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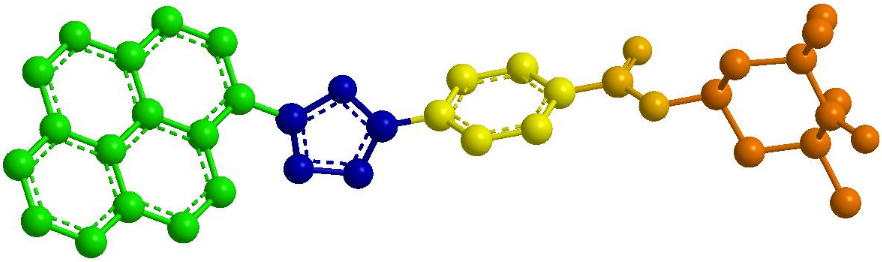

PhD THESIS

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PhD Supervisor:
Prof. dr. Petre Ioniță

2020

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PHOTOLUMINESCENT AND PARAMAGNETIC ORGANIC SWITCHES

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2020

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(corresponding to the PhD thesis)

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Introduction

Fluorophores are organic molecules with special optical properties that give them applications in fields such as materials chemistry, biochemistry, biology or environmental chemistry. The modification of the emission characteristics, through the structural variation, but also of the experimental conditions can be the result of different intermolecular and intramolecular processes such as: intramolecular proton transfer (ESIPT), molecular aggregation (AIE, ACQ), isomerization, etc.

The doctoral thesis “Photoluminescent and paramagnetic organic switches” presents the results obtained regarding the synthesis and study of optical properties, especially regarding fluorescence emission, of various substituted derivatives from the classes of *N*-acylhydrazones, oxadiazole heterocycles and profluorescent nitroxides. For this purpose, the fluorescence absorption and emission properties were investigated, in different experimental conditions and various applications were developed, based on the switching capacity of the synthesized compounds between the emitted colors and, respectively, the switching ability between fluorescence and paramagnetism.

The first chapter, entitled "*Fluorescence of organic compounds*", describes the results published in the literature on fluorophores, the mechanisms of occurrence and extinction of fluorescence, synthesis and applications of *N*-acylhydrazones, oxadiazole heterocycles and profluorescent nitroxides.

The second chapter, "*Synthesis of new bis-N-acylhydrazones with fluorescent properties*", describes the synthesis of new hydroxy-bis-*N*-acylhydrazones whose structural changes can lead to different properties and applications. The synthesized hydroxy-*N*-acylhydrazones can be used as molecules able of emitting fluorescence by excited intramolecular proton transfer (ESIPT). Also, the conformational changes resulting from the phenomenon of molecular aggregation, under the influence of water, lead to fluorescence emission (AIE).

The third chapter, "*Synthesis of new nitroxides with dual properties: luminescence and paramagnetism*", presents the synthesis of new profluorescent nitroxides derived from oxadiazoles. Fluorescence experiments of profluorescent nitroxides indicate the extinction of fluorescence in the presence of free radical and its recovery by reaction with a reducing agent (phenylhydrazine, sodium ascorbate). Also, the chapter presents the synthesis of fluorophore-aza-ether-crown compounds and, respectively, fluorophore-aza-ether-crown-nitroxide, for which the structure, photophysical properties and their behavior in the presence of alkaline metal ions were investigated.

The research results aim to be a contribution to the development of new fluorescent systems that can be modified in a controlled and reversible way, that can find applications as sensors for monitoring different chemical or biochemical systems.

CHAPTER 1. Fluorescence of organic compounds

1.1. General fluorescence considerations

Luminescence represents spontaneous emission of photons from the ultraviolet, visible or infrared domain of excited chemical species.[1] The process by which, following light absorption (photons), a molecule passes from the ground state (S_0) to the excited state (singlet - S_1 or triplet - T_1) and returns to the ground state by photon emission is called photoluminescence and can be defined as fluorescence ($S_1 \rightarrow S_0$), phosphorescence ($T_1 \rightarrow S_0$) or delayed fluorescence ($T_1 \rightarrow S_1 \rightarrow S_0$). Fluorescence is the phenomenon of spontaneous emission of electromagnetic radiation (radiative process) following an electronic transition between two states with the same spin multiplicity (singlet).[2]

Molecules that pass into an excited state by the absorption of a photon can return to the ground state by fluorescence emission or other non-radiative mechanisms (without radiation emission) such as: Internal Conversion (IC), Intersystem Crossing (ISC - maybe be followed by the process of phosphorescence), vibrational relaxation, intramolecular charge transfer or conformational changes.[1]

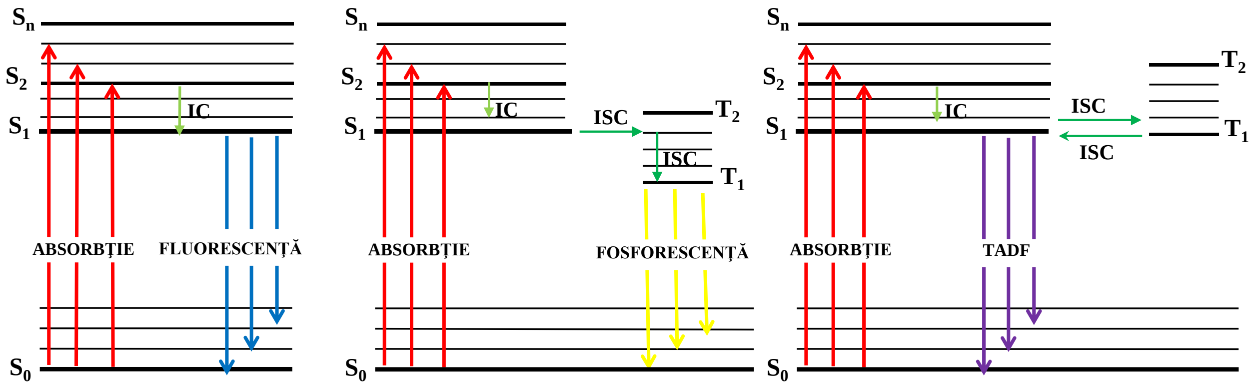
Excited species can interact with other molecules, generating processes such as: proton transfer, electron transfer, energy transfer, formation of excimers (unstable complexes that are formed in the excited state by the interaction of two identical molecules[3]) or exciplexes (unstable complexes that are formed in the excited state by the interaction between two different molecules[3]). Such processes can compete with the fluorescence process and can lead to the formation of new species with higher fluorescence than the original species.[1] Fluorescence emission can be influenced by physical or chemical factors such as: environmental polarity, pH, formation of hydrogen bonds, pressure, viscosity, temperature, electric potential, ions, etc.[1]

1.1.1. UV absorption and emission. Electronic transitions

Through absorption of a photon upon irradiation of a molecule, takes places an electron transitions from an occupied orbital to a higher energy free orbital, and the molecule changes from the ground state (S_0) to the excited state (S_1). Depending on the structure of the molecule, two types of electronic transitions can be encountered: $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ (for molecules containing non-participating electrons) (Figure 1).

Electronic transitions are allowed between energy levels with the same spin multiplicity, for example singlet-singlet or triplet-triplet transitions, but also many transitions can occur between levels with different multiplicities, such as singlet-triplet or triplet-singlet. These transitions occur with the inversion of the electronic spin and are called forbidden transitions.[1] Fluorescence emission can be observed after a singlet-singlet transition, and phosphorescence following a triplet-singlet spin reversal transition.

The Jablonski[1] diagram (Figure 2) is the simplest way to understand the transitions that can occur in the processes of excitation and emission of fluorescence or phosphorescence.



S – singlet T – triplet IC – conversie internă ISC – încrucișare intersistem (intersystem crossing) TADF - fluorescență întârziată activată termic

Figure 1. Jablonski diagram - schematization of electronic transitions that take place in the processes of absorption, fluorescence, phosphorescence and thermally activated delayed fluorescence

1.1.2. Parameters characteristic of fluorophores

Fluorophores have different and characteristic fluorescent properties determined by: emission wavelength, emission band intensity, excited fluorophore lifetime and quantum fluorescence efficiency. The determination of these parameters can be done by recording the excitation and emission spectra of the fluorophore of interest.

1.1.3. Structure of fluorophores. Mechanisms of occurrence and extinction of fluorescence

The characteristic parameters of a fluorophore may vary depending on the structure (nature of the substituents) and the experimental conditions (solvent, pH, viscosity, etc.) used to determine them. The variation of the structural and experimental parameters can lead to the dramatic improvement or decrease of the fluorescence emission and implicitly of the quantum fluorescence efficiency of a fluorophore.

1.1.3.1. Structural parameters that influence fluorescence

Fluorophores are generally aromatic compounds that contain in their structure conjugated electron systems. The presence of extended conjugation leads to a shift of the absorption and emission maxima to longer wavelengths.[4]

Replacement of fluorophores with groups such as -OH, $-NH_2$, -COOH can lead to changes in the emission color (wavelength) and thus the characteristics of the fluorophore. The nature of the substituent and its position can cause major changes in the absorption and fluorescence spectra.[4] Substitutes with a large atomic number, such as bromine or iodine, can also cause fluorescence quenching because it increases the probability that the non-radiative ISC transition to occur. However, there may be cases where such substituents do not influence fluorescence, especially when the quantum yield fluorescence is high.[4]

1.1.3.2. Experimental parameters that influence fluorescence

The characteristics of fluorescence emission are strongly influenced by the experimental conditions (solvent, viscosity, temperature). The interaction of the fluorophore with the solvent depends on the polarity of the solvent and on the value of the dipole moment of the fluorophore.

In addition, in the case of protic solvents such as water, the solvent can donate or accept a proton, favoring the phenomenon of protonation or deprotonation of the fluorophore in the excited state.[1] For example, in water, the acidic character of a phenolic group or the basic character of a nitrogen from a heterocycle, may increase after irradiation due to protonation/deprotonation processes that may occur.[1] Previous studies[1] have shown that the acidic form of a fluorophore has a maximum emission at shorter wavelengths compared to its basic form, which leads to the idea that the absorption and emission of fluorescence for fluorophores that contain functions susceptible to acidic or basic environment are dependent on pH.

