SOME UNUSUAL SPECTRAL PROPERTIES OF 6-AZAURACIL DERIVATIVES

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Prin intermediul spectroscopiei de fluorescență au fost obținute proprietățile optice ale câtorva derivați 6-azauracil. Această metodă a permis o caracterizare rapidă şi precisă, cu un grad ridicat de specificitate şi sensibilitate.

The optical properties of several new 6-azauracil derivatives have been obtained by means of fluorescence spectroscopy. This method allowed a rapid and accurate characterization, having high specificity and sensitivity.

Keywords: 6-azauracil, fluorescence, 6-azauracil derivatives

1. Introduction

In recent years, many applications have arisen for substances and compounds possessing specific luminescence properties. Such applications include photodynamic therapy, non-linear optics, micro- and nano-optoelectronics, sensors etc. [1],[2]

Luminescence is generated by the emission of a photon when a molecule returns from an excited electronic state to the ground state. One of the most important luminescence properties is represented by fluorescence, the process of photon emission when an electron returns from the lowest vibrational level of an excited singlet state to the ground state. It is important to note that the fluorescence phenomenon occurs only after the absorption of a suitable energy quantum, promoting the molecule to an excited state.[3]

In fluorescence spectroscopy, the wavelength of emitted light on the irradiation of a sample with a monochromic light can be easily recorded (emission spectra), as well as the variation of sample's absorption of excitation light at a

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fixed wavelength, similar to absorption spectra (excitation spectra). Recording all the emission spectra for an interval of excitation wavelengths, the 3D spectra can be obtained, which fully characterizes a given sample in terms of fluorescence.

The fluorescence properties of compounds can be of vital importance in medical applications, where fluorescence compounds can be used as markers and be readily traced in the human body, in a non-invasive manner. An emerging prospect in photodynamic therapy is the use of fluorescent photosensitizers that can be quantitatively dosed *in vivo*.[4]

In the present paper, we have investigated the fluorescence properties of several 5-substituted-6-azauracil derivatives.

2. Experimental

2.1. Materials

The synthesis of the 6-azauracil derivatives has been previously described.[5] The 6-azauracil derivatives have been obtained through nucleophilic substitutions of the bromine atom of 5-bromo-6-azauracil with various aromatic amines and phenols.

The fluorescence spectra have been investigated at room temperature, using dilute ethanol solutions. The 3D fluorescence spectra have been acquired using a JASCO FP-6500 fluorescence spectrophotometer, at 1 nm intervals for emission and 5 nm intervals for excitation, a response time of 0.02 seconds, high sensitivity, and a scanning speed of 2000 nm/minute.

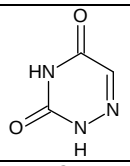
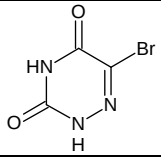
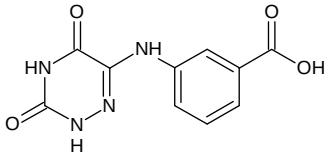
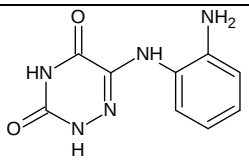
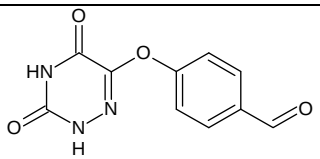
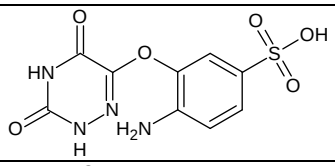
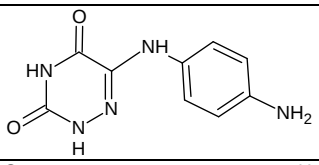
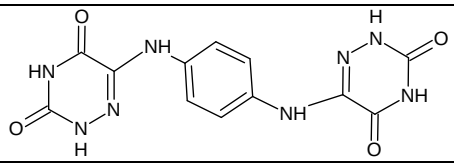
Unless otherwise stated, all he samples consisted in ethanol solutions of 0.0005 M concentration.

3. Results and discussion

The spectral properties of eight compounds have been investigated using the method described in section 2 of this paper. The compounds include 6-azauracil (**1**), 5-bromo-6-azauracil (**2**), four derivatives obtained by the nucleophilic substitution of 5-bromo-6-azauracil with various aromatic amines (**3**,**4**,**7**,**8**) and two compounds obtained by substitution with phenols (**5**,**6**). Their respective structures are presented in table 1.

Table 1

6-Azauracil derivatives analyzed

Compound	Structure
1	
2	
3	
4	
5	
6	
7	
8	

The fluorescence spectra recorded for compounds **1** - **8** show that all of the investigated compounds present fluorescence properties. The 3D fluorescence spectra consist in emission intensity versus emission wavelength and excitation wavelength. A typical spectrum of 6-azauracil is shown in Fig. 1.

