_3	3.99 (3H, s)
OSO_2CH_3	3.53 (3H, s)

¥ Values in ppm (δ_H) relative to Me_4Si as an internal reference. *J* values are in Hz.

Table 8. ^{13}C NMR data for compound **X2**



X2	δ_C
C-1	162.2
C-2	104.6
C-3	154.8
C-4	101.6
C-4a	156.2
C-5	148.1
C-6	117.5
C-7	124.8
C-8	115.8
C-8a	120.6

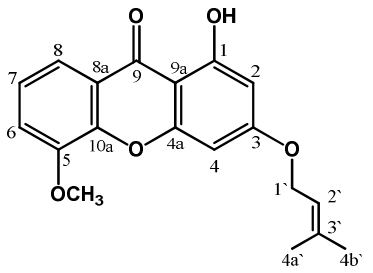
C-9	181.1
C-9a	107.3
C-10a	145.7
OCH_3	56.4
OSO_2CH_3	37.9

¥ Values in ppm (δ_C).

The ^{1}H NMR spectra of compounds **X1** and **X2** showed five signals corresponding to aromatic protons, namely H-2 and H-4 and H-6, H-7, H-8. The group OCH_3 was confirmed by the existence of a singlet due to three protons at 3.97 and 3.99 ppm, respectively 3.9 ppm. The signal for the protons of hydroxyl groups (OH_1) appeared as a singlet at 12.82 and 12.79 ppm, respectively. In compound **X2**, the protons of the group OSO_2CH_3 appeared as a singlet at 3.53 ppm.

The ^{13}C NMR spectra of **X1** and **X2** revealed signals that correspond to the carbon atom of the carbonyl group (δ_C 179.8 and 181.1, respectively) and to two aromatic rings (values of δ_C between 98.3 and 145.4 ppm for compound **X1** and between 104.6 and 145.7 ppm for compound **X2**; corresponding to twelve carbons). The signal for the carbon of OCH_3 group appeared at δ_C 56.2 and 56.4 ppm, respectively, and in compound **X2** the carbon of the mesyl group appeared at 37.9 ppm.

Table 9. ^{1}H NMR data for compound **P1**

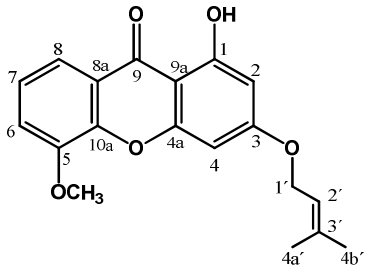


P1	δ_H
H-2	6.36 (1H, d, *J*= 2.3)

H-4	6.54 (1H, d, *J*= 2.3)
H-6	7.22 (1H, dd, *J*=8.0 and 1.7)
H-7	7.29 (1H, t, *J*=8.0)
H-8	7.81 (1H, dd, *J*=8.0 and 1.7)
O-H_1	12.81 (1H, s)
OCH_3	4.03 (3H, s)
H-1’ (H prenyl)	4.59 (2H, d, *J*=6.7)
H-2’ (H prenyl)	5.50 (1H, t, *J*=6.7)
H-4a’ and H-4b´ (H prenyl)	1.82 (3H, s) and 1.76 (3H, s)

¥ Values in ppm (δ_H) relative to Me_4Si as an internal reference. *J* values are in Hz.

Table 10. ^{13}C NMR data for compound **P1**



P1	δ_C
C-1	163.2
C-2	98.2
C-3	166.1
C-4	93.3
C-4a	157.5

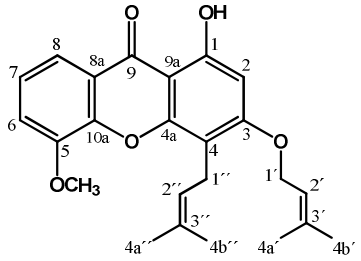
C-5	148.2
C-6	115.6
C-7	123.5
C-8	116.7
C-8a	121.5
C-9	180.7
C-9a	103.8
C-10a	146.3
OCH_3	56.4
C-1’ (prenyl)	65.5
C-2’ (prenyl)	118.5
C-3’ (prenyl)	139.3
C-4a’ and C-4b’ (prenyl)	25.8 and 18.3

¥ Values in ppm (δ_C).

The ^{1}H NMR spectrum of **P1** showed five signals corresponding to aromatic protons, namely H-2 and H-4 and H-6, H-7, H-8 (values of δ_H between 6.36 and 7.81 ppm). The group OCH_3 was confirmed by the existence of a singlet due to three protons at 4.03 ppm. The signal for the protons of hydroxyl group appeared as a singlet (δ_H 12.81 ppm). The presence of the prenyl group was confirmed by the existence of a duplet and a triplet corresponding to the protons H-1’ and H-2’ (δ_H 4.59 and 5.50 ppm, respectively) and the signal for the protons of two methyl groups appearing as a singlet (δ_H 1.82 and 1.76 ppm).

The ^{13}C NMR spectrum revealed signals that correspond to the carbon atom of the carbonyl group (δ_C 180.7 ppm) and to two aromatic rings (values of δ_C between 98.2 and 146.3 ppm; corresponding to twelve carbons). The signal for the carbon of OCH_3 group appeared at δ_C 56.4 ppm. The spectrum also revealed signals of the five carbons of the prenyl group.

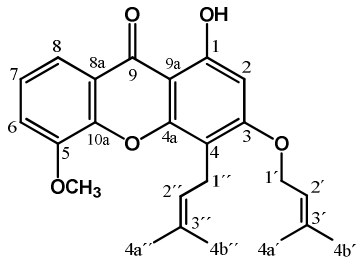
Table 11. ^{1}H NMR data for compound **P2**



P2	**δ_H**
H-2	6.39 (1H, s)
H-6	7.21 (1H, dd, *J*=7.9 and 1.8)
H-7	7.27 (1H, t, *J*=7.9)
H-8	7.79 (1H, dd, *J*=7.9 and 1.8)
O-H_1	12.96 (1H, s)
OCH_3	3.99 (3H, s)
H-1’ (H prenyl)	4.63 (2H, d, *J*=6.6)
H-2’ (H prenyl)	5.50 (1H, t, *J*=6.6)
H-4a’ and H-4b’ (H prenyl)	1.81 (3H, s) and 1.76 (3H, s)
H-1” (H prenyl)	3.55 (2H, d, *J*=7.4)
H-2” (H prenyl)	5.30 (1H, t, *J*=7.4)
H-4a” and H-4b” (H prenyl)	1.87 (3H, s) and 1.67 (3H, s)

¥ Values in ppm (δ_H) relative to Me_4Si as an internal reference. *J* values are in Hz.

Table 12. ^{13}C NMR data for compound **P2**



	P2 δ_C
C-1	161.7
C-2	95.2
C-3	163.7
C-4	108.4
C-4a	153.8
C-5	148.7
C-6	115.6
C-7	123.2
C-8	116.5
C-8a	121.2
C-9	181.3
C-9a	103.4
C-10a	146.7
OCH_3	56.2
C-1’ (prenyl)	65.7
C-2’ (prenyl)	119.0
C-3’ (prenyl)	138.5

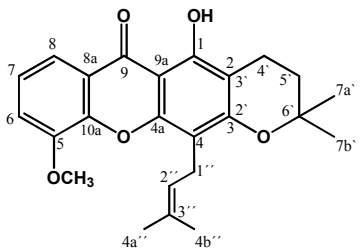
C-4a’ and C-4b’ (prenyl)	25.8 and 18.3
C-1” (prenyl)	21.8
C-2” (prenyl)	122.2
C-3” (prenyl)	131.7
C-4a” and C-4b” (prenyl)	25.9 and 17.6

¥ Values in ppm (δ_C).

