Mao inhibitory activity: 3-phenylcoumarins versus 4-hydroxy-3-phenylcoumarins

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Abstract: Coumarins constitute an important class of benzopyrones of different origin. Due to their structural variability they occupy an important place in the realm of natural products and synthetic organic chemistry. Coumarins exhibit a broad range of biological activities such as anticoagulants, antimicrobials, antibacterials, anticancers, and anti-HIV. Furthermore several studies have paid special attention to their antioxidative and enzyme inhibitory properties.

In particular some natural coumarins have shown a low monoamine oxidase (MAO) inhibitory potency while properly modified natural coumarins have been characterized as potent and selective MAO inhibitors. MAO is a FAD-depending enzyme found in the outer mitochondrial membrane of neuronal, glial and other mammalian cells. This enzyme is responsible for catalysing the oxidative deamination of dietary amines and neurotransmitters, regulating intracellular levels of biogenic amines in the brain and the peripheral tissues.

Our research group has also reported high MAO-B selectivity with 6- or 8- substituted-3-arylcoumarin scaffolds. A variety of functional groups of diverse size, lipophilic and electronic properties were introduced in both aromatic rings, concluding that a small substitution at C6 or C8 of the coumarin nucleus is important if a phenyl group is located at C3. Substituents on the 3-phenyl ring also play a crucial role in activity and selectivity: *meta* and *para* substitutions are the most favorable for desired activity.

Based on the previous 3-phenylcoumarins experimental results and with the aim of finding novel and selective MAO inhibitors in this paper we describe a new project with a comparative study between 3-phenylcoumarins and 3-phenyl-4-hydroxycoumarins.

We have used the Perkin and Suzuki reaction as a key step of a good methodology for the efficient and general synthesis of a selected series of 3-phenylcoumarins and 4-hydroxy-3-phenylcoumarins. Most of the tested compounds exhibit a high affinity and selectivity for the MAO-B isoenzyme with values of IC_{50} better than reference compounds.

Keywords: MAO, 3-phenylcoumarins

Introduction

Coumarins constitute an important class of benzopyrones of different origin. Due to their structural variability they occupy an important place in the realm of natural products and synthetic organic chemistry.[1]

They are widely distributed throughout the plant kingdom but also they occur as secondary metabolites in the seeds, roots and leaves of many plant species.[2] Synthetic coumarins are widely used as aroma chemicals because of their odour strength, tenacity, stability to alkali and relatively cheap price; applications include use as a sweetener and fixative (in perfume); fragrance enhancers (for natural essential oils); blenders (in soaps and detergents); aroma enhancers (in tobacco); and for imparting pleasant odours to industrial products.[3] Their interesting pharmacological properties make them attractive targets in organic and medicinal chemistry. Coumarins exhibit a broad range of biological activities such as anticoagulants, antimicrobials, antibacterials, anticancers, and anti-HIV.[4-8] Furthermore several studies have paid special attention to their antioxidative and enzyme inhibitory properties.

In particular some natural coumarins have shown a low monoamine oxidase (MAO) inhibitory potency[9] while properly modified natural coumarins have been characterized as potent and selective MAO inhibitors.[10] This enzyme is responsible for catalysing the oxidative deamination of dietary amines and neurotransmitters, regulating intracellular levels of biogenic amines in the brain and the peripheral tissues.[11,12] It is well-known that MAO plays a critical role in the regulation of central nervous system activity and contributes to the pathogenesis of human neurodegenerative and depressive disorders. Recent findings revealed that affinity and selectivity for MAO-A and MAO-B can be efficiently modulated by appropriate substitutions on the coumarin moiety; the 3/4 and 6/7 positions are particularly suitable to these modifications.[13-17] Our research group has also reported high MAO-B selectivity with 6- or 8- substituted-3-arylcoumarin scaffolds. Substituents on the 3-phenyl ring also play a crucial role in activity and selectivity: *meta* and *para* substitutions are the most favorable for desired activity.[18-24]

Based on the previous 3-phenylcoumarins experimental results and with the aim of finding novel and selective MAO inhibitors in this paper we describe a new project with a comparative study between 3-phenylcoumarins and 3-phenyl-4-hydroxycoumarins.