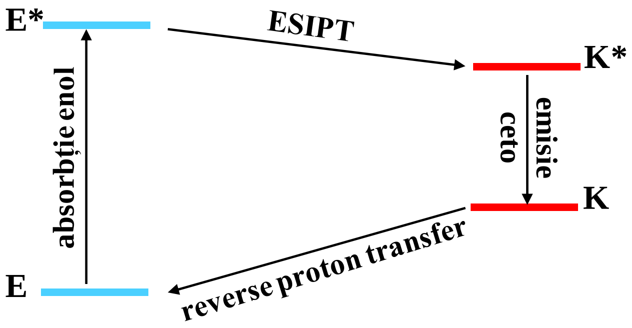
Changing the position of the maximum emission depending on the nature of the solvent used is called solvatofluorochromism, and the displacement of the maximum emission can take place towards longer (red) or shorter (blue) wavelengths.[1]

Fluorescence emission can also be influenced by steric factors such as the stiffness of the molecule and the viscosity of the solvent used. The use of a fluid solvent favors the relaxation phenomenon that accompanies the process of reorientation of the solvent dipoles.[4] The use of a high viscosity solvent will not allow the reorganization of the solvent molecules and, implicitly, will not lead to the change of the maximum emission position.[1]

1.1.3.3. Excited state intramolecular proton transfer (ESIPT)

The process of excited intramolecular proton transfer (ESIPT) requires the possibility of forming an intramolecular hydrogen bond between a donating proton group (–OH, –NH) and a proton acceptor group (–C=O, –N=) in the vicinity, favorably geometrically positioned, which determines, by irradiation, the transfer of the proton from one atom to another, by tautomerization.[5]

The most common examples of molecules showing the process of intramolecular transfer of protons contain in their structure the –OH group and may have keto-enol tautomerism, so that they are in the ground state in enol form, stabilized by the formation of the hydrogen bond between the two groups (Scheme 1).[5]



Scheme 1. Schematic representation of the intramolecular proton transfer process (ESIPT) for keto-enol species

1.1.3.4. Agreggation induced emision (AIE)

The molecular aggregation-induced emission process (AIE) is the phenomenon in which a low-emission fluorophore or non-fluorescent compound can exhibit intense emission due to the formation of molecular aggregates. The formation of these aggregates can be induced by dissolving the compound in an organic solvent, followed by the addition of water as a cosolvent.[9]

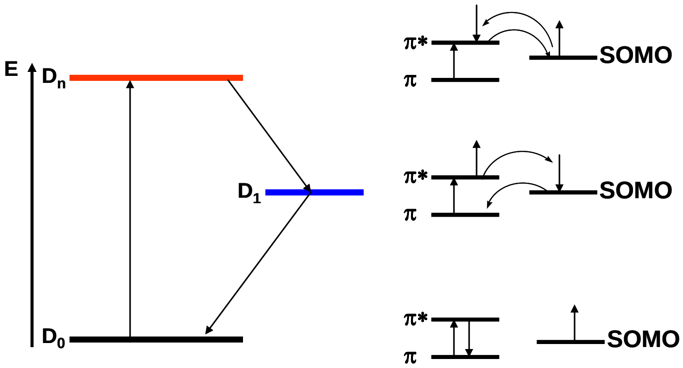
The mechanism of emission induced by the formation of molecular aggregates may involve: intramolecular rotational restrictions (RIR) or intramolecular vibration restrictions (RIV).[10]

1.1.3.5. Aggregation-caused quenching (ACQ)

Fluorescence quenching due to molecular aggregation (ACQ) can occur in both solution and solid state. Usually, classical fluorophores contain in their structure several conjugated aromatic rings, and in concentrated or solid solutions the phenomenon of fluorescence quenching occurs due to the π-π interactions and the planarity of the molecule.[11]

1.1.3.8. Fluorescence quenching in the presence of a paramagnetic center

Fluorescence quenching in the presence of a paramagnetic center is achieved by covalently binding a fluorophore to a stable free radical, and is due to the exchange of electrons between the two species that form the hybrid molecule.[12] The process generates the change of spin multiplicity of energy states (Figure 2). For example, in the case of a hybrid molecule containing in its structure a fluorophore and a nitroxide, the singlet ground state (S_0) and the singlet excited state with the lowest energy (S_1) of the fluorophore pass into the doublet state due to the presence of the free electron from the free radical structure.[12] The unpaired electron from SOMO (Singly Occupied Molecular Orbital) interacts with the conjugated electrons system of the fluorophore, the forbidden transitions from the singlet excited state (S_1) to the triplet state (T_2) and the transition at the singlet ground state (S_0) are allowed, the process taking place with a decrease in the quantum yield fluorescence (Figure 2).



Scheme 2. Transitions that occur at the interaction of the fluorophore conjugate electron system with the unpaired electron in the free radical structure

1.2. Organic fluorophores. *N*-acylhydrazones and oxadiazole heterocycles

In the present doctoral thesis were studied molecules belonging to the classes of *N*-acylhydrazone and oxadiazole heterocycles. Such molecules can generate materials known as smart fluorescent materials, defined as materials that can change their photophysical properties (eg fluorescence) in the presence of several stimuli such as temperature, pressure, vapors, solvents, applicable as optical devices, sensors, imaging substances, etc.[13]

N-acylhydrazones can show configurational and conformational changes under the action of various physical or chemical stimuli, changes that can lead to major changes in luminescent properties. They are also used for synthesis new 1,3,4-oxadiazoles.

Oxadiazole heterocycles have luminescence as their main property, this class of heterocycles being intensively studied in order to find new molecules with improved fluorescent or phosphorescent properties, known mainly for the manufacture of electronic devices such as organic LEDs.[14]

The design and synthesis of new organic molecules with fluorescent properties such as *N*-acylhydrazones or 1,3,4-oxadizoles is a challenge for organic chemists, given the multitude of synthesis methods by which they can be obtained but also the many applications they can have.

1.3. Stable free radicals. Profluorescent nitroxides

Free radicals are kinetically stable species and are characterized by the presence of an unpaired electron.[15] Stable free radicals are species that do not react with oxygen and can be isolated, handled and stored as pure compounds under normal conditions.[15]

The covalent bonding of a stable free radical and a fluorophore leads to the loss of fluorescence, which can be regained by annihilating the radical species (see below).[16] Compounds of this type can therefore have two properties - fluorescence and paramagnetism - between which they can switch depending on the targeted applications.

1.4. Conclusions

Aspects of - *i)* general considerations of the occurrence of photoluminescence phenomena and the structural diversity of fluorophores and factors influencing light emission, *ii)* description of structural properties, synthesis, reactivity and applications of *N*-acylhydrazones and oxadiazole heterocyclic derivatives, and *iii)* presentation of the structure of free radicals of nitroxide type and profluorescent nitroxide derivatives, synthesis and the main applications in which they find their utility - are the premises from which we started in the elaboration of the research results described in the doctoral thesis and which are presented in Chapters 2-4.

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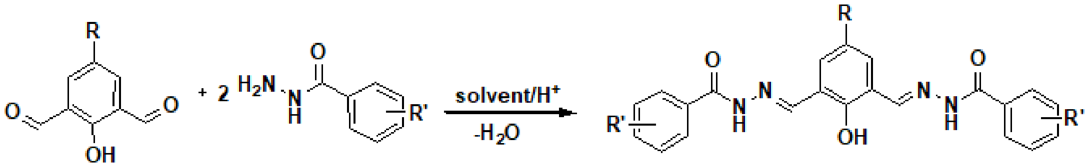
Chapter 2. Synthesis of new bis-*N*-acylhydrazones with fluorescent properties

In this doctoral thesis we have focused on hydroxy-bis-*N*-acylhydrazones, due to their structural features, but also to the many applications described in the literature, especially in terms of the ability to form metal complexes.[1, 4] Although they were intensively studied, there are few references to the investigation of optical properties (absorption and emission) or to the ability of conformational and configurational modification.

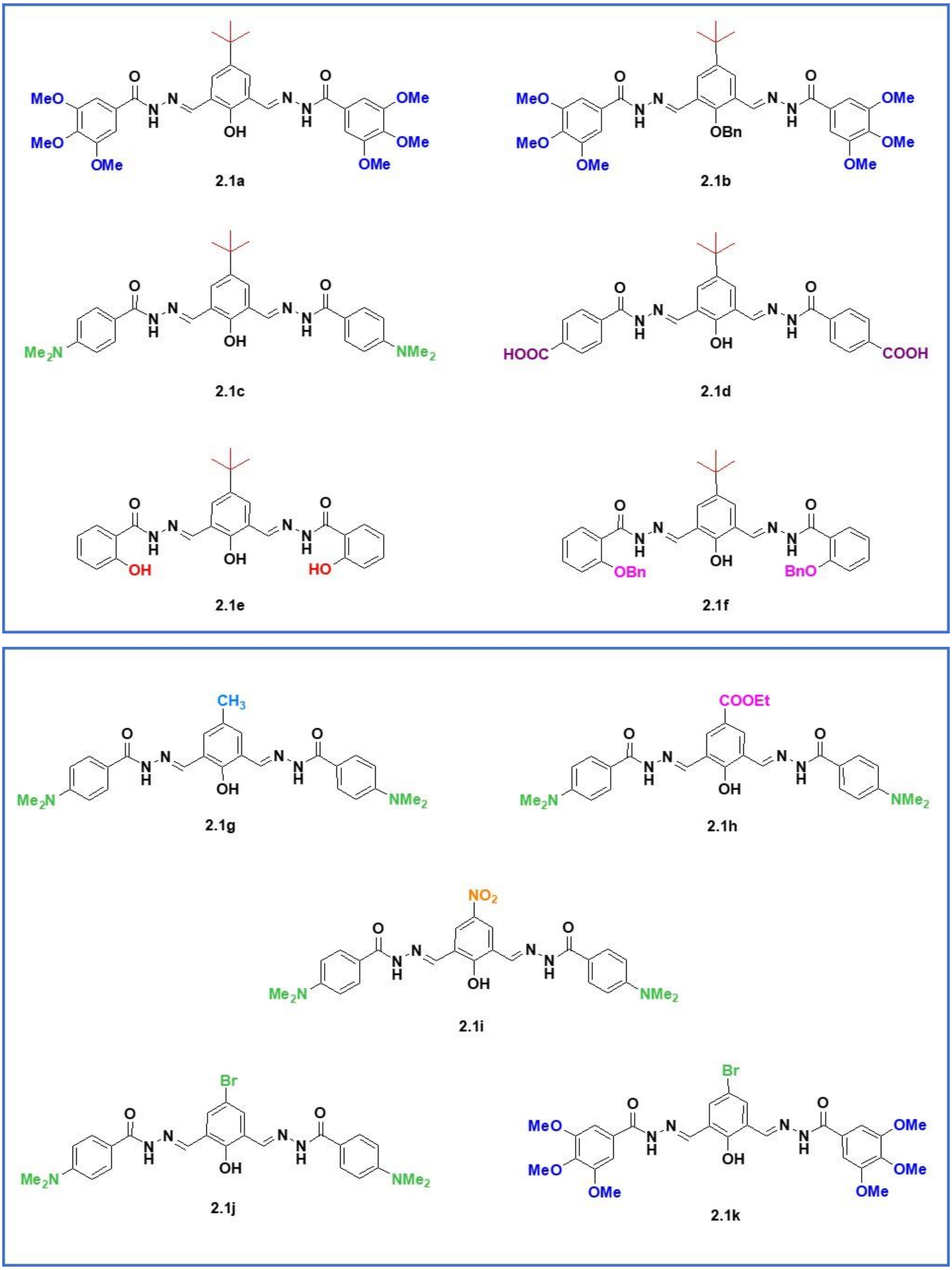
Hydroxy-bis-*N*-acylhydrazones (Scheme 2) contain numerous groups able of coordinating metal ions such as copper, zinc, molybdenum or lanthanides,[1] form complexes having applications as fluorescent sensors[1] for various analytes or catalysts[2] in the oxidation reactions of alcohols. Also, such compounds can be used to obtain gels with luminescent properties[1] as a result of molecular aggregation, which occurs after the addition of water to the system.



