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

OPTICAL AND ANTIOXIDATIVE PROPERTIES OF 5-(2,6-DIMETHYLPHENYL)-6-HYDROXY-4-METHYL-2-OXO-1,2-DIHYDROPYRIDINE-3-CARBOXAMIDE

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Abstract
A new heterocyclic azo dye 5-(2,6-dimethylphenyl)-6-hydroxy-4-methyl-2-oxo-1,2-dihydropyridine-3-carboxamide was synthesized and structurally characterized by elemental analysis, FTIR, ^{1}H and ^{13}C NMR spectroscopy techniques.
These analysis have confirmed that synthesized dye exists in the *tinctorial strongest* tautomeric *form*, hydrazone form. The dye is characterized by reflection spectrum, while absorption and emisson spectra are recorded in nine different solvents.
The antioxidant activity of the synthesized dye has been chemically tested and has been shown to have great potential as an antioxidant molecule.

Introduction
Azo compounds derived from 2-pyridone have been a special center of attraction due to their wide fields of application such as textile, pharmaceutical, electronic and graphic industries.
Azo dyes are often used in the field of non-linear optics, dye fibers, optical storage data and dye sensitized solar cells [1,2].
A thorough knowledge of molecular structure is important for finding the structure-activity relationship.
This study describes the synthesis, molecular structure, solvatochromism, and evaluation of antioxidant activity of 5-(2,6-dimethylphenyl)-6-hydroxy-4-methyl-2-oxo-1,2-dihydropyridine-3-carboxamide (Fig. 1). The dye molecular structure was confirmed based on the results of elemental analysis, FTIR, ^{1}H and ^{13}C NMR spectroscopy. The optical properties of the newly synthesized dye was defined on the basis of UV/Vis spectroscopy by recording the reflection spectra and determining the color position in the chromaticity diagram. Considering that dyes generally need to be dissolved in order to combine with other materials, knowledge of their behavior in solutions is of great importance, and therefore the absorption and fluorescence spectra of dye solutions in solvents of different polarity were recorded. Antioxidative activity of the dye has been evaluated by ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) assay, expressed as IC_{50} value and compared to ascorbic acid.

Experimental
Synthesis

The investigated azo dye have been synthesized from the 2,6-dimethylphenyl diazonium salt and 6-hydroxy-4-methyl-2-oxo-1,2-dihydropyridine-3-carboxamide [3], using the classical reaction of diazotization and diazo-coupling [4]. The crude product was recrystallized from *N*,*N*-dimethylformamide. Elemental analysis was performed using a Vario EL III elemental analyzer. The IR spectra were recorded using a Bomem (Canada) MB-Series 100 Fourier

transform-infrared (FT-IR) spectrophotometer in the form of KBr pellets. The ^{1}H and ^{13}C NMR data were performed using a Varian Gemini 2000 (200 Hz and 50 Hz, respectively) in deuterated dimethyl sulfoxide (DMSO-d_6) with tetramethylsilane (TMS) as an internal standard. All spectral measurements were carried out at room temperature (25 °C). The dye reflection spectra were recorded on a Shimadzu 2600 spectrophotometer in the range of 220-1350 nm.

The ultraviolet-visible (UV/Vis) absorption spectra were recorded on a Shimadzu UV-Visible UV-2600 (Japan) spectrophotometer in the range 200-700 nm, while emission spectra were recorded on Fluorescence spectrophotometer Perkin Elmer precisely (LS 45 Luminescence Spectrometer).

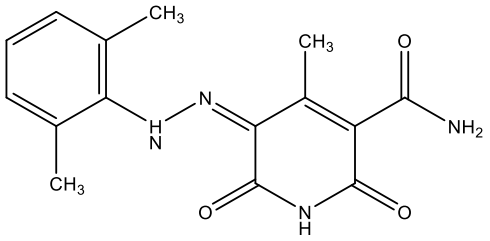

Fig. 1. *The chemical structure of the 5-(2,6-dimethylphenylazo)-6-hydroxy-4-methyl-2-oxo-1,2-dihydropyridine-3-carboxamide*

