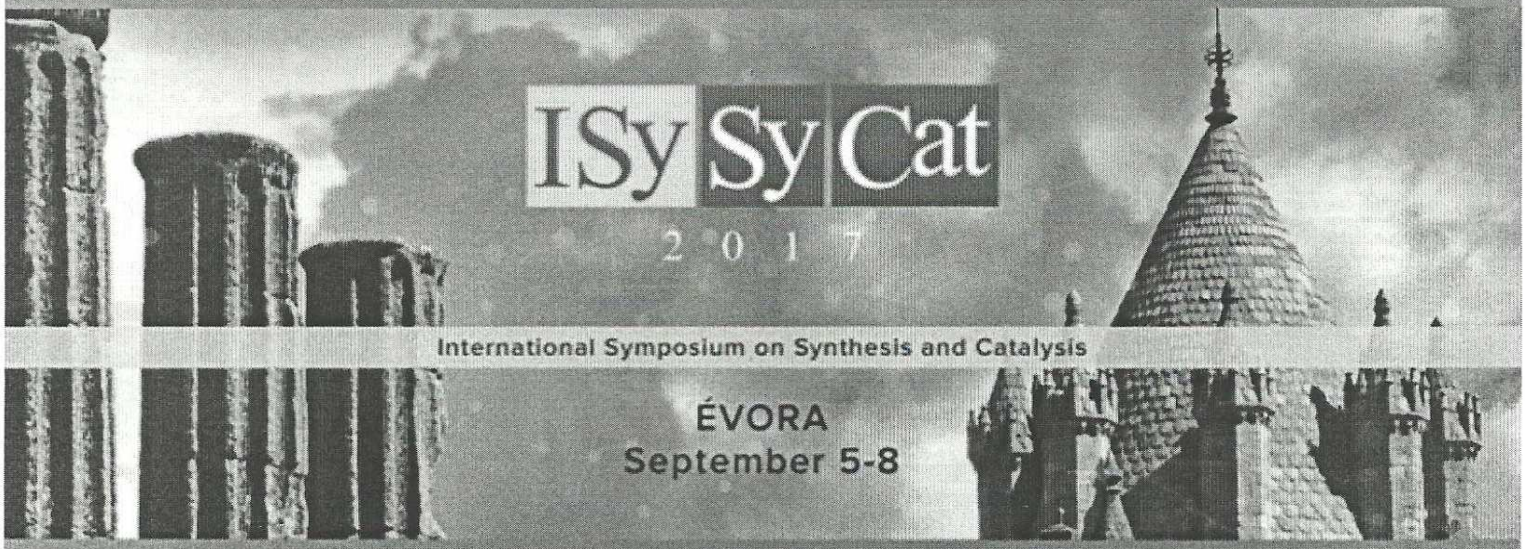

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Polyhydroxy Chalcones and Flavanones: Synthesis and Evaluation of Their Potential as Antioxidant and Anticholinesterasic Agents

Gonçalo P. Rosa,[a] Maria C. Barreto,[b] Diana C. G. A. Pinto,[c] Ana M. L. Seca[b,c]

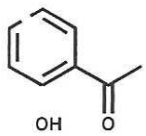
[a)] Faculty of Sciences and Technology, University of Azores, 9501-801 Ponta Delgada, São Miguel, Açores.; [b)] cE3c-Centre for Ecology, Evolution and Environmental Changes / Azorean Biodiversity Group & Faculty of Sciences and Technology, University of Azores, Rua Mãe de Deus, 9501-801 Ponta Delgada.; [c)] QOPNA- Organic Chemistry, Natural Products and Food Stuffs & Department of Chemistry, University of Aveiro,Campus de Santiago, 3810-193 Aveiro

Email: ana.ml.seca@uac.pt

Chalcones are one of the most important classes of natural products across the plant kingdom, belonging to the flavonoids family.[1] They have a wide range of pharmaceutical and industrial applications and are key precursors in the other flavonoids biosynthesis as well as in the synthesis of many biologically valuable heterocyclic compounds. Owing to the above stated reasons, the synthesis of chalcones and chalcone based functionalized derivatives had remained primary objectives and so, a number of procedures have been reported for their synthesis, although they are mostly different approaches of an aldol condensation.[2] The synthesis of polyhydroxylated chalcones involve hydroxyl groups protection and cleavage steps consequently the procedures are more expensive. Thus a search for new or improved routes towards the synthesis of polyhydroxychalcones is still a challenge.

