

# Encapsulation of (E)-N'-(1-(7-(diethylamino)-2-oxo-2H-chromen-3 yl)ethylidene)benzohydrazide (7-diEAHC) in $\beta$-cyclodextrins: Optimized synthesis of 7-diEACH and in silico ADME profiling, physical stability, antioxidant properties of encapsulated 7-diEAHC and bioavailability in rats

E. Khdhiri, S Abid, K. Athmouni, M. Abid, H. Ayadi, H. Ammar, Ludovic Paquin, Jean-Pierre Bazureau

▶ **To cite this version:**

E. Khdhiri, S Abid, K. Athmouni, M. Abid, H. Ayadi, et al.. Encapsulation of (E)-N'-(1-(7-(diethylamino)-2-oxo-2H-chromen-3 yl)ethylidene)benzohydrazide (7-diEAHC) in $\beta$-cyclodextrins: Optimized synthesis of 7-diEACH and in silico ADME profiling, physical stability, antioxidant properties of encapsulated 7-diEAHC and bioavailability in rats. Results in Chemistry, 2023, 5, pp.100681. 10.1016/j.rechem.2022.100681 . hal-03975255

**HAL Id: hal-03975255**
**https://hal.science/hal-03975255**

Submitted on 30 May 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Results in Chemistry 5 (2023) 100681

Contents lists available at ScienceDirect

Results in Chemistry

journal homepage: www.sciencedirect.com/journal/results-in-chemistry

# Encapsulation of (*E*)-*N*'-(1-(7-(diethylamino)-2-oxo-2*H*-chromen-3 yl) ethylidene)benzohydrazide (7-diEAHC) in β-cyclodextrins: Optimized synthesis of 7-diEACH and *in silico* ADME profiling, physical stability, antioxidant properties of encapsulated 7-diEAHC and bioavailability in rats

Emna Khdhiri [a,3], Souhir Abid [b], Khaled Athmouni [c], Majdi Abid [b], Habib Ayadi [c], Houcine Ammar [a], Ludovic Paquin [d,2], Jean-Pierre Bazureau [d,*,1]

[a] *Laboratoire de Chimie Appliquée : Hétérocycles, Corps Gras et Polymères (LCA-HCGP), Faculté des Sciences, Université de Sfax, Route de Soukra Km3, CP 3038 Sfax, Tunisia*

[b] *Chemistry Department, College of Science and Arts, Jouf University, Al Qurayyat, Al Jawf, Saudi Arabia*

[c] *Laboratoire de Biodiversité et des Écosystèmes Aquatiques, Écologie et Planctonologie UR 11 ES72, Faculté des Sciences, Département des Sciences de la Vie, Université de Sfax, Route de Soukra Km3,5, BP 1171, CP 3000 Sfax, Tunisia*

[d] *Institut des Sciences Chimiques de Rennes (ISCR), UMR CNRS 6226, Université de Rennes 1, Bât. 10A, Campus de Beaulieu, CS 74205, 263 Avenue du Général Leclerc, 35042 Rennes, France*

ARTICLE INFO

*Keywords:*
Hydrazone coumarin
β-Cyclodextrins
Optimization
ADME parameters
Micro-encapsulation
Bio-assay

ABSTRACT

The β-cyclodextrins (β-CD) as supramolecular have extremely attractive pharmaceutical applications. This study focused on the effects of macrocyclic systems complexation on stability, antioxidant activities, and bioavailability of the optimized synthesis coumarin incorporating hydrazone moiety. Optimal synthesis of (*E*)-*N*'-(1-(7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)ethylidene)benzohydrazide (7-diEAHC) was performed by using response surface methodology (RSM) with a temperature of 100 °C, a reaction time of 10 min in the presence of acetic acid (0.85 M). Physicochemical properties and *in silico* ADME profiling of 7-diEAHC were also determined. The encapsulation efficiencies of the 7-diEAHC were above 81 % for the micro-encapsulates. After sunlight exposure, the **7**-diEAHC-β-CD inclusion complex showed a photostability 19.4 % higher than free **7**-diEAHC. Additionally, the encapsulated 7-diEAHC exhibited better stability than free 7-diEAHC for the pH varied between 1 and 9. From the results, storage stability showed that the encapsulation method using β-CD conferred a greater ability of 7-diEAHC against temperature. The NMR and FT-IR techniques were employed to evaluate the chemical stability of 7-diEAHC after encapsulation. In addition, *in vivo* study showed that the micro-encapsulation approach increased the oral bioavailability of 7-diEAHC in treated rats.

## Introduction

Coumarin moiety plays an important key role in heterocyclic chemistry and forms an extensive research activity in medicinal chemistry [1,2]. Indeed, coumarins are a large group of compounds issued from natural products or obtained by synthetic methods [3]. They have been extensively studied during the two last decades due to their applications in biochemistry, pharmaceuticals, materials, physical fields, and others [4]. Therefore, coumarin derivatives have been used as fluorescent probes, charge-transfer agents, solar energy collectors, and nonlinear optical properties because of their high photo-stability, large stokes shift, and high fluorescence quantum yield [5,6]. Moreover, this series of compounds has an outstanding diverse range of biological properties including anti-HIV (human immunodeficiency virus) [7], anticoagulant [8], antimicrobial [9], anti-inflammatory [10] and antiviral activity [11,12]. Recently, coumarin derivatives are also used for

* Corresponding author.
*E-mail address:* jean-pierre.bazureau@univ-rennes1.fr (J.-P. Bazureau).
[1] https://orcid.org/0000-0003-3053-3425.
[2] https://orcid.org/0000-0001-8518-5363.
[3] https://orcid.org/0000-0002-8723-1615.

https://doi.org/10.1016/j.rechem.2022.100681
Received 9 October 2022; Accepted 23 November 2022
Available online 25 November 2022
2211-7156/© 2022 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

regulation of anticholinesterase, anti-5-lipoxygenase, anti-tyrosinase activities and also as anti-cancer agents [13].

Several studies have shown that these properties depend upon the pattern of substitution [2,14]. In particularly, various researches suggest that the presence of functional groups at the 3-position of coumarin skeleton includes specific activities [7]. A similar trend is seen for hydrazones, which are versatile synthetic intermediates showing activities in several pharmacological tests both *in vitro* and/or *in vivo* such as antibacterial and antimicrobial-antitumor agents [15]. For instance, indirubin-3-hydrazone has been found to be potent and selective for inhibition of protein kinase R (PKR) [16]. Recently, the Kaye's team reported the preparation of a series of 3-[1-(benzylidenehydrazono) ethyl]-4-hydroxycoumarins which exhibited encouraging activity against the trypanosomal parasite *T.b. brucei*; one of them, the 3-{[3,4-(dihydroxybenzylidene)hydrazono]ethyl}-4-hydroxycoumarin **A** exhibiting $IC_{50}$ values of 0.90 μM (Fig. 1) [17].

Due to knowledge of our laboratory in the field of derivatives of coumarins, we have explored recently a new approach for the synthesis of a novel class of coumarins incorporating hydrazone moieties in position 3 of the coumarin skeleton (compounds **B** in Fig. 1). This synthetic approach combines two distinct pharmacophores bearing potentially different biological properties [18]. The exploration of their cytotoxic properties against tumor cell lines as well as their ability to inhibit protein kinases is currently underway.

In contrast, the oral bioavailability coumarins isolated from medicinal plants was found to be low in animals [19]. Moreover, enhancement in the oral bioavailability of these compounds may play an important role in its future exploration. Furthermore, more attention has been focused on the β-cyclodextrin (β-CD) inclusion due to its effect on the improvement of oral bioavailability of active molecules [20]. Cyclodextrins (CDs) are cyclic oligosaccharides composed of hydrophilic faces and hydrophobic cavity [21]. Recently it has been shown that the positive impact of β-cyclodextrin inclusion technology in enhancing solubility and biological effect of drugs [22–24]. To the best of our knowledge, no comprehensive work was reported the in literature on (*E*)-*N'*-(1-(7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)ethylidene)benzohydrazide (7-diEAHC as code) as hydrazone coumarin derivative, for synthesis, micro-encapsulation, stability tests and oral bioavailability. In this context, the present study is aimed to optimize the 7-diEAHC synthesis using a Box-Behnken Design (BBD), to determine physicochemical properties and *in silico* ADME profiling, to explore the effects of β-CD inclusion complex on stability, to evaluate the antioxidant activities and also bioavailability in rats.

## Materials and methods

### *General information for organic synthesis*

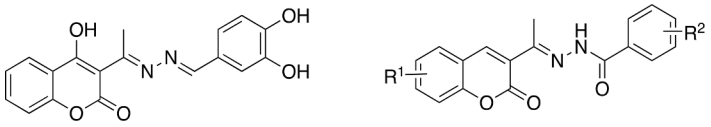
Melting points were determined on a Kofler melting point apparatus and were uncorrected. Thin layer chromatography (TLC) was accomplished on 0.2-mm precoated plates of silica gel 60F-254 (Merck). Visualization was made with ultraviolet light (254 and 365 nm) or with a fluorescence indicator. $^1$H NMR spectra were recorded on BRUKER AC 400 Avance III (400 MHz) spectrometer, $^{13}$C NMR spectra on BRUKER AC 400 Avance III (100 MHz) spectrometer using $CDCl_3$ with tetramethylsilane (TMS) as internal reference. Chemical shifts are expressed in parts per million downfield from TMS. Data are given in the following order: δ value, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), number of protons, coupling constants *J* is given in Hertz. High resolution mass spectra (HRMS) were recorded in positive mode using direct Electrospray infusion, on a Waters Q-Tof 2 spectrometer at the "Centre Régional de Mesures Physiques de l'Ouest (CRMPO)" ScanMAT UAR 2025 CNRS. Microwave reactions were carried out using an Anton Paar Monowave 200 microwave reactor (Anton Paar Tunisia) using 10-mL borosilicate glass vials equipped with snap caps. Elemental analyse was performed on a Flash Microanalyzer EA1112 CHNS/O Thermo Electron in the "Centre Régional de Mesures Physiques de l'Ouest" (CRMPO, Rennes). Solvents were evaporated using a BUCHI rotary evaporator. All reagents and solvents were purchased from Merck Sigma-Aldrich Chimie. Compound "acetyl-7-(diethylamino)-2*H*-chromen-2-one **3**" was synthesized according to a procedure described in literature [18].

