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New π-Extended Water-Soluble Squaraines as Singlet Oxygen Generators

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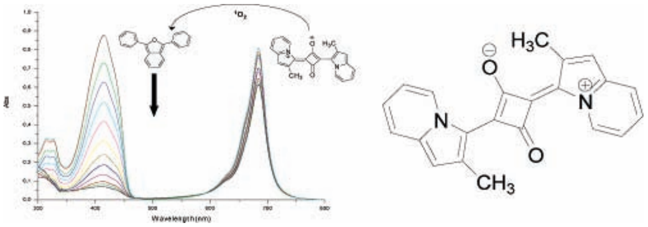
ABSTRACT



Condensation of squaric acid with a number of differently substituted 2-pyrrolyl derivatives afforded three new classes of squaraines. Their sharp and intense absorption bands in the biological window (700–900 nm), inherent singlet oxygen generation capabilities, together with proper functionalization allowing good water solubility make them suitable candidates as new non-porphyrinic singlet oxygen photosensitizers for photodynamic therapy (PDT).

Squaraine dyes (1,3-dicondensation products of squaric acid and electron-rich molecules)[1] are currently the object of intense investigation as molecular components for a number of technological applications. Among them, electrophotography,[2] optical data storage,[3] solar cells,[4] ion sensing,[5] and nonlinear optics[6] especially benefit from their peculiar photophysical and photochemical properties, namely, a sharp red near-IR absorption band and an extremely high extinction coefficient.[7] Recently,[8] squaraines have been proposed by Ramaiah et al. as a possible new class of photosensitizers for photodynamic therapy (PDT).[9] PDT is a noninvasive technique for the treatment of a number of tumors, including esophageal, bladder, lung, cervical, and skin cancer,[10] and other nontumoral diseases, such as age-related macular degeneration (AMD).[11] PDT involves the in vivo injection of photosensitizers that, upon selective irradiation at a suitable wavelength, generate, via an excitation transfer from its triplet excited state, the indirect production of singlet oxygen. The result is immediate and localized cellular damage, followed by necrosis and/or apoptosis.[12] An opti-

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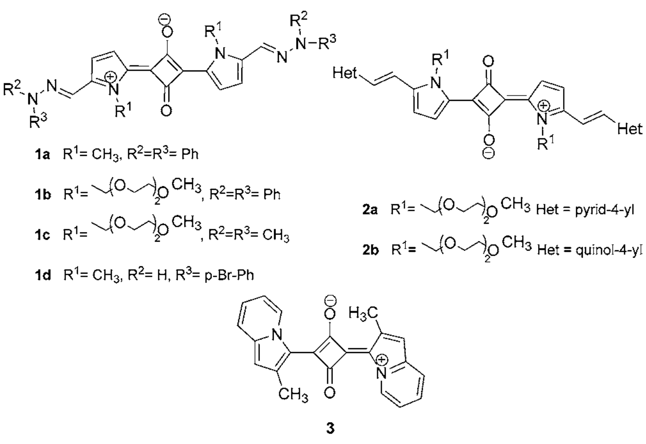
mized photosensitizer must, therefore, possess quite a unique collection of different properties, namely (i) a sharp and intense absorption band mainly localized in the biological tissues' transparency window (700−900 nm); (ii) good solubility in biological environment; (iii) an almost complete dark toxicity; (iv) high singlet oxygen sensitization quantum yield; (v) preferential localization within the tumor; and (vi) easy after-treatment removal from the body.

Porphyrin derivatives, and especially photophrin[13] and Vysudine[11] among them, represent the most common and widely used class of photosensitizers and the only ones already approved for human treatment by the FDA. Despite these achievements, porphyrins are far from being optimized photosensitizers.[13]

The aim of the present work is to convey some selected preliminary results that can exemplify the design, synthesis, and singlet oxygen production evaluation of three new classes of pyrrole-related squaraines able to address the previous (i)−(iv) issues (Scheme 1). To address item (i), it was necessary to increase the molecular conjugation length in order to bathochromically shift the absorption wavelength relative to previously described monomeric squaraines.[14] Our choice was to functionalize the pyrrole moiety attached to the squaric core either with arylhydrazone groups (**1a**−**1d**) or by elongating the chain with heteroaromatics (**2a**, **2b**) or else by incorporating the pyrrole ring in a π-conjugated and anellated structure (**3**).