5-(2,6-dimethylphenylazo)-6-hydroxy-4-methyl-2-oxo-1,2-dihydropyridine-3-carboxamide: Orange crystalline substance: m.p.: 243-245 ºC, yield 46 %; IR (KBr, ν/cm^{-1}): 3162 (NH), 3384 (NH), 1672, 1643 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6, δ/ppm): 2.24 (3H, s, CH_3), 2.48 (6H, s, CH_3), 7.16 (1H, t, J = 8,0 Hz, Ar–H), 7.24 (2H, d, J = 8,0 Hz, Ar–H), 7.55 (1H, s, NH_2), 7.75 (1H, s, NH_2), 11.75 (1H, s, NH on heterocyclic), 14.62 (1H, s, NH of hydrazone form); ^{13}C NMR (50 MHz, DMSO-d_6, δ/ppm): 166.9 ($CONH_2$), 162.3 (CO Py), 146.1 (Ar), 138.4 (Py), 130.1 (Ar+Py), 129.2 (Ar), 126.3 (Ar), 125.5 (Py), 124.3 (Ar), 19.7 (CH_3), 15.1 (CH_3). Anal. Calcd for $C_{15}H_{16}N_4O_3$: C, 59.99; H, 5.37; N, 18.66. Found: C, 60.12; H, 5.43; N, 18.73.

Evaluation of antioxidant activity

Antioxidant activity of the dye is determined by ABTS assay [5]. The test is based on the ability of the molecule to scavenge the $ABTS^{\cdot+}$ radical cation. The precentage inhibiton was calculated acording to the equation:

$$\text{Inhibition (\%)} = (Ac - As)/Ac * 100$$

where *As* is the absorbance of the sample solution and *Ac* is the absorbance of the control solution. Ascorbic acid was used as a standard antioxidant molecule. Furthermore, IC_{50} values of the dye and ascorbic acid were determined. The methanolic solutions of dyes and ascorbic acid were prepared at concentrations 3, 1.5, 0.75 and 0.15 mM, and obtained IC50 were compared.

Results and discussion

Pyridone azo dyes bearing –OH group in the position 6 of the pyridone ring are known to exhibit azo-hydrazone tautomerism, in both solid state and solutions. The IR and NMR spectra of the dye clearly show the existence of the hydrazone form in solid state and DMSO-d_6, respectively. The infrared spectra showed characteristic vibration of two carbonyl groups at

1672 and 1643 cm^{-1} indicating the presence of the hydrazone tautomeric form. Also, N–H stretching vibration from hydrazone group is observed at the 3384 cm^{-1}, while N–H stretching vibration of pyridone moiety is noted at the 3162 cm^{-1}.
The ^{1}H NMR spectrum of dye exhibits a signal at 14.62 ppm. This signal corresponds to N–H proton resonance of the hydrazone form. Also, ^{13}C NMR spectra confirmed the existence of the hydrazone form. Peak observed in the 166.9 ppm is ascribed to carbonyl group of 3-amido group, while peak originating from the C atom of carbonyl group, in the pyridone ring is observed at 162.3 ppm.

Reflection spectra
Figure 2 shows the reflection spectra, the photographed dye and its position in the color system CIE lab. The CIE lab color system determines color based on the dominant wavelength and mean reflectance [6].

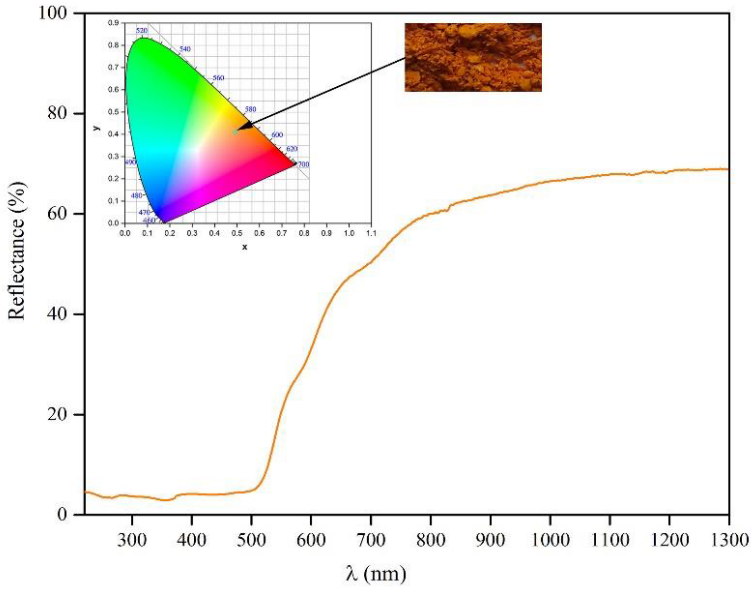

Fig. 2. *Reflection spectra of dye and its position in CIE lab color system*

The absorption and fluorescence spectra of the dye were recorded in solvents of different properties in order to see the influence of the solvent on the position of the absorption and fluorescent maxima, as well as on the shape of the bands. The strongest absorption band can be seen in the region from 350 to 500 nm, and corresponds to the π-π* transition of hydrazone form. The absorption maxima of the dye show little variation in different solvents. The Stokes shift in all tested solvents is about 80 nm.

