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"BABEŞ-BOLYAI" UNIVERSITY, CLUJ-NAPOCA
Faculty of Chemistry and Chemical Engineering
Doctoral School of Chemistry

PhD Thesis Abstract

Synthesis and biological activity assessments in series of new phenothiazine functionalized heteroaromatic compounds

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KEYWORDS: phenothiazine, alkylation, formylation, dipyrromethanes-, bis(indolyl)methanes-, benzothiazoles-, porphyrins- and metalloporphyrins with phenothiazine units, structural analysis by NMR, FT-IR, UV-Vis, fluorescence study, cytotoxicity against leukemia, phototoxic effects.

1. Phenothiazine derivatives

1.1. Introduction

The thesis is a study on the synthesis and structure characteristics of some heterocyclic compounds with biological activities. For this purpose, three essential heterocycles phenothiazine, benzothiazole and porphyrin were chosen and different synthetic methods were applied for the synthesis of the target molecules.

Phenothiazine has a three-ringed structure in which two benzene rings are connected by sulphur and a nitrogen atom (*Figure 1*). It is widely used as common building block for the synthesis of pharmaceuticals. Recent synthesis are based on the Cu-catalyzed rearrangement of 2-aminobenzenthiole in the presence of aryl *ortho*-dihalides[3].

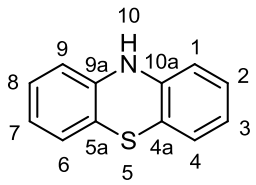


Figure 1. (10H-phenothiazine) atoms numbering system.

The research work presents the original results in the synthesis of mono- and poly functionalized phenothiazines and their use as intermediates in the synthesis of: dipyrromethanes, bis(indolyl)methanes, benzothiazoles, porphyrins.

The first part of the thesis includes the formylation of the phenothiazine. Synthesis and characteristics of novel phenothiazine based heteroarylmethanes forms the second part. Synthesis and biological evaluation of the new phenothiazine based benzothiazoles are presented in the third chapter. The last chapter proposed the structural elucidation of the new *meso*-phenothiazinyl-porphyrins (MPP) and metallo-MPPs by spectroscopic methods, the interpretation of reactivity and selectivity problems observed by molecular modelling; the results constitute baseline data for future research in the field of application.

1.2. Formylation of aromatic compounds

The development of new procedures of preparing these compounds, by the process of forming C-C bonds, from the very beginning there was and still is a challenge to organic chemists. Contrast to acylation of aromatic compounds that can be easily carried out by using acyl chlorides and Lewis acids using Friedel-Crafts techniques; there is no similar procedure

for the formylation of aromatic compounds, because the formyl halides have not been known. Earlier, chemists have attempted to overcome this problem through the following two strategies:

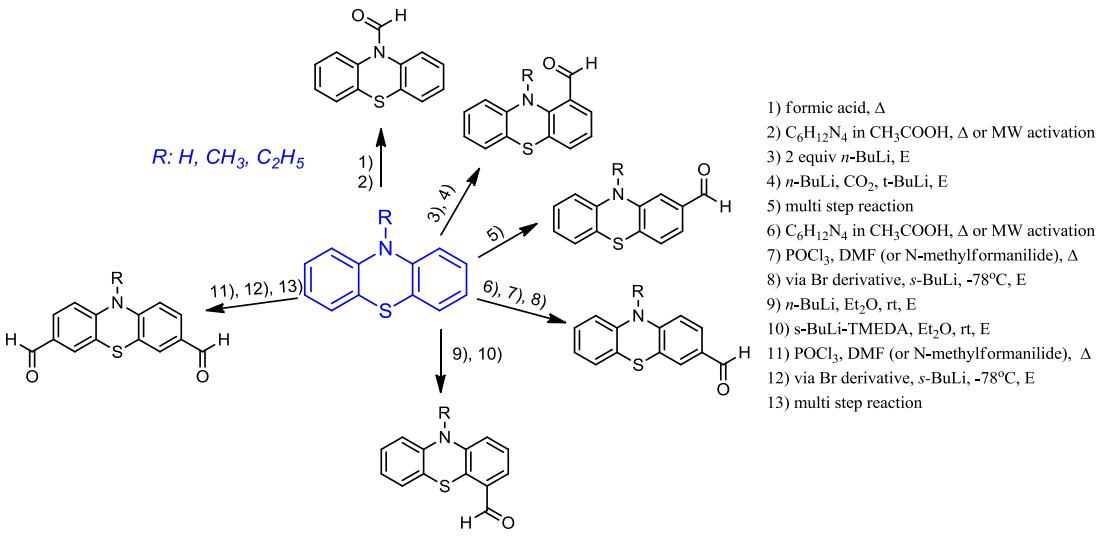
- the reactions of C-C bond forming occur reactions which give the hydroxymethyl or halomethyl-substituted aromatic compounds, and these were subsequently oxidized to produce the aromatic aldehyde. Approaches of Duff and Casiraghi are this type of reactions[13].

- on the other hand, chemists have tried to find direct formylation reagents, as a replacement for unstable formyl chloride, to permit direct introduction of the formyl group in the aromatic ring.

All classical direct formylation procedures suffer greater or lesser degrees of serious disadvantages. In some processes, highly toxic or corrosive compounds (carbon monoxide, hydrogen cyanide, phosgene, s-triazine, formyl fluoride, hydrogen chloride, phosphoryl chloride, etc.) are used. Some of these compounds are gaseous, not storage-stable (formyl fluoride), not easy to handle and difficult to dose. In all the above mentioned aldehyde synthesis, hydrolysis is the last step which can give rise to problems with wastewater. This question is particularly important if Vilsmeier-Haack reactions are carried out with DMF/$POCl_3$, the hydrolysed reaction mixture contains phosphoric acid, which is an environmental problem.

Direct lithiation and subsequent formylation with DMF is a facile procedure used to prepare heteroaromatic aldehydes with the formyl group adjacent to a heteroatom, regioselectively and in good yields. For example, the lithiation of furans/benzofurans[20] and benzothiophenes[21] always occurs at a free α-position.

1.3. Formylation of phenothiazines



Scheme 3. Formylation of the phenothiazine

Formylation of the phenothiazine can be achieved directly with the method of Vilsmeier-Haack, or indirect through the use of organo-lithium derivatives which upon treatment with DMF, are converted into the aldehyde.

In addition, phenothiazines substituted at C_1 or C_3 can be achieved from the corresponding *ortho*- or *para* substituted diphenylamines by cyclization using sulphur in the presence of iodine. The appropriate diphenylamines are synthesized from 2-chlorobenzoic acid and the appropriately substituted aminobenzene in two steps. When *meta*-substituted diphenylamines are subjected to cyclization using sulphur and iodine a mixture of C_2 and C_4 substituted phenothiazines are formed, the predominant isomers depends from the starting material[22].

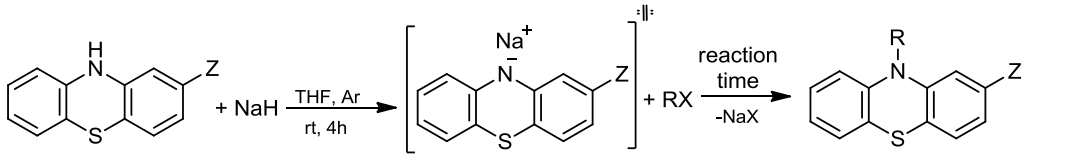
1.4. Original Contributions: Synthesis of 10-alkyl-phenothiazine carboxaldehyde regioisomers

A retrosynthetic approach 10-Alkyl-phenothiazine carbaldehyde regioisomers were found as versatile precursors for the synthesis of various heteroaromatic systems such as dipyrromethanes, bis(indolyl)methanes and porphyrins respectively, which were the target heterocyclic systems of this study. The synthetic pathway implies two reaction steps: first the N-alkylation in order to reduce the nucleophilicity of the heterocyclic nitrogen, followed by the regioselective formylation of the phenothiazine unit. A modulation of properties for the target compounds may be addressed by modifying these two parameters: the length of the

alkyl chain (with implication on the steric hindrance, hydrophobicity and solubility) and the position of the formyl substituent (with major influences on the extended π conjugated system).

1.4.1. *N*-alkyl-phenothiazines

To N-alkylate the phenothiazine ring with alkyl halides it is recommended to use an alkaline media, which can be achieved with the use of KOH, $NaNH_2$ or NaH. In presence of KOH in DMF the alkylation yields were low (less than 20%), for this reason we used $NaNH_2$ or NaH in THF. The reactions was carried out in an inert atmosphere by treating phenothiazine (**1a**, **1b**) with sodium hydride in THF to give 10-Na-phenothiazine (orange) which is subsequently reacted with an alkyl iodide or bromide to form the corresponding alkylphenothiazine in good yield (*Scheme 10*). By this procedure were synthesized 10-Me-, 10-Et-, 10-butyl-phenothiazine, 2-chloro-10-methyl-phenothiazine, 10-(6-bromohexyl)-phenothiazine, 1,6-bisphenothiazinyl-hexane (**2a-f.**).



