CHARLES UNIVERSITY IN PRAGUE, CZECH REPUBLIC

FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY AND DRUG CONTROL

UNIVERSITY OF PORTO, PORTUGAL

FACULTY OF PHARMACY

DEPARTMENT OF ORGANIC CHEMISTRY

DIPLOMA WORK- ERASMUS STAGE

"SYNTHESIS OF XANTHONE DERIVATIVES FOR *IN VITRO* AND *IN VIVO* BIOLOGICAL ACTIVITY STUDIES"

Porto, 2007/2008 **Jitka Široká**

This wok gave as a result a poster communication presented in the „First Meeting of Young Researchers of University of Porto 20-22 February 2008“; Faculdade de Arquitectura da Universidade do Porto, Portugal; Poster number 107: J. Siroka[1,2], E. Sousa[2,3] and M. Pinto[2,3].

„Synthesis of xanthone derivatives for *in vitro* and *in vivo* biological activity studies“

[1] Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic.

[2] Department of Organic Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

[3] Research Centre of Organic Chemistry, Phytochemistry and Pharmacology of the University of Porto (CEQOFFUP), Faculty of Pharmacy, University of Porto, Portugal.

See Abstract for this presentation on page 37.

Prohlašuji, že tato práce je mým původním autorským dílem, které jsem vypracovala samostatně. Veškerá literatura a další zdroje, z nichž jsem při zpracování čerpala, jsou uvedeny v seznamu použité literatury a v práci řádně citovány.

V Hradci Králové 29.2.2008 Jitka Široká

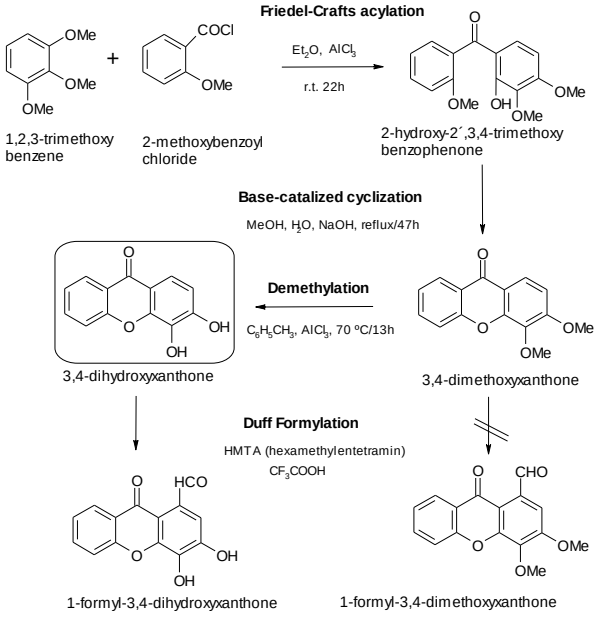
ABSTRACT

Synthesis of xanthone derivatives for *in vitro* and *in vivo* biological activity studies

J. Siroka

Xanthone derivatives are heterocyclic compounds with the dibenzo-γ-pyrone as the main molecular moiety. They contain different types of substituents in different positions, leading to a large variety of pharmacological activities. 3,4-Dihydroxyxanthone was revealed as a *hit* compound in a study involving the investigation of the inhibitory effect of oxygenated xanthones on several human tumor cell lines.

In order to obtain enough quantity for *in vivo* assays and for further molecular modifications, the synthesis of 3,4-dihydroxyxanthone was accomplished: the condensation of 1,2,3-trimethoxybenzene with the appropriate substituted benzoyl chloride (2-methoxybenzoyl chloride) afforded benzophenone (2-hydroxy-2´,3,4-trimethoxy benzophenone) which was further cyclized to give 3,4-dimethoxyxanthone.



The 3,4-dimethoxyxanthone was demethylated to furnish 3,4-dihydroxyxanthone.

Additionally, the synthesis of reactive formylated derivatives of xanthones, 1-formyl-3,4-dihydroxyxanthone and 1-formyl-3,4-dimethoxyxanthone, was attempted by Duff formylation. Only 1-formyl-3,4-dihydroxyxanthone was obtained. Due to a small amount of 3,4-dihydroxyxanthone obtained from the first synthesis, the synthesis was repeated without the formylation of 3,4-dimethoxyxanthone. 0,724 g of 3,4-dihydroxyxanthone obtained from the second synthesis will be used for *in vivo* antitumour activity studies.

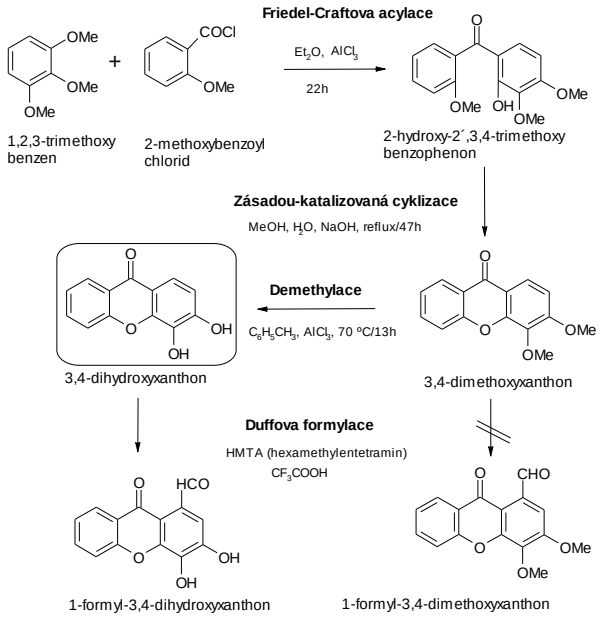
ABSTRACT (CZECH)

Syntéza xanthonových derivátů pro *in vitro* a *in vivo* studie biologické aktivity

J. Široká

Xanthonové deriváty jsou heterocyklické sloučeniny obsahující dibenzo-γ-pyronové jádro. V různých pozicích mohou mít umístěny různé substituenty, což vede k řadě farmaceutických účinků. 3,4-Dihydroxyxanthon se ukázal jako důležitá sloučenina ve studiích inhibičního efektu několika xanthonů obsahujících kyslík na různé druhy lidských nádorových buněk.

Pro potřebu *in vivo* výzkumů a pro potřebu dalších modifikací na molekule xanthonu byl stanoven způsob syntézy 3,4-dihydroxyxanthonu. Spojením 1,2,3-trimethoxybenzenu s vhodně substituovaným benzoyl chloridem (2-methoxybenzoyl chlorid) vznikl benzofenon (2-hydroxy-2´,3,4-trimethoxy benzophenon). Následnou cyklizací se vytvořil 3,4-dimethoxyxanthon. Demethylací 3,4-dimethoxyxanthonu byl získán 3,4-dihydroxyxanthon. Syntézy formylovaných derivátů xantinů 1-formyl-3,4-dihydroxyxanthon a 1-formyl-3,4-dimethoxyxanthon, byly prováděné Duffovou formylacíe vytvořil se jen 1-formyl-3,4-dihydroxyxanthon.



Kvůli malému výtěžku 3,4-dihydroxyxanthonu z první syntézy, byla celá syntéza opakována bez formylace 3,4-dimethoxyxanthonu. 0,724g 3,4-dihydroxyxanthonu získaných z druhé syntézy bude použito při *in vivo* studiích protinádorové aktivity.

ABBREVITATIONS:

^{13}C NMR	Carbon Magnetic Nuclear Resonance
^{1}H NMR	Proton Magnetic Nuclear Resonance
AQL	Aqueous layer
CNS	Central Nervous System
DMXAA	5,6-Dimethylxanthenone-4-Acetic Acid
EtOAc	Ethyl Acetate
HMTA	Hexamethylentetraamine
MAO	Monoamino Oxidase
MCF-7	Breast Adenocarcinome
MDR	Multidrug-Resistence
NPC 15437	2,6-diamino-N-[(1-tridecanoylpiperidin-2-yl)methyl]hexanamide
OL	Organic layer
P-gp	P-Glycoprotein
PHA	Phytohemaglutinin
PKC	Protein Kinase C
PMA	12-O-tetradecanoylphorbol-13-acetate
r.t.	room temperature
SAR	Structure- Activity Relationship
TFA	Trifluoroacetic Acid
TK-10	Renal Carcinomae
TLC	Thin Layer Chromatography
UACC-62	Melanoma

1.INTRODUCTION

Heterocycles play an important role in the design and discovery of new physiological-pharmacologically active compounds. Chemically, xanthones (9H-xanthen-9-ones) are heterocyclic compounds with the dibenzo-γ-pyrone framework (Fig. (1) [1]. The xanthone nucleus is numbered according to a biosynthetic convention with carbons 1–4 being assigned to acetate-derived ring A and carbons 5–8 to the shikimate-derived ring B. The other carbons are indicated as 4a, 10a, 8a, 9 and 9a for structure elucidation purposes (Fig. (1)) [2]. Naturally-occurring xanthones, with nearly one thousand known members, contain different types of substituents in different positions, leading to a large variety of pharmacological activities [1].

Natural xanthones can be sub-divided, depending on the nature of the substituents in the dibenzo-γ-pyrone scaffold, into: simple oxygenated xanthones, glycosylated xanthones, prenylated xanthones and their derivatives, xanthone dimers, xanthonolignoids and miscellaneous. On the other hand, the xanthones of a synthetic origin can have simple groups such as hydroxyl, methoxyl, methyl, carboxyl, as well as the more complex substituents such as epoxide, azole, methylidene-butyrolactone, aminoalcohol, sulfamoyl, methylthiocarboxylic acid, and dihydropyridine in their scaffold [3].

As their biosynthetic pathways are a limiting factor for the structural variation of naturally-occurring xanthones, the synthesis of new derivatives can help rationalize the relation of structural features versus activity [1].

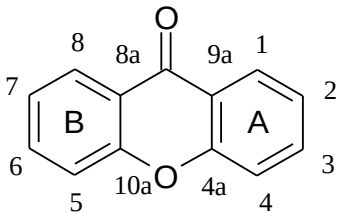


Fig. (1) Xanthone

The growing interest in this class of compounds has been associated with the pharmacological properties demonstrated by both natural and synthetic derivatives [3].

The first natural compound of xanthonic structure was described as a 2,3,4-trioxygenated xanthone isolated from *Kielmeyra* species. Other compounds have been isolated from the plants of Clusiaceae family (Guttiferae) especially from the genera *Cratoxylumr, Caraipa, Hypericum, Psorospermum, Vismia, Harungana and Calophylum*. They were investigated for the biological activity and as a result they were considered as chemotaxonomically useful compounds [4].

Pharmacological investigations of xanthones date back to 1968, when Bhattacharya's group reported the diuretic and cardiotonic actions of the natural glycoside mangiferin (**2**, Fig. (2)). Later, Da Re *et al.* described, for the first time, central stimulating and analeptic activities of synthetic aminoalkylxanthone derivatives. Further examination of this activity led to the report of a remarkable central nervous system (CNS) stimulating effect of mangiferin and *in vitro* experiments have shown that the above effect was caused by an inhibition of the enzyme monoamine oxidase (MAO). Meanwhile, in a following study with aminoalkylxanthones, new aminopropanoxy derivatives were described in 1972 as β-adrenergic blocking agents. In the same year, xanthone derivatives developed by isosteric substitution of benzo[*b*]acronycine were investigated for their *in vivo* antitumour activity while xanthone-2-carboxylic acids were shown to be effective in allergy. At the end of the 70's, much was achieved in the recognition of biological activity with more than twenty pharmacological studies being carried out for natural and synthetic xanthones [3].

At present, xanthones are of documented relevance to human diseases. The most remarkable example is dimethylxanthenone-4-acetic acid or DMXAA (**1**, Fig. (2)). This compound is currently undergoing clinical trials as an antitumour agent. On the other hand, the aqueous extracts of *Mangifera indica* (Vimang®) and *Garcinia mangostana* (Xango®), commercialized as antioxidants with human health promotion properties, were also found to contain xanthones. Interestingly,

the main constituent of Vimang® is mangiferin (**2**, Fig. (2)), while Xango® is rich in oxygenated and prenylated xanthones (**3-5**, Fig. (2)) [3].