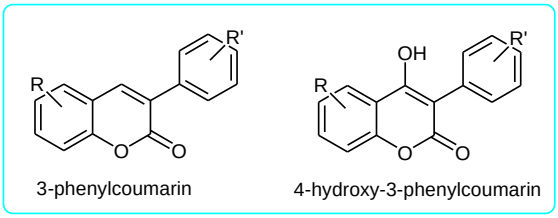


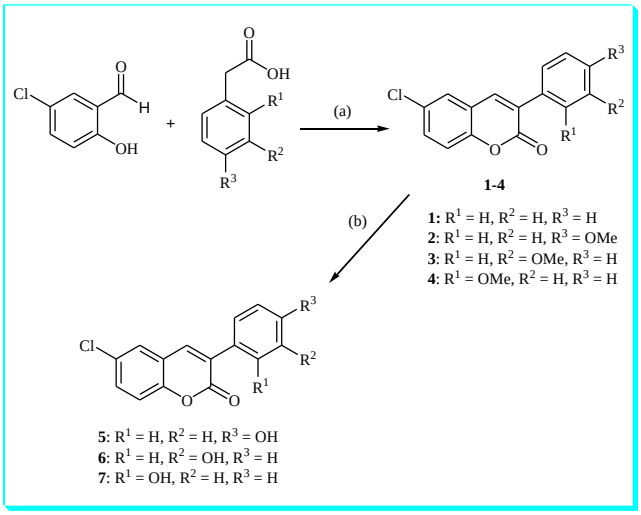
Figure 1

Based on the previous 3-phenylcoumarins experimental results and with the aim of finding novel and selective MAO inhibitors in this paper we describe a new project with a comparative study between 3-phenylcoumarins-and 3-phenyl-4-hydroxycoumarins (Figure 1).

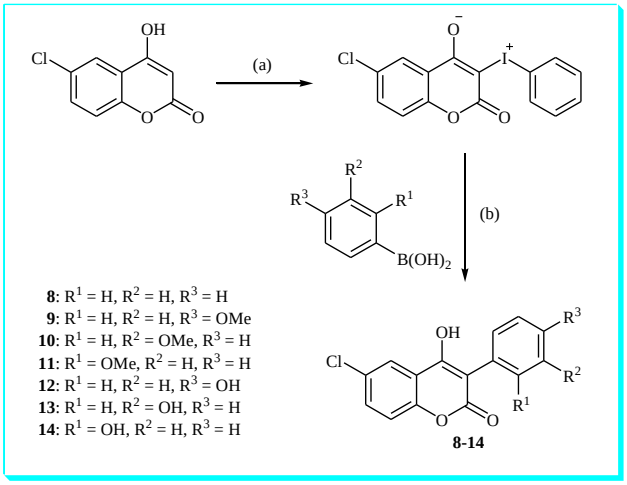
Results and discussion

3-phenylcoumarins, were prepared by Perkin[25,26] reaction Treatment of the corresponding chlorosalicylaldehyde and the conveniently substituted phenylacetic acids with dicyclohexylcarbodiimide (DCC) as dehydrating agent, in dimethyl sulfoxide (DMSO) at 110°C during 12h, afforded the 3-phenylcoumarins **1-4**.[27,28] Hydrolysis of the methoxy groups[29] by treatment with HI in acetic acid/acetic anhydride gave the hydroxy derivatives **5-7** (Scheme 1).[28]

The synthesis of 4-hydroxy-3-phenylcoumarins **8-14**[17,30-32], was achieved by the preparation of phenyliodonium coumarinate species, starting from the corresponding commercial 6-chloro-4-hydroxycoumarin. Then we carried out the palladium-catalyzed Suzuki coupling reaction between phenyliodonium zwitterion species and the conveniently substituted phenyl boronic acids to afford the final compounds (Scheme 2).



Scheme 1. *Reagents and conditions*: (a) DCC, DMSO, 110 °C, 12 h; (b) 57% HI/AcOH/Ac_2O, 0 °C to r.t., 3h.