On the other hand, acetylcholinesterase (AChE) inhibitors are a class of drugs used in clinic therapy to treat the Alzheimer's disease (AD) symptoms while antioxidants have also an important role in the control of degenerative and aging effects and they are tangled with AD.

In this work, we report for the first time the one-pot synthesis of polyhydroxychalcones using lithium bis(trimethylsilyl)amide (LiHMDS) as base (**Scheme 1**), as well as the compounds characterization by spectroscopic methods (1D and 2D NMR and MS). The radical scavenging activity and AChE inhibitory activities of the pure compounds were assayed by well-known methods.[3] The results showed that the 2',4',4-trihydroxychalcone radical scavenging activity is similar to the one observed with quercetin, a member of the flavonoid family with industrial application as antioxidant. Moreover, chalcones have superior radical scavenging activity than the corresponding flavanones. Some structure/activity relationships will also be discussed. Concerning the anticholinesterasic activity it seems that flavanones are more active than chalcones, maybe due to their affinity with the enzyme active site.



Scheme 1: Proposed one-pot synthesis of polyhydroxychalcones using LiHMDS as base.

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References:

1. Ferreyra M.L.F.; Rius S.P.; Casati P. *Front Plant Sci.* **2012**; 3, 222.
2. Bukhari S.N.A.; Jasamai M.; Jantan I.; Ahmad W. *Mini Rev. Org. Chem.* **2013**, *10*, 73.
3. Barreto M.C.; Arruda M.; Rego E.; Gouveia V.; Medeiros, J.; Rainha, N. *In*: Barreto M.C.; Simões, N. (eds.) (**2012**). pp. 65-81.

Polyhydroxy Chalcones and Flavanones: Synthesis and Evaluation of Their Potential as Antioxidant and Anticholinesterasic Agents

Rosa, G. P.;[a] Barreto, M. C.;[b] Pinto, D. C. G. A.;[c] Seca, A. M. L.[b,c]

[a] Faculty of Sciences and Technology, University of Azores, 9501-801 Ponta Delgada, São Miguel, Azores, Portugal; [b] cE3c- Centre for Ecology, Evolution and Environmental Changes / Azorean Biodiversity Group & Faculty of Sciences and Technology, University of Azores, 9501-801 Ponta Delgada, Portugal; [c] QOPNA- Organic Chemistry, Natural Products and Food Stuffs, University of Aveiro, 3810-193 Aveiro, Portugal.

Introduction

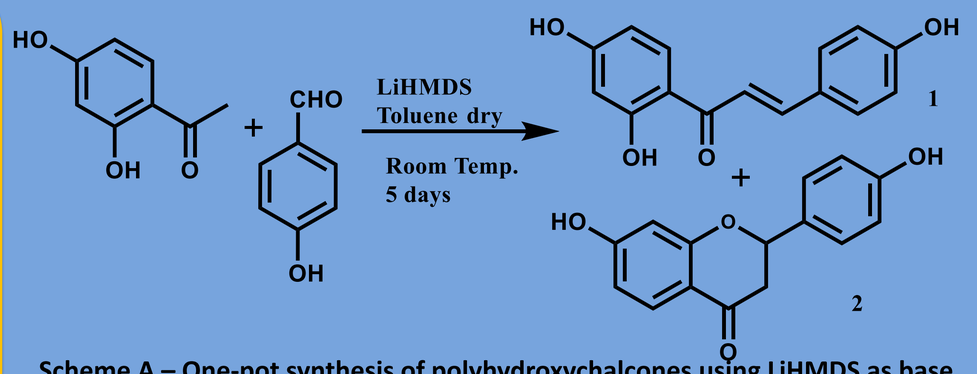
Chalcones belong to the flavonoids family, one of the most important classes of natural products across the plant kingdom.[1] They have a wide range of pharmaceutical and industrial applications and are key precursors in the biosynthesis of other flavonoids as well as in the synthesis of many biologically valuable heterocyclic compounds. Owing to the reasons stated above, the synthesis of chalcones has remained a primary objective and so a number of procedures have been reported for their synthesis, although they are mostly different approaches of an aldol condensation.[2] When it comes to synthesize polyhydroxylated chalcones by the most common methodologies, both steps for hydroxyl groups protection and for the cleavage of protecting groups are necessary, which consequently turns these procedures much more expensive and time-consuming. Thus, a search for new or improved routes towards the synthesis of polyhydroxychalcones is still a challenge. An improvement on the synthesis of these polyhydroxylated molecules can also create an opportunity to find new bioactive compounds as well as to understand the structure activity relationship .

