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Synthesis, spectroscopic studies and biological evaluation of acridine derivatives: The role of aggregation on the photodynamic efficiency

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ABSTRACT

Two new photoactive compounds (**1** and **2**) derived from the 9-amidoacridine chromophore have been synthesized and fully characterized. Their abilities to produce singlet oxygen upon irradiation have been compared. The synthesized compounds show very different self-aggregating properties since only **1** present a strong tendency to aggregate in water. Biological assays were conducted with two cell types: hepatoma cells (Hep3B) and human umbilical vein endothelial cells (HUVEC). Photodynamic therapy (PDT) studies carried out with Hep3B cells showed that non-aggregating compound **2** showed photoxicity, ascribed to the production of singlet oxygen, being aggregating compound **1** photochemically inactive. On the other hand suspensions of **1**, characterized as nano-sized aggregates, have notable antiproliferative activity towards this cell line in the dark.

Introduction

Acridine derivatives are well known biologically active compounds, widely used, for instance, as topical antibacterial and antiparasitic agents.[1] They have been used also as anticancer drugs since the planar structure of this chromophore permits the intercalation into the major groove of DNA and hence disruption of the replication process.[2] Further understanding of their molecular mode of action showed that this biological effect is not only due to their intercalating ability but also can be explained by targeting of overexpressed biomolecules in tumoral cells, such as telomerase, protein kinase and topoisomerases I and II.[3] An abundant collection of acridine-based antiproliferative compounds can be found in the literature, with plethora of structural variants designed to enhance not only their binding abilities at the site of action but also the membrane crossing features and transportation properties of the molecules through the cellular millieu.[4] For example, Delcros et al. have described a series of 9-substituted aminoacridines and amidoacridines linked to polyamine chains capable of inhibiting the growth of L1210 and CHO cells with IC_{50} values in the micromolar range.[5] One strategy followed to enhance the DNA binding efficiency is the introduction of a second acridine moiety in the structure, which improves the stacking to the nucleic acids and hence potentiates the biological effect.[6] Apart from applications in oncology, acridines have also found utility in other therapeutic fields, for instance as antimalarials[7] and as cholinesterase inhibitors for Alzheimer's disease therapy.[8]

In parallel with this conventional approach there is another therapeutic strategy also using acridine-derived compounds and light to inhibit the proliferation of cancerous cells[9] and also to kill microorganisms.[10] Photodynamic therapy (PDT) is a clinical tool that uses a photosensitizer in combination with visible or UV light to produce cytotoxic reactive oxygen species (ROS) including superoxide radical anion ($O_2^{\bullet-}$) and singlet oxygen ($^1O_2$). Numerous types of compounds have been employed so far for the generation of $^1O_2$, not only for photobiological applications,[11,12] but also for synthetic purposes.[13]

The use of acridines in PDT dates back to the very origin of this discipline[14] and currently there is a renewed interest in the development of acridine derivatives for photodynamic applications. In principle, acridine chromophore is an excellent candidate to develop a bioactive photosentitizer, taking into account the very efficient population of the triplet excited state upon irradiation.[15] Energy transfer to molecular triplet oxygen gives rise to very high yields of $^1O_2$ (Φ_Δ) both in polar and apolar medium. For instance, Ogilby et al.[16] have reported Φ_Δ (benzene) = 0.84 and Φ_Δ (acetonitrile) = 0.97. However, compared to the number of acridine derived compounds reported as DNA binding agents, the number of PDT active

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compounds based on this chromophore is relatively low. The aim of this study is to compare the ability of two new 9-amidoacridine compounds (**1** and **2** in Fig. 1) to generate $^1O_2$ upon irradiation. The hypothesis of this work is that the superstructure adopted by the photosensitizing molecules affects dramatically their photobiological efficiency, in such a way that aggregation can favour deactivation pathways of the excited states, leading to quenching of the photosensitizer (like molecule **1**) and hence their invalidation as PDT agents. In this regard, a simpler structure (like molecule **2**) avoiding this aggregation phenomenon would be more favourable for the photogeneration of $^1O_2$ in aqueous medium, and then their use for clinical application more recommendable.

Compounds **1** and **2** were synthesized by coupling carbobenzyloxy-L-valine with the corresponding amine or diamine, as depicted in Scheme 1, followed by deprotection of the Cbz group and reaction with acridine-9-carbonyl chloride. Compounds were purified by subsequent filtration and washing steps, and were fully characterized by means of HRMS, ^1H and ^{13}C NMR spectroscopy (see Supporting information).

