Journal of Photochemistry and Photobiology A: Chemistry 348 (2017) 102–109

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

Invited feature article

Synthesis of new triazole based imidazo[1,2-*a*]pyrazine-benzimidazole conjugates: H-bonding assisted FRET efficient ratiometric detection of pyrophosphate

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ARTICLE INFO

Article history:
Received 16 May 2017
Received in revised form 4 August 2017
Accepted 6 August 2017
Available online 16 August 2017

Keywords:
Imidazo[1,2-*a*]pyrazine
Benzimidazole
Click reaction
Suzuki reaction
FRET
Pyrophosphate

ABSTRACT

Triazole tethered imidazo[1,2-*a*]pyrazine-benzimidazole conjugates **13-38** has been synthesized by click and Suzuki-Miyaura cross coupling reactions at C-8 and C-6 positions, respectively. The research findings clearly predicted that by modification of electronic structure of the receptor, the sensitivity of the recognition process could be modified. Compound **24** with hydroxyphenyl substituent, showed stronger binding to the pyrophosphate than other compounds. Compound **24** has been used as selective probe for ratiometric detection of pyrophosphate amongst the other anions. The binding event of compound **24** toward PPi has been successfully evaluated by absorption and emission spectroscopy as well as NMR titration method. The compound **24** showed H-bonding assisted facilitation of FRET phenomenon in the presence of PPi.

1. Introduction

Phosphate-containing anionic species [1] are ubiquitous in biological systems and play important mediatory roles in signal transduction pathways, and carrying genetic informations [2]. For example, pyrophosphate ions (PPi) are involved in energy transduction in organisms, and controlling metabolic processes via participation in enzymatic reactions [3]. Various important biochemical reactions like DNA polymerization, synthesis of cyclic AMP and formation of activated intermediates in protein synthesis, are catalyzed by DNA polymerase, adenylate cyclase and amino-acyl-tRNA synthetase, respectively. While the hydrolysis of ATP with concomitant release of PPi is an important factor in biochemical pathways [4–6]. Therefore, several research groups have focused on the detection of this biological anion. The design of fluorescent chemosensor for detection of pyrophosphate ion remains a challenge [7–9]. Most of the developed phosphate anion sensors are based on transition metal complexes where the cavity formed by the metal ion with the receptor provides a cooperative binding site for phosphate derivative [10]. In this context, Zn(II) complex has been frequently used for detection of PPi [11–16]. But complexes with other metal ions like Tb(III) [17], Cd(II) [10], Mn(II) [18], Cu(II) [19–25] and Eu(III) [26] have also been employed. Some of these sensors displayed remarkable selectivity and sensitivity. However, considerable synthetic efforts are required for their preparation. Moreover, sensing molecules for detection of PPi are limited in literature and the potential for the development of these PPi sensors are at the primitive stage for bioanalytical applications. Until now, few chemosensors are reported for detection of PPi in the absence of metal ions [27–30].

Amongst different approaches proposed for ratiometric ion sensing, Förster resonance energy transfer (FRET) is a non-radiative energy transfer process in which the excitation energy of the donor is transferred to nearby acceptor via long-range dipole–dipole interaction and/or short-range multipolar interaction. FRET is generally designed as fluorescence sensor to adopt the photophysical changes produced on complexation [31–33]. Recently, FRET based probes [34,35] have been used in cell physiology, optical therapy and selective as well as specific sensing toward target analytes [36–38]. However, despite many advantages, ratiometric sensing of PPi using FRET phenomenon is not known. Imidazo[1,2-*a*]pyrazine is known to exhibit fluorescence properties such as chemiluminescence [39] and bioluminescence [40], found in the scaffold of the Coelenterazine and a bioluminescent compound isolated from the Jellyfish Aequorea Vistoria. Benzimidazole moiety has been commonly utilized as the molecular recognition site for cation, anion and neutral molecules due to its unique spectral properties. Besides their medicinal uses,

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http://dx.doi.org/10.1016/j.jphotochem.2017.08.009
1010-6030/

imidazopyrazine and benzimidazole derivatives have found technical applications as dyes, electroluminescent materials, organic semiconductors and as suitable ligand in coordination chemistry [41,42]. Keeping above points in consideration, in the present manuscript the two fluorescent moieties have been combined through a spacer as imidazo[1,2-*a*]pyrazine-benzimidazole conjugates for FRET based ratiometric determination of pyrophosphate (PPi). Moreover, conjugates based on imidazo[1,2-*a*]pyrazine and benzimidazole moieties as sensors have not been reported so far. Herein, we have synthesized these conjugates by implementing click reaction and Suzuki-Miyaura cross coupling reaction at respective C-8 and C-6 positions of imidazo[1,2-*a*] pyrazine and studied their photophysical properties to determine the FRET based ratiometric detection of pyrophosphate. The motivation behind the synthesis of these compounds was to synthesize sensor for biologically important anions. Thus, criterion for choosing the organic moiety was fluorescent electron deficient moiety having nitro group so that it can interact with electron rich anions. In continuation to search for an efficient compound, a series of organic compounds were synthesized to fine tune the FRET efficiency. The compound 24 showed the best quantum yield and FRET efficiency and was chosen for the further study.

