University of Bucharest
Faculty of Chemistry
Doctoral School in Chemistry

Doctoral Thesis

Synthesis and study of some nitrogen centered free radicals of aminyl and hydrazyl type

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Introduction

The doctoral thesis titled “ **Synthesis and study of some nitrogen centered free radicals of aminyl and hydrazyl type**” aims to synthesize and investigate the properties of hydrazyl and aminyl free radicals, their precursors (hydrazines), but also the anions derived from them. Such compounds arouse interest due to their properties, such as acid-base or redox reversible behavior accompanied by color change, antioxidant capacity, biological properties, etc., thus finding applications in various fields of interest. The thesis comprises 5 chapters, the first being represented by the literature data, offering an overview of free radical chemistry, and the next 4 by the original experimental data, followed finally by the experimental related part.

Literature data

Free radicals are chemical species that contain at least one unpaired electron [1] in the outer layer of the electron shell. Its presence gives them increased instability and reactivity. The first free radical synthesized was the inorganic potassium nitroso disulfonate, or "Fremy's salt," obtained by Edmond Fremy [2] in 1845. The year 1900 marked another important moment in free radical chemistry, being synthesized the first free organic free radical, triphenylmethyl or trityl [3], by Moses Gomberg, a professor at the University of Michigan. In 1929 Paneth and Hofeditz [4] obtained the methyl radical, concluding now that many reactions in organic chemistry are following a radical mechanism. In recent decades, free radical chemistry has developed dramatically, finding applications in a number of fields of interest: biology, biochemistry, medicine, industry, and especially in various branches of chemistry (mention only the halogenation of methane and other hydrocarbons, an industrial process of major importance).

Free radicals can be classified according to several criteria, the most important being the lifetime and the type of atom on which the free electron is centered. Depending on the lifetime we can classify free radicals as stable, persistent, and short-lived. The stable ones are, according to Ingold, those that can be isolated, stored, and handled [5] in a pure state for a long time in the laboratory, without the need for special conditions, not reacting with oxygen or moisture, while a persistent radical is one that has a lifetime sufficient to allow its investigation by spectroscopic techniques, but cannot be isolated. The stability of a free radical is due to electronic effects, steric hindrances, and conjugation. Another classification of free radicals could be made according to the nature of the element on which the odd electrons are found: i) free radicals of oxygen, ii) free radicals of nitrogen, iii) free radicals of carbon, iv) free radicals of sulfur, v) free radicals of phosphorus, etc.

The usual free radical detection and characterization technique [6a, b] is electron paramagnetic resonance (EPR), a non-destructive spectroscopic technique that is based on the absorption of electromagnetic radiation (from a microwave radiation source) by a paramagnetic sample located in a magnetic field. Its most important feature is the interaction of the free electron with the nuclear spin (hyperfine interaction). The number of lines present in the EPR spectra is given by the relation 2I + 1, where I is the nuclear magnetic moment, and in the case of a number of n identical nuclei, the relation will become 2nI + 1, where n is the number of identical nuclei of atoms with which free electrons interact (and have I different from 0). Examples of atoms with $I \neq 0$ are represented by hydrogen with $I = 1/2$; nitrogen with $I = 1$, phosphorus with $I = 1/2$. Thus, the spectrum of nitroxide radicals consists of three main lines due to the interaction of the free electron with the nitrogen atom (2x1 + 1 = 3).

Also, free radicals can be analyzed by ultraviolet-visible spectroscopy, because they are colored compounds, and even by infrared spectroscopy, we can obtain information about the functional groups in their structure. Spin electron resonance spectroscopy has two main methods by which can be employed in the study of biological systems: the spin labeling method [6], which involves the covalent binding of a paramagnetic residue in that system, and the spin-trapping one. Various information can be obtained about the spatial arrangement and dynamics of biomolecules, an example being the study of membrane proteins, but also about the properties of the environment in which the radicals were introduced. The other method used is spin trapping [7], through which it is possible to detect and characterize unstable free radicals, with a very short lifetime, in chemical or biological systems. The highly reactive radical reacts with a diamagnetic compound, which contains double bonds (named spin trap), generating a stable or persistent free radical called 'spin adduct', which can be analyzed by EPR.

The free radicals studied in this thesis will have the following characteristics: *they are free radicals of nitrogen* (the unpaired electron is centered on the nitrogen atom), *they have a lifetime that varies from a few minutes to indefinite*, *they are colored* (they have chromophore groups), *they can be involved in redox and acid-base processes,* *highlighted by color change*. The general structures and the illustration of the processes in which they can be involved are represented in Figure 1.1 and Scheme 1.1.