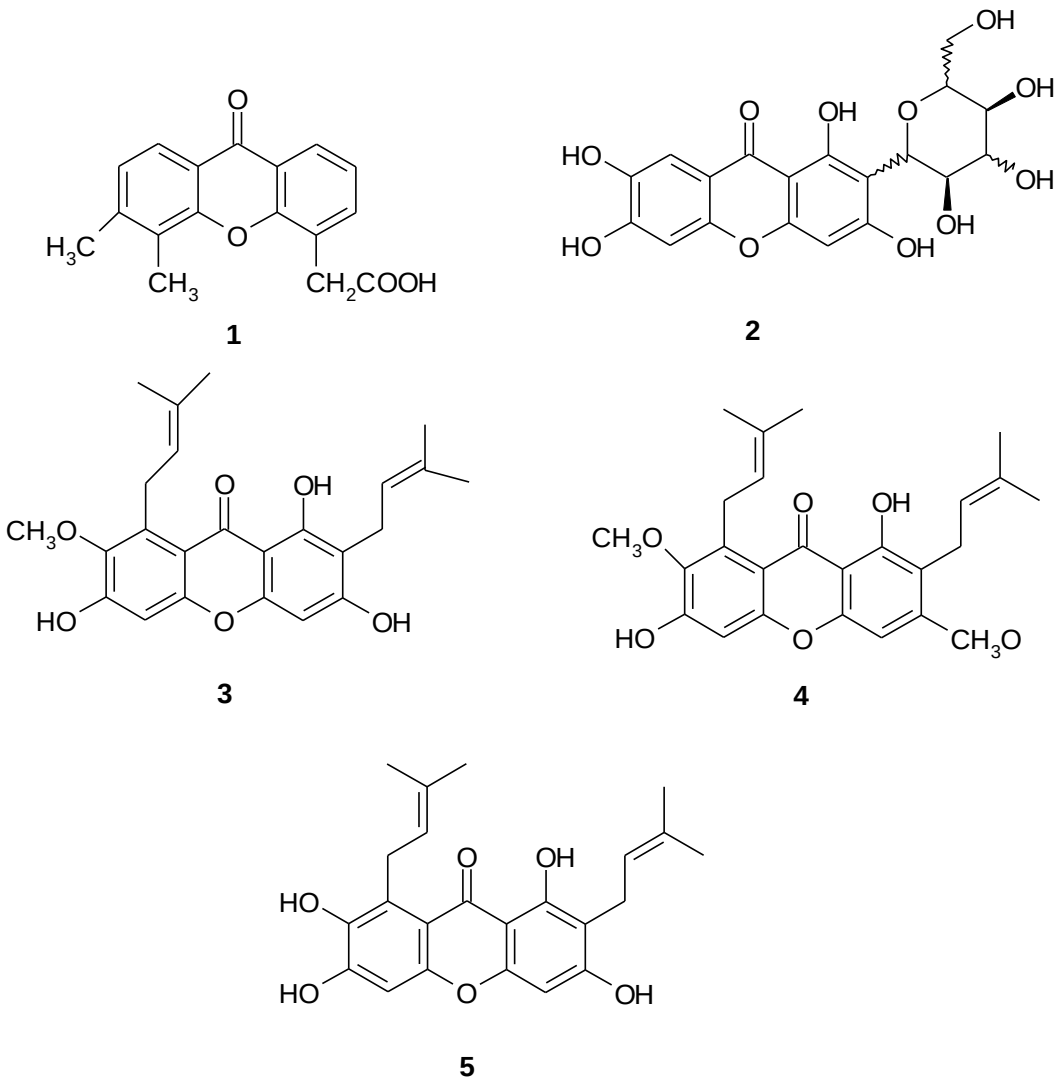
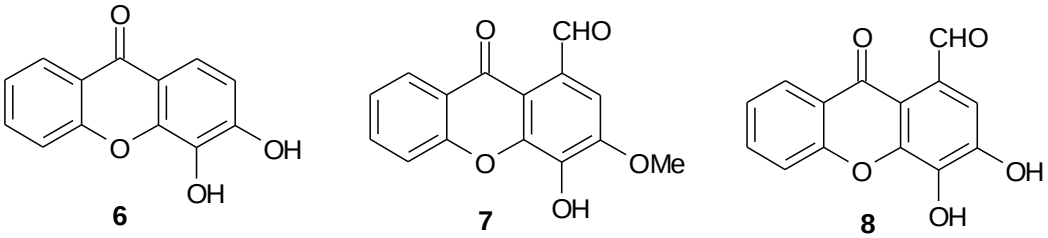


Fig. (2) Xanthones used in treatment of human diseases [3]

Natural and synthetic xanthones have been reported to mediate several important biological activities namely antitumour, anti-inflammatory, antithrombotic, neuropharmacological [5] and immunomodulatory effects. Although the anti-inflammatory activity of this group of compounds is documented, little information has been accumulated about its interference with the effectors cells of the specific immunity [2].

Recently, it has been suggested that these compounds may act, at least in part, by interacting with protein kinase C (PKC), and cause effects compatible not only with PKC activation, but also with PKC inhibition [5].



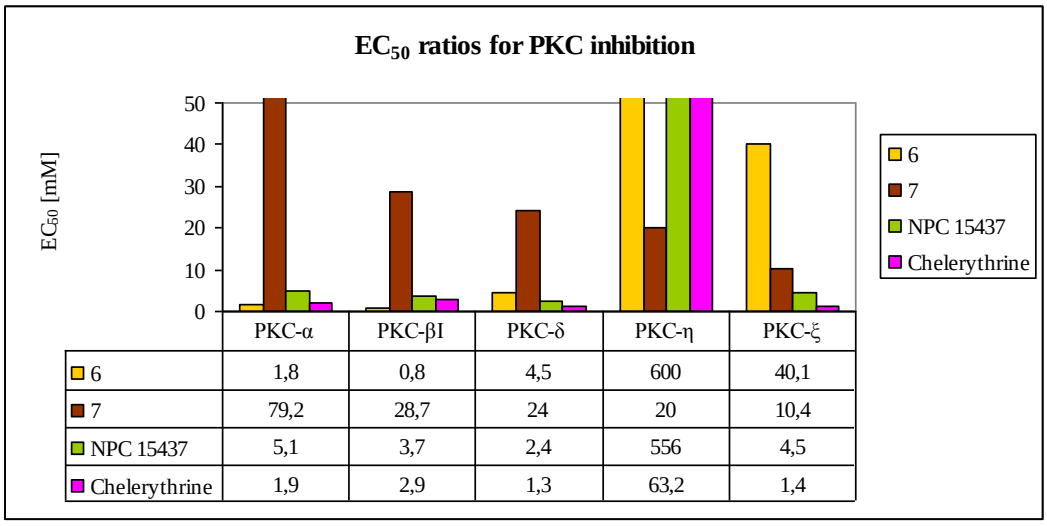
3,4-dihydroxyxanthone | 1-formyl-4-hydroxy-3-methoxy dihydroxyxanthone | 1-formyl-3,4-xanthone

Fig. (3) Xanthones with an effect compatible with PKC inhibition, with an effect on human tumor cell lines and human lymphocytes [2;5]

The modulatory activity of 3,4-dihydroxyxanthone (**6**, Fig. (3)) and 1-formyl-4-hydroxy-3-methoxyxanthone (**7**, Fig. (3)) on isoforms α, βI, δ, η and ξ of PKC was evaluated using an *in vivo* yeast phenotypic assay. In this assay, PKC activators caused growth inhibition of transformed yeast, while PKC inhibitors blocked the growth inhibition caused by a PKC activator. The majority of simple xanthones caused an effect compatible with PKC activation. The 3,4-dihydroxyxanthone (**6**) and 1-formyl-4-hydroxy-3-methoxyxanthone (**7**) caused an effect compatible with PKC inhibition, similar to that elicited by known PKC inhibitors (chelerythrine and NPC 15437). The PKC inhibition caused by (**6**-**7**) was confirmed using an *in vitro* kinase assay. 3,4-Dihydroxyxanthone (**6**), 1-formyl-4-hydroxy-3-methoxyxanthone (**7**), chelerythrine and NPC 15437 were used on the lowest concentration tested (10^{-8} M), the same used to obtain the EC_{50} ratios presented in (Graph 1.). The EC_{50} values were considered as the concentration of PKC activator that caused half of the growth inhibition caused by 10^{-5} M of standard PKC activators: PMA for α, βI, δ and η or arachidonic acid for PKC- ξ. Compounds (**6**-**7**) presented differences on their potency towards the individual PKC isoforms tested [5].

Xanthones **6** and **7** were able to revert the effect of the endogenous PKC activator with the effects similar to those caused by chelerythrine and NPC 15437.

Furthermore, for some isoforms, it showed potencies even higher than those of the PKC inhibitors used. Therefore, 3,4-dihydroxyxanthone (**6**) and 1-formyl-4-hydroxy-3-methoxyxanthone (**7**) were proposed to be used as a PKC inhibitors for characterization of PKC-mediated effects and for the development of new PKC isoform-selective inhibitors. It was concluded that xanthones (**6**) and (**7**) could become useful PKC inhibitor and xanthone derivatives can be explored to develop new isoform-selective PKC inhibitors [5].

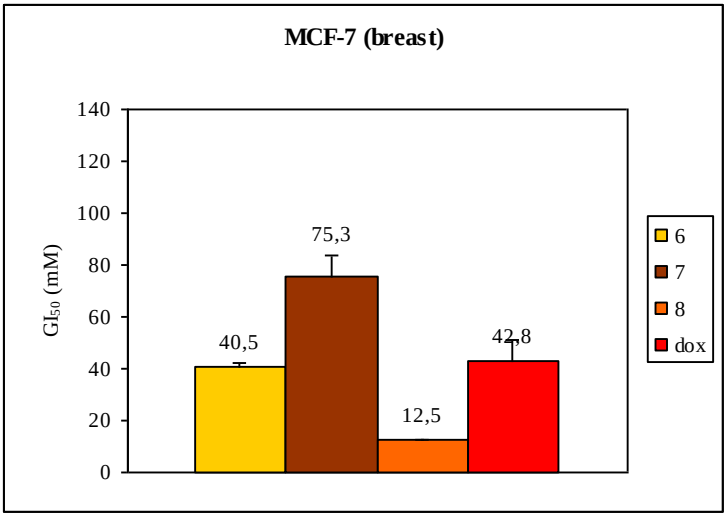


	PKC-α	PKC-βI	PKC-δ	PKC-η	PKC-ξ
6	1,8	0,8	4,5	600	40,1
7	79,2	28,7	24	20	10,4
NPC 15437	5,1	3,7	2,4	556	4,5
Chelerythrine	1,9	2,9	1,3	63,2	1,4

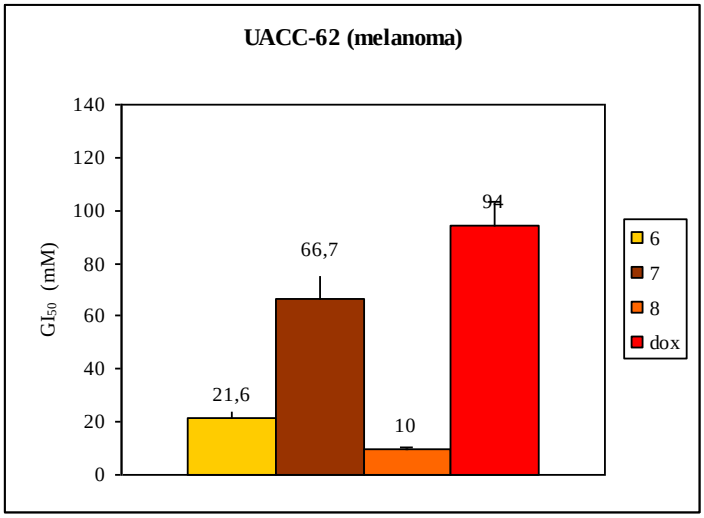
Graph 1 [5] EC_{50} ratios for xanthones **6**, **7**, NPC 15437 and chelerythrine on the individual PKC isoforms tested

3,4-Dihydroxyxanthone (**6**; Fig. (3)) was revealed as a hit compound in a study involving the investigation of the inhibitory effect of simple oxygenated xanthones on human tumour cell lines. In experiments of the effect on the growth of three human tumor cell lines: MCF-7 (breast cancer) (Graph 2), UACC-62 (melanoma) (Graph 3), TK-10 (renal cancer) (Graph 4), given in concentrations that were able to cause 50% of cell growth inhibition (GI_{50} (μM)), the 3,4-dihydroxyxanthone (**6**) was significantly ($p<0.01$) much more active to the melanoma UACC-62 (21.6± 2.6) cell line than to MCF-7 (40.5± 1.5) and TK-10 (59.2). Growing inhibition effect of 1-formyl-4-hydroxy-3-methoxyxanthone (**7**; Fig. (3)), MCF-7 (75.3±8.6), TK-10 (73.1±9.0), UACC-62 (66.7±8.5) was also observed [2]. In another study, 1-formyl-3,4-dihydroxyxanthone (**8**; Fig. (3)) showed a potent effect on the growth inhibition against UACC-62 (10.0) and MCF-7 (12.5) tumour cell lines [6]. In this tests doxorubicin was used as positive

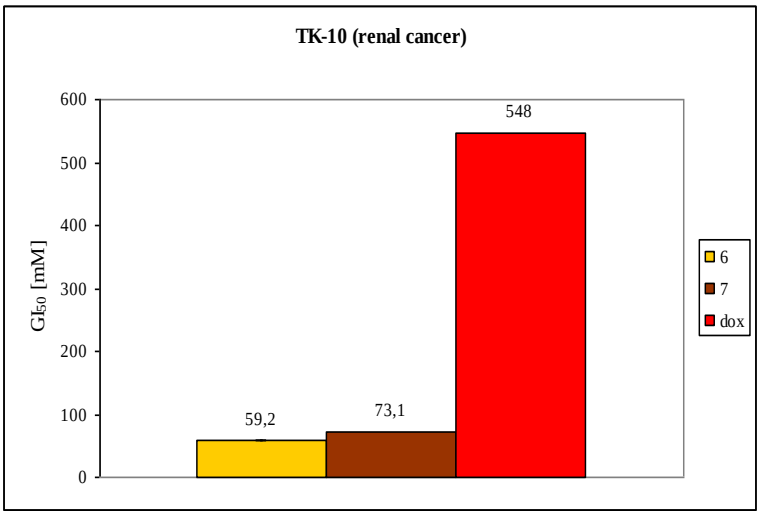
control in cancer cell lines growth GI_{50}: MCF-7 (42.8± 8.2 nM); GI_{50} : TK-10 (548.0± 60.0 nM); GI_{50} : UACC 62 (94.0± 9.4 nM) [2].



