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Nanoencapsulation of 3-Chloropropylaminobenzoate Derivatives with Potential Insecticidal Activity †

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Abstract: Aminobenzoic acid derivatives have shown various pharmacological properties, one of which is pesticide activity, giving these compounds the ability to work as alternatives to current pesticides. Nanotechnology could efficiently support the use of these compounds by encapsulating them in stable nanoformulations to improve their stability and effectiveness. In the present work, 3-chloropropylaminobenzoate derivatives were synthesised and evaluated against their effect on the viability of the insect cell line *Sf9* (*Spodoptera frugiperda*), and nanoencapsulation studies of the most active compound were carried out. The most potent molecules reduced insect cell viability by around 40% at 100 μg/mL.

Keywords: aminobenzoic acid derivatives; aminobenzoates; nanoencapsulation; *Sf9* (*Spodoptera frugiperda*); insecticides

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1. Introduction

According to the FAO (Food and Agriculture Organization), the world population will reach 9.1 billion by 2050, which represents a 34% increase in the current population [1] and, consequently, a 70% increase in food production will be required [1,2]. Agrochemicals have been fundamental to produce food as well as for the control of disease vectors [3]. To control weeds, insects, and various disease-carrying pest infestations in agricultural feeds, pesticides are widely used [3,4]. The intensive use of pesticides promotes an insecticide resistance that impacts the effectiveness and utility of pest protection compounds [5]. Thus, an alternative is necessary to develop pest control options, especially those with new mechanisms of action [3,5].

Aminobenzoic acid derivatives are of fundamental interest because different relative positions of the functional groups on the aromatic ring (*para*, *meta* and *ortho*) can produce significant differences in chemical properties. *ortho*-Aminobenzoic acid 9 (sometimes called anthranilic acid) and its analogues have a privileged profile as pharmacophores for the development of deliberate drugs for the management of pathophysiology and the pathogenesis of various diseases. The structure substitution of anthranilic acid provides a variety of compounds, which allow a comprehensive assessment of structure-activity relationship analysis for the identification of hits and leads in a typical drug development paradigm. Anthranilic acid derivatives exhibit interesting antimicrobial, antiviral, and insecticidal properties [6].

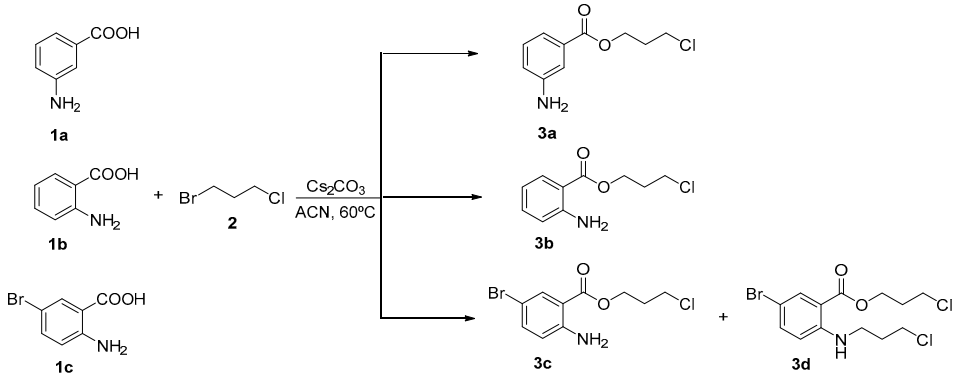
Nanostructured materials have recently received increasing attention due to their unique properties and wide range of vital applications [7,8]. Nanotechnology could efficiently support the use of several biologically active compounds by encapsulating them in stable nanoformulations, such as nano-emulsions, to improve their stability and effectiveness [9].

Taking these facts into account, the synthesis of 2- and 3-aminobenzoic acid derivatives were carried out in order to further evaluate them as potential alternative insecticides, and nanoencapsulation studies were performed with the most active compound.

2. Results and Discussion

2.1. Synthesis of Aminobenzoic Acid Derivatives **3a–d**

Esterification of the aminobenzoic acids **1a–c** with the intended alkyl bromide was carried out with cesium carbonate, since, compared to other alkali metal carboxylate salts, cesium salts have been shown to be especially efficient in esterification under non-aqueous conditions. Acetonitrile is a convenient reaction solvent due to its appropriate boiling point as well as its high dielectric constant and polar nature, which provides good solubility for the cesium carboxylate salts that are favorable for the esterification [10]. Thus, the reaction of 3-aminobenzoic acid **1a**, 2-aminobenzoic acid **1b**, and 2-amino-5-bromobenzoic acid **1c** with 1-bromo-3-chloropropane **2** was carried out in presence of cesium carbonate in acetonitrile at 60 ºC. After silica gel column chromatography purification, the corresponding esters derivatives, namely, 3-chloropropyl 3-aminobenzoate **3a**, 3-chloropropyl 2-aminobenzoate **3b**, and 3-chloropropyl 2-amino-5-bromobenzoate **3c** accompanied with 3-chloropropyl 5-bromo-2-((3-chloropropyl)amino)benzoate **3d**, respectively, were obtained in yields up to 41% (Scheme 1) and characterized by NMR (^{1}H and ^{13}C) spectroscopies.