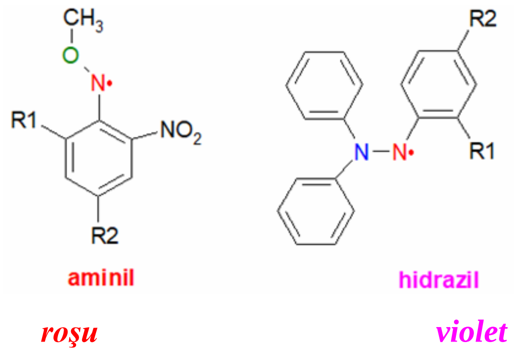
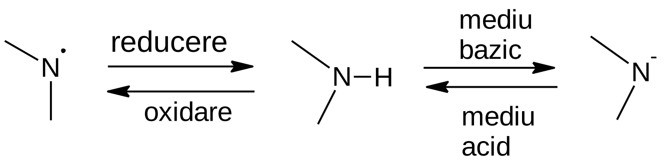


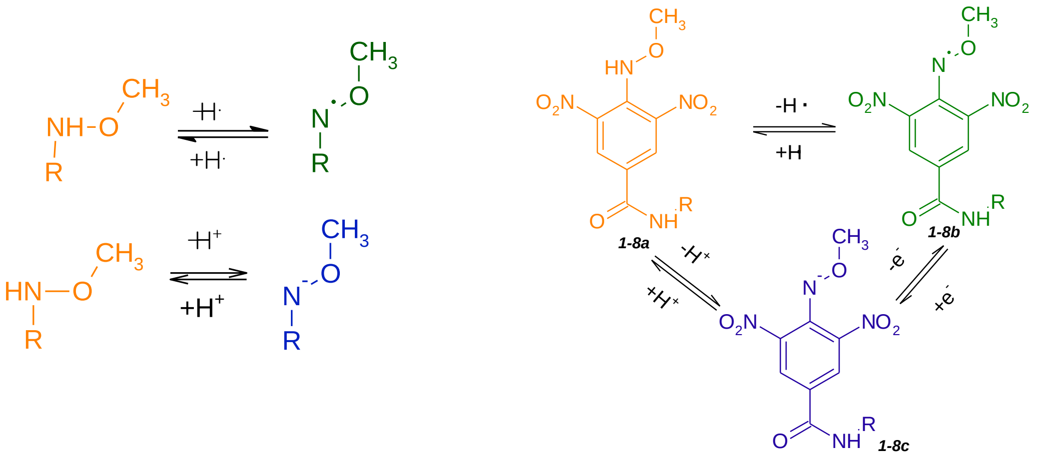
Fig.1.1. General structures of some aminyl and hydrazyl radicals



Schema 1.1. Processes in which nitrogen-centred free radicals can be involved

Original data

Chapter 2, entitled "***Synthesis and structural characterization of some novel methoxyamine derivatives***", aimed to synthesize and characterize new 3,5-dinitro-4-methoxy-aminobenzoic acid derivatives, which can generate methoxyaminyl radicals by oxidation. The structure of the compounds synthesized following these procedures was confirmed by nuclear magnetic resonance and other common techniques of organic chemistry. Free radicals derived from methoxyamines are obtained by treating them with PbO_2 (Scheme 1.2.). The corresponding anions were obtained by base treatment (KOH).



Schema 1.2. Reversible transformations of the *N*-alkoxy-3,5-dinitro-amino-benzoic derivates in free radical, anions respectively

Suitable crystals for X-ray diffraction analysis were obtained for two of the compounds (Fig. 1.2. and Fig. 1.3.), **2a** and **4a**.

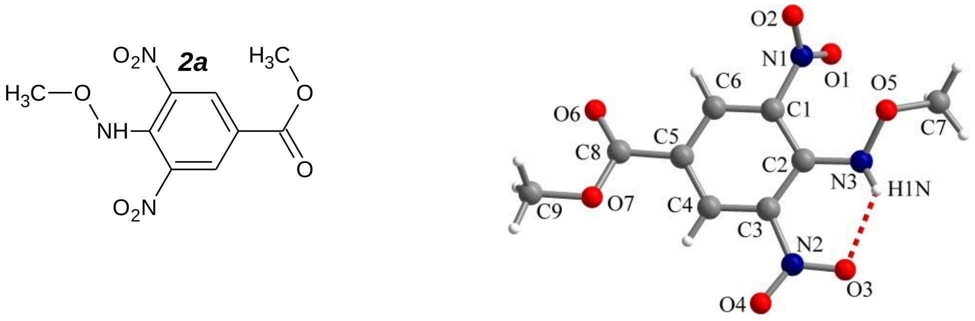


Fig. 1.2. Molecular structure of compound ***2a*** obtained by single-crystal X-Ray-diffraction

4a

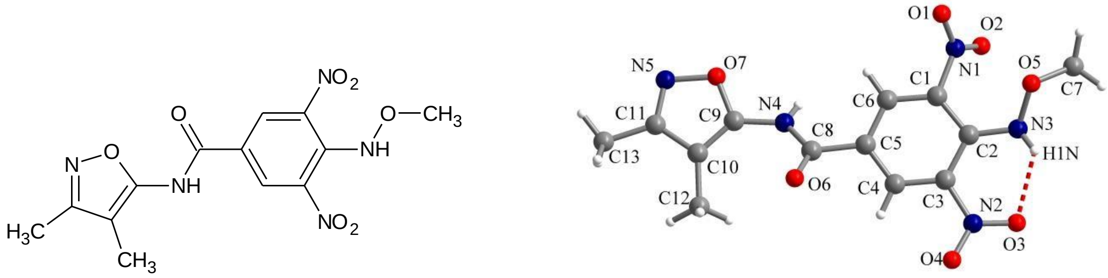


Fig. 1.3. Molecular structure of compound ***4a*** obtained by single crystal X-Ray-diffraction

Methoxyamine compounds are yellow solid derivatives, a general feature common to polynitroamine compounds. In the UV-Vis spectra, an intense absorption band is observed at approximately 380-390 nm. In basic media, this band is shifted bathochromically by about 200 nm and, as a result, the color of the compounds turns blue (Fig. 1.4.), due to the formation of the corresponding anions.