### *Optimized procedure for the preparation of (E)-N'-(1-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)ethylidene)benzohydrazide (acronym: 7-diEAHC)*

In a 10-mL glass tube (for microwave synthesis) were placed successively acetyl-7-(diethylamino)-2*H*-chromen-2-one **3** (0.199 g, 0.77 mmol, 1 equiv), commercial benzohydrazide **4** (0.105 g, 0.77 mmol, 1 equiv) and 1 mL of absolute ethanol. The glass tube was sealed with a snap cap and introduced in the microwave cavity of a Monowave 200 Anton Paar reactor (P = 800 Watt). The reaction mixture was irradiated at 100 °C for 10 min under vigorous magnetic stirring (500 rpm) using the software package of the microwave reactor. After microwave dielectric heating, the crude reaction mixture was allowed to cool down at room temperature, and the insoluble material was collected by filtration in a Buchner funnel (porosity N°4), washed successively with cooled ethanol (2 × 5 mL), dry ether (5 mL) and dried under high vacuum ($10^{-2}$ Torr) at 25 °C for 20 min. Recrystallization from absolute ethanol gave 0.232 g of the desired 7-diEAHC as a pale orange powder (80 % yield). Mp = 236–238 °C. $^1$H NMR ($CDCl_3$) $\delta = 1.21$ (t, $J = 7.1$ Hz, 6H, $H_{7''}$), 2.39 (s, 3H, $CH_3$), 3.42 (q, $J = 7.1$ Hz, 4H, $H_{7'}$), 6.46 (s, 1H, $H_8$), 6.58 (d, $J = 7.7$ Hz, 1H, $H_6$), 7.34 (d, $J = 7.7$ Hz, 1H, $H_5$), 7.46 (t, $J = 6.6$ Hz, 2H, $H_{12}$), 7.53 (d, $J = 6.6$ Hz, 1H, $H_{13}$), 7.86 (d, $J = 6.6$ Hz, 2H, $H_{11}$), 8.18 (s, 1H, $H_4$), 9.09 (s, 1H, NH). $^{13}$C NMR ($CDCl_3$) $\delta = 12.4$ ($C_{7''}$), 14.8 ($C_{CH3}$), 44.9 ($C_{7'}$), 96.7($C_8$), 108.4 ($C_6$), 109.4 ($C_{4a}$), 120.3 ($C_3$), 127.3 ($C_{11}$), 128.7 ($C_{12}$), 130.2 ($C_5$), 133.7 ($C_{10}$), 143.2 ($C_4$), 151.5($C_7$), 151.9($C_{3'}$), 157.0 ($C_{8a}$), 161.0 ($C_2$), 164.0 ($C_9$). HRMS ($ES^+$, MeOH), *m/z:* 400.1632 found (calculated for $C_{22}H_{23}N_3O_3Na$ $[M+Na]^+$ requires 400.1631). Anal. Calcd for $C_{22}H_{23}N_3O_3$: C, 70.01; H, 6.14; N, 11.13. Found: C, 70.05; H, 6.16; N, 11.14.

### *Preparation of 7-diEAHC complex with β-CD*

The preparation of 7-diEAHC complex with β-CD was realized according to a procedure described by Athmouni et al. [25]. The 7-diEAHC was reacted with β-CD in 50 mL deionized water at room temperature using an equimolecular mixture by stirring during 3 h. The homogenate solution was concentrated at 100 °C under reduced pressure of a rotatory evaporator. After concentration, the resulting 7-diEAHC/β-CD complex was stored at 4 °C (refrigerator) in a closed vessel under




**A:** *T.b. brucei* $IC_{50}$ 0.90 μM **B:** hydrazone coumarins

**Fig. 1.** Coumarin derivatives bearing benzylidenehydrazono- or hydrazone- fragments.

nitrogen for further analysis.

*Micro-encapsulation efficiency*

The micro-encapsulation efficiency of 7-diEAHC in the inclusion complex β-cyclodextrin was determined using the method described by Paramera's team [26]. Briefly, 10 mg of 7-diEAHC-β-CD complex were diluted in 10 mL of ethanol and the resulting solution was submitted for filtration (0.45 μm). The absorbance of the resulting solution was measured at 417 nm using T60 UV–Visible spectrophotometer apparatus. The following equation was used to determine the complexation efficiency (CE):

$$\text{Complexation efficiency}\,(\%) = \frac{C_E}{C_T} \times 100$$

$C_E$ refers to the mass of complexed 7-diEAHC and $C_T$ to the total mass of 7-diEAHC added initially.

*Inclusion complex characterization by FT-IR and NMR spectroscopies techniques*

The stability of encapsulated 7-diEAHC using β-cyclodextrins was evaluated by NMR spectroscopies (BRUKER AC 400P Spectrometer). $^{1}$H NMR (400 MHz) and $^{13}$C NMR (100 MHz) of 7-diEAHC before and after encapsulation with β-CD are presented respectively in Figs. S10 and S11 of the Supplementary Material. The comparison between two $^{1}$H NMR spectra (Fig. S10A, B) of the 7-diEAHC before and after encapsulation with β-CD showed the same signals. The absence of any new signal thus confirmed the stability of the 7-diEAHC. Further, the presence of the peak at $\delta_C$ 151.9 ppm corresponding to imidic carbon (C3′) and the methine carbon peak at $\delta_C$ 143.2 (C4) in $^{13}$C NMR spectra (Fig. S11A, B) confirmed in the first step the structure of 7-diEAHC and in the second step the stability of the 7-diEAHC after encapsulation with β-CD. The FT-IR spectra (Perkin Elmer FTIR 100 Spectrometer) of 7-diEAHC before and after encapsulation with β-cyclodextrin are also presented in Fig. S12 of the Supplementary Material. The infrared spectra of the two 7-diEAHC before and after encapsulation showed the same absorption bands ($\nu$, $cm^{-1}$) $\nu$ = 3060 (NH), 1685 (C═O, carbonyl of coumarin fragment), 1618 (C═O, carbonyl of hydrazone fragment) and 1603 (C═N). The presence of these absorption bands before the encapsulation confirmed the structure of the new compound and their presence after encapsulation with β-CD confirmed the stability of the product after synthesis.

*Stability studies*

*Natural light stability*

The photochemical stability of free and encapsulated 7-diEAHC was determined using the method developed by Paramera's team [26]. The free and encapsulated 7-diEAHC were exposed to sunlight in enclosed Petri dish. After exposure for 5, 10, 15, 20, 25 and 30 days, samples were collected and the percentage retention of free and encapsulated 7-diEAHC was measured at 430 nm using a T60 UV–Visible spectrophotometer.

*pH stability*

The free 7-diEAHC and complexed 7-diEAHC were diluted in a water/ethanol solution (70:30 v/v). A volume (4 mL) of each solution was adjusted in pH values in the range of 1–9 using commercial appropriate buffer solution [27]. The buffers used were respectively: phosphate-citrate buffer in the range from pH 1.0 to 5.0, sodium phosphate buffer from pH 6.0 to 8.0 and Tris-HCl buffer pH 9.0. After exposure, the absorbance was measured at 430 nm (a T60 UV–Visible spectrophotometer apparatus).

*Thermal stability*

The thermal stability of free and encapsulated 7-diEAHC were determined using the method developed by Paramera et al. [26]. Briefly, 10 mg of each sample were heated at 100 °C for 15, 30, 60 and 120 min. After thermal exposure, the samples were diluted in absolute ethanol and the 7-diEAHC content was measured at 430 nm using a T60 UV–Visible spectrophotometer.

*Antioxidant activity*

Antioxidant activities of free and complexed 7-diEAHC were investigated using DPPH scavenging, ABTS, NO scavenging capacity and FRAP (Ferric-Reducing Antioxidant Power).

*Assay of DPPH radical-scavenging activity*

The 1-diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging capacity was performed using the method described in literature [27]. Briefly, 4 mL of DPPH (0.1 mM) solution was added to 1 mL of various concentrations (0–1 $mg.mL^{-1}$) of free and complexed 7-diEAHC and the solution mixture was incubated for 30 min at 25 °C in dark conditions. The results were determined at 517 nm. The scavenging activity against DPPH radical was determined according to the following equation:

$$\%\,\text{DPPH scavenging} = \left[\frac{A_{cont} - A_{test}}{A_{cont}}\right] \times 100$$

$A_{cont}$ is the absorbance at 517 nm of the DPPH solution with absolute ethanol, and $A_{test}$ is the absorbance of the DPPH solution with the extract reaction.

*ABTS radical scavenging activity*

The 2,2-hydrazine-bis(3-ethyl-benzo-thiazoline-6-sulfonic acid) diamine (ABTS) radical scavenging activity was determined according to a method reported in literature [28]. The ABTS radical cation was prepared by the reaction between ABTS (7 mM) with potassium persulfate (2.45 mM) at room temperature in the dark for 16 h. The ABTS + solution was then diluted with ethanol to obtain an absorbance of 0.700 ± 0.01 at 734 nm. Reaction mixtures containing 20 μL of sample and 3 mL of reagent were incubated for 30 min. The inhibition rate was determined according to the following formula:

$$\%\,\text{Inhibition} = \left[\frac{(A_{cont} - A_{test})}{A_{cont}}\right] \times 100$$

where $A_{con}$ is the absorbance of the control reaction and $A_{test}$ is the absorbance of the extract reaction.