Graph 2 [6] Effect of xanthones **6**, **7**, **8** and doxorubicin on the growth of human breast cancer cell lines

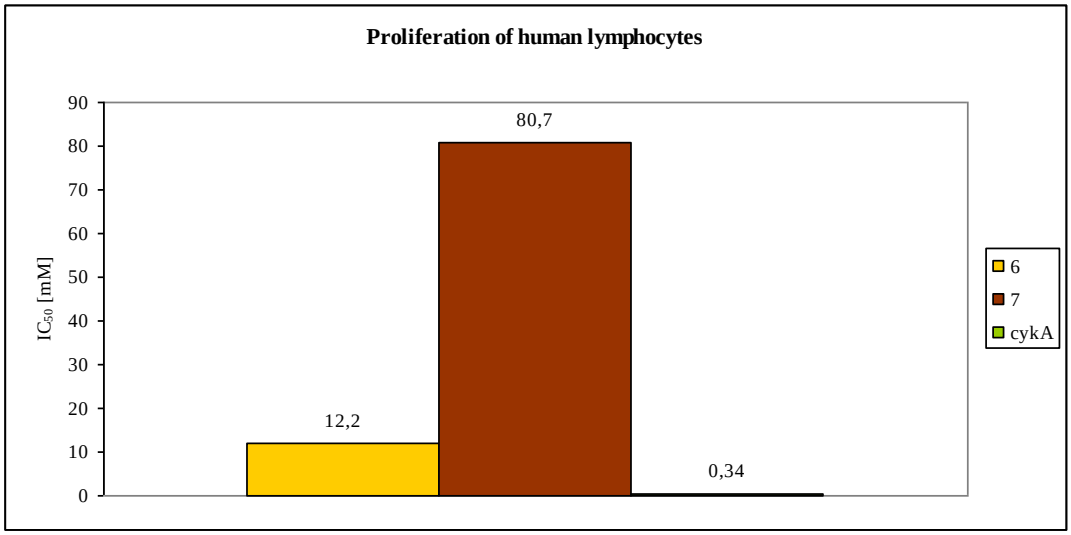


Graph 3 [6] Effect of xanthones **6**, **7**, **8** and doxorubicin on the growth of melanoma cell lines



Graph 4 [2] Effect of xanthones **6**, **7** and doxorubicin on the growth of human renal cancer cell lines

In studies of the *in vitro* effect of xanthones against the proliferation of human lymphocytes inducted by phytohemaglutinin (PHA), given in concentrations that were able to cause 50% inhibition of proliferation-IC_{50} (μM). 3,4-Dihydroxyxanthone (**6**; Fig. (3)) was found to possess a pronounced inhibitory activity (IC_{50}= 12.2± 1.3μM) on the mitogenic response of human lymphocytes to PHA. 1-Formyl-4-methoxy-3-hydroxyxanthone (**8**; Fig. (3)) exhibited weaker inhibitory effect (IC_{50}= 80,7± 11.7μM). The cyclosporin A was used as positive control in lymphocytes proliferation (IC_{50}= 0.34± 0.04 μM) (Graph 5). No lymphocytoxicity was observed when the human lymphocytes were exposed to the IC_{50} concentrations of this xanthone (cell viability >70%), which leads to the conclusion that their inhibitory activity was associated with cell proliferation rather than to a toxic effect. 3,4-Dihydroxyxanthone (**6**) showed the most interesting results for this antiproliferative effect when compared with other oxygenated xanthones [2].



Graph 5 [2] Effect of xanthones **6**, **7** and cyclosporin A on the proliferation of human lymphocytes

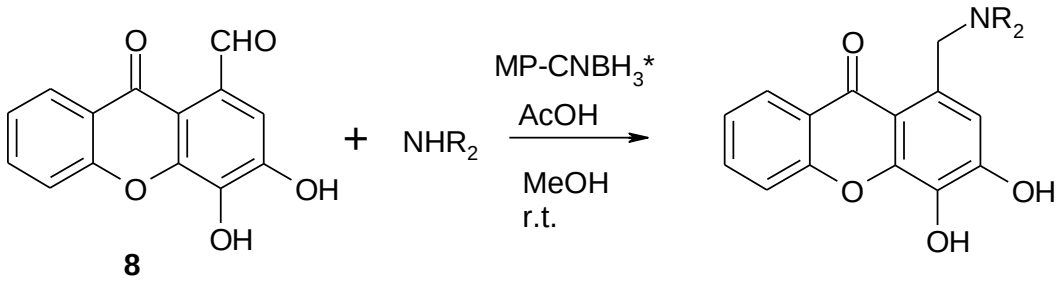
Recently, there has been an increase in the interest in new xanthonic structures, partly due to diverse pharmacological activities exhibited by this type of compounds. Also, in the field of medicinal chemistry, the groups of compounds that can bind to different classes of receptors have attracted much attention and xanthones can be considered as potential structures in this field. The main objectives of xanthone syntheses are not only for the development of more diverse and complex bioactive compounds for biological activity and structure-activity relationship (SAR) studies, but also for other applications in medicinal chemistry, such as preparation of fluorescence probes, due to photochemical properties of xanthone [1].

2. AIMS

Thus the main goal of this project was the synthesis of **3,4-dihydroxyxanthone** (**6**) in a larger scale in order to:

-obtain enough quantity for *in vivo* assays, concerning melanoma investigation,

-serve as a building block for further molecular modification to develop a library of compounds that could reveal simultaneously antitumour activity and P-glycoprotein (P-gp) inhibition. This further process should embrace synthesis of amines from 1-formyl-3,4-dihydroxyxanthone (**8**) (Scheme 1.) and the *in vitro* biological evaluation of MDR (multidrug resistance) modulation conducted by testing the ability of the molecules to reduce P-gp protein expression.



*MP-CNBH3 = solid supported cyanoborohydride

Scheme 1. Synthesis of amines

In industrialized countries, cancers are the second cause of death. Although enormous progress has been achieved in the field of cancer therapy, only approximately 50% of all cancers are susceptible to chemotherapy. From these, more than 50% rapidly develop drug resistance during therapeutical treatment [7]. Most often, this drug resistance is a multiple drug resistance (MDR) phenotype caused by overexpression of P- glycoprotein (P- gp), a membrane-bound efflux pump which transports a wide variety of anticancer agents out of the tumour cells [8]. Inhibition of P- gp is a powerful approach to reverse MNR both

in vitro and *in vivo*; however, the so far existing P- gp inhibitors have demonstrated limited clinical success due to limitations in potency and specifity.

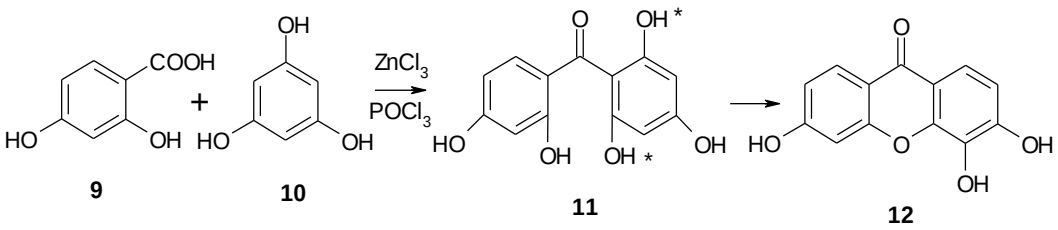
3. RESULTS AND DISCUSSION

3.1. Methodology for the synthesis of xanthones

One of the earliest and simplest methods for the synthesis of xanthones was introduced by Michael and Kostanecki. It involved the distillation of a mixture of a phenol, a *o*-hydroxybenzoic acid, and acetic anhydride. Since then, several other routes with higher yields, less drastic experimental conditions and without side reactions have been developed [9].

There are three traditional methods that can be applied for the synthesis of simple xanthones. The Grover, Shah and Shah (GSS) reaction, the synthesis *via* benzophenone, and the synthesis *via* diphenyl ethers intermediates [1].

In the Grover, Shah and Shah classic method the xanthones are obtained by condensation between an *ortho*-oxygenated benzoic acid (**9**) and an activated phenol (**10**), in phosphorous oxychloride and zinc chloride (Scheme 2.) [2]. It offers a convenient method for preparing hydroxyxanthones and still enjoys a great popularity, due to easily accessible materials. GSS method can afford the xanthone skeleton (**12**) directly only if the benzophenone intermediate (**11**) carries another hydroxyl group at the 6 or the 6' position (*), *i.e.*, if an alternative site for cyclization is available. Due to a number of limitations of this process other methods have taken over this one-pot synthesis of simple xanthones [1].



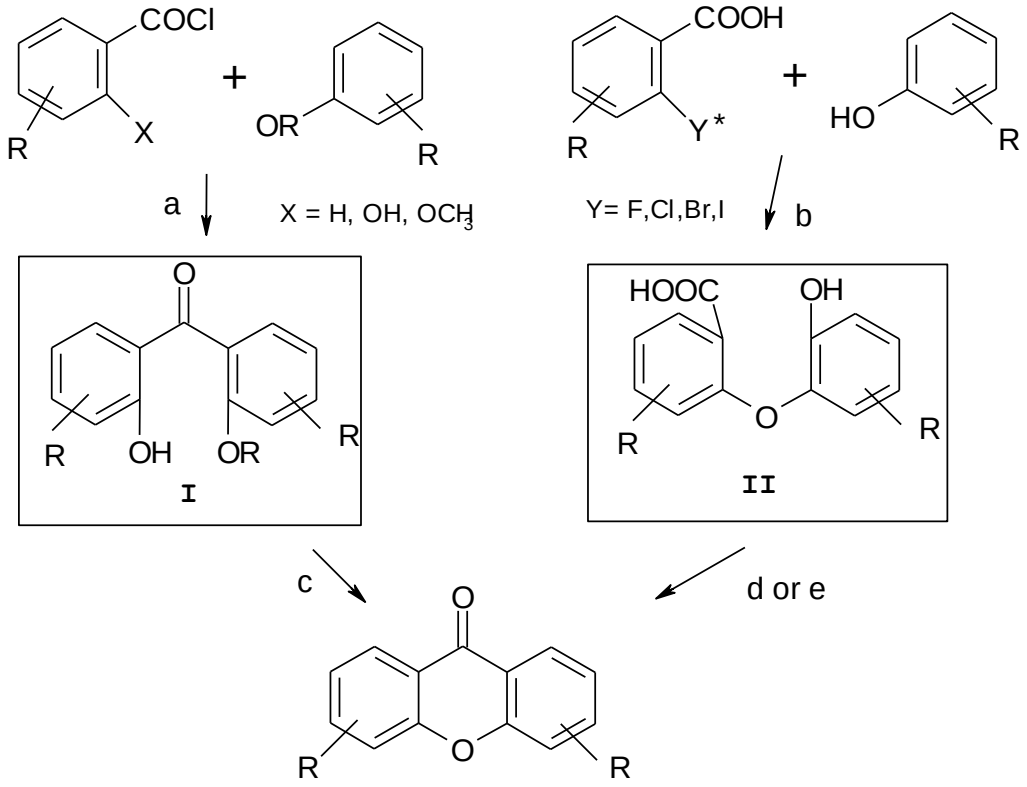
Scheme 2. Grover, Shah and Shah reaction

The benzophenone derivatives (**I**) are commonly accessible through condensation by Friedel–Crafts acylation (Scheme 3 (a)) of an appropriately substituted benzoyl chloride with a phenolic derivative and is followed by the cyclization step (Scheme 3 (c)) that involves a nucleophilic substitution or a

nucleophilic addition-elimination of 2,2'-dioxygenated benzophenones, or an oxidative process.

The diaryl ether synthesis (**II**), Ullmann condensation (Scheme 3 (b)), uses the reaction of phenols with benzoic acids bearing halogen in the *ortho* position (*), and the ring formation is accomplished by electrophilic cycloacylation of the 2-aryloxybenzoic acids.

Because intermolecular acylation reactions give generally higher yields than Ullmann ether syntheses, the most prevalent strategy for xanthone synthesis is acylation, followed by cyclization to form the heterocyclic ring [1].



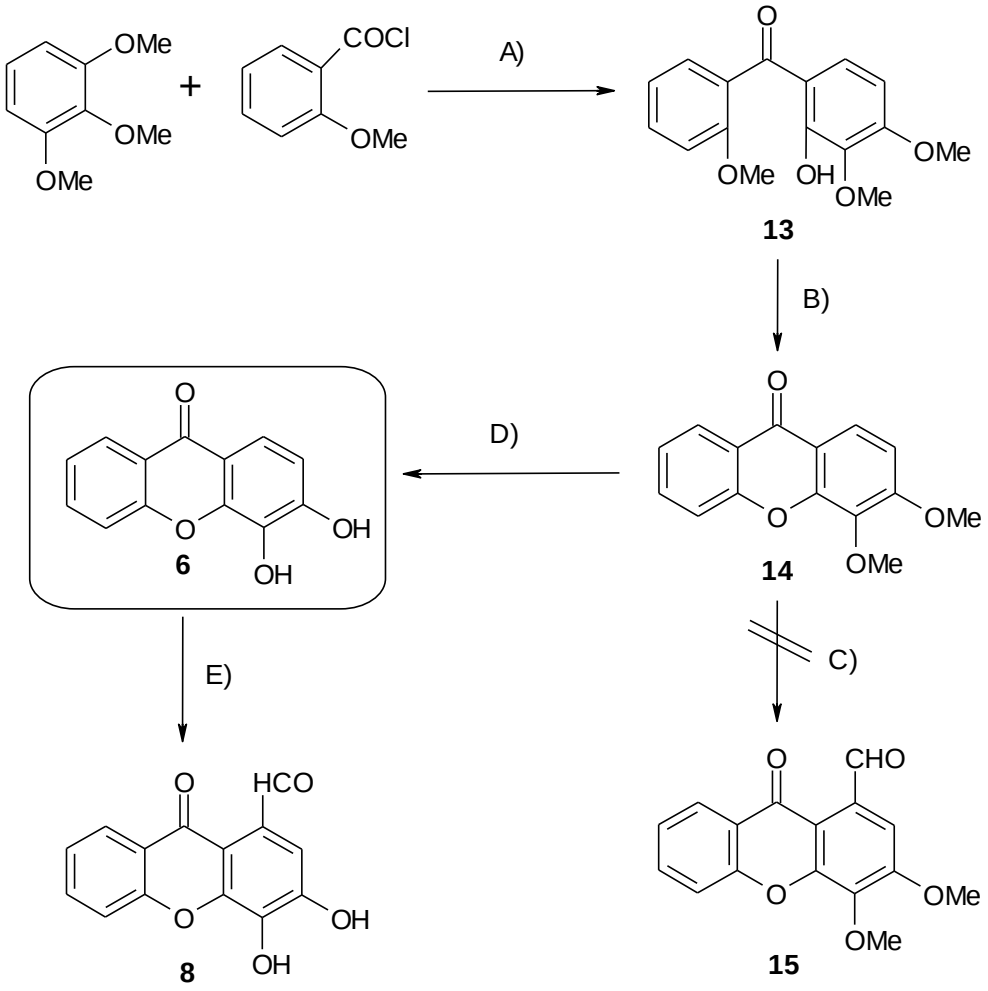
Scheme 3. Methods for the synthesis of xanthones

Some less conventional methods for the synthesis of xanthones have also been reported. These include aryl anion addition to salicylaldehyde, followed by reduction to xanthene and eventual oxidation to xanthone (Tanase method), synthesis of benzophenones involving ketimine intermediates (Robinson and Nishikawa synthesis), synthesis from a thioxanthen-9-one-10,10-dioxide nucleus, from extended poly-ß-ketides, and from the nucleophilic addition of phenols to

alkoxycarbonyl-*p*-benzoquinones (method of Muller and coworkers) followed by reduction [1].