1a Z=H
1b Z=Cl

2a	$R=CH_3$	X=I	Z=H
2b	$R=CH_2CH_3$	X=I	Z=H
2c	$R=(CH_2)_3CH_3$	X=I	Z=H
2d	$R=CH_3$	X=I	Z=Cl
2e	$R=(CH_2)_6Br$	X=Br	Z=H
2f	$R=(CH_2)_6{}^{N}Ptz$	X=Br	Z=H

Scheme 10. Reaction steps in the preparation of alkyl phenothiazines

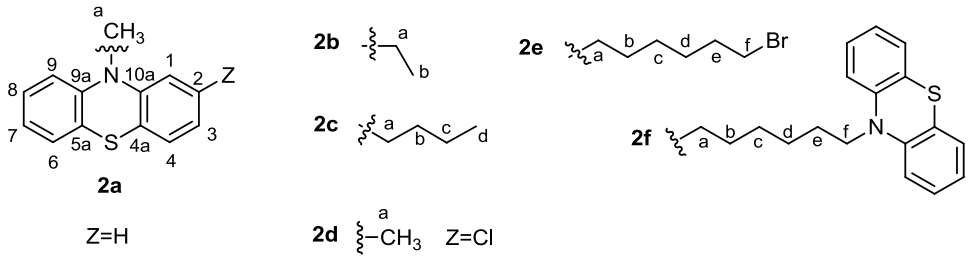
Purification of the N-alkyl-phenothiazine was achieved by recrystallization from ethanol (**2a**, **2b**, **2f**), by vacuum distillation (**2c**) or column chromatography (**2e**). Typical reaction parameters, to achieve high yields: alkylating agent, reaction time, yield, melting point, are presented in *Table 1*.

*Table 1: Reaction parameters to obtain **2a-f***

Cpd	Alkylating agent	Reaction time (h)	Yield (%)	m. p. (^oC)
2a	CH_3-I	4rt	95	99
2b	CH_3-CH_2-I	4rt	87	104
2c	CH_3-$(CH_2)_3$-I	12rt	76	<25
2d	CH_3-I	4(50^oC)+12(rt)	94	88
2e	Br-$(CH_2)_6$-Br	12rt	50	<25
2f	Br-$(CH_2)_6$-Br	12rt	44	151

The structures of the synthesized compounds were investigated by spectroscopic methods: FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectrometry (MS).

The 1D- and 2D-NMRs were recorded in $CDCl_3$ confirm the structure of the alkylated phenothiazines **2a-f**, the corresponding chemical shifts of the aliphatic and aromatic protons are in accordance with literature data.



Scheme 11. Structure of the alkylated phenothiazines

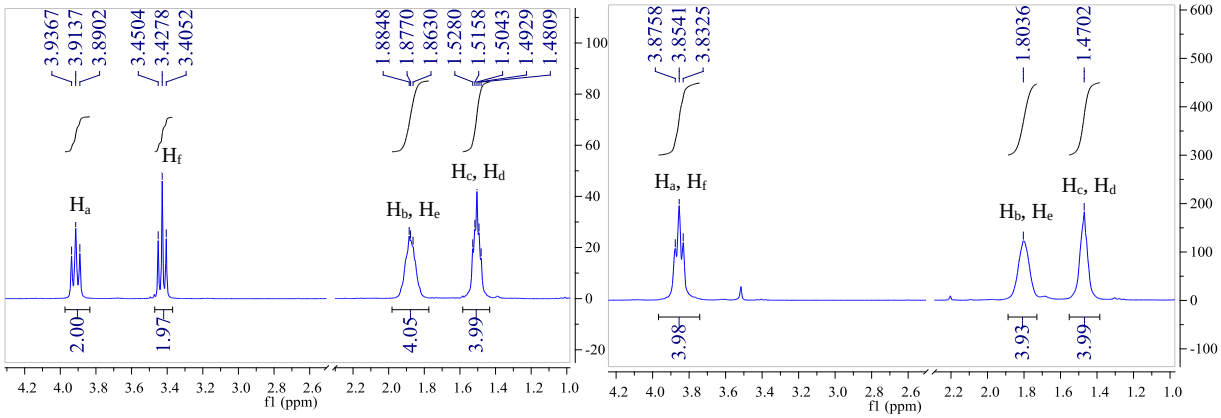


*Figure 2. ^{1}H-NMR, aliphatic part of **2e** (left) and **2f** (right) in $CDCl_3$ at 300 MHz*

Because of the symmetry of phenothiazine molecule, the two benzene rings are identical and thus a reduced number of signals was observed for **2a**, **2b**, **2c**, **2e** also even in the case of **2f** (*Figure 2, right*). Exception is the **2d**, the chlorine at the position *2* on the ring disrupt the symmetry (*Figure 3, right*).

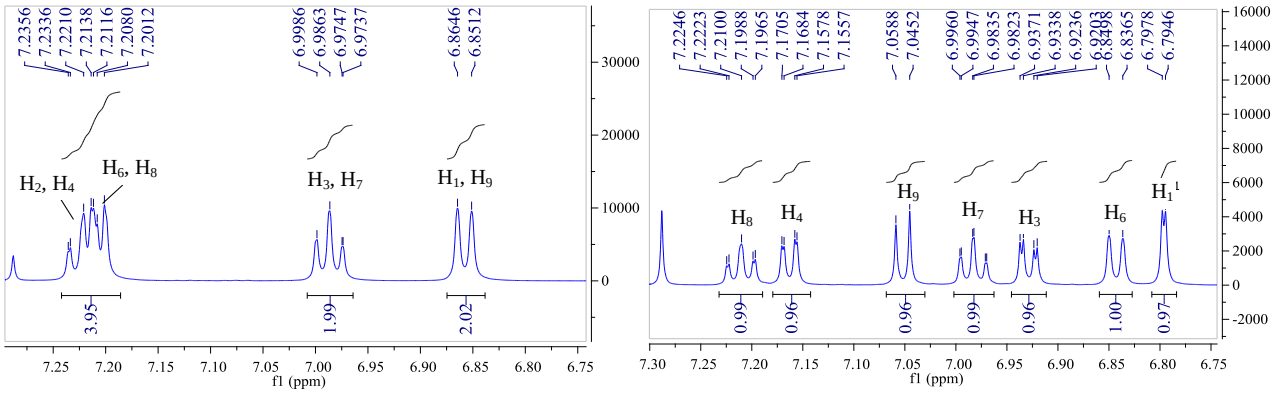
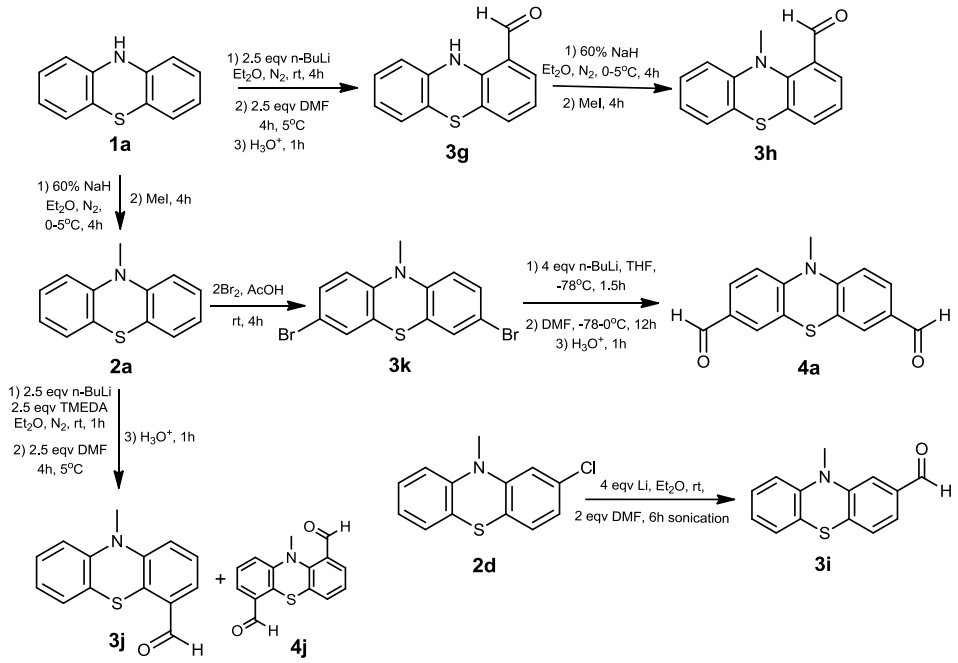


*Figure 3. ^{1}H-NMR, aromatic part of **2a** (left) and **2d** (right) in $CDCl_3$ at 400 MHz*

The MS (EI) spectra show some characteristic fragments for this type of compounds. In the MS spectra the molecular ion occur as the base peak, also we can observe with variable intensity fragments at values m/z 198 (phenothiazine radical cation), 184 (carbazole radical cation).

