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Preparation and in vitro biological evaluation of tetrapyrrole ethanolamide derivatives as potential anticancer agents

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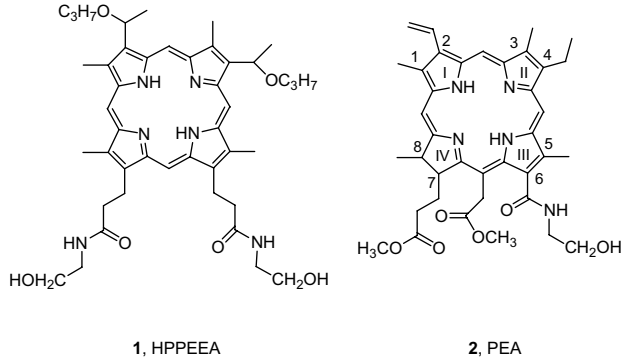
Abstract—Tetrapyrrole ethanolamide derivatives, **1** and **2**, were prepared from hematoporphyrin IX (HPIX, **3**) and methyl pheophorbide *a* (mPheo, **6**). These were evaluated for their dual action as chemotherapeutics and photosensitizers in treatment of cancer. The novel compounds showed significant in vitro anticancer activity as measured in different cell lines using the MTT assay and photodynamic activity measured by erythrocytes' photohemolysis.

The cyclic tetrapyrrole based dyes (e.g. porphyrins) have a potential use in medical applications, not only as a disease diagnostic tool, due to their fluorescence properties, but also as anticancer agents due to their photosensitization properties.[1] Photodynamic therapy (PTD) is a process that uses photosensitizer (PS) molecules that are capable of light absorption and generation of reactive oxygen species (ROS) such as singlet oxygen.[2] After administration of PS followed by irradiation with light the tumor cells are damaged. For examples, porphyrins, Photofrin®, Foscan®, and Visudyne® are clinically approved photosensitizers used in PTD to treat cancer and other diseases.[3]

The nitrogen mustards are a diverse class of compounds which share a common structural moiety and form a family of drugs useful in cancer therapy.[4] The principal antitumor reactivity of nitrogen mustards involves their interaction with DNA molecules.[5] A S_N2 type nucleophilic attack by the N-7 of the guanine base of DNA on the aziridinium ion intermediate (the active intermediate of N-mustard drugs) leads to the alkylation of DNA.[6,7] The result of the nitrogen mustard binding to the guanine is a defect in the DNA strand ultimately leading to cell death. A cross-link between two guanine bases on adjacent DNA strands can also occur if the nitrogen mustard contains two alkylating moieties. Rapidly proliferating cells, such as those found in neoplastic tissues, are the most sensitive to these DNA cross-linking agents, thus some selective tumor toxicity can be achieved.

Keeping all this in view, we have prepared two tetrapyrrole derivatives, hematoporphyrin propylether ethanolamide (HPPEEA, **1**) and pheophorbide *a* ethanolamide (PEA, **2**) (Fig. 1) from hematoporphyrin IX (HPIX, **3**) and methyl pheophorbide *a* (mPheo, **6**), respectively. The former derivatives were designed to incorporate an ethanolamide moiety, a known pharmacophore present in biologically active derivatives showing anticancer activity.[8] Moreover, the ethanolamide function was



1, HPPEEA **2**, PEA

Figure 1. Structure of hematoporphyrin propylether ethanolamide (HPPEEA, **1**) and pheophorbide *a* ethanolamide (PEA, **2**).

Keywords: Photodynamic therapy; Hematoporphyrin; Pheophorbide ethanolamide; Hematoporphyrin propylether ethanolamide.
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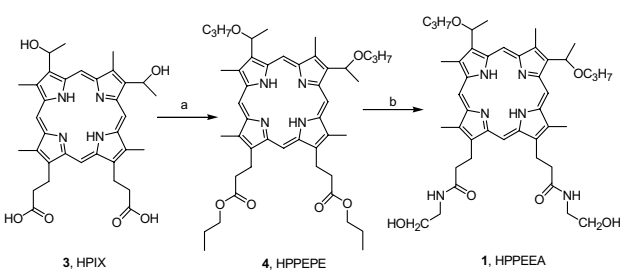
selected as it presents some structure similarities with the nitrogen mustards. Thus, compounds **1** and **2** could show interesting biological properties as dual action anticancer molecules. Consequently, these compounds were evaluated as chemotherapeutics as well as photosensitizers for the treatment of several types of cancers. The precursor derivatives, HPIX (**3**) and methyl pheophorbide *a* (mPheo, **6**), bearing no ethanolamide moiety were also tested on the same cancer cell lines in order to properly evaluate the real cytotoxic effects of the ethanolamide moiety on the final products, derivatives **1** and **2**.

Hematoporphyrin IX (**3**) was commercially available while methyl pheophorbide *a* (**6**) was obtained after isolation of chlorophyll *a* (**5**) from fresh spinach. The pheophorbide natural product was chosen because of its ease of preparation and its great capacity of singlet oxygen production (one of the most powerful ROS).