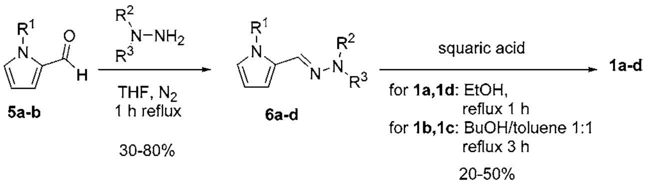
Scheme 1



Class **1** derivatives were chosen to evaluate the substituent effect on different aspects connected with PDT—absorption wavelength, water solubility, and the singlet oxygen production. Arylhydrazonomethylpyrroles represent a class of versatile electron-rich derivatives possessing a number of favorable features relevant for PDT. They are readily accessible simply by reacting in refluxing THF suitable N-substituted pyrrole-2-carboxaldehydes with a *N*-aryl or *N*,*N*-diaryl hydrazine directly as hydrochloride salts.

Hydrazones **6a**−**d** smoothly react with squaric acid, affording the corresponding squaraines often in an already suitable purity, simply by filtration from the reaction mixture (Scheme 2). Most notably, the high number of further possible functionalization sites in this class **1** squaraine is especially appropriate for the preparation of a variety of structurally related chromophores with finely tuned properties.

Scheme 2



We studied (1) the influence of the flexible triethyleneglycolic chain on the solubility in protic solvents[15] (**1a** and **1b**) and (2) the effect of a dialkylhydrazone on the tuning of the optical gap (**1c**). Finally, we introduced a bromine (**1d**), although in remote position with respect to the main conjugation pathway, to exploit the well-known heavy atom effect in the singlet oxygen production efficacy enhancement.[8a,16]

In class **2** squaraines, we extended the conjugation length by introducing a mild electron-withdrawing group as an electron-deficient heteroaromatic ring, instead of a donating one, as in class **1**. The use of the pyridine moiety in **2a** was also prompted by our previous experience[17] in the design of efficient noncytotoxic fluorescent markers with specific affinity for mitochondria,[18] based on the 1-(4-pyridyl)-2-(*N*-methylpyrrol-2-yl)ethylenic skeleton. The extension to a quinoline ring in **2b** was considered in order to provide additional functionalization sites. Interestingly, we found that in the reaction of derivatives **7a**−**b** with squaric acid the presence of a stoichiometric amount of pyridine is highly beneficial both by significantly reducing reaction times and by improving yields. Compounds **7a**−**b** were prepared in high yield by a crotonic condensation of *N*-triethyleneglycolpyrrole-2-carboxaldehyde and the corresponding methyl-substituted heterocycle (Scheme 3a).

Compound **3** represents the first term of a completely new squarainic skeleton endowed with a number of favorable features. This dye is readily accessible in good yield by

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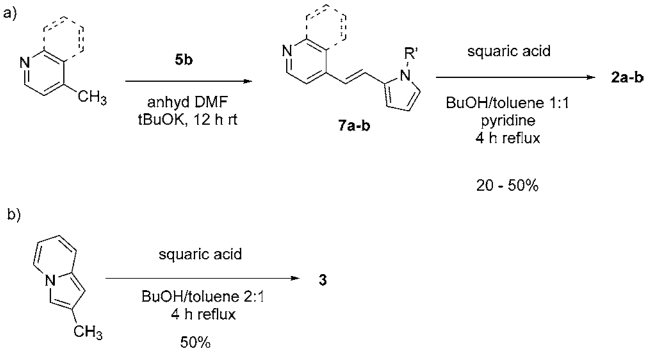
Scheme 3



refluxing the corresponding substituted indolizine[19] in a BuOH/toluene 2:1 mixture in the presence of squaric acid (Scheme 3b). In analogy with the reported indolizine electrophilic substitution regiochemistry,[20] we observed condensation at the α-pyrrolyl position.[21] Despite its remarkable structural simplicity, **3** displays a sharp and intense absorption spectrum, close to the biological window and very similar to that of class **2** dyes. The structure of the indolizine ring's intrinsic tailored nature allows facile and diverse substitutions on both the hexa- and pentatomic ring. We plan to fully exploit soon the wide variety of accessible indolizinic derivatives having derivative **3** as a parent representative.