*Nitric oxide (NO) scavenging activity*

The ability of free and encapsulated 7-diEAHC to scavenge capacity $NO^{\bullet}$ radicals was performed according to the method described in literature [29]. Briefly, 0.2 mL of sodium nitroprusside (10 mM) in phosphate buffer solution (pH 7.4) was mixed with different concentrations of free and encapsulated 7-diEAHC. Then, different tube tests were incubated at 37 °C for 3 h. After incubation, a volume of 1 mL of Griess reagent (1 % sulfanilamide, 2 % $H_3PO_4$ and 0.1 % *N*-(1-naphthyl) ethylenediamine was mixed with 1 mL of each solution test. The absorbance was determined at 542 nm. Ascorbic acid was used as standard. The nitric oxide scavenging activity was calculated using the following equation:

$$\%\,\text{Inhibition} = \left[\frac{(A_{cont} - A_{test})}{A_{cont}}\right] \times 100$$

where $A_{cont}$ was the absorbance of the control and $A_{test}$ was the absorbance of the sample.

*Assay of ferric reducing activity power (FRAP)*

The ability of free and encapsulated 7-diEAHC to reduce $Fe^{+3}$ to $Fe^{+2}$ was recorded according to the method developed in literature [30]. Briefly, 2.5 mL of free and encapsulated 7-diEAHC (0–1 mg $mL^{-1}$) was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (1 %). The solution mixture was incubated for 20 min at 50 °C. After incubation, 2.5 mL of trichloroacetic acid (10 %) was added in each reaction tube. After incubation at 3000 rpm, the 2.5 mL supernatant of each tube was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1 %). The absorbance was measured at 700 nm. Quercetin was used as a standard.

*Oral bioavaibility analysis*

A total of ten male Wistar albino rats weighting 175 g on the average, and ten weeks age were allocated randomly into two experimental groups of five rats each:

- Group I: treated with of free 7-diEAHC (15 mg/kg)
- Group II: treated with 7-diEAHC-β-CD complex (equivalent 15 mg/kg of 7-diEAHC).

The 7-diEAHC and the 7-diEAHC/β-CD complexes were administrated to rats by gastric gavage. Animals were anesthetized by exposure to diethyl ether and blood sample (0.3 mL) was collected from the *retro*-orbital plexus at 5, 10, 15, 20 and 25 h following drug administration. Plasma samples were collected and acidified with 10 mM acetic acid. Finally, plasma samples were stored at −80 °C until HPLC analysis. Plasma from rats treated with free 7-diEAHC and 7-diEAHC-β-CD complex were spiked with 6.7-dimethoxycoumarin used as standard. The methodology used, herein, for 7-diEAHC quantification in plasma samples was done according to a study described recently in literature [31] with some modifications. During the experimental period, all animals were checked daily for behavior change and general health conditions. They were caged individually and kept under standard laboratory conditions (temperature 25 ± 1 °C, natural light–dark cycle). The rats had free access to drinking water and commercial standard laboratory diet (SICO, Sfax, Tunisia). At the end of the experimental period, all animals were anesthetized and euthanatized for blood and tissue collections. The experimental protocols were conducted in accordance with the guide for the care and use of laboratory animals issued by the University of Sfax, Tunisia, and approved by the Committee of Animal Ethics of University of Sfax.

*HPLC analysis for bioavaibility study*

Apparatus used HPLC analysis linked to bioavaibility study: Agilent 1260, Agilent Technologies, Germany. The HPLC system comprising a vacuum degasser, an autosampler and a binary pump with a maximum pressure of 400 bar. The HPLC apparatus was equipped with a reversed phase C18 analytical column of 4.6 × 100 mm and 3.5 μm particle size (Zorbax Eclipse XDB C18). The DAD detector was set to a scanning range of 200–400 nm. Column temperature was maintained at 25 °C. The injected sample volume was 2 μL and the flow-rate of mobile phase was 0.4 mL/min. for each data of Fig. 9. The mobile phase composition was a mixture of MeOH/water 8:2 (v/v).

*Statistical analysis*

All the data were expressed as the mean of three replicate determinations. Analysis of variance (ANOVA, analysis of variance) was conducted using the stratigraphic software to make the statistical comparison and estimate the response of each experimental design and, optimized conditions. Statistical significance was set at $P < 0.05$.

**Results and discussion:**

*Single factor experiments for the second step of (E)-N'-(1-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)ethylidene)benzohydrazide (7-diEAHC) synthesis*

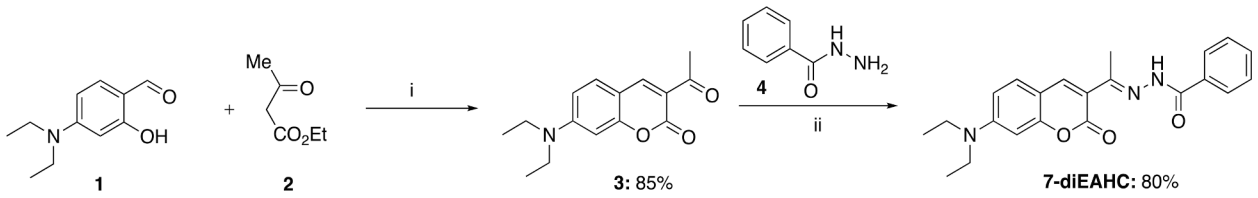
Access to the planned (*E*)-*N'*-(1-(7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)ethylidene)benzohydrazide (7-diEAHC) is outlined in Scheme 1. For the preparation of (*E*)-*N'*-(1-(7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)ethylidene)benzohydrazide (7-diEAHC), the first step is based on a facile and classical heterocyclisation according to a protocol developed in our laboratory [18] which involves the reaction between 4-diethylamino-2-hydroxybenzaldehyde **1** and methyl 3-oxobutanoate **2** catalyzed with piperidine 0.5 % mol. in refluxed ethanolic solution during 35 min., which produced the intermediate **3** in 85 % during 35 min. after work-up. With the 3-acetyl-7-(diethylamino)-2*H*-chromen-2-one **3** in hand, the second step involved the nucleophilic addition of benzohydrazide **4** to the electrophilic acetyl site in position C-3 of intermediate **3**. To optimize the reaction conditions for access of the desired 7-diEAHC compound, the major parameters influencing the chemical yield are respectively - the reaction temperature, - the reaction time and also the appropriate percentage of acetic acid (AcOH molarity) as catalyst. For this reaction, we used as a polar and protic solvent (ethanol) because most of the functionalized coumarin derivatives become insoluble in the reaction medium which facilitates the separation of the expected compound by a simple filtration. To monitor this reaction, we used thin layer chromatography (TLC) with 0.2-mm precoated plates of silica gel (60 F-254 Merck) and visualization was made with ultraviolet light (254 and 365 nm). All experiments were realized under microwave dielectric heating because microwave irradiation increases the kinetics of many reactions in organic synthesis [32]. From a practical point of view, we used a mono-mode microwave cavity (Monowave® 200 Anton-Paar apparatus operating at a frequency of 2.45 GHz from 0 to 800 Watt and with a maximum of 260 °C for IR fiber measurement of temperature).

According to the Box-Behnken Design (BBD) method, the single factor design was used to evaluate the preliminary range of the synthesis factors including $\mathbf{X_1}$ (temperature: 50, 60, 80, 90, 100, 110, 120), $\mathbf{X_2}$ (time: 2, 5, 7, 10, 15, 20) and $\mathbf{X_3}$ (acetic acid % mol. or molarity: 0.2, 0.5, 0.8, 1, 1.5, 2). The retention factor for 7-diEAHC formation analyzed by TLC was the dependent variables.

In Fig. 2 are reported successively impacts of reaction temperature (**A**), reaction time (**B**) and %mol. of AcOH (**C**) in reaction medium for the kinetic of 7-diEAHC formation analyzed by TLC. In Fig. 1A, effect of reaction temperature on the enhancement of **7**-diEAHC formation is noticeable from 80 to 100 °C. Any further increment resulted in the reduction of progress factor of this compound. In addition, reaction time is also one of the important parameters which affect the compound synthesis. Effects of time on the progress of reaction were presented in Fig. 2B. From this TLC analysis, the progress of **7**-diEAHC formation was significant when the reaction time is more than 8–10 min. The maximum is observed in the 10–15 min. range. Further increase in time beyond 15 min resulted in a significant decrease. In Fig. 2C, we have studied impact of AcOH % mol. in reaction medium on the progress of reaction with fixed parameters of reaction time and reaction temperature. From this study, the progress of the reaction is increased when working beyond 0.80 % mol. acetic acid in reaction medium and maximum was observed with 0.85 % mol.

*Analysis of response surface plot and contour for the second step of 7-diEAHC synthesis*

The influence of temperature, reaction time and concentration of acetic acid for the second step of 7-diEAHC synthesis was evaluated using Box-Behnken Design (BBD). In the statistical model, the coded variables were defined as follows: $\mathbf{X_1}$ = temperature, $\mathbf{X_2}$ = reaction time,




**Scheme 1.** Synthesis of (*E*)-*N*'-(1-(7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)ethylidene)benzohydrazide (**7-diEAHC**). *Reagents and reaction conditions:* i) piperidine, EtOH, reflux, 35 min. ii) AcOH, EtOH, MWI, 100 °C, 10 min.

