„*BABEŞ-BOLYAI”* **UNIVERSITY, CLUJ NAPOCA**

Faculty of Chemistry and Chemical Engineering

Doctoral School of Chemistry

PhD Thesis Abstract

Aza Functional Derivatives of (Hetero) Aromatic Compounds with Biological Activity

Petkes Hermina Iulia

Scientific Advisor:
Prof. Dr. Luminița SILAGHI-DUMITRESCU

CLUJ NAPOCA
2014

***"BABEŞ-BOLYAI"* UNIVERSITY, CLUJ NAPOCA**

Faculty of Chemistry and Chemical Engineering

Doctoral School of Chemistry

PhD Thesis Abstract

Aza Functional Derivatives of (Hetero) Aromatic Compounds with Biological Activity

Petkes Hermina Iulia

Scientific Advisor: **Prof. Dr. Luminița SILAGHI-DUMITRESCU**	" Babeș Bolyai University, Faculty of Chemistry and Chemical Engineering, Cluj Napoca"
President: **Conf. Univ. Dr. Ing. Castelia Cristea**	" Babeș Bolyai University, Faculty of Chemistry and Chemical Engineering, Cluj Napoca"
Reviewers: **Prof. Univ. Dr. Valentin Zaharia**	"Iuliu Hațieganu University of Medicine and Pharmacy, Cluj Napoca"
Prof. Univ. Dr. Antal Csámpai	"Eötvös Lóránd University, Faculty of Science, Budapest, Hungary"
Conf. Univ. Dr. Ing. Luminița David	" Babeș Bolyai University, Faculty of Chemistry and Chemical Engineering, Cluj Napoca"

CLUJ NAPOCA
2014

Table of Contents

Keywords: microwave assisted synthesis, phenothiazinyl nitrones, IR, fluorescence, NMR, MS, vasodilatation, antiradical activity, antimicrobial activity, nitriles, Strecker reaction, chalcones, Claisen Schmidt condensation, kinetic thiol assay, second order rate constant (k_2)

1. Introduction

The thesis is a study on the synthesis and structure characteristics of some heterocyclic, aromatic and aliphatic compounds with biological activities. For this purpose, two essential heterocycles phenothiazine and coumarin were chosen and different synthetic methods, including both microwave and conventional heating were applied for the synthesis of the target molecules.

Heterocycles have an enormous potential as the most promising molecules lead structures for the design of new drugs[1]. Phenothiazine is a tricyclic heterocycle with sulphur and nitrogen atoms, which are essential in biological activity[2]. Coumarin is an oxygen containing heterocycle and has clinical medical value by itself as edema modifier[3].

Microwave assisted synthesis became essential in organic and medicinal chemistry. The main advantage of microwave synthesis compared to convectional heating, is that it allows the use of condition required by green synthesis and keep or even enhance the efficiency of the reactions[4].

The first part of the thesis includes: synthesis, structure investigations and biological evaluation of aliphatic and aromatic nitrones as well as the first time synthesis of phenothiazinyl nitrones. Synthesis and characteristics of novel phenothiazine and ferrocene based nitriles forms the second part. A kinetic study on diphenyl chalcones and chalcones with coumarin moiety is presented in the last chapter.

Nitrones are valuable free radical trapping agents, and are succesfully applied for *in vitro* and *in vivo*[5] experiments. Due to this ability are considered as the more important and efficient antioxidants[6] and vasodilators[7]. They are versatile starting materials for large area of applications in cycloaddition reactions with alkynes, alkenes[8] resulting isoxazolines and isoxazolidine[9].

Nitriles are crucial in production of some pharmaceutical products such as anti-diabetic, and anti-cancer drugs for treating breast cancer[10].

Chalcones have been reported to have many useful biological properties including anti-inflammatory[11], antifungal[12], antioxidant[13] and antitumor[14] activities. Chalcones possesses a highly electrophilic α,β-unsaturated cabonyl moiety[15], which has been reported to be important feature of their biological activities. Coumarinyl chalcones posses, anti-HIV[16], anticoagulant[17], antihypertensive[18] and antileshmanial[19] activities.

References

1. Bharati, V. B.; *Resonance*, **2006**, 11, 10, 40-48

2. Tanaka, .M. J.; Kidd, S.; *Anticancer Research*, **1997**, 17, 1A, 381-385

3. Manidhar, D.; Maheswara, K. U. R.; Bakthavatchala, R. N.; Syama, S. Ch.; Suresh, R. C.; *Journal of the Korean Chemical Society,* **2012**, Vol. 56, No. 4, 459-463

4. Katritzky, Alan R.; Cai, C., Collins, Meghan D.; Scriven, E. F. V.; Singh, S. K; *Journal of Chemical Education*, **2006**, 83, 4, 634

5. Hensley, K.;. Carney, J. M.; Steward, A. Ch.; Tebatabaie, T.; Pye, Q.; Floyd, A. R.; *International Rewiev of Neurobiology*, **1996**, 40, 299-317

6. Chakraborty, K. B.; Scott, G.; Yaghmour, H.; *Journal of Applied Polymer Science*, **1985**, 30, 3267-3281

7. Camehn, R.; Rehse, K.; *Archiv Der Pharmazie (weinheim),* **2000**,. 333, 5, 130-134

8. Stanley, L. M.; Sibi, M. P., *Chem. Rev*., **2008**, 108, 2887-2902

9. Rück-Braun, K; Freysoldt, T. H. E.; Wierschem, F; *Chem. Soc. Rev*., **2005**, 34, 507-516

10. Assikis, V. J.; Buzdar, A.; *Seminars in Oncology*, **2002**, 29, 3, pp 120-128

11. Jianzhang, W.; Jianling, L.; Yuepiao, C.; Yong, P.; Faqing, Y.; Yali, Z.; Yunjie, Z.; Shulin, Y; Xiaokun, L.; Guang, L.; *J. Med. Chem.,***2011**, 54, 8110−8123

12. Lahtchev, K.L.; Batovska, D.I.; Parushev, St.P.; Ubiyvovk, V.M.; Sibirny, A. A.; *European Journal of Medicinal Chemistry*, **2008**, 43, 2220-2228

13. Venkatachalam H. and Nayak; Yogendra, N.; Jayashree, B. S.; *International Journal of Chemical Engineering and Applications,* **2012***, 3, 3, 216-219*

14. Kumar, D.; Kumar, N. M.; Akamatsu, K.; Kusaka, E.; Harada, H.; Ito, T.; *Bioorganic & Medicinal Chemistry Letters*, **2010**, 20, 3916-3919

15. Yadav, V. R.; Prasad, S.; Sung, B.; Aggarwal, B. B.; *Int Immunopharmacol.,* **2011**; 11(3), 295–309

16 Nakagawa-Goto, K.; Lee, K.-H.; *Tetrahedron Letters* , **2006**, 47, 8263–8266

17. Abdelhafez, O. M.; Amin, K. M.; Batran, R. Z.; Maher, T. J.; Nada, S. A.; Sethumadhavan, S.; *Bioorganic & Medicinal Chemistry*, **2010**, 18, 3371–3378

18. Amin, K. M.; Awadalla, F. M.; Eissa, A. A. M.; Abou-Seri, S. M.; Hassan, G. S.; *Bioorganic & Medicinal Chemistry*, **2011**, 19, 6087–6097

19. Firoozpour, L.; Edraki, N.; Nakhjiri, M., Emami, S.; Safavi, M., Kabudanian A. S.; Khoshneviszadeh, M.; Shafiee, A.; Foroumadi A.; *Arch Pharm Res ,* **2012**,35, 12, 2117-2125

2. Microwave assisted synthesis

Microwave synthesis became a powerful tool in the past few years for rapid and efficient synthesis of a variety of compounds because of selective absorption of microwave energy by polar molecules[1].

By convective heating, reactants are slowly activated by a conventional external heat source. In contrast, microwave irradiation produces efficient internal heating by direct coupling of microwave energy with the molecules (solvents, reagents, catalysts) that are present in the reaction mixture[2]. Thus, solvents play an important role in microwave

synthesis. Since the ability of a molecule to couple with the microwave radiation is a function of its molecular polarisability (i.e. a function of its dipole moment), only polar molecules interact with microwave energy. Therefore the more polar a reaction mixture is, the greater its ability to couple with the microwave energy and this interaction leads to a rapid rise in temperature and faster reaction rates[3].

References

1. Surati, M. A.; Jauhari, S.; Desai, K. R.; *Arch. Appl. Sci. Res.,* **2012**, 4 (1), 645-661

2. Kappe, C. O.**;** *Angew. Chem. Int. Ed*., **2004**, 43, 6250 –6284

3. Martínez-Palou, R.**;** *J. Mex. Chem. Soc*., **2007**,51(4), 252-264

3. Phenothiazines

Phenothiazine (*Figure 1.)* and related derivatives are commonly used in medicinal chemistry for their neuroleptic[1] and antihistaminic[2] properties. In addition phenothiazines and related compounds have been reported to posess biological activities such as tranquilizer[3], antiinflammatory[4], antimalarial[5], antipsychotropic[6], antimicrobial[7], antitubercolar[8], antitumor[9] and analgesic[10].

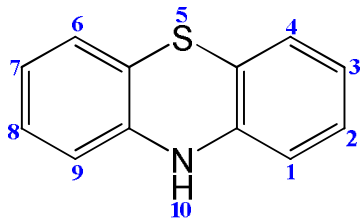


Figure 1. *Phenothiazine*

It has been suggested that the pharmacologycal activity of phenothiazines might be somehow related to their antioxidant or radical trapping ability. For example, recent studies have been proved that phenothiazine protects DNA against radical induced oxidation[11].

References

1. Ondarza, R. N.; HernAndez, E.; Iturbe, A.; Hurtado, G.; *Biotechnol, Appl. Biochem*, .**2000**, 32, 61-7

2. Shibl, A. M.; Hammouda, Y.; Al-Sowaygh, I.; *J. Pharm. Sci*., **1984**, 73, 6, 841-3

3. Hanlon, T. E.; Wiener, G.; Kurland, A. A., *Journal of Nervous & Mental Disease*, **1960**, 130, 1, 61-71

4. Saggiomo, A. J.; Sutton, B. M., *J Med Chem*., **1968**, 11, 5 ,1089-90

5. Kalkanidis, M.; Klonis, N.; Tilley, L.; Deady, L. W., *Biochemical Pharmacology*, **2002**, 63, 833-842

6. Basavaiah, K.; Krishnamurthy, G., *Microchim. Acta*, **1999**, 130, 197-201

7. Zieba, A.; Czuba, Z. P.; Krol, W., *Acta Poloniae Pharmaceutica-Drug Research*, **2012**, 69, 6, 1149-1152

8. Warman, J. A.; Rito, S. T.; Fisher, E. N.; Moss, D. M.; Berry, N. G.; O'Neill P. M.; Ward, S. A.; Biagini, G. A.; *J. Antimicrob. Chemother.*, **2013**, 68, 4, 869-880

9. Motohashi, N.; Kawase, M.; Saito, S.; Sakagami, H.; *Curr drug Targets*., **2000**,1, 3,237-45

10. N. K. Barkov, *Bulletin of Experimental Biology and Medicine*, **1961**, 50, 3, 950-952
11. Liu, Z-Q.; Tang, Y-Z.; Wub, D.; *J. Phys. Org. Chem*., **2009**, 22, 1009–1014

4. Nitrones

Nitrones are useful tools due to their succesful application as building blocks in the synthesis of various natural and biologically active compounds[1,2,3] of stable nitroxyl radicals[4] and of other important products for special purposes such as spin traps[5] for the study of radical processes[6], including those that take place in biological systems. Particularly the α-aryl nitrone have found extensively usages in these areas, since chemists showed in 1967 that some free radicals will react with nitrones to produce nitroxide free radicals[7]. Spin traps were developed in order to accumulate ("trap") highly reactive, primary radicals which cannot otherwise be observed directly. One of the most widely known nitrone is PBN (*Figure 2.*).

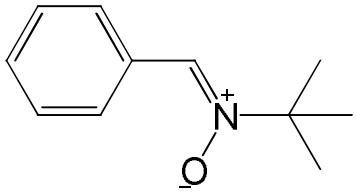


Figure 2*. Structure of α-phenyl tert-butyl nitrone*

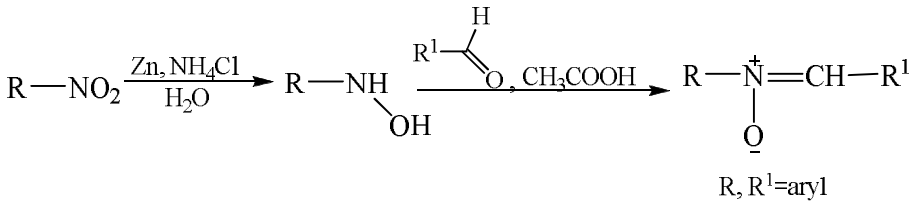
The advantage of nitrones uses as spin traps consists in the fact that in usually form stable adducts with a wide variety of radicals, not terribly toxic, so amenable to *in vivo* and *in vitro* spin trapping.

Chamulitra[8] showed in 1993, followed by Saito *et al*. [9] in 1988 that nitric oxide could be formed by PBN in aqueous environment under UV light decomposition. Nitric oxide is an important second messenger neurotransmitter[10,11,12] which may operate in part through an activation of guanylate cyclase activity. Due to this ability it is considered as an efficient vasodilator and play significant role in inflammation[13]. As a vasoldilator agent[14, 15, 16, 17] keeps the blood pressure in normal range playing an important role in the pathophysiological functions of the vascular system. The possibility exists that the vasoldilatory effect of spin traps is caused by their transformation to nitric oxide by enzimatic cleavage of the spin traps[18].

Consequently, nitric oxide donors may be useful in treatment of a variety of vascular disorders[19] Since nitrones are able to release nitric oxide and to trap free radicals they could become important vasodilator agents.

4.3. Strategies toward the synthesis of nitrones. A literature survey.

For the preparation of nitrones, the most popular method is the condensation of aldehydes or ketones with N-monosubstituted hydroxylamines[20] or hydroxylamine hydrochlorides and direct oxidation of secondary amines[21]. The synthesis of nitrones by condensation can be achieved in two steps: a.) *in situ* synthesis of hydroxylamines; b.) condensation between the resulted hydroxylamine and aldehyde (*Scheme 1.*) applying both microwave and convective heating[22, 23].



Scheme 1. *Synthesis of nitrones by condensation reaction*

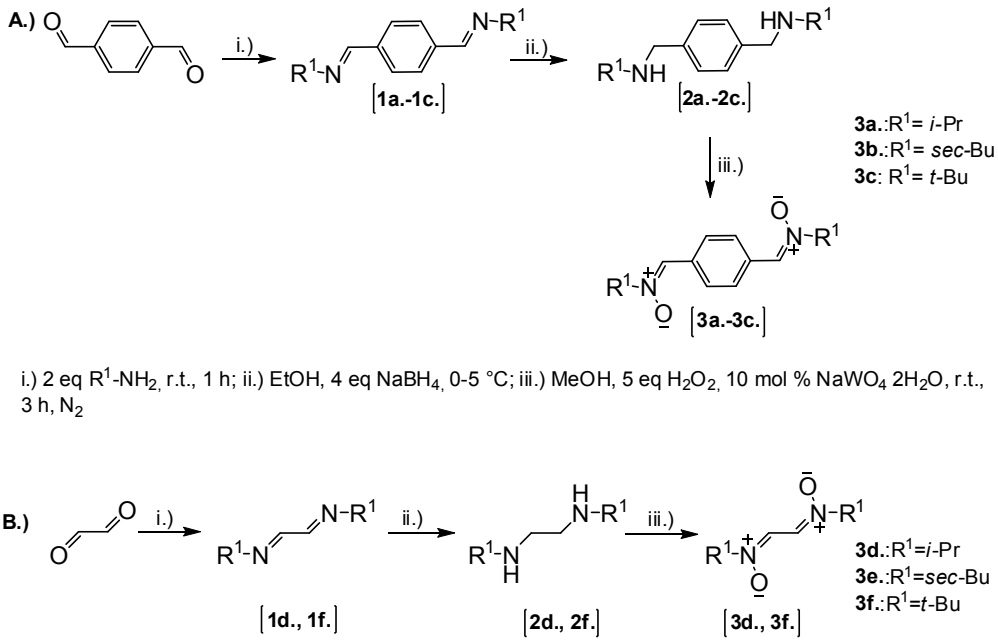
Oxidation of secondary amines to nitrone became a facile and rapid method in the last few years. The suitable oxidizing agents for this are *m*-chloro perbenzoic acid, reported by A. H. Becket *et. al*[24] or the commercialy available hydrogen peroxide[25], where the oxidation take place in presence of sodium tungstate (Na_2WO_4) catalyst in a polar solvent, at 0 ° .

4.4. Personal contributions

This part of the thesis it is designated for presenting the synthesis, the structural and spectroscopic characteristics of various nitrones with biological activities. The synthesis of nitrones was accomplished by different oxidation and condensation processes according to their structure. Biological activities of the prepared novel and known nitrones in vasodilatation, as antioxidant, antifungal and/or antibacterial agents was determined and presented as the secondary objective of this topic.

4.4.1. Synthesis of C-terephthal-, and C-glyoxal-*N*-alkyl *di*nitrones

C-terephthal-, or glyoxal- *N*-alkyl *di*nitrones [**3a.-3e.**] were easily achieved through oxidation of a secondary amine with H_2O_2, in presence of a catalytic amount of Na_2WO_4 using methanol as solvent[26]. Secondary amines [**2a.-2e.**] were synthesized by reduction with $NaBH_4$, of the previously prepared Schiff bases [**1a.-1e.**] by condensation of a commercially available aldehyde and amine. Reactions and conditions employed to prepare the C-aryl/alkyl, *N*-alkyl *di*nitrones are presented in *Scheme 2[A., B.]*.