The objective of this work is to report for the first time: i) the one-pot synthesis of polyhydroxychalcones using lithium bis(trimethylsilyl)amide (LiHMDS) as deprotonating agent; ii) the anticholinesterasic (AChE) and antioxidant activities of the synthesized compounds, bioactivities which have an important role in the control of degenerative and aging effects that are tangled with Alzheimer's disease (AD).

Methodology

2',4'-Dihydroxyacetophenone was dissolved in dry toluene in nitrogen atmosphere and under ice-bath, LiHMDS 1 mol.dm^{-3} (6.6 eq.) was added. At room temperature and after 30 minutes, 4-hydroxybenzaldehyde (1.3 eq.) was added and the reactional mixture was stirred for 5 days (Scheme A). After that, the reactional mixture was poured over ice/water, acidified to pH < 2.0 with HCl 37%, extracted with CH_2Cl_2. The solvent was evaporated and the residue purified by TLC using a mixture of hexane and ethyl acetate (1.3:1.1) as eluent (twice), affording compounds 1 and 2. The structural characterization was performed by 1D and 2D nuclear magnetic resonance.

The antioxidant activity (DPPH and ABTS) and AChE inhibitory activity of the compounds 1 and 2 were evaluated by previously described methods .[3,4]



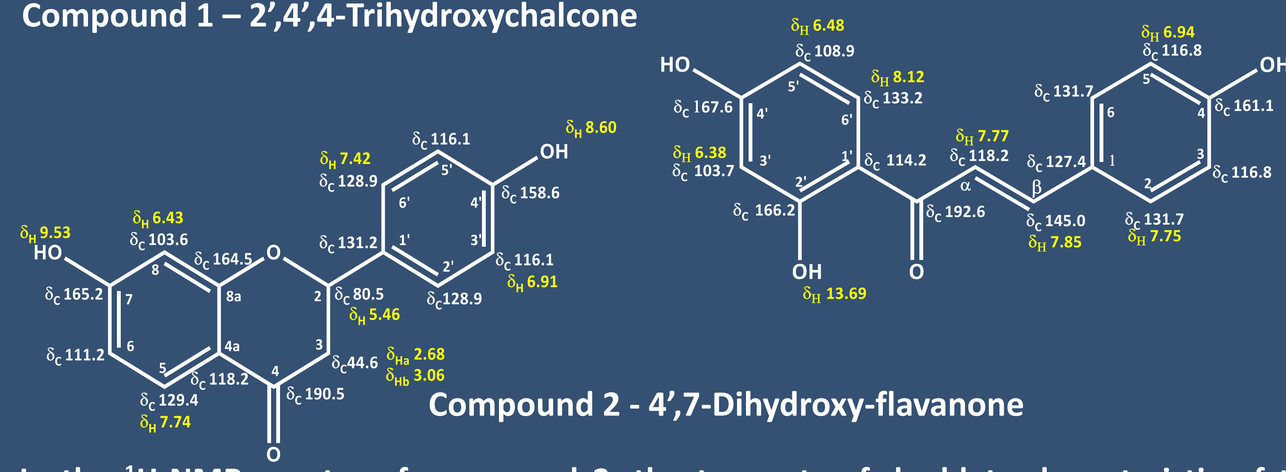
Scheme A – One-pot synthesis of polyhydroxychalcones using LiHMDS as base.

Results and Discussion

The 2',4',4-Tri-hydroxychalcone (compound 1) and 7,4'-dihydroxyflavanone (compound 2) were synthesized with 4.8 and 2.2% of yield, respectively, using the 2',4'-dihydroxyacetophenone and the 4-hydroxybenzaldehyde as starting material, and LiHMDS as base (Scheme A).

^{1}H-NMR of compound 1 contained two sets of doublets at δ 7.85 and 7.77 ppm (*J* = 15.4 Hz), signals characteristic of the *trans* configuration of chalcone vinylic protons. The difference between the chemical shifts of the H-α and H-β protons is due to deshielding mesomeric effect of the carbonyl group (C=O), whose presence is confirmed by the ^{13}C-NMR signal at δ 192.6 ppm. The signal for 2'-O<u>H</u> appears at 13.69 ppm ppm since it is in hydrogen bridge with C=O group. The signals for the aromatic protons appear in the range of δ 6.38-7.75. The signals for the resonance of C-2', C-4 and C-4' appear in the ^{13}C-NMR at chemical shifts in the range of δ 160-165 ppm, which indicates that they are connected to hydroxyl groups. This data is indicative of a tri-hydroxylated chalcone.