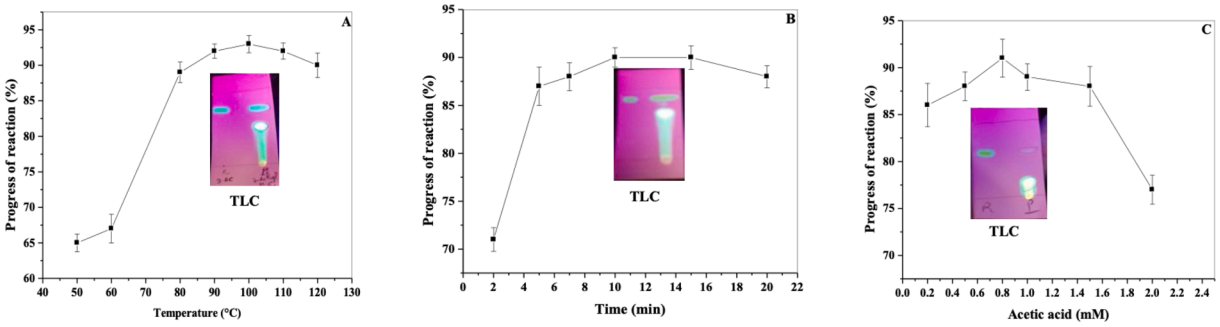



**Fig. 2.** Effect of temperature (A), reaction time (B) and % mol. of acetic acid (C) on the progress of reaction. Data are the mean ± SD (n = 3).

$\mathbf{X_3}$ = concentration of acetic acid (Table 1). The response variable, 7-diEAHC synthesis can be expressed as a function of the independent process variables according to the following response surface quadratic model:

$$\mathbf{Y} = -181.926 + 4.2405.\mathbf{X_1} + 10.6455.\mathbf{X_2} + 45.6061.X_3 - 0.0212406.(\mathbf{X_1})^2 + 0.0003.\mathbf{X_1}.\mathbf{X_2} - 0.204038.\mathbf{X_1}.\mathbf{X_3} - 0.45595.(\mathbf{X_2})^2 - 0.192308.\mathbf{X_2}.\mathbf{X_3} - 14.7604.(\mathbf{X_3})^2$$

where **Y** is retention factor of 7-diEAHC determined by the regression model, $\mathbf{X_1}$, $\mathbf{X_2}$ and $\mathbf{X_3}$ are the coded variables.

The different retention factor of 7-diEAHC synthesis according the Box-Behnken Design (BBD) is given in the Table 2. Our analysis showed that the progress of reaction (%) of 7-diEAHC synthesis varied from 71.7 to 100 %.

Fig. 3 shows the contour and surface response plots of different experimental conditions. For this second step of 7-diEAHC synthesis, the temperature and reaction time are major. The process variables for best combination of response function are reaction temperature (100 °C), reaction time (10 min.) and acetic acid molarity (0.85 % mol.). The p-value was 0.0003 for progress of reaction of 7-diEAHC synthesis, which indicated that the suitability of the model was significant. The coefficient of determination ($R^2$) of the model was 0.952, indicating the model confirmed sufficiently the real interaction between the independent and response variables. From analysis, it can be found in Table 3, reaction temperature had an important positive linear effect on the retention factor ($P < 0.0006$). The reaction time was another major important factor for the retention factor of this reaction ($P < 0.00001$).

**Table 1**
Independent variables and their levels in quadratic orthogonal rotation combination design.

| Factors | Parameters | Coded level | | |
|---|---|---|---|---|
| | | −1 | 0 | +1 |
| $X_1$ | Temperature (°C) | 80 | 100 | 120 |
| $X_2$ | Time (min) | 5 | 10 | 15 |
| $X_3$ | Acetic acid (mol) | 0.2 | 0.85 | 1.5 |

**Table 2**
Quadratic orthogonal rotation combination design and results.

| Run | Temperature (°C) | Time (min) | Acetic acid (M) | Progress of reaction (%) | |
|---|---|---|---|---|---|
| | | | | Experimental | Predicted |
| 1 | 100 | 10 | 0.85 | 100 | 100 |
| 2 | 100 | 10 | 0.85 | 100 | 100 |
| 3 | 120 | 10 | 0.2 | 84.2 | 85.5 |
| 4 | 80 | 15 | 0.85 | 88.4 | 90.6 |
| 5 | 120 | 10 | 1.5 | 77.1 | 77.9 |
| 6 | 80 | 10 | 0.2 | 88.1 | 87.4 |
| 7 | 100 | 5 | 1.5 | 73.4 | 74.8 |
| 8 | 80 | 5 | 0.85 | 76.8 | 76.7 |
| 9 | 120 | 15 | 0.85 | 83.4 | 83.5 |
| 10 | 100 | 15 | 0.2 | 92.6 | 91.1 |
| 11 | 100 | 10 | 0.85 | 100 | 100 |
| 12 | 100 | 5 | 0.2 | 75.1 | 75.9 |
| 13 | 100 | 10 | 0.85 | 100 | 100 |
| 14 | 100 | 10 | 0.85 | 100 | 100 |
| 15 | 80 | 10 | 1.5 | 91.6 | 90.3 |
| 16 | 100 | 15 | 1.5 | 88.4 | 87.5 |
| 17 | 120 | 5 | 0.85 | 71.7 | 69.5 |

*Physicochemical properties and in silico ADME study of 7-diEAHC*

The physicochemical properties of compound 7-diEAHC were calculated with the Swiss ADME [33] server platform [34]. These physicochemical properties were evaluated based on Lipinski's rules [35], considering log P (octanol/water partition coefficient) which indicates the concentration ratio of a neutral substance between two immiscible solvents in equilibrium and reveals the differential solubility of this substance in the two solvents. Log P is a very important parameter because it influences the bioavailability, permeability and toxicity of a potential drug candidate or a drug. Molecular weight (MW), aqueous solubility (log S), topological polar surface aera (*t*PSA), hydrogen bond donors (HBD), hydrogen bond acceptors (HBA) [36], number of rotatable bonds (RB), the fraction of $Csp^3$ and drug-likeliness (violation of the 5 Lipinski rules LV) completed this *in silico* analysis (Table 4).

The log P value of compound 7-diEAHC was acceptable and in agreement with Lipinski's rules ($-0.4 < \log P_{o/w} < +5.6$). According to the calculated value of aqueous solubility ($\log S_{\text{7-diEAHC}}$: − 4.73), compound 7-diEAHC is moderately soluble in water ($-6 < \log S_{\text{7-diEAHC}}$

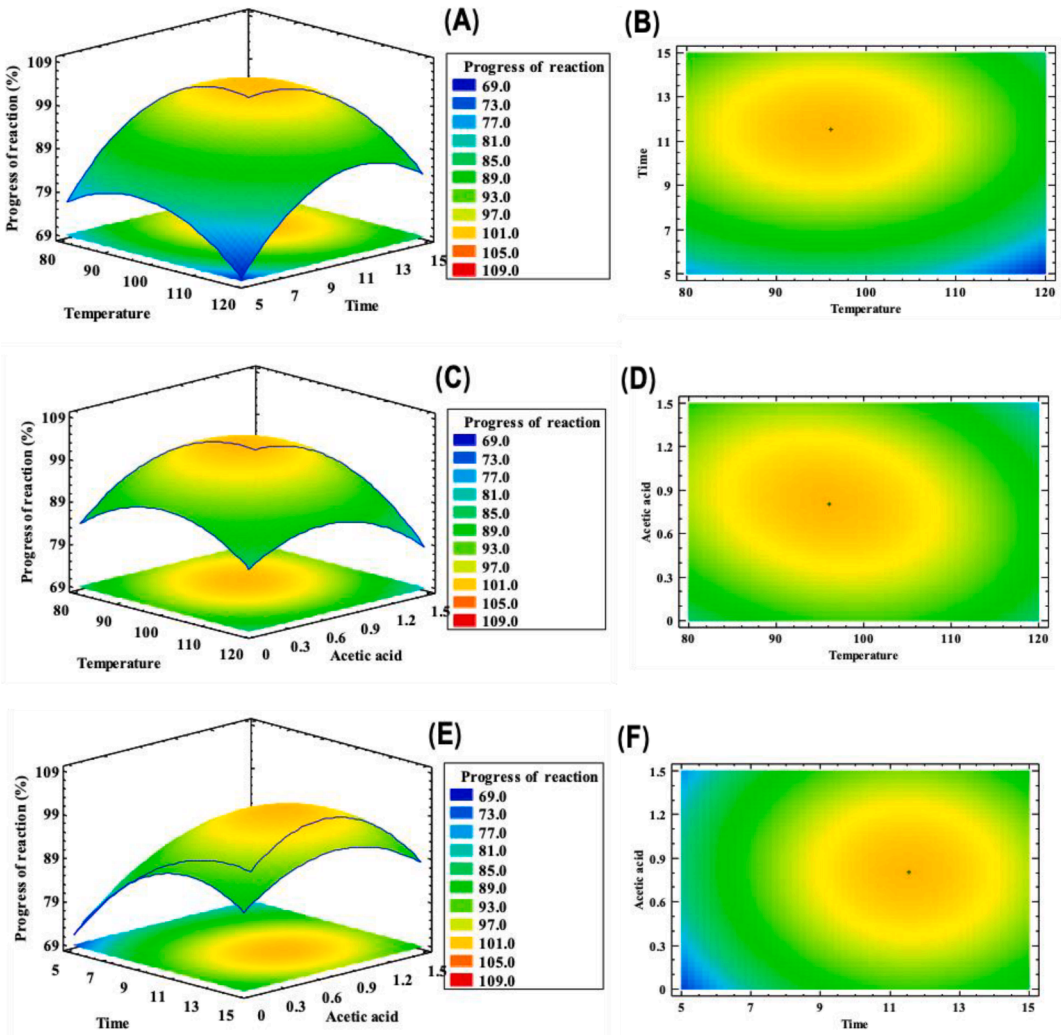



**Fig. 3.** Response surface (A, C and E) and contour plot (B, D and F) of different variables including the reaction temperature, the reaction time and AcOH % mol. in reaction medium for the second step of **7**-diEAHC synthesis.

