Chalcones: Synthesis, Bioactivities and Biotransformation

Dissertação de Mestrado

Gonçalo Pereira da Rosa

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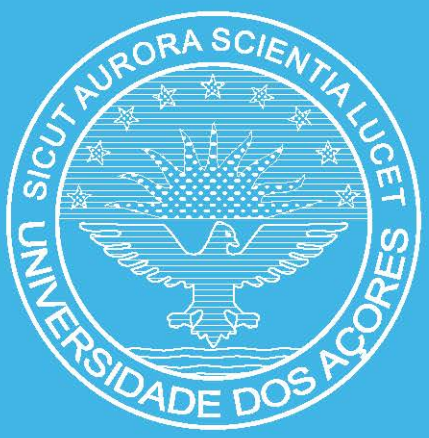

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Gonçalo Pereira da Rosa

Orientadoras

Doutora Ana Maria Loureiro da Seca
Doutora Maria do Carmo Barreto

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Abstract

Chalcones are naturally occurring α,β-unsaturated carbonyl compounds presenting a wide range of pharmaceutical and industrial applications. Due to our society's increasing attention to environmental problems, the application of eco-friendly methods in synthesis and transformation reactions is higbly desirable. One green approach is biotransformation, which makes use of the capabilities of enzymes and/or microorganisms to modify physicochemical and biological properties of compounds. These facts stimulate vast interest in synthesis, activities and biotransformation of chalcones.

In this work, 2'-hydroxychalcones with different hydroxyl and methoxyl substitution patterns were synthesized by aldol condensation, for the first time using sodium hydride (NaH) or LiHMDS as base. The most efficient method for chalcone synthesis was the one where NaH is the base, but deprotection of hydroxyl groups exerts a marked decrease in the final yield. LiHMDS allowed the one-pot synthesis of polyhydroxychalcones, which is an advantage, but the yields were low.

The synthesized compounds were evaluated for antioxidant, anticholinesterasic, antibacterial and antitumor activities, and structure/activity relationships were established, with 2',4',4-trihydroxychalcone being the most active compound in the antioxidant, anti-butyrylcholinesterase and antibacterial activities. 4'-7-Dihydroxyflavanone is able to inhibit both cholinesterases, which is very interesting in terms of Alzheimer's Disease therapy.

The extraction of a peroxidase rich enzymatic extract from *Brassica rapa* L. and optimization of its working parameters was also performed. This enzymatic extract is not able to biotransform any chalcone tested but catalyzes the biotransformation of the acetophenones, precursors of chalcones ring A, by hydroxylation of C-6' and an undetermined modification of C-2 methyl group, when the C-4' is hydroxylated.

Key words: Chalcone; aldol condensation; NaH; LiHMDS; bioactivities; biocatalysis; peroxidase

Resumo

As calconas são compostos carbonílicos α,β-insaturados que ocorrem na natureza e que apresentam uma ampla variedade de aplicações farmacêuticas e industriais. Devido à cada vez maior atenção que a sociedade tem mostrado pelos problemas ambientais, é altamente recomendável que sejam aplicados métodos de síntese e de transformação mais amigos do ambiente. Um dos métodos ecológicos que pode ser aplicado é a biotransformação, que recorre às capacidades das enzimas e/ou microrganismos para modificar as propriedades biológicas e físico-químicas dos compostos. Todos estes factos têm estimulado um grande interesse na síntese, nas atividades biológicas e na biotransformação de calconas.

Neste trabalho, foram sintetizadas por condensação aldólica, 2'-hidroxicalconas com diferentes padrões de substituição com grupos hidroxilo e metoxilo, usando pela primeira vez hidreto de sódio (NaH) ou LiHMDS como base. O método mais eficiente para a síntese de calconas foi aquele em que NaH foi utilizado como base, mas a desproteção dos grupos hidroxilo diminui acentuadamente o rendimento final. O LiHMDS permitiu a síntese de polihidroxicalconas em apenas um passo, o que é uma vantagem, mas os rendimentos foram baixos.

Os compostos sintetizados foram avaliados pelas suas atividades antioxidante, anticolinesterásica, antibacteriana e antitumoral, e foram estabelecidas relações estrutura/atividade, com a 2',4',4-trihidroxicalcona a mostrar ser o composto mais ativo em termos de atividade antioxidante, anti-butirilcolinesterase e antibacteriana. A 4',7-dihidroxiflavanona inibe ambas as colinesterases, o que é bastante interessante em termos de terapia da Doença de Alzheimer.

Também foi efetuada a preparação de um extrato enzimático rico em peroxidase, extraído de *Brassica rapa* L. bem como a otimização dos seus parâmetros operacionais. Este extrato enzimático não foi capaz de biotransformar nenhuma das calconas testadas, mas catalisa a biotransformação de acetofenonas, que são precursoras do anel aromático A das calconas. Esta biotransformação consiste na hidroxilação do C-6' numa modificação indeterminada no grupo metilo do C-2, as quais só ocorrem se o C-4' já estiver hidroxilado.

