Unsaturated oxazolones as nonlinear fluorophores

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ABSTRACT

Unwanted bleaching of two-photon absorption microscopy probes can be reduced by designing more efficient nonlinear fluorophores. Here the linear and nonlinear photophysical properties of unsaturated oxazolones are studied. A set of push–pull oxazolone derivatives have been designed by substituting the 2-phenyl-4-benzylidene-1,3-oxazol-5-one unit in two different positions with a combination of electron donor (dimethylaniline) and/or acceptor (nitro, nitrile and benzimidazole) groups. By engineering this green fluorescent protein analogue core we have prepared molecules with high two-photon absorption cross-sections 200–500 GM and fluorescence quantum yields up to 0.75. The factors determining the overall nonlinear emission efficiency of the new unsaturated oxazolones are discussed in terms of the nature of the substituents.

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1. Introduction

Two-Photon Absorbing (TPA) chromophores are molecules that absorb simultaneously two photons and release half of the absorbed photons with twice the energy. This type of compound has attracted significant interest in the last few decades due to their optical properties and subsequent wide range of applications such as microfabrication, data storage, photodynamic therapy, optical power limiting, optical imaging and sensing [1]. The multiphoton excitation requires high excitation powers due to the low probability of the nonlinear absorption process (typically two-photon absorption cross-section values, σ_2, are of the order of $\sim 10^{-51}$–10^{-46} cm^4 s, while linear absorption cross-sections are in the range of $\sim 10^{-19}$–10^{-16} cm^2). The high power causes significant bleaching in the focal plane. The unwanted bleaching can be reduced by designing more efficient nonlinear materials that allow the use of comparatively lower excitation powers.

Bioimaging is by far the most popular applications of nonlinear fluorophores. However, despite the efforts that have been put into developing suitable nonlinear dyes for bioimaging, the combination of high TPA and emission quantum yield with water solubility and biocompatibility are difficult to meet simultaneously. In general, the compromise to fulfill the requirements for bioimaging results in fluorophores with small σ_2 values (<20 GM, 1 GM = 10^{-50} cm^4 s), with the highest reported values for a few fluorophores around 200 GM. [2]. The need of biocompatible fluorophores for sensors and bioimaging was partially resolved by the discovery of fluorescent proteins. Fluorescent proteins can be genetically encoded in living organisms in order to target specific organelles, cells and tissues. Combining fluorescent proteins with nonlinear emission appears as a powerful tool for deeper tissue penetration, high three-dimensional resolution, reduced autofluorescence and out of focus photodamage. In a recent study, the best nonlinear fluorescent proteins have been reported to have TPA cross-sections around 300 GM and effective TPA cross-section ($\sigma_2\phi$, where ϕ is the fluorescence quantum yield) of 210 GM [3].

Optimization of TPA fluorophores relies on understanding their molecular spectroscopy, dynamics, and structure. This paper presents a systematic study of the structural effects on the linear and nonlinear photophysical properties of unsaturated oxazolones, which can be seen as analogues of the imidazolinone chromophore of the green-fluorescent protein (GFP). Fig. 1 compares the hydroxybenzylidene-imidazolinone chromophore with
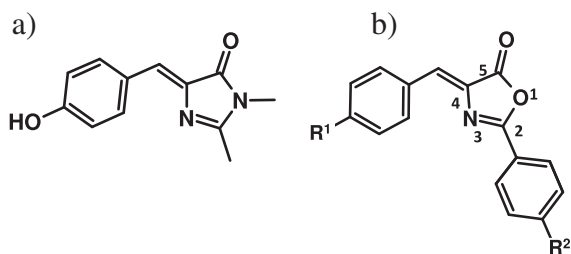
Fig. 1. GFP chromophore and analogous oxazolone core used as building block to the push–pull compounds studied in this paper.

the 4-benzylidene-2-phenyl-1,3-oxazol-5-(4H)-one core (hereafter referred to as oxazolone core).

Oxazolones are valuable precursors in the synthesis of non-natural aminoacids [4] and biologically active heterocyclic molecules [5]. Their reactivity has motivated extensive work on different types of transformations to obtain highly substituted scaffolds [6–8]. An exocyclic double bond at position 4 of the ring (Fig. 1b) gives new reactivity to unsaturated oxazolone and allows the construction of interesting structures and highly delocalized π-systems [9–11]. Oxazolones are also intermediates in the synthesis of the imidazolinone GFP chromophore [12]

One of the main issues regarding application of oxazolones is related to their poor photochemical stability. This issue can be circumvented in the solid phase due to steric hindrance of the ring-opening process that leads to photochemical decomposition. An interesting application of oxazolones in the solid phase (dispersed in sol–gel and polymer matrices) is that of optical pH-sensors [13,14]. In addition, the emission quantum yields of oxazolones with an exocyclic double bond at position 4 (Fig. 1b) are usually low. A reversible ring-opening process is believed to compete with emission from the singlet state leading to an efficient nonradiative deactivation in solution [15]. The low emission quantum yields have also been associated with intersystem crossing (ISC) to the triplet manifold. The triplet manifold is believed to be connected with H-abstraction and Z/E geometrical isomerization [16]. Encapsulation in the solid matrix prevents geometrical isomerization, thus increasing the radiative quantum yield. On the other hand, geometrical isomerization can be explored in photoswitching applications. The substantial conformational changes associated with E/Z isomerization have attracted attention to 4-benzylidene-oxazolones as potential molecular switches in biomolecular photocontrol [17]. An efficient photoswitching process has been recently demonstrated for (Z)-4-benzylidene-2-methyloxazol-5(4H)-one [18].