**Table 3**
Statistical analysis.

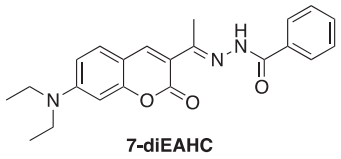
| Source | DF | 7-diEAHC | | |
|---|---|---|---|---|
| | | Sum of Squares | F value | P value |
| Model | 9 | 1674.93 | 4.75 | 0.00034[a] |
| $X_1$ | 1 | 101.46 | 35.78 | 0.0006[a] |
| $X_2$ | 1 | 388.09 | 136.85 | 0.00001[a] |
| $X_3$ | 1 | 11.11 | 3.92 | 0.088[b] |
| $X_1X_2$ | 1 | 0.0036 | 0.00 | 0.972[b] |
| $X_1X_3$ | 1 | 28.14 | 9.92 | 0.0161[a] |
| $X_2X_3$ | 1 | 1.56 | 0.55 | 0.482[b] |
| $X_1^2$ | 1 | 303.94 | 107.18 | 0.0001[a] |
| $X_2^2$ | 1 | 547.08 | 57.74 | 0.0001[a] |
| $X_3^2$ | 1 | 163.75 | 26.01 | 0.0001[a] |
| Lack of Fit | 3 | 19.85 | 2.75 | 0.132 |

[a] Means significance ($P < 0.05$).
[b] Not significance ($P > 0.05$).

$<-4$). For predicting a potential drug candidate solubility, there is another effective descriptor for transport properties of a molecule is the topological surface aera (*t*PSA) which described the sum of the surfaces of its polar atoms (N and O in our case), and the hydrogen atoms attached to them. *t*PSA is an interacting indicator of gastrointestinal absorption and penetration of the brain-blood barrier (BBB). For compound 7-diEAHC, *t*PSA value of 74.91 $Å^2$ evidenced an acceptable permeability. Complete analysis of the data in Table 4 shows that the compound 7-diEAHC is in agreement with Lipinski's rules of five (R05) [37] for the following requirements: (a) molecular weight <500 g/mole; (b) hydrogen bond donors HBD < 5; (c) hydrogen bond acceptors HBA < 10 [38]; (d) Log P < 4.15 and (e) *t*PSA ≤ 140 $Å^2$. This means that 7-diEAHC should have efficient absorption and good oral potential bioavailability [39].

Using the SwissADME server [34], the scale of similarity of synthesized 7-diEAHC with a drug was used to evaluate criteria integrating lipophilicity (LIPO), size (MW), polarity (tPSA), solubility (log S), flexibility (FLEX, see also the number of rotatable bonds RB in Table 4), and saturation (INSATU). The ideal situation is that when all properties of this compound is located in the pink region. That is to say when the basic constant properties are on the radar chart. The biocompatibility radar graphs and bioavailability of compound 7-diEAHC is reported in Fig. 4. Except for saturation (a very slight gap), the radar plot shows that compound 7-diEAHC is in the pink area. It should be noted that the bioavailability score % (%Abs) of this compound is 55 %.

**Table 4**
Results of physicochemical properties for compound **7-diEAHC**.




| MW (g/mole) | Log P | Log S | *t*PSA (Å$^2$) | HBA | HBD | RB | Fraction Csp$^3$ | LV |
|---|---|---|---|---|---|---|---|---|
| 377.17 | 3.72 | −4.73 | 74.91 | 4 | 1 | 7 | 0.23 | 0 |

MW, molecular weight; Log P, partition coefficient; Log S, water solubility; *t*PSA, topological surface aera; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor: RB, rotatable bonds, LV, number of Lipinski's rule of 5 violations.

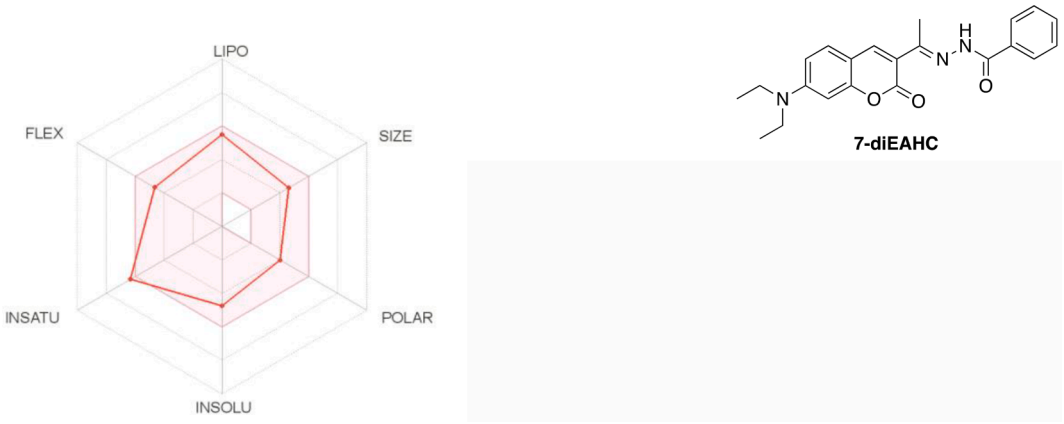



**Fig. 4.** Bioavailability radar related to the physicochemical properties of compound **7di-EAHC**. The pink colored zone is the suitable physicochemical space for oral bioavailability. LIPO (Lipophilicity): −0.7 < Log P < +5.0; SIZE: 150 g/mol < MW < 500 g/mol; POLAR (Polarity): 20 Å$^2$ < *t*PSA < 130 Å$^2$; INSOLU (Insolubility): −6 < Log S < 0; INSATU (Insaturation): 0.25 < Fraction Csp$^3$ < 1; FLEX (Flexibility): 0 < Number rotatable bonds < 9.

*Encapsulated 7-diEAHC with β-CD and stability studies of the complex*

Cyclodextrins (CDs) are macrocyclic carbohydrate polymers possessing 6–8 glucose units for α-, β- and γ-CDs, respectively [21]. They resemble a hollow, truncated cone and are well recognized as encapsulating agents for improvement of stabilities, activities, and solubilities of various bioactive compounds [20]. For the preparation of encapsulated 7-diEAHC/β-CD complex, we applied a method described in literature [25]. The encapsulation efficiencies of the (*E*)-N'-(1-(7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)ethylidene)benzohydrazide (7-diEAHC) was above 81 % for the micro-encapsulates. The excellent encapsulation efficiencies could be associated with the film forming property of 7-diEAHC and the non-thermal electrospray-based

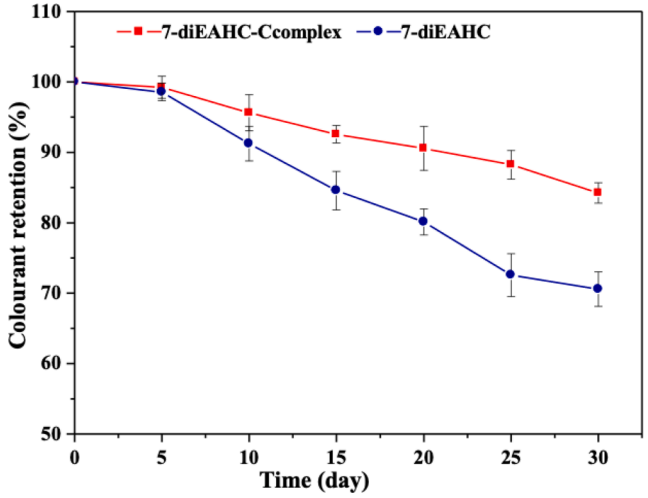



**Fig. 5.** Effect of natural light on the free and microencapsulated 7-diEAHC.

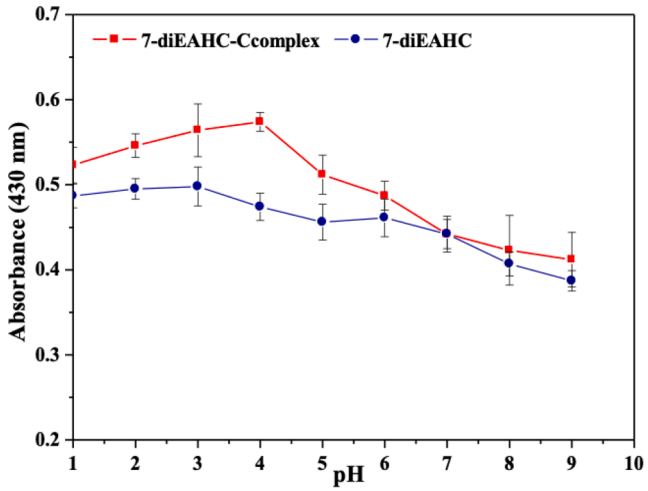



**Fig. 6.** Effect of pH on the free and microencapsulated 7-diEAHC.

encapsulation process.

*Effect of light on free and encapsulated 7-diEAHC*

Fig. 5 below, shows the effect of light on the stability of free and microencapsulated 7-diEAHC. After 30 days of sunlight exposure, the **7**-diEAHC-β-CD inclusion complex showed a photostability 19.37 % higher than free **7**-diEAHC. The colorant retention of free and microencapsulated **7**-diEAHC was 70.56 % and 84.23 %, respectively. From analysis, it can be concluded that **7**-diEAHC microcapsules with β-cyclodextrins should have better photostability to compare with free **7**-diEAHC.