Scheme 2. *Reactions and conditions for synthesis of C-terephthal-N-alkyl dinitrones (**A.**) and C-glyoxal-N-alkyl dinitrones (**B.**)*

The structures of novel (**3c**., **3e**) and known nitrones (**3a**, **3b**, **3d**, **3f**) were confirmed by ^{1}H and ^{13}C NMR, MS and FT-IR spectra. In ^{1}H-NMR spectra the characteristic singlet generated by the imine proton appear around 7.7 ppm. Due to the chiral centrum located in glyoxal and terepthal-*N*-*sec*-Bu dinitrones (**3b., 3d.**) in the ^{1}H-NMR spectra, protons of CH_2 became diastereotopic and their signals occurs at different chemical shifts around 1.4 and 2.0 ppm as septets (*Figure 2.*). In the amine the same splitting pattern was recognized, while in the parent Schiff base (**1b.**) these protons generated a quintet *(Figure 3.)*

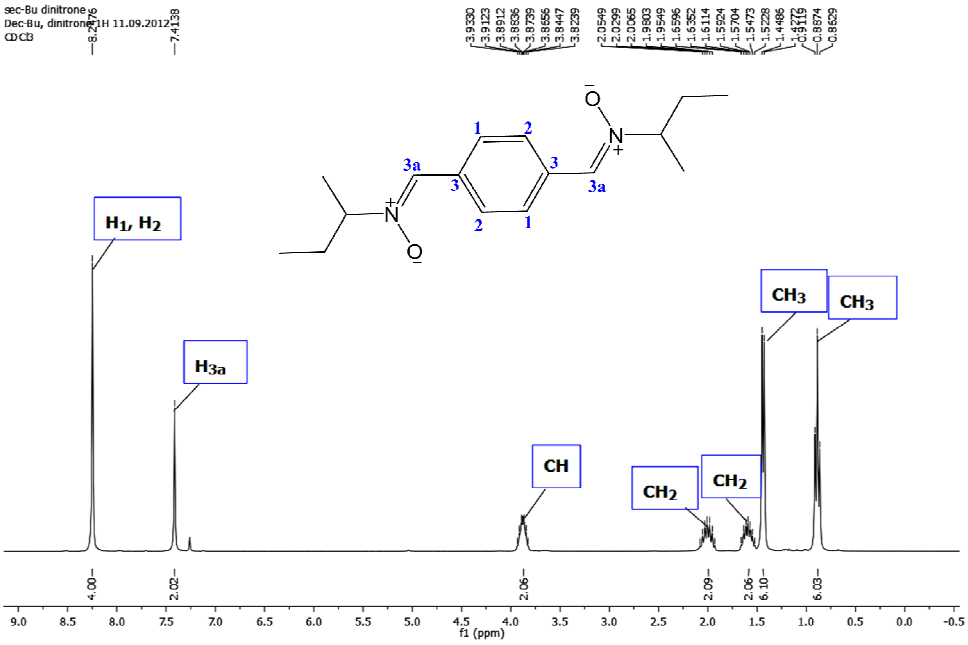


Figure 2. *^{1}H-NMR spectrum of nitrone **3b.** recorded in CDCl at 300 MHz*

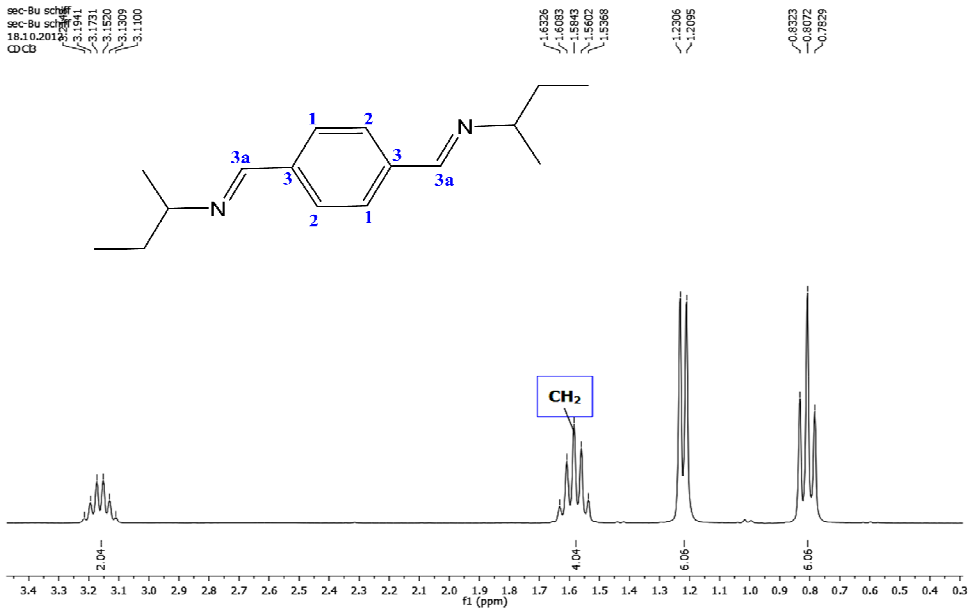
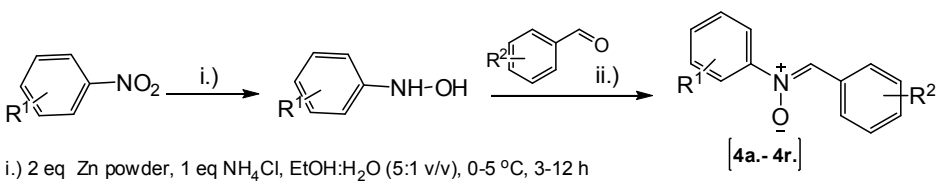


Figure 3. *Aliphatic region from ^{1}H-NMR spectrum of Schiff base* ***1b.*** *recorded in $CDCl_3$ at 300 MHz*

4.4.2. Synthesis of diphenyl nitrones

The following section presents the synthesis of diphenyl nitrones by condensation reaction between *N*-monosubstituted aromatic hydroxylamines and aldehydes according to the literature procedure[27] (*Scheme 3.*).



i.) 2 eq Zn powder, 1 eq NH_4Cl, EtOH:H_2O (5:1 v/v), 0-5 °C, 3-12 h
ii.) 1 eq benzaldehyde, CH_3COOH, r.t, 1-2 h

4a.:R^1=H,R^2=*o*-NO_2
4b.:R^1=H,R^2=*m*-NO_2
4c.:R^1=H,R^2=*m,p*-diNO_2
4d.:R^1=H,R^2=*p*-Br
4e.:R^1=H,R^2=*m,p*-diMeO
4f.:R^1=H,R^2=*p*-OMe
4g.:R^1=H,R^2=*m*-Cl
4h.:R^1=H,R^2=*p*-OH
4i.:R^1=*p*-Cl,R^2=*m*-Cl
4j.:R^1=*p*-Cl,R^2=*p*-Br
4k:R^1=*p*-Cl,R^2=*o*-NO_2
4l.:R^1=*p*-Cl,R^2=*m*-OH
4m.:R^1=*p*-Cl,R^2=*p*-OH
4n.:R^1=*p*-Cl,R^2=*p*-OMe
4o.:R^1=*p*-Cl,R^2=*m,p*-diMeO
4p.: R^1=*p*-Cl,R^2=*p*-Cl
4r.:R^1=*p*-Br,R^2=*m*-OH
4s.:R^1=*p*-Br,R^2=*p*-MeO
4t.:R^1=H,R^2=*t*-Bu

Scheme 3*. Synthesis of diphenyl nitrones by condensation reaction*

Synthesis of hydroxylamines is being carried out *in situ* via reduction of nitro compounds with zinc powder in the presence of weak acids (NH_4Cl or AcOH). Furthermore, reacted the resulted hydroxylamines with an aldehyde gave the desired nitrones in high yields. Reducing of nitro compounds to hydroxylamines was strongly dependent on the electron nature of the substituent present on the molecule. It was noted, that electron donating substituents in benzaldehyde, such -OMe, -OH increased the reaction rate, unlike electron withdrawing groups (Cl, Br, NO_2) decreased the yield of reaction.

Known nitrones (**4a; 4b; 4d-4h; 4j-4p; 4s; 4t)** showed spectroscopic data in good agreement with those reported in the literature. The structures of the newly-synthesized nitrones (**4c**, **4i**, **4r**) were confirmed by their ^{1}H-NMR, MS and FT-IR spectra. In the ^{1}H-NMR spectra the characteristic is the singlet generated by the imine proton in the range of 7.9-8.5 ppm (*Figure 4*).

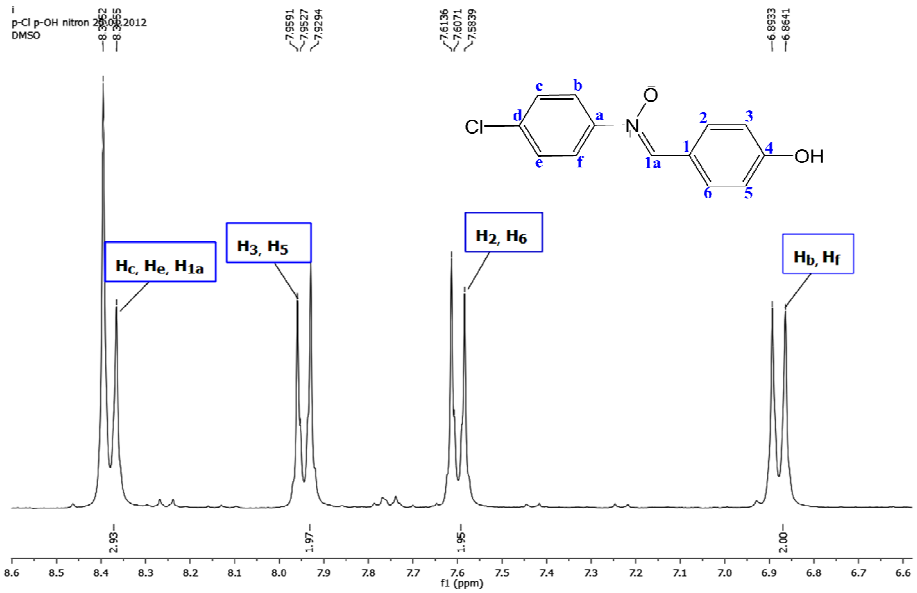


Figure 4. *^{1}H-NMR spectrum of nitrone* ***4m.****, recorded in $CDCl_3$ at 300 MHz,*

In the mass spectra of diphenyl nitrones all compounds gave molecular ion peaks under EI, althought the intensity varied considerably. The most characteristic feature in fragmentation of nitrones under EI is the elimination of the O$^{\bullet}$ from the nitrone group. It was estabilished that the presence of strong electron withdrawing groups in nitrones cause a decrease of molecular peak. A fragmentation scheme of compound **4m.** recorded with EI method at 70 eV is presented in *Figure 5*. The m/z =230, 231 are the abundant peaks, generated from elimination of the hyroxyl group or an oxigen molecule, respectively. The molecular peak, m/z= 247 is also diagnostic. Nitrones with hydrogen in the R position (**4h.**) produced a strong molecular peak (*Figure 6.)* in mass spectrum. The fragmentation of compound **4h.** is presented in *Scheme 23*.

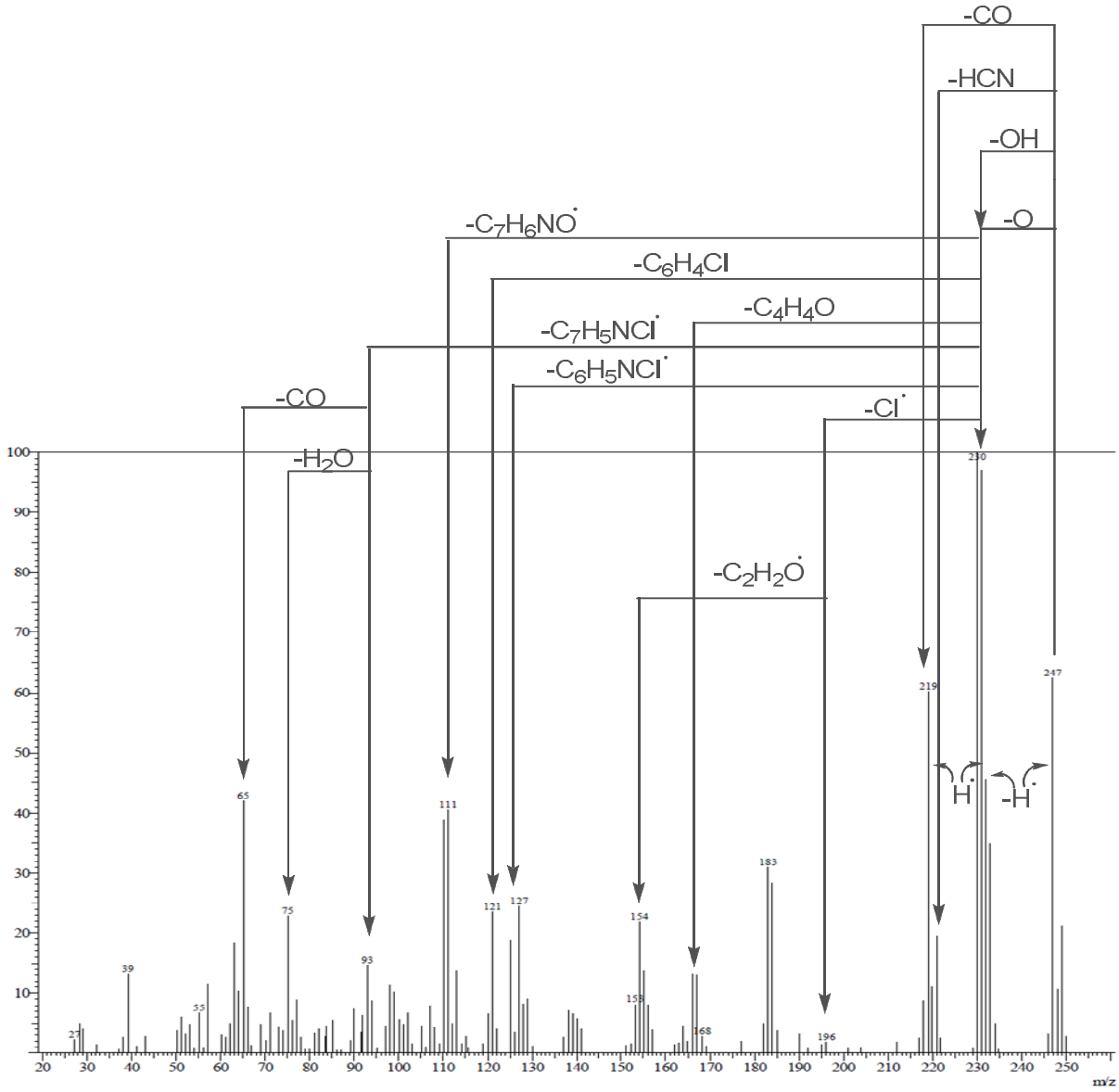
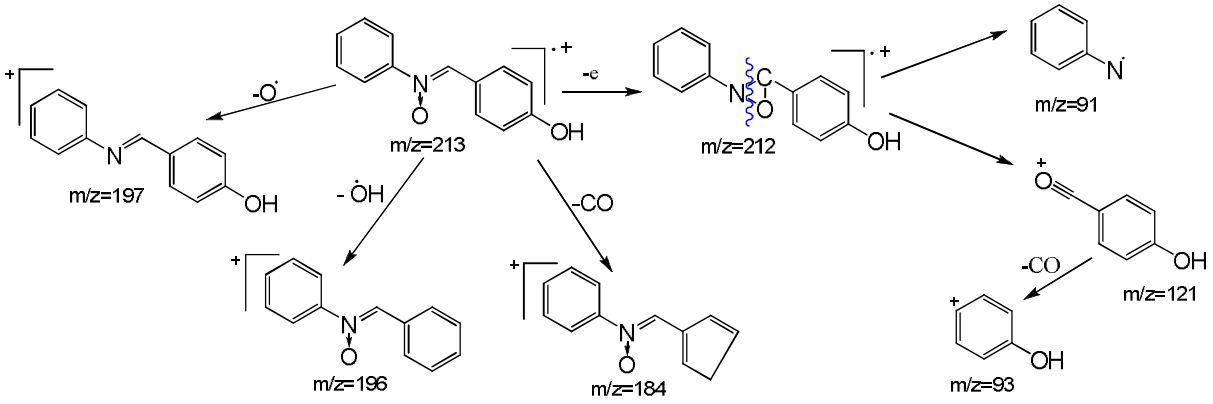


