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Synthesis and Tumor Cell Growth Inhibitory Effects of New Flavonosides and Xanthonosides

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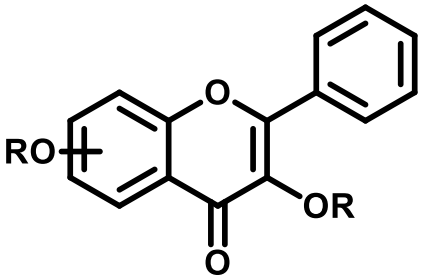
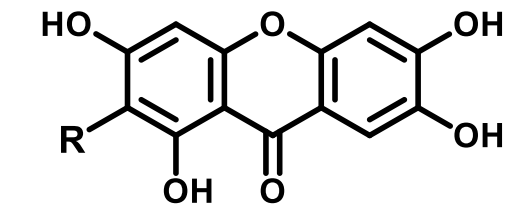
Synthesis and Tumor Cell Growth Inhibitory Effects of New Flavonosides and Xanthonosides



Glycosylation

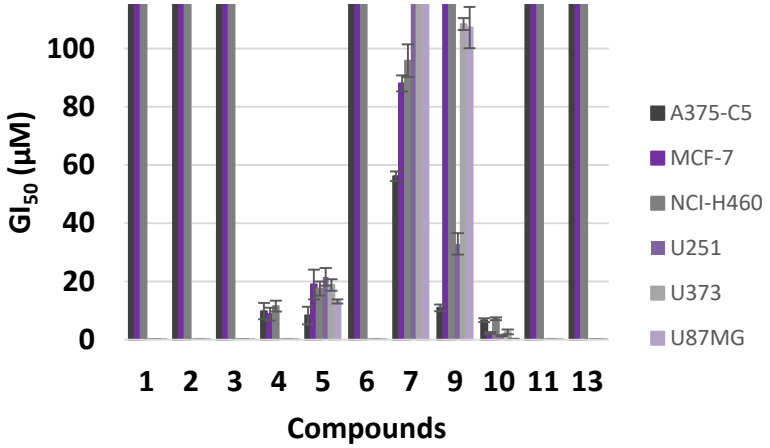
CuAAC



Acetylation

In vitro screening

Sulphorhodamine B assay

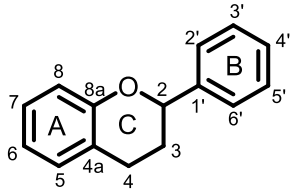


Abstract: Natural flavonoid and xanthone glycosides display several biological activities, with the glycoside moiety playing an important role in the mechanisms of action of these metabolites. Herein, to give further insights into the inhibitory cell growth activity of these classes of compounds, the synthesis of new flavonoid and xanthone derivatives containing one or more acetoglycoside moieties was carried out to evaluate their *in vitro* cell growth inhibitory activity in human tumor cell lines. The introduction of one or two acetoglycoside moieties in the framework of a hydroxylated flavonoid was performed using three synthetic methods: Michael reaction, Koenigs-Knorr reaction, and through a copper catalyzed azide-alkyne cycloaddition. Acetyl groups were introduced in rutin, diosmin, and mangiferin using acetic anhydride under microwave irradiation. The *in vitro* cell growth inhibitory activity of seven synthesized compounds was investigated in six human tumor cell lines: A375- C5 (malignant melanoma IL-1 insensitive), MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), U251 (glioblastoma astrocytoma), U373 (glioblastoma astrocytoma), and U87MG (glioblastoma astrocytoma). The most active compound in all tumor cell lines tested was a flavonoside and showed GI_{50} values below 10 µM.

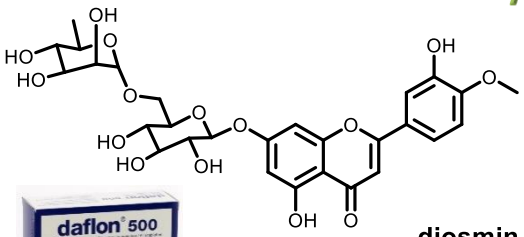
Keywords: Flavonoids; xanthones; growth inhibitory activity, acetylation, glycosylation.

