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Microwave Activated Synthesis of Benzalacetones and Study of Their Potential Antioxidant Activity Using Artificial Neural Networks Method

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Abstract: The α,β-unsaturated ketones known as benzalacetones are an interesting class of compounds frequently used as key intermediates in organic synthesis. Due to their conjugated system, benzalacetone and derivatives have been described as radical scavengers with potential antioxidant properties. We report here a simple and direct method to prepare functionalized α,β-unsaturated ketones via a microwaves activated Claisen-Schmidt reaction. The experimental protocol developed selectively produces benzalacetones without self-condensation product in very short reaction times and good yields. Interested in the biological properties of benzalacetones, we also studied the antioxidant potential of these compounds using an *in silico study* based on the DPPH• radical scavenging ability. The built mathematical model was based on the 0-3D DRAGON molecular descriptors and the artificial neural networks technique showing a correlation coefficient for the training set $(R^2) = 0.71$, an external correlation coefficient $(Q^2_{ext}) = 0.65$. Unfortunately, the results obtained in the *in-silico* study revealed that

synthesized benzalacetones have no antioxidant activity. The predicted results have been confirmed experimentally by an *in vitro* assay of DPPH• scavenging capacity.

Keywords: benzalacetones; artificial neural networks; antioxidant; QSAR; DPPH; free radical scavengers, microwaves-assisted synthesis

1. Introduction

As part of our studies involving the synthesis of bioactive compounds structurally related with the coumarin skeleton we have recently focused our attention on the synthesis of α,β-unsaturated ketones known as benzalacetones, which possess interesting properties for organic synthesis. Due to their conjugated system, benzalacetone and derivatives have been described as radical scavengers with potential antioxidant properties [1]. Various methods of synthesis for this type of compounds have been described in the literature. The Claisen-Schmidt is one of the simplest condensation methods. This reaction is typically catalyzed by acids ($AlCl_3$ or HCl) and more often by bases with or without solvent at room temperature or under conventional heating [2-4]. In order to increase the yield and to avoid the formation of by-products, several protocols relative to Claisen-Schmidt condensation have also been reported using different catalysts, sonochemical activation or microwaves irradiation. However, in all these conditions, side reactions start decreasing the yield of the desired product and entail further purification steps [5-20]. Consequently, we were particularly interested in developing an efficient preparation of benzalacetones from acetone and aromatic substituted aldehydes in basic conditions under microwave-activation.

Secondly we were interested in the study of the antioxidant activity of synthesized benzalacetones. To achieve our goal we developed an *in silico* study using the OD-3D DRAGON molecular descriptors (MDs) and the artificial neural networks (ANN) method. The ANN is one of the artificial intelligence techniques applied to Quantitative Structure Activity Relationships (QSAR) evaluations. In this study we develop an ANN in order to relate scavenging ability of the DPPH• radical and molecule features defined by established MDs. Finally the theoretical results were confirmed experimentally by the *in vitro* assay of DPPH• scavenging capacity.

2. Results and Discussion

2.1 Synthesis

Microwave activation for the synthesis of benzalacetones has not been widely described in the literature. Kappe et *al* reported the aldol condensation of *p*-methoxybenzaldehyde with acetone using microwave activation but could not prevent self-condensation. [21]

As a wide variety of aryl aldehydes is commercially available, microwave activation would provide a higher degree of flexibility with respects to functional groups which may be introduced in the benzalacetone skeleton. The details of the synthesis were previously described by our group **Figure 1**[22].

Following our interest in establishing an efficient, rapid and selective access to benzalacetones and considering our results previously obtained under conventional heating (dibenzalacetone formation in yields between 4-

39%), the Claisen-Schmidt condensations were carried out under controlled microwave activation. The reactions were performed with 1.5 equiv of NaOH, in a Discover™ microwave synthesizer. The compounds were mixed in a sealed microwave reaction tube and irradiated for 10 to 30 minutes (5 W) with stirring at 50 °C or 40°C. After irradiation, reactions were controlled by GC-MS analysis, and the purity of the desired products was evaluated by NMR spectroscopy. All synthetic details of microwave activation procedure and the extension of these conditions to the synthesis of various benzalacetones have been previously described by our research group [22].

The use of microwave activation resulted in a dramatic decrease of reaction times. The reactions were generally achieved within 10-15 min. The desired compounds were isolated with excellent yields (typically higher than 79% and often quantitative) and clean enough to be further used without any purification. These microwave-assisted condensation reactions could be "directly scalable". Identical yields were obtained on 50 mg and 500 mg scale [22].