Figure 5. *Electron impact (70 eV) mass spectrum of compound* ***4m***



Scheme 23. *Fragmentation scheme of compound* ***4h.***

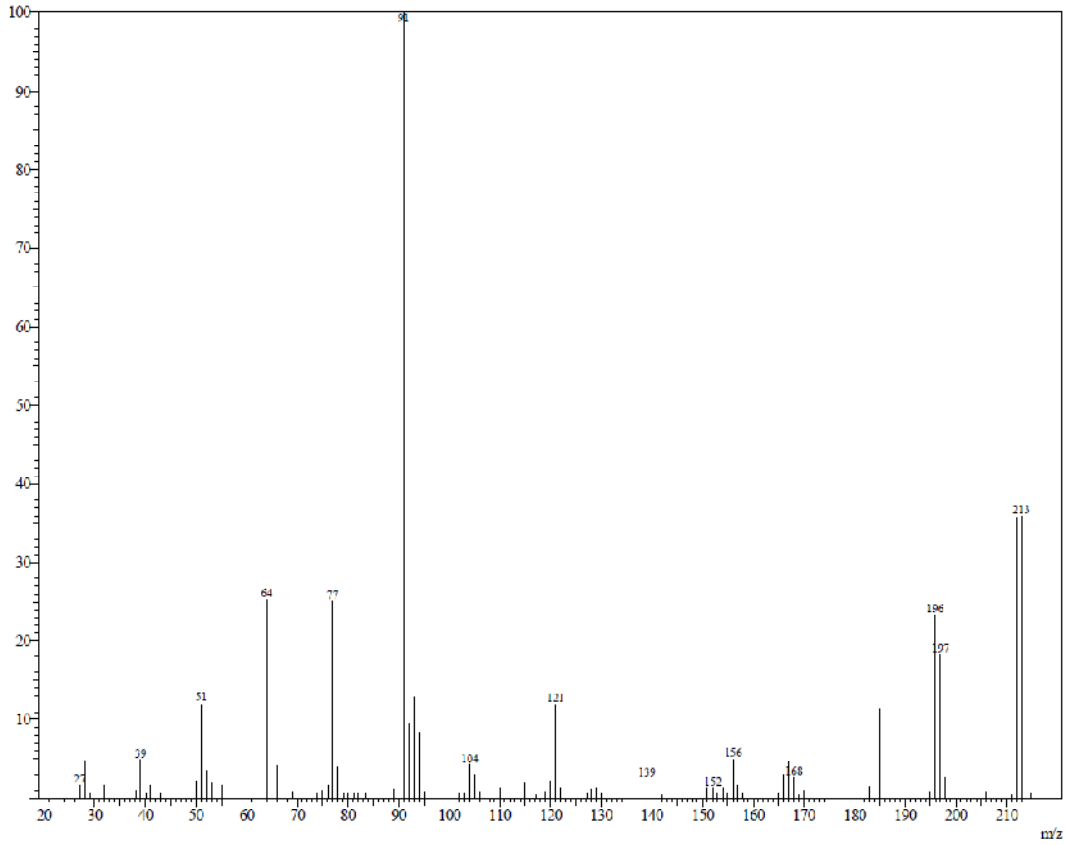
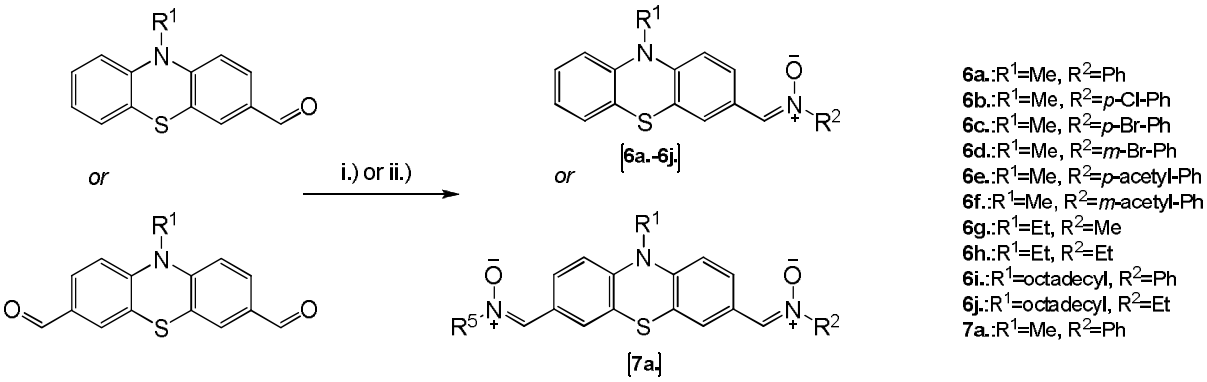


Figure 6. *Electron impact (70 eV) mass spectrum of compound* ***4h***

4.4.3. Synthesis of phenothiazinyl nitrones

The aims of this task were to prepare nitrones containing at least one phenothiazine unit, to develop and optimize a reproducible process for their synthesis using both microwave and convective heating.

Phenothiazinyl nitrones, a completely new class of nitrones were synthesized as potentially biologically active compounds. The synthesis approach was based on condensation reactions, by employing microwave assisted irradiation and convective heating. The synthesis pathway is a modification of a recently published method by M. M. Andrade *et. al*[23]. N-aryl or N-alkyl nitrones (**3.a.-3.j., 4.)** were obtained from low to hight yield (16-84%) by reacting an appropriate phenothiazine carbonyl derivative with an equimolar amount of hydroxylamines (*Scheme 4.*).



i.) 1 eq R^2-NHOH (prepared *in situ*), EtOH-H_2O (5:1), 1,2 eq NaOAc, MW, 100 °C, 10 min; ii.) 1 eq R^2-NHOH (prepared *in situ*), EtOH-H_2O (5:1), 1,2 eq NaOAc, 100 °C, 1-6 h

Scheme 4. *Synthesis of phenothiazinyl nitrones*

Microwaves significantly decreased the reaction time compared with classical syntheses. A comparison beetwen the reaction parameters under microwave assisted and conventional heating conditions is summarized in *Table 1*.

Table I. *The synthesis of phenothiazinyl-nitrones by microwave irradiation and by convective heating.*

Entry	Comp.	R^1	R^2	Convection heating		Microwave	
				Yield [%][a]	Reaction time [min]	Yield [%][a]	Reaction time [min]
1	**6.a.**	Me	C_6H_5	20	120	37	10
2	**6.b.**	Me	4-Cl-C_6H_5	16	60	30	10
3	**6.c.**	Me	4-Br-C_6H_5	21	60	28	10
4	**6.d.**	Me	3-Br-C_6H_5	35	480	50	10
5	**6.e.**	Me	4-acetyl-C_6H_5	30	90	44	10
6	**6.f.**	Me	3-acetyl-C_6H_5	41	60	56	10
7	**6.g.**	Et	Me	65	75	84	10
8	**6.h.**	Et	Et	77	75	81	10
9	**6.i.**	Octadecyl	C_6H_5	52	120	74	10
10	**6.j.**	Octadecyl	Et	70	75	84	10
11	**7a.**	Me	C_6H_5	34	60	61	10

[a]Isolated yield after column chromatography

This new class of phenothyazinyl nitrones appear to be highly sensitive for heating and decomposes immediately at higher temperatures (120-130 C°) without melting. It was observed that the electron withdrawing substituents in the aryl moiety destabilize the imine bond and these compounds are more instable compared to those containing an electron donatig group. Nitrones **6b.** and **6c.** bearing the highly electron withdrawing chloro and bromo atoms were isolated with lower yield 16 %, and 21%, respectively. In contrast the presence of an alkyl groups stabilized the imine bond. A significant stability was observed in case of nitrones

6g., 6h., **6j.**, which were obtained in high yields after purification and were resistant on air and heating. This might be attributed to the electron donating nature of the alkyl groups.

In the UV spectra of phenothiazinyl-nitrones, an intense absorption band is situated around ~390nm, and correspond to $\pi - \pi^*$ molecular transitions (Table 2). The *para* substitution of the phenyl rings in the nitrone **6b., 6c.** and **6d.** with halogen and **6e.**, **6f.** with acetyl group does not produce noticeable effects upon the absorption properties of nitrones. The optical properties of the newly synthesized nitrones are presented in *Table 2.*

Table 2. *Optical properties of phenothiazinyl-nitrones*

Comp.	λ_{abs} [nm] (ε mol^{-1}cm^{-1})[a)]	λ_{em} [nm]	Stokes shift $\Delta\nu$ [cm^{-1}]	Φ_F[b)]
6a.	315, 272, 393(9517)	551	7255	7.83
6b.	316, 274, 385(8277)	521	7142	5.59
6c.	316, 275, 380(10048)	518	7103	5.01
6d.	317, 275, 397(9561)	555	7535	5.43
6e.	312, 241, 398(8480)	550	7211	7.77
6f.	314, 271, 402(9398)	542	7197	8.33
6g.	302, 377(14026)	519	7275	3.50
6h.	302, 377(21778)	515	7076	4.70
6i.	314, 275, 382(14010)	569	7888	1.90
6j.	302, 376(22408)	518	7235	4.20
7a.	318, 419(16280)	554	7595	5.43

a) UV-Vis absorption in CH_2Cl_2.
b) Quantum yields against perylene standard in cyclohexane.

All phenothiazinyl-nitrones display light-blue fluorescence. Upon irradiation with the λ max in dichloromethane solution, nitrones display fluorescence with large Stokes shifts ($\Delta\nu$ =7076-7888 cm^{-1}) and quantum yields between 1.90 and 8.33 when measured against perilene standard in ciclohexane, and calculated with *Equation I.*

$$\Phi_f = \Phi_{f(std)} * \frac{A_{std} * d_x * F_x}{A_x * d_{std} * F_{std}} * \frac{n_x^2}{n_{std}^2} \qquad \text{Eq. (I)}$$

Where A_{std} and A_x represents the absorbance of the standard and the sample at the excitation wavelenght, d_{std} and d_x are the dilution quotient of concentration used in UV-Vis and in flourescence, F_x and F_{std} are the areas under the flourescence curves of the samples and the standard, and n_x and n_{std} are the refractive indices of the solvents used for sample and for standard.

The characteristic singlet generated by the imine proton in ^{1}H NMR spectra of phenothiazinyl nitrones was typicaly situated beetween 8.0-8.2 ppm. (*Figure 7.*),

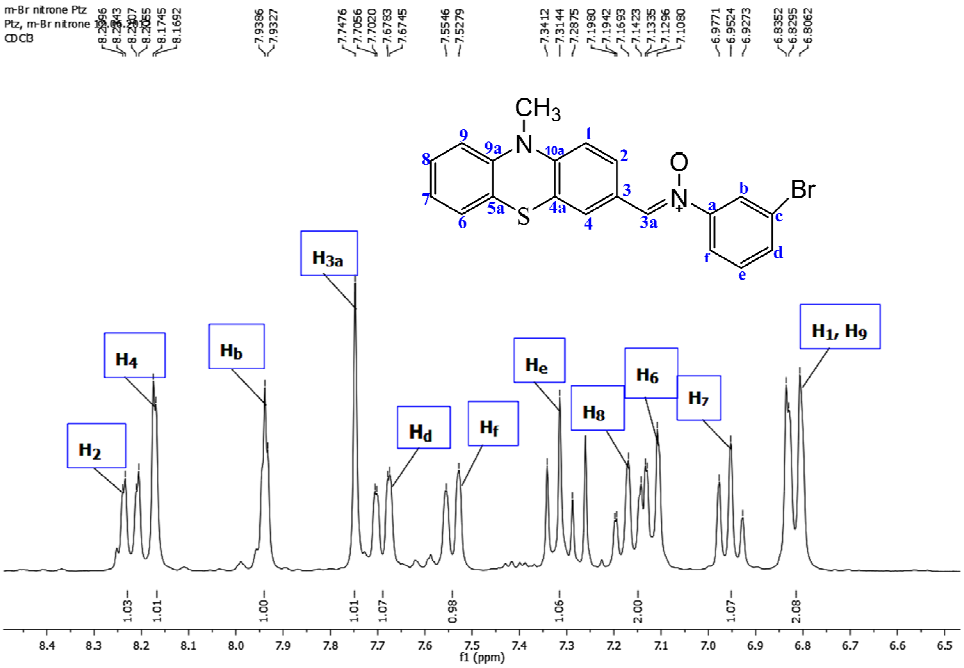


Figure. 7. *Detail aromatic region from [1]H-NMR spectrum of nitrone* ***6d.*** *recorded in $CDCl_3$ at 300 MHz*

In the [13]C-NMR spectra, the signal of the carbon in the imino group occured mostly at 150 ppm, a chemical shift similar to the one reported in the literature for phenothiazinyl Schiff bases[28].

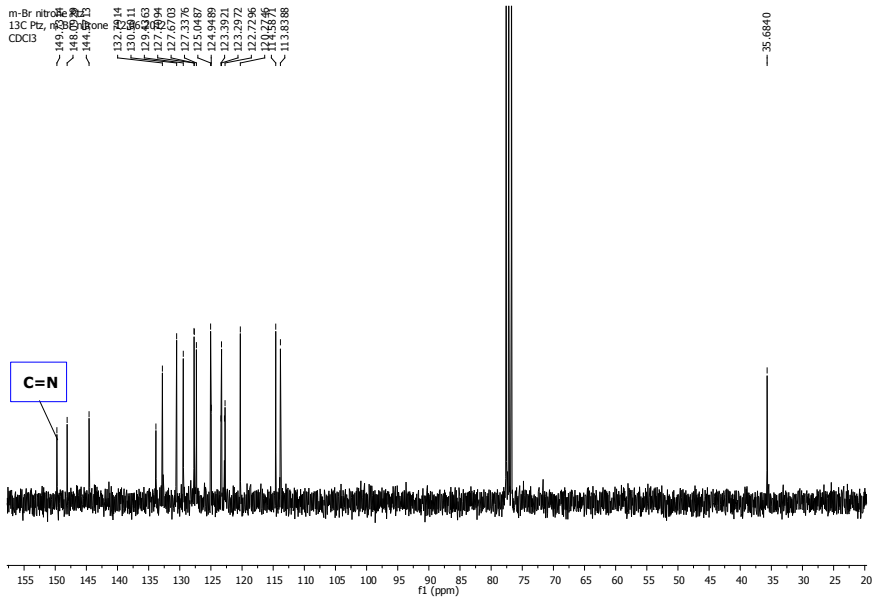


Figure 8*.Detail aromatioc region from [13]C-NMR spectrum of nitrone* ***6d.****,recorded in $CDCl_3$ at 75.5 MHz*

In mass spectrometry studies of phenothiazinyl nitrones the molecular peak was the one of the most abundant peak. A practical example of this is presented by mass spectrum of nitrone **6a** (*Figure 9*). Further peaks were assigned to the fragment resulted from elimination of $O^{\bullet}$ at m/z=316 as well m/z=301 for the imine, which formed as byproduct in the reaction

and it is detectable even after purification. The fragmentation pattern of phenothiazinyl nitrone **6a.** is proposed in *Scheme 5*.

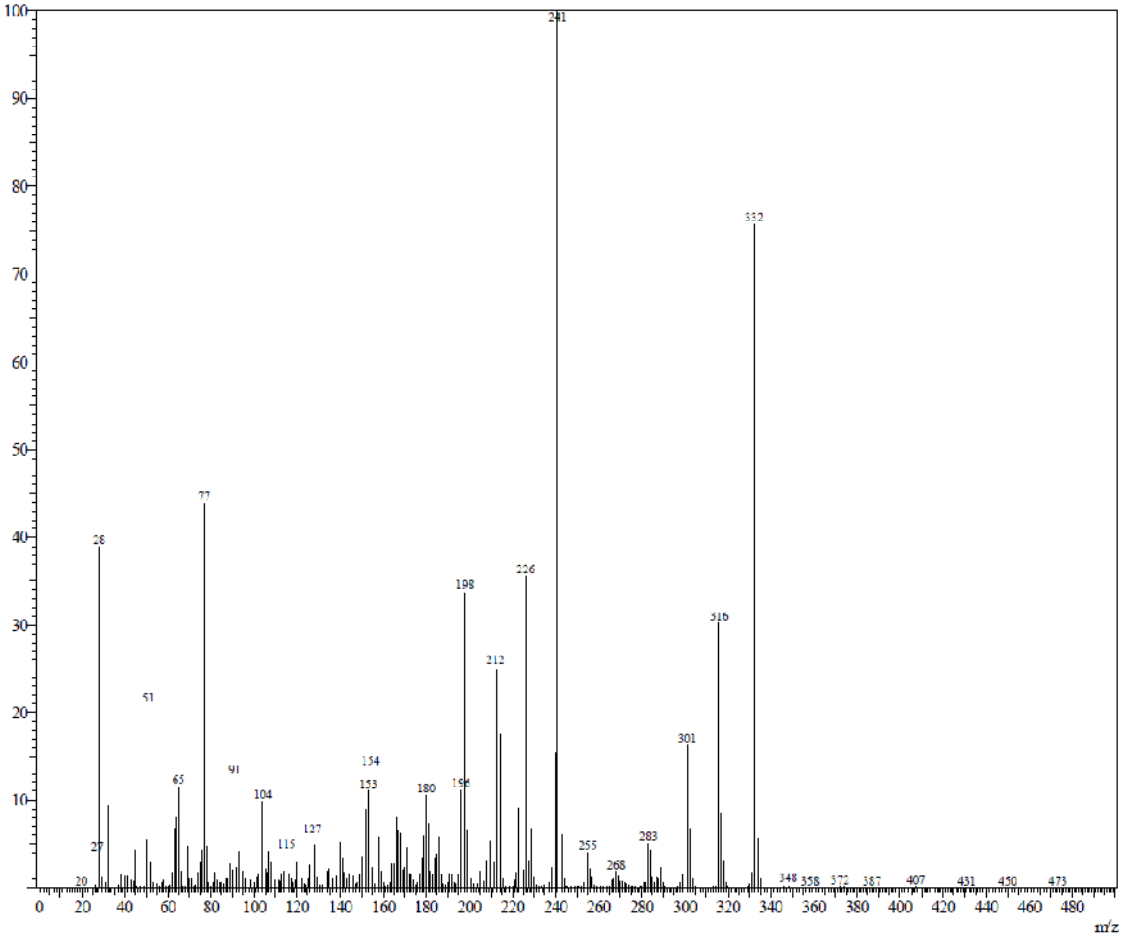
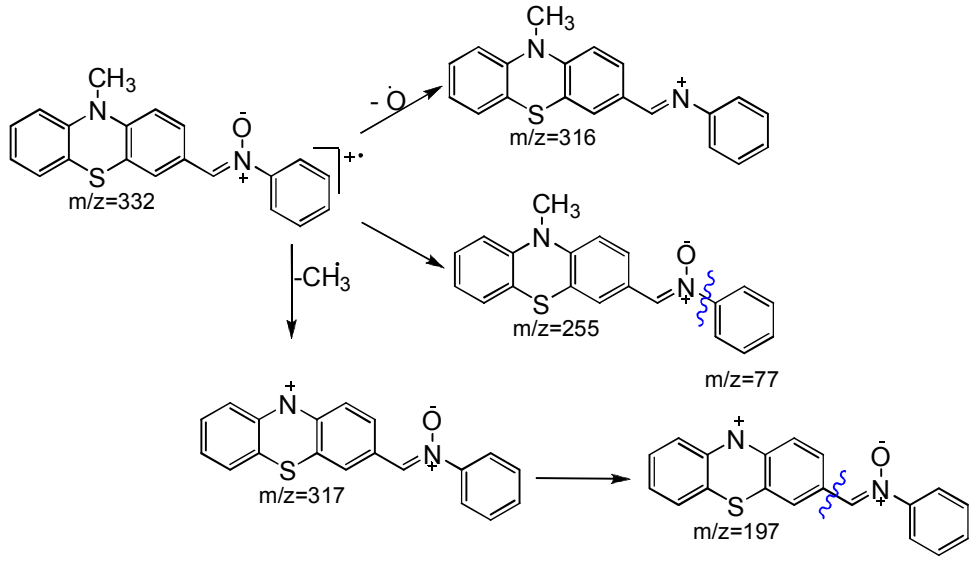


Figure 9**. Electron impact (70 eV) mass spectrum of nitrone **6a.