The ^{1}H NMR spectrum of **P2**, a diprenylated xanthone, showed four signals corresponding to aromatic protons of the phenyl rings, namely H-2 and H-6, H-7, H-8 (values of δ_H between 6.39 and 7.79 ppm). The group OCH_3 was confirmed by the existence of a singlet due to three protons at 3.99 ppm. The signal for the protons of hydroxyl group appeared as a singlet (δ_H 12.96 ppm). The presence of the prenyl groups was confirmed by the existence of two duplet corresponding to the protons H-1’ and H-1” (δ_H 4.63 and 3.55 ppm), of two triplet corresponding to the protons H-2’ and H-2” (δ_H 5.50 and 5.30 ppm) and the signal for the protons of two methyl groups appearing as a singlet (δ_H 1.87 and 1.67 ppm).

The ^{13}C NMR spectrum of **P2** revealed signals that correspond to the carbon atom of the carbonyl group (δ_C 181.3 ppm) and to two aromatic rings (values of $\boldsymbol{\delta}_C$ between 95.2 and 146.7 ppm; corresponding to twelve carbons). The signal for the carbon of OCH_3 group appeared at $\boldsymbol{\delta}_C$ 56.2 ppm. The spectrum also revealed signals of the ten carbons of the two prenyl groups present in this compound.

Table 13. ^{1}H NMR data for compound **P3**

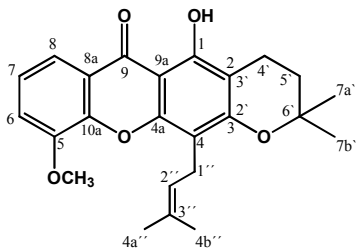


P3	$\boldsymbol{\delta}_H$
H-6	7.22 (1H, dd, *J*=7.8 and 1.8)

H-7	7.30 (1H, t, *J*=7.8)
H-8	7.81 (1H, dd, *J*=7.8 and 1.8)
O-H_1	13.10 (1H, s)
OCH_3	4.01 (3H, s)
H-4’ (H dihydropyran)	2.17 (2H, t, *J*=6.8)
H-5’ (H dihydropyran)	2.01 (2H, t, *J*=6.8)
H-7a’ and H-7b’ (H dihydropyran)	1.38 (6H, s)
H-1” (H prenyl)	3.98 (2H, d, *J*=7.4)
H-2” (H prenyl)	6.26 (1H, t, *J*=7.4)
H-4a” and H-4b” (H prenyl)	1.75 (6H, s)

¥ Values in ppm (δ_H) relative to Me_4Si as an internal reference. *J* values are in Hz.

Table 14. ^{13}C NMR data for compound **P3**



P3	δ_C
C-1	163.6
C-2	95.1
C-3	164.2
C-4	108.1

C-4a	152.7
C-5	148.4
C-6	115.8
C-7	123.4
C-8	116.7
C-8a	121.4
C-9	180.8
C-9a	103.4
C-10a	146.1
OCH_3	56.6
C-4’ (dihydropyran)	15.9
C-5’ (dihydropyran)	31.6
C-6’ (dihydropyran)	76.7
C-7a’ and C-7b’ (dihydropyran)	26.2
C-1” (prenyl)	28.6
C-2” (prenyl)	94.3
C-3” (prenyl)	135.4
C-4a” and C-4b” (prenyl)	18.9

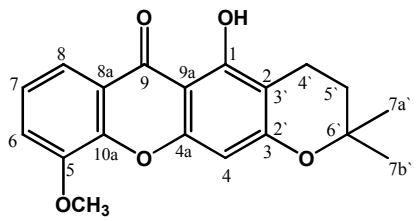
¥ Values in ppm (δ_C).

In the ^{1}H NMR spectra of **P3 t**he signal for the protons of hydroxyl group appeared as a singlet (δ_H 13.10) and the group OCH_3 was confirmed by the existence of a singlet due to three protons at 4.01 ppm. The spectra also show three signals corresponding to aromatic protons H-6, H-7, H-8 (values of δ_H between 7.22 and 7.81 ppm).

The presence of the prenyl group was confirmed by the existence of a duplet and a triplet corresponding to the protons H-1'' and H-2'' (δ_H 3.98 and 6.26 ppm, respectively) and the signal for the protons of two methyl groups appearing as a singlet (δ_H 1.75 ppm). The presence of a fused dihydropyran ring was confirmed by the existence of two triplets due to two methylene protons (H-4' and H-5': δ_H 2.17 and 2.01 ppm) and the signal for the protons of two methyl groups appearing as a singlet (δ_H 1.38 ppm).

The ^{13}C NMR spectra of **P3** revealed signals that correspond to the carbon atom of the carbonyl group (δ_C 180.8 ppm) and to two aromatic rings (values of δ_C between 95.1 and 164.2 ppm). The signal for the carbon of OCH_3 group appeared at δ_C 56.6 ppm. The spectrum revealed signals of the ten carbons of the two prenyl groups present in this compound.

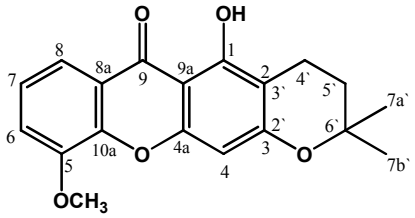
Table 15. ^1H NMR data for compound **P4**



P4	δ_H
H-4	6.46 (1H, s)
H-6	7.21 (1H, dd, *J*=8.0 and 1.8)
H-7	7.27 (1H, t, *J*=8.0)
H-8	7.81 (1H, dd, *J*=8.0 and 1.8)
O-H_1	13.20 (1H, s)
OCH_3	4.01 (3H, s)
H-4' (H dihydropyran)	2.74 (2H, t, *J*=6.8)
H-5' (H dihydropyran)	1.85 (2H, t, *J*=6.8)
H-7a' and H-7b' (H dihydropyran)	1.38 (6H, s)

¥ Values in ppm (δ_H) relative to Me_4Si as an internal reference. *J* values are in Hz.

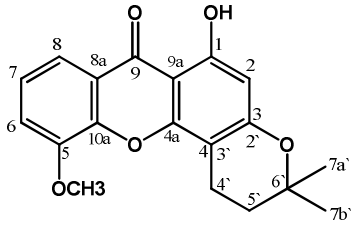
Table 16. ^{13}C NMR data for compound **P4**



P4	δ_C
C-1	160.4
C-2	104.2
C-3	161.9
C-4	95.4
C-4a	155.3
C-5	148.2
C-6	115.5
C-7	123.1
C-8	116.7
C-8a	121.4
C-9	180.8
C-9a	102.8
C-10a	146.4
OCH_3	56.4
C-4’ (dihydropyran)	16.0
C-5’ (dihydropyran)	31.7
C-6’ (dihydropyran)	76.3
C-7a’ and C-7b’ (dihydropyran)	26.7

¥ Values in ppm (δ_C).