Scheme 2. *Reagents and conditions*: (a) $PhI(OAc)_2$, Na_2CO_3, H_2O, r.t., 14 h; (b) $Pd(OAc)_2$, P(*t*-Bu)$_3$, LiOH, DME/H_20, r.t., 24-48 h.

All synthesized compounds **1-14** were evaluated as MAO-A and MAO-B inhibitors using clorgyline, moclobemide and R-(-)deprenyl as reference compounds. The inhibitory MAO activity was evaluated in *vitro* by the measurement of the enzymatic activity of human recombinant MAO isoforms expressed in BTI insect cells infected with baculovirus. The inhibitory activity MAO-B and the corresponding IC_{50} values are shown in Table 1.

Compounds	IC_{50} hMAO-B (µM)	**Compounds**	IC_{50} hMAO-B (µM)
1	0.56 ± 0.04	**8**	2.00 ± 0.13
2	0.004 ± 0.0003	**9**	***
3	0.001 ± 0.0001	**10**	6.20 ± 0.42
4	***	**11**	0.63 ± 0.04
5	0.133 ± 0.020	**12**	23.42 ± 1.56
6	0.212 ± 0.022	**13**	75.74 ± 3.36
7	0.443 ± 0.055	**14**	0.36 ± 0.02
Clorgyline	63.41 ± 1.20		
R-(-)-deprenyl	0.017 ± 0.0019		

Table 1: Inhibitory effects of compounds **12-19** and kojic acid on mushroom tyrosinase activity.

From the experimental results, it can be observed that most of the tested compounds are selective MAO-B inhibitors in the micro and nanomolar range. As a rule, the 3-phenylcoumarins (compounds **1-3** and **5-7**) without the OH group in 4 position, were found to be the most active molecules among the compounds tested as MAO-B inhibitors.

The results revealed that the most active compounds as hMAO-B were the coumarins **2** and **3** with IC_{50} of 0.004 and 0.001 μM. They have a better IC_{50} value than the R-(-)-deprenyl, used as reference MAO-B inhibitor compound (IC_{50} = 0.017 μM). Both coumarins **2** and **3** have a methoxy group in the 3-phenyl ring, respectively in *para* and *meta* position. These data exhibited that the MAO-B inhibitory activity improves significantly when in these positions is introduced a small lipophilic group. Effectively, when we changed this group with an hydroxyl substituent (compounds **5** and **6)** the MAO-B inhibitor activity decreases.

With regard the compounds **8-14**, as we said before, they are less active than the corresponding coumarins not hydroxylated in the 4 position, with the exception of 6-chloro-4-hydroxy-3-(2'-hydroxyphenyl)coumarin **14**.

General experimental procedure

Starting materials and reagents were obtained from commercial suppliers and were used without purification. Melting points (mp) are uncorrected and were determined with a Reichert Kofler thermopan or in capillary tubes in a Buchi 510 apparatus. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded with a Varian Inova 500 spectrometer using DMSO-δ6 or $CDCl_3$ as solvent. Mass spectrometry was carried out with a Saturn 2000 ion-trap coupled with a Varian 3800 gas chromatograph (Varian, Walnut Creek, CA) operating under EI conditions (electron energy 70 eV).

Chemistry

*General procedure for the preparation of 3-phenylcoumarins **1-4***: to a solution of *o*-hydroxybenzaldehyde (7.34 mmol) and the corresponding phenylacetic acid (9.18 mmol) in DMSO (15 mL), N,N'-dicyclohexylcarbodiimide (11.46 mmol) was added. The mixture was heated at 110°C for 24 h. Then, ice (100 mL) and AcOH (10 mL) were added to the reaction mixture.

After keeping it at room temperature for 2 h, the mixture was extracted with Et2O (3x25 mL). The organic layers were combined and washed with sodium bicarbonate solution (50 mL, 5%) and water (20 mL). The solvent was then evaporated under vacuum, and the dry residue was purified by flash chromatography (hexane/EtOAc 9:1) to give the desired compounds.