Introduction

FLAVONOIDS



2-phenylchromane



diosmin

hesperidin

Fruits and vegetables

Biological activities

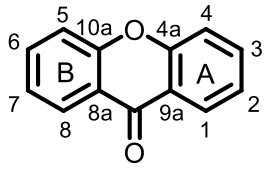
Anti-inflammatory

Antioxidant

Antitumor

Antimicrobial

XANTHONES



dibenzo-gamma-pirone

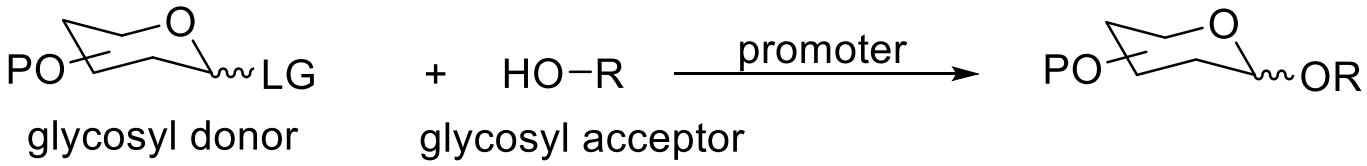
Higher plants

Fungi

Lichens

L.M.M. Vieira and A. Kijjoa. *Current Medicinal Chemistry,* 2005, *12,* 2413-2446; M.M.M. Pinto *et al.*,*Current Medicinal Chemistry*, 2005, 12, 2517-2538. ; J. S. Negi et al., *Journal of Applied Chemistry* Volume 2013, Article ID 621459; Kumar, S. and A. K. Pandey. The Scientific World Journal, 2013, 2013: 16.

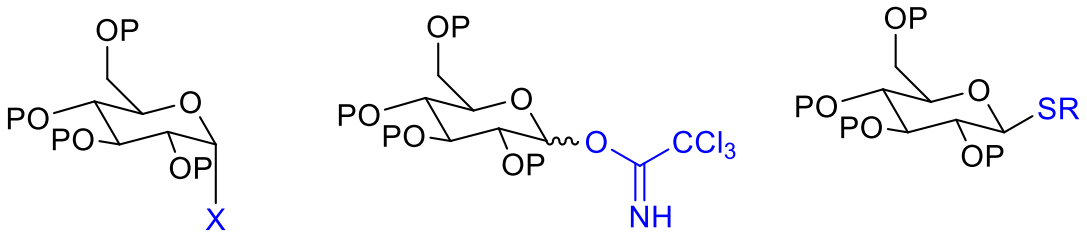
Introduction – Glycosylation methods



glycosyl donor glycosyl acceptor

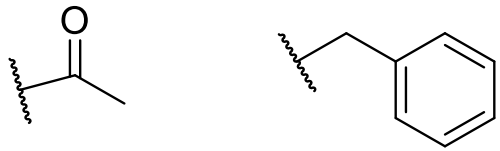
LG = leaving group
P = protecting group
R = aromatic or aliphatic

Glycosyl donors



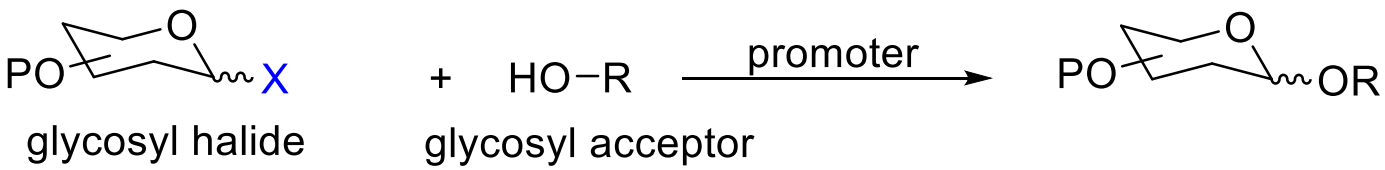
X=Br, I, Cl
P=protecting group
R=alkyl

Protecting groups



Brito-Arias, M., 2007, Springer US: Boston, MA. p. 68-137. Jensen, K.J., *Journal of the Chemical Society, Perkin Transactions 1* **2002**, 2219-33.