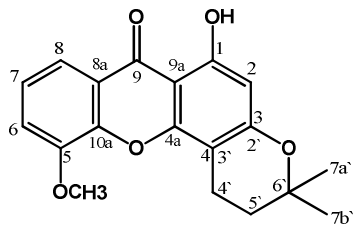
Table 17. ^{1}H NMR data for compound **P5**



P5	δ_H
H-2	6.26 (1H, s)
H-6	7.22 (1H, dd, *J*=7.9 and 1.7)
H-7	7.29 (1H, t, *J*=8.0)
H-8	7.82 (1H, dd, *J*=7.9 and 1.7)
O-H_1	12.61 (1H, s)
OCH_3	4.02 (3H, s)
H-4’ (H dihydropyran)	2.93 (2H, t, *J*=6.8)
H-5’ (H dihydropyran)	1.89 (2H, t, *J*=6.8)
H-7a’ and H-7b’ (H dihydropyran)	1.39 (6H, s)

¥ Values in ppm (δ_H) relative to Me_4Si as an internal reference. *J* values are in Hz.

Table 18. ^{13}C NMR data for compound **P5**



P5	δ_C
C-1	160.8

C-2	99.5
C-3	161.61
C-4	100.1
C-4a	154.5
C-5	148.5
C-6	115.6
C-7	123.5
C-8	116.7
C-8a	121.5
C-9	180.8
C-9a	103.6
C-10a	146.3
OCH_3	56.5
C-4' (dihydropyran)	16.2
C-5' (dihydropyran)	31.8
C-6' (dihydropyran)	76.3
C-7a' and C-7b' (dihydropyran)	26.7

¥ Values in ppm (δ_C).

In the ^{1}H NMR spectra of **P4** and **P5** the group OCH_3 was confirmed by the existence of a singlet due to three protons at 4.01 and 4.02 ppm, respectively. The signal for the protons of hydroxyl group appeared as a singlet (δ_H 13.20 and 12.61 ppm, respectively).

The multiplicity and coupling constants for the protons observed in the ^{1}H NMR spectra of **P4** and **P5** showed four signals corresponding to aromatic protons, namely H-4 or H-2 respectively, and H-6, H-7, H-8 (values of δ_H between 6.46 and 7.81 ppm (P4); 6.26 and 7.82 ppm

(P5)). The presence of a fused dihydropyran ring was confirmed by the existence of two triplets due to two methylene protons (H-4' and H-5': δ_H 2.74 and 1.85 ppm (P4); 2.93 and 1.89 ppm (P5)) and the signal for the protons of two methyl groups appearing as a singlet (δ_H 1.38 ppm (P4); 1.39 (P5)).

The ^{13}C NMR spectrum of **P4** and **P5** revealed signals that correspond to the carbon atom of the carbonyl group (δ_C 180.8 ppm) and to two aromatic rings (values of δ_C between 95.4 and 161.9 ppm (P4); 99.5 and 161.6 ppm (P5)). The signal for the carbon of OCH_3 group appeared at δ_C 56.4 and 56.5 ppm, respectively. The spectrum revealed signals of the five carbons of the prenyl group present in these compounds.

- **NMR – 2D**

The position of the substituents on the xanthone skeleton was determined on the basis of HSQC and HMBC spectral analysis (**Figures 27 and 28**).

In HMBC spectra of all prenylated xanthones, the hydrogen-bonded hydroxyl group (OH-1) correlated with C-1, C-2 and C-9a, allowing the assignment of these carbon resonances. The H-1´ of prenyl group in compound **P1** and H-1´ and H-1´´ of prenyl group in compound **P2**, correlated with C-3 of xanthone ring indicating that all the prenylated xanthones had a 3,3-dimethylallyloxy group at C-3. For the diprenylated xanthones **P2** and **P3** it was also observed that the H-1´´ of the prenyl group correlated with C-4 and C-4a.

In the case of dihydropyranoxanthones, it was observed that the H-4´ of the pyran ring of compounds **P3** and **P4** correlated with C-1, C-2, C-3, C-5´, and C-6´ indicating the presence of a 2,3-dihydropyran ring. The H-4´ of the pyran ring of compound **P5** correlated with C-3, C-4, C-4a, C-5´, and C-6´ indicating the presence of a 3,4-dihydropyran ring.

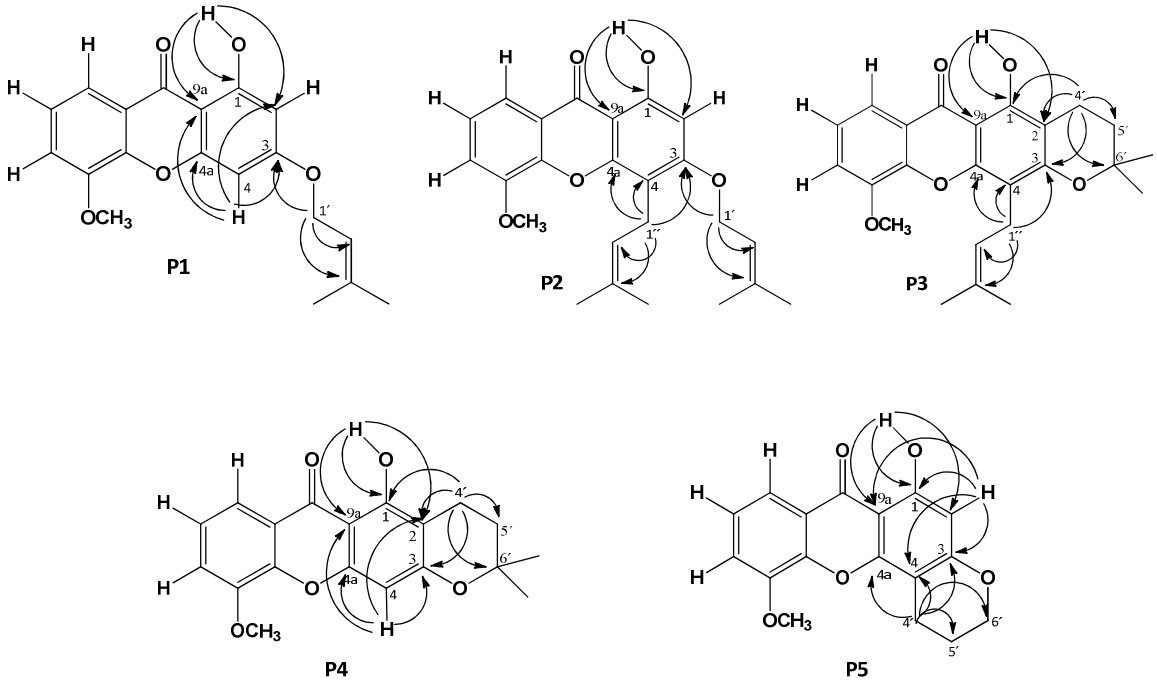


Figure 27. Main connectivities found in HMBC of prenylated xanthone **P1-P5**

(a)