Scheme 5.** Fragmentation scheme for phenothiazinyl nitrone **6a.

4.4.4. Biological evaluation

The biological activity of the synthetized nitrones was investigated; the phenothiazinyl nitrones also participated in the biological evaluation, showing beneficial activities as antifungals, antimicrobials, antioxidants as well as vasodilators.

4.4.5. Antimicrobial (antifungal and antibacterial) activities

The antifungal and antibacterial activity of the new and known nitrones mentioned above was evaluated against several *Candida* species and gram negative bacteria such as *E. Coli*, *Citrobacter spp*, *Morganella spp*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (± ESBL), *Proteus spp, Acinetobacter spp* and gram positive bacteria *Staphylococcus aureus*.

Antifungal and antibacterial activities were investigated in triplicate, by disk diffusion method at 8 μM nitrone concentration. Nitrones, **3a.**, **4a.** and **4r** were sensitive to *Candida* strains *Candida albicans* and *Candida krusei*.

In case of phenothiazinyl nitrones **6a.**, **6h.** and **4t.** the tests were repeated at higher concentration 25μg/disk. Only the *Candida parpsilosis* was sensitive to phenothiazinyl nitrone with the inhibition diameter of 6mm for nitrone **6a**., 7mm for nitrone **6h**. and 6mm for nitrone **4t**.

4.4.6. Vasodilating activity of nitrones

Nitrones, presented here, were evaluated *in vitro* as nitric oxide donors using UV radiation as hydroxyl anion source and the reaction with rat hepatic homogenates. Afterwards the most active compounds were evaluated *in vivo* using rabbits as experimental animals. The amount of NO was determined from aqueous environment by indirect method, measuring the amount of decomposition products of nitric oxide: the nitrate and nitrite ions.

In vitro testing on nitrones showed a significant ability for them to release nitric oxide upon reaction with hydroxyl radical generated by UV light. Especially terephthal and glyoxal dinitrones showed a considerable capacity to release nitric oxide in high quantities.

Experiments with rat hepatic homogenase was used to verify if there are hepatic enzymes able to metabolize nitrones with release of NO. During the experiments only in case of nitrones **3b., 3e., 6g.** was found to increase of nitrite concentration in rate hepatic homogenates.

In *in vivo* experiments with rabbits terepthal and glyoxal, *N*-alkyl dinitrones showed the best results. Administration of nitrones to animals have been shown intense vasodilatation such, tachycardia, dilated blood vessels in the ear, easy blood sampling procedure. Nitrones **3e.** and **3c.** also acted as excellent nitric oxide modulators, and **3f.** significantly increased the

nitrite concentration but only 7 hours after administration. Similar behaviour was observed with nitrone **3e.**, where higher nitrite levels were detected after 2 hours after administration.

4.4.7. Antioxidant activity of nitrones

Antioxidants are substances with the ability to inhibit or even prevent the oxidation of another molecule by sacrificing their own electrons to feed free radicals. Free radicals are molecules, atoms or molecular fragments containing one or more unpaired electrons in its outermost atomic or molecular orbital[29]. This unpaired electron results in molecular instability and therefore, radicals are highly reactive molecules that promote oxidative damage to cellular components[30]

Nitrones are well known spin traps and are widely used for their radical scavenging activity as antioxidants and anti inflammatory agents. Spin traps have been shown to affect cellular oxidation states and oxidatively sensitive enzyme systems[31]. Thereby, nitrones are promising therapeutic agents for pathological conditions involving free radical-driven oxidative stress[32].

Antioxidant activity of nitrones were assessed by DPPH procedure. The DPPH antioxidant assay is based on the reaction of DPPH with an other radical (*Scheme 2*), or radical scavenger, where the reaction can be monitored even visually. Since, DPPH has a strong absorption band centered at 520 nm, has a deep violet color in solution. When DPPH will be neutralized by a radical scavenger the color will become pale yellow. The outcome of the process can be detected by UV-Vis spectroscopy and the number of initial radicals can be counted from the optical absorption at 520 nm. The water-chloroform partition coefficient ($P_{o/w}$) of nitrones were determined spectrophotometrically[33]. Both characteristics of nitrones, the antiradical capacity and partition coefficient values were closely related with their structural form and evaluated in function of structure- activity relationship.

The antiradical activity of nitrones was calculated as a percentage of DPPH decoloration using the following equation:

$$\text{Antiradical activity} = 100 \times [1-(A_s/A_{ref})]$$

where, A_s represents the absorbance of sample and A_{ref} is the absorbance of reference.The antiradical activities of nitrones are summarized in Table 3 and Table 4..

Table 3. Antioxidant activity of diphenyl nitrones

Nitrone	R^1	R^2	AO activity (%) (t_0, t_{60}, t_{120}) (min)
4a.	H	*o*-NO_2	14, 81, 88
4b.	H	*m*-NO_2	10, 51, 55
4c.	H	*m*,*p*-diNO_2	4, 31, 38
4d.	H	*p*-Br	20, 90, 91
4e.	H	*m*,*p*-diMeO	11, 86, 88
4f.	H	*p*-OMe	59, 91, 90
4g.	H	*m*-Cl	44, 92, 92
4h.	H	*p*-OH	23, 93, 93
4i.	*p*-Cl	*m*-Cl	2, 71, 79
4j.	*p*-Cl	*p*-Br	11, 28, 35
4k.	*p*-Cl	*o*-NO_2	47, 93, 93
4l.	*p*-Cl	*m*-OH	45, 93, 93
4m.	*p*-Cl	*p*-OH	49, 92, 93
4n.	*p*-Cl	*p*-OMe	52, 92, 92
4o.	*p*-Cl	*m*,*p*-diMeO	48, 90, 91
4p.	*p*-Cl	*p*-Cl	22, 69, 73
4r.	*p*-Br	*m*-OH	0.5, 35, 44
4s.	*p*-Br	*p*-OMe	25, 33, 49

AO-antioxidant activity

Table 4. Antioxidant activity of phenothiazinyl nitrones

Nitrones	R^3	R^4	AO activity (%) (t_0, t_{60}, t_{120}) (min)
6a.	Me	Ph	0, 0, 8
6b.	Me	*p*-Cl-Ph	4, 9, 11
6c.	Me	*p*-Br-Ph	3, 10, 14
6d.	Me	*m*-Br-Ph	5, 8, 6
6e.	Me	*p*-acetyl-Ph	3, 14, 19
6f.	Me	*m*-acetyl-Ph	6, 7, 8
6g.	Et	Me	[a]
6h.	Et	Et	3, 5, 22
6i.	Octadecyl	Ph	5, 5, 0
6j.	Octadecyl	Et	3, 4, 3,5
7a.	Me	Ph	1,5, 8, 18

[a]No antioxidant activity was observed.

The highest antioxidant activities were estabilished for diphenyl nitrones. The antioxidant activity of these compounds was strongly dependent to the substituents found in the molecule. Especially hydroxyl groups (nitrones **4h.**, **4l.** and **4m)** located in *para* or *meta* positions showed to enhance scavenging of free radicals.. Furthermore, methoxy (**4n.**, **4o.**)

and nitro groups showed a good tendency to increase the antiradical activity of nitrones. Slightly lower, but still high antiradical activity was found with halogenated nitrones (**4g.**, **4d.**, **4i.**, **4p.**) in range of 88 % and 79 % activity.

Water/chloroform partition coefficients of nitrones was determined using the shake-flask technique[34], where the maximum absorption wavelength of nitrones was determined earlier. The obtained partition coefficient results for the two class of nitrones are summarized in Table 5. and Table 6.

Table 5. Partition coefficients and maximum absorption wavelenght of diphenyl nitrones

Nitrone	R^1	R^2	Partition coefficient ($K_{w/c}$)	λ_{max}[nm]
4a.	H	*o*-NO_2	76,99	276
4b.	H	*m*-NO_2	7,8	317
4c.	H	*m,p*-diNO_2	2,14	384
4d.	H	*p*-Br	4,03	315
4e.	H	*m,p*-diMeO	18,41	342
4f.	H	*p*-OMe	7,97	331
4g.	H	*m*-Cl	21,68	319
4h.	H	*p*-OH	80,1	324
4i.	*p*-Cl	*m*-Cl	37,52	311
4j.	*p*-Cl	*p*-Br	40,21	314
4k.	*p*-Cl	*o*-NO_2	7,47	276
4l.	*p*-Cl	*m*-OH	21,6	310
4m.	*p*-Cl	*p*-OH	8,65	333
4n.	*p*-Cl	*p*-OMe	80,1	328
4o.	*p*-Cl	*m,p*-diMeO	17,03	313
4p.	*p*-Cl	*p*-Cl	11,04	326
4r.	*p*-Br	*m*-OH	nd	
4s.	*p*-Br	*p*-OMe	20,22	338

nd-Not detected

The partition coefficients obtained for diphenyl nitrones were above one, which indicates that their solubility is greater in organic phase than in aqueous phase. The affinity to water of nitrone **4c.** can be explained by the sparing affinity of the two nitro groups to water. Similar results were detected for other nitro substituted nitrones, excluding the the *ortho* substituted nitrone **4a.**, which showed very high affinity to the organic phase. Even though, if in literature hydroxyl groups enhance the hydrophilicity of a compound, in our case nitrone **4h.** with a hydroxyl group in *para* position displayed high lipophilicity, with the highest partition coefficient value of 80.1.

Table 6. Partition coefficients and maximum absorption wavelength of phenothiazinyl nitrones

Nitrone	R^3	R^4	Partition coefficient ($K_{w/c}$)	λ_{max} [nm]
6a.	Me	Ph	nd	
6b.	Me	*p*-Cl-Ph	nd	
6c.	Me	*p*-Br-Ph	nd	
6d.	Me	*m*-Br-Ph	nd	
6e.	Me	*p*-acetyl-Ph	nd	
6f.	Me	*m*-acetyl-Ph	nd	
6g.	Et	Me	23,19	377
6h.	Et	Et	19,92	377
6i.	Octadecyl	Ph	nd	
6j.	Octadecyl	Et	nd	
7a.	Me	Ph	nd	

nd-Not detected

Unfortunatelly, phenothiazine nitrones were completely inappropriate to determine their water-chloroform partition coefficients. The presence of the long aliphatic chain on phenothiazine gave poor water solubility for this series of nitrones. Only N-aliphatic nitrones **6g.** and **6h.**, which are soluble,were characterized with partition coefficient values and showed moderate affinity toward water.

References

1. Floyd, R. A.; *Aging Cell*, **2006**, 5, 51-57

2. Romeo, G.; Iannazzo, D.; Piperno, A.; Romeo, R.; Corsaro, A.; Rescifina, A.; Chiacchio, U.; *Mini-Reviews in Organic Chemistry,* **2005**, 2*,* 59-77

3. Mallesha, H.; Ravi Kumar, K. R.; Vishu Kumar, B. K.; Mantelingu, K.; Rangappa, K.S.; *Proc. Indian Acad. Sci*., **2001**, 113, 4, 291-296

4. Rozantsev, E. G.; Sholle, V. D.; *Russ. Chem. Rev*., **1971**, 40, 3, 233

5. Barriga, G.; Olea-Azara, C.; Norambuena, E.; Castro, A.; Porcad, W.; Gerpe, A.; González, M.; Cerecetto, H.; *Bioorganic & Medicinal Chemistry*, **2010**, 18, 795–802

6. Floyd, R. A.; Hensley, K.; Forster, M. J.; Kelleher-Andersson, J. A.; Wood, P. L., *Mechanisms of Ageing and Development*, **2002**, 123, 1021–1031

7. Fu, Y.; Zhang, H. Y., Liu, Y. H.; Feng Li, X.; Huang, D. F., Hu, Y. L., *Chinese Chemical Letters*, **2010,** 21, 1075–1078

8. Chamulitra,t W.; Jordan S.J.; Mason R.P.; Saito K.; Cutler R.G.; *J. Biol. Chem.,* **1993**, 5, 268, 16, 11520-7

9. Saito K.; Yoshioka H.; Kazama S.; Cutler R. G., *Biol. Pharm. Bull*., **1988**, 21,4, 401-404

10. Vincent, S. R.; *Prog Neurobiol.,***2010**, 90, 2, 246-255

11. Hirsch, D. B.; Steiner, J. P.; Dawson, T. M.; Mammen, A.; Hayek, E.; Snyder, S. H.; *Curr Biol.,***1993**, 3, 11, 749-754

12. Kuriyama, K.; Ohkuma, S.; *Jpn J Pharmacol.,***1995**, 69, 1, 1-8

13. Saito K.; Gutler, R. G.; *Oxidative Stress and Aging Molecular and Cell Biology Updates*, **1995**, 379-386

14. Pepke-Zaba, J.; Higenbottam, T.W.; Dinh-Xuan, A.T.; Stone, D.; Wallwork, J.; *The Lancet*, **1991**, 338, 8776, 1173–1174

15. Palmer, R. M. J.; Ferrige, A. G.; Moncada, S.; *Nature,* **1987**,327, 524-526

16. Huang, P. L.; Huang, Z.; Mashimo, H.; Bloch, K. D.; Moskowitz, M. A.; Bevan, J. A.; Fishman, M. C.; *Nature*, **1995**, 377, 239-242

17. Roberts Jr, J. D.; Polaner, D. M.; Zapol, W. M.; Lang, P.; *The Lancet*, **1992**, 340, 8823, 818–819

18. Neuberger, A.; Van Deenen, L. L. M.; *Elsevier Science B.V.:New Comprehensive Biochemistry,*Volume 28, **1994**, Free Radical Damage and Its Control

19. Bivalacqua T. J.; Champion, H. C.; De Witt, B. J.; Saavedra, J. E.; Hrabie, J. A.; Keefer, L. K.; Kadowitz, P.J.; *J Cardiovasc Pharmacol*., **2001**, 38, 1, 120-129

20. Zhao, H.; Zhao, W. B.; Zhu, J. S.; Jia, Y. M.; Yu, C. Y.**;** *Molecules* **2013**, 18, 6021-6034

21. Goti, A.; Cardona; F.; Soldaini, G.; *Organic Syntheses*, **2005**, 81, 204-212, *Coll. Vol*., **2009**, 11, 114-120

22. Dondoni, A., Franco, S., Junquera, F., Merchan, F., Merino, P., Tejero, T., *Sinth. Commun.*, **1994**, 24, 2537

23. Andrade, M. M.; Barros, M. T.; Pinto, R. C.; *Tetrahedron*, **2008**, 64, 10521-10530

24. Mitsui, H.; Zenki, S.; Shiota, T.; Murahashi, S. I.; *J. Chem. Soc. Chem. Commun*., **1984**, 874-875

25. Murahashi, S. I.; Mitsui, H.; Shiota, T.; Tsuda, T.; Watanabe, S.; *J. Org. Chem.* **1990**, *55,* 1736-1744

26. Kumar, R. K. R.; H. Mallesha, K. S. R.; *Synthetic Communications*, **2003**, 9, 33, 1545

27. Al-Hashimi, A. F.; Faik, H. M.; Al-Khazragi, A. S.; *Saudi Med J.,* **2004**; 25, 9,: 1186-1192

28. Powers, S.; Nelson, W. B.; Larson-Meyer, E., *Journal of Sports Science*, **2011**, 29, S1, S47-S55

29. Trudeau-Lame, M. E.; Kalgutkar, A. S.; LaFontaine, M.; *Drug Metabolism and Disposition*, **2003**, 31, 2, 147-152

30. Ginsberg, M. D.; Becker, D. A.; Busto, R.; Belayev, A.; Zhang, Y.; Khoutorova, L.; . Ley, J. J.; Zhao, W.; Belayev, L.; *Ann Neurol*, **2003**, 54, 3, 330-342

31. Murahashi, S. I.; Shiota, T.; *Tetrahedron Letters*, **1987**, 28, 21 2383-2386

32. Andrade, M. M.; Barros, M. T.; Pinto, R. C.; *Tetrahedron*, **2008**, 64, 10521-10530

33. Burda, S.; Oleszek, W.; *J Agric Food Chem*, **2001**, 49, 2774-2779

4.4.8. Conclusion

- Aliphatic therepthal/glyoxal dinitrones were obtained by oxidation of secondary amines and showed the best results in vasodilatation experiments with rabbits
- Diphenyl nitrones were excellent free radical scavengers. The highest antiradical activity was estabilished to nitrones with a hydroxyl group located in the structure.
- The novel phenothiazinyl nitrones were obtained by condensation of *N*-monosubstituted hydroxylamines with phenothiazine carbaldehyde under both microwave and classical thermal activation. Microwave assisted synthesis significantly decreased the reaction time. All phenothiazinyl nitrones display light-blue fluorescence with large Stokes shifts ($\Delta\nu$ =7076-7888 cm-1) and the quantum yields (ΦF) between 1.90 and 4.7%. Notable activity in biological studies for phenothiazinyl nitrones was observed for nitrone **6a.**, and **6h.** in antimicrobial studies against *Candida parpsilosis*.