Introduction – Glycosylation methods



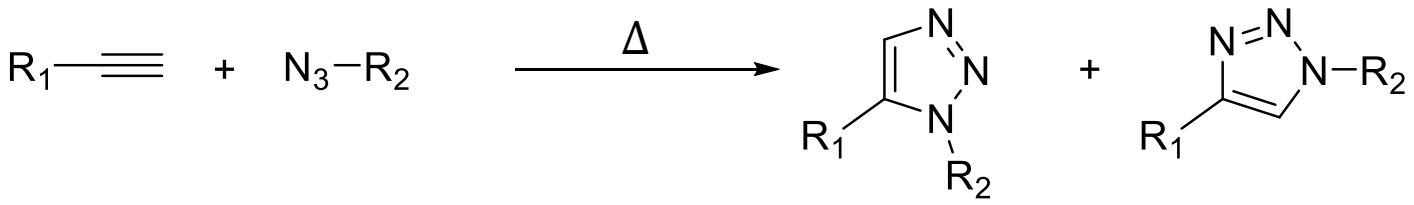
X = F, Cl, Br, I
P = protecting group
R = aromatic or aliphatic

Michael Reaction	Fischer Reaction	Koenigs-Knorr Reaction
• Protected glycosyl donor • Basic conditions • Produces exclusively *β*-glycosides	• Unprotected glycosyl donor • Acid conditions • Produces a mixture of *α* and *β*-glycosides	• Protected glycosyl donor • Silver salts or Lewis acids

Brito-Arias, M., 2007, Springer US: Boston, MA. p. 68-137. Jensen, K.J., *Journal of the Chemical Society, Perkin Transactions 1* **2002**, 2219-33.

Introduction – Click Chemistry

Huisgen 1,3-dipolar cycloaddition

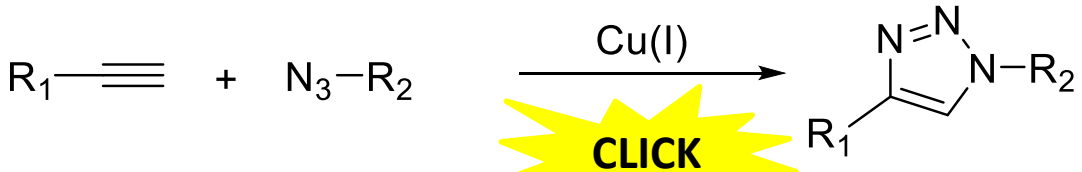


Lack of selectivity

Two regioisomers difficult to separate

Requires heating and long reaction times

Cu (I)-Catalyzed Azide-Alkyne Cycloaddition (CuAAC)



Regiospecific

Benign solvents

Short reaction times

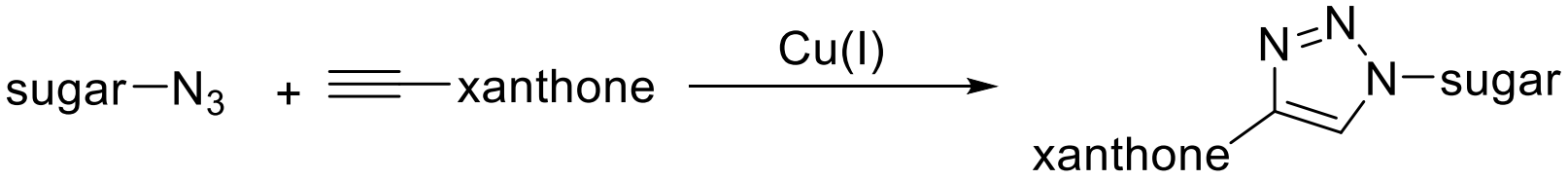
Simple reaction conditions and purification

High yields

Kolb, H.C., M.G. Finn, and K.B. Sharpless, Angewandte Chemie, 2001. **40**(11): p. 2004-2021.