According to literature [40], the inclusion complex formation enhances the photostability of curcumin compounds. Recently, it has been shown that the curcumin complexed with β-cyclodextrins showed light stability tests of their microcapsules in solution [41]. In this context and according to literature [42], phycocyanin complexed with chitosan polymer showed greater stability against sunlight exposure. Moreover, Castillo et al. [43] indicated that the γ-cyclodextrins protected curcumin against photodegradation.

*Effect of pH on free and microencapsulated 7-diEAHC*

The effects of pH on the stability of free and encapsulated 7-diEAHC are reported in Fig. 6. From these results, the encapsulated 7-diEAHC exhibited better stability than free 7-diEAHC for a range of pH varying between 1 and 9. However, when pH is 7, no obvious difference can be observed from the values of the absorbance of the free and encapsulated 7-diEAHC. The use of microencapsulation provided a protective layer to our compound 7-diEAHC except for neutral pH. Similar to present study, Yan and coworkers [42] also found that encapsulation method increased phycocyanin stability against variation of pH. The protection action of β-cyclodextrins is usually associated with their complexation strength.

*Effect of temperature on free and microencapsulated 7-diEAHC*

The temperature is considered as a significant factor influencing the stability of a compound [42]. Fig. 7 presents the effects of temperature on the stability of free and encapsulated 7-diEAHC**.** The thermal stability of free and encapsulated was evaluated after a 2 h isothermal heating at 100 °C. The colorant retention rate of free and encapsulated 7-diEAHC was 80.2 % and 90.4 %, respectively. Our results indicated that the formed complex can efficiently lake the degradation of 7-diEAHC upon temperature exposure. Mongolin's team [40] indicated that the complexed curcumin with β-cyclodextrins exposed to 100 °C and after 2 h showed greater storage stability against temperature. Additionally, the phycocyanin pigment complexed with chitosan polymer indicated

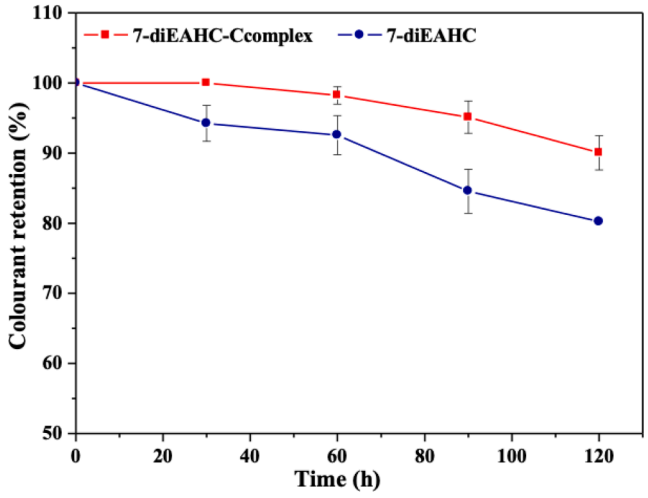



**Fig. 7.** Effect of temperature on the free and microencapsulated 7-diEAHC after 2 h using isothermal heating at 100 °C.

greater stability against temperature [42]. Moreover, recently it has been shown that β-CDs have potential application in the control and preservation of natural color to preserve the food quality [44]. Moreover, Ho and coworkers indicated that catechin encapsulated with β-CD provided protection against temperature up to 120 °C [45].

*Comparison of antioxidant activities of 7-diEAHC before and after encapsulation with β-CD.*

ABTS and DPPH methods are generally classified as single electron transfer reactions. These radical indicators may be neutralized by direct reduction *via* electron transfer or by radical quenching *via* hydrogen atom transfer [46]. The $DPPH^{\bullet}$ scavenging assay is the most method used for *in vitro* evaluation of antioxidant capacity of samples [47]. Fig. 8A shows the $DPPH^{\bullet}$ radical-scavenging activity of free and encapsulated 7-diEAHC. The antiradical activity values for investigated samples varied between 22.11 and 85.9 % for the 7-diEAHC and 26.94 to 92.63 % for the encapsulated 7-diEAHC. According to literature [22], β-cyclodextrin encapsulation elevates antioxidant potential of catechin isolated from tea. Therefore, the anti-radical enhancement of encapsulated 7-diEAHC can be associated with the cooperation between the host–guest hydrogen bonding interactions and the shielding of encapsulated 7-diEAHC OH group in the β-CD cavity. In addition, the improved stabilities of 7-diEAHC upon inclusion complexation correlate with their increased antioxidant activities. Aree's team [22] obtained similar results to those of this investigation.

ABTS radical cation ($ABTS^{\bullet+}$) is reactive towards most antioxidants, and it is soluble in both aqueous and organic solvents. The $ABTS^{\bullet+}$ assay is a useful tool in determining the antioxidant activity of both lipophilic and hydrophilic antioxidants in different samples [48]. The ABTS assay is based on the generation of a blue/green $ABTS^{\bullet+}$ that can be reduced by antioxidants. The values of the $ABTS^{\bullet+}$ radical scavenging activity ranged from 27.56 to 81.42 % for the 7-diEAHC and 34.36 to 89.06 % for the encapsulated 7-diEAHC (Fig. 8B).

The ability of the free and encapsulated 7-diEAHC to inhibit the $NO^{\bullet}$ radical was determined and compared with ascorbic acid as positive control (Fig. 8C). The values of $NO^{\bullet}$ scavenging activity ranged from 82.26 % in the free 7-diEAHC to 90.78 % in the encapsulated 7-diEAHC.

The Reducing Antioxidant Power Test (RAPT) is a simple and straightforward method for assessing "antioxidant potency". The antioxidant capacity of the free and encapsulated 7-diEAHC based on the ability to reduce ferric ions is presented in Fig. 8D: it can be observed that the encapsulated 7-diEAHC showed higher ability to reduce $Fe^{+3}$ than in the free form.

*β-Cyclodextrin microparticles as a potential approach to enhance 7-diEAHC bioavaibility*

Numerous studies showed that most of the hydrazone derivatives have poor bioavaibility. Due to that compound 7-diEAHC presented a moderate solubility in water (Log $S_{\mathbf{2\text{-}diEAHC}}$: −4.73), the use of macrocyclic complex, such as β-CD, offered an opportunity to enhance the absorption of 7-diEAHC in the plasma of a rat as a model for a first approach. To the best of our knowledge, this approach has not been addressed so far for a coumarin derivative bearing hydrazone fragment, which moreover has moderate solubility in the aqueous medium. The mean plasma concentration–time profiles of free and encapsulated 7-diEAHC in rats after oral administration is shown in Fig. 9. The HPLC technique (Agilent 1260, Agilent Technologies, Germany) has been employed to evaluate the pharmacokinetics of free 7-diEAHC (15 mg.$kg^{-1}$) and encapsulated 7-diEAHC (mg.$kg^{-1}$) using rats as animal model. Also, the value of $T_{max}$ was obtained to be earlier (5 h) for the encapsulated 7-diEAHC than that of the free 7-diEAHC (10 h). For the analysis, the maximum concentration ($C_{max}$) in rat plasma was determined 4.56 μg.$mL^{-1}$ for encapsulated 7-diEAHC and 2.45 μg.$mL^{-1}$for free 7-diEAHC. In this context, Gidwani's team [49] found that the

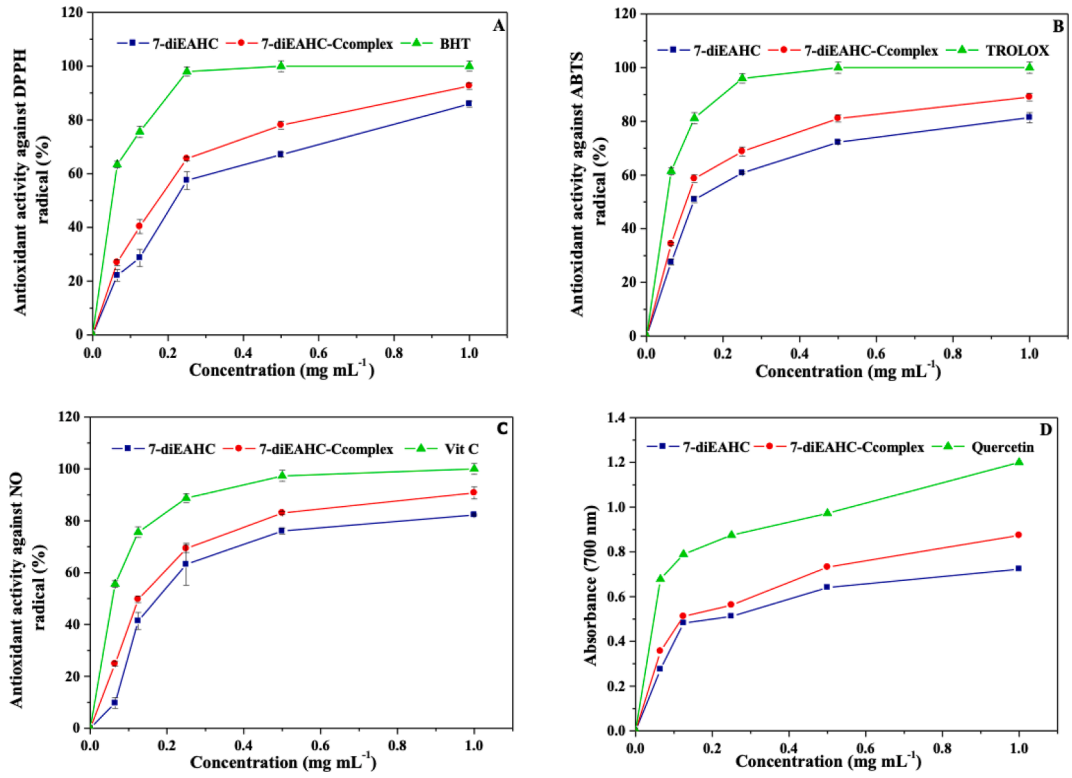



**Fig. 8.** *In vitro* antioxidant potential of free and encapsulated 7-diEAHC. Scavenging activity of free and encapsulated 7-diEAHC against DPPH (**A**), ABTS (**B**) and NO (**C**) radicals. Reducing power capacity of free and encapsulated 7-diEAHC against free $Fe^{+3}$ (**D**). The data are presented as a mean value ± SD.