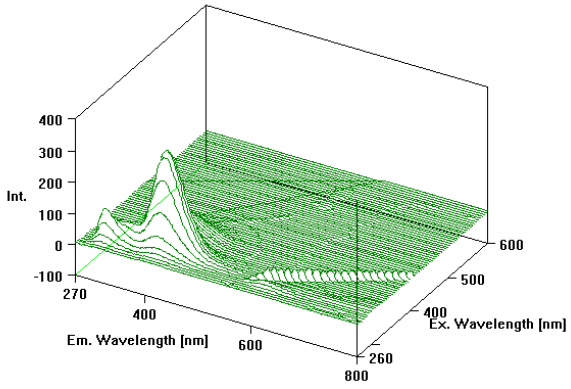


Fig. 1. 3D fluorescence spectra of 6-azauracil

Covering a high range of ultraviolet and visible spectra, a 3D fluorescence can be used to differentiate between compounds with similar structures. A comparison between the fluorescence of compound **4** and **7**, whose structure differ only through the position of an amino group on the aromatic ring, can be seen in Fig. 2. The fluorescence spectra show an emission maximum of compound 4 is found at 409 nm for an excitation of 345 nm while for compound 7 the emission maximum of 636 nm is found, for an excitation of 550 nm.

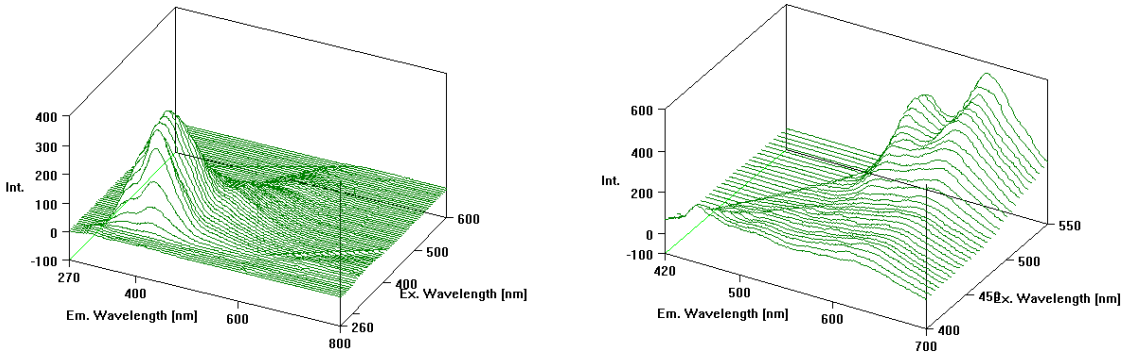


Fig. 2. Comparison between the florescence spectra of **4** (left) and **7** (right)

Similarly, compounds **7** and **8** are mono- and di- substituted phenylendiamine derivatives. The difference between the two compounds is easily spotted in 3D fluorescence spectra, 7 showing an emission maximum of 636 nm for an excitation wavelength of 550 nm, while 8 presents an emission maximum of 605 nm for an excitation of 590 nm (see Fig. 3).

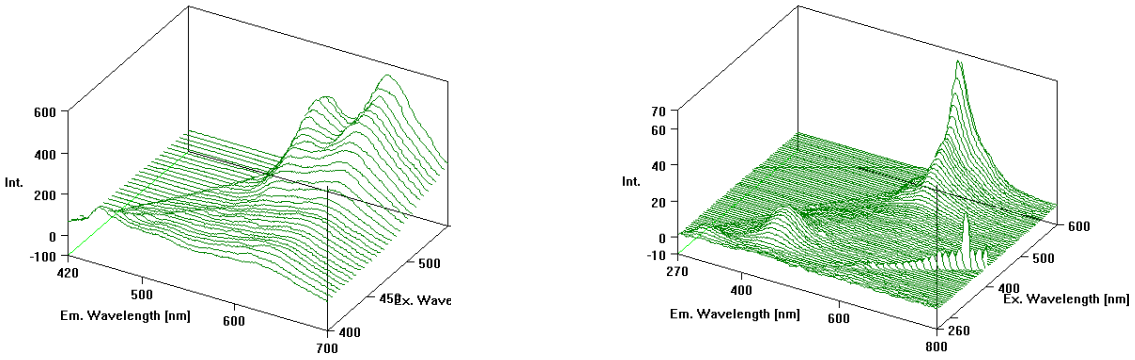


Fig. 3. Comparison between the florescence spectra of **7** (left) and **8** (right)

Using the *JASCO Spectral Manager* software, the emission and excitation maxima for every compound have been found from the "Emission search view" function. The difference between the emission maximum and excitation maximum is due to the fact that the energy of emitted quantum after an absorption process is lower than that of the absorbed photon, part of its energy being lost through non-radiative relaxation processes. This fact causes the emission maximum to be found at higher wavelengths than the excitation maximum. This difference is known as the Stokes shift.