3.2. Pathway for the synthesis of 3,4-dihydroxyxanthone (6) and derivatives

Based on described methods (for the synthesis of oxygenated xanthones) [1], the pathway presented in Scheme 4. was proposed for the synthesis of 3,4-dihydroxyxanthone (**6**) in a larger scale. This strategy proceeded *via* benzophenone as the intermediate to obtain such substituted xanthone.



A) **Friedel-Crafts acylation** Et_2O, $AlCl_3$, reflux, room temperature/ 96h

B) **Base-catalized cyclization** MeOH, H_2O, NaOH, reflux, 40ºC/ 40h

C) **Duff Formylation** HMTA, TFA, reflux, 72°C/ 12h

D) **Demethylation** $C_6H_5CH_3$, $AlCl_3$, reflux, 110 ºC/ 7h

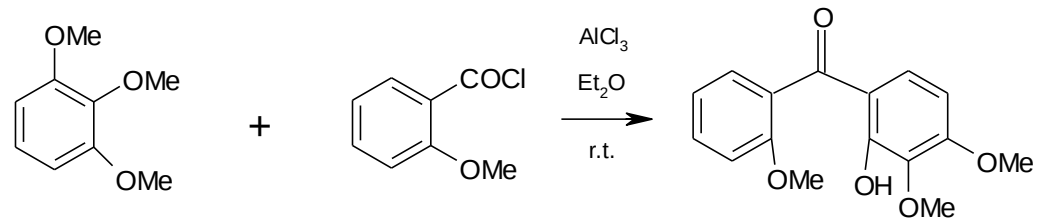
E) **Duff Formylation** HMTA, TFA, reflux, 72°C/ 9h

Scheme 4. Synthesis of 3,4-dihydroxyxanthone and the 1-formylated derivatives

3.3. Synthesis of 3,4-dihydroxyxanthone (6)

The first step of the pathway involves the connection of an appropriate trimethoxybenzene with another appropriate methoxybenzoyl chloride by Friedel-Crafts acylation. This acylation follows the rules of the aromatic electrophilic substitution and allows the formation of 2-hydroxy-2´,3,4-trimethoxybenzophenone (**13**) as the major product for further cyclization. Also under these conditions the methoxy group in position *ortho* to carbonyl suffers demethylation due to the easy access of $AlCl_3$. The cyclization process involves a base-catalyzed reaction. The further purpose of the pathway is the demethylation of 3,4-dimethoxyxanthone (**14**) [10,11]. Removal of the methoxy groups gives 3,4-dihydroxyxanthone (**6**). All the reaction results/mechanisms are described below.

3.3.1. Synthesis of 2-hydroxy-2´,3,4-trimethoxybenzophenone (13); (A)



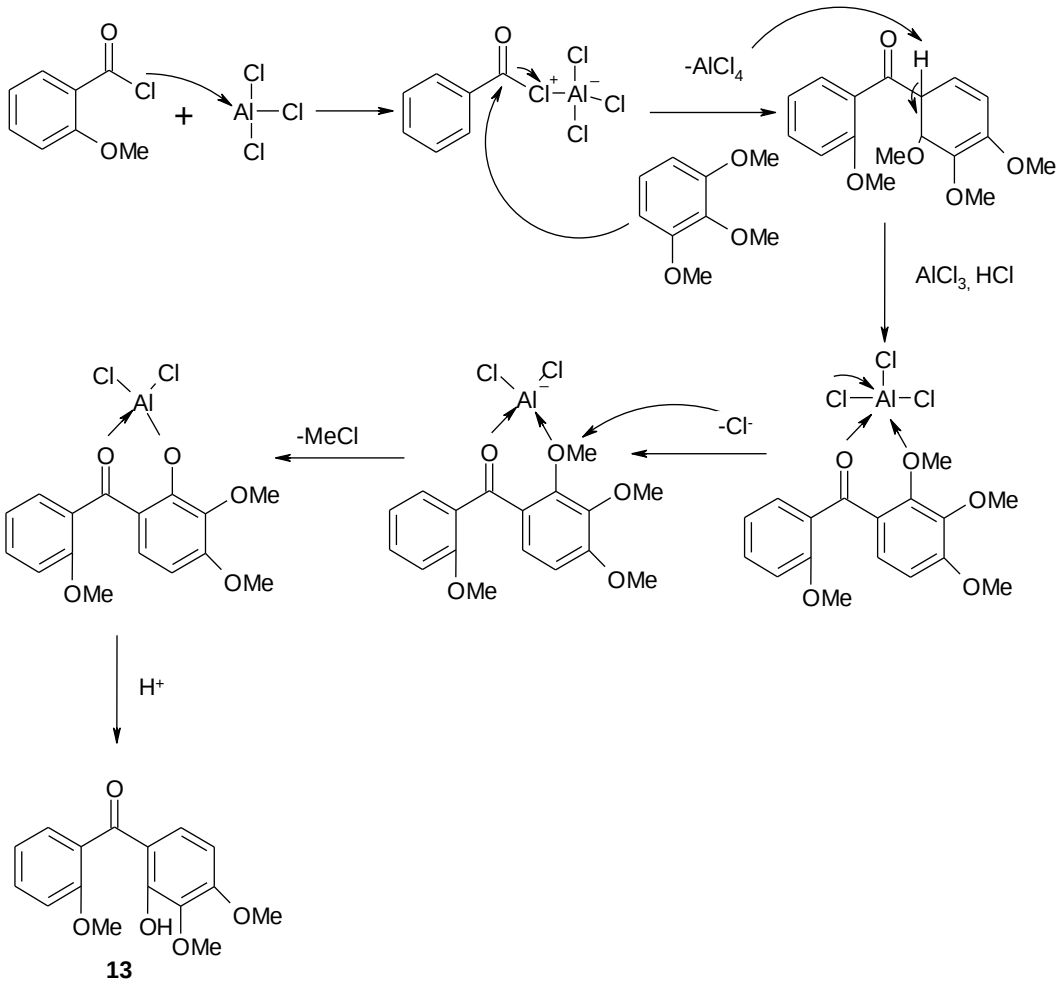
1,2,3-trimethoxybenzene

2-methoxybenzoylchloride

2-hydroxy-2´,3,4-trimethoxy benzophenone

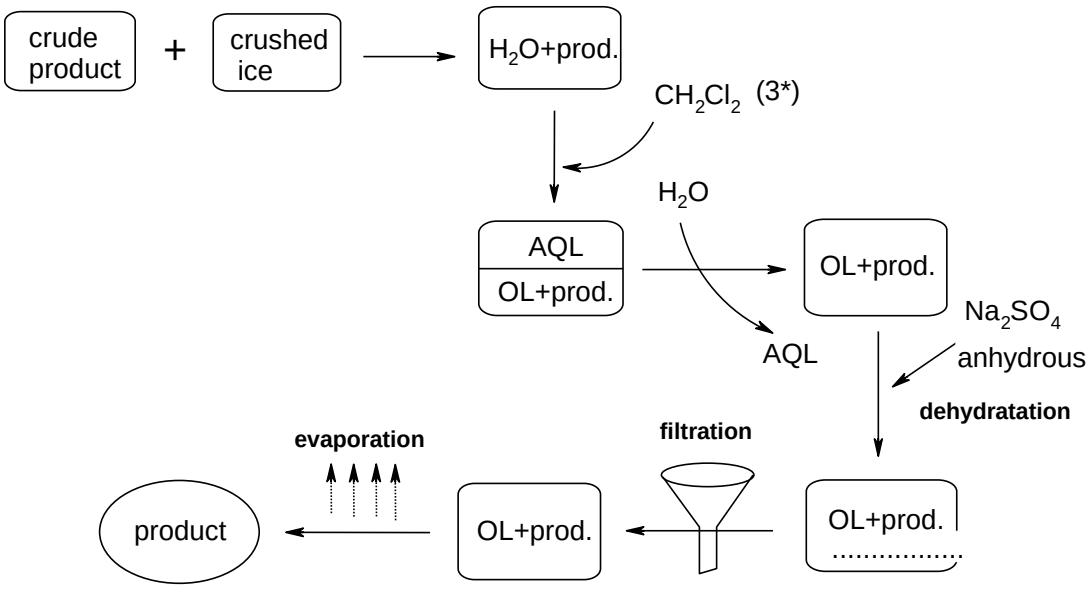
13

By a Friedel Crafts acylation, the building blocks 1,2,3-trimethoxybenzene (10 g) and 2-methoxybenzoylchloride (9.2 g) were allowed to react at room temperature for 4 days in the presence of $AlCl_3$ and anhydrous diethylether to give 2-hydroxy-2´,3,4-trimethoxybenzophenone (**13**). The mechanism of reaction is represented in Scheme 5.



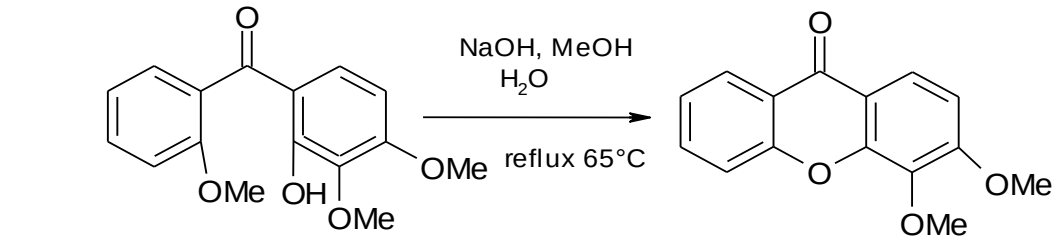
Scheme 5. The mechanism of Friedel Crafts acylation

The crude product was purified by a chemical extraction represented in Scheme 6. 22.1 g of a brown solid were obtained and confirmed to be 2-hydroxy-2´,3,4-trimethoxybenzophenone (**13**) by thin layer chromatograpy (silica; *n*-hexane: EtOAc 6:4) by comparison with a standard. The brown solid was used in the next reaction (3.3.2.) without further purification procedures.



Scheme 6. Purification of crude 2-hydroxy-2′,3,4-trimethoxybenzophenone (**13**)

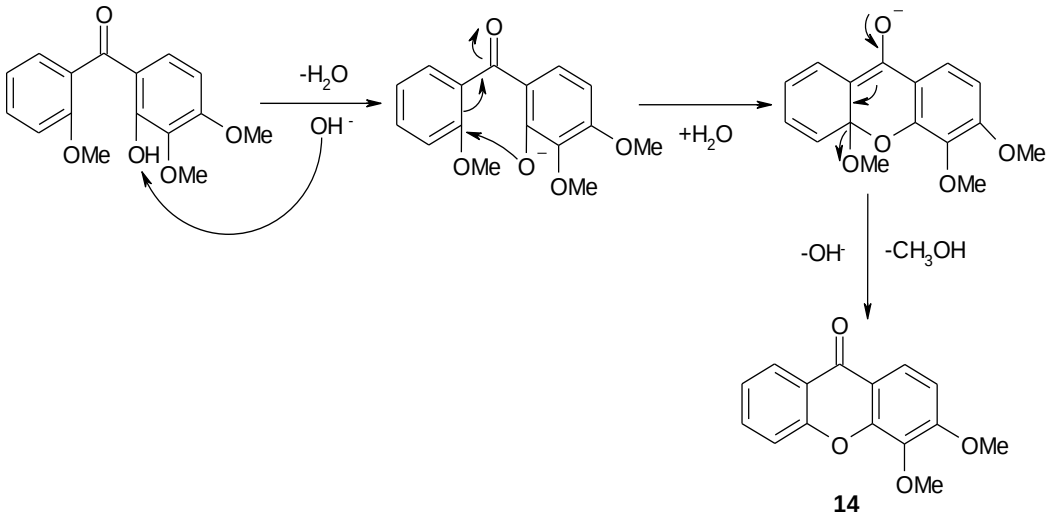
3.3.2. Synthesis of 3,4-dimethoxyxanthone (14); (B)



2-hydroxy-2′,3,4-trimethoxybenzophenone **13**

3,4-dimethoxyxanthone **14**

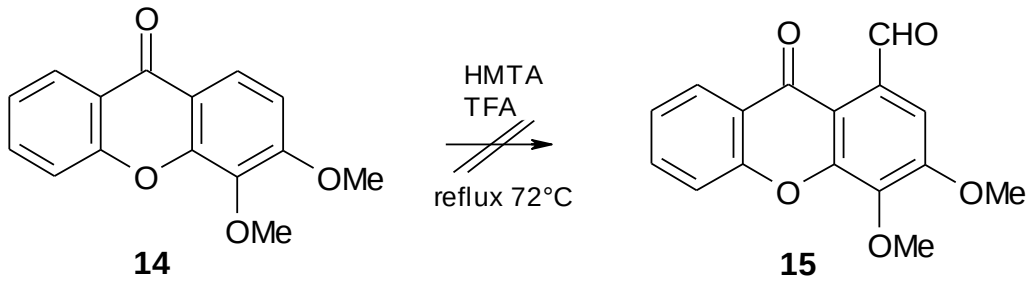
The crude product of 2-hydroxy-2′,3,4-trimethoxybenzophenone (**13**) (22.1 g) was allowed to react with sodium hydroxide in methanol and water under reflux (65˚C) for 40 hours in order to cyclize into a xanthone skeleton (**14**). The mechanism of reaction is presented in Scheme 7.