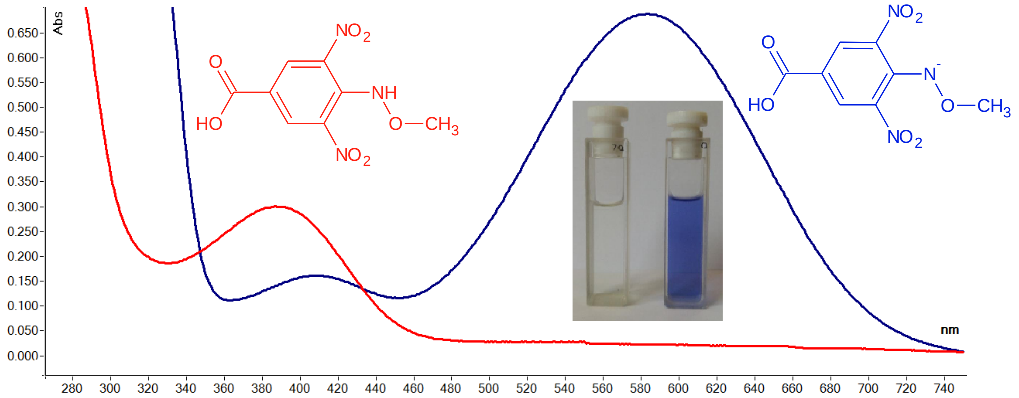


Fig. 1.4. UV-Vis spectra of compounds ***1a*** and ***1c***

Because compounds ***1a-8a*** may be involved in redox reactions, their electrochemical behavior has been studied, and it is known that such *N*-alkoxyl derivatives generate persistent free radicals by oxidation. The recorded cyclic voltammograms showed an irreversible oxidation peak, with a potential value between 1.05-1.35 V (Fig. 1.5.), attesting to the short lifetime of the methoxyaminyl radicals formed.

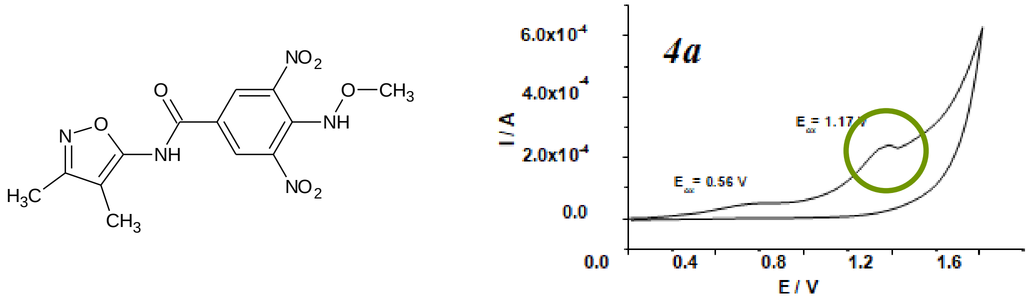


Fig. 1.5. Cyclic voltammogram of a methoxyaminyl compound

The corresponding ESR spectra were recorded for all compounds ***1b-8b*** (Fig. 1.6.). As a general rule, a triplet is observed, together with the appearance of hyperfine splitting lines.

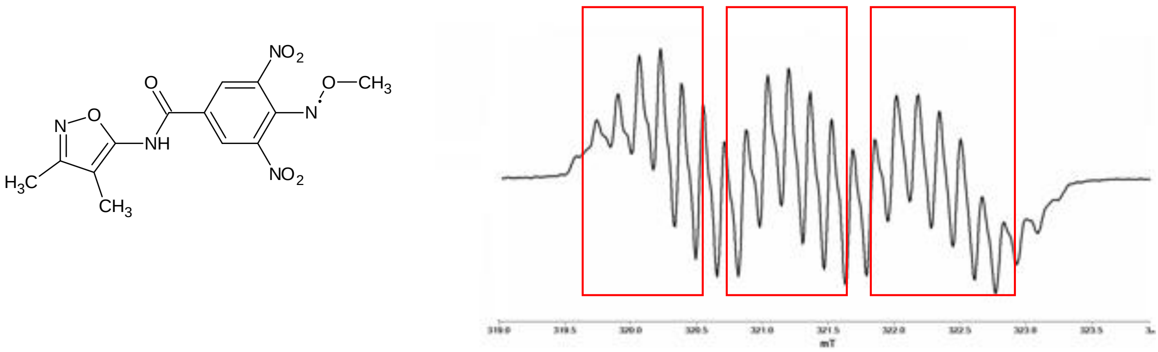


Fig. 1.6. EPR spectra of ***4b*** radical

Hydrophobicity was also determined by calculating the molecular descriptors: partition coefficient (log P) and total polar surface area (PSA), which are considered [10] to be extremely important in determining the biological activity of chemical compounds. The values obtained indicated a good lipophilicity in the case of compounds ***8a*** and ***7a***, which contain a residue of antipyrine and benzocaine, respectively.

The antimicrobial and anti-proliferative activities were evaluated for the synthesized compounds. Antimicrobial activity was tested on cells embedded in biofilm but also on those in suspension, both on *Gram-positive* and fungal bacterial cells, and anti-proliferative activity on HCT8 cells (colon tumor cells).