1.4.2. 10-Alkyl-*10H*phenothiazin-carbaldehyde regioisomer series

In this work, the regioselective C-formylation of 10-methyl-*10H*-phenothiazine substrate was achieved by modulating the chemical reactivity of the heterocycle towards electrophilic substitution. Phenothiazine 1-, 2- and 4-carbaldehyde regioisomers were successfully obtained by a two-steps procedure involving a phenothiazine-lithium intermediate further trapped by the treatment with dimethylformamide (DMF) as electrophile (*Scheme 12*). A careful selection of the reaction conditions substrate, lithium reagent, and reaction temperature (*Table 2*) was required in order to obtain satisfactory yields of each target regioisomer.



Scheme 12. Regioselective formylation of 10-methyl-10Hphenothiazine derivatives

Table 2: Selected reaction conditions to obtain the regioisomers.

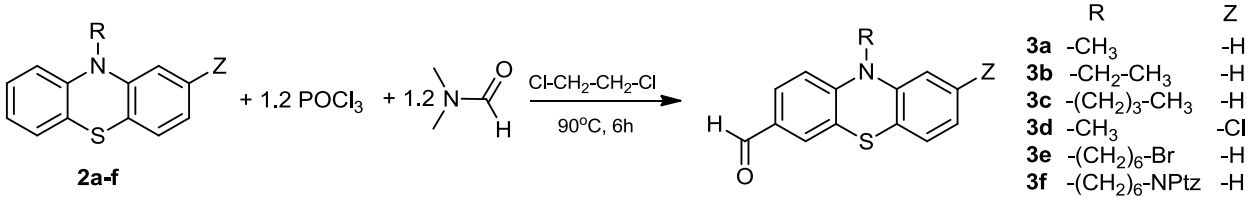
Substrate	Cpd.	Solvent	Deprotonation agent / Electrophile (equiv)	Temperature (°C)*	Reaction time*	Yield (%)**
1a	**3g**	Et_2O	2.5 *s*-BuLi/DMF	rt/0	45min/4h	24
1a	**3g**	Et_2O	2.5 *n*-BuLi/DMF	rt/0	45min/4h	10
1a	**3g**	Et_2O	2.5 *n*-BuLi/DMF	rt/0	4h/4h	18
1a	**3g**	Et_2O	2.5 *n*-BuLi/DMF	rt/0	10h/4h	25
2a	**3j**	Et_2O	2.5 *s*-BuLi-TMEDA/DMF	rt/0	45min/4h	61

2a	**3j**	Et_2O	2.5 *n*-BuLi-TMEDA/DMF	rt/0	45min/4h	36
2a	**4j**	Et_2O	2.5 *s*-BuLi-TMEDA/DMF	rt/0	45min/4h	3
2a	**4j**	Et_2O	2.5 *n*-BuLi-TMEDA/DMF	rt/0	45min/4h	7
2d	**3i**	Et_2O	2 Li/DMF	rt	6h	4
2d	**3i**	Et_2O	4 Li/DMF	rt	6h	20
3k	**4a**	THF	4 *n*-BuLi/DMF	-5/rt	2h/4h	55
3k	**4a**	THF	4 *n*-BuLi/DMF	-78/rt	1.5h/12h	83

*before and after adding the electrophile
**isolated yields after column chromatography

Literature data indicate the phenothiazine-3-carbaldehyde as the most widely employed regioisomer because it can be easily reached by subjecting *N*-alkyl-phenothiazine to the formylation procedures Vilsmeier, Bergman, or Duff based on aromatic electrophilic substitution reactions governed by the electron donor effect of the heterocyclic N atom.

In this work, the N-protected alkyl phenothiazines was formylated by the method proposed by Bodea[54] (1965) with minor modifications (*Scheme 13*) to obtain phenothiazinyl aldehydes in 1,2-dichloroethane at position *3* in high yield (*Table 3*).



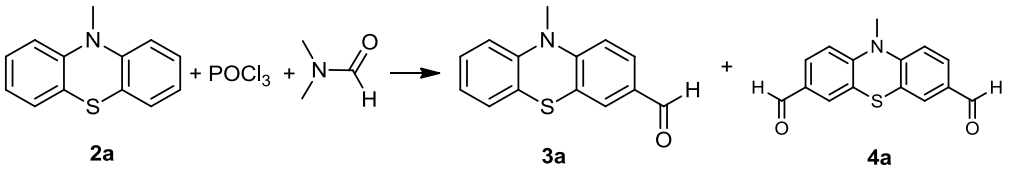
Scheme 13. Formylation of N-protected phenothiazines

*Table 3: Reaction parameters to obtain **3a-f***.*

Cpd.	Reaction time (h)	Yield (%)**	m.p. (^{o}C)
3a	6h	80	89
3b	6h	78	82
3c	6h	47	51
3d	6h	35	165
3e	6h	65	72
3f	6h	70	95

* Vilsmeier agent (1.2 $POCl_3$, 1.2 DMF); 90^{o}C in 1,2-dichloroethane
** isolated yields after column chromatography

The reaction gives low yields in the case of **3d** where the position *2* is already occupied indicating that the presence of the adjacent bulky atom reduces the efficiency of the aldehyde formation at position *3*. The chlorine in *meta* position to the nitrogen, directed the aldehyde to the other *para* position (position *7*), although the position *3* is free.



Scheme 14. Formation of mono-and diformyl 10-methyl-phenothiazines

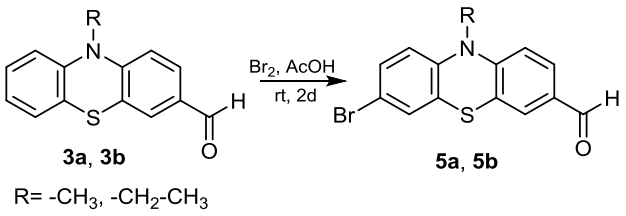
The mechanism proposed by Bodea explains the formation of mono- and diformyl-derivatives, regardless of the *Vilsmeier* reagent excess. In this work due to the low yield of the diformyl products, were isolated only the 10-alkyl-3-formylphenothiazines. Exception was in case of compound **2a** (*Scheme 14*), the ratio between the mono- and diformyl species was controlled in the reaction by using of formylating agent and different solvents in the reaction. A comparison between the reaction parameters is summarized in *Table 4*.

*Table 4: The summarized reaction parameters for preparing **3a** and **4a***

***Precursor* / Product**	**Vilsmeier agent (equiv.)**	**Solvent**	**Temperature (^oC)**	**Reaction time (h)**	**Yield (%)***
***2a*/3a**	1	DMF	100	6	64
***2a*/3a**	1	DCE	90	6	80
***2a*/3a**	1	$CHCl_3$	70	6	40
***2a*/3a**	4	DCE	90	6	63
***2a*/4a**	1	DMF	100	6	14
***2a*/4a**	1	DCE	90	6	6
***2a*/4a**	1	$CHCl_3$	70	6	0
2a/4a	4	DCE	90	6	16

* isolated yields after column chromatography

In some special cases the usual method involving the use of $POCl_3$ has to be modified, as halogen exchange takes place in some instances. To eliminate the halogen exchange it was used the method described by Krammer[55], to obtain 7-bromo-10-alkylphenothiazine-3-carbaldehydes up to 80% yield (*Scheme 15*).



Scheme 15. Preparation of unsymmetrically functionalized phenothiazines

With these unsymmetrically functionalized phenothiazines in hand now a selective functionalization of the bromo- or the formyl moiety could be successfully performed.

The design of the symmetrically functionalized phenothiazine **3k**, **4a** and the unsymmetrically functionalized compounds **3d**, **4d**, **5a**, **5b** ^{1}H-NMR spectra distinct from the mono substituted phenothiazine derivatives.