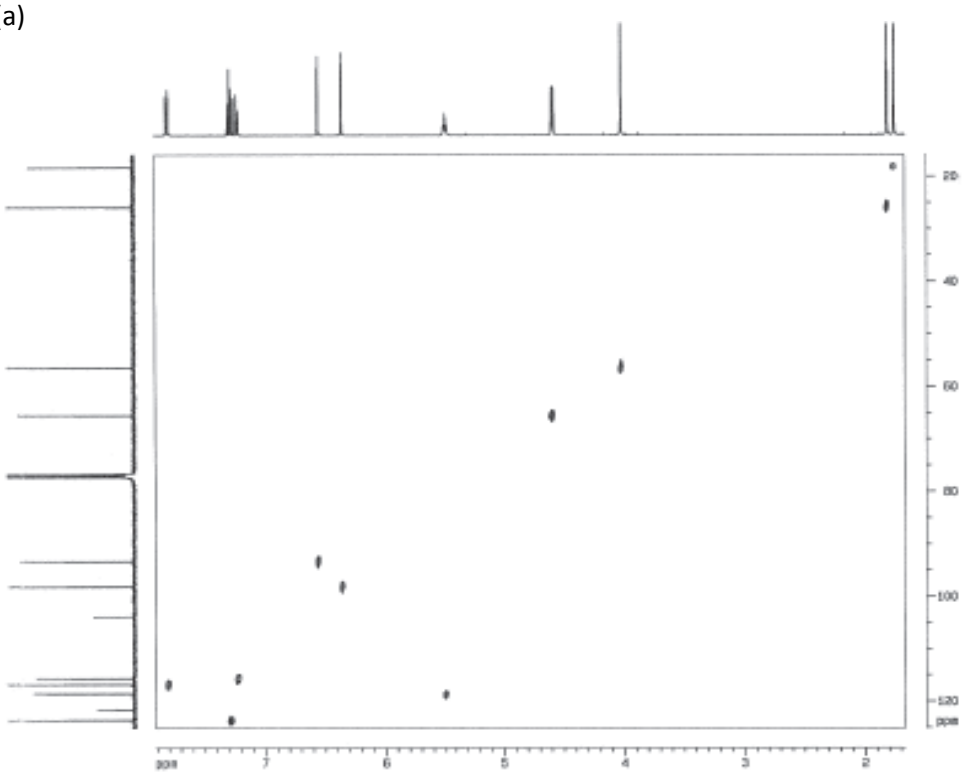


Figure 28. (a) Example of HSQC spectrum of prenylated xanthone **P1**

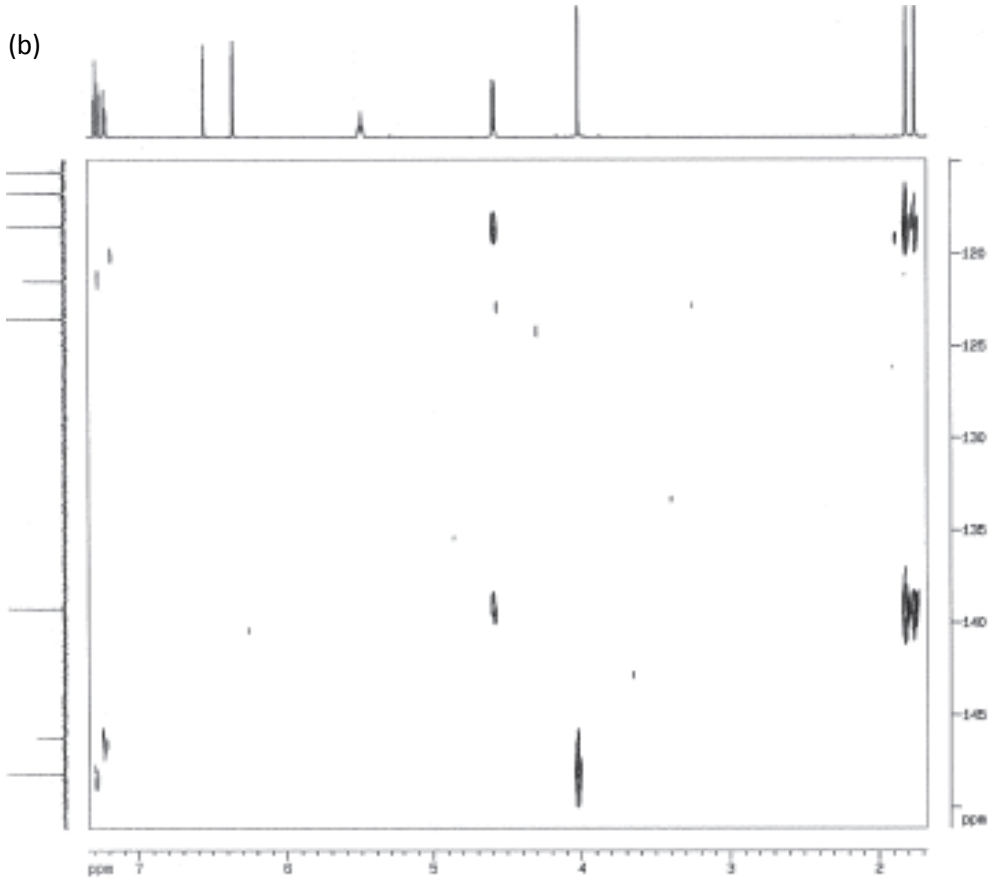


Figure 28. (b) Example of HMBC spectrum of prenylated xanthone **P1**

3. BIOLOGICAL ASSAYS

In CEQUIMED-UP, a research area in increasing development has been the investigation of xanthones as potential antitumor agents. In this context, the synthesized compounds **X1, X2, P1, P2, P3, P4** and **P5** were evaluated by their effect on the *in vitro* growth of three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer) and A375-C5 (malignant melanoma).

In **table 19**, the results of the evaluated activity are presented.

Table 19. Effects of the synthesized compounds on the growth of human tumor cell lines

Xanthone	GI_{50} (μM)		
	MCF-7	**NCI-H460**	**A375-C5**
X1	>110	>90	N.R.
X2	>150	>150	>150
P1	>112.5	>112.5	>112.5
P2	>12.4	>12.4	>12.4
P3	>7.5	>12.4	>12.4
P4	>150	>150	>150
P5	>37.5	>37.5	>37.5

Results expressed as GI_{50}, concentration of compound that cause 50% inhibition of tumor cell lines growth, are means ± SEM (standard error of the mean) of 3 independent experiments performed in duplicate. Doxorubicin was used as positive control, GI_{50}: MCF-7 = 0,065 ± 0,0085 μM; NCI-H460: 0,064 ± 0,0068 μM; A375-C5: 0,145 ± 0,0098 μM. NR, not reproducible. Final concentrations of DMSO (≤0,75%) did not interfere with the biological activity tested.

In this essay, prepared in the laboratory according to the established in National Cancer Institute (NCI) (Monks, A*. et al*., 1991; Vichai, V. and Kirtikara, K., 2006), the compounds were tested in several concentrations until a maximum of 150 μM. The reasons why tests are not executed with superior concentrations are the following: i) the vehicle of compounds (dimethyl sulfoxide, DMSO) becomes toxic to the cells and ii) components with GI_{50} values above 150 μM, in principle, do not have a therapeutical interest. In fact, the majority of compounds with therapeutical interest present GI_{50} values in the scale of nanomolar. So that the compounds can be tested until a maximum concentration of 150 μM, without achieving the toxicity limit of DMSO,

they should be dissolved in a stock concentration of 60 mM. When such is not possible, they can only be tested in concentrations that do not hit the toxicity limit of DMSO and therefore not in all the desired concentrations. By analyzing table 19, none of the components hit the GI_{50} in the tested concentrations.