They showed good antimicrobial activity, but also cytotoxicity. The structure of the most active compounds is illustrated in Figs. 1.7. and 1.9.

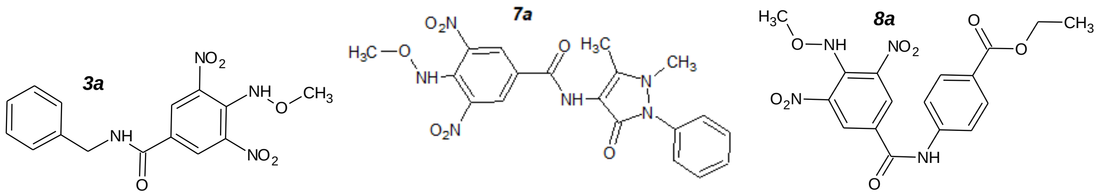


Fig.1.7. Structures of the compounds with the most potent antimicrobial effect

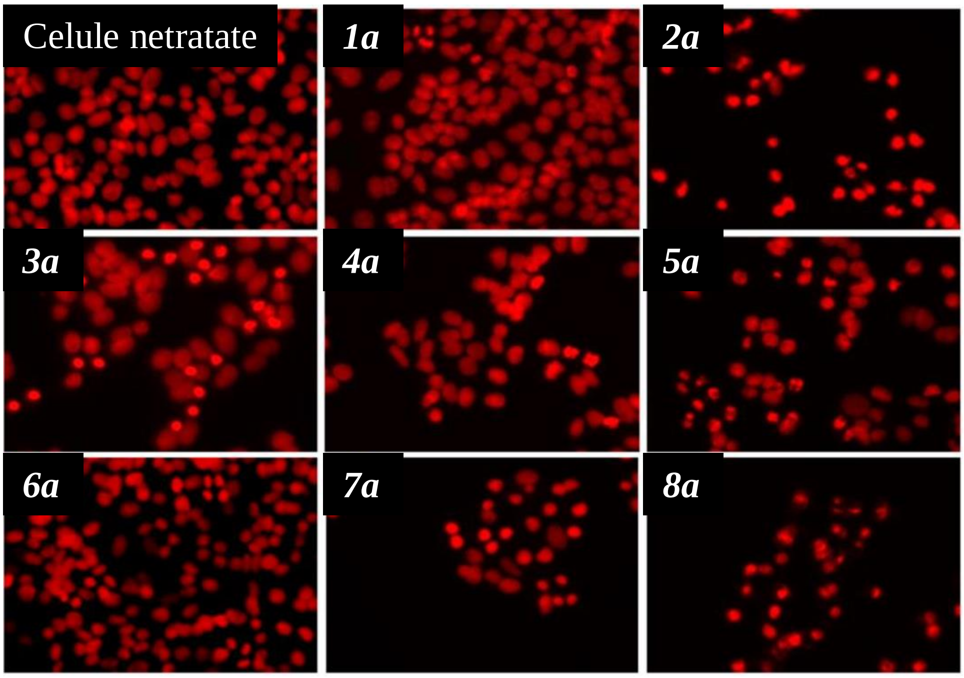


Fig. 1.8. Effect of 5 mg/mL compounds ***1a-8a*** on the morphology of HCT8 cells

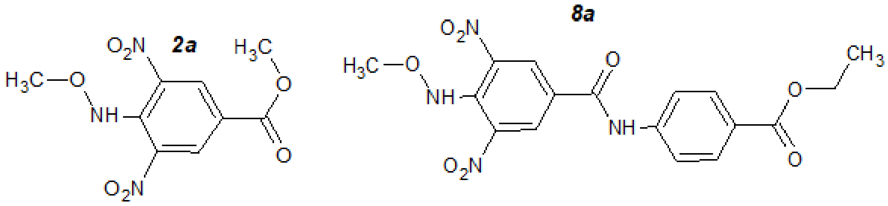


Fig. 1.9. The most anti-proliferative compounds

Chapter three, titled ***"Synthesis and characterization of some hydrazyl free radicals"*** presents a series of five hydrazyl compounds. This series contains four compounds known in the literature (but not commercially available) and, in addition, a new one, compound ***12a*** (Fig. 1.10.). These hydrazyl radicals were synthesized together with their precursors (hydrazines) and the corresponding anions.

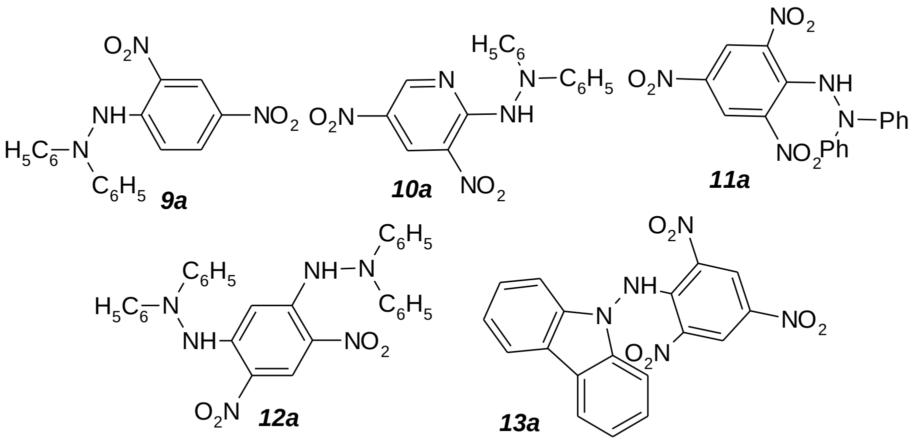


Fig. 1.10. Structures of hydrazines ***9a-13a***

Compounds ***9a-13a*** are obtained from activated halogen-nitrobenzene derivatives and the corresponding hydrazines [9], in the presence of a base. By oxidation with PbO_2 and by treatment with a base the corresponding anions are obtained (Fig. 1.11).