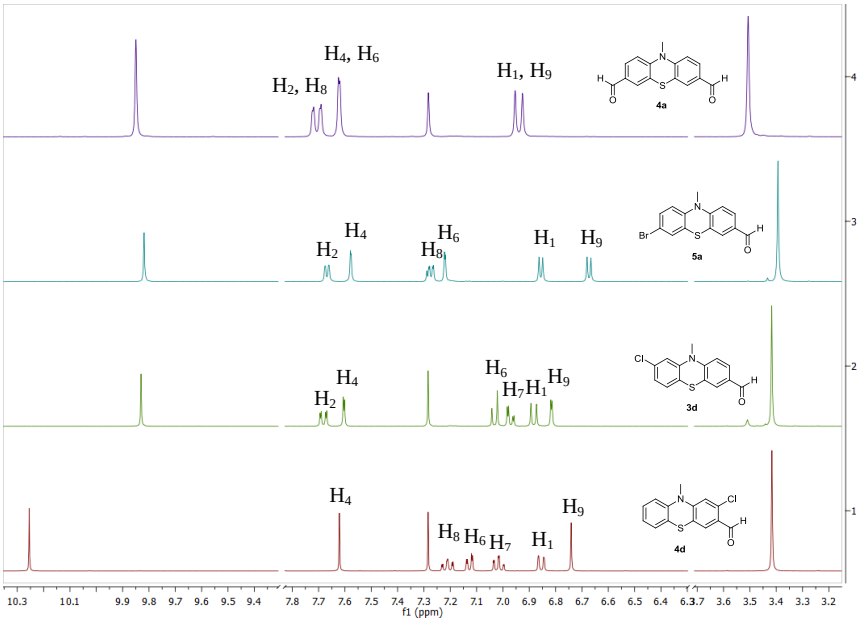
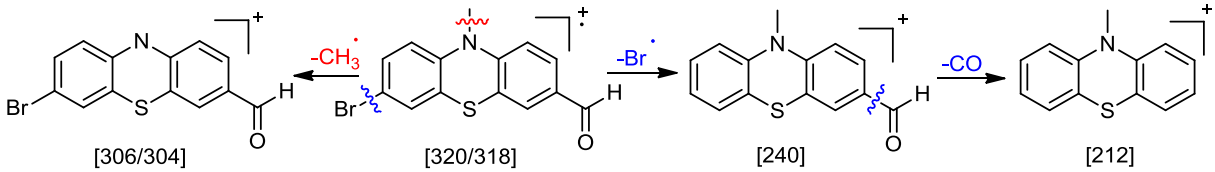


*Figure 5. ^{1}H-NMR spectra of **4a**, **5a**, **3d**, **4d** at 400 MHz in $CDCl_3$*

Mass spectrometry studies carried out on formyl phenothiazines confirmed their molecular weight by recording the corresponding molecular peak and fragmentation peaks with different intensity occurring at values m/z 240, 226, 198. *Sheme 16* shows the fragmentation of 10-methyl-3-formyl-7-bromo-phenothiazine **5a**.



*Scheme 16. Fragmentation of compound **5a**.*

Each phenothiazine carbaldehyde show two absorption bands, situated in the UV region at 277-282 nm and 381-419 nm respectively. These absorption bands appear less affected by the substitution pattern, except for the case of **3a** which reveals a hypsochromic shift of approx. 30 nm for the band situated at longer wavelength. Upon excitation with the typical absorption wavelength, the phenothiazine carbaldehyde show day light fluorescence emission with low quantum yields but large stokes shift (*Table 5*).

Table 5: Electronic properties of phenothiazine carbaldehyde regioisomers determined by UV-Vis absorption/emission spectroscopy 10^{-4} M in DCM

Cpd.	λ_{abs} (nm)	ε ($m^{-1}cm^{-1}$)	λ_{em} (nm)	Stokes shift (cm^{-1})
3h	277*, 411	54400, 3180	543	9400
3i	282*, 418	109200, 4730	551	5700
3a	282*, 381	60950, 5900	567	8600
3j	277*, 419	58600, 6170	571	6300

*Absorption maximum, excitation wavelength

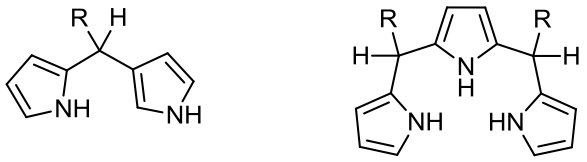
2. Heteroarylmethane derivatives containing phenothiazine units

2.1. Dipyrromethane derivatives.

Dipyrromethanes are compounds known for more than a century and are widely being used as important building blocks in organic synthesis, occupy a central place in porphyrin chemistry. In the past decade, dipyrromethanes lacking *β*-substituents but substituted at the *meso*-position have come to play a valuable role in the preparation of synthetic porphyrins and porphyrin analogs (dipyrrins, calix[4]pyrroles, chlorins, corroles), which have recent applications as chiral catalysts, chiral sensors, synthetic receptors for small molecular devices, potential sensitizers for photodynamic cancer therapy[56]. Moreover, this dipyrrolic systems are the precursors of BODIPY dyes (4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene or boron dipyrromethene), which are currently receiving increasing attention due to their valuable properties, such as the relatively high absorption coefficients and fluorescence quantum yields, high photochemical stability, and improved synthetic availability.

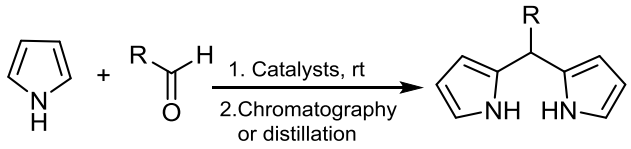
Synthesis of dipyrromethanes

Dipyrromethanes are usually prepared via a one-flask method based on the acid-catalysed condensation of an appropriate aldehyde (or ketone) with an excess of pyrrole (used as solvent) and flash chromatography is required in most cases to obtain the dipyrromethanes in high purity. The drawback of this approach is the competitive formation of tripyrromethanes (*Scheme 18*) and higher oligopyrromethanes, as well as *N*-confused (α, β-linked) dipyrromethane derivatives.



Scheme 18. N-confused dipyrromethane (left), tripyrane or tripyrromethane (right)

Lewis acids are excellent catalysts for the condensation of pyrrole with aldehydes[66,67]. Using anhydrous indium chloride ($InCl_3$) for unhindered aromatic and aliphatic aldehydes and magnesium bromide ($MgBr_2$) for hindered aromatic aldehydes (mesitaldehyde and 2,6-dichlorobenzaldehyde), the expected 5-substituted dipyrromethanes could be obtained in good yields. Several research groups have applied the acid-catalysed condensation of aldehydes with pyrrole to obtain 5-aryldipyrromethanes (*Scheme 21*, *Table 6*), which were used for the synthesis of various functionalized porphyrins[60,61,65,66].



Scheme 21. Synthesis of 5-aryldipyrromethanes

Table 6: The acid-catalysed condensation of aldehydes with pyrrole

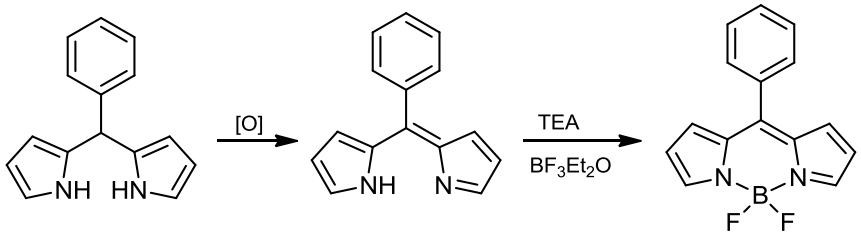
Pyrrole:aldehyde ratio	**40:1**	**25:1**	**60:1**	**100:1**	**100:1**
Catalist:	***TFA*[60]** **Error! Bookmark not defined.**	***TFA*[61]**	***BF_3·Et_2O*[65]**	***$InCl_3$*[66]**	***$MgBr_2$*[66]**
R=					
Ph	49%	53%	85%	82%	-
p-Me-C_6H_4-	76%	33%	81%	-	-
p-I-C_6H_4-	57%	57%	-	-	-
p-Cl-C_6H_4-	-	-	73%	-	-
o,o',p-Me_3-C_6H_2-	55%	27%	-	49%	53%
o,o'-Cl_2-C_6H_3-	-	39%	-	-	60%
C_6F_5-	-	65%	-	80%	-
p-CF_3-C_6H_4-	-	-	85%	-	-
p-CN-C_6H_4-	-	-	64%	-	-
p-NO_2-C_6H_4-	-	56%	74%	-	-
p-$MeCO_2$-C_6H_4-	-	-	-	75%	-
CH_3-$(CH_2)_4$-	60%	-	-	62%	-
H-	-	41%[a]	-	63%[b]	-

a reaction mixture heated to 50°C
b reaction carried out at 55°C for 2.5h

Dipyrromethanes are unstable compounds, and as they are sensitive to light, air and acid, best used immediately after preparation. Oxidation of the dipyrromethane yields a

dipyrromethene, or dipyrrin (*Scheme 24*). Only a few examples where the aldehyde is not an aromatic aldehyde, as the oxidation tends to fail in other cases[80].

Subjecting the dipyrrin to base (TEA) and boron trifluoride etherate ($BF_3 \cdot Et_2O$) affords the borondifluoride complex in high yield.



Scheme 24. Oxidation of the dipyrromethane

They are neutral and their characteristics are mostly independent of solvent polarity. The complexes are stable in physiological pH-range, only decomposing in strong acidic and basic conditions[82]. These desirable properties combine with a low toxicity[83], to make them excellent probes for use in biological systems and novel materials. Dipyrrins provide the basis for the boron-dipyrrin dyes and also have a rich chemistry with diverse transition metals.