Scheme 1. Synthesis of esters derived from amino benzoic acids **3a–d**.

In the ^{1}H NMR spectra, signals related to the methylene protons of the new substituent in all derivatives are shown as a quintet (δ 2.21–2.24 ppm) and triplets (δ 3.67–4.44 ppm). For compound **3d**, it was also visible the presence of methylene protons linked to the amine group, also as a quintet (δ 2.24 ppm) and triplets (δ 3.41–3.70 ppm). The presence of aromatic protons was detected by the presence of three signals for all derivatives (δ 6.32–7.86 ppm). The ^{13}C NMR spectra showed the carbons of the methylene groups for all derivatives (δ 31.65–61.51 ppm) as well as the aromatic carbons (δ 107.28–161.43 ppm). The confirmation of the presence of the newly formed ester linkage in all compounds was also supported by ^{13}C NMR spectra, which displayed signals of the carbonyl group (δ 166.54–167.39 ppm).

2.2. Toxicity of Aminobenzoic Acid Derivatives **3a–d**

The impact of aminobenzoic acid derivatives **3a–d** in the viability of *Sf9* cells was evaluated at 100 µg/mL, following 24 h of exposure. As shown in Figure 1, compound **3c**, containing, simultaneously, a bromine and an amine group in the benzen ring, was completely devoid of toxicity. On the other hand, the derivatives **3a**, **3b**, and **3d** elicited a significant reduction in viability, with compound **3b** being the most potent, causing ca. 40%

cell death. For this reason, **3b** was chosen for further nanoencapsulation assays, keeping in mind a future application as an insecticide.

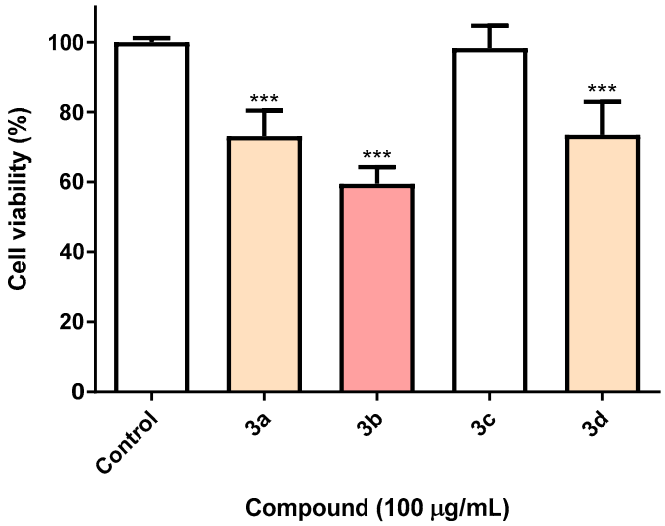


Figure 1. Viability of *Sf9* cells after incubation with the indicated molecules (100 μg/mL) for 24 h. *** $p < 0.001$.

2.3. Nanoencapsulation Studies

Before encapsulation, a preliminary study of the photophysical properties of compound **3b** was carried out by measuring its absorption and emission spectra in solution. This study was needed for determination of the encapsulation efficiency and release kinetics, and the results are displayed in Figure 2. The absorption spectrum shows two peaks, the first at 250 nm and the other at 340 nm. The fluorescence spectra revealed a band between 360 nm and 500 nm, with a maximum around 410 nm.

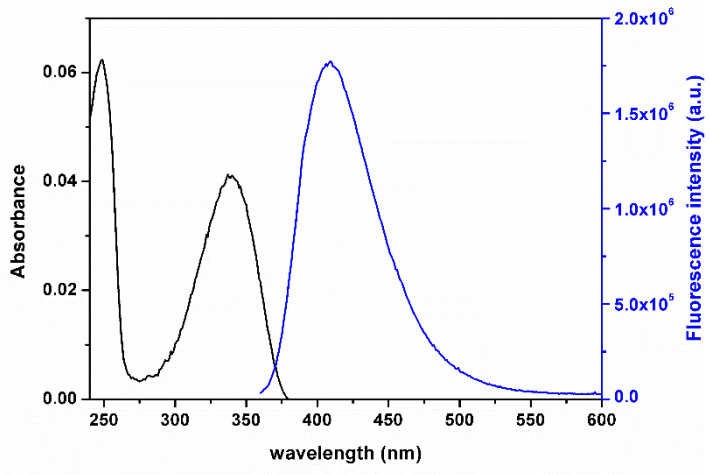


Figure 2. Absorption and fluorescence emission (excitation at 340 nm) spectra of compound **3b** in ethanol (1×10^{-5} M for absorption and 1×10^{-6} M for emission).

