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Research paper

Glucose-functionalized amino-OPEs as biocompatible photosensitizers in PDT

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ABSTRACT

Photodynamic therapy (PDT) is a minimally invasive procedure that can provide a selective eradication of neoplastic diseases by the combined effect of a photosensitizer, light and oxygen. New amino oligo(phenylene-ethynylene)s (OPEs), bearing hydrophilic glucoside terminations, have been prepared, characterized and tested as photosensitizers in PDT. The effectiveness of these compounds in combination with UVA light has been checked on two tumor cell lines (HEp-2 and HeLa cells, derived from a larynx carcinoma and a cervical carcinoma, respectively). The compounds triggered a mitotic blockage that led to the cell death, being the effect active up to 3 μm concentration. The photophysical properties of OPEs, such as high quantum yield, stability, singlet oxygen production, biocompatibility, easy cell-internalization and very good response even at low concentration, make them promising photosensitizers in the application of PDT.

1. Introduction

Phototherapy is a minimally invasive therapeutic procedure already approved for the treatment of various oncological and non-oncological pathologies of skin [1,2]. Variants of phototherapy include targeted ultraviolet B (UVB) phototherapy, topical psoralen plus ultraviolet A (PUVA), and photodynamic therapy (PDT). All of them are localized forms of phototherapy, either as first-line treatment or as complementary treatment for those that do not respond to other topical treatments [3,4]. PDT is based on the specific accumulation of a photosensitizer (PS) in the target tissue, followed by irradiation with light at a wavelength matching the absorption spectrum of the PS. Upon light absorption, the PS transits from its ground state to an unstable excited singlet state, from which it can decay, either to the ground-state or to the excited triplet state. In this long-lived excited triplet state, the PS is able to produce singlet oxygen ($^1O_2$) which, in turn, gives rise to other reactive oxygen species (ROS), such as peroxides, superoxide anions or hydroxyl radicals. Taking into account that $^1O_2$ has a lifetime of approximately 40 ns, it acts near to the site where it is generated. ROS are able to react directly with a biological substrate, oxidizing vital cellular components inducing an acute cell stress response culminating in cellular death, mainly by apoptosis and/or necrosis. The cell death pathway depends, among other factors, on the nature and intracellular localization of the PS and on tumor properties [1,5].

Due to their selectivity towards tumor tissue in PDT, porphyrins and porphyrin-related macrocycles are at the forefront of PDT [6]. Several compounds belonging to a first generation of PSs, such as the hematoporphyrin derivative HpD (Photofrin) [1,5], are already

Abbreviations: OPE, oligo(phenylene ethynylene); PDT, phothodynamic therapy; PS, phothosensitizer; UV, ultraviolet light; UVA, ultraviolet light A; UVB, ultraviolet light B; PUVA, psolaren plus ultraviolet A therapy; ROS, reactive oxygen species; LD50, lethal dose 50%; LD90, lethal dose 90%; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; AO-EtBr, acridine orange-ethidium bromide; MTs, microtubules.

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in clinical use or in clinical trials to treat cancer patients and are approved by the Food and Drug Administration for the palliative treatment of obstructive lung and esophageal cancers. A second-generation of PSs, with improved pharmacokinetics and reduced skin photosensitivity, includes aminolevulinic acid (ALA; a pro-PS), widely used for the treatment of non melanoma skin cancer (NMSC), benzoporphyrin derivative (BPD), silicon phthalocyanine (Pc4) or *m*-tetrahydroxyphenylchlorin (mTHPC) [1,2]. In the case of PUVA, psoralens are excited by UVA light at suberythemogenic doses and have been used to treat determined skin diseases, including psoriasis, vitiligo and mycosis fungoides [7–9].

Photosensitizers based on amphiphilic skeletons, bearing lipophilic and hydrophilic moieties, are in continuous development due to their biological applications. For instance, symmetric and asymmetrically substituted phthalocyanines bearing hydrophilic D-galactose units have shown to be efficient PSs towards HeLa cells [10], while glucose-appended Iron(III) complexes caused HeLa cells apoptosis by the generation of ROS on irradiation [11].

The ideal photosensitizers [12] should possess high photoactivity and selective photocytotoxicity for sick skin cells but low dark toxicity, activation at discrete wavelengths, efficient and fast distribution and elimination from tissues and chemical and physical stability. Although a significant number of PS are synthesized each year, the development of new targets that fulfill all the requirements continues to be an appealing area of research in phototherapy [13]. Oligo(phenylene-ethynylene)s (OPEs) are luminescent linear oligomers with extended conjugated aromatic and ethynylenic moieties. Due to their intriguing optical and electronic properties [14], OPEs have lately gained a prominent role in the design of organic materials such as sensing or electronic organic devices. On the other hand, there are only few examples of their use in biological fields. For instance, OPEs incorporating cationic ends have been shown to behave as light-activated biocides, killing some specific kind of bacteria, as a result of the production of singlet oxygen after irradiation [15]. Galactose-functionalized oligomers have been shown to act as inhibitors of *Pseudomonas aeruginosa* lectin LecA [16]. *N*-Hydroxy succinimidoyl esters incorporated to OPEs were reported to behave as luminescent labeling probes for proteins [17]. Aryleneethynylene compound bearing two polar sulfonate groups has been reported as effective fluorescent markers for liposomes and mammalian cell membranes [18]. In spite of the encouraging electronic properties displayed by the OPEs, to our knowledge, there are no precedents of their use as photosensitizers in photodynamic therapy.