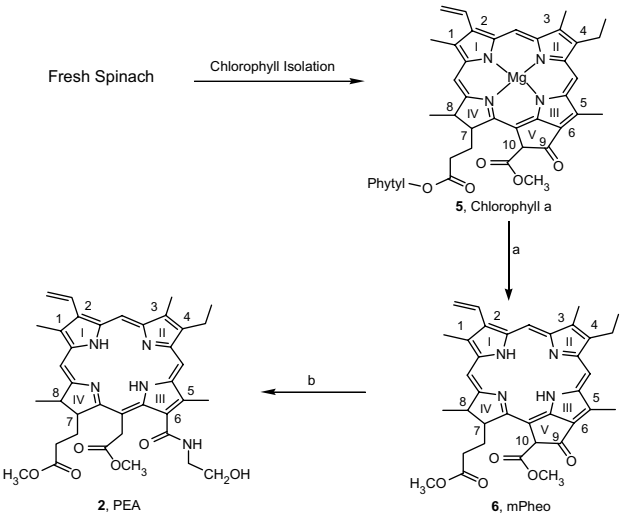
The synthesis of compound **1** (HPPEEA) was carried out starting from HPIX (**3**) (Scheme 1). Alkylation of **3** was done under strong acidic condition which yielded compound **4** (hematoporphyrin propylether-propylester, HPPEPE) in good yield. Condensation of compound **4** with ethanolamine in dioxane at reflux gave compound **1** (HPPEEA) in 76% isolated yield.

The synthesis of compound **2** was started from methyl pheophorbide *a* (**6**) which was initially obtained from chlorophyll *a* (**5**) isolated from fresh spinach (Scheme 2).[13] For this, chlorophyll *a* (**5**) was stirred for 2 h with acidic methanol at room temperature under an inert atmosphere of nitrogen which yielded methyl pheophorbide *a* (**6**) in good yield. The condensation of methyl pheophorbide *a* (**6**) with ethanolamine in dioxane was achieved with stirring at room temperature under dry nitrogen that gave compound **2** (PEA) in 45% yield upon ring opening of cyclopentenone ring (ring V). This ring opening is known in the literature[9].

The various compounds were characterized by the use of IR and NMR spectroscopy. In addition, the purity of methyl pheophorbide *a* (**6**) and its ethanolamide derivative PEA (**2**) was further assessed by HPLC which showed, in both cases, a purity exceeding 95%. This level of purity is comparable to other commercially available porphyrin products (Porphyrin Products, Logan, Utah, USA) that we tested.



Scheme 1. Reagents and conditions: (a) dry propanol, H^+, heat; (b) 2-aminoethanol, dioxane, reflux.



Scheme 2. Reagents and conditions: (a) H^+, CH_3OH, 21 °C; (b) 2-aminoethanol, dioxane, 21 °C.

The cytotoxic activity of the compounds was evaluated using the MTT cell proliferation assay.[10–12] The MTT assay was performed over an incubation period of 96 h under deem light to avoid decomposition. Different human neoplastic cell lines were tested to evaluate the chemotherapeutic activity of the ethanolamide derivatives HPPEEA (**1**), PEA (**2**) and their parent compounds, HPIX (**3**) and mPheo (**6**). Adriamycin, a chemotherapeutic drug of known activity, was used as a positive control and polyhematoporphyrin (pHP) which is a noncytotoxic PDT drug was used as a negative control. Table 1 gives the cell survival of various neoplastic cell lines at various drug concentrations. These are expressed as a percentage of untreated control cells (blank) which indicates that 100% survival indicates a noncytotoxicity situation.

For Adriamycin, the positive control, cytotoxicity starts at 0.01 μM in some of the cells and at 100 μM the survival in all cell lines was near 0%. pHP, the negative control, showed some cytotoxicity in the cell lines at about 50 μM and at the highest concentration (100 μM) the maximum response was about 20–60% cell survival. The drug concentrations required to cause a 50% survival (IC_{50}) are presented in Figure 2. These results confirm that Adriamycin is highly toxic, while pHP is essentially noncytotoxic.

HPIX (**3**) exhibited very little cytotoxic activity (Table 1 and IC_{50} in Fig. 2). However, at the highest concentration tested (100 μM) HPIX showed a little cytotoxicity. For example, the cell line K562 showed 30% survival. HPPEEA (**1**) exhibited significant cytotoxic activity (Table 1 and IC_{50} in Fig. 2). When 20 μM of HPPEEA was added to the cell lines, the survival dropped to near 10%. Below 20 μM, very little cytotoxicity is observed (Fig. 2).

Methyl pheophorbide *a* (mPheo, **6**) showed very little cytotoxicity (Table 1 and IC_{50} in Fig. 3). At the highest concentration of mPheo (100 μM) tested, the cell survival was near 80%. PEA (**2**) exhibited significant