Studies of Encapsulation of a New Potential Antitumoral Indole Derivative in Nanoliposomes for Drug Delivery Applications

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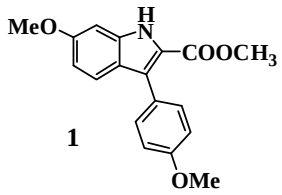
INTRODUCTION: Nanosized liposomes are among nanotechnological delivery methods for chemotherapeutic drugs in the treatment of cancer. This technique can potentially overcome many common pharmacologic problems, such as those involving solubility, pharmacokinetics, *in vivo* stability and toxicity. Liposomes are closed spherical vesicles consisting of a lipid bilayer that encapsulates an aqueous phase in which hydrophilic drugs can be stored, while water insoluble compounds can be incorporated in the hydrophobic region of the lipid bilayer [1,2]. In this work, a potential antitumoral fluorescent indole derivative **1**, previously synthesized by us [3], has been encapsulated in nanoliposomes of DPPC (dipalmitoyl phosphatidylcholine), egg-yolk phosphatidylcholine (Egg-PC) and dioctadecyl-dimethylammonium bromide (DODAB).



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Methyl 6-methoxy-3-(4-methoxyphenyl)-1*H*-indole-2-carboxylate

METHODS: Nanoliposomes were prepared by injection of an ethanolic solution of the lipid in an aqueous media under vigorous stirring, above the lipid melting transition temperature. Mean liposome size was measured by dynamic light scattering (DLS). The encapsulation of compound **1** in the nanoliposomes was assessed by fluorescence resonance energy transfer (FRET) between the fluorescent compound **1** and the fluorescent labelled phosphatidylethanolamine NBD-PE, included in the liposome formulation (with NBD-PE/lipid ratio of 1:250).

RESULTS: The nanoliposome hydrodynamic diameters, obtained by DLS, are 87 ± 11 nm for DPPC, 51 ± 2 nm for Egg-PC and 268 ± 37 nm for DODAB. All samples are monodisperse.

Antitumoral evaluation: The effect of compound **1** on the *in vitro* growth of three human tumor cell lines, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and a melanoma cell line (A375-C5), was evaluated after a continuous exposure of 48 h (Table 1).

*Table 1. Values of compound **1** concentration needed for 50% of cell growth inhibition (GI_{50})*

	GI_{50} (μM)		
	MCF-7	**NCI-H460**	**A375-C5**
1	0.37 ± 0.02	0.33 ± 0.03	0.25 ± 0.02

Fluorescence measurements showed the possibility of FRET between the electronic excited compound (acting as donor) and the labelled lipid NBD-PE (with NBD acting as energy acceptor). In Figure 1 it is possible to observe a strong NBD emission upon excitation of compound **1** incorporated in nanoliposomes.

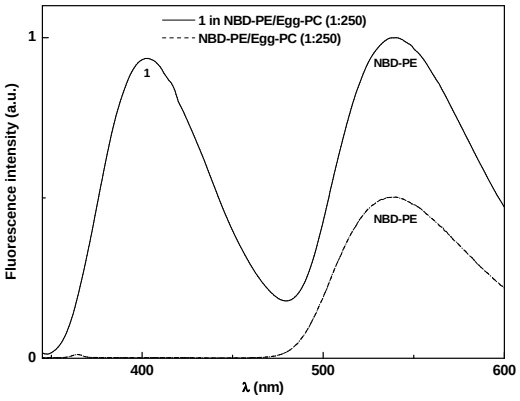

*Fig. 1: Fluorescence spectra of compound **1** in Egg-PC liposomes labelled with NBD-PE and labelled Egg-PC liposomes alone (λ_{exc}=325 nm).*

DISCUSSION & CONCLUSIONS: Compound **1** shows excellent antitumoral properties, exhibiting very low GI_{50} values in the three tumor cell lines. FRET assays indicate that compound **1** molecules and the NBD-labelled lipids are in close proximity in nanoliposomes. These results are important for drug delivery purposes, considering the antitumoral properties of compound **1**.

REFERENCES: [1] T.L. Andresen et al (2005) *Prog Lipid Res* **44**:68-97. [2] Y. Malam et al (2009) *Trends Pharmacol Sci* **30**:592-599. [3] M.-J.R.P. Queiroz et al (2007) *Tetrahedron* **63**:2215-2222.

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