Scheme 3. Synthesis of hydroxy-bis-*N*-acylhydrazones

The main objective was the synthesis of a series of new hydroxy-bis-*N*-acylhydrazones, containing different substituents and the study of behavior under the action of various physical or chemical stimuli, in order to be used as molecular switches. Hydroxy-bis-*N*-acylhydrazones with various structures were synthesized, containing electron-attracting or repulsive substituents. Thus, the secondary objectives consisted in the study of the influence of substituents on the absorption and emission properties, but also the structural changes that hydroxy-bis-*N*-acylhydrazones can present under the action of different stimuli (for example: light, solvent), in order to develop possible applications.

The chapter presents the synthesis, structural analysis and investigation of photophysical properties of hydroxy-bis-*N*-acylhydrazones **2.1a-k** (Figure 3) as well as studies on the behavior of acylhydrazones in the presence of factors such as water, light or pH and their applications in biochemistry.

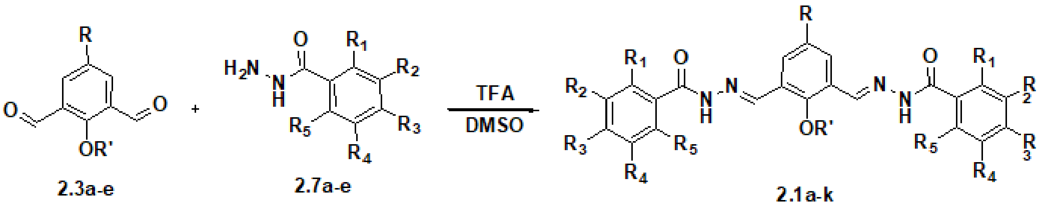


2.1a **2.1b** **2.1c** **2.1d** **2.1e** **2.1f** **2.1g** **2.1h** **2.1i** **2.1j** **2.1k**

Figure 2. Structures of hydroxy-bis-*N*-acylhydrazones **2.1a-k**

2.2. Synthesis of bis-*N*-acylhydrazones

The synthesis of hydroxy-bis-*N*-acylhydrazones (Scheme 3) was performed by the condensation reaction between aldehydes **2.3a-e** and hydrazides **2.7a-e** using DMSO as solvent, in the presence of a catalytic amount (2-3 drops) of trifluoroacetic acid.



Scheme 4. Synthesis of hydroxy-bis-*N*-acylhydrazones

Hydroxy-bis-*N*-acylhydrazones **2.1a-k** are solid, white or yellow compounds (orange in the case of the compound containing in its structure the group -NO_2), having melting points between 150-306°C.

2.3. Structural analysis

The structure of the compounds was confirmed by nuclear magnetic resonance spectroscopy (^{1}H, ^{13}C, two-dimensional spectra) and high-resolution mass spectrometry, using Chemical Ionization at Atmospheric Pressure (APCI-HRMS) as the ionization source. NMR spectra were recorded in deuterated solvent (DMSO-d_6) at a frequency of 500 MHz or 300 MHz for proton spectra and 125 MHz or 75 MHz for carbon spectra. Thin layer chromatography and melting temperature were used to confirm the purity of the compounds.

2.3.1. Solid state structure

Additional data on the structure of the compounds were obtained by single crystal X-ray diffraction for compounds **2.1c** and **2.1j.** Compound **2.1c** (Figure 4) was crystallized from different solvents, in both cases crystallizing together with the organic solvent and the structures obtained are similar.

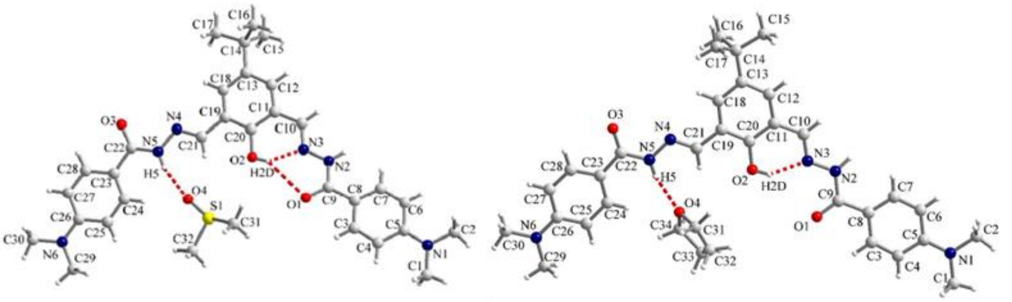


Figure 3. Structures of compound **2.1c-DMSO** (left), **2.1c-THF** (right) obtained by single crystal X-ray diffraction

2.4. Absorption and emission properties

The photophysical properties of the synthesized compounds were studied by UV-Vis spectroscopy and by fluorescence spectroscopy, in solution and in solid state.

In general, *N*-acylhydrazones show low solubility in most organic solvents, but hydroxy-bis-*N*-acylhydrazones have been shown to be readily soluble in solvents such as DMSO, THF, DMF[5] and sparingly soluble in MeOH. The experiments were performed in DMSO or DMSO/water mixtures in varying proportions at micromolar concentrations.

2.4.1. Emission spectra of solid state hydroxy-bis-N-acylhydrazones

In the solid state, hydroxy-bis-*N*-acylhydrazones emit in the green and blue regions, with emission maximum values between λ_{em}= 420 nm and λ_{em}= 565 nm, after excitation at λ_{ex}= 370 nm or λ_{ex}= 480 nm (**2.1 e**) and relatively large Stokes displacements, except for compound **2.1b** which contains phenolic -OH groups protected with benzyl groups.

2.4.2. Absorption spectra in solution

Absorbtion spectra of hydroxy-bis-*N*-acylhydrazones **2.1a-k** were recorded in a first step in DMSO at a concentration of $2x10^{-5}$ M.

Compounds **2.1a-f** contain in their structure the same substituent (*t*-butyl) grafted on the dialdehyde ring, have a maximum absorption around λ_{max}=370 nm, except for compound **2.1d** which has the hydroxyl group protected by a benzyl group and presents a shoulder at λ_{max}=336 nm. Also, from the absorption spectra of compounds **2.1a**, **2.1b**, **2.1d** a second absorption maximum can be observed around λ_{max}=305 nm, and for compounds **2.7c**, **2.7e**, **2.7f** the second absorption maximum was recorded at different values.

The UV-Vis spectra of compounds **2.1a**, **2.1b**, **2.1d**, **2.1e**, **2.1f**, **2.1k** have a similar profile due to the similar structure, given the presence of the substituent grafted on the rest of the aldehyde (except **2.1k** similar to **2.1a** by the presence of groups methoxy grafted on the rest of the hydrazide).

The absorption maxima recorded for compounds **2.1a-k** ($2x10^{-5}$ M in DMSO) are due to the π-π * transitions that may occur within the molecule (eg on the aromatic nucleus, the -C=N bond, etc.).

2.4.2. Fluorescence spectra

Screening of the luminescence of the compounds by exposure to UV light (356nm) of solutions of different concentrations in DMSO showed that at a high concentration (10^{-4} M) the emission is in the orange region, and at low concentrations the emission is green (Figure 4). All compounds exhibit similar behavior, except **2.1b**, **2.1f** and **2.1i**.

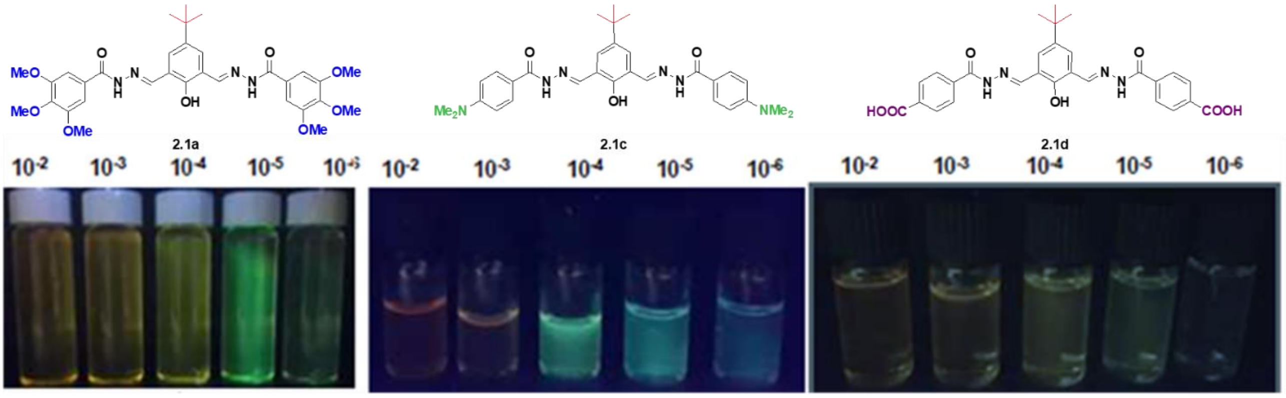


Figure 4. Solutions of different concentrations of compounds **2.1a** (left), **2.1c** (middle) and **2.1d** (right), under the UV lamp (365nm)

The fluorescence spectra of the **2.1a-k** compounds, recorded at a concentration of 10^{-4} M in DMSO, showed wide emission bands, with maxima between λ_{em}=440-590 nm, by excitation at λ_{ex}=370 nm (Figure 5).

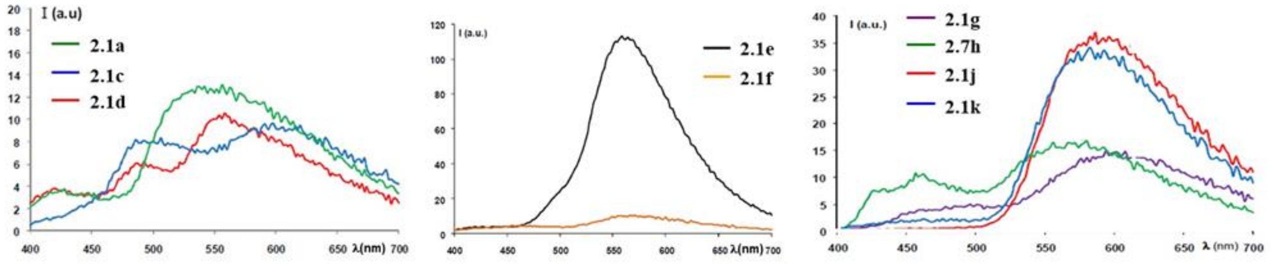


Figure 5. Emission spectra of compounds **2.1a-d** (left), **2.1e-f** (middle) and **2.1j-k** (right) in solution (10^{-4} M in DMSO) obtained by excitation at λ_{ex}=370 nm

The excitation at wavelengths between λ_{ex}=420-460 nm, led to the shift of the emission maxima to blue, these being located around λ_{em}=500 nm (Figure 6).