Introduction – Click chemistry

Cu (I)-Catalyzed Azide-Alkyne Cycloaddition (CuAAC)



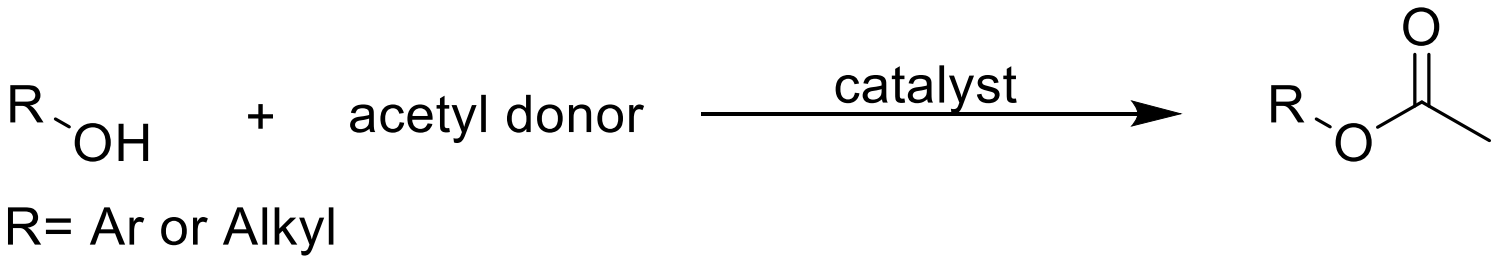
Cu (II) salts ($Cu_2SO_4 \cdot 5H_2O$) *in situ* to form Cu (I) salts

(with a reducing agent)

Cu (I) salts like CuBr or CuI

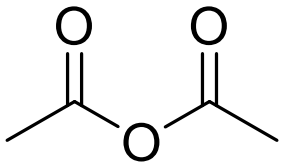
Kolb, H.C., M.G. Finn, and K.B. Sharpless, Angewandte Chemie, 2001. **40**(11): p. 2004-2021; Correia-da-Silva, M., *et al*., *Scientific Reports* **7**, Article number: 42424 (2017).

Introduction – Acetylation methods



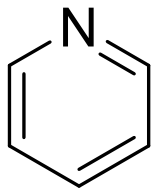
R= Ar or Alkyl

Acetyl donors



Acetic anhydride

Catalysts



Pyridine

Na—F

Sodium flouride

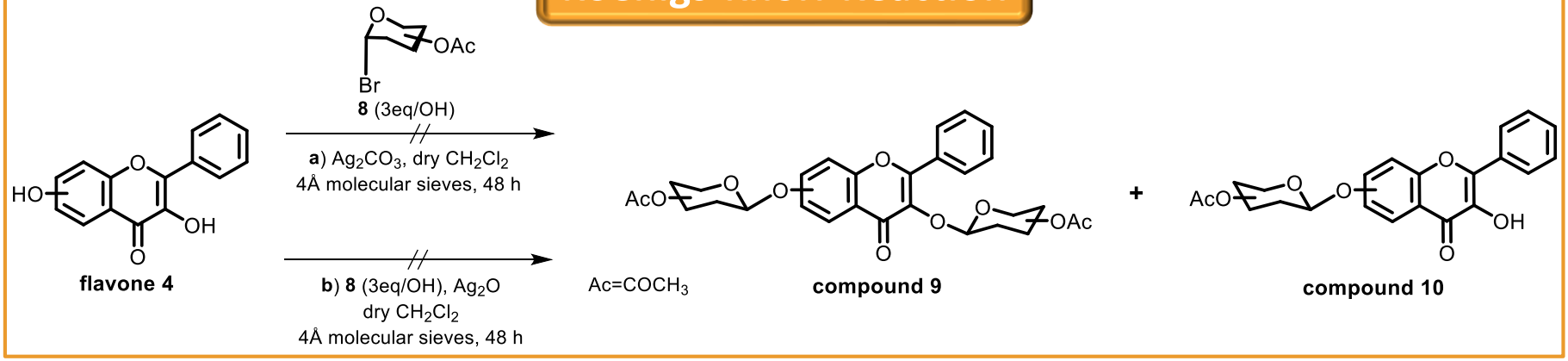
I—I

Molecular iodine

Bosco, J. W. J., *et al.*, *Tetrahedron Letters.* **2006**, *47* (24), 4065-4068; Ahmed, N.; van Lier, J. E., *Tetrahedron Lett.* **2006**, *47* (30), 5345-5349.