All of the compounds possess absorption bands partially or completely localized in the biological window together with remarkably high extinction coefficients (Table 1). We observed that the use of *N*-aryl-substituted hydrazones in class **1** compounds generally leads to red-shifted absorption maxima (**1a**, **1b**, **1d**) and increased stabilities with respect to the alkyl ones (**1c**). While the introduction of a bromine substituent on the phenyl *para* position (**1d**) does not significantly affect the main absorption band, an unprecedented high-energy absorption band arises in the near-UV region.

Table 1. Linear Absorption and Emission Parameters of Compounds **1**−**3** in CH_2Cl_2

compound	λ_{max}(abs) [nm]	λ_{max}(em) [nm]	ϵ [mol^{-1} cm^{-1}]
1a	728	-	241000
1b	728	757	239000
1c	688	721	252000
1d	717		154000
2a	678		254000
2b	688	716	260000
3	684	699	178000

Also, we observed that the nature of the substitution at the pyrrolic nitrogen does not significantly affect the optical properties, an especially important feature on considering that the parent squaraines are usually almost insoluble in polar solvents. In fact, while the methyl-substituted squaraine **1a** is only sparingly soluble in chlorinated solvents, all of the glycol-substituted derivatives show high solubility in polar media and, at least for compounds **1c** and **2a**, almost complete water solubility. Aqueous solutions, while chemically stable in general, do show some aggregation, details of which will be reported elsewhere. A comparison of **2a** and **2b** spectra clearly shows that the benzofusion on the azine ring only marginally affects the optical properties, making **2b** suitable for derivatization, for example, with heavy atoms.

Having addressed both the solubility and optical absorption issues, we turned to the singlet oxygen sensitization efficiency evaluation. The long tail of the rather strong and broad NIR emission shown by derivatives pertaining to all of the three classes prevented us from a direct evaluation by means of singlet oxygen excitation−transfer-induced fluorescence (Figure 1).[23] A qualitative comparative study was carried out by monitoring the reaction of 1,3-diphenylisobenzofuran (DPBF) with photosensitizer-promoted singlet oxygen.[24] DPBF is known to form an endoperoxide upon cycloaddition with singlet oxygen, resulting in the complete disappearance of its characteristic absorption band at 415 nm. We treated a solution of DPBF (50 μM) and photosensitizers (5 μM) with a filtered light source of wavelength $\lambda > 600$ nm over a period of 60 min.[25]

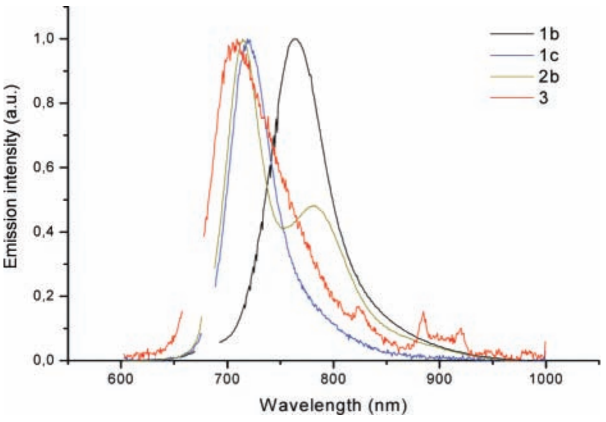


Figure 1. Photoluminescence spectra (CH_2Cl_2) of squaraines **1b**, **2b**,[22] **1c**, and **3**.