5. Nitriles

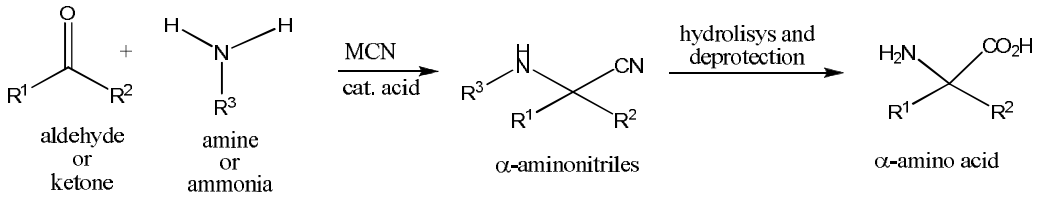
5.1 General introduction and background

Nitriles, organic cyanides, are a group of compounds based on a common structure formed by nitrogen and carbon atoms. Cyanides are produced by certain bacteria, fungi, and algae, and may be found in food and plants[1]

5.2. Strategies toward the synthesis of nitriles. A literature survey.

Aromatic nitriles one of the most important synthetic precursors of organic synthesis, due to the fact that they can be easily transformed into esters, amides, carboxylic acides, amines, amidines, ketones and nitrogen containing heterocycles, such as tetrazoles, oxazoles, and natural products[2].

Strecker reaction is one of the simplest and most economical methods for the synthesis of racemic α-aminonitriles. One pot synthesis of the α-aminonitriles by Strecker reaction consist in the reaction of an aldehyde, an amine (or ammonia) and a cyanide source, which after hydrolizing give the corresponding amino acid[3] (*Scheme 5.)*



Scheme 5. *Formation of α-amino acid by Strecker reaction*

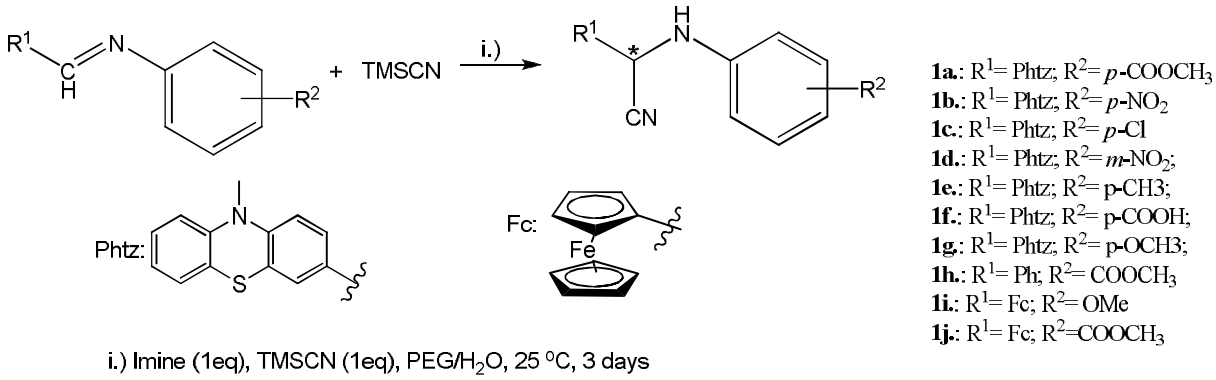
A tipical example of this is reaction of an aldehyde with ammonia in presence of alkali metal cyanides (KCN, NaCN)[4]. Recent modifications to the traditional Strecker reaction have seen the replacement of the cyanide source from toxic hydrogen cyanide to the mild TMSCN[5,] where TMSCN has a powerful impact in the catalytic asymmetric Strecker type reaction[6].

5.3. Original results

This fragment of the thesis presents the synthesis and structure analysis of some aromatic nitrile derivatives bearing a phenothiazine or ferrocene unit.

5.3.1. Synthesis of nitriles by Strecker reaction

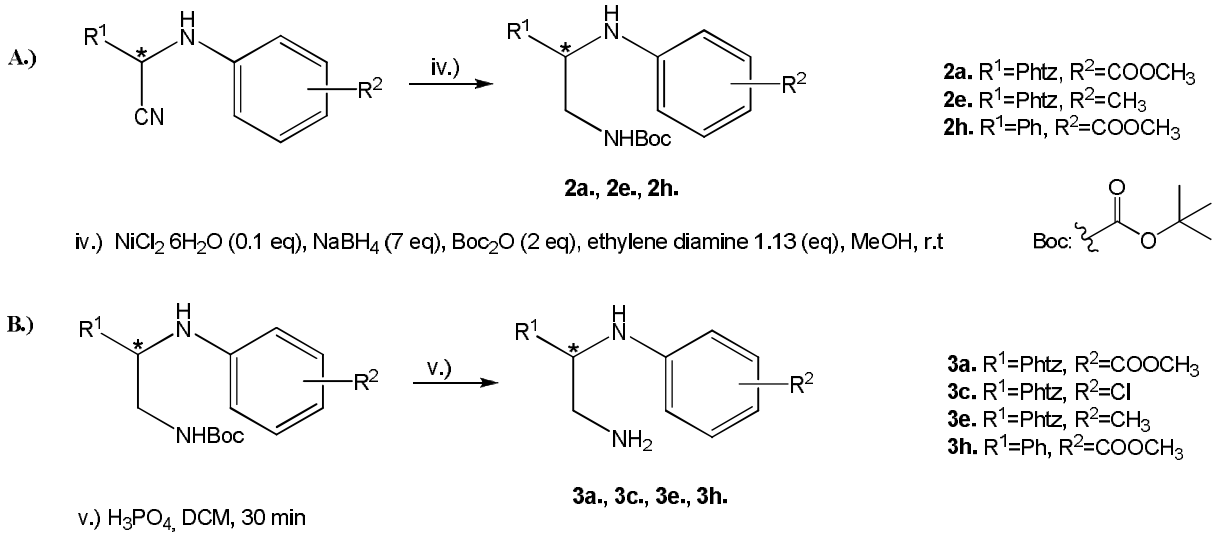
The principal aim for the synthesis of novel nitriles was a latter reduction of the nitrile function to amine, which has quite good complexing ability for certain metals. The novel nitriles were readily available by Strecker reaction under environment-friendly conditions. Eco-friendly cyanation of the imines (Schiff bases) was performed with TMSCN, at room temperature in water/PEG medium, affording nitriles in excellent yield *(Scheme 6.*)



Scheme 6. *Synthesis of nitriles by Strecker reaction*

5.3.2. Reduction of nitrile group to primary amine

Reduction of phenothiazinyl nitriles were achiewed by nichel catalyzed hydrogenation, using $NaBH_4$ as hydrogen source by the method reported by Caddick, S. et al.[7]. The reaction take place in presence of Boc-protecting groups, where the protected primary amines were firstly obtained (*Scheme 7.**A.***)). The final product was accomplished by a subsequent deprotection treatment (*Scheme 7.**B.**)*).



Scheme 7. A.) *Reduction-protection of nitriles;* ***B.)*** *Deprotection of amines*

The structures of nitriles and amines were determined on the basis of spectral data (^1H, ^{13}C NMR and MS). HRMS studies of nitriles gave two characteristic peak, the molecular peak and a fragment generated by elimination of the cyano group. (*Figure 10.*). In case of Boc protected amines the m/z spectrum shows dominant peaks higher then the expected molecular mass, which can be identified as the sodium adduct ions $(M+Na)^+$ or $(2M+Na)^+$. In *Figure 11.* is illustrated the HRMS ESI spectrum of the Boc-protected amine **2e.**, where the sodiuadduct ion have been found at m/z 484.2029.

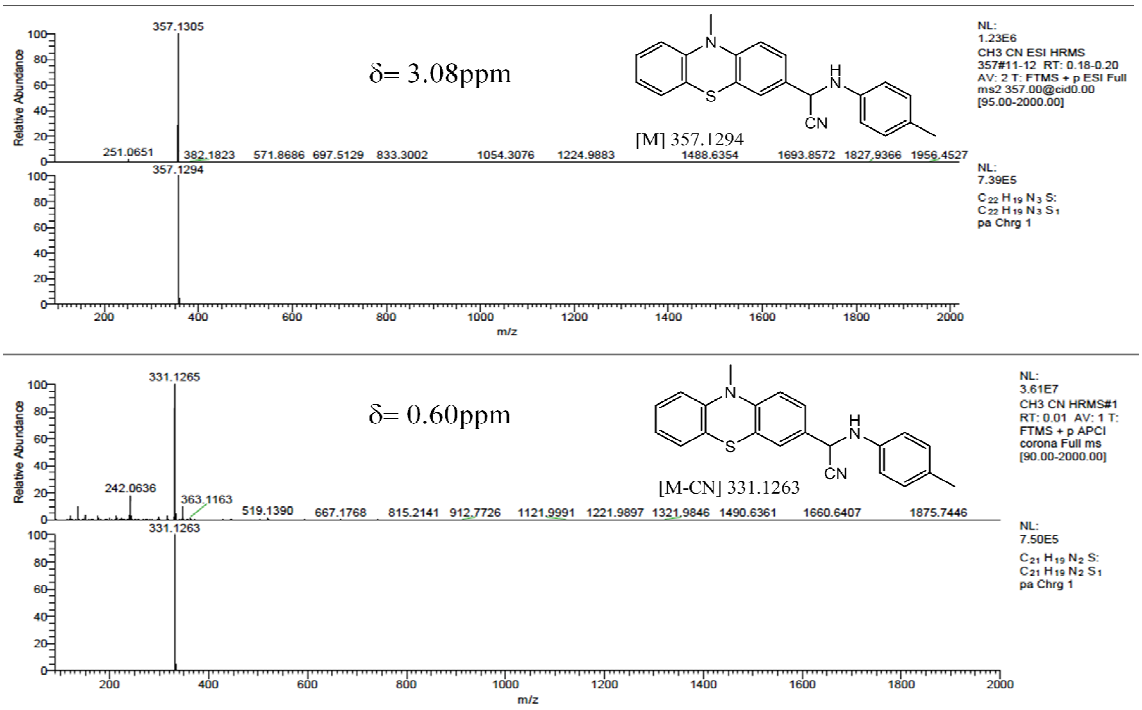


Figure 10**. HRMS APCI(+) spectrometry of compound **1e.

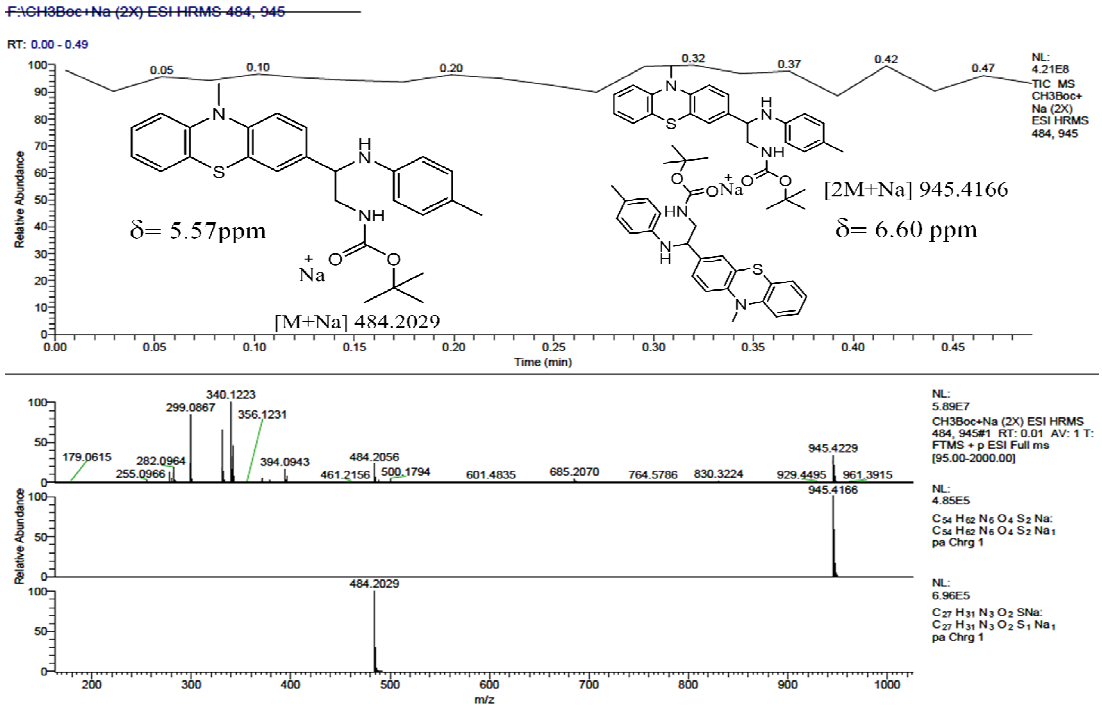


Figure 11**.HRMS APCI (+) spectrum of compound **2e.

Further investigation on the newly synthetized nitrile's structure was performed with ^{1}H and ^{13}C NMR studies. In ^{1}H NMR spectrum of nitriles, proton of NH appear as doublet (*Figure 12.*) at 4.45 ppm, instead of singlet as was expected, splited in two by the proton of the adjacent carbon atom, with a coupling constant of $J_{NH\text{-}CH}$=7.44Hz. Consequently, the chiralic

proton (CH) gave a further doublet at 5.36 ppm, with the adequate coupling constant of J_{NH-CH}=7.44Hz. Protons of the phenyl ring appear as doublets and protons of phenothiazine unit in general gave characteristic signals like doublet for H_1 and H_9, triplet for H_7 and H_8, singlet for H_4 and doublet-doublet for H_2 (*Figure 13.*)

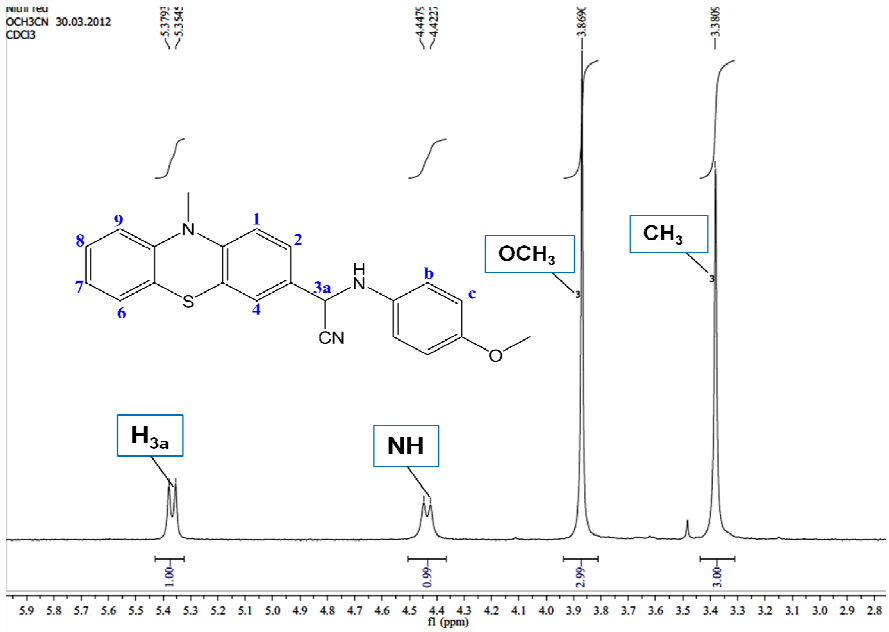


Figure 12. *Aliphatic range of ^{1}H NMR of compound* ***1g.****, recorded in $CDCl_3$, at 300 MHz*

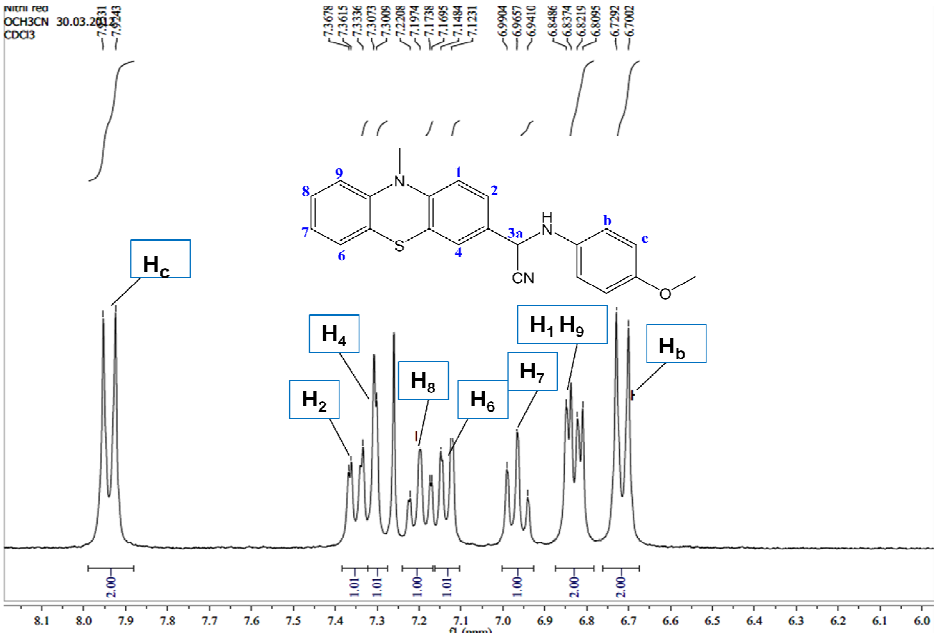


Figure 13. *Aromatic range of ^{1}H NMR of compound* ***1g.****, recorded in $CDCl_3$, at 300 MHz*

Structures of protected amines, was also confirmed with ^{1}H NMR studies. Protons of CH_2-β appear as multipet in range of 3.38-3.55 ppm, which is attributed to the adjacent chiralic center located in the molecule. Proton of the chiralic center in this case display at 4.48 ppm but as a quartet due to the presence of the newly appeared protons. The two amino groups

located in the Boc-protected amine were also observable, where the NH-γ of carbonyl group as more shielded giving a singlet at 5.79 ppm, while NH appear similarly as in case of nitriles at 4.89 ppm (*Figure 14.*).