Results and discussion - Glycosylation

Koenigs-Knorr Reaction

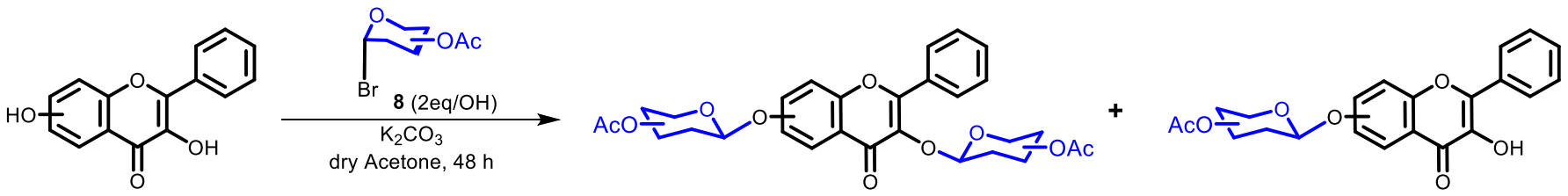


flavone 4

compound 9

compound 10

Michael Reaction



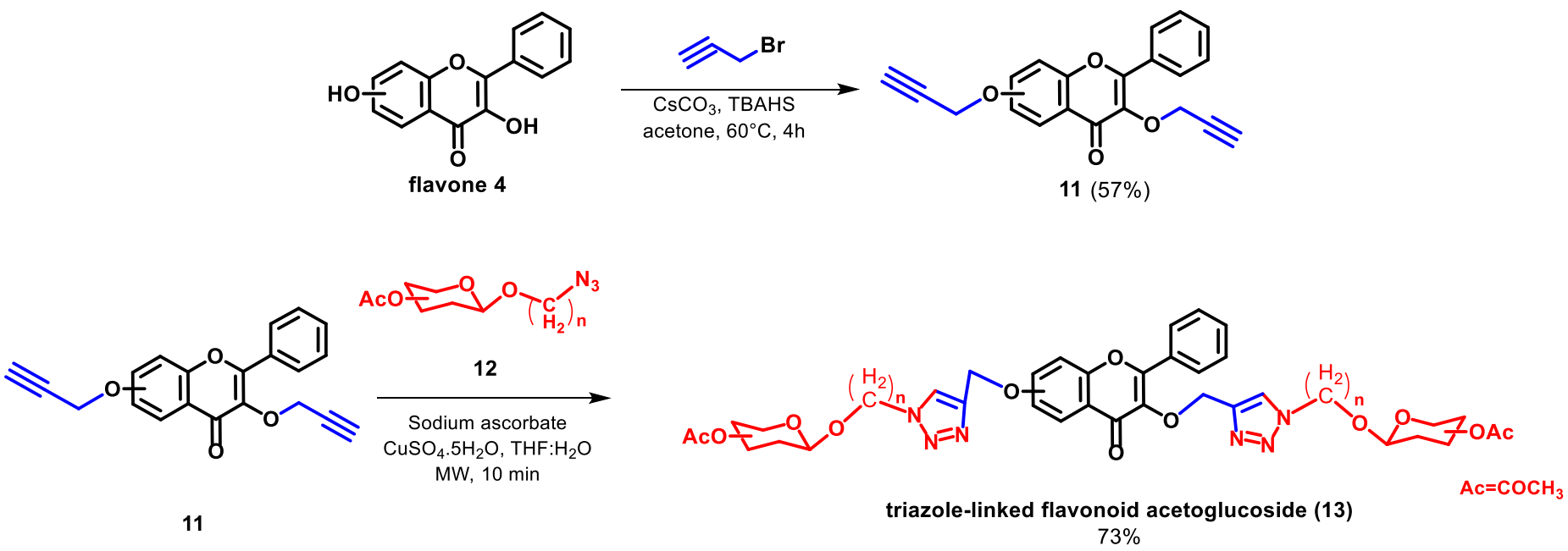
flavone 4

compound 9 (11%)

compound 10 (2%)