2.1 Modeling

The 0D-3D DRAGON MDs were computed for an in-house dataset of 1329 compounds whose DPPH• scavenging capacity has been experimentally determined and reported in the literature. Using a wrapper based variable selection procedure; a subset of 14 variables was obtained and posteriorly used as the ANN input. The mathematical model constructed showed a correlation coefficient (R^2) for the training set of 0.71. The predictive ability of the models was assessed using the external validation procedure yielding a correlation coefficient obtain (Q^2_{ext}) of 0.65. Both values are above the limits established for model acceptance, which is an indicator of the robustness and predictive power of the obtained MLP model.

2.2. Prediction:

Virtual screening allows for prior assessment of the potential bioactivity of chemical compounds, and thus providing key guidelines in posterior experimental work. In this study the MLP model previously obtained was used to predict the DPPH• scavenging capacity of a series of functionalized benzalacetones (Bz-der). The results of the predictions are shown in **Table 1**.

As it can be noticed the Bz-der seem to be less effective in DPPH• radical capturing, since their values of pIC_{50} are much higher than the reference compound, butylated hydroxytoluene (BHT, experimental pIC_{50} of 2.10). The exception is 1-naphtalene, in which the substituent is benzene fused to the benzalacetone moiety (pIC_{50} of 2.97).

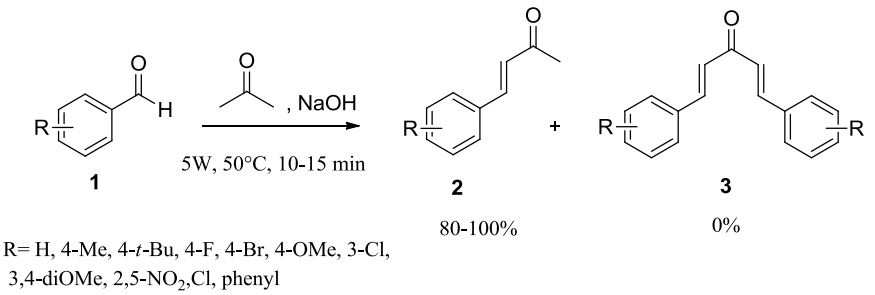
According to conventional understanding of antioxidant activity, increasing of the number of phenolic hydroxyl groups enhances the compounds' antioxidant effectiveness. That's why many efforts have been carried out to synthesize antioxidants containing phenolic hydroxyl groups [23].

Consequently, we suggest that low antioxidant activity of the benzalacetones studied here could be due to the absence of hydroxyl groups linked to the aromatic system. On the other hand, several compounds used as references in the evaluation of antioxidant activity contain the phenolic group, for instance, Trolox, Gallic Acid and BHT.

2.3. In vitro Assay: The result obtained with the *in silico* modeling was corroborated using the experimental study of DPPH• scavenging capacity for the non-substituted compound, *(E)*-4-phenylbut-3-en-2-one whose pIC_{50} was previously predicted (4, 21), and experimentally

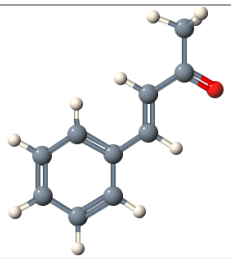
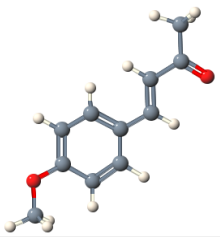
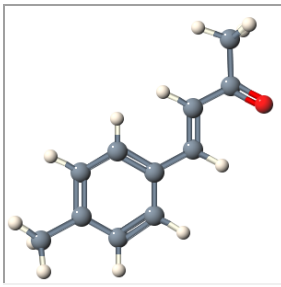
obtained (4.97). These values of IC_{50} were indicative of very low scavenging activity, compared with the value obtained for BHT (2.10). The results demonstrate the predictive power of the designed ANN model and its possible applicability in the study of benzalacetones as antioxidant compounds. These results also suggest the need to synthesize benzalacetone derivatives bearing hydroxyl groups which would improve the antioxidant activity of these compounds