Scheme 7. The mechanism of base catalized cyclization of 2-hydroxy-2′,3,4-trimethoxybenzophenone (**13**)

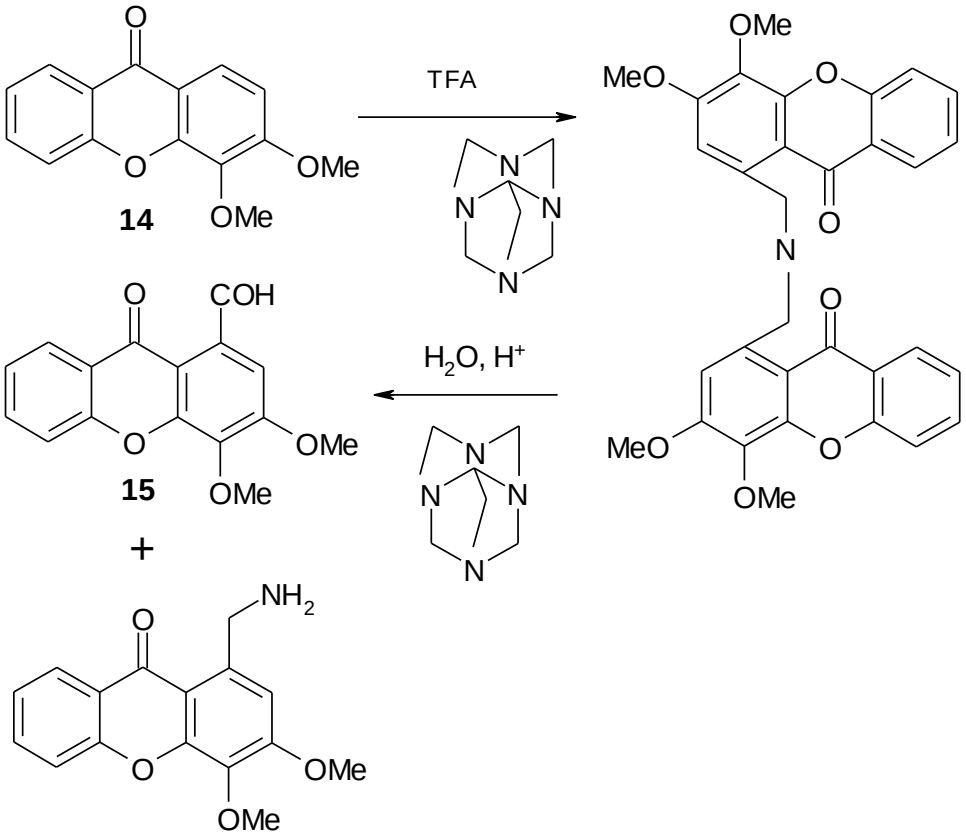
The 9.24 g of a brown oily product obtained by filtration of the crude product was confirmed to contain as a main component 3,4-dimethoxyxanthone (**14**) by thin layer chromatography (silica; 100% CH_2Cl_2). This oil was used in the next reaction (3.3.3.) without further purifications.

3.3.3. Formylation of 3,4-dimethoxyxanthone (14); (C)



The Duff formylation of 3,4-dimethoxyxanthone (**14**) was chosen as an uncommon strategy of preparing 1-formyl-3,4-dihydroxyxanthone (**8**). The plan was to prepare 1-formyl-3,4-dimethoxyxanthone (**15**) and then demethylate it to compound **8**. With this strategy the 1-formyl-3,4-dihydroxyxanthone (**8**) would not be obtained. 1-Formyl derivatives of compounds **6** and **14** are potent inhibitors of growth of human cancer cell lines [6].

The 9.24 g of crude 3,4-dimethoxyxanthone (**14**), HMTA and TFA were allowed to react for 29 hours to form the derivate with aldehyde group in position 1 on the xanthonic framework. The supposed mechanism is presented in the Scheme 8.

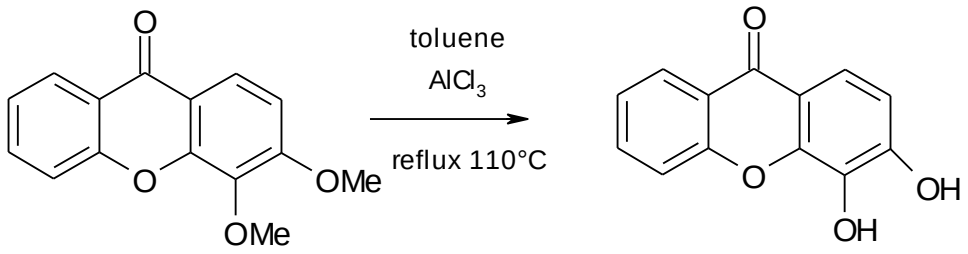


Scheme 8 [12] The mechanism of formylation of 3,4-dimethoxyxanthone (**14**)

The product of reaction was purified by column chromatography and by the cross proof thin layer chromatography it was concluded that the reaction didn‘t produce any 1-formyl-3,4-dimethoxyxanthone (**15**). 2.58 g of 3,4-dimethoxyxanthone (**14**) were recovered as white crystals after purification and recrystalization in 10% yield. This pure product was used for further demethylation procedure (3.2.4.1.).

3.3.4. Demethylations of 3,4-dimethoxyxanthone (14) and a mixture 4-hydroxy-3-methoxyxanthone and 3-hydroxy-4-methoxyxanthone; (D)

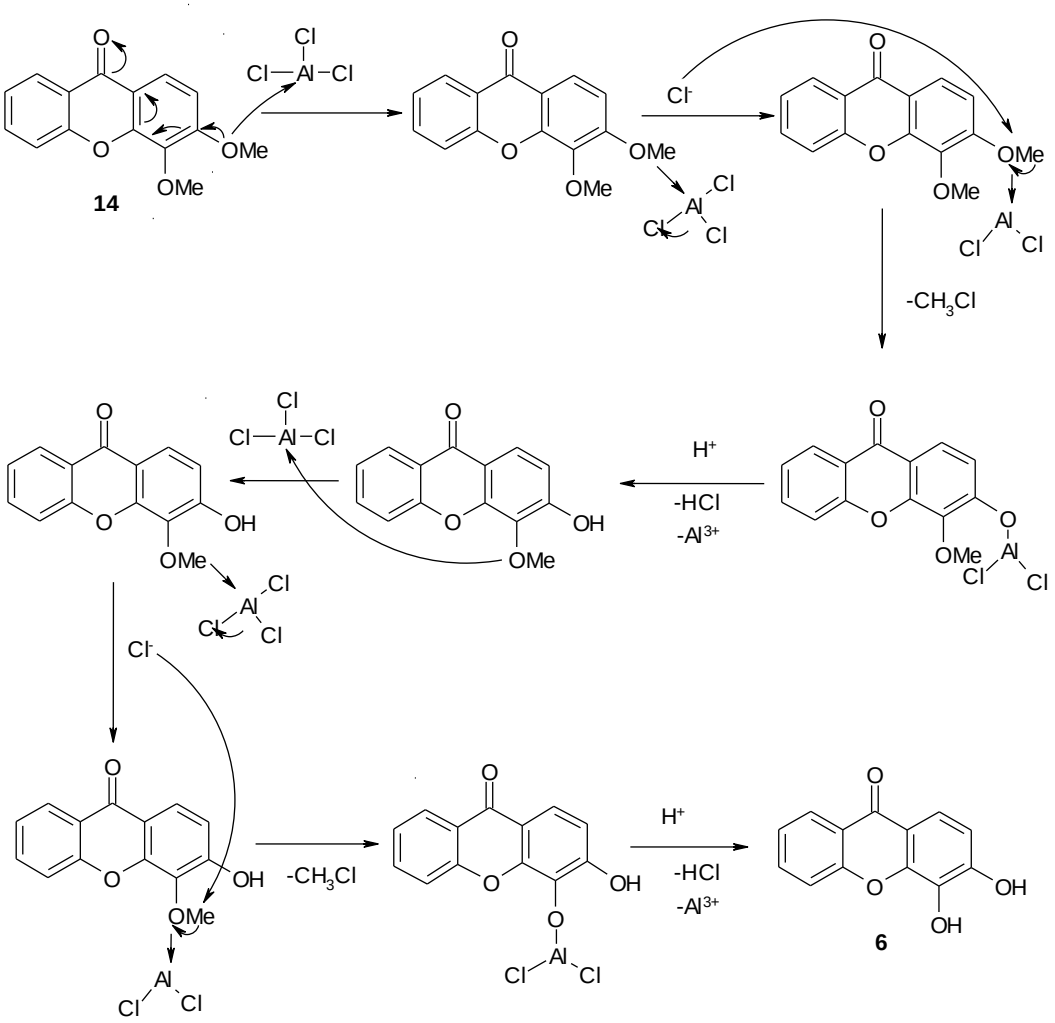
3.3.4.1. Demethylation of 3,4-dimethoxyxanthone (14)



3,4-dimethoxyxanthone **14**

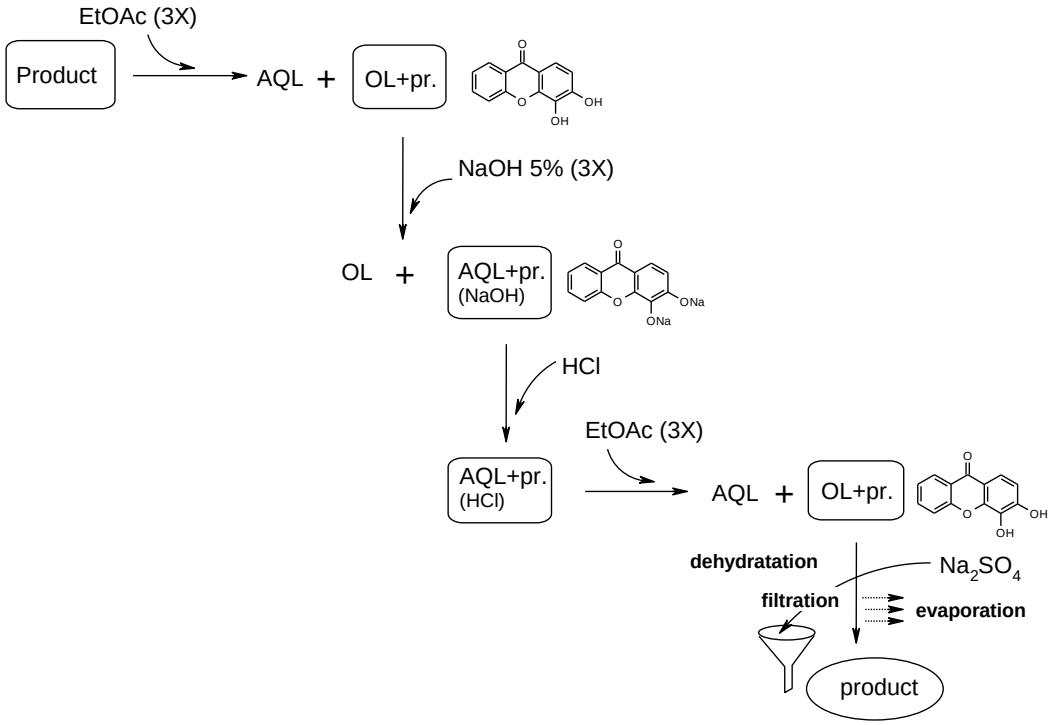
3,4-dihydroxyxanthone **6**

2.7 g of 3,4-dimethoxyxanthone (**14**) were demethylated in anhydrous toluene using $AlCl_3$ under reflux (110˚C) for 7 hours by the mechanism represented in Scheme 9.