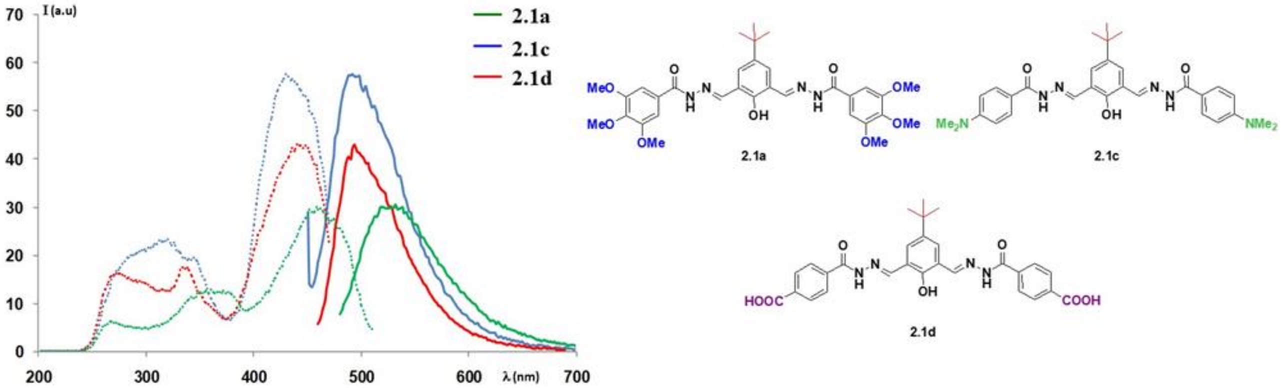


Figure 6. Excitation (dotted line) and emission (line) spectra for compounds **2.1a**, **2.1c** and **2.1d** in DMSO (10^{-4} M)

Significant differences between excitation spectra and absorption spectra (Figure 7) indicate that fluorescence is due to the *Excited-State Intramolecular Proton Transfer* (ESIPT) process.

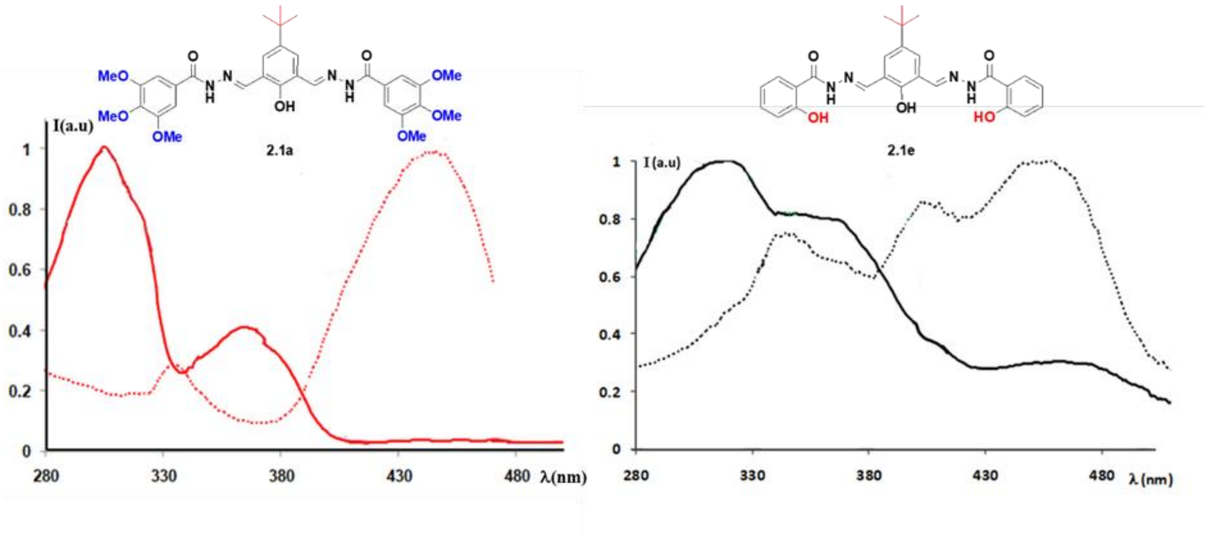


Figure 7. Excitation (dotted line) and emission (line) spectra for compounds **2.1a** and **2.1e** in DMSO (10^{-4} M)

2.4.3. Investigation of the molecular switching properties of hydroxy-bis-*N*-acylhydrazones under the influence of water

In the context of the existing literature[4] and our own observations on compounds **2.1a-k**, we studied their behavior when adding water over organic solvent solutions and investigated the photophysical properties in the presence of variable amounts of water (up to 90%) of bis-*N*-acylhydrazones **2.1a-k**.

The results obtained by recording the absorption and fluorescence emission spectra of compounds **2.1a-k** in DMSO and DMSO/water mixtures indicate the presence of the phenomenon of intramolecular proton transfer (ESIPT) up to a quantity of water of 50%. At percentages of water higher than 50% the fluorescence emission is due to the phenomenon of molecular aggregation (AIE). The compounds show light emission in DMSO, which varies from yellow-orange to green depending on the concentration (Figure 8). In DMSO/water mixtures the intensity of fluorescence emission decreases, and the emission maxima are shifted to longer wavelengths compared to those recorded for solutions in DMSO (Figure 8).

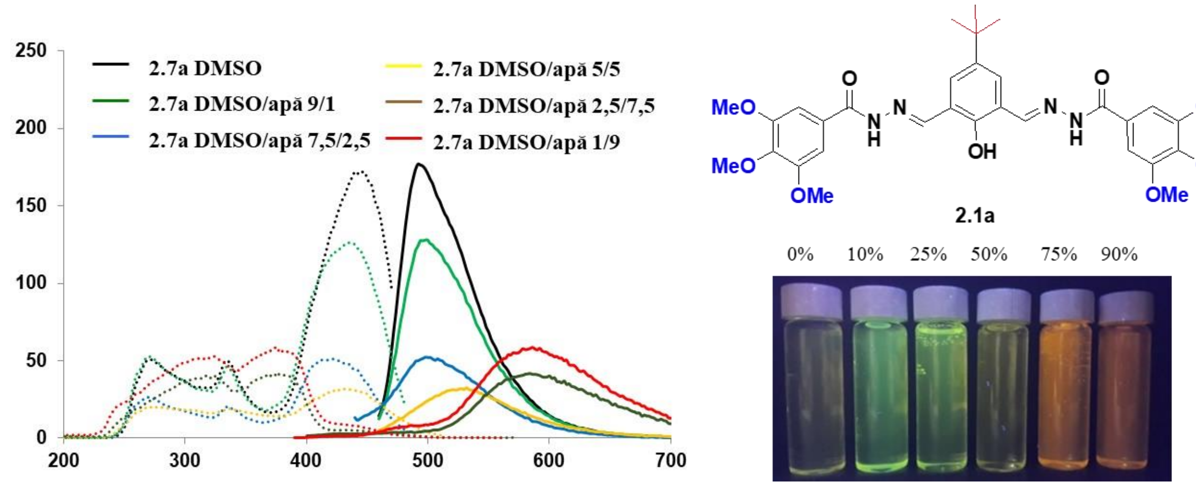


Figure 8. Excitation (dotted line) and emission (line) spectra for compound **2.1a** in DMSO and DMSO/water (10^{-4} M)

2.4.3.1. Molecular switches

a) Behavior in the presence of water

The molecular switching character of hydroxy-bis-*N*-acylhydrazones under the influence of water was studied by performing an experiment which consisted in adding and removing water in a solution in DMSO of compound **2.1a**, which causes the emission color change from orange to green (Figure 9). Changing the color of the fluorescence emission is caused by conformational changes that can occur by adding or removing water from the system.

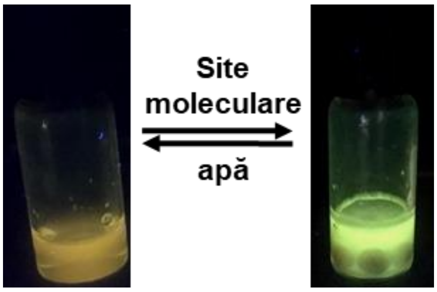


Figure 9. Solutions of compound **2.1a** in DMSO/water = 1:9 and reversible change of fluorescence under UV lamp after modification of the amount of water by the action of molecular sieves

Switching between the most likely species, under the influence of water, was studied by NMR spectroscopy.

b) Light

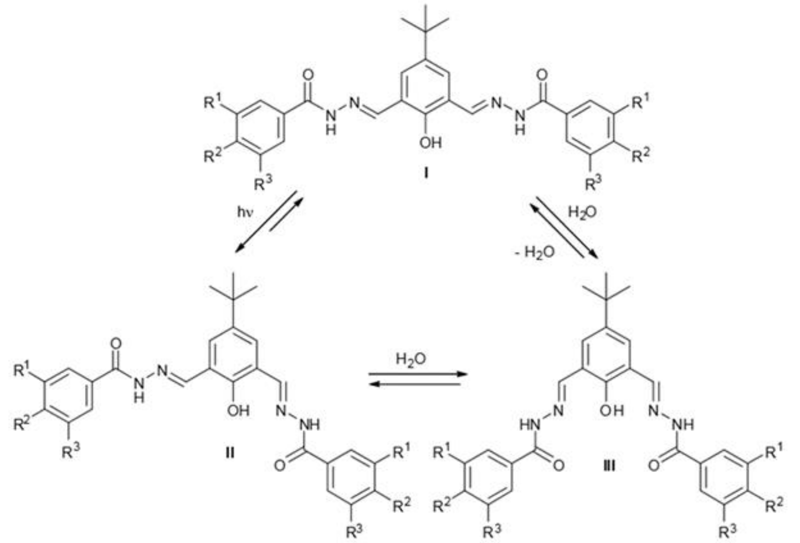
The ability of the -C=N group in the structure of *N*-acylhydrazones to isomerize by rotating the double bond out of the plane or the inversion of the nitrogen atom in the plane,[5] are the basis for the conceptual definition of their switching properties under the influence of light, redox processes or metal ions.[6] In general, isomerization occurs when the most unstable of the isomers can be stabilized by a non-covalent interaction, such as hydrogen bonding.

In order to verify whether the synthesized *N*-acylhydrazones have such a behavior, solutions of compounds **2.1a-d** in DMSO (10^{-4} M) and DMSO/water mixtures (10^{-4} M) after irradiation at λ=356 nm were studied. Irradiation of solutions in DMSO, followed by recording of absorption spectra indicates a decrease in the intensity of absorption maxima and highlights the effect of light on the structure of the compounds.