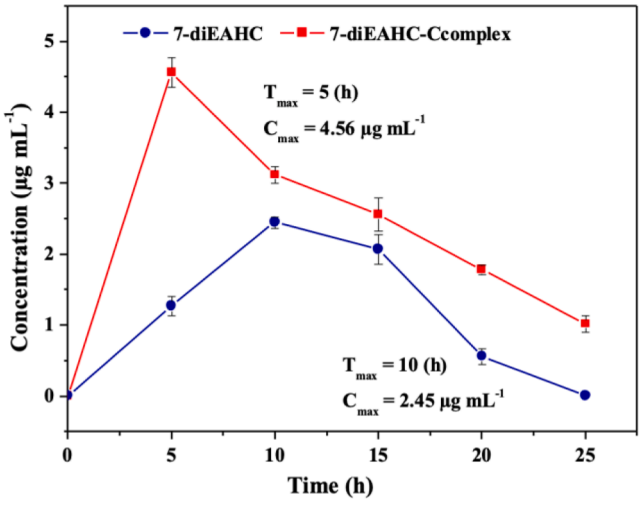



**Fig. 9.** Variation concentration in rat plasma after oral administration of free **7-diEAHC** and encapsulated **7-diEAHC** with β-CD. Data were represented as mean ± SD, (n = 5).

β-cyclodextrin enhanced altretamine solubility and oral bioavailability. Therefore, the microencapsulation increased the cell permeability and bioavailability of compounds [50]. This phenomenon would be associated with the high solubility of cyclodextrin complexes.

## Conclusion

In summary, the optimized reaction conditions under microwave dielectric heating for the synthesis (*E*)-*N*'-(1-(7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)ethylidene)benzohydrazide (7-diEAHC) as a coumarin platform bearing hydrazone moiety were fruitful by using the Box-Behnken Design (BBD) method. Analysis of response surface and contour plot of different variables including the reaction temperature, reaction time, and acetic acid %mol. in the reaction medium showed that optimal synthesis factors are: 100 °C under microwave for 10 min. with a concentration of AcOH 0.85 % mol. as catalyst. According to calculated ADME properties, the compound 7-diEAHC is in agreement with Lipinski's rules of five (R05). This means that 7-diEAHC should have efficient absorption and good oral potential bioavailability. Encapsulation of 7-diEAHC with β-cyclodextrins (81 % of efficiency) provided protection against sunlight (a gain of 19.4 % for photostability), pH and temperature associated to the enhancement of the antioxidant capacity of 7-diEAHC. The complex 7-diEAHC/β-cyclodextrin increased the solubility and the oral bioavailability in rats. These results presented in this work showed that the use of microencapsulation is a relevant approach in pharmacotherapy.

## CRediT authorship contribution statement

**Emna Khdhiri:** . **Souhir Abid:** Resources. **Khaled Athmouni:** . **Majdi Abid:** . **Habib Ayadi:** . **Houcine Ammar:** Resources, Supervision, Conceptualization, Funding acquisition. **Ludovic Paquin:** Methodology. **Jean-Pierre Bazureau:** Supervision.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

The authors acknowledge the "Ministry of Higher Education, Scientific Research and Technology, Tunisia", for the support of this study. The authors are grateful to the assistance of the staff of the CRMPO

analytical chemistry core facility for HRMS analysis (CRMPO platform, ScanMAT UAR 2025 CNRS at University of Rennes 1, Building 11A, Campus de Beaulieu, Rennes, France). The authors wish to thank Miss Leila Mahfoudh for his valuable feedback while proof reading this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rechem.2022.100681.

## References

[1] F. Borges, F. Roleira, N. Milhazes, L. Santana, E. Uriarte, Simple coumarins and analogues in medicinal chemistry: occurrence, synthesis and biological activity, Curr. Med. Chem. 12 (2005) 887–916, https://doi.org/10.2174/0929867053507315.

[2] S. Emami, S. Dadashpour, Current developments of coumarin-based anti-cancer agents in medicinal chemistry, Eur. J. Med. Chem. 102 (2015) 611–630, https://doi.org/10.1016/j.ejmech.2015.08.033.

[3] I. Raad, R. Terreux, P. Richomme, E.L. Matera, C. Dumontet, J. Raynaud, D. Guilet, Structure-activity relationship of natural and synthetic coumarins inhibiting the multidrug transporter P-glycoprotein, Bioorg. Med. Chem. 14 (2006) 6979–6987, https://doi.org/10.1016/j.bmc.2006.06.026.

[4] J.M. Li, X. Zhang, X. Wang, Y.C. Xie, L.D. Kong, Protective effects of cortex fraxini coumarines against oxonate-induced hyperuricemia and renal dysfunction in mice, Eur. J. Pharmacol. 666 (2011) 196–204, https://doi.org/10.1016/j.ejphar.2011.05.021.

[5] K. Khemakhem, H. Ammar, S. Abid, R. El Gharbi, S. Fery-Forgues, Spectroscopic study of 3-aryl-7-methoxy-coumarin, iminocoumarin and bis-iminocoumarin derivatives in solution, Dyes Pigm. 99 (2013) 594–598, https://doi.org/10.1016/j.dyepig.2013.06.003.

[6] J. Sun, M. Zheng, J. Jia, W. Wang, Y. Cui, J. Gao, New Coumarin-benzoxazole derivatives: Synthesis, photophysical and NLO properties, Dyes Pigm. 164 (2019) 287–295, https://doi.org/10.1016/j.dyepig.2019.01.010.

[7] R.S. Keri, B.S. Sasidhar, B.M. Nagaraja, M.A. Santos, Recent progress in the drug development of coumarin derivatives as potent antituberculosis agents, Eur. J. Med. Chem. 100 (2015) 257–269, https://doi.org/10.1016/j.ejmech.2015.06.017.

[8] S.F. Razavi, M. Khoobi, H. Nadri, A. Sakhteman, A. Moradi, S. Emami, A. Foroumadi, A. Shafiee, Synthesis and evaluation of 4-substituted coumarins as novel acetylcholinesterase inhibitors, Eur. J. Med. Chem. 64 (2013) 252–259, https://doi.org/10.1016/j.ejmech.2013.03.021.

[9] G.X. Pang, C. Niu, N. Mamat, H.A. Aisa, Synthesis and in vitro biological evaluation of novel coumarin derivatives containing isoxazole moieties on melanin synthesis in B16 cells and inhibition on bacteria, Bioorg. Med. Chem. Lett. 27 (2017) 2674–2677, https://doi.org/10.1016/j.bmcl.2017.04.039.

[10] K. Fylaktakidou, D. Hadjipavlou-Litina, K. Litinas, D. Nicolaides, Natural and synthetic coumarin derivatives with anti-inflammatory/antioxidant activities, Curr. Pharm. Design 10 (2005) 3813–3833, https://doi.org/10.2174/1381612043382710.

[11] G. Liu, C. Wang, H. Wang, L. Zhu, H. Zhang, Y. Wang, C. Pei, L. Liu, Antiviral efficiency of a coumarin derivative on spring viremia of carp virus in vivo, Virus Res. 268 (2019) 11–17, https://doi.org/10.1016/j.virusres.2019.05.007.

[12] Y. Hu, W. Chen, Y. Shen, B. Zhu, G.X. Wang, Synthesis and antiviral activity of coumarin derivatives against infectious hematopoietic necrosis virus, Bioorg. Med. Chem. Lett. 29 (2019) 1749–1755, https://doi.org/10.1016/j.bmcl.2019.05.019.

[13] C. Karthikeyan, V.R. Solomon, H. Lee, P. Trivedi, Design, synthesis and biological evaluation of some isatin-linked chalcones as novel anti-breast cancer agents: A molecular hybridization approach, Biomed. Prev. Nutr. 3 (2013) 325–330, https://doi.org/10.1016/j.bionut.2013.04.001.

[14] D. Yiğit, Y.A. Udum, M. Güllü, L. Toppare, Electrochemical and spectroelectrochemical studies of poly(2,5-di-2,3-dihydrothieno[3,4-*b*][1,4]dioxin-5-ylthienyl) derivatives bearing azobenzene, coumarine and fluorescein dyes: Effect of chromophore groups on electrochromic properties, Electrochim. Acta 147 (2014) 669–677, https://doi.org/10.1016/j.electacta.2014.09.053.

[15] J. Lasri, M.M. Aly, N.E. Eltayeb, B.A. Babgi, Synthesis of symmetrical and asymmetrical azines from hydrazones and/or ferrocenecarboxaldehyde as potential antimicrobial–antitumor agents, J. Mol. Struct. 1164 (2018) 1–8, https://doi.org/10.1016/j.molstruc.2018.03.030.

[16] M.P. Udumula, A. Bhat, S. Mangali, J. Kalra, I. Dhar, D. Sriram, A. Dhar, Pharmacological evaluation of novel PKR inhibitor indirubin-3-hydrazone in-vitro in cardiac myocytes and in-vivo in wistar rats, Life Sci. 209 (2018) 85–96, https://doi.org/10.1016/j.lfs.2018.07.055.