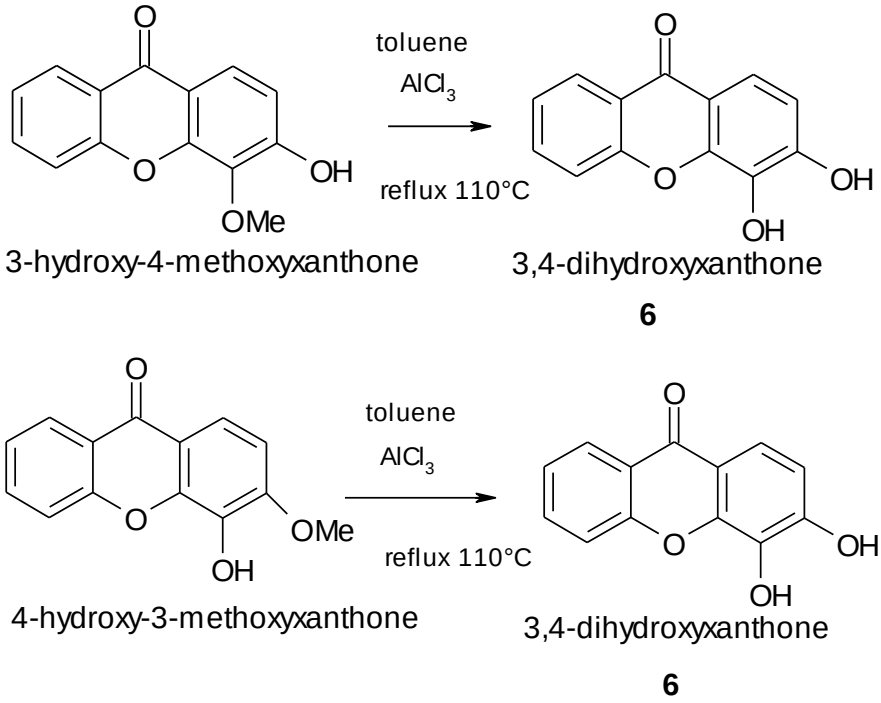
Scheme 9. The mechanism of demethylation of 3,4-dimethoxyxanthone (**14**)

The crude product of the reaction was purified by chemical extraction (Scheme 10.) and recrystallized from methanol and water (4:1) to furnish 0.450 g of pure yellow crystals, that were confirmed to be 3,4-dihydroxyxanthone (**6**) by thin layer chromatography (silica; $CHCl_3$: Acetone 8:2). Yield 18.7%.



Scheme 10. Purification of crude product of 3,4-dihydroxyxanthone (**6**)

3.3.4.2. Demethylation of a mixture 4-hydroxy-3-methoxyxanthone and 3-hydroxy-4-methoxyxanthone



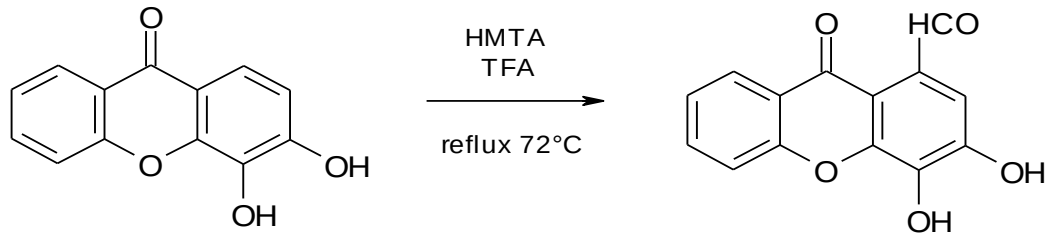
2.18 g of a mixture 4-hydroxy-3-methoxyxanthone and 3-hydroxy-4-methoxyxanthone (which were synthetized by CEQOFF-UP previously and given to me) were demethylated in anhydrous toluene using $AlCl_3$ under reflux (110˚C) for 18 hours. The methoxy groups were removed by the mechanism described in Scheme 9.

The crude product of reaction was purified by chemical extraction (Scheme 10) and recrystallized from methanol and water to obtain 0.7495 g of a yellow crystals, that were confirmed to be 3,4-dihydroxyxanthone (**6**) by thin layer chromatography (silica; $CHCl_3$: Acetone 8:2). The yield of this reaction was 36,4%.

1.1995 g of a yellow solid of 3,4-dihydroxyxanthone (**6**) were obtained from both demethylations and used for further formylation steps.

3.4. Formylation of 3,4-dihydroxyxanthone (6); (E)

The purpose of this reaction was to obtain 1-formyl-3,4-dihydroxyxanthone (**8**) as an appropriate building block for the preparation of tertiary amines for a library of compounds with antitumor and P-glycoprotein activity.

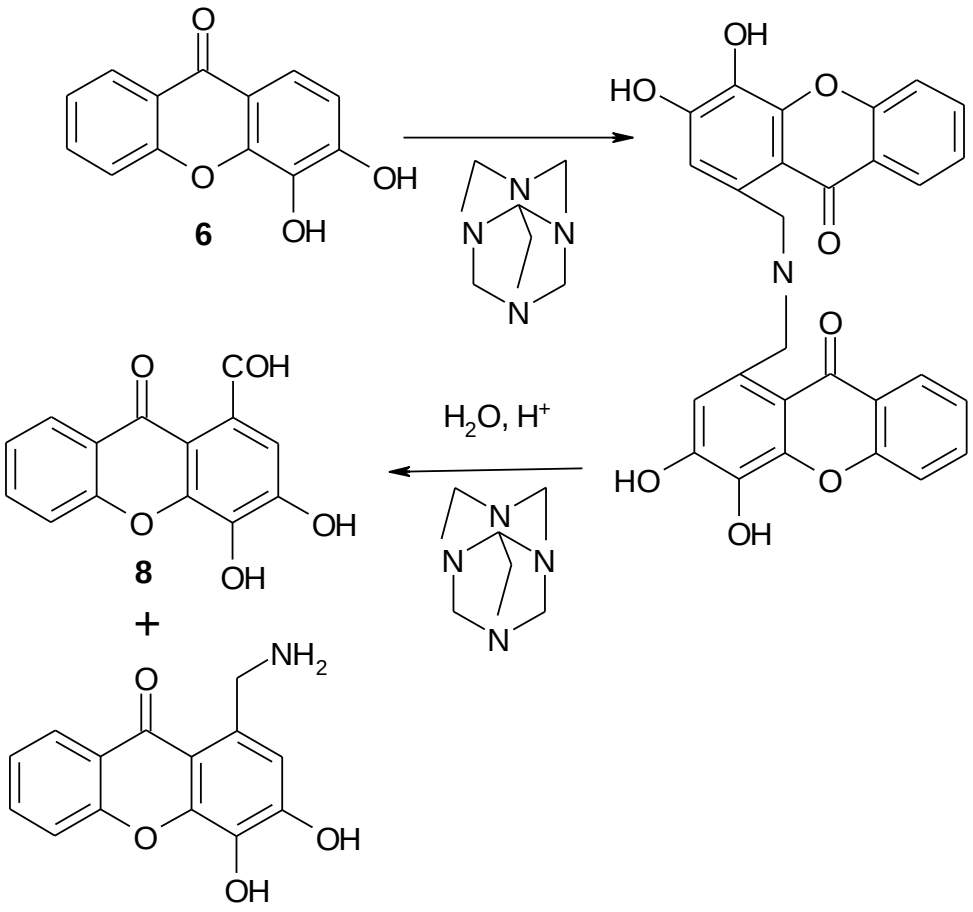


3,4-dihydroxyxanthone
6

1-formyl-3,4-dihydroxyxanthor
8

The Duff formylation was selected due to the group experience [2,6] as a proper process to obtain 1-formyl-3,4-dihydroxyxanthone (**8**). The formylation step was carried out three times. A mixture of compound **6**, HMTA and TFA by rate of **6**: HMTA: TFA→ 1 : 1 : 20.1, was allowed to react under reflux (72˚C) for 9 hours in order to obtain the aldehyde derivative in the position 1 on the xanthonic framework. The mechanism proposed [12] for this reaction is described

in Scheme 11. In the first experiment 10 mg of compound **6** were allowed to react in a sealed tube in the hot air oven. The presence of compound **8** was confirmed by cross proof TLC using a standard of compound **8**. A scale-up of this reaction was done in a small round bottom flask with 0.446 g of compound **6**. The presence of compound **8** was again confirmed by TLC cross proof. The third reaction was done with the remaining 0.7435 g of compound **6** following the rate of compounds and conditions presented former.



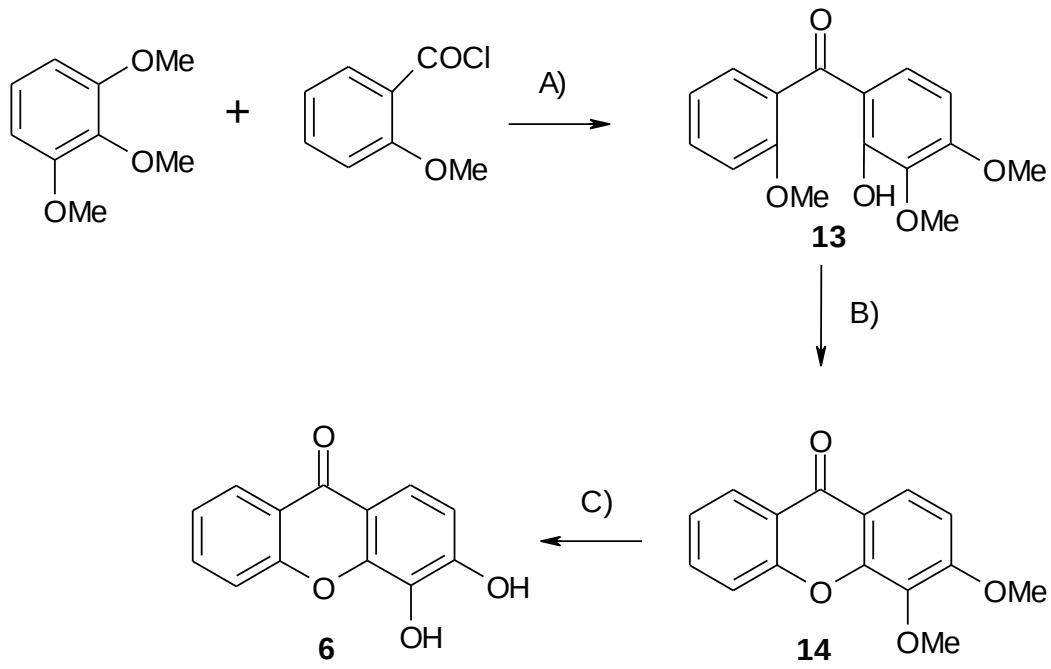
Scheme 11. [12] The mechanism of formylation of 3,4-dihydroxyxanthone (**6**)

0.63 g of an impure brown solid obtained from the gathering of the third and second formylation crude products were purified by the column chromatography ($CHCl_3$: *n*-hexane 5 : 5) and the fractions that contained a mixture of compounds **6** and **8** were collected for further purification by the plate preparative chromatography.

3.5. Repetition of the synthesis of the 3,4-dihydroxyxanthone (6)

The low amount of 3,4-dihydroxyxanthone (**6**) obtained from the first synthesis due to large losses in the formylation steps, conduced this project into the second synthesis in order to obtain 3,4-dihydroxyxanthone (**6**) for *in vitro* and *in vivo* studies regarding melanoma investigations.

The global synthetic pathway was similar to the first synthesis (Scheme 12.): a Friedel-Crafts acylation (A) followed by base-catalyzed cyclization (B) and a demethylation (C). Some modifications were applied in comparison with the first synthesis.



A) **Friedel-Crafts acylation** Et_2O, $AlCl_3$, room temperature/ 92 h
B) **Base-catalized cyclization** MeOH, H_2O, NaOH, reflux 65 ºC/ 64 h
C) **Demethylation** $C_6H_5CH_3$, $AlCl_3$, 70 ºC/ 22 h

Scheme 12. Synthesis of 3,4-dihydroxyxanthone (**6**)

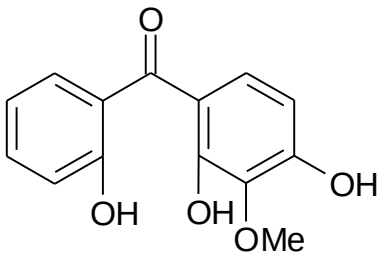
In the first step (=3.3.1.), 22.16 g of a brown solid containing compound **13** were obtained. This impure product was used for the second step, base catalyzed cyclization (=3.3.2.), without further purification and 17.89 g of a brown oil of compound **14** were obtained. This impure product was directly

demethylated (=3.3.3.). The reaction was finished when no more compound **14** neither 3-hydroxy-4-methoxyxanthone and 4-hydroxy-3-methoxyxanthone were observed by thin layer chromatography.

A significant difference was observed by TLC in the crude product of this reaction when compared to the first synthesis (3.3.3.); three main compounds could be obtained after purification by the column chromatography that were shown to be a result of compound **14** decomposition.

The first compound, designated as **J1**, corresponded to 0.144 g of a pure light yellow solid; infrared and ^{1}H and ^{13}C NMR data (not shown) of the pure compound (**J1**) revealed this to be a benzoic acid derivative and compound **J1** structure was not further elucidated.

The secondnd compound, designated as **J2**, was recrystallized from methanol and water (4:1) to give 1.14 g of a pure bright yellow crystals. Infrared and ^{1}H and ^{13}C NMR data (not shown) of the pure compound (**J2**) revealed this to be a benzophenone with the following structure:



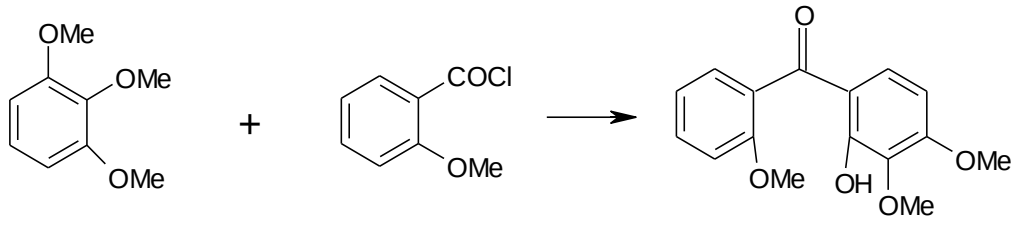
J2

The third compound was recrystallized from $CHCl_3$/ n-hexane (3:1) and methanol/ water (4:1) to obtain 0.724 g of a yellow solid of 3,4-dihydroxyxanthone (**6**).