The fluorescence spectra of the irradiated solutions show an increase in the band intensity corresponding to the enol form, but also a decrease in the fluorescence intensity when is used as the excitation wavelength λ_{ex}=440 nm. Spectra modifications can be explained by structural changes that lead to the formation of new species that cannot achieve the hydrogen bonds required for the excited intramolecular proton transfer process (ESIPT) to take place. However, nuclear magnetic resonance studies performed under these conditions do not show significant changes in spectra indicating a very low stability of species generated by irradiation, most likely due to steric hindrance and lack of a stabilizing element in the molecular structure (eg a group able of making hydrogen bonds that stabilize the less stable species so that it can be studied on the time scale of NMR).

Irradiation of the solutions made in the DMSO/water mixture showed, through the measurements of absorption and emission of fluorescence, that the structural changes can take place up to a quantity of 50% of water. After this percentage the phenomenon of molecular aggregation becomes active, and the light can no longer induce any conformational/configurational change.

Therefore, following the results obtained, it can be stated that derivatives ***2.1a-k*** *have the ability to convert from W shape (structure I - Scheme 5) to S or U shapes (structure II, structure III - Scheme 5) and can operate in solution as molecular switches due to conformational changes under the action of light or solvent (water or glycerol).*



Scheme 5. Conformational changes that may occur under the influence of light and/or solvent for compounds **2.1a-k**

2.4.4. pH response of compound 2.1e

Compound **2.1e** has in its structure three hydroxyl groups able of coordinating metal ions or anions, the great ability of coordination and the use of these types of hydroxy-bis-*N*-acylhydrazones as metal ion ligands to be known in the literature.[2] In this context, a series of anionic analytes were tested qualitatively: carbonate, bicarbonate, acid phosphate, phosphate, sulfate, thiocyanate, cyanate, thioacetate, acetate, chloride, iodide, fluoride, bromide, nitrate, nitrite, iodate, bromate, azide which were added individually to a solution of compound **2.1e** in DMSO/water mixture in a 1: 9 (v/v) ratio.

By exposing the mixtures to the UV lamp (365 nm) we noticed the color change from yellow-orange to green when carbonate, bicarbonate, phosphate or fluoride was added, but also an increase in fluorescence emission in the presence of hydroxyl ions. The change in emission color is due to the deprotonation of the hydroxyl groups in the structure of compound **2.1e** by carbonate, bicarbonate, phosphate, fluoride or hydroxide ions, which have a basic character.

Titration with aqueous sodium hydroxide solution resulted in an emission maximum shift from λ_{em}=590 nm to λ_{em}=545 nm (λ_{ex}=370 nm) and an increase in fluorescence intensity of approximately 100-fold after exceeding the stoichiometry (Figure 10).

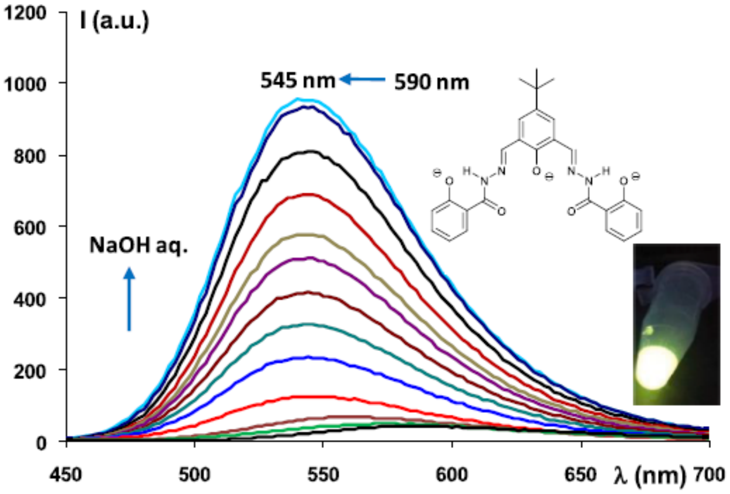


Figure 10. Emission spectra (λ_{exc}=370 nm) of compound **2.1e** (10^{-4} M final concentration in 1% DMSO in water) after addition of various amounts of NaOH (up to 80 equivalents relative to compound **2.1e**). Inset: the proposed structure for compound **2.1e** at basic pH; Photo: UV (365nm) exposure of the solution of compound **2.1e** after the addition of NaOH

The study of the variation of fluorescence as a function of pH was performed by recording the fluorescence emission spectra of some solutions of compound **2.1e** for pH values between 5.8-8.0 and, respectively, for pH values between 8.6 to 10.6. With the increase of the pH value, the displacement of the maximum emission was observed simultaneously with the increase of the maximum emission intensity, but also of the emission band from λ_{em}=420 nm. The graphical representation of the ratio of emission intensities I_{590}/I_{520} as a function of pH, led to the obtaining of two inflection points approximately at pH=7.0 and pH = 8.9 which correspond to the two ionization steps.

In order to verify the cause of the emission color change, observed by the addition of sodium hydroxide, NMR spectra were recorded in the presence of D_2O and NaOD.

According to the obtained results, the possible conformation in which the double bond C=N has the *E* configuration is stabilized by the addition of water in solution, which results from the two-dimensional spectra recorded for solutions with a water content of 30% in DMSO in which different amounts of deuterated sodium hydroxide (NaOD) were added. The spectra show a large coupling distance between phenyl and imine protons.

Therefore, the experiments show that the species presented above (Scheme 5) are most likely to exist in solution after the addition of sodium hydroxide, and green fluorescence is most likely the consequence of negative net charge that can induce intramolecular proton transfer in the excited state (ESIPT), following photoiradiation, by means of the hydrogen bond formed between the –NH group and the rest of the hydrazide.

2.5. Applications in cell labeling of compound 2.1e

Verification of cell staining ability using compound **2.1e** was performed in a first step for eukaryotic *Saccharomyces cerevisiae* cells, which were used as a model.

Cells exposed to UV radiation become fluorescent after about 10 seconds, the fluorescence profile indicating following simultaneous staining experiments with **2.1e** and $DiOC_6$ (3,3′-dihexyloxacarbocyanin iodide), the accumulation of compound **2.1e** in the membrane of the endoplasmic reticulum.

Simultaneous staining with *N*-acylhydrazone **2.1e** and $DiOC_6$ indicates the accumulation of both dyes on the membrane of the endoplasmic reticulum. The experiments indicated the presence of hydroxyl groups as an essential condition for visualizing the endoplasmic reticulum.

2.6. Conclusions

The chapter describes the synthesis of new hydroxy-bis-*N*-acylhydrazones whose structural changes can lead to different properties and applications. Experiments show that hydroxy-*N*-acylhydrazones can be used in applications based on molecules capable of emitting fluorescence by excited intramolecular proton transfer (ESIPT), the properties being influenced by the polarity of the solvent and the mesomeric effects dictated by the structural features that these compounds present them. Conformational changes and phototautomerization lead to fluorescence emission.

An essential structural condition of the synthesized compounds, for the occurrence of the phenomenon of fluorescence emission due to molecular aggregation (AIE), in the solutions made in DMSO/water mixture refers to the existence of two *N*-acylhydrazone groups, given that the experiments prove that the presence of a single group cannot lead to the occurrence of the AIE process. The presence of a protecting group such as the benzyl group induces the gelling process in the presence of water, providing a blue fluorescent gel that once again confirms the versatility of this class of compounds.

Most of the synthesized *N*-acylhydrazones show solid fluorescence in the yellow to orange region. The color of the emission does not depend on the number of *N*-acylhydrazone groups, but the substituents influence it, and the structural diversification can lead to new molecules that can find different applications in the chemistry of materials.

The color of the emission may change depending on the concentration or amount of water added and is due to conformational changes that may occur. This property of *N*-acylhydrazones can be used to develop new applications such as sensors for different ions or molecular switches.

Studies on the behavior of these compounds after prolonged exposure to UV lamp (365 nm) have concluded that photoisomerization is difficult for bis-*N*-acylhydrazone, but this can be optimized by structural changes so that the isomerization process to take place and the *Z* isomer to be stabilized.

The fluorescence emission of bis-*N*-acylhydrazones at different pH values was associated with conformational changes, investigated by nuclear magnetic resonance experiments.

In addition, the synthesized bis-hydroxy-bis-*N*-acylhydrazone (**2.1e**) was used to label the endoplasmic reticulum of eukaryotic cells (*S. cerevisiae*) or mammals (CCD fibroblasts and HeLa cells), while experiments showed that this compound has a reduced cytotoxic effect, is chemically stable in aqueous medium and inert to intracellular activity.

Therefore, fluorescence generated by conformational or configurational changes is a useful strategy for the development of new fluorescent systems that can be modified in a controlled and reversible manner, in contrast to structural diversification through chemical changes that provide only a change in emission.

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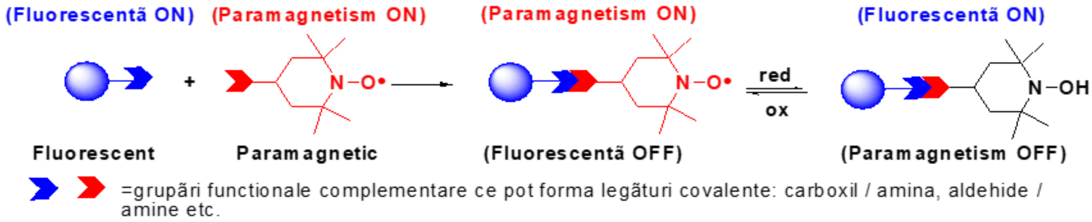
Chapter 3. Synthesis of new nitroxides with dual properties: luminescence and paramagnetism

The basic concept (Scheme 6) for the design of profluorescent nitroxides consists in the covalent binding of a molecule that shows fluorescence emission (fluorescence ON) to a stable free radical (paramagnetism ON).



Scheme 6. Schematic representation of the synthesis of profluorescent nitroxides; regaining fluorescence by reducing profluorescent nitroxide to the corresponding hydroxylamine

The hydride molecule obtained loses its fluorescence (fluorescence OFF) but retains its paramagnetic properties (paramagnetism ON) (Scheme 6). Reduction of the free radical or its reaction with another species reverses the two properties, and the derivative regains its fluorescence (fluorescence ON), but loses its paramagnetic properties (paramagnetism OFF) (Scheme 6) .[1]

The derivation with stable free radicals of oxadiazoles allows to obtain new multifunctional hybrid compounds, whose properties can be easily controlled by various physical or chemical processes (pH variation, light, redox potential, etc.). All of these different properties generate the emergence of new characteristics, which can be exploited by using these compounds as sensors or markers in different chemical or biochemical systems.

In this chapter, we have focused on two major objectives:

- synthesis of new profluorescent nitroxides derived from 1,3,4-oxadiazoles;
- synthesis of new profluorescent nitroxides derived from 1,2,5-oxadiazoles.