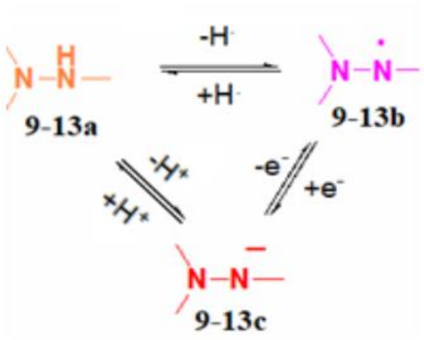


Fig. 1.11. Reversible transformations hydrazines-free radicals-anions

Suitable crystals were obtained for compound ***13a*** for X-ray diffraction analysis (Fig. 1.12.).

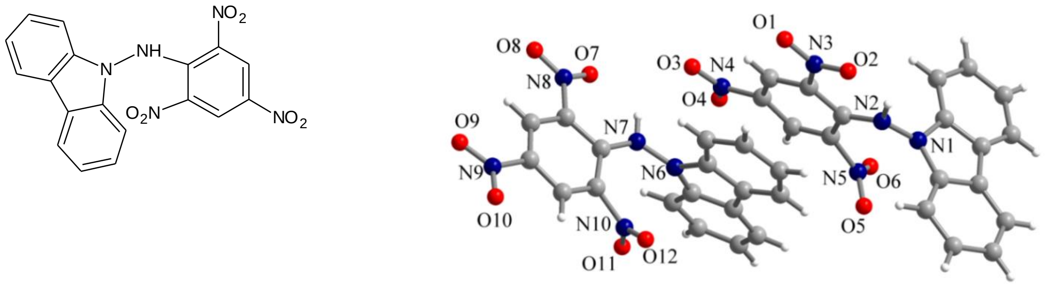


Fig. 1.12. View of the asymmetric unit in the crystal structure of compound ***13a***

UV-Vis spectra of hydrazines ***9a-13a*** (blue) and corresponding anions **9c-13c** (red) were recorded in methanol (Fig. 1.13.). The synthesized compounds have different colors. As a general rule, compounds ***9a-13a*** have the maximum absorption in the range of ***320-360 nm*** (yellow color), by treatment with a base the hydrazines are transformed into the corresponding anions, reddish-brown color, with the maximum absorption located in the range ***360- 440 nm***. Free radicals have a narrower range of absorption values of ***500-530 nm*** (purple color) (Fig. 1.14.).

