Studies of encapsulation of new antitumoral fluorescent compounds in nanoliposomes for drug delivery purposes

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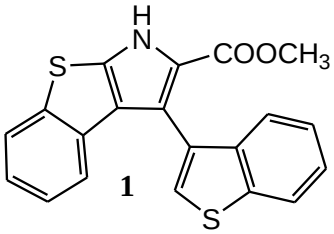
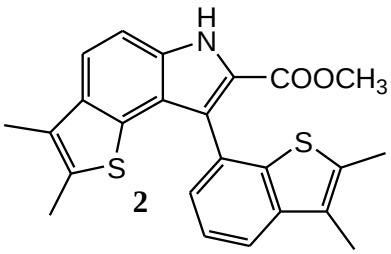
Nanosized liposomes are among nanotechnological delivery methods for chemotherapeutic drugs in the treatment of cancer. This nanotechnology can potentially overcome many common pharmacologic problems, such as those involving solubility, *in vivo* stability, pharmacokinetics, tumor uptake and toxicity [1,2]. 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 [3].

In this work, new synthetic fluorescent antitumoral compounds **1** and **2** [4] were encapsulated in nanosized liposomes of DPPC (dipalmitoyl phosphatidylcholine), egg lecithin (phosphatidylcholine from egg yolk) and DODAB (dioctadecyldimethylammonium bromide). The phospholipids DPPC and egg lecithin (Egg-PC) are neutral components of biological membranes, while cationic liposomes based on the synthetic lipid DODAB have been used as vehicles for DNA transfection and drug delivery [5].



1

Methyl 3-(benzo[*b*]thien-3-yl)benzothieno[2,3-*b*]pyrrole-2-carboxylate



2

Methyl 8-(2,3-dimethylbenzo[*b*]thien-7-yl)-2,3-dimethyl-*6H*-thieno[2,3-*e*]indole-7-carboxylate

Monodisperse and nanosized liposomes were prepared by injection of an ethanolic solution of the lipid in an aqueous media under vigorous stirring, above the lipid melting transition temperature (T_m ~ 41 ºC for DPPC and 45 ºC for DODAB). The hydrodynamic diameters of 87 ± 11 nm for DPPC, 51 ± 2 nm for Egg-PC and 268 ± 37 nm for DODAB were obtained by dynamic light scattering.

The effect of compounds **1** and **2** on the *in vitro* growth of three human tumor cell lines, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268), was evaluated after a continuous exposure of 48h. Values of compound concentrations able to inhibit 50% of cell growth (GI_{50}) are shown on Table 1. The benzothieno[2,3-*b*]pyrrole **1** showed the best results, exhibiting lower GI_{50} values than compound **2** in the three tumor cell lines, being significantly more potent against the MCF-7 and NCI-H460 tumor cell lines.

Table 1 . Effect of compounds **1** and **2** on the growth of three human tumor cell lines

Compounds	GI_{50} (μM)		
	MCF-7	**NCI-H460**	**SF-268**
1	7.9 ± 0.1	7.9 ± 1.8	14.1 ± 3.0
2	20.1± 16.7	16.7 ± 8.6	16.5 ± 9.4

Results represent means ± SEM of 3-4 independent experiments performed in duplicate. Doxorubicin was used as positive control, GI_{50}: MCF-7 = 42.8 ± 8.2 nM; NCI-H460 = 94.0 ± 8.7 nM and SF-268 = 94.0 ± 7.0 nM.

The intrinsic fluorescence of compounds **1** and **2**, due to its high sensitivity and dependence on the solvent, was used to obtain information about compounds location in the nanoliposomes prepared. Fluorescence emission and anisotropy measurements were performed below (gel phase) and above (liquid crystalline phase) the lipid transition temperature (figure). The results indicated that compound **1** preferential location is at the lipid bilayer, near the polar head groups, while compound **2** prefers generally a more fluid environment. These encapsulation experiments are relevant for further studies involving drug delivery applications in cancer treatment.

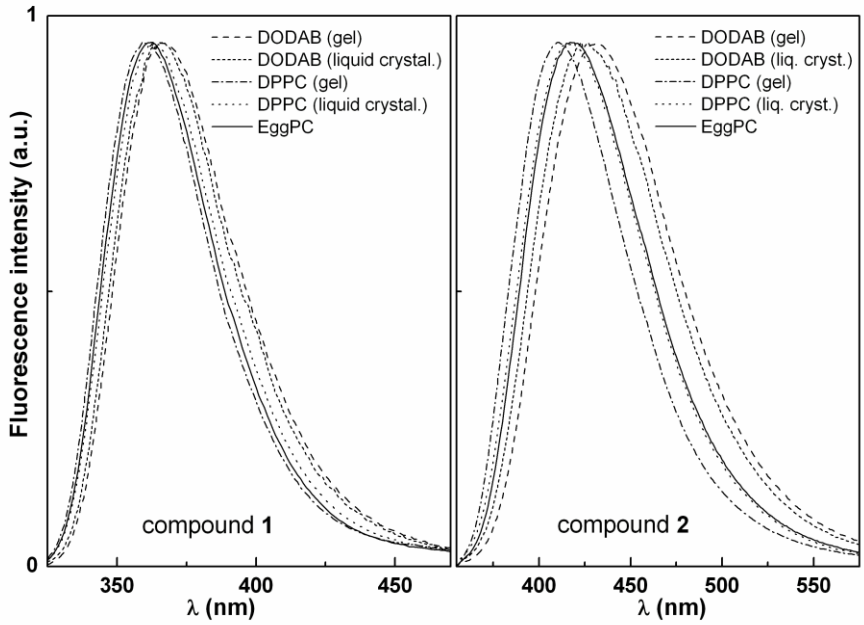


Figure: Normalized fluorescence emission spectra of compounds **1** and **2** incorporated in nanoliposomes of DPPC, Egg-PC and DODAB.

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