The compounds **X2** and **P4** present concentrations of GI_{50} superior to 150μM, indicating that they are components that do not achieve the GI_{50} in the tested concentrations for this essay, in the three used cellular lines. Regarding the compounds **X1** and **P1**, even though they have not been tested until the maximum desired concentration (150 μM), they were tested until concentrations close to 100 μM, not being able to achieve the GI_{50}. Therefore, xanthones **X1, X2, P1** and **P4** are not active, showing results for GI_{50} above 100 μM.

The evaluation of the other compounds (**P2, P3** and **P5**) was limited by problems associated with their solubility; it was not possible to prepare stocks with the desired concentrations (60 mM) to test and determine the concentration value able to hit the GI_{50}. It was only possible to determine a low limit of concentration until which the GI_{50} was not achieved. Therefore, we cannot conclude regarding the activity of **P2**, **P3** or **P5**.

It is also important to mention that the component **X1** has not showed reproducible results. Even though it was possible to determine an amount of concentration of GI_{50} in the first two essays (medium value 45 μM), when new essays were executed the result has not been reproducible. This lack of reproducibility could be explained by the lack of stability of the compound that can have suffered alteration during the evaluation.

IV. EXPERIMENTAL PART

IV. EXPERIMENTAL PART

GENERAL METHODS

Purifications of compounds were performed by flash chromatography using Merck silica gel 60 (0.040-0.063 mm), chromatography flash cartridge (GraceResolv®, Grace Company, Deerfield, IL, USA) and preparative thin layer chromatography (TLC) using Merck silica gel 60 (GF_{254}) plates. TLC was used for monitoring reactions.

MW reactions were performed using glassware setup for atmospheric-pressure reactions and also 12mL or 50mL closed glass reactors (internal reaction temperature measurement with a fiber-optic probe sensor) and were carried out using an Ethos MicroSYNTH 1600 Microwave Labstation from Millestone (ThermoUnicam, Portugal).

Melting points were obtained in a Köfler microscope and are uncorrected. IR spectra were measured on an ATI Mattson Genesis series FTIR (software: WinFirst v.2.10) spectrophotometer in KBr microplates (cm^{-1}). ^{1}H and ^{13}C NMR spectra were taken in DMSO-*d6* or $CDCl_3$ at room temperature, on Bruker Avance 300 (300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C) or Bruker DRX-500 (500.13 and/or 300.13 MHz for ^{1}H and 125.77 and/or 75.47 MHz for ^{13}C) spectrometers. Chemical shifts are expressed in δ (ppm) values relative to tetramethylsilane (Me_4Si) as an internal reference and assignment abbreviations are the following: singlet (s), doublet (d), triplet (t) and doublet of doublets (dd). ^{13}C NMR assignments were made by 2D HSQC and HMBC experiments (long-range C, H coupling constants were optimized to 7 and 1 Hz).

HRMS spectra were recorded as electronic impact (EI) mode on a VG Autospec M spectrometer (*m/z*) at C.A.C.T.I. (Vigo, Spain).

2-hydroxy-3-methoxybenzoic acid, phloroglucinol (1,3,5-trihydroxybenzene), prenyl bromide 95% and montmorillonite K10 clay were purchased from Sigma-Aldrich and were grade *pro analysis.*

Part I - Synthesis of 1,3-dihydroxy-5-methoxyxanthone (X1)

A) General procedure for the synthesis of 1,3-dihydroxy-5-methoxyxanthone (**X1**) through Grover, Shah and Shah (GSS) classical method

A mixture of phloroglucinol (1.2 g, 9.6 mmol), 2-hydroxy-3-methoxybenzoic acid (1.25 g, 7.4 mmol) anhydrous zinc chloride (3 g), and phosphoryl chloride (12 mL) were heated, under stirring, at 70°C for 4h30. The deep-red reaction mixture obtained was poured onto crushed ice. The resulting red brownish solid was filtered, washed with water and dried. The crude product was then purified by flash chromatography (SiO_2; a gradient of $CHCl_3/Me_2CO$, starting with 95:5). The product was crystallized from methanol to afford yellow crystals of xanthone. Compound 1,3-dihydroxy-5-methoxyxanthone (**X1**) (39% yield) was identified by their spectroscopic and analytical data.

B) General procedure for the synthesis of 1,3-dihydroxy-5-methoxyxanthone (**X1**) through modified GSS applying Eaton's reagent

To obtain Eaton's reagent, a mixture of phosphorus pentoxide (6.5 g) and methanesulfonic acid (40 mL) was heated at 100°C for 30 min, under stirring, until a clear solution was obtained. Phloroglucinol (2,0 g, 16 mmol) and 2-hydroxy-3-methoxybenzoic acid (1.61 g, 9.6 mmol) were added to this mixture and heating continued for 1h. The reaction mixture was poured into ice-water. The resulting solid was collected by filtration, washed with water and dried. The crude product was then purified by flash chromatography (SiO_2; a gradient of $CHCl_3/Me_2CO$ with crescent polarity). The products were crystallized from methanol to afford yellow crystals of both xanthones (**X1 and X2**).

The compounds 1,3-dihydroxy-5-methoxyxanthone (**X1**) (23% yield) and 1-hydroxy-3-mesyl-5-methoxyxanthone (**X2**) (15% yield) were identified by their spectroscopic and analytical data.

1,3-dihydroxy-5-methoxyxanthone (X1) - mp 219-222°C (methanol); IR (KBr) ν_{max}: 3465, 3093, 2936, 1654, 1605, 1567, 1499, 1450, 1362, 1176, 1107, 992 cm^{-1}; ^{1}H-NMR (300.13 MHz; DMSO-*d6*): δ = 12.82 (1H, s, O-H_1), 7.66 (1H, dd, *J* = 8.0 and 1.4 Hz, H-8), 7.50 (1H, dd, *J* = 8.0 and 1.4 Hz, H-6), 7.38 (1H, t, *J* = 8.0, H-7), 6.41 (1H, d, *J* = 2.1, H-4), 6.22 (1H, d, *J* = 2.1, H-2), 3.97 (3H, s, OCH_3) ppm; ^{13}C-NMR (75.47 MHz; DMSO-*d6*): δ = 179.8 (C-9), 166.1 (C-3), 162.8 (C-1), 157.2 (C-4a), 147.9 (C-5), 145.4 (C-10a), 124.1 (C-7), 120.6 (C-8a), 116.7 (C-6), 115.7 (C-8), 102.1 (C-9a), 98.3 (C-2), 94.2 (C-4), 56.2 (OCH_3) ppm. EI-MS *m/z* (%): 259 (18), 258 (100) $[M]^+$, 244 (10), 243 (62), 229 (5), 215 (6), 187 (26). EI-HRMS m/z calc for $C_{14}H_{10}O_5$: 258.0528, found: 258.0530.