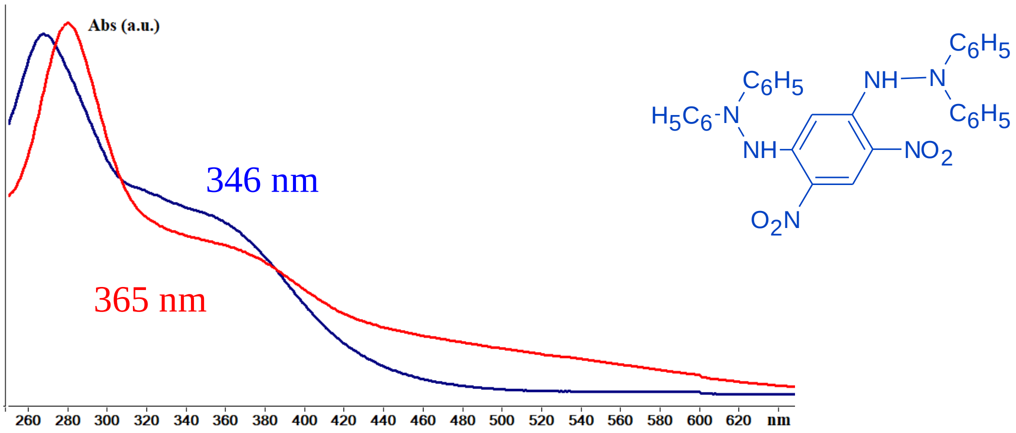


Fig. 1.13. UV-Vis spectra of compounds ***12a,b***

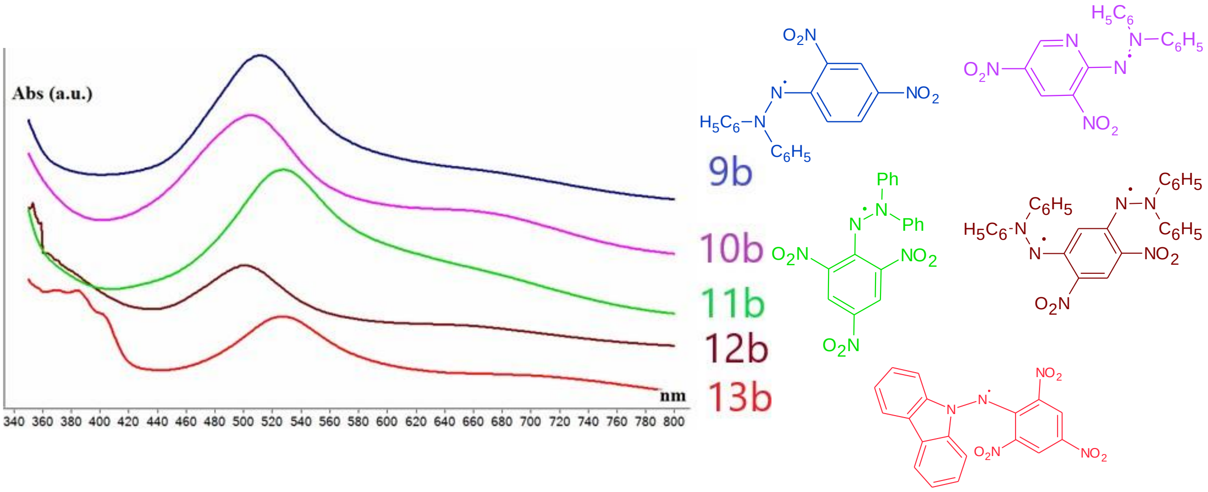


Fig.1.14. UV-Vis spectra of radicals ***9b-13b***

Cyclic voltammetry demonstrated the long life of these compounds (***9b-13b***). By oxidizing them with a single electron, hydrazyl free radicals ***9b-13b*** were obtained. By removing the second electron, cation radicals are obtained, resulting in the second oxidation peak. The process of electro-oxidation of anions to free radicals is reversible (Fig. 1.15.).

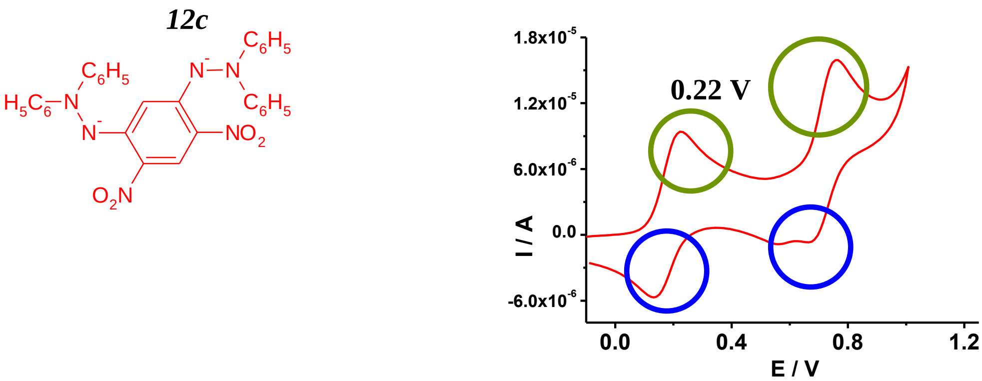


Fig 1.15. Cyclic voltammogram of compound ***12c***

Compounds ***9b-13b*** are persistent free radicals, a fact proved by ***EPR*** (Fig. 1.16.**).** As a general rule, it can be observed the apparition of five large lines in the spectra, due to the interaction of the unpaired electron with two equivalent nitrogen atoms.

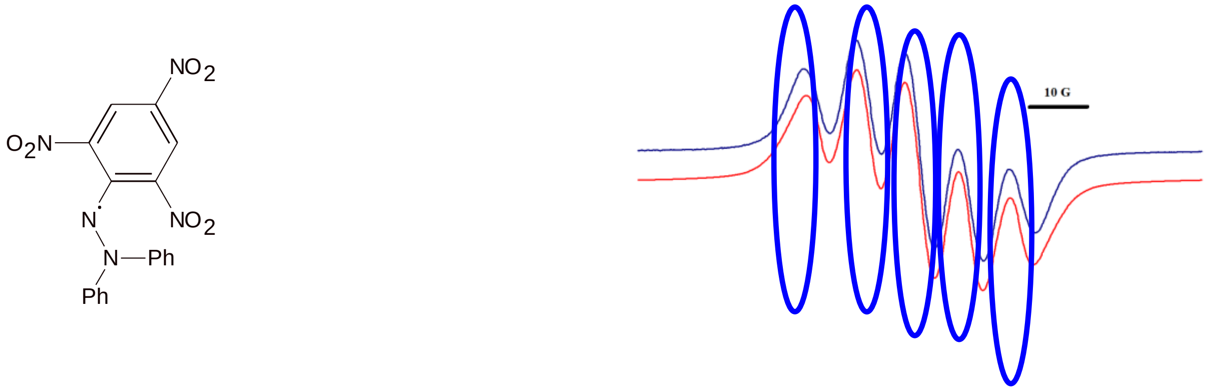


Fig. 1.16. EPR spectra of ***11b*** compound

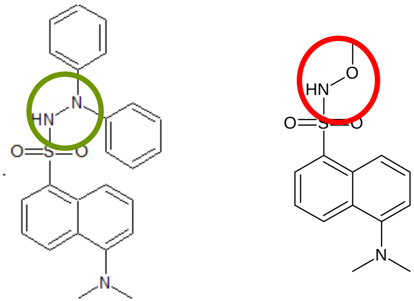
The hydrophobicity was also determined by the MolInspiration program, calculating the partition coefficient and the total polar surface.