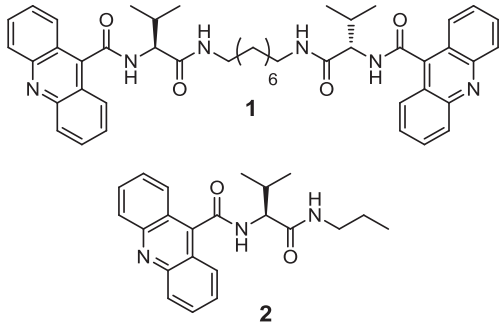


Fig. 1. Synthesized compounds based on the 9-amidoacridine chromphore.

In acetonitrile, compounds **1** and **2** show the typical absorption of the acridine chromophore at 360 nm, displaying optical features appropriate for UVA excitation and weak fluorescence at 419 nm (Fig. 2).[17] In a polar solvent such as water, absorption maximum undergoes opposed shifts (365 nm for **1** and 357 nm for **2**) and fluorescence emission batochromic shifts (439 nm for **1** and 435 nm for **2**). The emission quantum yield in water is notably different: very low for compound **1** and moderate for the mono-acridine derivative **2**. Summarized photophysical properties of the synthesized compounds are shown in Table 1.

The generation of $^1O_2$ upon irradiation was tested using a well-known benchmark reaction like the oxygenation of 1,5-dihydroxynaphthalene (DHN) to juglone, depicted in Scheme 2.[18] Compounds **1** and **2** were irradiated in quartz cuvettes with light of 365 nm. The analysis of the reaction was performed by UV-vis absorption measurements following the decreasing absorption of DHN at 298 nm.

An illustrative example of the spectral changes occurring upon irradiation of acridine derivatives in the presence of DHN can be found in Fig. 3. As it can be seen, the absorption bands of DHN at 298 nm disappear when increasing the irradiation time and,

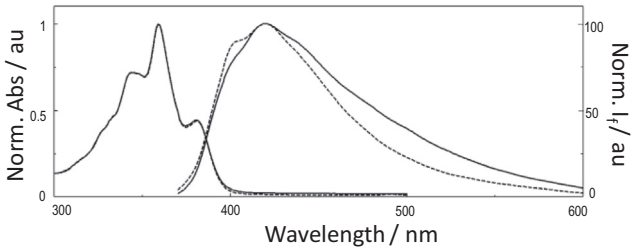
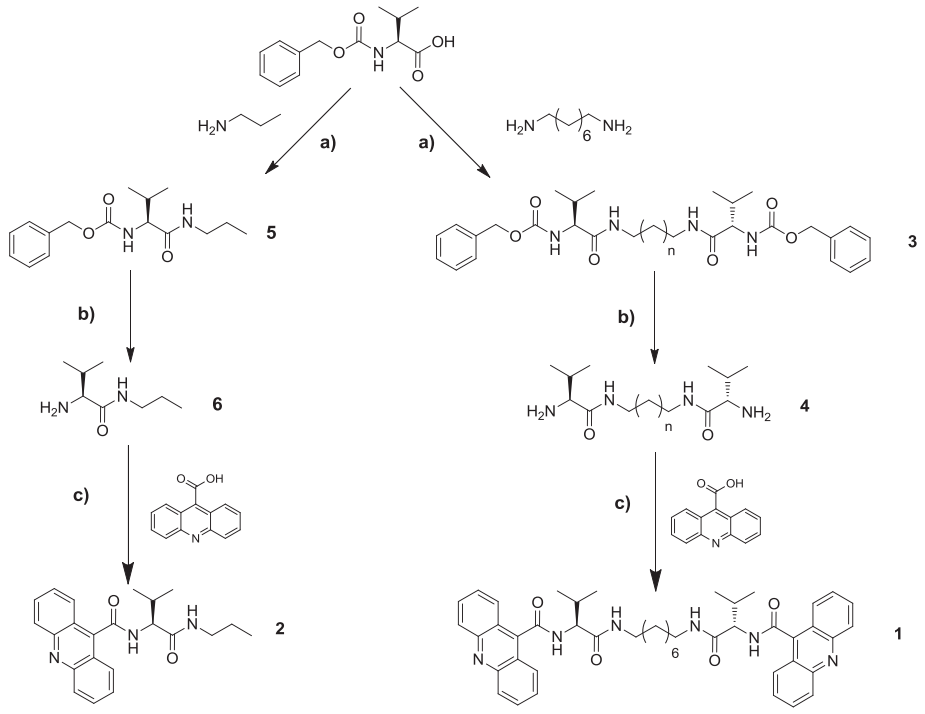


Fig. 2. Absorption and emission spectra of synthesized compounds in CH_3CN. **1** continuous line, **2** dashed line.



Scheme 1. Synthetic route for compounds **1** and **2** a) THF, Et_3N ClCOOEt, r.t., 12 h b) CH_3OH, Pd/C H_2, r.t., 6 h c) for acridine-9-carboxylic acid activation: CH_2Cl_2, oxalyl chloride, DMF(cat). For activated acid and amine coupling reaction: THF, Et_3N, 16 h.