Recently, our group reported the photophyiscal properties of a set of push–pull molecules with either D−π−A or A−π−A arrangements based on the 2-phenyl-1,3-oxazol-5(4H)-one by changing the nature of the substituent at position 4 [19]. The highest TPA cross-sections found, between 80 and 100 GM, were either for oxazolone derivatives with strong electron donating groups (dimethyl-amine) or mild electron accepting groups (benzimidazole) that behaved as electron donors with respect to the oxazolone ring. The best performing nonlinear fluorophore had only a modest effective TPA cross-section of 25 GM. These results have guided our efforts into designing more efficient nonlinear fluorophores based on the oxazolone core. The push–pull oxazolone derivatives in the present work are prepared by substituting the 2-phenyl-4-benzylidene-1,3-oxazol-5-one unit in two different positions (R[1] and R[2] in Fig. 1) with a combination of electron donor (dimethylaniline) and/or acceptor (nitro, nitrile and benzimidazole) groups. The different donor and acceptor groups are introduced by substituting either the phenyl

group attached directly to position 2 of the oxazolone ring (R[2]) or the phenyl group attached to the oxazolone core via the vinyl bridge at position 4 (R[1]). The different types of combinations used allow us to change the direction of charge transfer and manipulate the involvement of the oxazolone core in the process. A systematic investigation of the linear and nonlinear photophysical properties of this set of oxazolone derivatives is made and the results are discussed with the aid of electronic structure calculations.

2. Experimental and computational details

2.1. Material

All the reagents used were purchased from either Aldrich or Merck and were used without further purification. The aldehyde used in the synthesis of compounds **IX**, **X**, **XI** and **XII** (see Section 2.3) was prepared accordingly to the literature procedure [20]. The reaction time evolution was followed by TLC using silica Merck Kieselgel 60 F254 plates, and revealed by ultraviolet light at 254 nm and 325 nm or stain solutions of phosphomolybdenum acid in ethanol.

2.2. Instrumentation

The compounds were characterized by NMR (Bruker Avance II 400 and 300 Ultrashield Plus), by elemental analysis performed at the services of Universidade Nova de Lisboa and Faculty of Pharmacy of the University of Lisbon, by infrared spectroscopy (Shimadzu IRAffinity-1 FTIR spectrometer, using KBr platelets), by measuring the melting point (Stuart SMP10 apparatus) and by mass spectrometry in a quadrupole ion trap mass spectrometer (Varian Inc. Palo Alto CA, USA, equipped with an ESI source) and in a triple quadrupole mass spectrometer (Micromass Quattro Micro API Waters). High Resolution mass spectra were obtained by using a Finnigan FT/MS 2001 DT, FT-ICR/MS mass spectrometer, operating at EI positive mode.

The linear absorption spectra were recorded on a Shimadzu UV-3101PC UV−vis−NIR spectrophotometer. The emission spectra were recorded on a Horiba Jobin Yvon Fluorolog 3-22 Spectrofluorimeter. The two-photon absorption spectra were measured by two-photon excited fluorescence using Rhodamine 6G in methanol (fluorescence quantum yield of 0.93, λ_{em} = 500−600 nm) [21] as a standard to account for collection efficiency and pulse characteristics. A modified setup that follows closely the one described by Xu and Webb [22] was used. In order to select a narrow bandwidth of emission wavelengths an H20Vis Jobin Yvon monochromator was placed at the entrance of a PMC-100-4 photomultiplyer tube (Becker and Hickl GmbH). The excitation source was a Ti:Sapphire laser (Tsunami BB, Spectra-Physics, 710−990 nm, 1.7 W, 100 fs, 82 Mz). In general, solutions of 10 μM concentration in tetrahydrofuran (THF) were used. The TPA cross-sections of more challenging derivatives with low TPA values were evaluated in more concentrated solutions (2−0.7 mM). The two-photon absorption cross-section was calculated from the relation:

$$\sigma_2 = \left(\frac{F_2}{\phi C n}\right)_{ox} \left(\frac{\phi C n \sigma_2}{F_2}\right)_{ref} \tag{1}$$

where F_2 stands for fluorescence intensity, ϕ is the one-photon excited fluorescence quantum yield, n refers to the refractive index of the solution, C is the concentration, and ox and ref are relative to the oxazolone derivatives and the Rhodamine 6G reference, respectively. The emission intensity dependence of the excitation power was checked to be quadratic at the excitation maximum for all the compounds.