2. Experimental

2.1. General experimental conditions

All commercially available compounds (Avra, Spectrochem, Aldrich, Merck etc.) were used without further purification. Final reactions were carried\ out in an oil bath using Microwave Vials (10–15 ml). Melting points were determined in open capillaries and were uncorrected. ^{1}H and ^{13}C NMR spectra were performed on Jeol ECS 400 NMR spectrometer, which was operated at 400 MHz for ^{1}H nuclei and 100 MHz for ^{13}C nuclei, using $CDCl_3$, DMSO-d_6 and trifluoroacetic acid (TFA) as solvents. Chemical shifts are reported in parts per million (ppm) with TMS as internal reference and *J* values are given in hertz. 2D NOE studies were performed on same instrument. Mass Spectra of the synthesized compounds were recorded at Water Micromass-Q-T of Micro. Reactions were monitored by thin layer chromatography (TLC) with silica plate coated with silica gel HF-254 and column chromatography was performed with silica gel 60–120/100–200 mesh. Ethylacetate and methanol were adopted solvent systems.

2.1.1. General procedure for synthesis of compounds ***4*** *and* ***5***

2-Bromoethylamine hydrobromide/3-bromopropylamine hydrobromide (4.90 mmol) was dissolved in distilled water with stirring. Sodium azide was added (13.84 mmol) carefully to this solution in succession with continuous stirring. The reaction mixture was refluxed for 12 h. After completion of reaction, reaction mixture was cooled to room temperature and was quenched by addition of sodium hydroxide (17.5 mmol) and further stirred for 30 min at room temperature. Thereafter, mixture was extracted with diethyl ether and dried over sodium sulphate to obtain ether extract and stored at low temperature. The ether extract being volatile in nature, was used as such for next reaction.

2.1.2. General procedure for synthesis of compounds ***6*** *and* ***7***

To the ether extract containing **4** or **5** was added to the solution of 6,8-dibromoimidazo[1,2-*a*]pyrazine **1** (1.80 mmol) in acetonitrile in the presence of diisopropylethylamine (DIPEA). The reaction mixture was refluxed for 24 h. After completion of reaction, the mixture was extracted with chloroform and water. Organic layer was separated, dried over sodium sulphate, filtered and concentrated under vacuum. The crude mixture was then purified by silica gel chromatography 60–120 mesh using hexane: ethyl acetate (8:2) as eluents.

2.1.3. Synthesis of 2-(3-nitrophenyl)-1-(prop-2-ynyl)-1H-benzo[d] imidazole (***9***)

To 2-(3-nitrophenyl)-1*H*-benzo[*d*]imidazole **8** (4.08 mmol) was added 80% solution of propargyl bromide in toluene (8.40 mmol) in the presence of potassium carbonate (4.08 mmol) and DMF. The mixture was stirred at room temperature for 12 h. Completion of reaction was monitored by TLC. Reaction was quenched by addition of ice cold water. The solid product was filtered to obtain pure off white solid **9**.

2.1.4. General procedure for synthesis of compounds ***10*** *and* ***11***

To a stirred solution of 2-(3-nitrophenyl)-1-(prop-2-ynyl)-1*H*-benzo[*d*]imidazole **9** (3.6 mmol) and **6** or **7** (3.6 mmol) in 50 ml ethanol:water (8:2), copper sulphate pentahydrate (5 mol%) and sodium ascorbate (10 mol%) were added and stirred at room temperature for 2 h. After completion of reaction, water was added and extracted with chloroform. Chloroform layer was dried over anhydrous sodium sulphate, filtered and concentrated in vacuum to obtain pure solid **10** or **11**.

2.1.5. General procedure for synthesis of compounds ***13-38***

To a solution of **10** or **11** (0.178 mmol) in mixture of 1,4-dioxane: water (9:1) in a sealed tube, boronic acid (0.178 mmol) and K_2CO_3 (0.178 mmol) were added under inert atmosphere. Then, [Pd(PPh_3)$_4$] (5mol%) was added with continued nitrogen purging. Sealed the tube and refluxed the reaction mixture for 6–8 h. Completion of reaction was determined by TLC. The mixture was extracted with chloroform and water. Organic layer was dried over sodium sulphate to obtain crude product which was further purified by column chromatography to get pure products **13-38**.

2.2. Photophysical measurements

All photophysical measurements were performed in acetonitrile. Absorption spectra were measured with a UV-2500, Shimadzu spectrophotometer. Fluorescent measurements were performed with a Carry Eclipse spectrophotometer. The quantum yields of the imidazo[1,2-*a*]pyrazine analogues were determined relative to anthracene.

2.3. Fluorescence quantum yield

The fluorescence quantum yield Φ_{fs} for all compounds was determined at room temperature in analytical grade CH_3CN using anthracene (Φ_{fr} = 0.22) in acetonitrile as the standard. The quantum yield was calculated by using eqn–1, in which Φ_{fs} is the radiative quantum yield of the sample, Φ_{fr} is the radiative quantum yield of reference, A_s and A_r are the absorbance of the sample and the reference, respectively, D_s and D_r are the areas of emission for the sample and reference respectively, L_s and L_r are the lengths of the absorption cells, and N_s and N_r are the refractive indices of the respective sample and reference solutions (pure solvents were assumed).

$$\phi_{fs} = \phi_{fr} \times \frac{1 - 10^{-A_r L_r}}{1 - 10^{-A_s L_r}} \times \frac{N_s^2}{N_r^2} \times \frac{D_s}{D_r} \quad (1)$$