Compound 1 – 2',4',4-Trihydroxychalcone



Compound 2 - 4',7-Dihydroxy-flavanone

In the ^{1}H-NMR spectra of compound 2, the two sets of doublets characteristic of the vinylic protons and the strong hydrogen bonds involving the carbonyl group and the proton of the 2'-hydroxyl substituent were absent. Instead of that, a doublet of doublets at δ 3.08 (*J* = 13.8 and 16.7 Hz, H-3b) and 2.68 (*J* = 2.8 and 16.7 Hz, H-3a) is observed, indicating that the heterocyclic C of flavanone was formed. There are two signals at δ 9.53 and 8.60 assigned respectively to 7- OH and 4´-OH. The ^{13}C-NMR spectra display a signal at δ 190.5 that is characteristic of the carbonyl group (C=O). The aromatic protons appear in the range of δ 5.46 and 7.74. This data is indicative of a di-hydroxylated flavanone.

This reaction was carried out in same conditions but during 8 days, and there were no significant changes in the yield obtained, meaning that it is not the time of reaction that is limiting the efficiency of this method. Also, about 80% of the acetophenone was recovered from the reaction mixture, which indicates that there is lack of energy for the reaction to occur. Thereby, some of the reaction parameters need to be optimized. One possible change is the use of microwave irradiation as source of energy, which could increase the yield obtained with this method, and also decrease exponentially the time of reaction.

- **Biological Activities**

Table 1 – Antioxidant and anticholinesterasic activities of the synthesized compounds

Compounds and References	DPPH scavenging activity		ABTS scavenging activity		AChE inhibitory activity	
	% Activity (150 µg/mL)	IC_{50} (µg/mL)	% Activity (150 µg/mL)	IC_{50} (µg/mL)	% Inhibition (150 µg/mL)	IC_{50} (µg/mL)
1	86.92 (±0.43)	26.47 (±0.70)	80.33 (±1.59)	12.72 (±0.92)	-	-
2	25.64 (±2.45)	-	44.51 (±0.18)	-	47.11 (±3.58)	-
Quercetin	87.71 (±0.33)	-	84.88 (±0.70)	0.57 (±0.02)	n.t	n.t
Galanthamine	n.t	n.t	n.t	n.t	98.40[a] (±1.50)	0.43(±0.09)

a - % Enzyme Inhibition at 50 µg/mL; **n.t.** – Not tested

The results (Table 1) show that, in the DPPH scavenging test, compound 1 (chalcone) presented higher antioxidant activity than the corresponding flavanone (2), meaning that the conjugated double bond is responsible for the molecule's ability to scavenge the DPPH radical. The activity of this compound is comparable to the one of quercetin, a well-known antioxidant from the flavonoid family. In the ABTS scavenging assay, the compounds tested presented better results than the ones obtained for the DPPH assay. Again, compound 1 was the one presenting the best activity, with an IC_{50} of 12.72 µg/mL, which shows that the presence of three hydroxyl groups increases the ability of the compound to reduce the ABTS radical.

Compound 2 presents acetylcholinesterase (AChE) inhibitory activity at the maximum tested concentration, although it is less pronounced than the inhibition obtained for galanthamine, which was used as control. It appears that the flavanone scaffold is better for the inhibition of AChE activity than the chalcone vinylic system. In fact, the ring closure seems to increase the inhibitory activity, since compound 2, which is a flavanone, is more active than compound 1, the respective chalcone.

References

1. Ferreyra M.L.F.; Rius S.P.; Casati P. *Front Plant Sci.* **2012**, 3, 222.
2. Bukhari S.N.A.; Jasamai M.; Jantan I.; Ahmad W. *Mini Rev. Org. Chem.* **2013**, 10, 73.
3. Barreto M.C.; Arruda M.; Rego E.; Gouveia V.; Medeiros, J.; Rainha, N. **2012** *In*: Barreto M.C.; Simões, N. (eds.) (2012). pp. 65-81.
4. Re R.; Pellegrini N.; Proteggente A.; Pannala A.; Yang M.; Rice-Evans C. *Free Radical Bio. Med.* **1999**, 26, 1231.

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