1-hydroxy-3-mesyl-5-methoxyxanthone (X2) - mp 201-204°C (methanol); IR (KBr) ν_{max}: 3437, 3086, 2937, 1651, 1618, 1581, 1495, 1439, 1363, 1283, 1184, 1111, 973 cm^{-1}; ^{1}H-NMR (300.13 MHz; DMSO-*d6*): δ = 12.79 (1H, s, O-H_1), 7.71 (1H, dd, *J* = 8.0 and 1.5 Hz, H-8), 7.58 (1H, dd, *J* = 8.0 and 1.5 Hz, H-6), 7.45 (1H, t, *J* = 8.0, H-7), 7.18 (1H, d, *J* = 2.1, H-4), 6.83 (1H, d, *J* = 2.1, H-2), 3.99 (3H, s, OCH_3), 3.53 (3H, s, OSO_2CH_3) ppm; ^{13}C-NMR (75.47 MHz; DMSO-*d6*): δ = 181.1 (C-9), 162.2 (C-1), 156.2 (C-4a), 154.8 (C-3), 148.1 (C-5), 145.7 (C-10a), 124.8 (C-7), 120.6 (C-8a), 117.5 (C-6), 115.8 (C-8), 107.3 (C-9a), 104.6 (C-2), 101.6 (C-4), 56.4 (OCH_3), 37.9 (OSO_2CH_3) ppm. EI-MS *m/z* (%): 337 (12), 336 (85) $[M]^+$, 258 (100), 243 (43), 229 (71), 187 (17), 97 (25), 83 (22), 69 (31). EI-HRMS m/z calc for $C_{15}H_{12}O_7S$: 336.0304, found: 336.0305.

Part II - Synthesis of prenylated xanthones (P1-P5) under MW irradiation

A) General procedure for the synthesis of prenylated xanthones (**P1** and **P2**) under MW irradiation in alkaline medium

A mixture of 1,3-dihydroxy-5-methoxyxanthone (**X1**) (2 mmol), prenyl bromide (4 mmol), Cs_2CO_3 (4 mmol) in dry acetone (200 mL), in a two-necked glassware apparatus, provided with magnetic stirring bar, fiber-optic temperature control and reflux condenser, was heated for 1h according to the following microwave program: Power:200W; temperature: 62°C; ramp time: 5 min; hold time: 55 min; final temperature 59°C. After cooling, the mixture was filtered and washed with acetone. The solvent was removed under reduced pressure and the collected crude product was purified by flash chromatography (SiO_2; a gradient of hexane/ethyl acetate, with crescent polarity). The isolation of the components of the mixture was then carried out by preparative TLC (SiO_2; hexane/ethyl acetate 75:25). Prenylated xanthones **P1** and **P2** were crystallized from CH_2Cl_2/petroleum ether (60-80) to afford yellow crystals. These two compounds were shown to possess spectroscopic and analytical data as next described. Compounds yields were 15% for **P1** and 5% for **P2**.

B) General procedure for the synthesis of prenylated xanthones (**P3 - P5**) under MW irradiation with Montmorillonite K10 clay

A slurry of the K10 clay (20 equiv by weight, 10 g) in $CHCl_3$ (30mL) was treated with the 1,3-dihydroxy-5-methoxyxanthone (**X1**) (2 mmol), followed by the addition of prenyl bromide (4 mmol) in a 100mL closed microwave reactor, provided with magnetic stirring bar and fiber-optic temperature control. The mixture was irradiated at 180W for 45 min (ramp time: 5 min; hold time: 40 min) and the final temperature was 96°C. After cooling, the reaction mixture was filtered under vacuum, washed with CH_2Cl_2, Me_2CO and MeOH, and the solvent evaporated under reduced pressure. The recovered clay was reactivated by washing with MeOH. The crude product was purified by flash chromatography (SiO_2; hexane/ethyl acetate 95:5) and the isolation of the components of the mixture was then carried out by preparative TLC (SiO_2; hexane/ethyl acetate 6:4). Prenylated xanthones were then crystallized from n-hexane/ethyl acetate to obtain yellow crystals. The prenylated xanthones **P3**, **P4** and **P5** were identified by their spectroscopic and analytical data as next described. Compounds yields were 0,3% for **P3**, 3% for **P4** and 13% for **P5**.

1-hydroxy-5-methoxy-3-(3-methylbut-2-enyloxy)xanthone (P1) – mp 158-160°C (CH_2Cl_2/petroleum ether (60-80)); IR (KBr) ν_{max}: 3427, 2965, 2914, 2843, 1650, 1614, 1570, 1488, 1431, 1283, 1153, 1094, 955 cm^{-1}; ^{1}H-NMR (300.13 MHz; $CDCl_3$): δ = 12.81 (1H, s, O-H_1), 7.81 (1H, dd, *J* = 8.0 and 1.7 Hz, H-8), 7.29 (1H, t, *J* = 8.0, H-7), 7.22 (1H, dd, *J* = 8.0 and 1.7 Hz, H-6), 6.54 (1H, d, *J* = 2.3, H-4), 6.36 (1H, d, *J* = 2.3, H-2), 5.50 (1H, t, *J* = 6.7, H-2'), 4.59 (2H, d, *J* = 6.7, H-1'), 4.03 (3H, s, OCH_3), 1.82 and 1.76 (3H, s, H-4a' and H-4b') ppm; ^{13}C-NMR (75.47 MHz; $CDCl_3$): δ = 180.7 (C-9), 166.1 (C-3), 163.2 (C-1), 157.5 (C-4a), 148.2 (C-5), 146.3 (C-10a), 139.3 (C-3'), 123.5 (C-7), 121.5 (C-8a), 118.5 (C-2'), 116.7 (C-8), 115.6 (C-6), 103.8 (C-9a), 98.2 (C-2), 93.3 (C-4), 65.5 (C-1'), 56.4 (OCH_3), 25.8 and 18.3 (C-4a' and C-4b') ppm. EI-MS *m/z* (%): 326 (6) $[M]^+$, 309 (11), 259 (9) , 258 (100), 243 (25), 187 (9). EI-HRMS m/z calc for $C_{19}H_{18}O_5$: 326.1154, found: 326.1157.

1-hydroxy-5-methoxy-4-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (P2) – mp 116-118°C (CH_2Cl_2/petroleum ether (60-80)); IR (KBr) ν_{max}: 3447, 2956, 2920, 2849, 1648, 1610, 1579, 1493, 1439, 1273, 1170, 1096, 963 cm^{-1}; ^{1}H-NMR (300.13 MHz; $CDCl_3$): δ = 12.96 (1H, s, O-H_1), 7.79 (1H, dd, *J* = 7.9 and 1.8 Hz, H-8), 7.27 (1H, t, *J* = 7.9, H-7), 7.21 (1H, dd, *J* = 7.9 and 1.8 Hz, H-6), 6.39 (1H, s, H-2), 5.50 (1H, t, *J* = 6.6, H-2'), 5.30 (1H, t, *J* = 7.4, H-2"), 4.63 (2H, d, *J* = 6.6, H-1'), 3.99 (3H, s, OCH_3), 3.55 (2H, d, *J* = 7.4, H-1"), 1.87 and 1.67 (3H, s, H-4a" and H-4b"), 1.81 and 1.76 (3H, s, H-4b' and H-4a') ppm; ^{13}C-NMR (75.47 MHz; $CDCl_3$): δ = 181.3 (C-9), 163.7 (C-3), 161.7 (C-1), 153.8 (C-4a), 148.7 (C-5), 146.7 (C-10a), 138.5 (C-3'), 131.7 (C-3"), 123.2 (C-7), 122.2 (C-2"), 121.2 (C-8a), 119.0 (C-2'), 116.5 (C-8), 115.6 (C-6), 108.4 (C-4), 103.4 (C-9a), 95.2 (C-2), 65.7 (C-1'), 56.2 (OCH_3), 25.9 and 17.6 (C-4b" and C-4a"), 25.8 and 18.3 (C-4b' and C-4a'), 21.8 (C-1") ppm. EI-MS *m/z* (%): 394 (6) $[M]^+$, 377 (17), 326 (21), 322 (17), 311 (45), 309 (100), 294 (26), 271 (17), 258 (17). EI-HRMS m/z calc for $C_{24}H_{26}O_5$: 394.1780, found: 394.1769.

