PROCEEDINGS OF THE

27th International Symposium on Analytical and Environmental Problems

Szeged, Hungary

November 22-23, 2021

University of Szeged

Edited by:
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Publisher:
University of Szeged, H-6720 Szeged, Dugonics tér 13, Hungary

ISBN 978-963-306-835-9

2021.
Szeged, Hungary

The 27th International Symposium on Analytical and Environmental Problems

Organized by:
SZAB Kémiai Szakbizottság Analitikai és Környezetvédelmi Munkabizottsága

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Lecture Proceedings

2-OXO-1,2,3,4-TETRAHYDROPYRIMIDINE-AZO-PYRIDONE DYE: A POTENTIAL APPLICATION AS NEW GREEN-EMITTING FLUORESCENT PROBE

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Abstract
Molecular imaging is a relatively new research field, which has demonstrated great potential, especially in clinical oncology – from drug development to cancer early detection. The key of fluorescence imaging is the construction of fluorescent probe which is composed of two parts, the recognition groups to recognize cancer cells, and fluorophores to signal the recognition events. In this research, the structure of new fluorescent azo dye based on 2-oxo-1,2,3,4-tetrahydropyrimidin and 2-pyridone moieties has been reported. The absorption and emission properties of the investigated azo dye have been studied using UV-Vis and fluorescence spectroscopy. The obtained results suggest that studied dye meets the requirements for new green-emitting fluorescent probe, suitable for further application in biomedical researches.

Introduction
Fluorescent probes are one of the major driving force of the molecular imaging. They are the agents used to visualize, characterize and measure biomolecules and biological processes in living systems [1,2]. Fluorescent molecules absorb light of a specific wavelength and emit light of a longer wavelength. Emission variations of the bound fluorescent compound are indicators of changes in the conformation of biomolecules, providing a useful tool for tracking biological pathways [3]. For surface applications, such as detecting tumors on epithelial surface, lower wavelength (e.g., blue, green, yellow) emitters, with high quantum efficiency, may produce as good or better results compared to the NIR emitters [4]. In this research, structure of new fluorescent azo dye, and its absorption and emission properties have been reported. In order to design compound with fluorophores and pharmacophores, 2-oxo-1,2,3,4-tetrahydropyrimidin based diazonium salt has been coupled with 3-cyano-6-hydroxy-4-methyl-1-phenyl-2-pyridone, resulting in a new fluorescent azo dye. The structure, absorption and emission properties of the investigated azo compound have been studied using ATR-FTIR, NMR, UV-Vis and fluorescence spectroscopic measurements.

Experimental
Synthesis
The synthesis of reported azo dye has been in detail described in our published study [5]. In brief, 2-oxo-1,2,3,4-tetrahydropyrimidine-azo-pyridone dye (PHPD) was prepared within diazo-coupling reaction. Pyrimidine derivate, 4-(4-aminophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate was utilized as diazo component, and 3-cyano-6-hydroxy-

1-phenyl-2-pyridone was a coupling component (Fig. 1). The structure of the dye was confirmed by spectroscopic data [5]. The FTIR-ATR spectra were recorded using a Nicolet™ iS™ 10 FT-IR Spectrometer (Thermo Fisher Scientific). The ^{1}H NMR and ^{13}C NMR spectral measurements were performed on a Bruker Ascend 400 instrument (400 Hz and 100 MHz, respectively) in deuterated dimethylsulfoxide (DMSO-d_6).

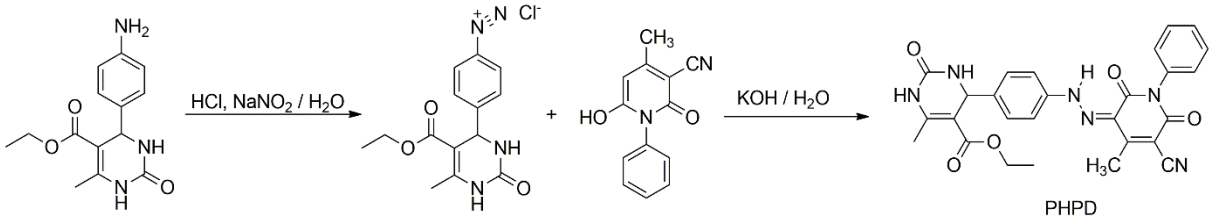

Fig. 1. Synthesis of PHPD

Optical measurements

The optical measurements were conducted in four solvents of different features. The UV-Vis absorption spectra were recorded on Shimadzu 1700 spectrophotometer, at concentration 4×10^{-5} mol L^{-1}, while emission spectra were recorded, at the same concentration, on Shimadzu RF-1501 PC spectrofluorometer. All spectroscopic measurements were carried out at room temperature (25 °C).