4. EXPERIMENTAL PART

Purifications of compounds were performed by column chromatography using Merck silica gel 60 (0.50– 0.20 mm) and preparative thin layer chromatography (TLC) using Merck silica gel 60 (GF_{254}). The infrared spectra were recorded on a Hitachi model 260-30 IR spectrophotometer in KBr. ^{1}H and ^{13}C NMR spectra were taken in deuterated chloroform or dimethylsulfoxide at room temperature, on Bruker DRX 300 instrument. The standards used were obtained previously in the CEQOFF-UP.

4.1. Synthesis of 2-hydroxy-2´,3,4-trimethoxybenzophenone (13)

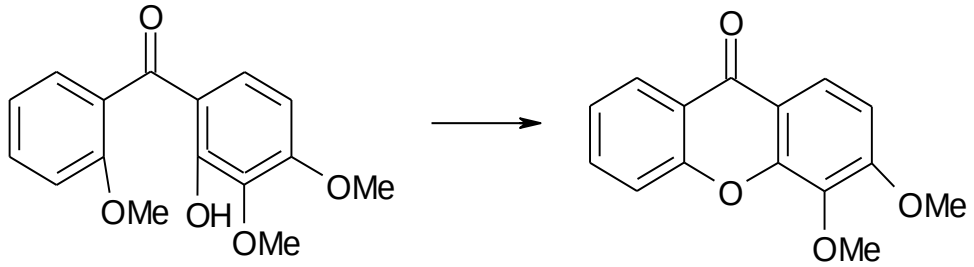


1,2,3-trimethoxybenzene 2-methoxybenzoylchloride 2-hydroxy-2´,3,4-trimethoxy benzophenone

13

In a round bottom flask 10 g of 1,2,3-trimethoxybenzene (60 mmol, 168.19 g/mol) were dissolved in 435 ml of anhydrous diethylether (74.122 g/mol) and then 7.25 ml of 2-methoxybenzoylchloride (54 mmol, 170.6 g/mol) were added. To this solution 29 g of $AlCl_3$ (133.34 g/mol) were added carefully and the reaction was kept at room temperature with magnetic stirring. The reaction finished when by TLC control (silica; *n*-hexane:EtOAc 6:4) no more 2-methoxybenzoylchloride was observed. The reaction was considered finished after 118 hours. The solvent was evaporated and the crude product was poured into ice (50 ml) and neutralized with 10% HCl (100 ml). The final product was extracted from water with (3×50 ml) of dichloromethane. The organic layer was dried with anhydrous sodium sulphate and the solvent was evaporated to give 22.1 g of a brown solid, which was confirmed to be 2-hydroxy-2´,3,4-trimethoxybenzophenone (**13**) (228.3 g/mol) by cross proof TLC (silica; *n*-hexane:EtOAc 6:4) when comparing to a standard.

4.2. Synthesis of 3,4-dimethoxyxanthone (14)



2-hydroxy-2´,3,4-trimethoxy benzophenone

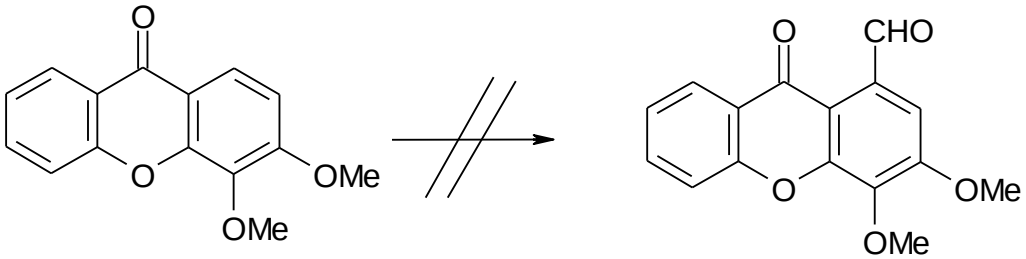
13

3,4-dimethoxyxanthone

14

22.09 g of the crude product (**13**) (76.3 mmol, 288.3 g/mol) from the former reaction (4.1.) were dissolved in 180 ml of methanol. After careful addition of NaOH (40 g) dissolved in 120ml of water, the reaction was stirred under reflux (65˚C) for 40 hours. The reaction was considered finished when no benzophenone was observed by TLC cross proof control (silica; 100%CH_2Cl_2) using the standard of 3,4-dimethoxyxanthone (**14**). Filtration of the suspension gave a brown solid, which was washed with water and dried in vacuum and hot air to give 9.24 g of a brown solid of impure 3,4-dimethoxyxanthone (**14**) (256.26 g/mol).

4.3. Formylation of 3,4-dimethoxyxanthone (13)



3,4-dimethoxyxanthone

13

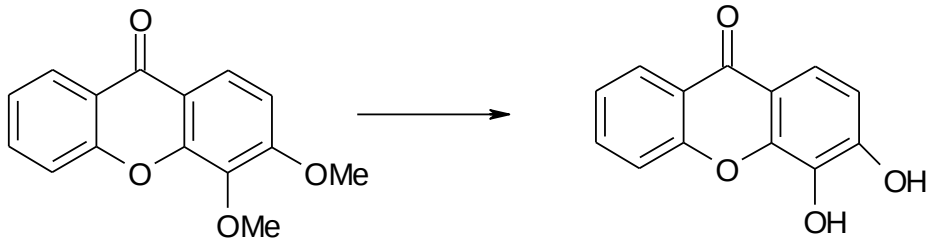
1-formyl-3,4-dimethoxyxanthone

15

A mixture of 9.24 g of the crude (**14**) (30.98 mmol, 256.26 g/mol) from the previous reaction (4.2.) and 5.83 g of HMTA (140.19 g/mol) in 62.4 ml of TFA (114.03 g/mol) were kept stirring at 90-100˚C for 29 hours. The reaction was controlled by cross proof TLC (silica; CH_2Cl_2:acetone 9:1). After pouring into ice

(200 ml) the suspension was basified with Na_2CO_3 and extracted with ethylacetate (organic layer 1). The aqueous layer was acidified by HCl and the organic compounds were extracted with ethylacetate (organic layer 2). The ethylacetate was evaporated and the solids from both organic layers (1 and 2) were purified by column chromatography (silica; CH_2Cl_2:*n*-hexane 5:5) and recrystallized from methanol and water (4:1) to obtain 2.58 g of a pure white solid which was confirmed to be 3,4-dimethoxyxanthone (**14**) (10.5 mmol, 256.26 g/mol). The yield of the recovered product (**14**) was 10%.

4.4. Demethylation of 3,4-dimethoxyxanthone (14)



3,4-dimethoxyxanthone

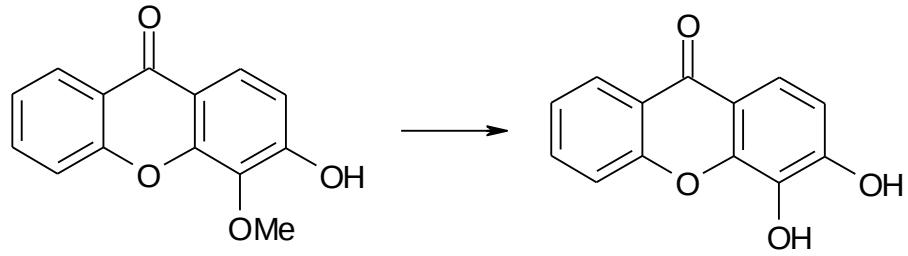
14

3,4-dihydroxyxanthone

6

2.7 g (10.5 mmol, 256.26 g/mol) of 3,4-dimethoxyxanthone (**14**) obtained from the previous reactions (4.2.) and (4.3.) were dissolved in 100 ml of anhydrous toluene. Then, 4.2 g of $AlCl_3$ (30 mmol, 133.5 g/mol) were added carefully. The reaction was kept under reflux (110˚C) and controlled by TLC (silica; $CHCl_3$: acetone 8:2). After 7 hours, when no more 3,4-dimethoxyxanthone (**14**) was observed in the reaction, the reaction was stopped by addition of 50-100 ml of an aquous solution of HCl 5N. The product was extracted with ethylacetate (3×40 ml), then the organic layers were joined and washed with aqueous NaOH 5N (3×40 ml). The aqueous layers (NaOH) were washed with ethylacetate (2×30 ml), acidified with HCl 5N. 3×40 ml of ethylacetate were used to extract the product. The organic layers were dried with anhydrous sodium sulphate and the solvent was evaporated to give 1g of a brown solid. The solid was crystallized from a mixture of methanol and water (4:1) to give 450 mg of a yellow solid of 3,4-dihydroxyxanthone (**6**) (1.97 mmol, 228.21 g/mol). Yield: 18.7%.

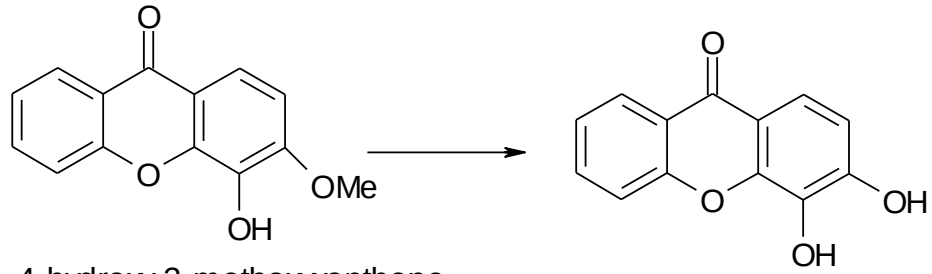
4.5. Demethylation of a mixture 4-hydroxy-3-methoxyxanthone and 3-hydroxy-4-methoxyxanthone



3-hydroxy-4-methoxyxanthone

3,4-dihydroxyxanthone

6



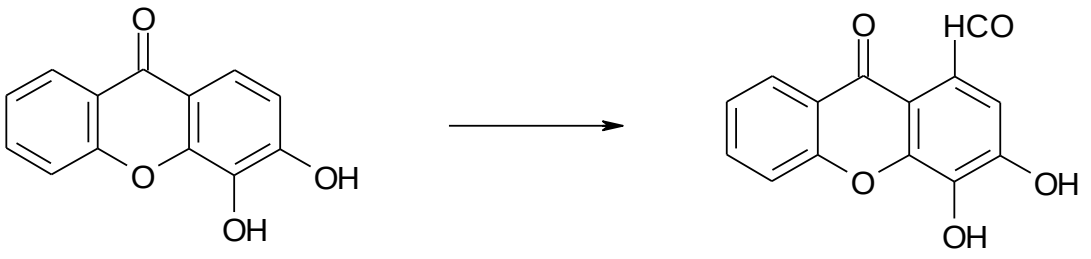
4-hydroxy-3-methoxyxanthone

3,4-dihydroxyxanthone

6

The 2.18 g (9 mmol, 242 g/mol) of a mixture of 4-hydroxy-3-methoxyxanthone and 3-hydroxy-4-methoxyxanthone were dissolved in 100 ml of anhydrous toluene. Then, 3.6 g of $AlCl_3$ (27 mmol, 133.5 g/mol) were added carefully. The reaction was kept under reflux (110˚C) and controlled by TLC (silica; $CHCl_3$:acetone 8:2). After 18 hours, when no more 3,4-dihydroxyxanthone (**6**) was observed in the reaction, the reaction was stopped by addition of 50-100 ml HCl 5N. Then, it was treated like in reaction (4.4.). After recrystallization from methanol and water (4:1) 0.7495 g of a yellow solid 3,4-dihydroxyxanthone (**6**) (3.28 mmol, 228.21 g/mol) were obtained. Yield: 36.4%.

4.6. Formylation of 3,4-dihydroxyxanthone (6)



3,4-dihydroxyxanthone

6

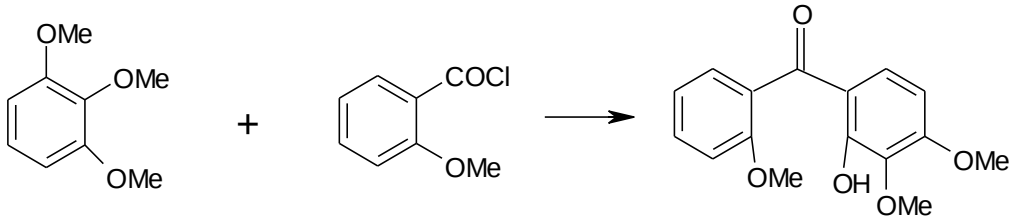
1-formyl-3,4-dihydroxyxanthon

8

A mixture of 1.19 g of 3,4-dihydroxyxanthone (**6**) (5.25 mmol, 228.21 g/mol) from reactions (4.4.) and (4.5.), 0.4567 g of HMTA (3.26 mmol, 140.19 g/mol) and 4.8 ml of TFA (65.48 mmol, 114.03 g/mol) were allowed to react under reflux (72˚C) for 9 hours. The reaction was controlled by cross proof TLC (silica; $CHCl_3$:MeOH:HCOOH 93:7:0.1). After cooling, 44.5 ml of 15% HCl were added and the reaction was kept under reflux for 60 minutes. After this period, the reaction was cooled, poured on ice and left for 8 hours in the refrigerator. The solid thus obtained was filtrated, washed with water and dried. Purification of the crude product was performed by the column chromatography (silica; $CHCl_3$:*n*-hexane 5:5). Eleven fraction of a mixture of compound **6** and **8** were collected for further plate chromatography purification.