*General procedure for the preparation of 3-phenylcoumarins **5-7***: a solution of the corresponding methoxy-3-phenylcoumarin (0.50 mmoli) in acetic acid 5.0 mL) and acetic anhydride (5.0 mL) at 0 °C, was prepared. Hydriodic acid 57% (10.0 mL) was added drop-wise. The mixture was stirred under reflux temperature for 3 h. The solvent was evaporated under vacuum and the dry residue was purified by FC (hexane/ethyl acetate 8:2) to give the desired compound.

General procedure for the preparation of 3-phenyliodonium coumarinates: iodobenzene diacetate (10 mmol) was suspended in a solution of Na_2CO_3 (10 mmol) in water (100 mL) and was stirred for 30 min at room temperature. To this solution was added a mixture of the corresponding 4-hydroxycoumarin (10 mmol) and Na_2CO_3 (10 mmol) in water (100 mL). After the mixture was stirred at room temperature for 14 h, the precipitate was collected by filtration, washed with water (5 x 20 mL) and dried under vacuum. The resulting white solid was used without further purification.

*General procedure for the preparation of 3-phenyl-4-hydroxycoumarins **8-14***: a degassed solution of appropriated phenyl boronic acid (1.21 mmol) and P(*t*-But)$_3$ (0.109 mmol) in DME and H_2O (4:1, 12.5 mL) was added to a mixture of iodonium ylide (0.55 mmol), LiOH/H_2O (1.65 mmol) and

$Pd(OAc)_2$ (0.027 mmol) under argon at room temperature. After being stirred at the same temperature for 24-48 h. The resulting mixture was purified by FC (hexane/ethyl acetate, 7:3) to give the desired compound.

Biological assays

The effects of the tested compounds on hMAO isoform enzymatic activity were evaluated by a fluorimetric method. Briefly, 0.1 mL of sodium phosphate buffer (0.05 M, pH 7.4) containing the tested drugs in several concentrations and adequate amounts of recombinant hMAO-A or hMAO-B required and adjusted to obtain in our experimental conditions the same reaction velocity [165 pmol of *p*-tyramine/min (hMAO-A: 1.1 µg protein; specific activity: 150 nmol of *p*-tyramine oxidized to *p*-hydroxyphenylacetaldehyde/min/mg protein; hMAOB: 7.5 µg protein; specific activity: 22 nmol of *p*-tyramine transformed/min/ mg protein)] were placed in the dark fluorimeter chamber and incubated for 15 min at 37 °C. The reaction was started by adding (final concentrations) 200 µM Amplex Red reagent, 1 U/mL horseradish peroxidase and 1 mM *p*-tyramine. The production of H_2O_2 and, consequently, of resorufin was quantified at 37 °C in a multidetection microplate fluorescence reader (FLX800TM, Bio-Tek Instruments, Inc., Winooski, VT, USA) based on the fluorescence generated (excitation, 545 nm, emission, 590 nm) over a 15 min period, in which the fluorescence increased linearly. Control experiments were carried out simultaneously by replacing the tested drugs with appropriate dilutions of the vehicles. In addition, the possible capacity of the above tested drugs for modifying the fluorescence generated in the reaction mixture due to non-enzymatic inhibition (e.g., for directly reacting with Amplex Red reagent) was determined by adding these drugs to solutions containing only the Amplex Red reagent in a sodium phosphate buffer. The specific fluorescence emission (used to obtain the final results) was calculated after subtraction of the background activity, which was determined from vials containing all components except the hMAO isoforms, which were replaced by a sodium phosphate buffer solution.

Conclusion

We have used the Perkin and Suzuki reaction as a key step of a good methodology for the efficient and general synthesis of a selected series of 3-phenylcoumarins and 4-hydroxy-3-phenylcoumarins. Most of the tested compounds exhibit a high affinity and selectivity for the MAO-B isoenzyme with values of IC_{50} better than reference compounds. Only the 6-chloro-4-hydroxy-3-(2'-hydroxyphenyl)coumaris **14** has an inhibitor MAO-A activity.

Acknowledgements

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