Table 2

Excitation and emission maximum and Stokes shift for the compounds analyzed

Compound	Excitation maximum [nm]	Emission maximum [nm]	Stokes shift [nm]
1	305	409	104
2	315	406	91
3	360	445	85
4	345	409	64
5	415	456	41
6	505	560	55
7	550	636	86
8	590	605	15

From all the analyzed compounds, 6-azauracil **1** shows the greatest Stokes shift, with a difference of 104 nm between the excitation and emission maximum. The heavy atom (Br) quenching effect can be seen by the decrease of 13 nm in Stokes shift for 5-bromo-6-azauracil 2 in comparison with 6-azauracil.

Likewise, the replacement of a bromine atom with an aromatic moiety in compounds **3** – **8** leads to a quenching effect in comparison with 6-azauracil **1**, having Stokes shifts between 86 nm (compound **7**) and 15 nm (compound **8**).

Concerning the excitation maxima, **1** has the lowest excitation wavelength maxima. Compound **2** shows an increase of 10 nm, while **4**, **3** and **5** have excitation maxima of 345, 360 and 415 nm, respectively. From all compounds, only compounds **6** – **8** present excitation maxima above 500 nm, with **6** having a maximum at 505nm, **7** at 550 nm and **8** at 590 nm. Excitation maxima above 500 nm are a requirement of photodynamic therapy, as human skin is more permeable for red light. [6]

From the 6-azauracil derivatives, compounds **3** and **7** show the highest fluorescence properties, as seen from the Stokes shifts. The 3D spectra and emission and excitation maxima for compound **3** are presented in Fig. 4 and Fig. 5, respectively.

The 3D fluorescence spectrum of compound **3** shows an intense peak corresponding to the emission maximum of 445 nm for a excitation wavelength of 360 nm. A second peak can be seen at a lower excitation wavelength of 290 nm. It appears at the same emission wavelength as the main peak. The excitation spectrum for a constant emission wavelength equal to that of the emission maximum is represented by the left side graph in Fig. 4. Likewise, the emission spectrum is obtained for constant excitation wavelength equal to the excitation maximum, and is represented by the right side graph in Fig. 5.

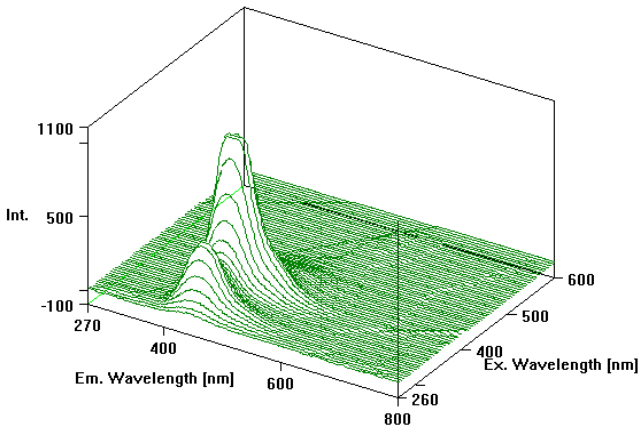


Fig. 4. 3D Fluorescence spectra for compound **3**

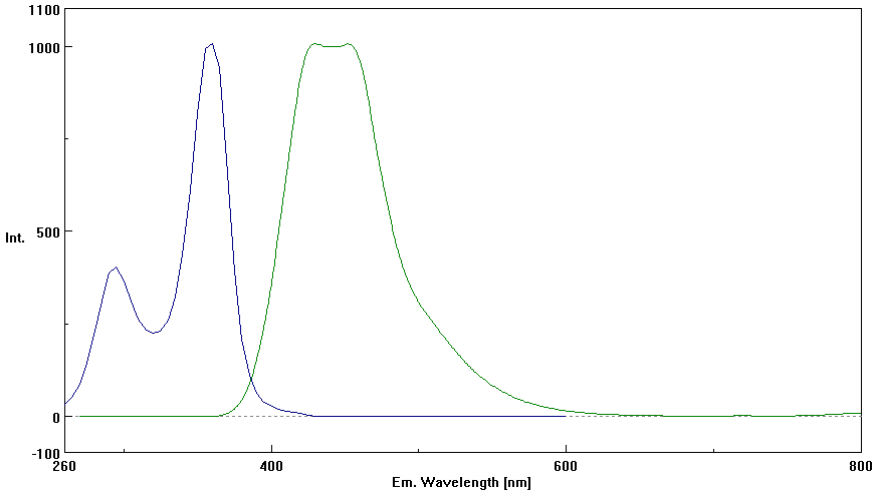


Fig. 5. 3D Excitation and emission spectra for compound **3**

4. Conclusions

The fluorescence spectra of 6-azauracil and seven of its derivatives have been investigated. All compounds show distinct fluorescence spectra, which can be used to easily distinguish between similar 6-azauracil derivatives. The 3D fluorescence spectrography allows for a qualitative and quantitative determination of 6-azauracil compounds.

From all compounds, only compounds **6** – **8** present excitation maxima above 500 nm, which is required in photodynamic therapy.

Acknowledgements

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R E F E R E N C E S

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