4.7. Repetition of the synthesis

4.7.1. Synthesis of 2-hydroxy-2´,3,4-trimethoxybenzophenone (13)



1,2,3-trimethoxybenzene

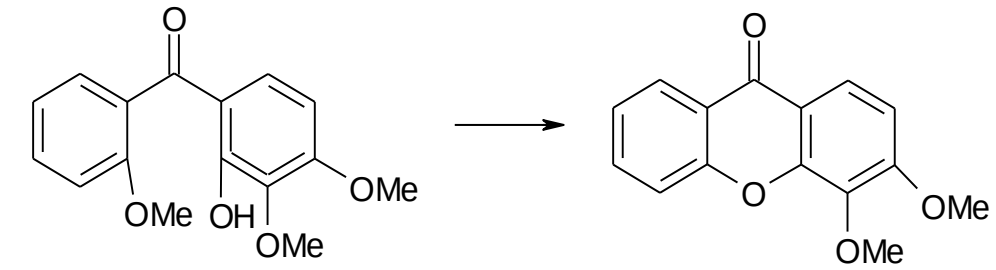
2-methoxybenzoylchloride

2-hydroxy-2´,3,4-trimethoxy benzophenone

13

In a round bottom flask 10 g of 1,2,3-trimethoxybenzene (60 mmol, 168.19 g/mol) were dissolved in 435 ml of anhydrous diethylether (74.122 g/mol) and then 7.25 ml of 2-methoxybenzoyl chloride (54 mmol, 170.6 g/mol) were added. To this solution 29 g of $AlCl_3$ (133.34 g/mol) were added carefully and the reaction was kept at room temperature with magnetic stirring. The reaction was followed by TLC control (silica; *n*-hexane:EtOAc 6:4) and was stopped when no more 2-methoxybenzoylchloride was observed. The reaction was considered finished after 92 hours. The solvent was evaporated and the crude product was poured into ice (50 ml) and neutralized with 10% HCl (100 ml). The final product was extracted with dichloromethane (3×50 ml). The organic layer was dried with anhydrous sodium sulphate and the solvent was evaporated to give 22.16 g of a brown solid, which was confirmed to be 2-hydroxy-2´,3,4-trimethoxybenzophenone (**13**) (228.3 g/mol) by cross proof TLC (silica; *n*-hexane:EtOAc 6:4) when comparing to a standard.

4.7.2. Synthesis of 3,4-dimethoxyxanthone (14)



2-hydroxy-2´,3,4-trimethoxy benzophenone

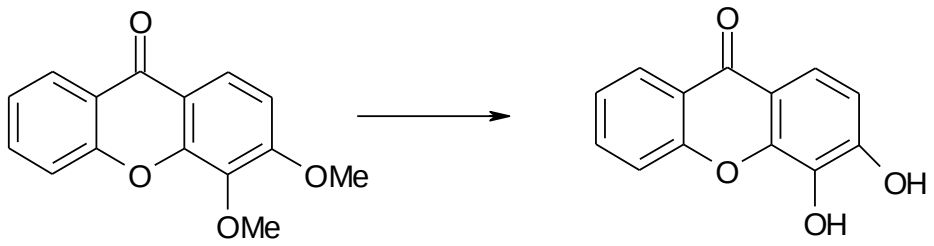
13

3,4-dimethoxyxanthone

14

22.16 g of the crude product (**13**) (76.86 mmol, 288.3 g/mol) from the previous reaction (4.7.1.) were dissolved in 180 ml of methanol. After careful addition of NaOH (40 g) dissolved in 120ml of water, the reaction was stirred under reflux (65˚C) for 64 hours. The reaction was considered finished when no benzophenone was observed by TLC cross proof control (silica; 100%CH_2Cl_2) using the standard of 3,4-dimethoxyxanthone (**14**). Then, the reaction was acidified with HCl 32% (10 ml) and product was extracted with dichloromethane. The dichloromethane was evaporated to give 17.89 g of a brown solid of impure 3,4-dimethoxyxanthone (**14**) (256.26 g/mol).

4.7.3. Demethylation of 3,4-dimethoxyxanthone (14)



3,4-dimethoxyxanthone **14**

3,4-dihydroxyxanthone **6**

The 17.89 g (58.53 mmol, 256.5 g/mol) of a brown solid containing 3,4-dimethoxyxanthone (**14**) from the previous reaction were dissolved in 300 ml of anhydrous toluene. Then, 23.44 g of $AlCl_3$ (175.58 mmol, 133.5 g/mol) were added carefully. The reaction was kept under reflux (110˚C) and controlled by TLC (silica; $CHCl_3$:acetone 8:2). After 22 hours, when no progress in the reaction was observed, *i.e.*, when no increase on the amount of 3,4-dihydroxyxanthone (**6**) was observed, the reaction was stopped by addition of 50-100 ml of an aqueous solution of HCl 5N. The reaction was filtrated and 18,85 g of a brown solid were obtained. This solid was purified by column chromatography. There were 0.144 g of a pure light yellow solid (**J1**) obtained with *n*-hexane:CH_2Cl_2 95:5. A compound designed **J2** was collected in fractions containing *n*-hexane:CH_2Cl_2 9:1. The solvent was evaporated and the solid thus obtained was crystallized from a mixture of methanol and water (4:1) to furnish pure bright yellow crystals of 1,3,1'-trihydroxy-2-methoxybenzophenone (**J2**, 1.14 g). Reunion of fractions containing *n*-hexane:CH_2Cl_2 8:2 gave 0.724 g of a yellow solid of 3,4-dihydroxyxanthone (**6**) (3.17 mmol, 228.21 g/mol) after crystallization with a mixture of $CHCl_3$ and *n*-hexane (3:1).

5. CONCLUSION

The synthesis of 3,4-dihydroxyxanthone (**6**) was achieved.

The yield of the first synthesis of 3,4-dihydroxyxanthone (**6**) was 4.57% (based on 1,2,3-trimethoxybenzene and 2-methoxybenzoylchloride), due to losses in the formylation attempt of 3,4-dimethoxyxanthone (**14**).

1,19 g of 3,4-dihydroxyxanthone (**6**) obtained by the first synthesis were used to obtain 1-formyl-3,4-dihydroxyxanthone (**8**). The presence of the 1-formyl-3,4-dihydroxyxanthone (**8**) was confirmed, but the compound was not isolated in a pure form. Future work with plate preparative chromatography should be accomplished. Compound (**8**) will serve as a building block to set a library of amines potential dual modulators of glycoprotein P with antitumor activity.

The yield of compound **6** from the second synthesis was only 2.78% (based on 1,2,3-trimethoxybenzene and 2-methoxybenzoylchloride), due to the opening of the xanthonic skeleton to a benzophenone (**J2**) during the demethylation process.

0,724 g of compound (**6**) will be used for *in vivo* studies on melanoma.

6. REFERENCES

[1] M.E.Sousa, M.M.M.Pinto; *Synthesis of Xanthones: An Overview*; Curr. Med. Chem.; (2005); 12, 2447-2479.

[2] M.Pedro, F.Cerqueira, M.E.Sousa, M.S.J.Nascimento, M.M.M. Pinto; *Xanthones as Inhibitors of Growth of Human Cancer Cell Lines and Their Effects on the Proliferation of Human Lymphocytes In Vitro*; Bioorg. Med. Chem.; (2002); 10, 3725–3730.

[3] M.M.M. Pinto, M.E.Sousa, M.S.J.Nascimento; *Xanthone Derivatives: New Insights in Biological Activities*; Curr. Med. Chem.; (2005); 12, 2517-2538.

[4] M.M.M.Pinto, M.E.Sousa; *Natural and Synthetic Xantholignoids: Chemistry and Biological Activities*; Curr. Med. Chem.; (2003); 10, 1-12.

[5] L.Saraiva, P.Fresco, E.Pinto, M.E.Sousa, M.M.M.Pinto, J.Goncalvesa; *Inhibition of Protein Kinase C by Synthetic Xanthone Derivatives*; Bioorg. Med. Chem.; (2003); 11, 1215–1225.

[6] M.E.Sousa, M.M.M.Pinto, N.Nazareth, R.Wilairat, M.Pedro, M.S.J.Nascimento; Poster: *Formylated xanthones: potent inhibitors of the growth of human tumour cell lines*; TRAMECH (2004) Transmediterranean Colloquium on Heterocyclic Chemistry, Marrakech, Morocco.

[7] G.F. Ecker, P.Chiba; *Developement of modulators of multi drug resistance: pharmacoinformatic approach* Pure Appl. Chem.; (2004); 76, 997.

[8] R.L.Juliano, V.Ling; *A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants*; Biochem. Biophys. Acta; (1976); 455, 152.

[9] P.K.Grover, G.D.Shah, R.C.Shah; *Xanthones. Part IV. A New Synthesis of Hydroxyxanthones and Hydroxybenzophenones*; J. Chem. Soc.; (1955); 3982.

[10] C.N.Lin, S.S.Liou, F.N.Ko, C.M.Teng; *Pyrone compounds II: Synthesis and antiplatelet Effects of Tetraoxygenated xanthones*; J. Pharm. Sci.; (1992); 81, 1109-1112.

[11] C.N.Lin, S.S.Liou, F.N.Ko, C.M.Teng; γ*-Pyrone compounds. IV: Synthesis and antiplatelet effects of mono- and dioxygenated xanthones and xanthonoxypropanolamine*; J. Pharm. Sci; (1993); 82, 11.

[12] A.M.A.G.Oliveira; *Analogos do psoraleno com um núcleo di benzofurano xanthona ou carbazole síntese a aplicacoes*; Tese do doutoramento em ciencias ramo de quemica; (2005); cap IV, 148.

7. SUPPLEMENT

Abstract for the presentation: „First Meeting of Young Researchers of University of Porto 20-22 February 2008“

Synthesis of xanthone derivatives for *in vitro* and *in vivo* biological activity studies

J. Siroka[1,2], E. Sousa[2,3] and M. Pinto[2,3]

[1] Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy in Hradec Králové, Charles University in Prague, Czech Republic.

[2] Department of Organic Chemistry, Faculty of Pharmacy, University of Porto, Portugal.

[3] Research Centre of Organic Chemistry, Phytochemistry and Pharmacology of the University of Porto (CEQOFFUP), Faculty of Pharmacy, University of Porto, Portugal

Xanthone derivatives are heterocyclic compounds with the dibenzo-γ-pyrone as the main molecular moiety. They contain different types of substituents in different positions, leading to a large variety of pharmacological activities [1]. 3,4-Dihydroxyxanthone (**1**, Fig. 1) was revealed as a *hit* compound in a study involving the investigation of the inhibitory effect of oxygenated xanthones on several human tumor cell lines [2].

In order to obtain enough quantity for *in vivo* assays an for further molecular modifications, the synthesis of 3,4-dihydroxyxanthone (**1**) was accomplished (Fig. 1): the condensation of 1,2,3-trimethoxyphenol (**2**) with the appropriate substituted benzoyl chloride **3** afforded benzophenone **4** which was further cyclized to give 3,4-dimethoxyxanthone (**5**). Compound **5** was demethylated to furnish 3,4-dihydroxyxanthone (**1**).

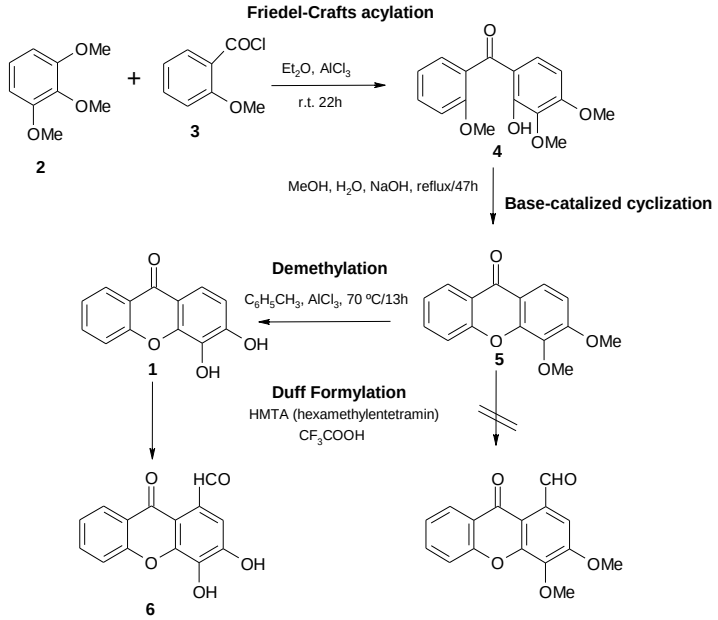


Fig. 1. Synthesis of compounds **1** and **2**.

Additionally, the synthesis of reactive formylated derivatives of xanthones, **1** and **5**, was attempted by Duff formylation. Only 1-formyl-3,4-dihydroxyxanthone (**6**) was obtained by this procedure. Compound **6** will be used as a building block on the construction of a library of amine xanthonic derivatives to investigate antitumour activity.

References:

[1] M.M.M.Pinto, M.E.Sousa, M.S.J.Nascimento; *Xanthone derivatives: new insights in biological activities*; Current Medicinal Chemistry; (2005); 12, 2517-2538.

[2] M.Pedro, F.Cerqueira, M.E.Sousa, M.S.J.Nacimento, M.M.M.Pinto; *Xanthones as inhibitors of growth of human cancer cell lines and their effects on the proliferation of human lymphocytes in vitro*; Bioorganic Medicinal Chemistry; (2002); 10 (12), 3725-3730.

Acknowledgments: FCT (I&D, nº226/94), FEDER, POCI for financial support.
The present work was done under supervision of Professor Maria Emília Sousa, in the Faculty of Pharmacy, under sponsoring of ERASMUS programme.

Porto, Faculty of Pharmacy, 27th of February,

(Maria Emília Sousa)