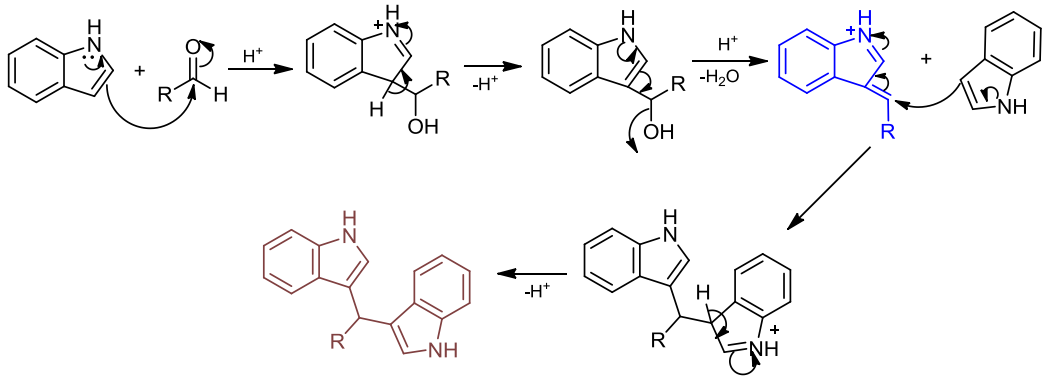
2.2. Bis-(indolyl)methane derivatives

Indoles have been widely identified as a privileged structure or pharmacophore, with representation in over 3000 natural isolates[87] and are known to possess broad spectrum of biological and pharmaceutical activities[88]. Indomethacin[89] and tenidap[90] are indole derivatives, which have beside anti-inflammatory activities, analgesic and antipyretic properties.

Bis(indolyl)methanes have received much attention in recent years[100]. Such compounds are prone to develop interesting bio-activity and find useful applications as anti tumoral[101,102] and anti-bacterial agents[103]. Non-conjugated bis-indolylmethane derivatives with a sp^3 hybridized carbon at *meso* position may even promote more significant colour changes and can act as a selective colorimetric sensor for F^- (or HSO_4^-)[104], CN^-[105] and also as a highly selective fluorescent molecular sensor for Cu^{2+}[106,107], and Hg^{2+}[108].

Synthesis of bis(indolyl)methanes

The reaction of indole with aldehydes or ketones produces azafulvanium salts which react further with a second indole molecule (*Scheme 27*) to form bis(indol-3-yl)methanes[110].



Scheme 27. Mechanism involving azafulvanium salts finally giving bis(indolyl)methane

The most of the existing synthetic methods involve toxic metal ions and solvents, corrosive reagents and have laborious work-up procedures. One of these methods, the acid-catalysed electrophilic substitution reaction of indoles with aldehydes, is the most simple and straight forward approaches for the synthesis. The electron rich indole nucleus shows an enhanced reactivity towards carbon electrophiles that generally results in the formation of three substituted indoles derivatives[111]. Synthesis of bis(indolyl)methanes is also achieved in the absence of catalyst using protic solvents but with long reaction time.

2.3. Original contributions: Synthesis of tris-heteroarylmethane derivatives containing phenothiazine units

2.3.1 Dipyrromethane derivatives containing phenothiazine units

Phenothiazinyl-carbaldehyde regioisomers and poly functionalized phenothiazines gave the corresponding (N-alkyl-phenothiazinyl)dipyrromethanes **11e-p** in 60-89% yields despite the length of the alkyl chain attached to the heterocyclic nitrogen atom, thus indicating the absence of steric hindrance at the reaction site. All reactions worked smoothly and compounds **11a-p** are freely soluble in common organic solvents.

The compound formation was confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, ESI-MS mass spectral analysis. In the ^{1}H-NMR spectra symmetrical pyrrol units gave three distinct signals situated at chemical shift ranging in 5.9-6.7 ppm area. The phenothiazine units produced more deshielded signals ranging in the 6.8-7.2 ppm area, while the acidic NH protons (H_{10}, H_{11}) gave downfield signals situated around 7.9 ppm (*Figure 8*). The phenothiazine units proved the most pronounced electron-donor tendency and thus, the recorded signals for the *meso* protons appeared slightly shielded.

The studies of ^{13}C-NMR spectra of substituted phenothiazinyl-dipyrromethanes revealed downfield shifts of phenothiazinyl carbon atoms, the most downfield shifts exhibited by C_{9a} and C_{10a} from the phenothiazine ring after than the *ipso* carbon atom above 136 ppm. In case of phenyl-dipyrromethanes and substituted phenyl-dipyrromethanes the most downfield shifts exhibited by *ipso* carbon atoms.

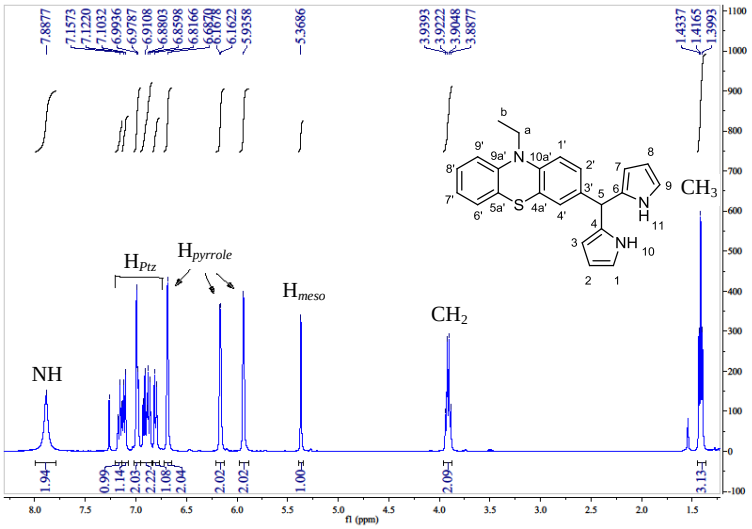
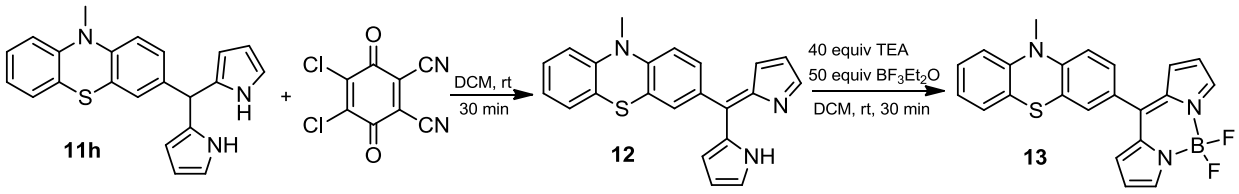


*Figure 8. ^{1}H-NMR of **11i** at 400MHz in $CDCl_3$ and its IUPAC numbering system*

In the EI-MS spectra of each product **11a-p** the molecular ion was recorded in high abundance. The main fragmentation pattern implies the loss of alkyl groups (attached to the phenothiazine core) and the loss of pyrrole units.

N-methyl-phenothiazinyl-dipyrrin **12** was prepared by the oxidation of **11h** and further complexation with boron trifluoride generated N-methyl-phenothiazinyl-borondifluoro-dipyrromethene (phenothiazinyl-BODIPY) dye **13** as shown in *Scheme 29*. After standard work-up, the crude compound was purified by silica gel column chromatography and afforded the BODIPY as black solid in 40% yield.



*Scheme 29. Synthesis of phenothiazinyl-BODIPY dye **13***

The complete structural assignments of **12** and **13** were performed based on 2D NMR homonuclear correlation (^{1}H-^{1}H COSY) and heteronuclear (HMQC) experiments. The 2D-NMR proton homonuclear correlation spectrum of **13** afforded the discrimination between the

coupling patterns of dipyrromethene δ= 6.5, 6.9 and 7.9 ppm and phenothiazine units δ= 6.8-7.4 ppm (*Figure 9*).