The profluorescent nitroxides derived from 1,3,4-oxadiazoles proposed to be synthesized are presented in Figure 11, their structure fulfilling *a priori* the necessary conditions for profluorescent nitroxides.

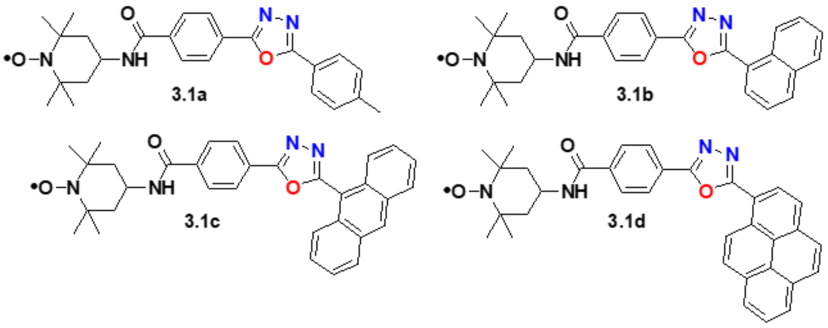


Figure 11. Structure of profluorescent nitroxides **3.1a-d**

Profluorescent nitroxide **3.2** (Figure 12) derived from 4-chloro-7-nitro-1,2,3-benzoxadiazole (NBD-Cl) contains in its structure a crown ether (Kryptofix 22).

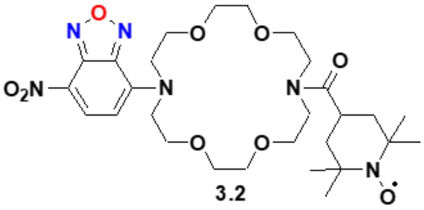
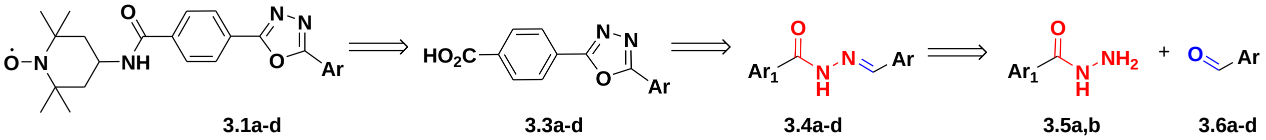


Figure 12. Structure of profluorescent nitroxides **3.1a-d**

3.2. Retrosynthesis of profluorescent nitroxides

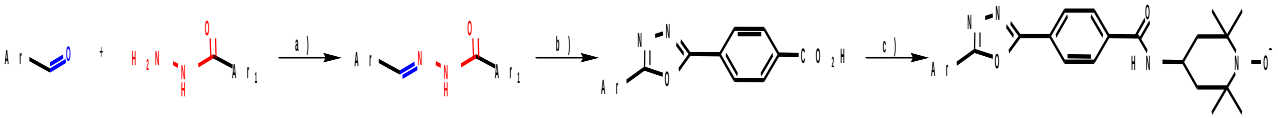
The profluorescent nitroxides **3.1a-d** proposed to be synthesized (Figure 13) contain in their structure an amide bond, which can be achieved by the reaction between oxadiazoles **3.3a-d** functionalized with carboxyl groups and a derivative of the stable radical TEMPO. The amide bond formed was chosen as the spacer between the fluorescent side and the paramagnetic side due to the high stability in the reduction reactions. Oxadiazole derivatives **3.3a-d** functionalized with carboxyl groups can be obtained by an oxidative cyclization reaction of *N*-acylhydrazones **3.4a-d**, that are formed following the reaction between aldehydes **3.6a-d** and hydrazides **3.5a,b**.



Scheme 7. Retrosynthetic scheme for obtaining profluorescent 1,3,4-oxadizoles

3.3. Synthesis of profluorescent nitroxides

Based on the retrosynthetic analysis we performed the synthesis of profluorescent nitroxides **3.1a-d** in three stages (Scheme 8). The first step consisted in the synthesis of *N*-acylhydrazones **3.4a-d** by a condensation reaction between aldehydes **3.6a-d** and hydrazides **3.5a, b** in acid catalysis. The next step was the synthesis of 1,3,4-oxadiazoles **3.3a-d** by oxidative cyclization reaction of *N*-acylhydrazones **3.4a-d**, using molecular iodine as the cyclizing agent. In the last step, 1,3,4-oxadiazoles **3.3a-d** were treated with 4-amino TEMPO, using the reaction conditions specific to peptide synthesis, to obtain profluorescent nitroxides **3.1a-d**.



Scheme 8. Synthesis scheme of profluorescent 1,3,4-oxadizoles: a) $CHCl_3$/DMF, reflux; b) I_2/DMSO, 100 °C; c) 4-amino TEMPO, PyBOP, DIPEA, DCM

3.4. Structural analysis of synthesized compounds

The structure of the synthesized compounds was confirmed using nuclear magnetic resonance spectroscopy (^{1}H, ^{13}C, two-dimensional spectra) and high-resolution mass spectrometry, using Chemical Ionization at Atmospheric Pressure (APCI-HRMS) as the ionization source. NMR spectra were recorded in deuterated solvent DMSO-d_6, at a frequency of 500 MHz for proton spectra and 125 MHz for carbon spectra.

The structure of compounds **3.1a-d** was confirmed using spin electron spectroscopy, nuclear magnetic resonance spectroscopy (^{1}H, ^{13}C, two-dimensional spectra) and high-resolution mass spectrometry using Chemical Ionization at Atmospheric Pressure (APCI-HRMS) as the ionization source. NMR spectra were recorded in deuterated solvent ($CDCl_3$) using a reducing agent (phenylhydrazine) at a frequency of 500 MHz for proton spectra and 125 MHz for carbon spectra. High resolution mass spectra were also performed in the presence of a reducing agent (trifluoroacetic acid). Thin layer chromatography and melting temperature determination were used to confirm the purity of the compounds.

3.4.1. Cyclic voltammetry

Cyclic voltammetry studies performed for synthesized profluorescent nitroxide compounds show that a reversible oxidation process takes place and corresponds to the formation of oxoammonium cations and has an anodic peak potential (Epa) with values between 0.5 V and 0.88 V.

3.4.2. Solid state structure of nitroxide 3.1d

Slow diffusion of nitroxide **3.1d** into dichloromethane/petroleum ether resulted in monocrystals which were analyzed by X-ray diffraction.

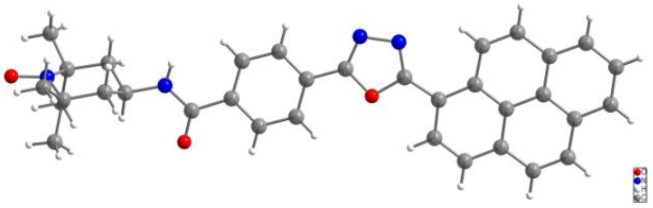


Figure 13. Structure of compound **3.1d** obtained by X-ray diffraction on a single crystal

3.5. Investigation of the photophysical properties of 1,3,4-oxadiazoles and profluorescent nitroxides

The investigation of the photophysical properties of the synthesized compounds was performed by UV-Vis spectroscopy recording the absorption spectra and, respectively, by fluorescence spectroscopy, both in solution and in solid state, recording the excitation and emission spectra. Oxadiazole precursors **3.2a-d**, nitroxides **3.1a-d** and corresponding hydroxylamines obtained from the reduction of nitroxides were studied. The experiments were performed using an aqueous solution at micromolar concentrations.

3.5.1. Absorption and emission properties of 2,5-diaryl -1,3,4-oxadiazoles

Absorption spectra (Figure 14) were recorded at a concentration of 2.7x10^{-5} M, in HEPES buffer (0.001 M, pH = 7.51) with a 15% DMSO content. The spectra show bands specific to the $\pi - \pi^*$ transitions due to the oxadiazole nucleus[5] but also to the characteristic transitions to the anthracene[6] or pyrenic nucleus[7], with absorption maxima between λ_{max}=294 nm and λ_{max}=390 nm.

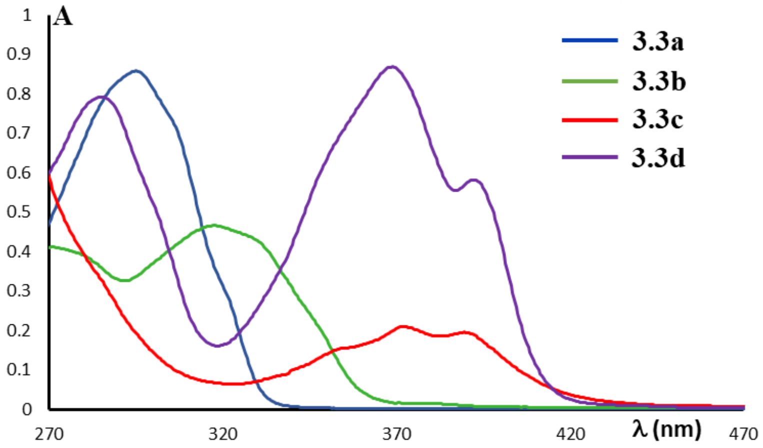


Figure 14. Absorption spectra (in solution) of oxadiazoles **3.3a-d**

Also, the excitation spectra (Figure 16) show bands that can be attributed to the π–π* transitions in the structure of the oxadiazole nucleus[5] for compounds **3.3a** (λ_{max}=294 nm) and **3.3b** (λ_{max}= 323 nm), but also bands that correspond to the specific transitions of the anthracene[8] or pyrenic nucleus[7] in the case of compounds **3.3c** (λ_{max}= 370 nm) and, respectively, 3.3d (λ_{max} = 360).

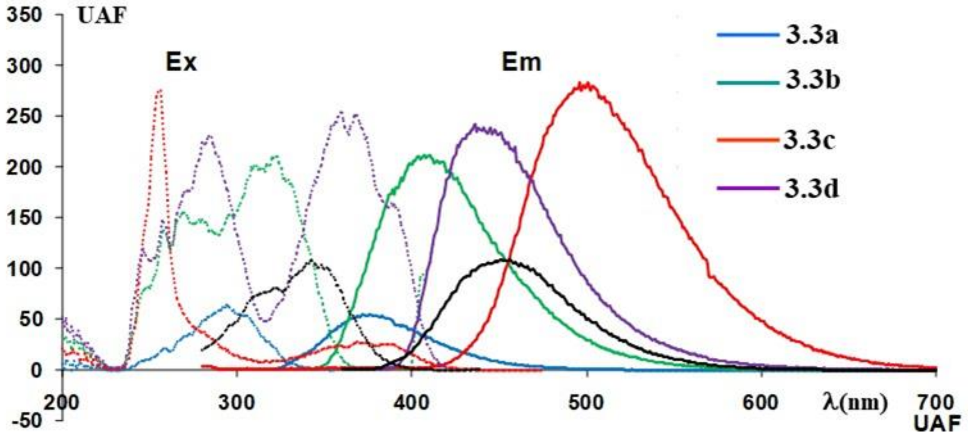


Figure 15. Fluorescence excitation (dotted lines) and emission (lines) spectra in solution of oxadiazoles **3.3a-d**

The emission spectra recorded for compounds **3.3a-d** in the solid state indicate a shift of the emission maxima towards the red region for all compounds, leading to very wide Stokes shifts.