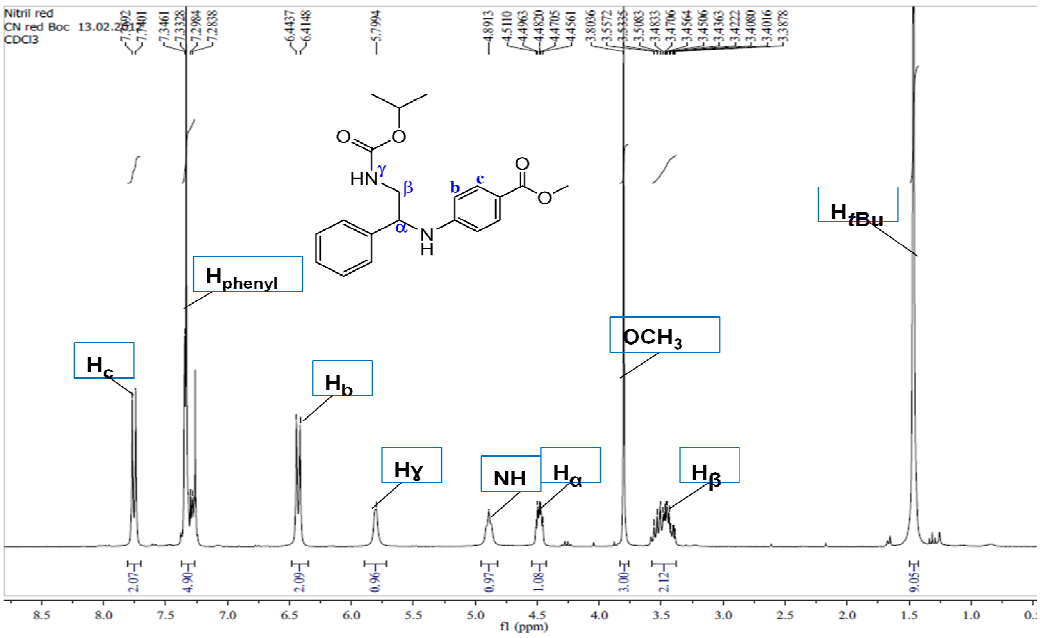


Figure 14. *^{1}H NMR spectra of compound **2h.** performed in $CDCl_3$, at 300 MHz*

The most important feature in ^{1}H NMR of deprotected amine was observed where the two amino groups switch their places in the spectra. Contrary to the protected amine, proton of NH group appear as doublet and more the more deshielded in the aliphatic range, at 5.90 ppm, while protons of primary amine function gave a large singlet at 5.00 ppm (*Figure 15.*)

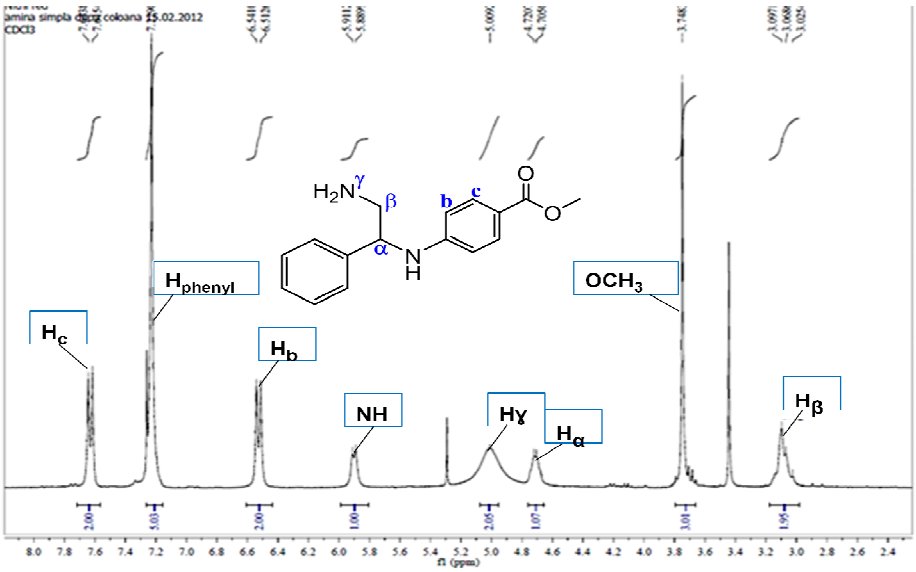


Figure 15. *^{1}H NMR of compound **3h.**, performed in $CDCl_3$, at 300 MHz*

5.3.3. Complexation of amine

Complexation of amines were performed with several metal salts (*Scheme 8.*) such Ga, Pd, Pt. Amines showed good complexation properties toward Pd and Pt, where primary and secondary amine function represents a potential coordination position for metal cations. No complexation was observed with Ga salts.

Scheme 8. *Complexation reaction of amines*

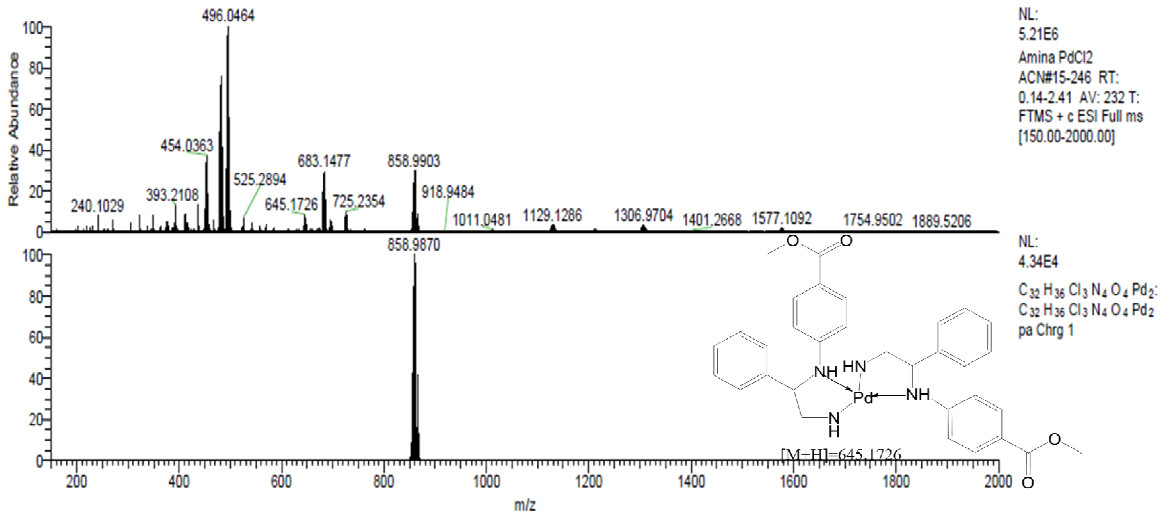


Figure 16. *HRMS APCI (+) of Pd complex of amine* ***3h.***

5.3.3. Conclusions

A series of novel phenothiazinyl and ferrocenyl nitriles were prepared by Strecker reaction, under eco-friendly conditions using TMSCN as cyanide source. Nitriles were obtained in excellent yields, above 90 %.

Nitriles **1a.**, **1c.**, **1e.**, **1h.** were successfully converted to primary amines by Ni-catalyzed reduction in presence of Boc_2O protecting groups, affording in this manner the Boc-amine, which after a subsequent deprotection treatment gave the target primary amines.

References

1. Nawaz, M. S.; Davis, J. W.; Wolfram, J. H.; Chapatwala, K. D.; *Applied Biochemistry and Biotechnology*, **1991**, 29 28, 865-866

2. Ishii, G.; Moriyama, K.; Togo, H.; *Tetrahedron Letters*, **2011**, 52, 18, 2404–2406

3. Jenner, G.; Salem, R. B.; Kimc, J. C.; Matsumoto, K.; *Tetrahedron Letters*, **2003**, 44, 447–449

4. Merino, P.; Marqués-López, E.; Tejero, T.; Herrera, R. P.; *Tetrahedron*, **2009**, 65, 7, 1219-1234

5. De, *S. K. Synth. Commun*. **2005**, 35, 653

6. Takamura, M.; Hamashima, Y.; Usuda, H.; Kanai, M.; Shibasaki, M.; Angew Chem., 2000, 112, 9, 1716-1718

7. Caddick, S.; Judd, D. B.; K.de K Lewis, A.; Reich, M. T.; R.V Williams, M.; *Tetrahedron,* **2003**, 59, 29, 5417–5423

6. Chalcones

6.1. Introduction and background

The α, β unsaturated carbonyl moiety is present in a large number of natural and synthetic products exhibiting a variety of biological properties[1]. Compounds containing such as unsaturated system are considered as potential drug candidates due to their ability to act as Michael acceptors for the addition of protein functional groups[2]. Especially the sulfhydryl group of cysteines in proteins plays a major role in Michael-addition-based activation processes through α,β-unsaturated carbonyl compounds[3].

Chalcones (*Figure 17.*) belonged to the class of α, β unsaturated carbonyl compounds and their beneficial biological activity is directly connected to the Michael acceptor activity.

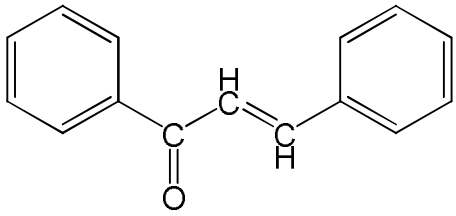


Figure 17. *Molecular structure of chalcone*

Coumarin derivatives constitute an important class of compounds with a wide range of biological properties.Coumarins and its derivatives exhibit antitumor, antioxidant and anti-inflammatory activities usually asociated with low toxicity [4].

6.2. Biological activities of chalcones

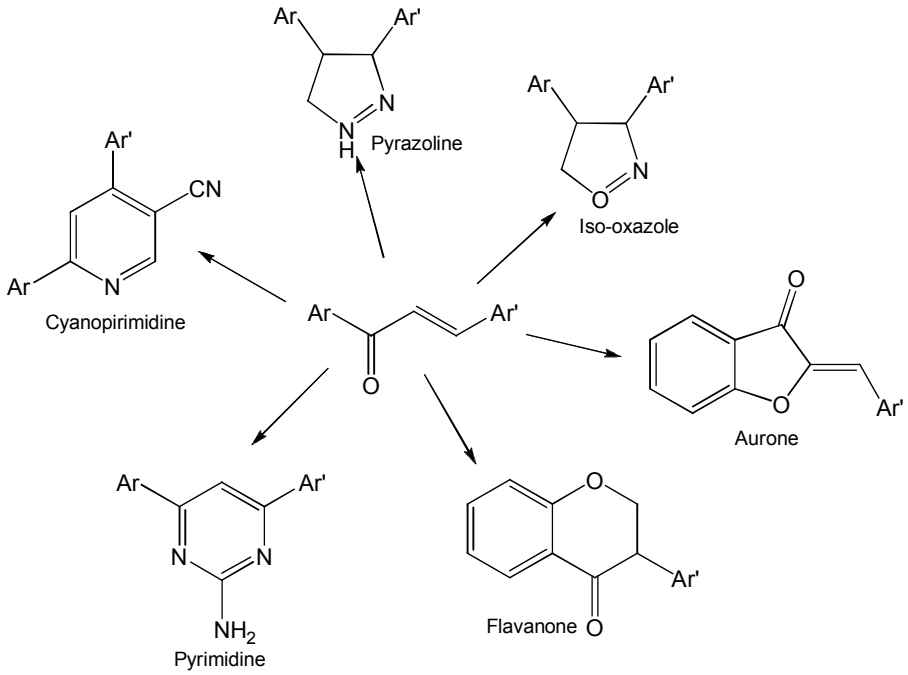
Chalcones containing heterocycles have been reported to have a wide range of biological properties[5]. Coumarins with chalcone as backbone have been reported to exhibit a wide variety of pharmacological effects, including anti-inflammatory, antiviral and antibacterial activities[6]

Chalcones possessing predominantly hydroxyl and phenyl substituents exhibit important antioxidant properties. Naturally occurring chalcones are mostly in the hydroxylated forms[7]. One of these hydroxyl chalcones, isoliquiritigenin have been shown to be potent inhibitors of skin carcinogenesis *in vivo*. Several studies have demonstrated that

chalcones act as chemopreventive agents, capable of inhibiting carcinogenesis induced by chemical agents through enhancement of reduced glutathione levels[8].

6.3. Strategies toward the synthesis of chalcones. A literature survey.

Chalcones serve as starting materials for the synthesis of various heterocyclic compounds such as aurones[9,] flavanones[10], pyrimidines[11], pyrazolines[12], flavones[13], cyanopirimidine[14], which are of some therapeutic importance (*Scheme 8.*).



Scheme 8. *Synthesis of various heterocycles from chalcone*

In addition, chalcones are very important compounds as a Michael acceptor in organic syntheses. The Michael addition reaction is one of the most fundamental C-C bond-forming reactions in the synthesis of 1, 5-dicarbonyl compounds.

The most convenient method is the acid or base catalyzed Claisen-Schimdt condensation of equimolar quantities of aryl-methyl-ketone with aryl aldehyde.

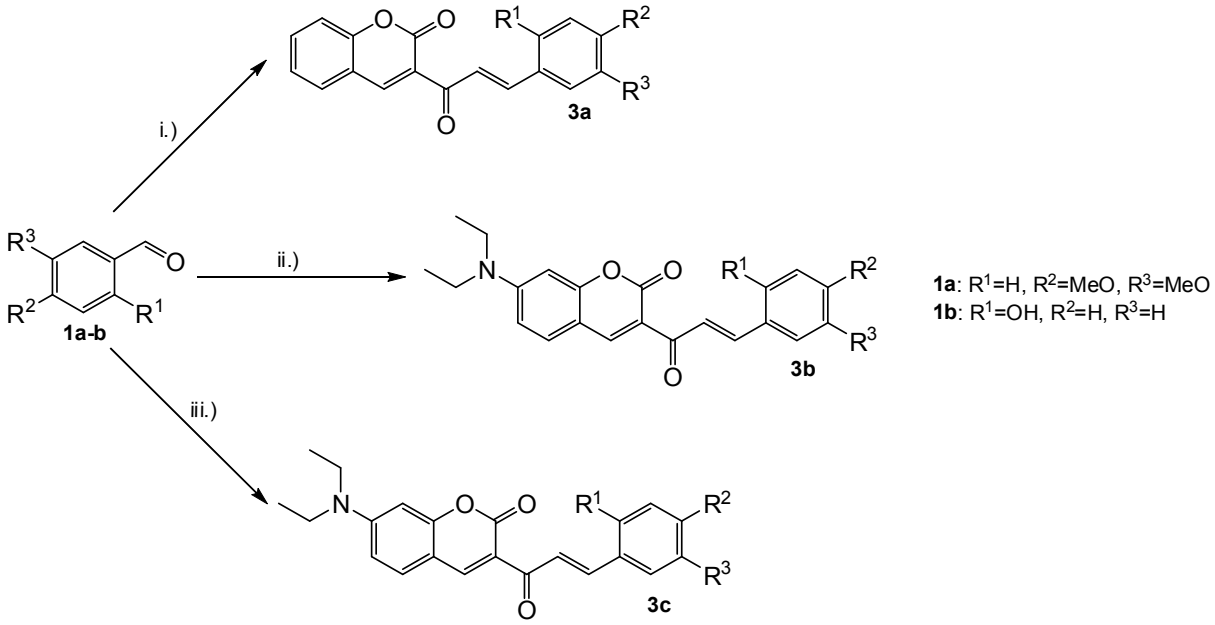
6.4. Personal contributions

The main objective of this part of the thesis was a study on the reactivity of α,β unsaturated carbonyl compounds through a kinetic thiol assay. The kinetic assay used to define the second order rate constant (k_2) in reaction of coumarinyl chalcones and other α,β unsaturated carbonyl compounds with cysteamine was based on their Michael acceptor activity.