PSA=106,91 ***log P=8,20***

Fig.1.17. Structures of compounds with good results on hydrophobicity determinations

The acidity constant was determined and the values of dissociation energy for the N-H bond were calculated, together with those of the oxidation potentials previously determined. The lowest value of the acidity constant is presented by compound ***11a***, which has three nitro groups in its structure. For compound **13a** the dissociation energy is 82 kcal / mol. This value is consistent with the strong oxidizing capacity [10-12] of radical ***13b***, which is correlated with the substituent's electronic effects.

Chapter 4 "***Dansyl chloride derived profluoresnt precursors of aminyl and hydrazyl free radicals***" presents a synthesis of new fluorescent compounds containing in the structure a dansyl residue and a free radical moiety of methoxyaminyl or hydrazyl type.



14 ***15***

Fig. 1.18. Structures of the newly synthesized precursors:

14-hydrazine
15-methoxamine

Compound ***15*** was suitable to be characterized by single crystal X-ray diffraction. Similar absorption maxima were obtained by characterization by ultraviolet-visible spectroscopy, confirming the absorption behavior of the dansyl part [13] (Fig. 1.19.).

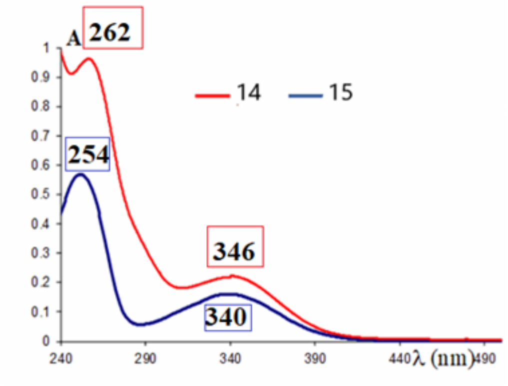
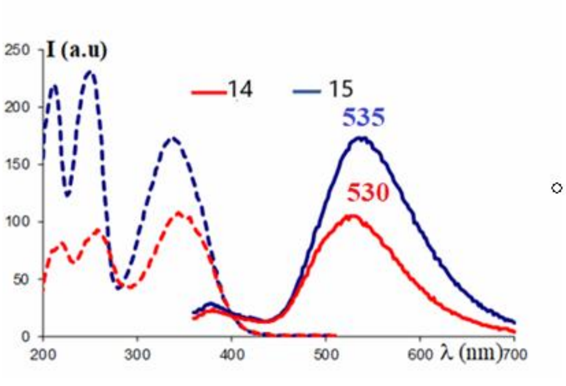


Fig. 1.19. UV-Vis spectra of compounds ***14*** and ***15***



Excitation (dotted lines) and emission (plain lines) of compounds ***14*** and ***15***

The fluorescence behavior could be assigned to the dansyl moiety, known for the typical charged-transfer band between the donor dimethylamino group and the sulfonyl unit, centered around λ_{em} = 540 nm.

Oxidation with lead dioxide of compound ***14*** led to the formation of radical ***16***, its presence being detected by EPR spectroscopy, the recorded spectrum being formed by five equidistant lines, a wide characteristic of hydrazys (Fig. 1.20.).

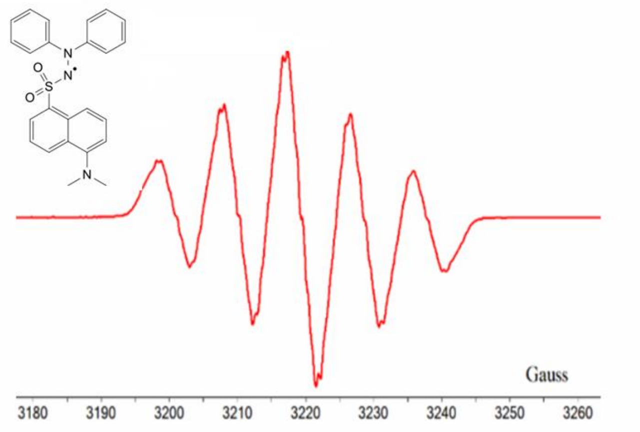


Fig. 1.20. EPR spectra of ***16***
$\alpha_{N1} = \alpha_{N2} = 9.25$ *Gauss,*
linewidth 4 G

Under the same conditions, the oxidation of compound ***15*** did not lead to the appearance of an EPR signal, but we can certify that the unstable radical **17** was formed (in the literature [14, 15, 16] such free radicals are described); on decomposition, a by-product is formed, the methoxy radical The spin-trapping method was used, using the tert-butyl-α -nitrone trap (Fig. 1.21.), for confirmation. The spectrum of the spin adduct consists of a triplet of doublets.