1-hydroxy-5-methoxy-4-(3-methylbut-2-enyl)-6',6'-dimethyl-4',5'-dihydropyran(2',3':3,2) xanthone (P3) – mp >300°C (decomp.); IR (KBr) ν_{max}: 3429, 2954, 2927, 2872, 1651, 1613, 1579, 1495, 1441, 1271, 1165, 1102, 803 cm^{-1}; ^{1}H-NMR (300.13 MHz; $CDCl_3$): δ = 13.10 (1H, s, O-H_1), 7.81 (1H, dd, *J* = 7.8 and 1.8 Hz, H-8), 7.30 (1H, t, *J* = 7.8, H-7), 7.22 (1H, dd, *J* = 7.8 and 1.8 Hz, H-6), 6.26 (1H, t, *J* = 7.4, H-2"), 4.01 (3H, s, OCH_3), 3.98 (2H, d, *J* = 7.4, H-1"), 2.17 (2H, t, *J* = 6.8, H-4'), 2.01 (2H, t, *J* = 6.8, H-5'), 1.75 (6H, s, H-4a" and H-4b"), 1.38 (6H, s, H-7a' and H-7b') ppm; ^{13}C-NMR (75.47 MHz; $CDCl_3$): δ = 180.8 (C-9), 164.2 (C-3), 163.6 (C-1), 152.7 (C-4a), 148.4 (C-5), 146.1

(C-10a), 135.4 (C-3”), 123.4 (C-7), 121.4 (C-8a), 116.7 (C-8), 115.8 (C-6), 108.1 (C-4), 103.4 (C-9a), 95.1 (C-2), 94.3 (C-2”), 76.7 (C-6’), 56.6 (OCH_3), 31.6 (C-5’), 28.6 (C-1”), 26.2 (C-7a’ and C-7b’), 18.9 (C-4a” and C-4b”), 15.9 (C-4´) ppm.

1-hydroxy-5-methoxy-6’,6’-dimethyl-4’,5’-dihydropyran(2’,3’:3,2)xanthone (P4) – mp 154-156°C (n-hexane/ethyl acetate); IR (KBr) ν_{max}: 3445, 2954, 2927, 2841, 1650, 1617, 1576, 1493, 1445, 1269, 1156, 1098, 992 cm^{-1}; ^{1}H-NMR (300.13 MHz; $CDCl_3$): δ = 13.20 (1H, s, O-H_1), 7.81 (1H, dd, *J* = 8.0 and 1.8 Hz, H-8), 7.27 (1H, t, *J* = 8.0, H-7), 7.21 (1H, dd, *J* = 8.0 and 1.8 Hz, H-6), 6.46 (1H, s, H-4), 4.01 (3H, s, OCH_3), 2.74 (2H, t, *J* = 6.8, H-4’), 1.85 (2H, t, *J* = 6.8, H-5’), 1.38 (6H, s, H-7a’ and H-7b’) ppm; ^{13}C-NMR (75.47 MHz; $CDCl_3$): δ = 180.8 (C-9), 161.9 (C-3), 160.4 (C-1), 155.3 (C-4a), 148.2 (C-5), 146.4 (C-10a), 123.1 (C-7), 121.4 (C-8a), 116.7 (C-8), 115.5 (C-6), 104.2 (C-2), 102.8 (C-9a), 95.4 (C-4), 76.3 (C-6´), 56.4 (OCH_3), 31.7 (C-5´), 26.7 (C-7a´ and C-7b´), 16.0 (C-4´) ppm. EI-MS *m⁄z* (%): 327 (7), 326 (41) $[M]^+$, 311 (10), 309 (33), 283 (21), 271 (100), 270 (21), 258 (5), 256 (9), 242 (11). EI-HRMS m⁄z calc for $C_{19}H_{18}O_5$: 326.1154, found: 326.1153.

1-hydroxy-5-methoxy-6’,6’-dimethyl-4’,5’-dihydropyran(2’,3’:3,4)xanthone (P5) – mp 215-217°C (n-hexane/ethyl acetate); IR (KBr) ν_{max}: 3424, 2967, 2933, 2851, 1657, 1612, 1577, 1492, 1432, 1268, 1158, 1099, 968 cm^{-1}; ^{1}H-NMR (300.13 MHz; $CDCl_3$): δ= 12.61 (1H, s, O-H_1), 7.82 (1H, dd, *J* = 7.9 and 1.7 Hz, H-8), 7.29 (1H, t, *J* = 8.0, H-7), 7.22 (1H, dd, *J* = 7.9 and 1.7 Hz, H-6), 6.26 (1H, s, H-2), 4.02 (3H, s, OCH_3), 2.93 (2H, t, *J* = 6.8, H-4’), 1.89 (2H, t, *J* = 6.8, H-5’), 1.39 (6H, s, H-7a’ and H-7b’) ppm; ^{13}C-NMR (75.47 MHz; $CDCl_3$): δ = 180.8 (C-9), 161.6 (C-3), 160.8 (C-1), 154.5 (C-4a), 148.5 (C-5), 146.3 (C-10a), 123.5 (C-7), 121.5 (C-8a), 116.7 (C-8), 115.6 (C-6), 103.6 (C-9a), 100.1 (C-4), 99.5 (C-2), 76.3 (C-6´), 56.5 (OCH_3), 31.8 (C-5´), 26.7 (C-7a´ and C-7b´), 16.2 (C-4´) ppm. EI-MS *m⁄z* (%): 327 (10), 326 (48) $[M]^+$, 311 (30), 309 (9), 272 (13), 271 (100), 270 (9), 258 (13), 256 (14), 242 (20). EI-HRMS m⁄z calc for $C_{19}H_{18}O_5$: 326.1154, found: 326.1157.

Part III – Tumor Cell Growth Assay

Stock solutions of compounds were prepared in DMSO (Sigma Chemical Co.) and stored at 4°C. The samples were freshly diluted with culture medium just prior to the assays.

TUMOR CELL GROWTH ASSAY: The effect of compounds on the growth of the human tumor cell lines was evaluated according to the procedure adopted by the National Cancer Institute (NCI, USA) in the *in vitro* anticancer drug discovery screen. This assay is based in the protein-binding dye sulforhodamine B (SRB) to indirectly assess cell growth (Monks, A*. et al*., 1991; Vichai, V. and Kirtikara, K., 2006). The following human tumor cell lines were used: MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer) and A375-C5 (malignant melanoma). Cells were routinely cultured in RPMI-1640 medium with ultraglutamine 1 (Lonza) supplemented with 5% heat-inactivated fetal bovine serum (FBS, PAA Laboratories), at 37°C in an humidified atmosphere containing 5% CO_2. For the SRB assay experiments, cells were plated in 96-well plates at the following densities: at 5000 cells/well for MCF7 and NCI-H460 cell lines and at 7500 cells/well for A375-C5. The cell density for each cell line was chosen based on previous studies (Neves, M.P. *et al.*, 2012 and 2011) in order to ensure cellular exponential growth throughout all the experimental period, ,. Cells were further incubated and allowed to adhere for 24 h and then treated with five serial dilutions of studied compounds (prepared in culture medium) for 48 h. In addition, cells were also treated with five serial dilutions of doxorubicin (Sigma Aldrich), as a positive control, and with the vehicle of the compounds, DMSO (at maximum concentration of used). Following this incubation period, cells were fixed *in situ* with 10% of trichloroacteic acid (TCA, Merck), washed with water, and incubated with SRB (Sigma Aldrich) for 30 minutes. After washing with 1% acetic acid (Merck), the bound dye was solubilized with 10 mM Tris-BASE (Sigma Aldrich) and the absorbance was measured at 510 nm in a microplate reader (Synergy Mx, Biotek). A dose–response curve was generated and GI_{50}, corresponding to the concentration of compound that inhibits 50% of the net cell growth, was determined as previously described (Monks, A. *et al*., 1991).

