

Water Soluble PEGylated phenothiazines. Synthesis, Characterization and Antitumor Properties [†]

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Abstract: The paper reports a series of three new PEGylated phenothiazine derivatives which keep the potential of valuable building blocks for preparing bio-materials. PEGylated phenothiazine derivatives were prepared by grafting PEG chains to the phenothiazine core by three different linkers: ether, ester or amide units. The structure of the targeted molecules was confirmed by FTIR and NMR spectra. The capacity of the synthesized compounds to self-assemble in water was studied by DLS and UV-vis techniques, which allowed finding the hydrodynamic diameter of the aggregates. The particularities of the formed aggregates were investigated by fluorescence spectroscopy, SEM, AFM, POM and UV light microscopy. Their biocompatibility was assessed on normal human dermal fibroblasts and five human cancer cell lines. The synthesized compounds showed the formation of luminescent aggregates which proved excellent biocompatibility on normal cells. A concentration dependent cytotoxicity against HeLa cancer cell lines was noticed for the PEGylated phenothiazine containing an ester unit and for direct PEGylated phenothiazine.

Keywords: phenothiazine; poly(ethylene glycol); luminescence; biocompatibility; antitumor activity

1. Introduction

Phenothiazine is a valuable building block for the synthesis of a wide range of compounds used in bio-medicine. Besides their neuroleptic activities [1], phenothiazines are used in the synthesis of many classes of drugs, such as bactericides, fungicides, antitumor, antiviral, anti-inflammatory, antimalarial, antifilarial, trypanocidal, anticonvulsant, analgesic and immunosuppressive agents [2]. In cancer treatment, phenothiazine based derivatives presented promising results as adjuvants and as inhibitors of Farnesyltransferase [3], Tubulin polymerization [4], etc.

Because of their low solubility in water, the biological activity of phenothiazine based drugs is limited [5]. Up to now, phenothiazine derivatives used in cancer treatment were administrated as ammonium salts to be water soluble [6]. However, besides the cytotoxicity against cancer cells, the cytotoxicity against normal cells wasn't declare.

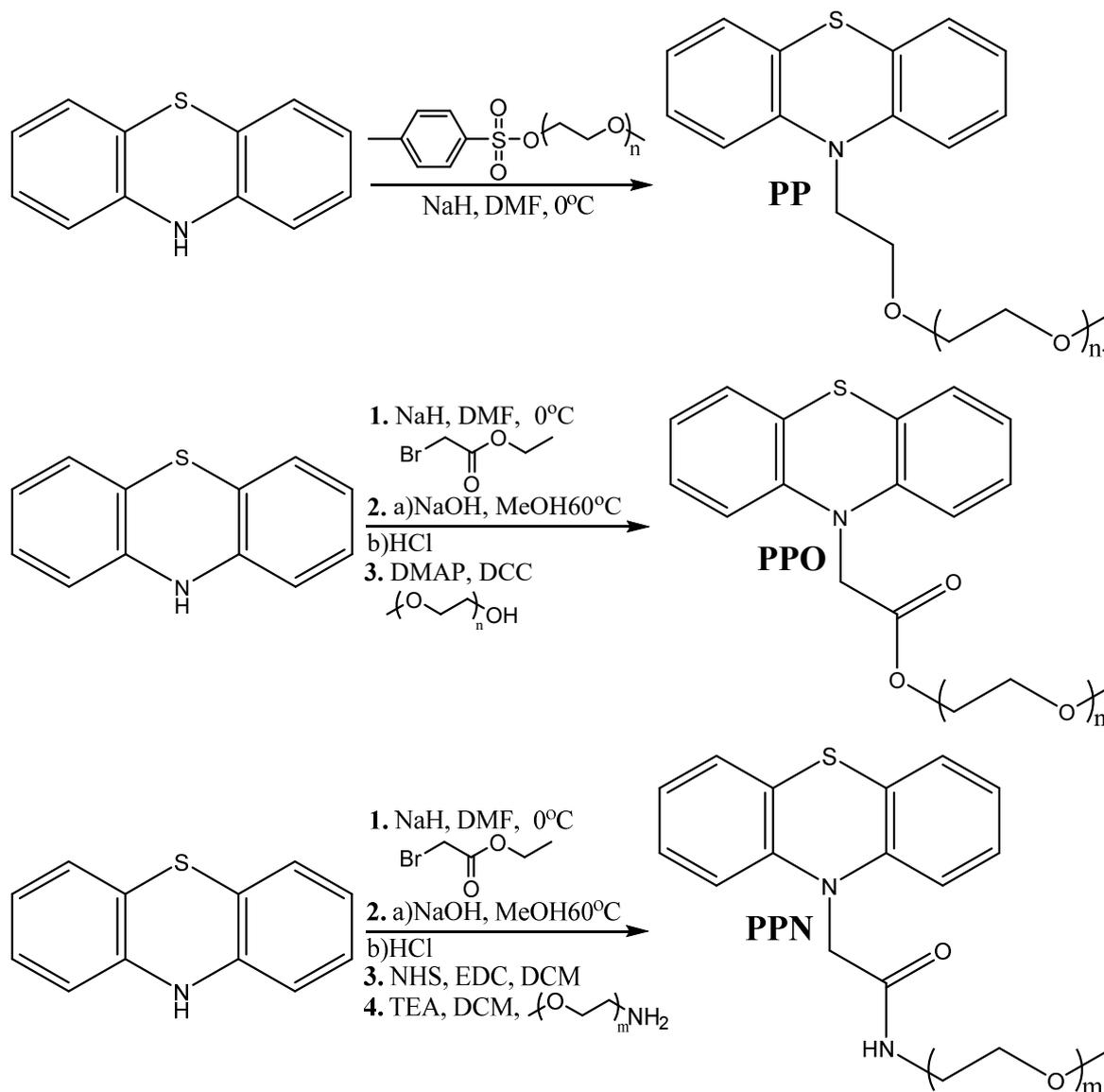
In this work our attention was focused on synthesis of three new phenothiazine derivatives by grafting a poly(ethylene glycol) chain at the nitrogen atom of phenothiazine core, using three different synthesis strategies to obtain biocompatible, low toxic and water soluble phenothiazine derivatives. The poly(ethylene glycol) unit was chosen because it is a water soluble, biocompatible FDA approved synthetic polymer, frequently used in bioapplication, especially for drug formulation [7]. The new synthesized derivatives showed an amphiphilic behavior, due to their hydrophobic-hydrophilic