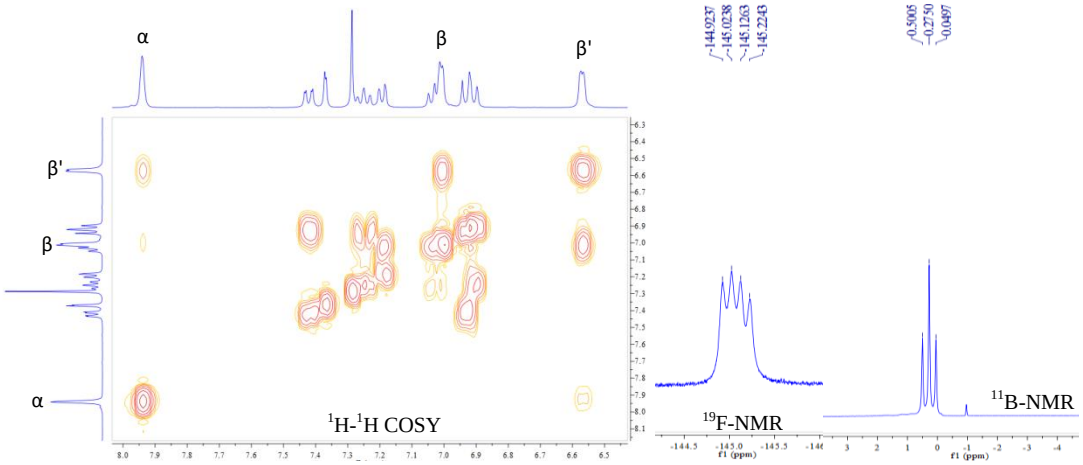


*Figure 9. ^{1}H-^{1}H COSY, ^{19}F- and ^{11}B-NMR details of compound **13** in $CDCl_3$*

The recorded ^{11}B-NMR shows a shielded signal splitted by the direct coupling of the boron atom with the two fluorine atoms, giving rise to a sharp triplet ($^{1}J_{B\text{-}F}$= 28.9 Hz, *Figure 9*) which can be assigned to the boron nucleus connected to the two pyrrole nitrogens. The ^{19}F-NMR spectrum shows a quartet, because of coupling of the magnetically equivalent ^{19}F (I = 1/2) atoms to the quadrupolar ^{11}B (I = 3/2) nucleus ($^{1}J_{F\text{-}B}$ = 28.2 Hz, *Figure 9*).

The UV-Vis absorption properties of the parent phenothiazinyl-dipyrrolylmethane **3c**, phenothiazinyl-dipyrrine **12** and phenothiazinyl-BODIPY dye **13** are very distinct as shown in *Figure 10*. While the parent **11h** had no absorbance in the visible range, the phenothiazinyl-dipyrrine **12** was characterized by a broad absorption band situated around 430 nm and a bathochromic shift of about 70 nm was achieved by complexation in **13**.

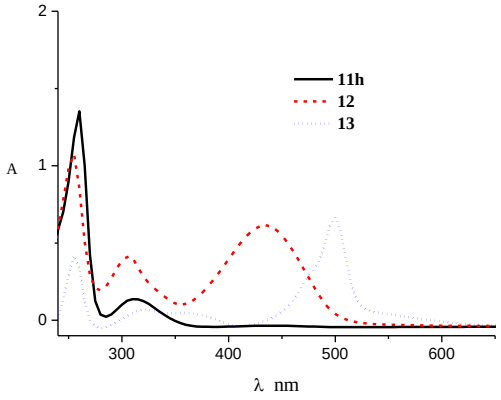
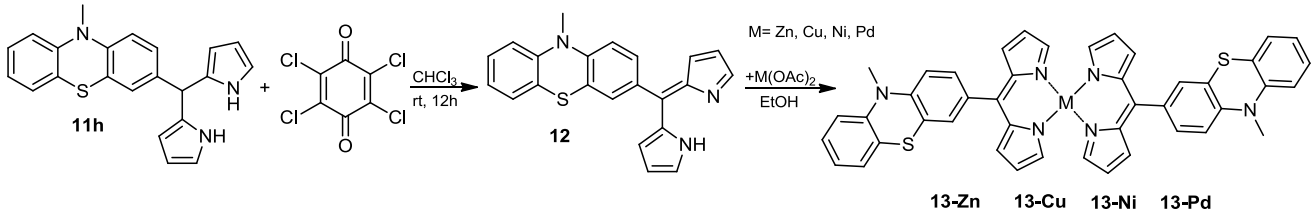


*Figure 10. UV-Vis spectra of phenothiazinyl-dipyrromethane derivatives (**11h**, **12**, **13**) in CH_2Cl_2*

Dipyrromethenes (dipyrrins) are monoanionic divalent ligands. They have unique properties and possess some distinct advantages in their coordination chemistry. Upon deprotonation, they chelate various transition metals to form isolable neutral complexes, with no counter anion required. The corresponding acetate (*Scheme 30*) was introduced into

ethanolic solution of **12**, to form metal complexes. The resulting complexes were purified by column chromatography on silica gel. The molecular weight of each metal complexes was confirmed by HRMS mass spectrometry.



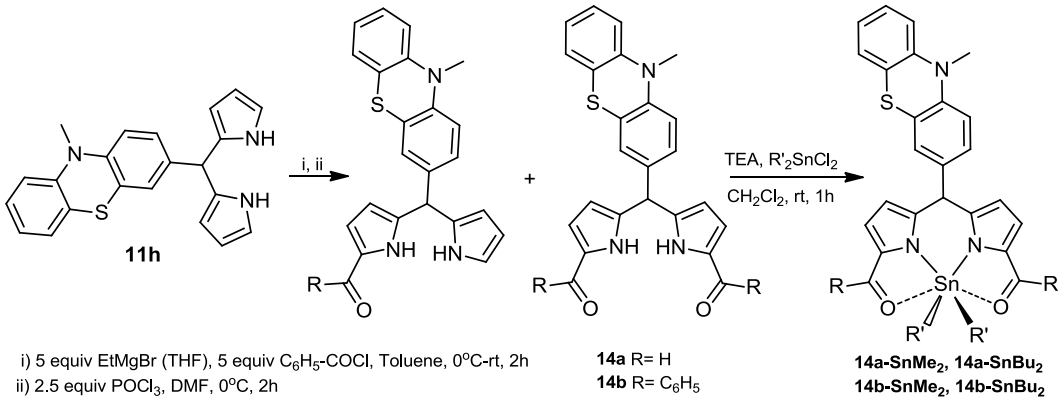
Scheme 30. Synthesis of homoleptic complexes

The phenothiazinyl-dipyrrine **12** was characterized by a broad absorption band situated around 430 nm. Metal dipyrrinato complexes typically display two intense absorption bands in the visible region of the electromagnetic spectrum. The more intense absorptions occur at absorption maxima ranging from 482 nm for **13-Cu** to 483 nm for **13-Pd**, together with the molar extinction coefficients the values are summarized in *Table 8*.

Table 8: Electronic properties of the phenothiazinyl-dipyrrin metal complexes determined by UV-Vis absorption spectroscopy in CH_2Cl_2

Cpd	λ_{max} (nm)	ε ($cm^{-1}\cdot mol^{-1}$)
11h	312	4365
12	430	22566
13-Zn	480	84374
13-Cu	463	23874
13-Ni	470	48762
13-Pd	483	16042

The acylation of dipyrromethanes to form 1,9-diacyldipyrromethanes is an essential step in the rational synthesis of porphyrins. Although several methods for acylation are available. Two methods (Grignard, Vilsmeier) were examined for the direct 1,9-diacylation of the dipyrromethane **11h**. To facilitate isolation of phenothiazinyl-1,9-diacyldipyromethane, the crude acylation mixture is treated with triethylamine and dialkyltin dichloride (Me_2SnCl_2 and Bu_2SnCl_2) to afford **14-SnR'$_2$** in moderate yield (*Scheme 31*).



Scheme 31. Selective dialkyltin complexation of the phenothiazinyl-1,9-diacyldipyrromethane

The tin-complexation process is selective for 1,9-diacyl species, yielding the hydrophobic phenothiazinyl-1,9-diacyldipyrromethane-dialkyltin complexes **14-SnR'$_2$**. This procedure proved viable for small-scale preparation, but partial decomplexation upon chromatographic separation limited the larger scale implementation.

The complete structural assignments of the phenothiazinyl-1,9-diacyldipyrromethane dialkyltin complexes were performed based on 2D-NMR homonuclear correlation (^{1}H-^{1}H COSY) and heteronuclear (HMQC) experiments.

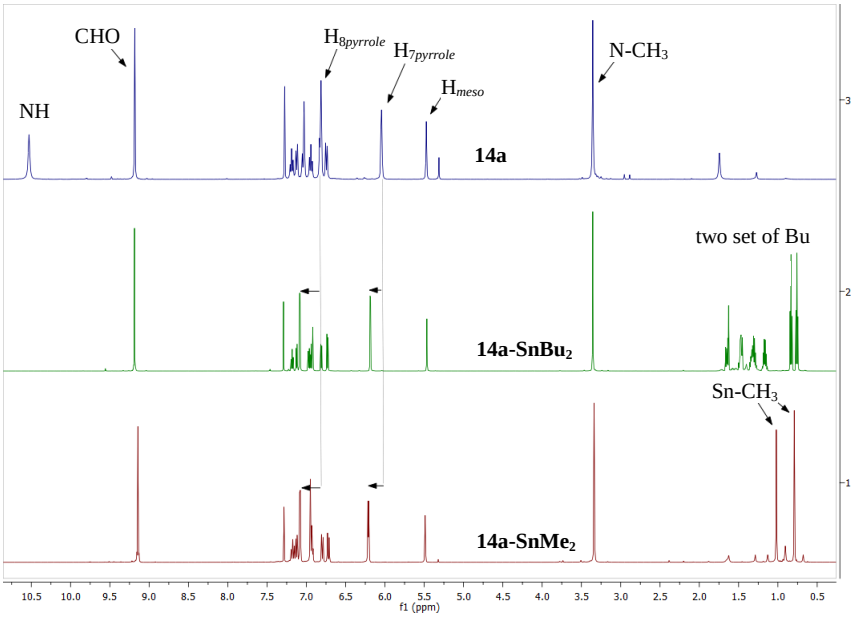
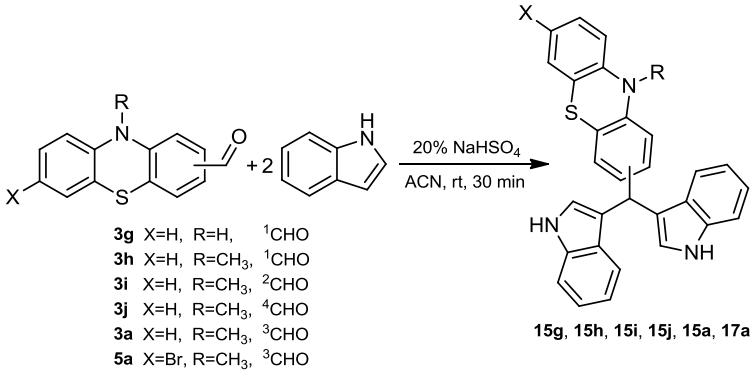