Results and discussion - CuAAC

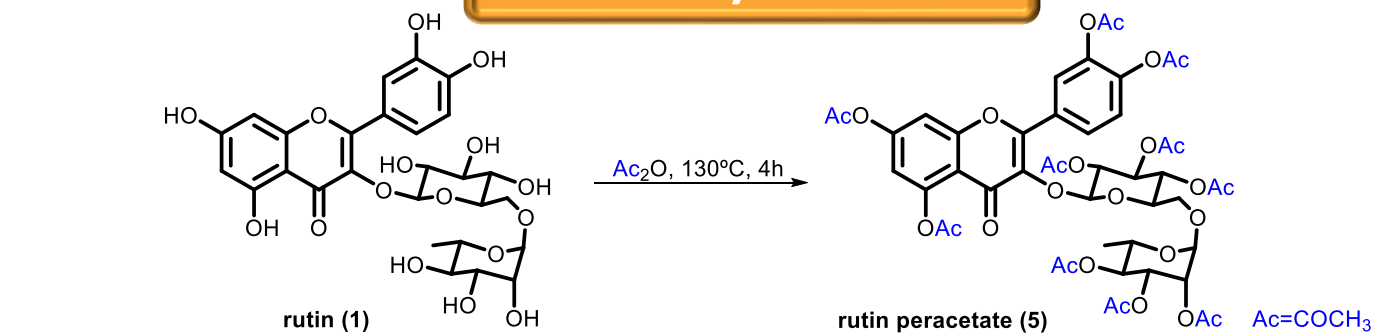
Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC)

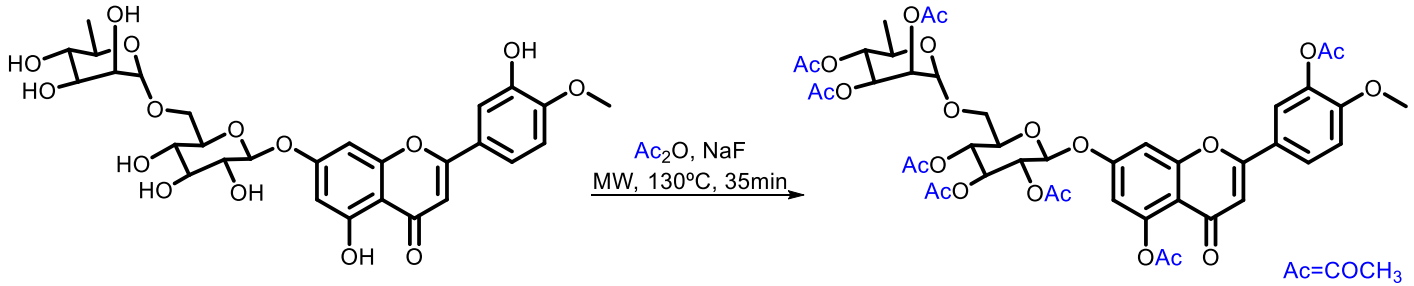


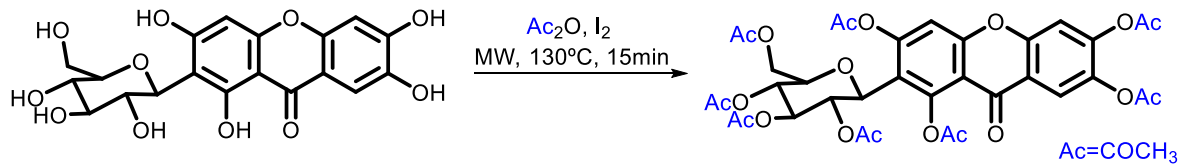
MW – microwave; TBAHS - Tetrabutylammonium hydrogen sulfate; THF – tetrahydrofuran