Results and discussion

The synthesized azo compound is a dark orange powder of high purity, and it was obtained in a good yield (about 70%). The spectral data of PHPD suggest the existence of the hydrazone tautomeric form (Fig. 1) in the solid state, as well as, in the DMSO-d_6 solution. The ATR-FTIR measurements displayed that N–H stretching vibrations of the hydrazone group appear at 3248 cm^{-1}. The bands at 1678 cm^{-1} and 1629 cm^{-1} are ascribed to the vibrations of carbonyl groups. The ^{1}H NMR spectrum showed the signal of hydrazone N–H group at 14.64 ppm, and ^{13}C NMR spectrum contained the signal at 161.11 ppm, confirming the existence of hydrazone tautomeric form [5].

The absorption and emission spectra were recorded in the range from 300 to 700 nm in following solvents: acetonitrile, DMSO, ethanol and chloroform (Fig. 2). From the presented optical spectra, it can be observed that used solvents had negligible effect on the position of absorption, as well as emission maxima. The obtained optical spectra suggest the existence of hydrazone tautomeric form in case of all used solvents. An intense band appearing in UV-Vis spectra, in the region of 370-550 nm, is ascribed to the intramolecular charge transfer (ICT) of the hydrazone tautomeric form [6]. The emission maxima of the investigated dye are in the region of 530 nm, and corresponding Stokes shifts are between 76 and 88 nm, indicating the recommendable properties for application as the potential new fluorescent probe.

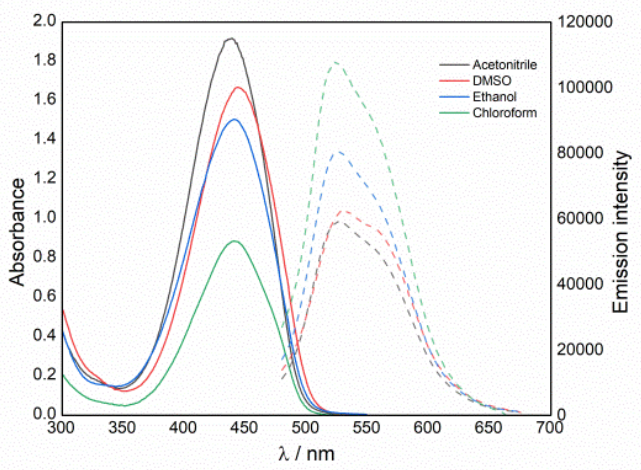

Figure 2. The UV-Vis and fluorescent spectra of the investigated PHPD dye

In addition, the biocompatibility assay, conducted within our previous study, demonstrated the non-toxic effect of the the investigated potential fluorescent probe (IC_{50} = 194.56±9.43 μM) to the normal human fibroblast cell line (MRC-5), suggesting that studied fluorescent azo dye is suitable for further investigation related to its potential application in different *in vitro* and *in vivo* models [5].

Conclusion

In this work, structure and optical properties of new potential fluorescence probe based on 2-oxo-1,2,3,4-tetrahydropyrimidine and 2-pyridone scaffolds have been presented. The FTIR-ATR and NMR analysis have shown that synthesized azo dye appeared in a solid state, as well as in the DMSO-d_6 solution, in hydrazone form. The absorption and emission spectra of the investigated dye were studied in solvents of different characteristics. The obtained absorption maxima were positioned in the region of 440 nm, while the emission maxima were in the green spectral region, with Stokes shifts ranking from 80 to 90 nm. Presented results may serve for further development of new green-emitting fluorescent probe and its potential application in fluorescence imaging.

Acknowledgements

This work was supported by Ministry of Education, Science and Technological Development of Republic of Serbia (Contract No. 451-03-9/2021-14/200017, 451-03-9/2021-14/200378 and 451-03-9/2021-14/200135).

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