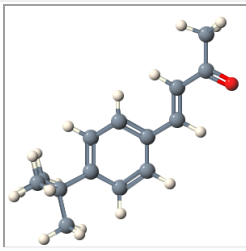
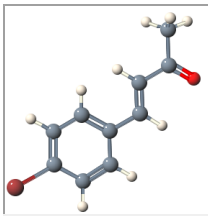
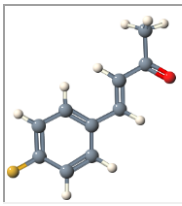
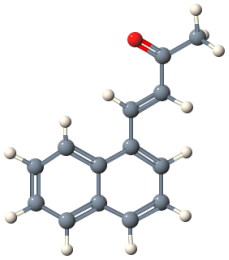
Figure 1: Preparation of benzalacetones under microwaves conditions



R= H, 4-Me, 4-*t*-Bu, 4-F, 4-Br, 4-OMe, 3-Cl, 3,4-diOMe, 2,5-NO_2,Cl, phenyl

Table 1: Predictions of the pIC_{50} values for Bz-der

Origin	IUPAC name	3D Structures	Predict pIC50
Bz-der		Benzalacetone	4,216405
Bz-der		4-OMeBenzalacetone	3,997319
Bz-der		4-MeBenzalacetone	4,228023

Bz-der		4-tBuBenzalacetone	3,931096
Bz-der		4-BrBenzalacetone	4,138268
Bz-der		4-FBenzalacetone	4,276980
Bz-der		1-naphtalenebenzalacetone	2,973071

3. Materials and Methods

3.1 General procedure for the microwave-assisted syntheses

In a capped 10 mL MW-vessel, the aldehyde (50 mg, 1 equiv) and acetone (13.6 equiv) were mixed and then an aqueous solution of NaOH (0.6 g /cm^3 of water) was added. The tube was positioned in the irradiation cavity and the mixture was stirred and heated at the temperature 40/50 °C (measured with an IR temperature sensor), in the monomode microwave oven (5 W) for 10/15 min. After completion, upon cooling to room temperature, the conversion was directly controlled by GC-MS analysis. The product was extracted with AcOEt. The organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to obtain the corresponding benzalacetone. The purity of the final products was controlled by NMR [22].

3.2 In silico study

Data: Experimental results of the scavenging ability of the DPPH• radical (expressed as IC_{50}) for 1329 molecules were extracted from over 170 scientific reports in the literature; and thus yielding a comprehensive and diverse database of compounds for the mathematical analysis. All the structures were optimized using the CORINA software. The response variable (IC_{50}) were transformed to their corresponding pIC_{50} values.

Molecular Descriptors: The parameterization of the structures was performed using 3224 molecular descriptors implemented in the DRAGON software. A wrapper based variable selection procedure was used to obtain a subset of variables for the ANN building.

Development of ANN model: The QSAR model was develop using as chemometric tool a Multilayer Perceptron Neural Network implemented in STATISTICA 8.0 software. For the modeling a Broyden-Fletcher-Goldfarb-Shanno algorithm was used as the optimization method; and the following network architecture was considered: fourteen inputs; eight neurons in hidden layer and one output.

Predictions of antioxidant activity: The benzalacetone derivatives were optimized following the same configurations previously used and the corresponding MDs computed.

3.3 In vitro DPPH• assay: The free radical scavenging activity of benzalacetone was measured using the stable DPPH radical, according to Blois' method [24]. Briefly, 0.1 mM solution of DPPH• in methanol was prepared and this solution (1 mL) was added to sample solution in methanol (3 mL) at different concentrations (250–1250 µg/mL). The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measure at 517 nm. Butylated hydroxytoluene (BHT) was used for comparison. Both determinations were due in triplicate. The capability to scavenge the DPPH• radical was expressed as IC_{50} (concentration of antioxidant that produces 50% of absorbance inhibition)

.

4. Conclusions

In conclusion, an efficient and selective general method has been developed for the synthesis of benzalacetones via a Claisen-Schmidt reaction using microwaves activation. The desired compounds were obtained in shorter times and in almost all cases in quantitative yields. No further purification was required. Under microwaves activation, no dibenzalacetone formation has been observed except in the case of electron-withdrawing substituted aldehydes. Moreover, an ANN based model was developed to predict the antioxidant activity of the synthesized benzalacetones. Unfortunately, the *in-silico* study showed that benzalacetones do not have antioxidant activity. Finally, the predicted results have been experimentally confirmed by the *in vitro* assay of DPPH• scavenging capacity.

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Author Contributions

The French team, A.M.R., M.S.-I.V. and C.F., is responsible for the synthesis and characterization of compounds, the Cuban team (E.G.R. and E.J.R.D.) and S.J.B. are responsible for the *in silico* study and the *in vitro* evaluation. All authors contributed to the drafting and revision of the article and approved the final version.

Conflicts of Interest

The authors declare no conflict of interest

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