Compound **3b** was encapsulated into nanoliposomes of egg lecithin/cholesterol (Egg-PC:Ch 7:3), aimed at allowing an effective release of the loaded compound. A high encapsulation efficiency of 97.1% was obtained, showing that derivative **3b** can be efficiently encapsulated into the nanoliposomes. The structural characterization of the prepared loaded nanoliposomes was performed by DLS by measuring their hydrodynamic diameter, polydispersity index, and zeta potential. As expected, liposomes with the size of 82.4 ± 1.3 nm, PDI of 0.16 ± 0.02 and with a zeta potential of −4.75 ± 1.28 mV were ob-

tained, similarly to previous results obtained for the same formulation loaded with other compounds [11].

The release kinetic profile of compound **3b** from the nanoliposomes was determined during 24 h and the obtained experimental data is displayed in Figure 3. The data of the release profile were fitted to two kinetic models, the Weibull model, and the first-order model, and the obtained parameters are summarized in Table 1.

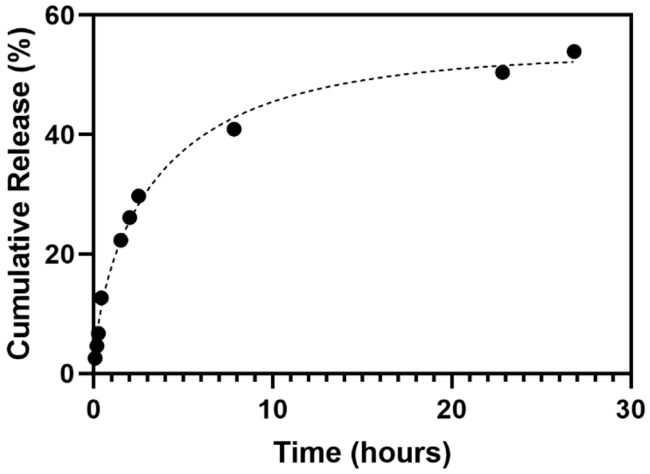


Figure 3. Cumulative release of compound **3b** from nanoliposomes of Egg-PC:Ch, fitted to the Weibull model.

Table 1. Parameters obtained by fitting the release profile to the first-order kinetic model and Weibull model, and the respective coefficients of determination (R^2).

First-Order		Weibull		
K (s^{-1})	R^2	b	a	R^2
0.38	0.97	0.41	0.67	0.99

A high release percentage of 54% was achieved, after 26 h, indicating that the Egg-PC:Ch formulation is suitable for the efficient release of compound **3b**. The Weibull model better fitted the experimental results, showing a higher coefficient of determination (Table 1). From the Weibull model fit, a Fickian diffusion is expected because the b value is below 0.75. The first-order model also fits the data quite well, although not as well as the Weibull model, allowing the determination of a rate constant of 0.38 s^{-1}.

3. Material and Methods

3.1. Typical Procedure for the Preparation of Compounds **3a–d** *(Illustrated for* **3b***)*

Cesium carbonate (2.38 g, 7.30 mmol) and 1-bromo-3-chloropropane **2** (0.173 mL, 2.56 mmol) were added to a solution of 2-aminobenzoic acid **1b** (0.200 g, 1.46 mmol) in acetonitrile (3 mL). The reaction mixture was stirred for 25 h at 60 °C, and was monitored by TLC (silica: dichloromethane/light petroleum ether 9:1). 3-Chloropropyl 2-aminobenzoate **3b** was obtained as a brown oil (0.098 g, 32%). Rf = 0.74 (dichloromethane). ^{1}H NMR δ_H ($CDCl_3$, 400 MHz): 2.22 (quint, J = 6.0 Hz, 2H, $OCH_2CH_2CH_2Cl$), 3.70 (t, J = 6.4 Hz, 2H, $OCH_2CH_2CH_2Cl$), 4.35 (t, J = 6.4 Hz, 2H, $OCH_2CH_2CH_2Cl$), 5.76 (s broad, 2H, NH_2), 6.63–6.68 (m, 2H, H-3 and H-5), 7.28 (dt, J = 8.8 and 1.6 Hz, 1H, H-4), 7.85 (d, J = 8.0 and 1.6 Hz, 1H, H-6) ppm. ^{13}C NMR δ_c ($CDCl_3$, 100.6 MHz): 31.65 ($OCH_2CH_2CH_2Cl$), 41.31 ($OCH_2CH_2CH_2Cl$), 60.86 ($OCH_2CH_2CH_2Cl$), 110.36 (C-1), 116.10 (C-3), 116.61 (C-5), 130.95 (C-6), 134.11 (C-4), 150.52 (C-2), 167.75 (C=O) ppm.