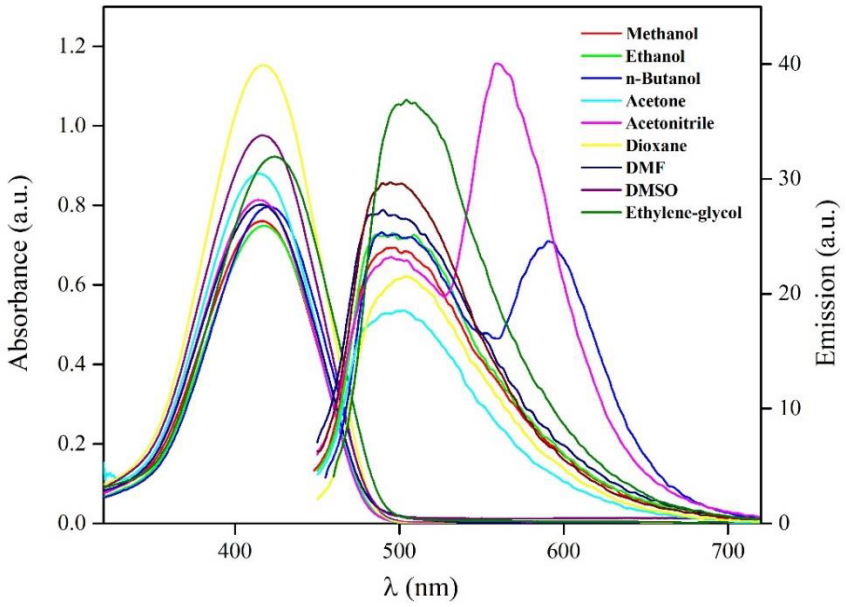

Fig. 3. *Apsorption and emission spectra of dye in different polarity solvent*

Antioxidative properties

Antioxidant activity of the dye is assayed using the ABTS method. The scavenging activity of azo dye was compared to the activity of ascorbic acid. The results have shown that dye exhibit remarkable activity (c = 3 mM) of the investigated dye (96.0%) compared to ascorbic acid (95.3%). Furthermore, IC_{50} values, which corresponds to the concentration of sample able to scavenge 50% of ABTS radicals in the solution, are evaluated. IC_{50} values of the dye (0.78 mM) and ascorbic acid (1.25 mM) have shown that the dye is more potent antioxidant molecule than ascorbic acid (Fig. 4) and could be considered as promising antioxidant candidate.

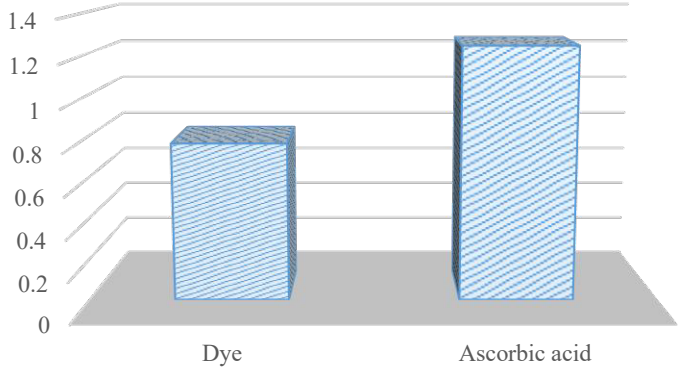

Fig. 4. *IC_{50} values of the dye and ascorbic acid*

Conclusion

The investigated dye was synthesized and according to the FTIR, ^{1}H and ^{13}C NMR spectral data, dye exists in the hydrazone form in the solid state and in DMSO-d_6. Analysis of the UV/Vis spectra confirmed that the analyzed dye appears in all solvents in hydrazone tautomeric

form, while the analysis of emission and absorption spectra determined that the Stokes shift in all tested solvents is about 80 nm.
Evaluation of the antioxidant activity of the dye and ascorbic acid have shown that dye is more potent scavenger of $ABTS^{\bullet+}$ radical cation than the stanard molecule. Furthermore, its IC50 value is lower than IC50 value of ascorbic acid indicating that this dye could be considered as promising antioxidant candidate.

Acknowledgements
The research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (contract number 451-03-9/2021-14/200017 and 451-03-9/2021-14/200135)

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