Palavras-chave: Calcona; condensação aldólica; NaH; LiHMDS; bioatividades; biocatálise; peroxidase

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Abbreviations

ABTS – 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)

ACh – acetylcholine

AChE – acetylcholinesterase

AD – Alzheimer's Disease

ATCI – acetylthiocholine iodide

BDTAC – benzyldimethyltetradecyl ammonium chloride

BSA – bovine serum albumin

BuChE – butyrylcholinesterase

BuTCI – butyrylthiocoline iodide

BY – Baker's yeast

d – doublet

dd – double doublet

DMF – dimethylformamide

DMSO – dimethylsulfoxide

DPPH – 1,1-diphenyl-2-picryl-hydrazyl

DTNB – 5,5′-dithiobis[2-nitrobenzoic acid]

ee – enantiomeric excess

FKA – flavokawain A

FKB – flavokawain B

FKC – flavokawain C

Glu – D-glucose

Gluc – glucuronosyl

HLM – human liver microsomes

HMBC – heteronuclear multiple bond correlation

HRP – horseradish peroxidase

HSQC – heteronuclear single-quantum correlation

LiHMDS – lithium bis(trimethylsilyl)amide

M. – *Morus*

MBC – 4'-*n*-butoxy-2,4-dimethoxychalcone

MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide

NMR – nuclear magnetic resonance

OBn – benzyloxy

PEG – polyethylene glycol

POD – peroxidase

PT – prenyltransferase

R. – *Rhodotorula*

ROS – reactive oxygen species

s – singlet

sp. – species

t – triplet

THF – tetrahydrofuran

TLC – thin layer chromatography

TMS – tetramethylsilane

UDP – uridine diphosphate

UGT – UDP-glucuronosyltransferase

UHP – urea-hydrogen peroxide

General Introduction and Objectives

Chalcones, or (*E*)-1,3-diphenylpropene-1-ones, are one of the most important classes of natural products across the plant kingdom, belonging to the flavonoids family and containing two aromatic rings joined by a three carbon α,β unsaturated carbonyl bridge, which are the C_{15} key intermediate in the biosynthesis of all the natural flavonoids. They are also used as precursor on the synthesis of many biologically valuable heterocyclic compounds (*e.g.* benzothiazepines and pyrazolines)

Chalcones are very interesting compounds with great pharmacological potential since they exhibit many biological activities, including antioxidant activities and can be used in the treatment of microbial and viral infections, cardiovascular diseases and their multiple risk factors, and cancer just to state a few.

All of these features of chalcones make them important objects of study in terms of their synthesis, transformation, pharmacologic effects and structure/activity relationships. In terms of synthesis and transformation, it is desirable that eco-friendly methods are chosen, due to our society's increasing attention to environmental problems. In that regard, many studies have been made about the synthesis of chalcones, mainly by aldol condensation. However, when these methods are applied in the synthesis of polyhydroxychalcones, there are limitations in terms of yield and complexity of the procedure that should be attenuated. Therefore, there is still space to the search for alternative methods and improvement of the existing ones, in order to overcome the current limitations.

Chalcones are well known for their pharmacological interest, and there are several works describing their numerous activities, however, some biological effects have not yet been studied for many chalcones, specially the hydroxylated ones and so, some of the structure/activity relationships are not completely established.

There is also a great interest in transforming the chalcone scaffold in order to obtain new bioactive derivatives. More eco-friendly ways to achieve that transformations include the use of microorganisms, enzymatic extracts, cell suspensions or pure enzymes as biocatalysts, microwave or ultrasound irradiation and green solvents. From all of these approaches, there is gap in the knowledge of the application of peroxidase enzymatic extracts in the biotransformation of chalcones, which is worth exploring.

The objectives of the present work are: a) to contribute to increase the scientific knowledge on modification of the known methods for synthesis of hydroxylated chalcones, by using for the first time NaH and LiHMDS as bases in the aldol condensation and evaluate the yield and time of reactions; b) to determine the biological activities of the compounds synthesized and establish their structure/activity relationships; c) to extract and optimize the optimal working parameters of a peroxidase rich enzymatic extract from a *Brassica rapa* and d) to apply the obtained extract in the biotransformation of chalcones and their precursors.

This thesis is divided in three different chapters. The first one presents a bibliographic revision on the eco-friendly methods used in the transformation of chalcones, where the versatility, efficiency, reactional conditions, advantages and disadvantages and main results obtained with the different approaches are discussed, in order to unveil the knowledge gaps and eventually guide future research on this area.

In the second chapter, a brief introduction on chalcone synthesis is presented and then the results of the different synthetic methods used are reported and discussed, in terms of yield, time of reaction and ease of its application. Then, the results of the antioxidant, anticholinesterasic, antibacterial and antitumor activities of the studied compounds are presented, and their structure/activity relationships are discussed.

The third chapter is about the extraction and optimization of an enzymatic extract and further application on biotransformation. Like in chapter II, this chapter also starts with an introduction on the use of enzymes as catalysts, the various factors affecting their activity, and the applications of type III peroxidases. Then the results for the preparation of the enzymatic extract and the optimization of its working parameters are presented and discussed. After that, the results of the various biotransformation experiments performed are shown and discussed.

Finally, the general conclusions of this work are presented, with an assessment of which objectives were accomplished and the discussion of some future perspectives.