V. CONCLUSIONS

CONCLUSIONS

Despite all the advances that science has had over the years, Nature remains today the larger source of new drugs.

Recently there has been an increased interest in xanthones, due to their diverse pharmacological activities and to their capacity of binding to different classes of receptors, which make them "privileged structures" in Medicinal Chemistry. Therefore, xanthones obtained by synthesis began to represent a significant part of the derivatives described in literature.

In this work, seven new compounds were obtained – X1, X2, P1, P2, P3, P4 and P5 - by the application of different methodologies and reaction conditions.

The synthesis of 1,3-dihydroxy-5-methoxyxanthone (**X1**) by GSS was a successful way to obtain **X1** in moderate yields (39%). When the GSS reaction was modified by the use of Eaton's reagent as condensation agent, another product **X2** was obtained in 15% yield and the yield of **X1** was lower (23%).

To obtained prenylated xanthones from **X1**, the synthetic approach used involved MAOS. The reaction was not very selective and different products were obtained. In an alkaline medium (Cs_2CO_3), four products were originated, but only two were in sufficient quantity to be isolated and identified – the prenylated xanthones 1-hydroxy-5-methoxy-3-(3-methylbut-2-enyloxy)xanthone (**P1**) and 1-hydroxy-5-methoxy-4-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (**P2**), obtained in 15% and 5% yield, respectively.

MAOS was also combined with heterogeneous catalysis, using montmorillonite K10 clay as catalyst being obtained three main products: 1-hydroxy-5-methoxy-4-(3-methylbut-2-enyl)-6',6'-dimethyl-4',5'-dihydropyran(2',3':3,2)xanthone (**P3,** 0,3% yield), 1-hydroxy-5-methoxy-6',6'-dimethyl-4',5'-dihydropyran(2',3':3,2)xanthone(**P4,** 3% yield), 1-hydroxy-5-methoxy-6',6'-dimethyl-4',5'-dihydropyran(2',3':3,4)xanthone (**P5,** 13% yield).

All the compounds were structurally elucidated using analytical techniques like IR, NMR and HRMS, and evaluated by their antitumor activity in human tumor cell lines.

According to the data (GI_{50}), the compounds xanthones **X1**, **X2**, **P1** and **P4** were not active, showing results for GI_{50} above 100 μM. Concerning the compounds **P2**, **P3** and **P5,** because it was only possible to determine a low limit of concentration until which the GI_{50} was not achieved, it was not possible to conclude regarding the activity of **P2**, **P3** or **P5**.

In the future, once the solubility of the compounds was a limiting factor of this evaluation, it must be improved to enable the preparation of solution in higher concentrations (60mM) that allow the determination of IG_{50}. For a future and more conclusive evaluation, the solubility can be improved by using co-solvents like Tweens, or even by the incorporation of the compounds in macromolecules like cyclodextrins (Castro, C.A., *et al.*, 1995).

Therefore, according to the aims mentioned before, the work developed enabled to:

- obtain the building block 1,3-dihydroxy-5-methoxyxanthone;
- apply different methodologies of synthesis resulting in five new prenylated xanthone derivatives – cyclic and open-chain;
- structurally elucidate the new compounds using analytical techniques;
- evaluate their antitumor activity.

The xanthone scaffold revealed to be promising to the development of new biological active compounds. In the future it will be important to optimize the experimental conditions for MAOS and heterogeneous catalysis, once they can be methodologies of election in the synthesis of xanthone derivatives.

Xanthones comprise an ever growing and considerably diverse group of compounds in terms of structure, occurrence, and bioactivity. They represent attractive targets for both total synthesis and associated exploration of analogues for the purpose of exploiting the infinity of diverse and specific bioactivities that this class of compounds possesses.

The synthesis of the xanthone derivatives is an exciting contemporary area of chemical research, and the work developed shows that the evolution of chemical synthesis, through the application of new “non-classical” methodologies, can be a powerful tool to create structural diversity, leading to new and improved bioactive compounds of great interest.

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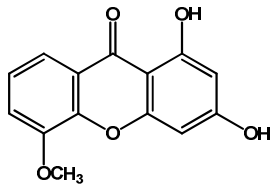
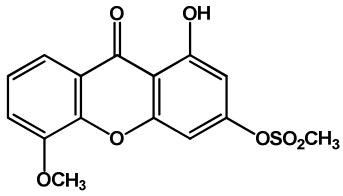
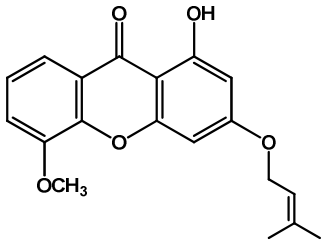
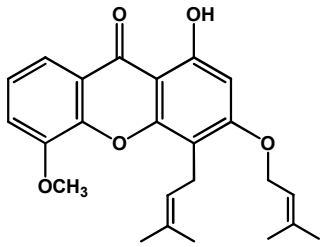
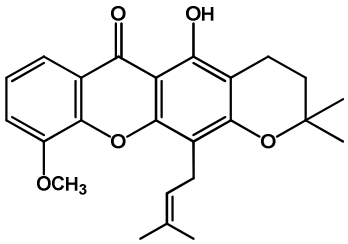
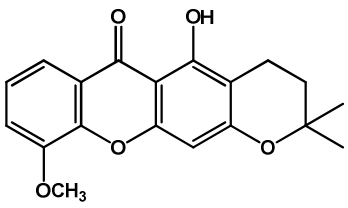
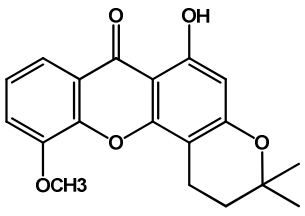
ATTACHMENTS

ATTTACHMENT I : COMPOUNDS STRUCTURES

NAME	STRUCTURE	ABBREVIATION
1,3-dihydroxy-5-methoxyxanthone		**X1**
1-hydroxy-3-mesyloxy-5-methoxyxanthone		**X2**
1-hydroxy-5-methoxy-3-(3-methylbut-2-enyloxy)xanthone		**P1**
1-hydroxy-5-methoxy-4-(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone		**P2**
1-hydroxy-5-methoxy-4-(3-methylbut-2-enyl)-6',6'-dimethyl-4',5'-dihydropyran(2',3':3,2)xanthone		**P3**
1-hydroxy-5-methoxy-6',6'-dimethyl-4',5'-dihydropyran(2',3':3,2)xanthone		**P4**
1-hydroxy-5-methoxy-6',6'-dimethyl-4',5'-dihydropyran(2',3':3,4)xanthone		**P5**