Results and discussion

Acetylation



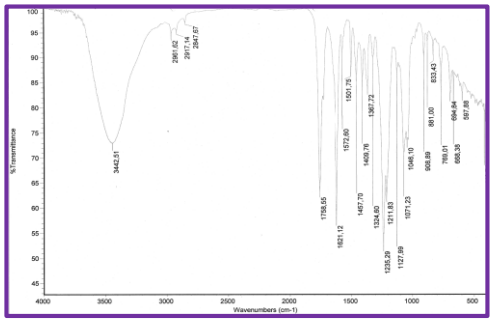




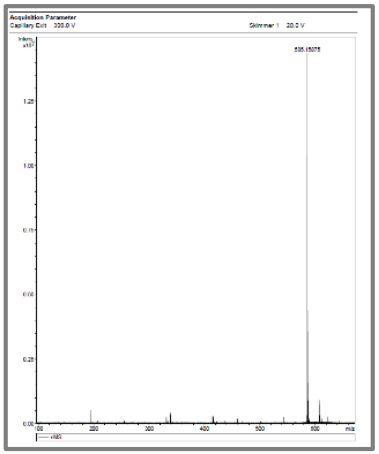
Ac_2O – anhydride acetic; MW - microwave

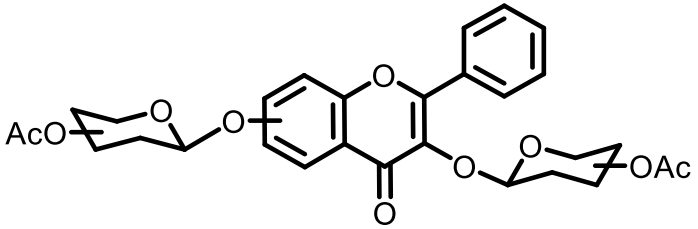
Results and discussion – Structure elucidation