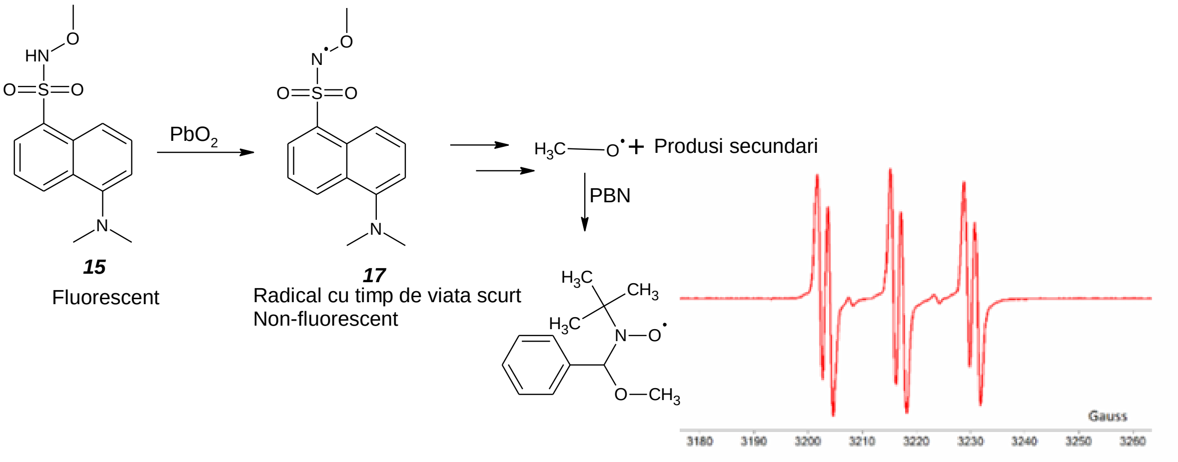


Fig. 1.21. Formation of the ***17*** spin adduct and its EPR spectrum

The differences and the general qualitative comparisons (mentioned in the past chapters) were also discussed from a quantitative point of view in chapter five entitled "***Comparative study of the properties of aminyl and hydrazyl radicals***".

General conclusions

In this thesis, two series of compounds were obtained, methoxyamines and hydrazines, precursors of free radicals such as aminyls and hydrazyls. Their choice was based on their properties, such as acid-base or redox behavior, reversible rections, accompanied by color change, antioxidant capacity, biological properties. The compounds were investigated by various techniques and methods (NMR, ESR, IR, MS, cyclic voltammetry, UV-Vis, log P, etc.). A comparison was made between the previously investigated properties of the two classes of compounds, being important to show what characterizes one in relation to the other. The study was also completed with the synthesis of two dansyl-derived fluorescent precursors. The multitude of techniques and the easiness with which such chemical species can be obtained and monitored, coupled with their biological properties, recommend them for use in (bio) chemical processes of interest.

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List of publications and participation at scientific manifestations:

Articles

Nr.	Title/ authors/ Magazine/ year/ volume/ pages
1.	*Synthesis and structural characterization of some novel methoxyamino derivatives with acid-base and redox behaviour,* M. Bem, R. Baratoiu, C. Radutiu, C. Lete, S. Mocanu, G. Ionita, S. Lupu, M. T. Caproiu, A. M. Madalan, **B. Pãtraşcu**, I. Zarafu, P. Ionita, *Journal of Molecular Structure*, **2018**, 1173, 291-299
2.	*Bioevaluation of the antimicrobial and antiproliferative potential of some derivatives of 3,5-dinitro-4-methoxyamino-benzoic acid,* I. Zarafu, **B. Pătraşcu**, L. Măruţescu, C. Bleotu, C. Limban, A. Tatibouet, M. C. Chifiriuc, D. C. Nuţă, P. Ioniţă, *Farmacia*, **2020**, 68, 8-14
3.	*Synthesis and spectral comparison of electronic and molecular properties of some hydrazines and hydrazyl free radicals***,** **B. Pătraşcu**, C. Lete, C. Popescu, M. Matache, A. Păun, A. Mădălan, P. Ioniţă, *Arkivoc*, **2020**, vi, 1-10
4.	*Synthesis of fluorescent dansyl derivatives of methoxyamine and diphenylhydrazine as free radical precursors,* **B. Pătraşcu**, S. Mocanu, A. Coman, A. M. Mădălan, C. Popescu, A. Păun, M. Matache, P. Ioniţă, *International Journal of Molecular Sciences*, **2020**, 21, 3559- 3568

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Nr.	Title/ authors/ manifestation
1.	*Synthesis and cytotoxicity of new aminopyrazolo-benzimidazoles derivates-* ***poster,*** C. Zãlaru, V. G. Preda, M. A. Strinoiu, **B. Pãtraşcu**, F. Dumitraşcu, C. Drãghici, I. A. Târcomnicu, M. Marinescu, M. Fierbinţeanu, A. Juncu, *Analytical and nano-analytical methods for biomedical and environmental sciences, IC-ANMBES* ***2018****, Brasov 23-25 Mai*
2.	*Novel substituted pyrazole hybrids with anti-tumor activity-****poster***, A. Tãtulescu, V. Cojocaru, **B. Pãtraşcu**, C. Zãlaru, F. Dumitraşcu, C. Drãghici, I. Tarcomnicu, M. Marinescu, R. Tatia, L. Moldovan, *21st Romanian International Conference on Chemistry and Chemical Engineering, Constanţa, Mamaia-România, 4-7 Septembrie* ***2019***
3.	*Synthesis and comparative study of some aminyl and hydrazyl free radicals and their congeners-* ***comunicare oralã****,* **B. Pãtraşcu**, C. Lete, C. Popescu, M. Matache, A. Pãun, A. Mãdãlan, I. Zarafu, P. Ioniţă, *SICHEM - International Symposium of Chemical Engineering and Materials, 17-18 Septembrie* ***2020****, Bucureşti*