In addition a synthesis on chalcones bearing a coumarin moiety is also presented.The synthesis of coumarinyl chalcones were performed with Claisen-Schmidt condensation and characterized by NMR an MS spectroscopies.

6.4.1. Synthesis and characterization of coumarinyl chalcones

Coumarinyl chalcones (**3a.-3c.**) were prepared by a reported procedure by Ha J-H. *et al*[15] through *Claisen Schmidt* condensation of a coumarine derivative (**2a.** or **2b.**) and an aldehyde (**1a.**or **1b.**) (*Scheme 9.*). The obtained chalcones fluoresce highly in solution, especially chalcones with diethyl-amino group at the coumarin mojety.



i.) **1a.** (3.5 eq), **2a.** (1eq), piperidine, EtOH, reflux, 4h; ii.) **1a.** (3.5 eq), **2b.** (1eq), piperidine, EtOH, reflux, 30h; iii.) **1b.** (3.5 eq), **2b.** (1eq), piperidine, EtOH, reflux, 24h

Scheme 9. *Synthesis of coumarinyl chalcones chalcones **3a**–**3c**; Reagents and conditions.*

A notable intramolecular rearragement was detected for chalcone **3a.**, which occurred spontaneously in solvents. On the LC-MS spectrum recorded for the mixtures of chalcone **3a.** and the resulted compound from the intramolecular rearregement, two peaks was observable with identical molecular weight. (*Figure 17.*).

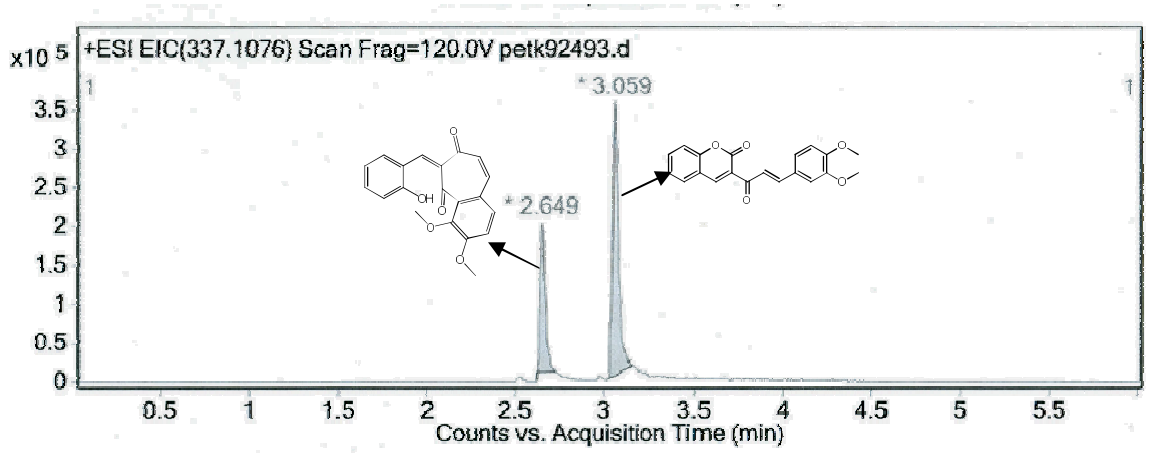


Figure 17. *HRMS spectrum for mixture of compouns **3a.** and **3a..-cy***

Based on NMR and LC-MS studies the proposed structure resulted from this rearragement gave a seven membered ring including the coumarinyl moiety as well as the α,β unsaturated system (*Figure 18.),* which is not reactive toward cysteamine in the UV-Vis kinetic thiol assay. A mechanism for the intramolecular rearragement is presented on *Scheme 4.*

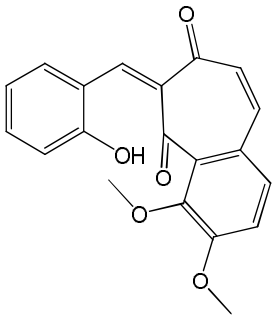
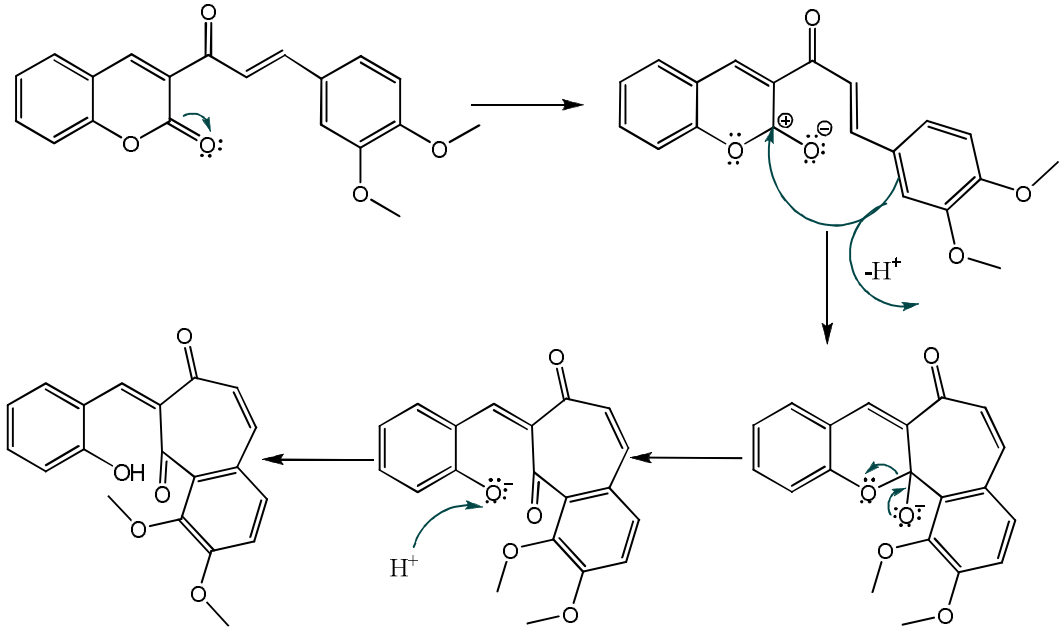


Figure 18. *Molecular structure of the seven membered cyclic compound*



Scheme 10. *Intramolecular rearrangement of **3a.** to **3a.-cy***

By in other hand this cyclization was observable on the time scale of ^{1}H-NMR experiments. *Figure 19.* presents the conversion of chalcone **3a.** in its seven membering isomer by dinamic ^{1}H-NMR study. *Spectrum 1.* and *Spectrum 5.* are the pure ^{1}H-NMR spectra of chalcone **3a.** and its cyclic isomer (**3a.-*cy***), respectively. *Spectrum 2.* recorded 3 days later, some extra signals was immediately observable in both aromatic and aliphatic range. Two singlets in aliphatic range for the methoxy groups of the newly formed compound, gave signals at different chemical shifts at 3.55 ppm and 3.83 ppm. Furthermore, additional signals can be observed at 4.96 ppm and 5.04 ppm on *Spectrum 3.* recorded 4 days later than *Spectrum 2*. On *Spectrum 5*. recorded one week later than the previous one, can be observed the dissapeareance of the singlet at 8.57 ppm, which belongs to H_4 from chalcone **3a**

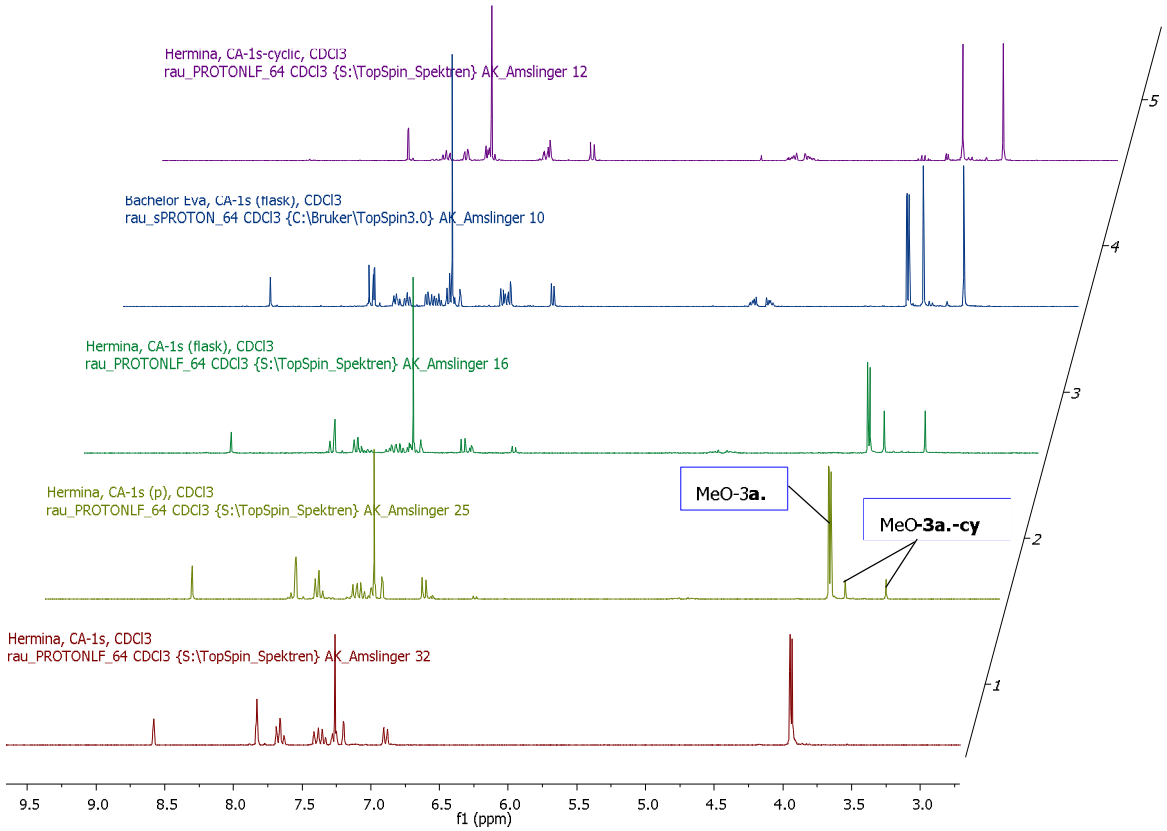
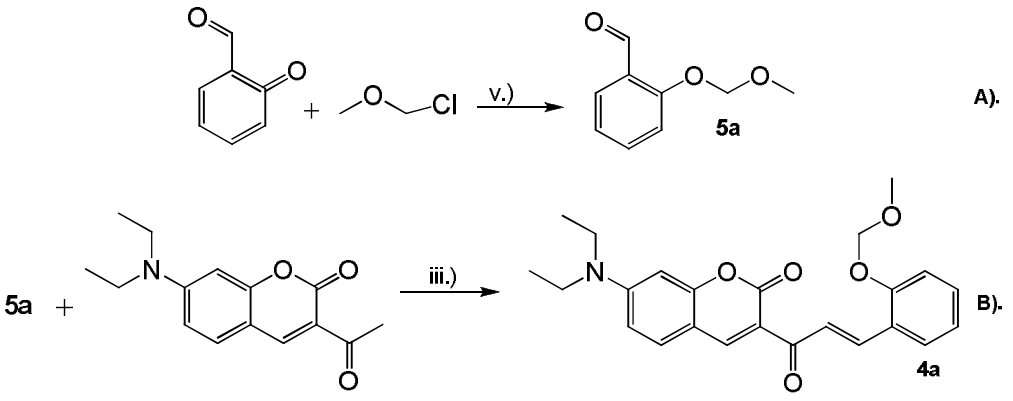


Figure 19. *^{1}H-NMR study on cylization of chalcone **3a.**, performed in $CDCl_3$ on 400 MHz*

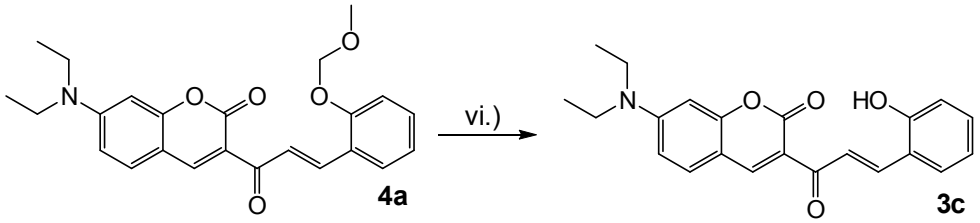
A pronounced instability was observed with chalcone **2c**. due to the hydroxyl group on the phenyl ring, which may decrease the stability of coumarins by its electron withdrawing nature. The hydroxyl group of salicylalehyde was protected with chloromethyl methyl ether according the method reported by Cumming, R. G. *et al*[16] (*Scheme 11. B.).*



v.) MOMCl (1.2 eq), DIPEA (1.6 eq), DCM, 16 h, 0 °C-r.t.

Scheme 11. *Synthesis of protected coumarinyl chalcone **4a. A).** Pprotection of salicylaldehyde ;**B.)** Synthesis of protected chalcone **4a.***

After chalcone **4c.** purification by column chromatography, the deprotection with HCl afforded chalcone **3c.** in pure form (*Scheme 12.*).



vi.) HCl, MeOH, reflux, 4 h

Scheme 12. *Deprotection of coumarinyl chalcone* ***3c.***

The structures of the synthesized coumarinyl chalcones were established by NMR spectroscopy and high resolution mass spectrometry. The characteristic signals for α and β protons in ^{1}H-NMR spectra appear at 7.80 and 8.20 ppm, with large coupling constant values of ~15 Hz (*J*=15.64 Hz for chalcone **3b.**) (*Figure 20.*) specific for this two **H-α** and **H-β** hydrogens.

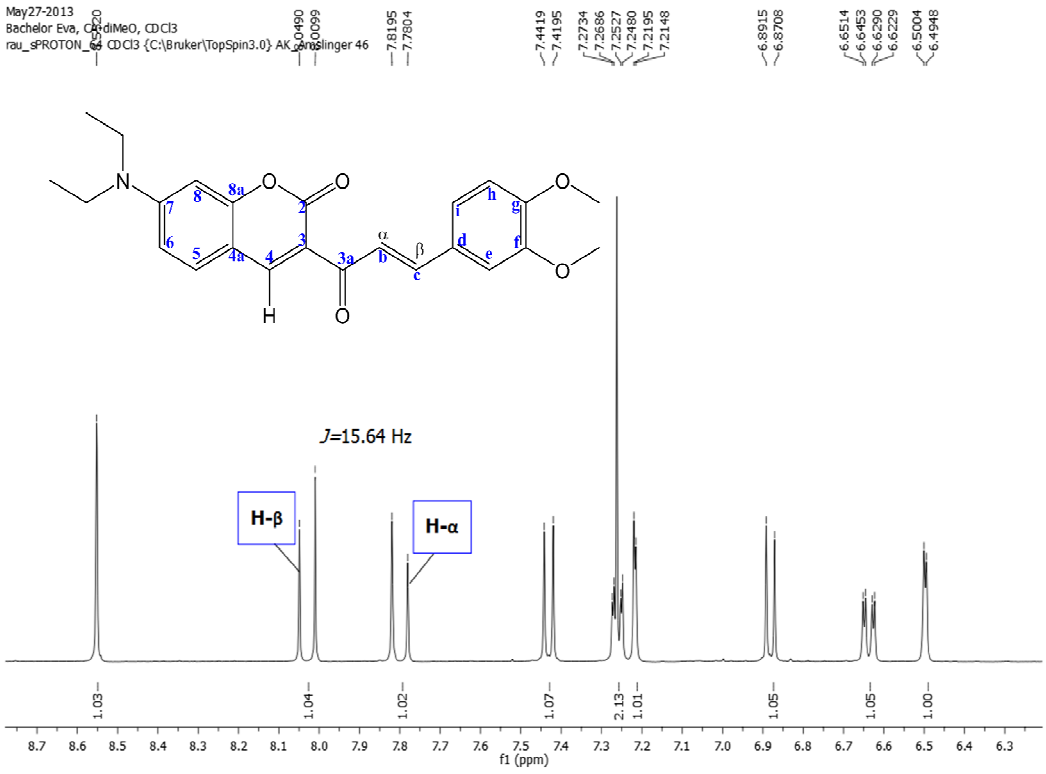


Figure 20. *Detailed aromatic region from* ^{1}*H-NMR spectrum of chalcone* ***3b.*** *recorded in* $CDCl_3$ *at 400 MHz*

6.4.2. Kinetic thiol assay of α,β unsaturated compounds

The assay is a first step to estabilish the ability of this class of compound as potential biologically active agents, since they might interact with sulfhydryl group of proteins and peptides. The most caracteristic feature of sulfhydryl groups is their nucleophilic addition to a double bond. If the double bonds are activated by conjugation, thiols add spontaneously to

them. The most important conjugated system is the α,β unsaturated carbonyl grouping[17] (*Figure 21.*).