Infrared spectroscopy

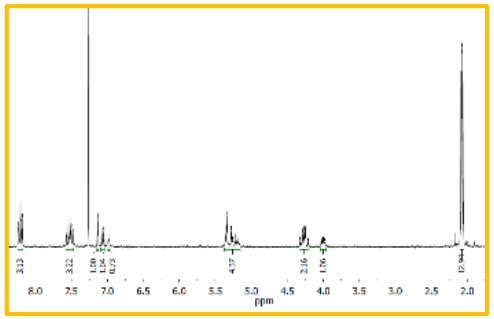


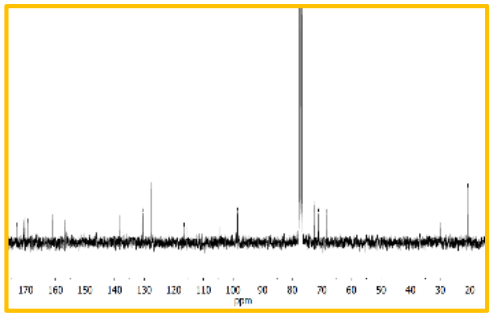
High resolution mass spectrometry





^{1}H and ^{13}C nuclear magnetic resonance





Results and discussion – Growth inhibitory activity

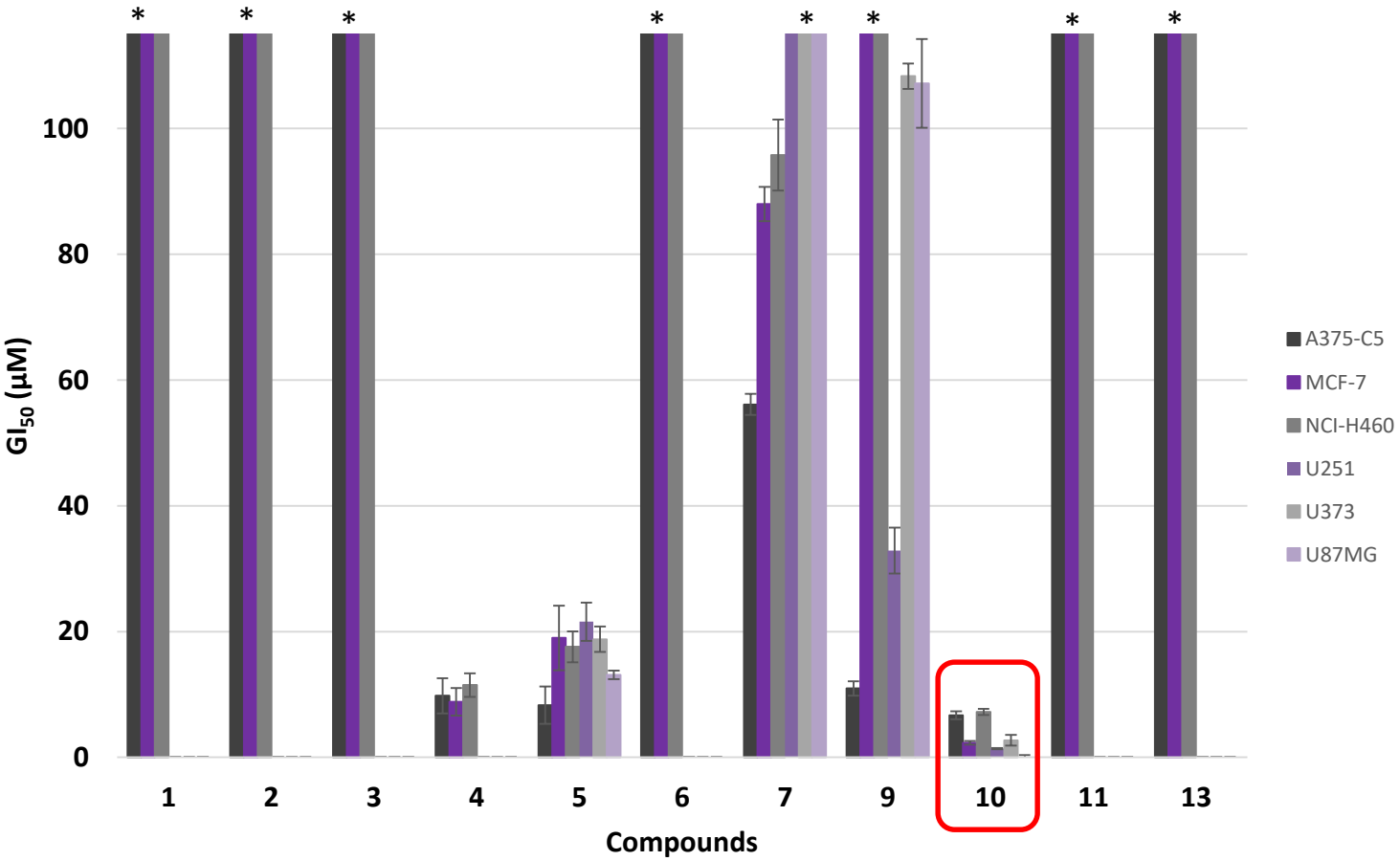


Figure 1 – Cell growth inhibitory activity displayed by compounds **1**-**7** and **9**-**13** on human tumor cell lines. Compounds **1**-**4**, **6, 11** and **13** were only tested on A375-C5, MCF-7, and NCI-H460 human tumor cell lines. * - values higher than 150 µM.

Conclusions

- Five acetylated flavonosides (**5**, **6**, **9**, **10**, and **13**) and one xanthonoside (**7**) were synthesized.
- The Michael reaction led to the glycosylation of flavone **4**.
- A high yield was obtained in the glycosylation of flavone **4** through the click chemistry reaction.
- Non-classic strategies were applied successfully in acetylation.
- Discovery of a flavonoid acetoglucoside **10** with a potent growth inhibition effect in human tumor cell lines.

Acknowledgments

This work was developed in Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia da Universidade do Porto. This research was developed under the projects Strategic Funding UID/Multi/04423/2013, PTDC/ MAR-BIO/4694/2014 and PTDC/AAG-TEC/0739/2014 supported through national funds provided by Fundação da Ciência e Tecnologia (FCT/MCTES, PIDDAC) and European Regional Development Fund (ERDF) through the COMPETE – Programa Operacional Factores de Competitividade (POFC) programme (POCI-01-0145-FEDER-016790 and POCI-01-0145-FEDER-016793), Reforçar a Investigação, o Desenvolvimento Tecnológico e a Inovação (RIDTI, Project 3599 and 9471), and INNOVMAR - Innovation and Sustainability in the Management and Exploitation of Marine Resources, reference NORTE-01-0145-FEDER-000035, Research Line NOVELMAR. The candidate performed this work with a doctoral fellowship (SFRH/BD/114856/2016) supported by FCT.