*Figure 12. ^{1}H-NMR of **14a**, **14a-SnBu$_2$**, **14a-SnMe$_2$** at 400MHz in $CDCl_3$*

The signal of NH protons at around 10.6 ppm in ^{1}H-NMR for 1,9-diacyldipyrromethanes disappears in tin complexes. The proton chemical shifts are not much influenced by the dialkyltin group indicating that tin complexation with the pyrrolic nitrogen atom and coordination to the carbonyl had only a negligible effect on the electronic environment of the *meso*-substituent.

2.3.2. Bis-(indolyl)methane derivatives containing phenothiazine units

In this study we wish to report a protocol for the rapid synthesis of a variety of biologically important bis(indolyl)methanes using a catalytic amount of sodium bisulphate under extremely mild and green conditions. Phenothiazinyl-carbaldehyde derivatives (**3g**, **3h**, **3i**, **3j**, **3a**, **5a**) gave the corresponding phenothiazinyl-bis(indolyl)methanes in 58-81% (*Scheme 33*).



Scheme 33. Condensation reaction of indole with phenothiazinyl-carbaldehyde

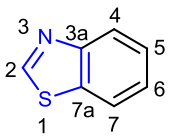
The yield was moderated when sterically hindered phenothiazinyl aldehydes were employed, but with the other type of the phenothiazinyl-carbaldehydes the reaction yields were slightly increased. The compound formation was confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, ESI-MS analysis.

3. Thiazole derivatives

3.1. Benzothiazole derivatives

Benzothiazoles are heterocyclic compounds, weak base, with electron rich sulphur and nitrogen atoms showing numerous biological activities such as antimicrobial[132], anticancer[133], anthelmintic[134], and anti-diabetic[135]. Various benzothiazoles such as 2-aryl benzothiazole received much attention due to unique structure and its uses as radioactive amyloid imagining agents[138], and anticancer agents[139]. Benzothiazole is also mentioned as fungicide[140].

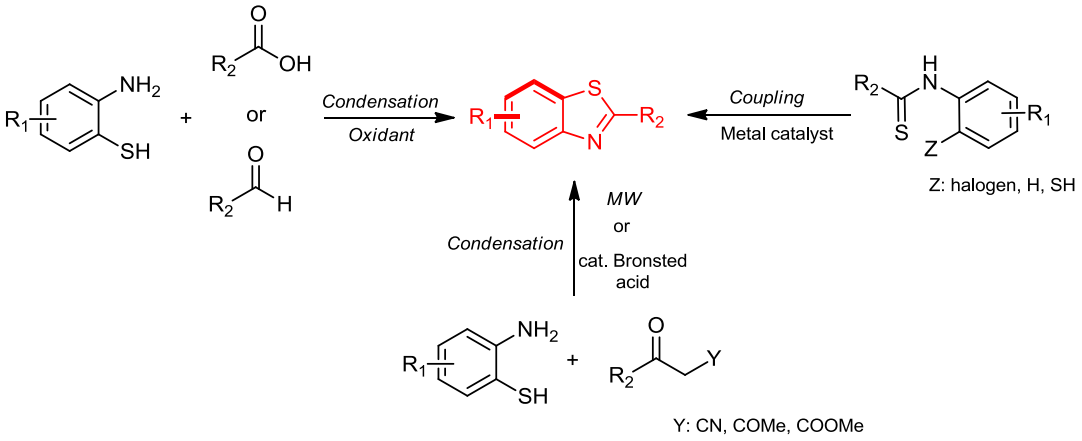
Sulphur and nitrogen atoms constitute the core structure of thiazole and any pharmacologically and biologically active compounds. The basic structure of benzothiazole consist of benzene ring fused with *4, 5* position of thiazole. The two rings together constitute the basic nucleus 1,3-benzothiazole (*Scheme 34*).



Scheme 34. Numbering of the benzothiazole ring

Synthesis of benzothiazoles

In order to synthesize benzothiazoles, unprotected SH or NH_2 groups are necessary.



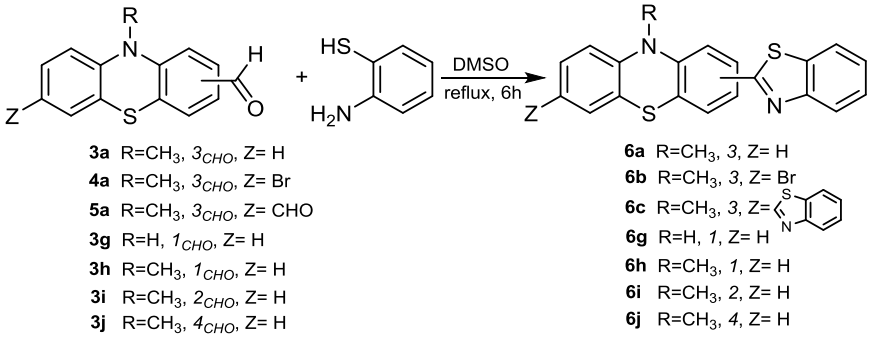
Scheme 36. Synthesis of benzothiazoles

In recent years, several main methods have been developed for the synthesis of benzothiazoles (*Scheme 36*). One method involves the condensation reactions of 2-aminothiophenols with carboxylic acids[149] or aldehydes[150,151] under oxidative conditions (oxidants: bromine, iodine, quinine, metal salts). Another method involves the transition-metal-catalysed intramolecular cyclization of anilides[152]. Most efforts were focused on noble metal catalysts, such as Ru, Rh, Pd. Due to the wide existence and low toxicity of iron compounds, more and more attention have been paid to the iron-catalysed[153] reaction for benzothiazole synthesis. Metal-free methods with alkyl amines[154] or aryl ketones[155] at high temperature under oxidative conditions were also reported. The last method involves the condensation reactions of 2-aminothiophenols with β-ketonitriles[156], β-ketoesters[157], or β-diketones[158] under microwave activation and high temperature or under Brönsted acid catalysed conditions.

3.2. Original contributions: Thiazole derivatives functionalized with phenothiazine unit

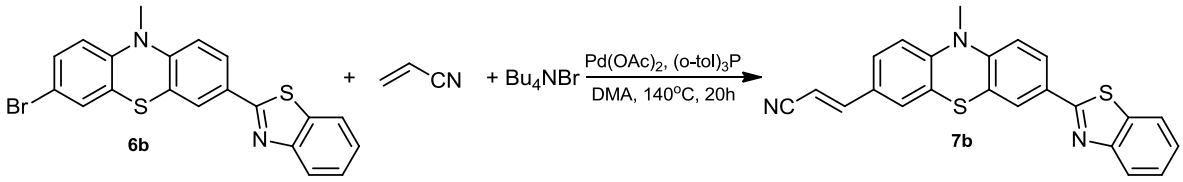
3.2.1. Benzothiazolyl-Phenothizine regioisomers

The first series started with formyl phenothiazine regioisomers, which were heated with *ortho*-aminobenzenethiol in DMSO for six hours, afforded the corresponding 10-methyl-10H-phenothiazinyl-1,3-benzothiazoles (*Scheme 37*) in good yields (60–80%).



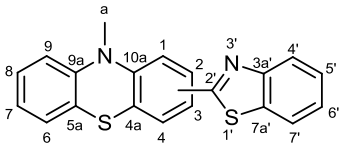
Scheme 37. Regioselective 2-phenothiazinyl-1,3-benzothiazoles

The cyanovinyl-substituted derivative (**7b**) was prepared by a palladium-catalyzed C-C coupling (Heck coupling) between the corresponding halogenated benzothiazolyl-phenothiazine (**6b**) and acrylonitrile in basic media at high temperature (*Scheme 38*).



