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Nonlinear optical response and two-photon biological applications of a new family of imidazole-pyrimidine derivatives

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ABSTRACT

A series of novel D–π–A type two-photon absorption (2PA) imidazole-pyrimidine derivatives (**EX-1~EX-4**) have been synthesized and characterized, with **EX-1** was crystallography confirmed. Based on systematic photophysical investigations, the structure–property relationships can be drawn as follows: (1) Both theoretical and experimental studies indicated that the different donor groups have large influences on the optical properties. (2) The 2PA cross-section values (σ) were obtained both by Z-Scan and two-photon excited fluorescence (2PEF) measurements. 2PA cross sections show an increasing trend with increasing electron-donating strength and the number of branches. (3) Comprehensively considered the optical performance, molecular volume, cytotoxicity and solubility, **EX-1** and **EX-2** were identified to be the best candidates for living cells (HepG2) imaging. Moreover, the 2PA excitable features of **EX-1** and **EX-2** are capable of imaging in fresh mouse's liver tissues with a depth of ca. 70 μm.

1. Introduction

Two-photon excitation fluorescence (2PEF) microscopy induced by spatially confined two-photon excitation exhibits several advantages over one-photon fluorescence, such as excitation with longer wavelength (low energy), higher special-resolution localization. Therefore, novel large two-photon-active chromophores [1] have attracted great attention due to a wide range of potential applications in 2PEF microscopy [2], three dimensional (3D) optical data storage [3], upconversion response, [4] photodynamic therapy [5] and microfabrication [6]. The previous studies revealed that the 2PA cross-section increased mainly due to the conjugation length, planarity and dimensionality of the π-center, the vibronic coupling, and the donor/acceptor strength. The recent applications of the 2PA materials in biology put forward to a new challenge. Apart from the high δ_{2PA} values, 2PA chromophores have to satisfy various requirements, depending on the specific application. For instance, chromophores with a large 2PA cross-section as well as a high fluorescence quantum yield in the long-wavelength range (near the ideal imaging window 650–900 nm) are required for bioimaging. Reconstruction of 3D images of living cell or tissues which need the chromophores with reduced photodamage towards cellular endogenous molecules, photostability, auto-fluorescence free, and along with 2PA across-sections in the NIR range, whereas the commonly used commercial dyes and fluorescent probes have small 2PA cross-sections (δ_{2PA}). To exploit new chromophores with large δ_{2PA} values for specific application is still one of the hot topics in the field of 2PA functional materials [7]. Further design criteria include excellent photostability, water solubility, and good cell permeability, whereby the latter is dependent upon the molecular weight and size. In this context, a convenient strategy to achieve the desired properties is to optimize the individual fragment and its position, resulting the best 2PA activity in a small molecular volume.

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According to these guidelines, the triphenylamine core has been selected as a privileged building unit for the design of symmetric octupoles as its strong electron donor, its C_3 symmetry and versatile chemistry represent major advantages. Indeed, a considerable number of triphenylamine derivatives have been successfully used in material chemistry and more recently appeared in a number of molecular systems devoted to photovoltaic applications [8]. Nonetheless, such molecular engineering considerations have led to high molecular weight aromatic molecules which are a primary non suitable for biological imaging. Solutions exist, such as micelle encapsulation [9,10], to address this issue and enable the use of such molecules in aqueous media, but there is still a lack of ready-to-use, low-molecular weight biological probes with optimized 2PA cross-sections.

It is noteworthy that the pyrimidine derivatives were intensively investigated as electroluminescent materials and biomaterials in the past [11] owing to the planarity they provide. Pyrimidine is π-electron deficient with an ionization potential value of 10.41 eV [12]. On the other hand, the pyrimidine ring has well-known reactivity in the positions 4 and 6, which can easily undergo reactions with an aromatic aldehyde under solvent-free conditions. There may be two main reasons for this reactivity [13]. According to our knowledge, little attention was paid to triphenylamine derivatives with pyrimidine group for their 2PA and related applications. Herein, 2-thiomethyl-4(6)6-dimethylpyrimidine central units, was used to tune the electronic delocalization along the conjugated backbone in the ground state by modulation of the twist angle between the two halves of the molecules [14-15].