Following our biological studies of carbohydrate based conjugated systems [19–21], we recently prepared several end-glucose functionalized OPEs and reported their ability to permeate the cellular cancer membrane and to localize in cytoplasmic organelles [22]. Few structural features were found to be crucial to reach an efficient cytoplasmatic OPE dye: three aryl ethenyl conjugated units, two hydrophilic glucose molecules at both ends of the OPE skeleton and, a dimethylamino substituent at the central aryl ring of the conjugated system (see compound **1** in Fig. 1). Preliminary biological evaluation tests demonstrated that these OPE derivatives were non-cytotoxic. Taking into account these precedents, we decided to study the behavior of two OPE derivatives, as biocompatible photosensitizers targets in PDT (Fig. 1). Herein we report full details of the preparation of end-glucose OPE derivative **1** and a new derivative **2**, the photophysical studies and *in vitro* toxicity, either in the absence of light or under light irradiation, against HeLa and HEp-2 tumor cell lines. The presence of hydrophobic (aryl conjugated fragments) and hydrophilic (glucose) moieties in these structures was expected to facilitate cellular membrane permeation. Moreover, the terminal glucose molecules were chosen considering the enhanced glycolytic process of cancer carbohydrate metabolism (Warburg effect) [23].

2. Results and discussion

2.1. Chemistry

The target dimethylamino-derived OPE glucosides **1** and **2**, having one or two *N,N*-dimethylamino substituted aryl moieties respectively, are depicted in Fig. 1.

The synthesis of compound **1** was completed following a strategy previously reported by us [22], based on copper-free Sonogashira type coupling to assemble the triple bonds to the adequately substituted aromatic rings, in a convergent manner (Scheme 1). Thus, compound **1** was prepared from the key intermediate *N,N*-dimethyl-2,5-bis[(trimethylsilyl)ethynyl] aniline **4**, which allowed a double Pd (0) catalyzed cross-coupling reaction with iodo aryl derivative **7**, incorporating the 2,5-dimethoxy aryl substituents as well as the acetylated glucoside methylene ethynyl moiety, leading to the final carbon skeleton in one step. Precursor **4** was prepared in 55% isolated yield, using a Pd(0) cross-coupling reaction between *N,N*-dimethyl-2,5-dibromoaniline **3** and an excess of ethynyltrimethylsilane, whereas compound **7** was efficiently formed by Pd mediated cross-coupling of 1,4-diiodo-2,5-dimethoxybenzene **5** [24] and tetraacetylpropynyl glucoside **6** [25]. The use of 2 equivalents of the diiododerivative **5** was essential to achieve a high yield for the preparation of **7**. Inspired by the work of Mori [26], and Naso [27] we successfully achieved the cross-coupling reaction between **4** and **7** (2 equiv) in 61% yield using $[Pd(PPh_3)_4]$ as catalyst in the presence of Ag_2O, which was known to act as activator facilitating coupling between aromatic iodides and silane derivatives. Final deprotection of acetylated glucoside moieties gave the desired gluco-OPE **1**.

Preparation of gluco-OPE **2** is based on a convergent strategy that allows a double assembly of a gluco-2-propynyl aryl ethynylene framework with the aryl central core 1,4-diiodo-2,5-dimethoxy benzene **5** (Scheme 2). It is noteworthy to mention that the Pd(0) mediated coupling reaction of **6** and 2,5-dibromo aryl derivative **3**, [28] in an appropriate molecular ratio (1:1), exclusively led to the mono substituted regioisomer **8**, [29] resulting from reaction on the less sterically hindered 5-bromo substituent situated *meta* to the bulky dimethylamino group of **3**, in a 60% yield. The palladium catalyzed cross-coupling reaction between **8** and an excess of commercially available trimethylsilylacetylene gave rise to the key intermediate **9** in high yield under very mild conditions. The final Ag_2O-modified Pd(0) mediated coupling [26,27] between **9** (2 equiv) and 1,4-diiodo-2,5-dimethoxy benzene **5** allowed a double assembly that directly lead to the desired carbon skeleton of OPE **10**. All the newly synthesized intermediates **8**, **9** and **10** could be purified by conventional flash column chromatography and are stable and easy to handle. Finally, quantitative deacetylation of **10** in the presence of an excess of aqueous ammonia gave the gluco-OPE **2** as a brilliant yellow solid, easily separated from residual acetamide by subsequent washings with MeOH, in almost quantitative yield.

2.2. Photophysical properties

The absorption and photophysical data of the final OPE glucosides **1** and **2** in aqueous solution are summarized in Table 1. For comparison purposes, the data of all 2,5-dimethoxy aryl ring gluco-OPE **11** [22] (Fig. 2), previously studied by us are also included.

The absorption spectra of the OPEs **1** and **2**, in aqueous solution (pH = 7.2, buffer phosphate, see Fig. 3), are characterized by intense broad low-energy absorption bands centered at 388 nm and at 392 nm, respectively with ε values in the range 10^4–10^5 M^{-1} cm^{-1}.