designe. Their aggregation ability and time stability was studied and biological activity on normal and cancer cells.

2. Experiments

2.1. Materials and Methods

2.1.1. Synthesis



Scheme 1. Synthesis of the PEGylated derivatives.

3. Results

3.1. Synthesis

Three PTZ derivatives were obtained using three different synthetic routes. The first derivative was obtained by direct alkylation of a tosylated poly(ethylene glycol)PEG chain resulting the (PP) compound. The other two were synthesized by grafting the PEG chain via an ester function (PPO), and an amide function (PPN), respectively (Scheme 1).

The successful synthesis of the compounds was confirmed by FTIR and NMR spectroscopy. The FTIR spectra (Fig. 1a) displayed the characteristic vibrations of the main groups present in the final

compounds. The NMR spectra (Figure 1b) showed the disappearance of the chemical shifting characteristic to the hydrogen linked to the nitrogen atom of phenothiazine, and chemical shifting characteristic to the new synthesized compounds in the right ratio of their integrals.

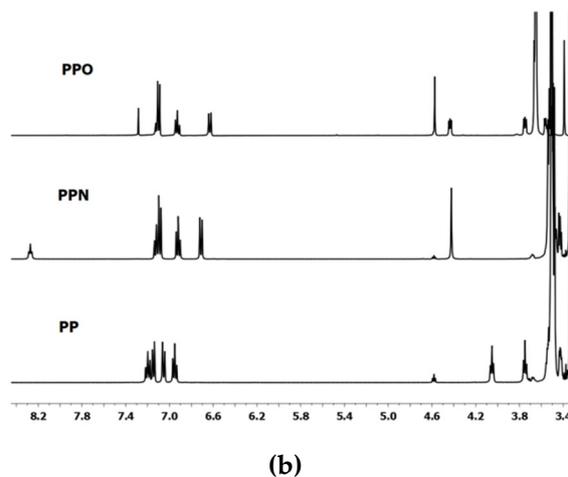


Figure 1. (a) FTIR spectra of the PEGylated phenothiazine derivatives, (b) ¹H NMR spectra of the PEGylated derivatives.

3.2. Photophysical Behavior

The photophysical behavior of the compounds was investigated by UV-vis spectroscopy in comparison with the pristine **PTZ**. The compounds absorption spectra (Figure 2a) showed the two absorption bands from phenothiazine, with the difference that the second one is bathochromic shifted with 25 nm. This is a consequence of aggregate formation, due to the amphiphilic nature of the compounds.