Here we have strategically designed and presented a similar one-step method for preparation of a series of new D–π–A type 2-imidazolyl-4(6) methyl-pyrimidine derivatives (**EX-1~EX-4**) with different directions of charge transfer (Scheme 1). Structurally, the triphenylamine and ethoxyphenyl triphenylamine groups have richer π-electron densities than the phenyl group. Imidazolium group can increase the solubility of the molecule, as well as enhance the extent of electron delocalization and ability of the 2PA chromophore to accept electrons. More discussion on the structure–property relationships of 2-imidazolyl-4(6) methyl-pyrimidine derivatives was presented in this article. Linear photophysical characterization and investigation of 2PA properties were described as a basis for potential applications in two-photon fluorescence imaging. Relying on their comprehensive studies, **EX-1** and **EX-2** were selected for bioimaging study using two-photon scanning microscopy. It was showed that HepG2 cells could be sufficiently stained with **EX-1** and **EX-2**, which exhibits higher selectivity, and is capable of monitoring the fluorescence signals for a long period of time (0–600 s) with minimal photobleaching. These results suggested that **EX-1** and **EX-2** could potentially applied as 2PA probes as *in vivo* and *in vitro* bioimaging material.

2. Results and discussion

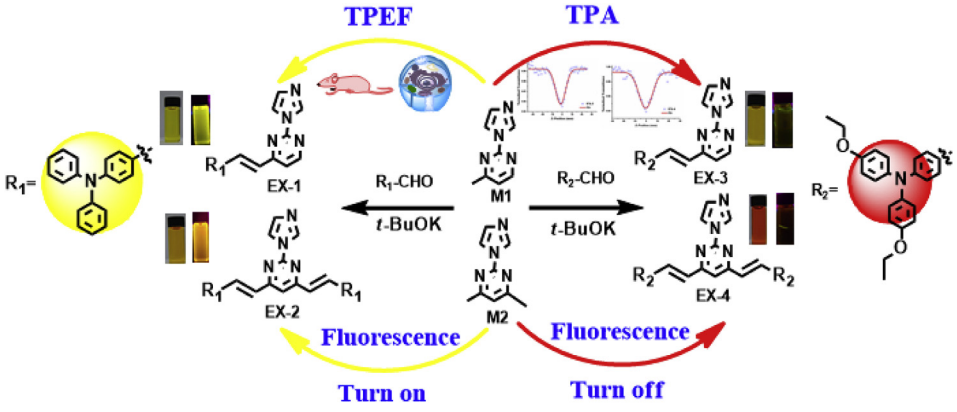
2.1. Synthesis

As shown in Scheme 1, **M1** and **M2** were prepared by Ulman reaction using imidazole and 2-iodide-4-methyl-pyrimidine et al. in the presence of *t*-BuOK which afforded the title product over 80% yield. **EX-1, EX-2, EX-3** and **EX-4** were synthesized by Witting reaction. Yellow crystals of **EX-1** suitable for single crystal X-ray diffraction analysis were obtained by slow evaporation of the remaining acetonitrile solution. The details of the synthesis and characterization data were given in the Experimental Section, the crystallography data of the chromophores were given in Table S2 and S3.

2.2. Crystal structure and DFT calculations

In order to obtain accurate structure information, single crystal of **EX-1** (Fig. 1) shows that the molecule looks like a goldfish with one moderately distorted tail 'triphenylamine', in which the dihedral terminal group between the terminal (P_1) and phenyl rings (P_2) is 62.57°, and the other (between P_1 and P_3) is 72.33°. The linkage bond is between the conjugated bond lengths of C(19)-C(21) [1.314 Å] (Table S2). These structural features suggest that a highly π-conjugated system is formed by all the non-hydrogen atoms, leading to a charge transfer from donor to acceptor over the π-bridge. To better understand the charge distribution, density functional theory (DFT) calculations on **EX-1** were carried out. The molecular geometry used for the calculation is obtained from X-ray diffraction crystallographic data.

Fig. 2 gives straightforward representations of the electron density distribution. Orbital analysis exhibits that the highest occupied molecular orbital (HOMO) is comprised of triphenylamine unit with a smaller contribution from pyrimidine ring. Also the lowest unoccupied molecular orbital (LUMO) distribution is localized in the pyrimidine orbitals with abundant conjugated π-bridge. Hereby, there are relatively strong π-donor interactions between triphenylamine and the pyrimidine center, and the electron density in each molecule is very similar. As shown in Fig. 2 and Table S4, the lowest-energy excitation bands of all the chromophores (λ: 434–501 nm) are assigned as the ICT transition [$\pi_{triphenylamine} \rightarrow \pi^*_{CH=CH}$] mixed with the [$\pi_{triphenylamine} \rightarrow \pi^*_{imidazole}$] (**EX-1~EX-4**) due to the H → L transition. The high energy band of **EX-1~EX-4** is mainly due to the H-n → L + n transition



Scheme 1. Synthetic routes for the chromophores.