Figure 2 shows, as an example, the family of spectra that we obtained for squaraine **1d** in CH_2Cl_2. The squaraine

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[25] With 230 V, 300 W halogen lamp filtered with a 3 wt % $K_2Cr_2O_7$ solution.

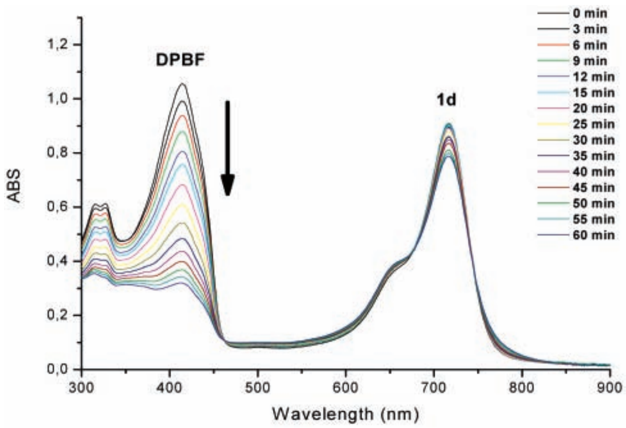

Figure 2. Time evolution of the UV−vis spectrum of a DPBF (50 μM) and **1d** (5 μM) in CH_2Cl_2 solution.

absorption does not significantly change until the majority of DPBF is decomposed. Afterward, when the sensitizers' and quenchers' concentrations become comparable, the squaraine self-induced suicidal singlet oxygen-mediated photobleaching becomes more probable. Figure 3 plots the decrease of DPBF absorption at 415 nm as a function of illumination time for each sensitizer compared to that of *meso*-tetraphenylporphyrin (TPP), a well-known and very efficient singlet oxygen generator. We restricted the analysis to the first 30 min because, during this time, the photosensitizer did not show any appreciable degradation.[26] A first general analysis shows that, despite the reported[2] low intersystem crossing efficiency of other squarainic derivatives, these squaraines do possess some inherent singlet oxygen generation abilities. In particular, within class **1** derivatives, the brominated compound **1d** shows peak performances, in accord with the anticipated heavy atom effect.

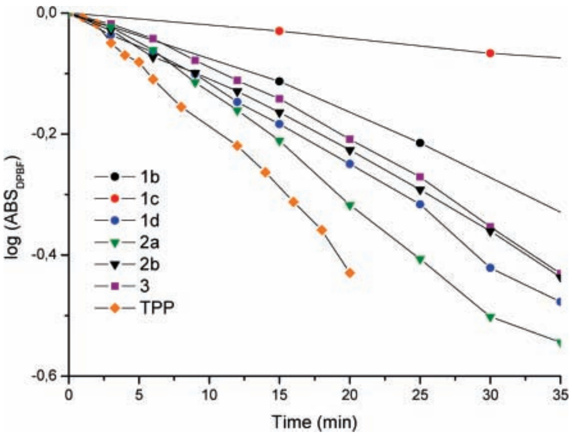

Figure 3. Comparative singlet oxygen generation of squaraines **1d**, **1c**, **1b**, and **4** in CH_2Cl_2 at 5 μM concentration.

In conclusion, we have designed and synthesized representative members of three new classes of squaraines, the intrinsic tailored nature of which allows the stepwise fulfillment of some of the many demanding requirements associated with the development of new photosensitizers for PDT: (a) all new dyes display absorption bands in the biological window; (b) the introduction of a triethyleneglycol chain results in significant solubility enhancement and achievement of high water solubility for **1c** and **2a**; (c) a qualitative estimate of singlet oxygen generation quantum yield shows that all of the derivatives possess intrinsic triplet yield. We plan to further enhance the singlet oxygen production by introduction of bromine and/or iodine atoms in suitable positions. Efforts are underway to evaluate the dark toxicity and preferential cellular localization for the most efficient derivatives.

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Supporting Information Available: Experimental procedures and characterization data for all of the new compounds. Singlet oxygen induced DPBF degradations for all of the squaraines. This material is available free of charge via the Internet at http://pubs.acs.org.

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[26] Only derivative **1c** readily decomposed under measurement conditions.