3.5.2. Absorption and emission properties of profluorescent nitroxides

The absorption spectra of profluorescent nitroxides **3.1a-d** were recorded under the same conditions as their precursors (**3.3a-d**). The profile of the absorption bands is similar to that of oxadiazoles 3.3a-d, with absorption maxima between λ_{max}=296 nm and λ_{max}=392 nm.

The excitation maxima show red shift compared to carboxy-derivatives, being 20 nm higher in the case of compounds **3.1b** and **3.1d**, by 12 nm for compound **3.1a**, while compound **3.1c** shows no change in maximum excitation. Stokes displacements have values between 92-124 nm, being approximately equal to those of carboxy-derivatives.

The attachment of the free radical to the previously obtained carboxy-derivatives leads to the extinction of fluorescence[9], and this aspect can be observed in the fluorescence emission spectra recorded for nitroxide solutions (Figure 16), but also in those on solid.

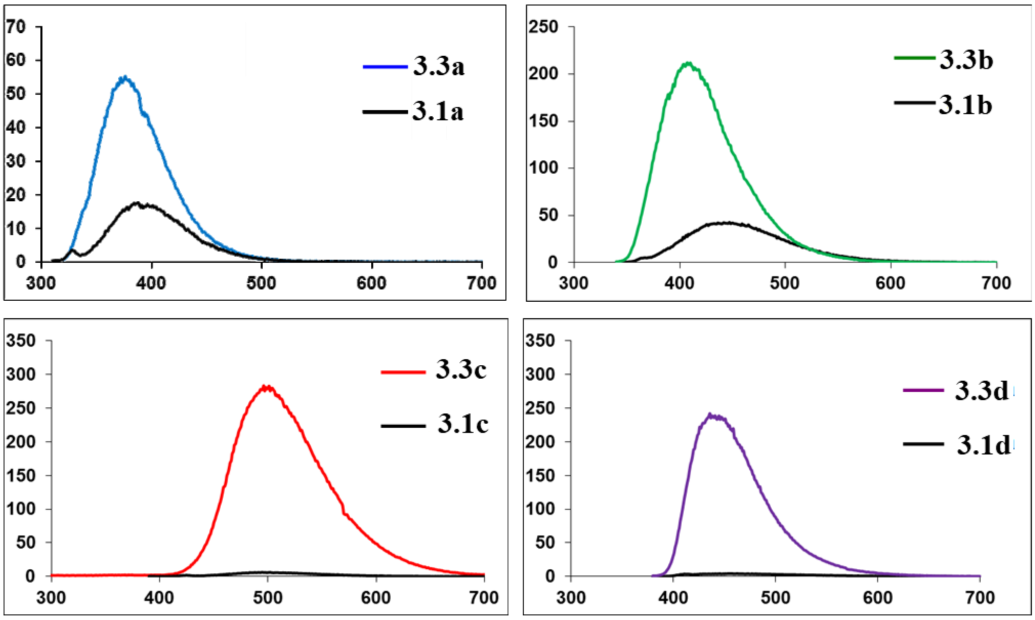


Figure 16. Emission spectra (in solution) of nitroxides **3.1a-d** (black lines) and emission spectra of carboxy-derivatives **3.3a-d**

3.5.2.1. Investigation of the dual properties of profluorescent nitroxides

Verification of the reduction of nitroxides to the corresponding hydroxylamines was performed by RES studies in the presence of sodium ascorbate[9] or phenylhydrazine in different molar ratios between nitroxides and reducing agents. The experiments were performed in a degassed solution of HEPES 0.01M pH = 7.51 with a content of 15% DMSO at a concentration of 5 x 10^{-5} M.

The use of sodium ascorbate as a reducing agent in a molar ratio of 1: 1 (nitroxide: reducing agent) led to the complete reduction of the radical within 45 minutes for compounds **3.1a** and **3.1d**, while compounds **3.1 b** and **3.1c** were completely reduced after 120 minutes.

Nitroxide fluorescence measurements in the presence of sodium ascorbate indicate a variable increase in fluorescence intensity: hydroxylamine corresponding to nitroxide **3.1a** shows an increase in fluorescence intensity five times higher than nitroxide, hydroxylamine corresponding to nitroxide **3.1b** shows an increase in fluorescence intensity four times higher, and that corresponding to nitroxide **3.1c** two times higher, while the hydroxylamine of nitroxide **3.1d** does not show a significant increase in fluorescence intensity.

The use of phenylhydrazine as a reducing agent led to the reduction of nitroxides after 30 minutes using a 1:10 molar ratio, while the use of a 1: 1 molar ratio did not lead to the reduction of nitroxides.

3.5.2.2. Effect of the solvent on the emission properties of profluorescent nitroxides

Given the results obtained for nitroxide **3.1d** was studied the effect of the solvent on the emission and absorption properties. The emission spectra of nitroxide **3.1d** (5 x 10^{-5} M) and the corresponding hydroxylamine (after treatment of the nitroxide solutions with 10 eq. phenylhydrazine for 30 minutes) were recorded in solvents such as: DMSO, MeOH, MeCN and $CHCl_3$.

The fluorescence spectra recorded for nitroxide **3.1d** in different solvents have a similar profile which is not influenced by the polarity of the solvent used.

The use of the water/DMSO solvent mixture leads to a dramatic decrease in the fluorescence emission of nitroxide **3.1d**.

After the reduction of nitroxide **3.1d**, the fluorescence emission intensity and implicitly the obtained quantum yields are considerably higher for the spectra recorded in organic solvents, compared to the one obtained in water/DMSO.

The experimental data obtained lead to the conclusion that the use of the polar water/DMSO mixture allows the formation of molecular aggregates due to the interactions that take place between the hydrophobic residues of the molecule, generating the extinction of fluorescence.[10]

The formation of supramolecular aggregates was also confirmed by NMR spectroscopy, by recording ^{1}H-NMR spectra in $CDCl_3$ and DMSO-d_6 in the presence of an excess of phenylhydrazine[11], for reduction to hydroxylamine.

3.5.2.3. Applications

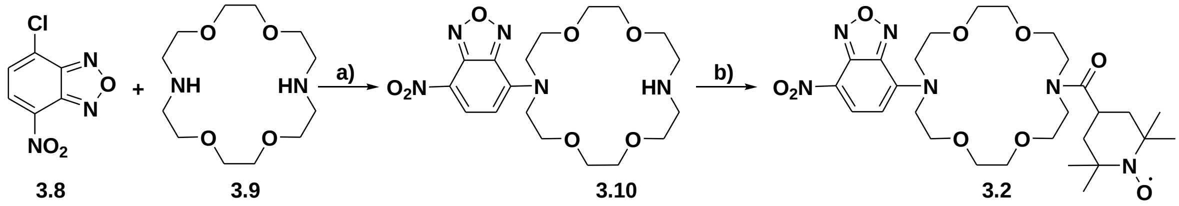
The literature presents examples of dual molecules such as profluorescent nitroxides as being used in various studies on the antioxidant properties of ascorbic acid.[12]

The experiments consisted in treating the solutions of nitroxide **3.1a** with different amounts of sodium ascorbate (between 0 and 2 equivalents) and recording the fluorescence emission spectra at intervals of 15 minutes.

The results confirmed that nitroxide **3.1a** is completely reduced to the corresponding hydroxylamine after 45 minutes, which could indicate its use for the detection of ascorbate in various biological systems.

3.7. Synthesis of a new profluorescent derivative of 4-chloro-7-nitro-2,1,3-benzoxadiazole

Synthesis of precursor **3.10** was performed by a substitution reaction starting from furazan **3.8** (4-chloro-7-nitro-2,1,3-benzoxadiazole) and crown azaether **3.9** (1,7,10,16-tetraoxa-4,13-diazacyclooctadecane), known as Kryptofix 22, the reaction taking place in dichloromethane using triethylamine as a base (Scheme 9). The previously synthesized compound **3.10** was further used in the amide coupling reaction with 4-carboxy-TEMPO, using PyBOP (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate) as the activating agent and DIPEA (*N*, *N*-Diisopropylethylamine).



Scheme 9. Synthesis of compounds **3.10** and **3.2**: a) CH_2Cl_2, Et_3N, rt, overnight, 62% b) 4-carboxy-TEMPO, PyBOP, DIPEA, DCM, rt, 4h, 81%

3.7.1. Structural analysis

For compound **3.10** the ^{1}H-NMR and ^{13}C-NMR spectra were recorded in DMSO-d_6 and $CDCl_3$ (500MHz for proton and 125MHz for carbon).

Compounds containing the TEMPO radical in their structure can be studied by means of nuclear magnetic resonance spectroscopy (^{1}H-NMR and ^{13}C-NMR), more precisely the ^{13}C-NMR spectra can provide information on atoms located at a considerable distance from the radical center.[13] Thus, in the case of nitroxide **3.10**, the ^{1}H-NMR spectrum shows broad and unresolved signals while the ^{13}C-NMR spectrum shows signals that have been attributed to furazan, crown ether and carbonyl group involved in the amide bond (δ=170.2 ppm), but also carbon atoms in the radical structure.

3.7.2. Complexation studies

Complexation studies in the presence of alkali metals (Li^+, Na^+, K^+) performed for compound **3.9** were followed by NMR spectroscopy and mass spectrometry (HR-MS) to determine its ability to form "host-guest" compounds.

NMR experiments indicate signal changes with the addition of a potassium perchlorate solution, while the addition of lithium or sodium perchlorate solutions did not result in significant changes.

Complexation experiments were also studied by mass spectrometry (ES(+)-MS). The complexation capacity of alkali metal ions ($LiClO_4$, $NaClO_4$ and $KClO_4$) by the crown ether was verified both by individual experiments and by competitive experiments in which mixtures of equimolecular solutions of the salts of the three metal ions used were used.

Individual experiments indicated the formation of supramolecular complexes between compound **3.10** and the metal ions used.

Complexation studies of nitroxide **3.2** with alkali metal cations such as $LiClO_4$, $NaClO_4$, $KClO_4$ and $CsClO_3$ indicated the formation of new host-host complexes with all four cations used.