3.2. Biological Assays of Aminobenzoic Acid Derivatives **3a–d**

The potential of compounds **3a–d** were evaluated as biopesticides in assays using the *Sf9* (*Spodoptera frugiperda*) insect cell line. Cells were maintained at 28 °C and cultivated in Grace's medium with 10% FBS. For the evaluation of viability, cells were plated at 3.0×10^4 cells/well and exposed to the molecules, after which resazurin was added, resulting in being read at 560/590 nm after 60 min of incubation.

3.3. Nanoencapsulation and Release Studies of Compound **3b**

The ethanolic injection method was used for the preparation of nanoliposomes loaded with compound **3b** [12]. Liposomes of 1,2-diacyl-sn-glycero-3-phosphocholine from egg yolk (egg phosphatidylcholine, Egg-PC) and cholesterol (Ch) (70% Egg-PC and 30% Ch) were used [10]. Briefly, an ethanolic solution of Egg-PC:Ch (7:3) and compound **3b** was injected, drop-by-drop, into an aqueous solution, under vortexing. For the determination of the encapsulation efficiency, *EE* (%), Equation (1) was used,

$$EE(\%) = \frac{C_{total} - C_{non\text{-}encapsulated}}{C_{total}} \times 100 \quad (1)$$

where c_{total} is the compound concentration used for the preparation of liposomes and $c_{non\text{-}encapsulated}$ is the compound concentration that was not encapsulated into the nanoliposomes. The separation of the compound-loaded liposomes from the non-encapsulated compound was performed using Amicon® Ultra centrifugal filter units of 100 kDa by centrifugation at 3000 rpm for 10 min. The emission of the non-encapsulated compound (the filtrate part) was measured for the determination of its concentration using a previously obtained calibration curve of fluorescence intensity vs. concentration.

The release kinetic profiles of compound **3b** from the nanoliposomes were obtained using Amicon® centrifugal filters, in which the upper compartment was filled with the **3b**-loaded nanoliposomes and the bottom with water. For the determination of the cumulative release, aliquots of 200 µL were collected from the bottom part of the Amicon® and replaced with an equal volume of water for 24 h. The concentration of the released compound was determined by measuring the emission of the aliquots, and the experimental data were fitted to the Weibull model [13] and first-order model [14]. The Weibull model expresses the compound fraction accumulated (m) in solution at time t, following Equation (2),

$$m = 1 - e^{\left[-(t-T_i)^b/a\right]} \quad (2)$$

where a defines the timescale of the process, T_i is a location parameter representing the latency time of the release mechanism, and b parameter denotes the curve type shape. For $b > 1$, the transport follows a complex release mechanism; $b \leq 0.75$ indicates Fickian diffusion (in either fractal or Euclidian spaces), and $0.75 < b < 1$ indicates a combined mechanism (Fickian diffusion and Case II transport).

The first-order model follows Equation (3), in which $F(\%)$ and M_0 are the percentage and the total amount of the compound released, respectively, in which k represents the first-order rate constant, and in which t is time.

$$F(\%) = M_0 \times \left(1 - e^{-kt}\right) \quad (3)$$

The emission spectra were collected in a Fluorolog 3 spectrofluorometer (HORIBA Jobin Yvon IBH Ltd., Glasgow, UK), and the UV-Vis absorption spectrum was obtained in a Shimadzu UV-3600 Plus UV-Vis-NIR spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The structural characterization of the nanoliposomes was performed by Dynamic Light Scattering (DLS), using a Litesizer 500 equipment from Anton Paar (Anton Paar GmbH, Graz, Austria) with a solid-state laser of 648 nm and 40 mW. For the hydrodynamic diameter, polydispersity index, and zeta potential, three independent measurements were performed.

4. Conclusions

In the present work, four 3-chloropropylaminobenzoate derivatives were synthesized and used in biological studies against the *Sf9* cell line, with the aim of evaluating their potential as insecticides.

The encapsulation in liposomes of Egg-PC:Ch allowed a high encapsulation efficiency and an effective release of the most active compound **3b**, being a suitable formulation for this potential insecticide.

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