[17] M.H. Manyeruke, H.C. Hoppe, M. Isaacs, R. Seldon, D.F. Warner, R.W.M. Krause, P. T. Kayea, Synthesis and exploratory biological evaluation of 3-[(*N*-4-benzyloxyphenyl)iminoethyl]- and 3-(1-hydrazonoethyl)-4-hydroxycoumarins. *Arkivoc* 2022, part V: 205-216. https://doi.org/10.24820/ark.5550190.p011.760.

[18] E. Khdhiri, K. Mnafgui, H. Ammar, S. Abid, Design, synthesis and biological evaluation of novel hydrozones coumarins as potent antiobesity, antioxydant and antidiabetic agents. 2022, submitted to publication.

[19] Y. Cai, N.D. Anavy, H.H. Sherry Chow, Contribution of presystemic hepatic extraction to the low oral bioavailability of green tea catechins in rats, Drug Metab. Dispos. 30 (2002) 1246–1249, https://doi.org/10.1124/dmd.30.11.1246.

[20] E. Pinho, M. Grootveld, G. Soares, M. Henriques, Cyclodextrins as encapsulation agents for plant bioactive compounds, Carbohydr. Polym. 101 (2014) 121–135, https://doi.org/10.1016/j.carbpol.2013.08.078.

[21] H. Dodziuk, Cyclodextrins and Their Complexes: Chemistry, Analytical Methods, Applications. 1st Edition. Wiley-VCH, Weinheim. 2006. https://doi.org/10.1002/3527608982.

[22] T. Aree, S. Jongrungruangchok, β-Cyclodextrin encapsulation elevates antioxidant capacity of tea: A closing chapter on non-epicatechins, atomistic insights from X-ray analysis, DFT calculation and DPPH assay, Carbohydr. Polym. 194 (2018) 24–33, https://doi.org/10.1016/j.carbpol.2018.04.016.

[23] P. Puligundla, C. Mok, S. Ko, J. Liang, N. Recharla, Nanotechnological approaches to enhance the bioavailability and therapeutic efficacy of green tea polyphenols, J. Funct. Foods 34 (2017) 139–151, https://doi.org/10.1016/j.jff.2017.04.023.

[24] T. Loftsson, M.E. Brewster, Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization, J. Pharm. Sci. 85 (1996) 1017–1025, https://doi.org/10.1021/js950534b.

[25] K. Athmouni, D. Belhaj, S. Gammoudi, A. El Feki, H. Ayadi, Nano-encapsulation using macrocyclic carbohydrate polymers (β-cyclodextrins) of Periploca angustifolia extract: Physical stability and protective effect against cadmium-induced alterations in HepG2 cells, Int. J. Biol. Macromol. 125 (2019) 711–720, https://doi.org/10.1016/J.IJBIOMAC.2018.12.044.

[26] E.I. Paramera, S.J. Konteles, V.T. Karathanos, Microencapsulation of curcumin in cells of Saccharomyces cerevisiae, Food Chem. 125 (2011) 892–902, https://doi.org/10.1016/j.foodchem.2010.09.063.

[27] H. Öztürk, U. Kolak, C. Meric, Antioxidant, anticholinesterase and antibacterial activities of jurinea consanguinea DC, Records Nat. Prod. 51 (2011) 43–51. https://www.researchgate.net/profile/Ufuk-Kolak.

[28] M. Ozgen, R.N. Reese, A.Z. Tulio, J.C. Scheerens, A.R. Miller, Modified 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2,2′-diphenyl-1- picrylhydrazyl (DPPH) methods, J. Agric. Food Chem. 54 (2006) 1151–1157, https://doi.org/10.1021/jf051960d.

[29] A. Shirwaikar, A. Shirwaikar, K. Rajendran, I.S.R. Punitha, *In vitro* antioxidant studies on the benzyl tetra isoquinoline alkaloid berberine, Biol. Pharm. Bull. 29 (2006) 1906–1910, https://doi.org/10.1248/bpb.29.1906.

[30] M. Oyaizu, Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine, Japan J. Nutr. Diet. 44 (6) (1986) 307–315.

[31] K. Athmouni, K.M. Hammi, A. El Feki, H. Ayadi, Development of catechin–phospholipid complex to enhance the bioavailability and modulatory potential against cadmium-induced oxidative stress in rats liver, Arch. Physiol. Biochem. 126 (2018) 82–88, https://doi.org/10.1080/13813455.2018.1493608.

[32] A. Bouattour, M. Fakhfakh, S. Abid, L. Paquin, H. Ammar, J.-P. Bazureau, Microwave mediated efficient syntheses of *N*-3 substituted 9-methoxy-4*H*- [1]-benzopyrano[2,3-*d*]pyrimidine-4(5*H*)-imine and formamidine derivatives from methyl 3-cyano-8-methoxy-4*H*-[1]-benzopyran-2-yl methanimidate, Synthesis (2017) 3768–3774, https://doi.org/10.1055/s-0036-1588825.

[33] A. Daina, O. Michielin, V. Zoete, SwissADME: a free web tool to. Evaluate pharmacokinetics, druglikeness and medicinal chemistry friendliness of small molecules, Nature Sc. Rep 7 (2017) 42717, https://doi.org/10.1038/srep42717.

[34] Swiss ADME of Swiss Institute of Bioinformatics. Accessed 9 September 2022. http://www.swissadme.ch/.

[35] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev. 46 (2001) 3–26, https://doi.org/10.1016/S0169-409X(00)00129-0.

[36] C.A. Lipinski, Lead- and drug-like compounds: the rule-of-five revolution, Drug Discov. Today Technol. 1 (2004) 337–341, https://doi.org/10.1016/j.ddtec.2004.11.007.

[37] A. Alex, D.S. Millan, M. Perez, F. Wakenhut, G.A. Whitlock, Intramolecular hydrogen bonding to improve membrane permeability and absorption in beyond rule of five chemical space, MedChemComm 2 (2011) 669–674, https://doi.org/10.1039/c1md00093d.

[38] C. Abad-Zapatero, A sorcerer's apprentice and the rule of five: from rule-of-thumb to commandment and beyond, Drug Discov. Today 12 (2007) 995–997, https://doi.org/10.1016/j.drudis.2007.10.022.

[39] B.C. Doak, B. Over, F. Giordanetto, F. Kihlberg, Oral druggable space beyond the rule of 5: Insights from drugs and clinical candidates, Chem. Biol. 21 (2014) 1115–1142, https://doi.org/10.1016/j.chembiol.2014.08.013.

[40] C.S. Mangolim, C. Moriwaki, A.C. Nogueira, F. Sato, M.L. Baesso, A.M. Neto, G. Matioli, Curcumin-β-cyclodextrin inclusion complex: Stability, solubility, characterisation by FT-IR, FT-Raman, X-ray diffraction and photoacoustic spectroscopy, and food application, Food Chem. 153 (2014) 361–370, https://doi.org/10.1016/j.foodchem.2013.12.067.

[41] Y. Wang, Z. Lu, F. Lv, X. Bie, Study on microencapsulation of curcumin pigments by spray drying, Eur Food Res Tech 229 (2009) 391–396, https://doi.org/10.1007/s00217-009-1064-6.

[42] M. Yan, B. Liu, X. Jiao, S. Qin, Preparation of phycocyanin microcapsules and its properties, Food Bioprod. Process 92 (2014) 89–97, https://doi.org/10.1016/j.fbp.2013.07.008.

[43] M.L. Castillo, R. Del, E. López-Tobar, S. Sanchez-Cortes, G. Flores, G.P. Blanch, Stabilization of curcumin against photodegradation by encapsulation in gamma-cyclodextrin: A study based on chromatographic and spectroscopic (Raman and UV-visible) data, Vibr. Spectr. 81 (2015) 106–111, https://doi.org/10.1016/j.vibspec.2015.10.008.

[44] J.M. López-Nicolás, A.J. Andreu-Sevilla, Á.A. Carbonell-Barrachina, F. García-Carmona, Effects of addition of α-cyclodextrin on the sensory quality, volatile compounds, and color parameters of fresh pear juice, J. Agric. Food Chem. 57 (2009) 9668–9675, https://doi.org/10.1021/jf901919t.
[45] S. Ho, Y.Y. Thoo, D.J. Young, L.F. Siow, Inclusion complexation of catechin by β-cyclodextrins: Characterization and storage stability, LWT 86 (2017) 555–565, https://doi.org/10.1016/j.lwt.2017.08.041.
[46] R.L. Prior, X. Wu, K. Schaich, Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements, J. Agric. Food Chem. 53 (2005) 4290–4302, https://doi.org/10.1021/jf0502698.
[47] I.N. Beara, M.M. Lesjak, D.D. Četojević-Simin, D.Z. Orčić, T. Janković, G. T. Anačkov, N.M. Mimica-Dukić, Phenolic profile, antioxidant, anti-inflammatory and cytotoxic activities of endemic Plantago reniformis G. Beck, Food Res. Int. 49 (2012) 501–507, https://doi.org/10.1016/j.foodres.2012.08.006.
[48] A. Cano, M. Acosta, M.B. Arnao, A method to measure antioxidant activity in organic media: application to lipophilic vitamins, Redox Rep. 5 (2000) 365–370, https://doi.org/10.1179/135100000101535933.
[49] B. Gidwani, A. Vyas, Pharmacokinetic study of solid-lipid-nanoparticles of altretamine complexed epichlorohydrin-β-cyclodextrin for enhanced solubility and oral bioavailability, Int. J. Biol. Macromol. 101 (2017) 24–31, https://doi.org/10.1016/j.ijbiomac.2017.03.047.
[50] M. Ahmad, P. Mudgil, A. Gani, F. Hamed, F.A. Masoodi, S. Maqsood, Nano-encapsulation of catechin in starch nanoparticles: Characterization, release behavior and bioactivity retention during simulated *in vitro* digestion, Food Chem. 270 (2019) 95–104, https://doi.org/10.1016/j.foodchem.2018.07.024.