3.7.3. Absorption and emission properties

The absorption spectrum of compound **3.10** recorded in DMSO at a concentration of $2x10^{-5}$ M shows two absorption maxima at 350 nm and 500 nm, corresponding to the transitions of nitrobenzofurazan[15] indicating that its covalent binding to the crown ether does not influence the absorption properties of oxadiazole. The absorption maxima vary depending on the solvent used, as shown in the literature[16], which confirms the results obtained and indicates a solvatochromic behavior of compound **3.10**. For compound **3.2** the absorption spectra in solution were recorded under the same conditions as mentioned above for compound **3.10** and showed a similar profile due to the presence of nitrobenzofurazane.

The spectra recorded in DMSO or DMSO/water mixture (1% DMSO in PBS buffer or 10% DMSO in ultrapure water) show a maximum emission λ_{em}=550 nm (λ_{ex}=500 nm) corresponding to nitrobenzofurazane. The intensity of the emission bands decreases with increasing polarity of the solvent, which suggests the extinction of fluorescence as a result of the formation of molecular aggregates, induced by the polarity of the solvent.

The fluorescence spectrum of compound **3.10** in solid state has a maximum emission at λ_{em}=564 nm (λ_{ex}=490 nm), slightly shifted to red compared to the spectra in solution and a Stokes displacement of 74 nm. In the case of nitroxide **3.2** a similar profile can be observed, the emission intensity being lower compared to that recorded for its precursor.

Also, through fluorescence studies it was observed that the addition of a five times higher amount of metal ion solution ($LiClO_4$, $NaClO_4$, $KClO_4$ and $CsClO_3$) to compound **3.2** decreases the fluorescence intensity.

3.8. Conclusions

In this chapter we have presented the synthesis of new 2,5-diaryl-1,3,4-oxadiazoles (**3.3a-d**) derived from *p*-tolyl, naphthyl, anthracenyl and pyrenyl which contain in their structure a carboxyl group that allows further functionalization. The compounds were characterized structurally, but also in terms of optical properties. Experiments have shown that naphthyl and pyrenyl derivatives show quantum yields and large Stokes displacements.

Proflorescent nitroxides **3.1a-d** were obtained by the reaction of 1,3,4-oxadiazoles-2,5-disubstituted **3.3a** and 4-amino-TEMPO and characterized by RES spectrometry, NMR, cyclic voltammetry and X-ray diffraction. RES specra registred for nitroxides **3.1a-d** confirmed the attachment of the radical to the previously obtained carboxy-derivatives. The presence of free radical gives nitroxides electrochemical properties, confirmed by cyclic voltammetry.

The fluorescence spectra of nitroxides showed that in the presence of the free radical the fluorescence is quenched. This can be regained by the reaction of nitroxides with a reducing agent (phenylhydrazine, sodium ascorbate), which made it possible to use the compound containing the tolyl unit for the detection of ascorbic acid.

Following these studies, the synthesis of fluorophore-aza-ether-crown and, respectively, fluorophore-aza-ether-crown-nitroxide compounds was performed, for which the structure, photophysical properties and behavior in the presence of alkaline metal ions were investigated. Confirmation of the identity of the compounds was performed by NMR spectroscopy (^{1}H-NMR, ^{13}C-NMR), high resolution mass spectrometry and RES spectroscopy (for nitroxide **3.2**). Also, the behavior of the compounds in the presence of metal ions Li^+, Na^+, K^+ and Cs^+ was studied through NMR spectroscopy and mass spectrometry. The fluorescence spectra obtained suggest a weak interaction between fluorophore and free radical, given that fluorescence is maintained in the presence of the paramagnetic center. Partial quenching of fluorescence in the solid state or by the addition of a metal ion solution may be useful in various fields, such systems consisting of a fluorophore, a crown ether and a nitroxide can find applications as sensors for monitoring the processes that take place in various chemical or biochemical systems.

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General conclusions

The thesis entitled “*Photoluminescent and paramagnetic organic switches*” aimed at the synthesis and study of the optical properties of some *N*-acylhydrazone derivatives, oxadiazole heterocycles and profluorescent nitroxides. *N*-acylhydrazones and oxadiazole heterocycles have luminescence as their main property, such derivatives being intensively studied in order to find molecules with improved fluorescent or phosphorescent properties, which can be useful in developing new applications in fields such as materials chemistry, biochemistry, biology or chemistry. environment. Profluorescent nitroxides are hybrid molecules made up of a fluorophore and a stable free radical, which works bimodally, with the possibility of switching between the two properties, fluorescence emission and paramagnetism.

The thesis is structured in four chapters, one dedicated to the presentation of literature data, two chapters describing the results obtained and a chapter containing the detailed experimental part.

The first chapter “*Fluorescence of organic compounds*” presents the literature data on fluorophores, the mechanisms of occurrence and quenching of fluorescence, as well as the synthesis and applications of *N*-acylhydrazones, oxadiazole heterocycles and profluorescent nitroxides.

The second chapter, "*Synthesis of new bis-N-acylhydrazones with fluorescent properties*", describes the synthesis of new hydroxy-bis-*N*-acylhydrazones whose conformational changes lead to fluorescence emission and various applications. Experiments show that hydroxy-*N*-acylhydrazones can be used as molecules able of emitting fluorescence by excited intramolecular proton transfer (ESIPT), the emission properties being influenced by the polarity of the solvent and the mesomeric effects dictated by the structural features that the compounds present. An essential structural condition for the occurrence of the fluorescence emission phenomenon due to molecular aggregation (AIE) in the case of synthesized compounds, refers to the existence of two *N*-acylhydrazone groups, experiments proving that the presence of a single group does not lead to AIE. Protection of the hydroxyl group induces the gelling process in the presence of water, providing a blue fluorescence gel that once again confirms the versatility of this class of compounds. In addition, the color of the emission may change depending on the concentration or amount of water added and is due to the conformational processes that take place. Studies on the behavior of synthesized hydroxy-bis-*N*-acylhydrazones after prolonged exposure to UV lamp (365 nm) have led to the conclusion that photoisomerization occurs with difficulty for bis-*N*-acylhydrazones, but this can be optimized by changes so that the isomerization process takes place and the Z isomer is stabilized. Also, the fluorescence emission of bis-*N*-acylhydrazones at different pH values has been associated with conformational changes, investigated by nuclear magnetic resonance experiments.

The applications of the synthesized compounds can be extended in biological chemistry and biochemistry, one of the synthesized compounds proving to be a good dye of a cellular organelle (endoplasmic reticulum), in experiments performed on *S. cerevisiae* cells and mammalian cells. Also, most of the derivatives obtained show emission of fluorescence in solid state, in the yellow-orange region and for two of the compounds were obtained monocrystals which were studied by X-ray diffraction, which could make possible their use in materials chemistry.

The synthesized compounds therefore behave as light-sensitive molecules, capable of changing the color emitted by the action of light and/or solvent. Structural changes imposed by grafting substituents with different electronic properties lead to compounds with different applications.

The third chapter, entitled "*Synthesis of new nitroxides with dual properties: luminescence and paramagnetism*", presents the synthesis of new profluorescent nitroxides derived from 2,5-diaryl-1,3,4-oxadiazoles and 1,2,5-oxadiazoles.

Profluorescent nitroxides derived from 1,3,4-oxadiazoles-2,5-disubstituted and 4-amino-TEMPO were structurally characterized by RES spectrometry, NMR, cyclic voltammetry and X-ray diffraction. The RES spectra of these derivatives confirmed the attachment to the carboxy derivatives obtained, and the presence of the free radical gives nitroxides electrochemical properties.

In the presence of the free radical the fluorescence is quenched, but can be regained by the reaction of nitroxides with a reducing agent (phenylhydrazine, sodium ascorbate), which makes it possible to use such compounds for the detection of ascorbic acid.

Also, the third chapter presents the synthesis of fluorophore-aza-ether-crown compounds and, respectively, fluorophore-aza-ether-crown-nitroxide derivatives obtained from 1,2,5-oxadiazoles, which have been characterized in terms of structural view, photophysical properties, but also of the behavior in the presence of alkali metals. The recorded fluorescence spectra suggest a weak interaction between the fluorophore and the free radical, as the fluorescence is maintained in the presence of the paramagnetic center, which is also observed in the solid state or by the addition of a metal ion solution. The behavior of the compounds in the presence of metal ions Li^+, Na^+ , K^+ and Cs^+ was studied by NMR spectroscopy and mass spectrometry, which showed the formation of supramolecular complexes, especially with potassium ions. Such systems consisting of a fluorophore, a crown ether and a nitroxide, which do not completely lose their fluorescence in the solid state or when adding a solution of a metal ion, can find applications as sensors for monitoring the processes that take place in different chemical or biochemical systems.

All synthesized compounds were characterized by physical and spectral methods (NMR spectroscopy, RES spectroscopy, mass spectrometry, absorption spectroscopy, fluorescence spectroscopy, cyclic voltammetry) to confirm purity, structure and physical properties. Molecular structures were also obtained by X-ray diffraction for two of the bis-N-acylhydrazones, and for one of the profluorescent nitroxides, respectively.

In conclusion, the results obtained in the doctoral thesis suggest that the emission of different fluorescence is generated by conformational or configuration changes and is a useful strategy for the development of new fluorescent systems such as bis-*N*-acylhydrazones that can be modified in a controlled and reversible manner. Profluorescent nitroxides are hybrid molecules made up of a fluorophore and a free radical that works bimodally, having the ability to switch between the two properties, fluorescence and paramagnetism.

The results obtained were published in five ISI-rated articles, as follows:

1. **A. G. Coman**, C. C. Paraschivescu, A. Paun, A. Diac, N. D. Hadade, L. Jouffret, A. Gautier, M. Matache, P. Ionita, *Synthesis of novel profluorescent nitroxides as dual luminescent-paramagnetic active probes, New J. Chem.,* **2017***,41,* 7472. **IF: 3,069**
2. **A. G. Coman**, A. Paun, C. C. Popescu, N. D. Hădade, C. C. Anghel, A. M. Mădălan, P. Ioniţă, M. Matache, *Conformation-induced light emission switching of N-acylhydrazone systems, New J. Chem.* **2018,** *42*, 14111. **IF: 3,069**
3. **A. G. Coman**, A. Paun, C. C. Popescu, N. D. Hădade, A. Hanganu, G. Chiritoiu, I. C. Farcasanu, M. Matache*, A novel adaptive fluorescent probe for cell labelling, Bioorg. Chem.,* **2019***, 92,* 103295. **IF: 3,926**
4. **A. G. Coman**, C. Stavarache, A. Paun, C. C. Popescu, N. D. Hadade, P. Ionita, M. Matache, *A novel profluorescent paramagnetic diaza-crown ether: synthesis, characterization and alkaline metal-ion complexation, RSC Adv.*, **2019** , *9*, 6078. **IF: 3,049**
5. **A. G. Coman**, A. Paun, C. C. Popescu, N. D. Hadade, A. Hanganu, A. M. Mădălan, M. Matache, *Examination of structure-activity relationship of new N-acilhydrazones, Rev. Roum. Chim.*, **2020**, *65*, 109. **IF: 0,395**