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 Xanthones

In vitro screening

Glycosylation

CuAAC

Acetylation

Sulphorhodamine B assay

RO

OR

HO

OH

GI_{50} (μM)

0

20

40

60

80

100

1

2

3

4

5

6

7

9

10

11

13

Compounds

A375-C5
MCF-7
NCI-H460
U251
U373
U87MG

3rd International Electronic Conference on Medicinal Chemistry
1-30 November 2017
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

Biological activities

**Anti-inflammatory**

**Antioxidant**

**Antitumor**

**Antimicrobial**

**FLAVONOIDs**

2-phenylchromane

diosmin

**XANTHONES**

dibenzo-gamma-pirone

**Fruits and vegetables**

**Higher plants**

**Fungi**

**Lichens**

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Introduction – Glycosylation methods

\[
\text{glycosyl donor} \quad \text{glycosyl acceptor}
\]

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

Glycosyl donors

Protecting groups

Introduction – Glycosylation methods

\[
\begin{align*}
\text{PO} & \quad \text{X} \\
\text{glycosyl halide} & \quad + \quad \text{HO–R} & \quad \text{promoter} \\
\text{PO} & \quad \text{OR} \\
\text{glycosyl acceptor} & \\
\end{align*}
\]

\( X = F, \ Cl, \ Br, \ I \)

P = protecting group

R = aromatic or aliphatic

**Michael Reaction**

- Protected glycosyl donor
- Basic conditions
- Produces exclusively \( \beta \)-glycosides

**Fischer Reaction**

- Unprotected glycosyl donor
- Acid conditions
- Produces a mixture of \( \alpha \) and \( \beta \)-glycosides

**Koenigs-Knorr Reaction**

- Protected glycosyl donor
- Silver salts or Lewis acids

Introduction – Click Chemistry

Huisgen 1,3-dipolar cycloaddition

\[
R_1\equiv + N_3-R_2 \xrightarrow{\Delta} \begin{array}{c}
  \text{Product 1} \\
  \text{Product 2}
\end{array}
\]

Lack of selectivity  Two regioisomers difficult to separate  Requires heating and long reaction times

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

\[
R_1\equiv + N_3-R_2 \xrightarrow{\text{Cu(I)}} \begin{array}{c}
  \text{Product 1} \\
  \text{Product 2}
\end{array}
\]

Regiospecific  Benign solvents

Short reaction times  Simple reaction conditions and purification  High yields

Introduction – Click chemistry

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

Cu (II) salts (Cu$_2$SO$_4$·5H$_2$O) in situ to form Cu (I) salts (with a reducing agent)

Cu (I) salts like CuBr or Cul

Introduction – Acetylation methods

\[ R_{\text{OH}} + \text{acetyl donor} \xrightarrow{\text{catalyst}} R_{\text{O}} \text{O} \]

R = Ar or Alkyl

**Acetyl donors**

- Acetic anhydride

**Catalysts**

- Pyridine
- Na–F (Sodium flouride)
- Molecular iodine

Results and discussion - Glycosylation

Koenigs-Knorr Reaction

\[ \text{flavone 4} \rightarrow \text{compound 9} + \text{compound 10} \]

- a) Ag$_2$CO$_3$, dry CH$_2$Cl$_2$
  - 4Å molecular sieves, 48 h
- b) 8 (3eq/OH), Ag$_2$O, dry CH$_2$Cl$_2$
  - 4Å molecular sieves, 48 h

Michael Reaction

\[ \text{flavone 4} \rightarrow \text{compound 9} (11\%) + \text{compound 10} (2\%) \]

- K$_2$CO$_3$, dry Acetone, 48 h

Ac=COCH$_3$
Results and discussion - CuAAC

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

MW – microwave; TBAHS - Tetrabutylammonium hydrogen sulfate; THF – tetrahydrofuran
Results and discussion

Acetylation

rutin (1) → Ac₂O, 130°C, 4h → rutin peracetate (5) 73%

rutil (1)

Ac₂O, NaF
MW, 130°C, 35min

diosmin (2) → diosmin peracetate (6) 62%

Ac₂O, I₂
MW, 130°C, 15min

mangiferin (3) → mangiferin peracetate (7) 78%

Ac₂O – anhydride acetic; MW - microwave
Results and discussion – Structure elucidation

Infrared spectroscopy

$^1\text{H}$ and $^{13}\text{C}$ nuclear magnetic resonance

High resolution mass spectrometry
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.