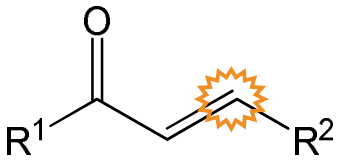
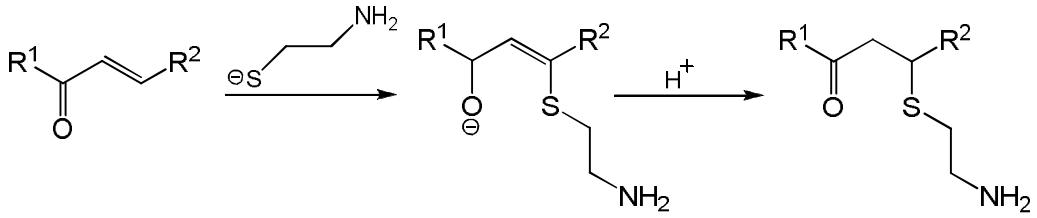


Figure 21. *α,β unsaturated compound*

The most important value in interaction of α,β unsaturated carbonyls with sulfhydryl groups is the anticancer effects by their preferential reactivity toward cellular thiols. α,β unsaturated carbonyls are important thiol-alkylators which play important role in inhibition of tumor growth[18] This kinetic thiol assay gives information about reactivity of α,β unsaturated carbonyls toward cysteamine. The nucleophilic Michael addition of cysteamine is presented on *Scheme 13*.



Scheme 13*. General reaction scheme of α,β unsaturated carbonyls with cysteamine*

In first step the k_{obs} (pseudo first order rate constants) values were obtained from the time dependent decay of the absorbance (A_t) of the α,β unsaturated carbonyls with thiol by fitting the data of individual experiments to the first order exponential equation (*Equation 1.*).]

$$A_t = A_0 e^{-k_{obs} * t} + C \quad with\ A_0 = A_t\,[\propto \beta]_0 \qquad (Equation\ 1.)$$

k_2 values were obtained by potting the individual k_{obs} values against the corresponding different thiol concentration (*Figure 8.),* where thiol concentration:

$$C_{thiol} = 0.00004 * fold\ thiol$$

where, 10^{-4} M is the final concentration of the solution of α,β unsaturated carbonyl after mixing with thiol expressed in Molarity.

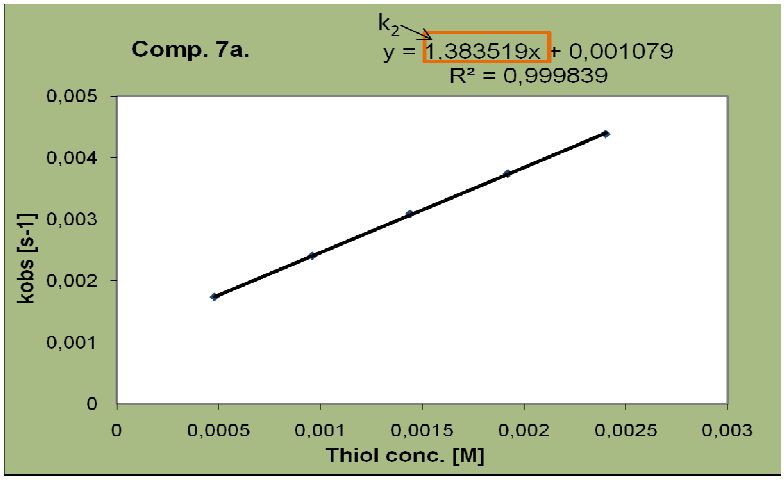


Figure 22. *Plot of experimental k_{obs} against thiol concentration*

Results:

The structure of the α,β unsaturated carbonyls used in the study are presented on *Scheme 14.* In case of cyclic chalcones no product was formed. The second order rate constants (k_2) of open chain chalcones varied significantly accordingly to the substituents presented. The second order rate constant values (k_2), suitable wavelenghts, the reaction time and the thiol concentration of α,β unsaturated carbonyls are summarized in *Table 7.*

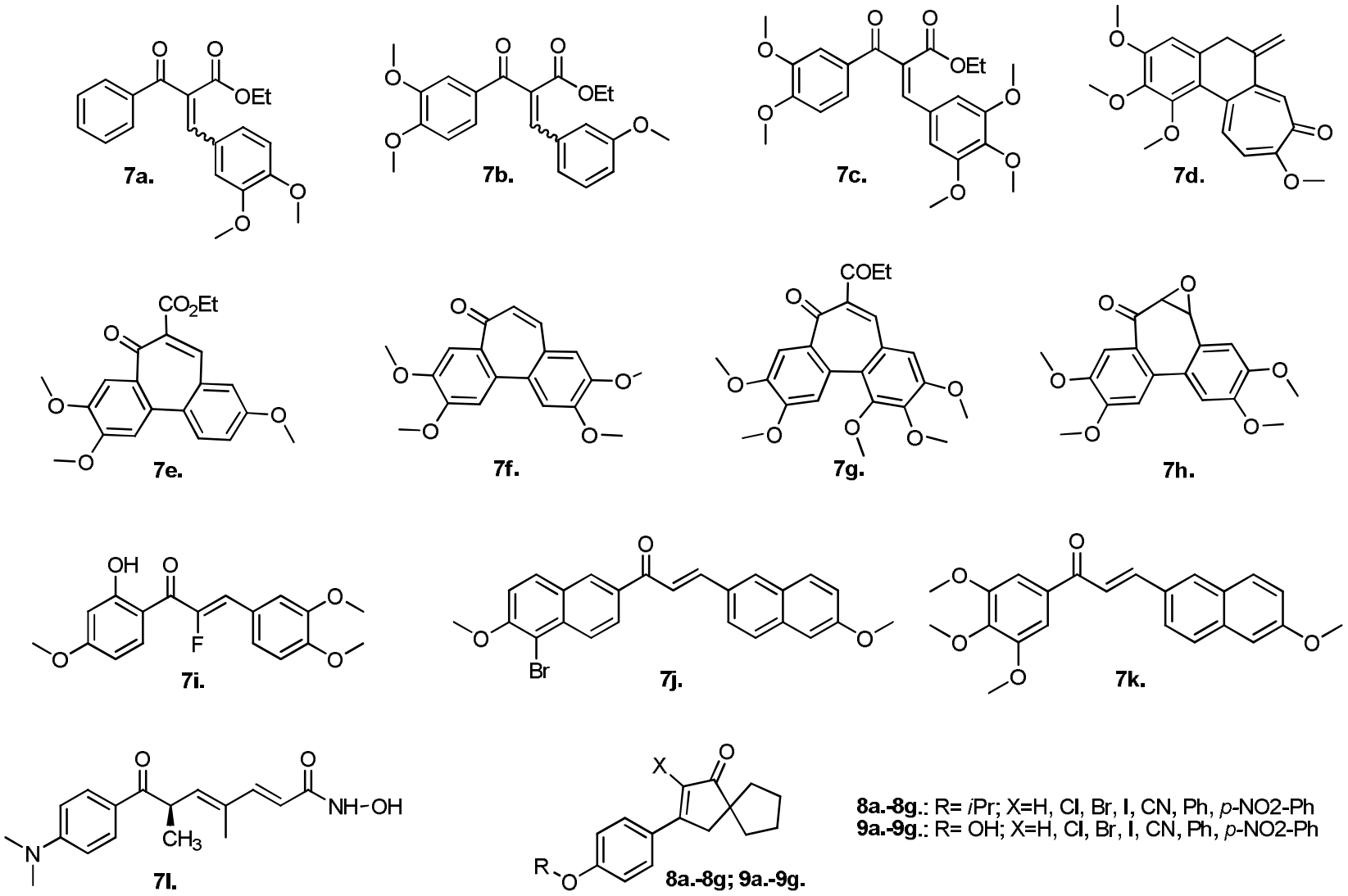
Table 7. Kinetic values of α,β unsaturated carbonyls

α,β unsaturated carbonyl	**Reaction time** (s)	**Fold thiol**	**Wavelenght** (nm)	k_2 [M^{-1} s^{-1}]
3a.			[a]	
3b.	11	12-60	490	0.446±0.0410
3c.	11	24-72	490	1.276±0.0249
7a.	11	12-60	330	1.301±0.0676
7b.	11	24-72	320	1.236±0.0988
7c.	16	12-60	320	1.004±0.0326
7d.	14h	300-500	410	0.00550±0.001
7e.				[b]
7f.				[b]
7g.				[b]
7h.				[b]
7i.	20	300-500	380	0.035±0.0028
7j.				[b]
7k.	11	60-108	365	0.900±0.0651
7l.				[b]
8a.-8g.				[b]
9a.-9g.				[b]

[a] No suitable wavelength was found. The UV of the starting material and the product are identical.
[b] Not active toward cysteamine. No product formation was observed by mass spectrometry

The reaction rates of chalcone **3b.** and **3c.** was significantly different. While, chalcone **3b.** gave a k_2 value of 0.446 M^{-1} s^{-1}, chalcone **3c.** was accelerated with a k_2 value of 1.276 M^{-1} s^{-1}. The main reason of this reaction rate acceleration with chalcone **3c.**is the presence of the

OH group in the phenil ring, which play an important role in this process. The OH group acts in two ways: it activates the carbonyl group through intramolecular H-bond and stabilizes the conjugation in the system[19]. In terms of chalcones **7a.**, **7b.**, **7c.**, the kinetic values were dependent on the presence of methoxy groups. Methoxy substituents with electron donating nature may stabilize the α,β unsaturated carbonyl system. Hence, compound **7a.** was the most reactive toward cysteamine with a k_2 value of 1.300 M^{-1} s^{-1}.



Scheme 14. *Structures of α,β unsaturated carbonyl compounds*

6.4.3. Conclusions

Synthesis of coumarinyl chalcones [**3a.**, **3b.**, **3c.**] was performed by Claisen Schmidt condensation. The prepared chalcones highly flouresce in solution, especially those with diethyl amino group [**3b.**, **3c.**].

Bromination of coumarinyl chalcones take place on the coumarin mojety instead of α position as expected.

The highest electrophile nature in case of coumarinyl chalcones, tested via nucleophilic Michael addition, was estabilished for chalcone **3c.**, with a second order kinetic rate constant of 1.301 M^{-1} s^{-1}, due to the presence of the hydroxyl group in *ortho* position, which activates the carbonyl group through intramolecular hydrogen bonding.

It was observed that cyclic α,β unsaturated carbonyls are not reactive toward cysteamine.The presence of electron donating groups, as methoxy groups located in diphenyl chalcones (**7a.**, **7b.**, **7c.**) may stabilize the unsaturated system, consequently decrease the reactivity toward sulfhydryl group.

References

1. Tran, T.-D.; Nguyen, T.-N.; Do, T.-H.; Thai, K.-M.; Tran, C.-D.; *15th International Electronic Conference on Synthetic Organic Chemistry,* **2011**

2. Spirtovic-Halilovic, S.; Zavrsnik, D.; Imamovic, B.; Becic, E.; *International Journal of Pharmacy Teaching & Practices*, **2013**, 4, 1, 498-491

3. Hsu, Y. L.; Kuo, P. L.; Tzeng, W. S.; Lin, C. C.; *Food and Chemical Toxicology*, **2006**, 44, 704–713

4. Achanta, G.; Modzelewska, A.; Feng, L.; Khan, S. R.; Huang, P.; Molecular Pharmacology**, 2006,** *70. 1, 426-433*

5. Garg, S. K.; Kumar, R.; Chakraborti, A. K.; *Tetrahedron Letters,* **2005**, 46, 1721–1724

6. Amslinger, S.; Al-Rifai, N.; Winter, K.; Wormann, K.; Scholz, R.; Baumeister, P.; Wild, M.; *Organic & Biomolecular Chemistry*, **2013**, 11, 4, 549-54

7. Sashidhara, K. V.; Kumar, A.; Kumar, M.; Sarkar, J.; Sinha, S.**;** *Bioorganic & Medicinal Chemistry Letters*, 7, 24, 7205-7211

8. Tran, T.-D.; Nguyen, T.-N.; Do, T.-H.; Thai, K.-M.; Tran, C.-D.; *15th International Electronic Conference on Synthetic Organic Chemistry,* **2011**

9. Spirtovic-Halilovic, S.; Zavrsnik, D.; Imamovic, B.; Becic, E.; *International Journal of Pharmacy Teaching & Practices*, **2013**, 4, 1, 498-491

10. Hsu, Y. L.; Kuo, P. L.; Tzeng, W. S.; Lin, C. C.; *Food and Chemical Toxicology*, **2006**, 44, 704–713

11. Achanta, G.; Modzelewska, A.; Feng, L.; Khan, S. R.; Huang, P.; Molecular Pharmacology**,** 2006**,** *70.* 1, *426-433*

12. Roussaki, M.; Costa Lima, S.; Kypreou, A.-M.; Kefalas, P.; Cordeiro da Silva, A.; Detsi, A.; *International Journal of Medicinal Chemistry*, **2012**

13. Cabrera, M.; Simoens, M.; Falchi, G.; Lavaggi, M. L., Piro, O. E.; Castellano, E. E.; Vidal, A.; Azqueta, A.; Monge, A.; Lopez de Cerain, A.; Sagrera, G.; Seoane, G.; Cerecettoa, H.; Gonzaleza, M.; *Bioorganic & Medicinal Chemistry,***2007**, 15, 3356–3367

14. Joshi, V. D.; Kshirsagar, M. D.; Singhal, S.; *Der Pharmacia Sinica*, **2012**, 3 3 , 343-348

15. Ha, J-H.; Yoon, D-H.; Park, S.; Kim, H-J.; *Tetrahedron*, **2011**, 67, 7759-7762

Chemistry, **2009**, 17, 3995-3998

16. Joshi, V. D.; Kshirsagar, M. D.; Singhal, S.; *International Journal of ChemTech Research*, **2012**, 4, 3, 971-975

18. Solankee, N.; Patel, K. P.; Patel, R. B.; *Journal of Chemical and Pharmaceutical Research*, **2012**, 4, 8, 3778-3782

19. Chausset-Boissarie, L.; Arvai, R.; Cumming, G. R.; Besnard, C.; Kundig, E. P.,; *Chem. Commun.,* 2010, **46**, 6264–6266î

7. General conclusions

The present doctoral thesis deals with the synthesis, structure characteristics of 73 compounds, from various classes, including:

- Schiff bases
- Secondary amines
- Aliphatic and aromatic mono-, and dinitrones, and phenothiazinyl nitrones
- Nitriles with a phenothiazine or ferroncene unit
- Primary amines
- Chalcones with coumarin moiety

From the 73 compounds, 38 were obtained and characterized for the first time in this work.

*Di*Schiff bases [**1a.-1f.**] were obtained in high yields under solvent-free conditions, by condensation of the corresponding aldeyde with an aliphatic amine.

Secondary amines [**2a.-2f.**], as precursors of nitrones were easily available by reducing the *di*Schiff bases with $NaBH_4$.

Terepthal [**3a.-3c.**] and glyoxal [**3d.-3f.**] *di*nitrones were obtained from secondary amines, by oxidation with H_2O_2 in presence of a catalytic amount of $NaWO_4$. Nitrones **3b.** and **3e.** were synthesized and characterized for the first time. In vasodilatation study terephthal and glyoxal dinitrones showed a considerable capacity to release nitric oxide in high quantities, where nitrone **3e.** was the most efficient as *in vivo* nitric oxide source.

4 novel [**4c.**, **4i.**, **4l.**, **4r.**] and 14 known [**4a.**, **4b.**, **4d.-4h.**, **4j.-4p.**, **4s.**, **4t.**] diphenyl nitrones were obtained by condensation between an aldehyde and hydroxylamine prepared *in situ*. All diphenyl nitrones were able to release NO in reaction with hydroxyl radical generetad by UV. In antimicrobial test, only nitrones **4a.** and **4r.** acted as fungicides in case of *Candida*

albicans and *Candida krusei.* Diphenyl nitrones were excellent antioxidant agents against DPPH$^{\bullet}$ free radical. The antiradical activity falls within the range of 38-93%, where the electron donating or withdrawing character of the substituents played the major role.

11 [**6a.-6j.**, **7a.**] novel phenothiazinyl nitrones were obtained and characterized for the first time. Microwave assisted synthesis proved to be more efficient than classical way, by decreasing the reaction time and increasing the yields of reaction. The antimicrobial study showed that nitrones **6a.** and **6h.** are sensitive for *Candida parpsilosis* with the inhibition diameter of 6mm**.** and 7mm, respectively**.**

In vasodilatation study, administration of nitrones to rabbits have been shown intense signs of vasodilatation such, tachycardia, dilated blood vessels in the ear, easy blood sampling procedure, which confirm that nitrones can act as *in vivo* NO donors. Overall it can be said, that nitrones could become a new class of vasodilators.

10 novel nitriles [**1a.-1j.**]: 7 with a phenothiazine, 2 with a ferrocene unit and 1 diphenyl nitrile were prepared by Strecker reaction under eco-friendly conditions. 4 amines [**3a**, **3c.**, **3e.**, **3h.**] were succesfully obtained by Ni-catalyzed reduction of the corresponding nitriles in presence of Boc_2O protected groups.

3 coumarinyl chalcones [**3a.**, **3b.**, **3c.**] were obtained by Claisen-Schmidt condensation under basic conditions. The second order rate constant (k_2) of the reaction with cysteamine was determined by an UV-Vis kinetic thiol assay, where the electrophile nature was strongly influenced by the substituents located in the molecule. Chalcone **3c.,** with an *ortho* hydroxyl group showed the fastest response toward cysteamine, since hydroxyl groups accelerate the reaction rate by an intramolcular hydrogen bond.

The electrofile nature of 12 α,β unsaturated carbonyls [**7a.-7l.**] were studied. It was clearly illustrated with compound **7a.**, **7b.** and **7c.**, that methoxy groups may stabilize the double bond of the α,β unsaturated system by electrondonating effects, and therefore decrease the electrophile nature and the reaction rate. No reactivity was observed toward cysteamine in case of cyclic α,β unsaturated carbonyl compounds [**7e.-7h.**] and spiro α,β unsaturated carbonyls [**8a.-8g.**; **9a.-9g.**].