On the other side, the samples were able to emit blue light under UV lamp illumination (Figure 3d). The recorded emission spectra (Figure 3b) confirmed the visual observations by the presence in the spectra of a band with a maximum in the blue region at 450 nm.



Figure 2. (a) UV-vis absorption spectra of PEGylated compounds and PTZ and (b) photoluminescence spectra of PEGylated compounds in water. Compounds water solution under (c) natural light and (d) UV light.

3.3. Self-assembling Behavior

The UV-vis findings, according to which the compounds are able to self assemble into aggregates, were confirmed by DLS (Figure 3) measurements.

In all cases the aggregates were nanometric with a mean diameter of 200 nm and a quite low dimensional polydispersity. The morphological investigations by SEM, AFM and POM techniques (Figure 4), demonstrated the spherical shape of the aggregates and their uniformity.

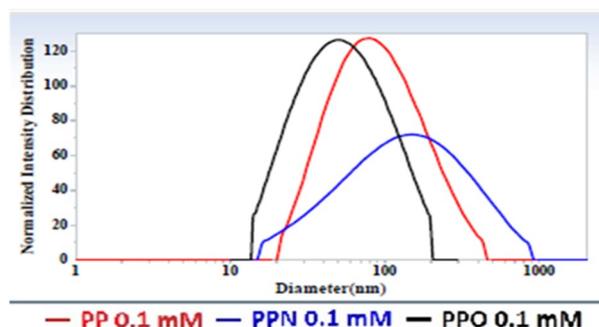


Figure 3. DLS graphs of the studied compounds.

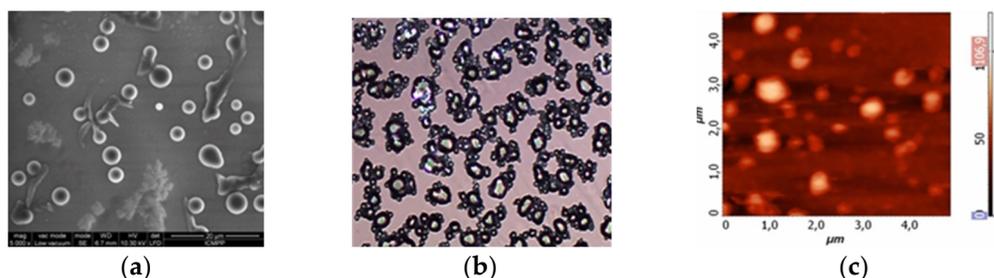


Figure 4. (a) SEM (b) POM images of the PP embedded into a solid PVAB matrix and (c) AFM images of the PP in pure form.

3.4 In vitro Biocompatibility

All three compounds presented a good biocompatibility on Normal Human Dermal Fibroblast (NHDF) cells for concentrations up to 0.1 mM, while for PPN the concentration increased up to 1 mM (Figure 5).

The PP and PPO presented a good antitumor activity on Human Cervical Cancer (HeLa) cells at concentration 0.1 mM, with a relative cell viability of 58 % for PP and 34 % for PPO (Figure 5).

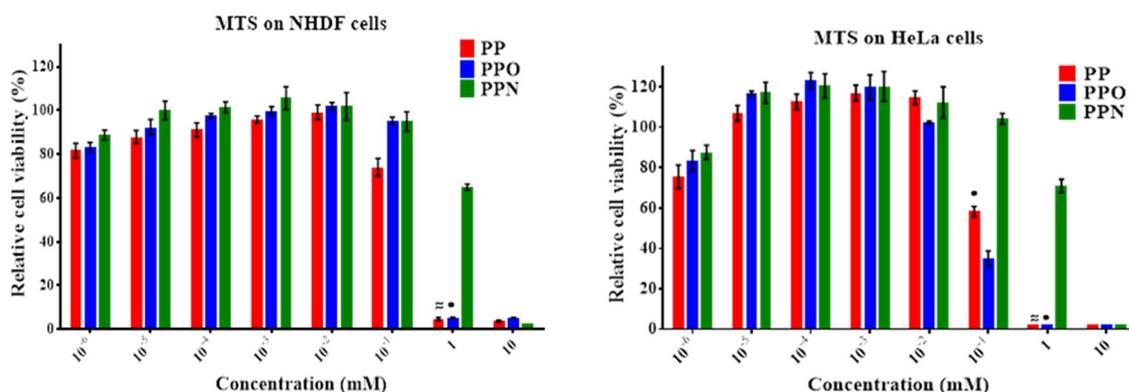


Figure 5. Cell viability on NHDF and HeLa cells.

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