Scheme 38. Palladium catalyzed Heck reaction affording cyanovinyl-benzothiazolyl-phenothiazine

In case of the phenothiazinyl-benzothiazoles the interpretation of the NMR measurements based on the starting regioisomer aldehydes.



Scheme 39. Numbering of the phenothiazinyl-benzothiazoles

The signals observed in the ^{1}H-NMR spectra of the phenothiazinyl-benzothiazoles under study are collected in *Figure 15*. The ^{1}H-NMR spectra of the benzothiazoles showed the characteristic pattern corresponding to the homocyclic couplings. The chemical shifts were not much influenced by the regioisomeric nature of the phenothiazine and the observed chemical shifts are 0.4–0.6 ppm higher for $H_{4'}$(d) and $H_{7'}$(d) as compared to $H_{5'}$(t) and $H_{6'}$(t).

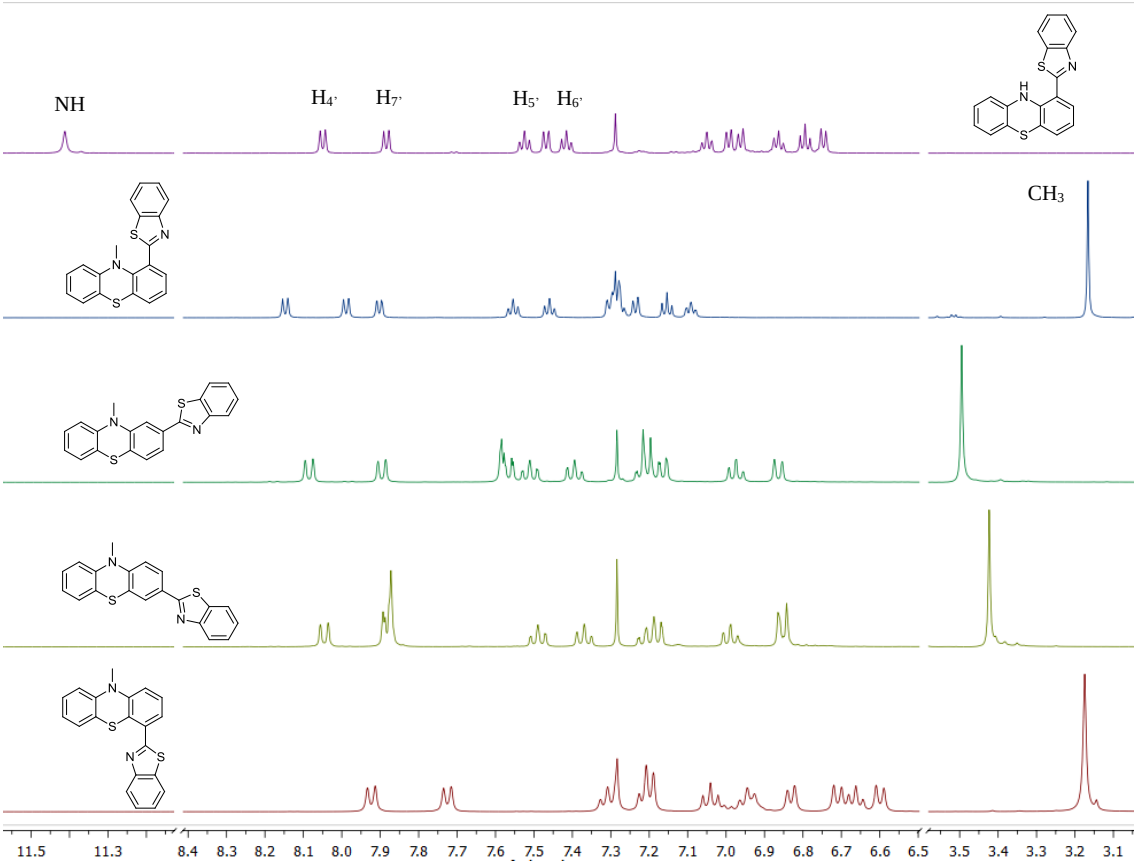
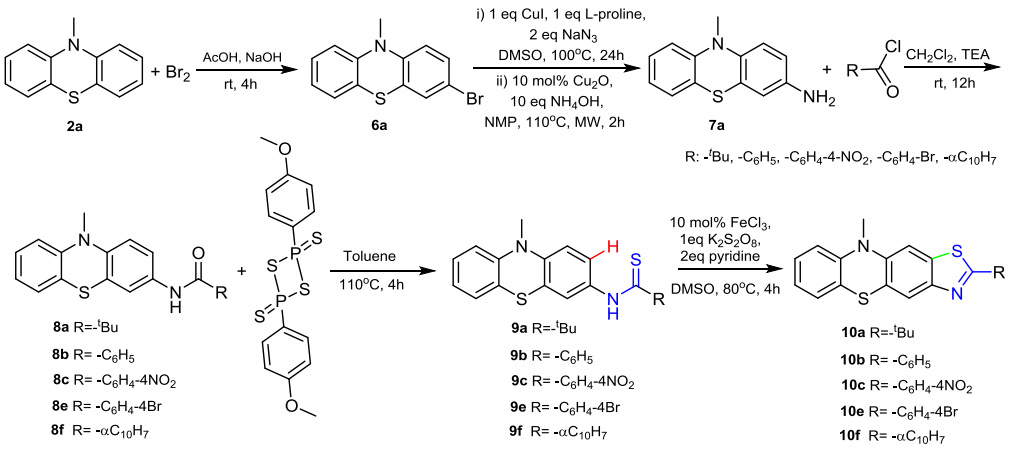


*Figure 15. ^{1}H-NMR of the phenothiazinyl-benzothiazoles (**6a**, **6g**, **6h**, **6i**, **6j**) at 400 MHz in $CDCl_3$*

3.2.2. Thiazolophenothiazine derivatives

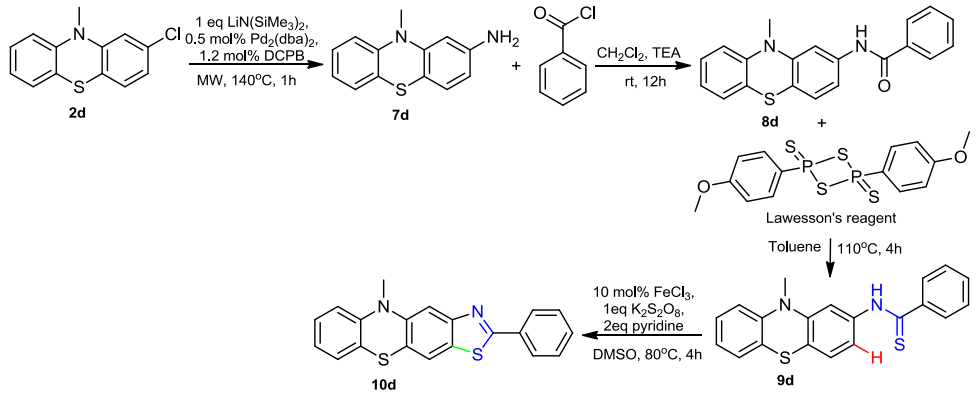
For the preparation of the second series of new phenothiazine derivatives with fused thiazole unit, the intramolecular Jacobson cyclization of N-phenothiazinyl-benzothioamide/analogues using cheap and environmentally acceptable catalyst Fe^{III} was developed.

The first step of synthetic path applied was the regioselective amination of halogeno-10-alkyl-phenothiazine substrate, palladium-catalyzed amination appears to be a convenient route for the preparation of 2-amino-10-alkyl-phenothiazine derivatives[159] (*Scheme 41*). The copper-catalyzed coupling of aryl halides with aqueous ammonia was alternatively applied for the microwave-assisted amination of **6a** (*Scheme 40*, ii), very good yield of 3-amino-10-methyl-10*H*phenothiazine (**7a**) were isolated after 2 h irradiation.



Scheme 40. Synthesis of 10-methyl-2-aryl-10H-thiazolo[5,4-b]phenothiazines

The amino phenothiazines were functionalized to aromatic amides, using substituted aromatic acid chlorides for driving the equilibrium to the product formation (**8a-f**).



Scheme 41. The synthetic process of 5-methyl-2-phenyl-5H-thiazolo[4,5-b]phenothiazine

The 2,4-bis(4-methoxyphenyl)-1,3-dithiaphosphetane-2,4-disulfide, known as Lawesson's reagent, has been used for the efficient conversion of oxygen functionalities into the thio- analogues in moderate yield (**9a-f**). After iron-catalysed C-H functionalization/C-S bond formation under mild conditions, produced moderate yields of **10a-f**, along with the formation of a small amount of by-product[153].

Structural study of the phenothiazinyl-benzothiazoles

The ^{1}H-NMR experiments in the series of phenothiazinyl-thiobenzamides **9a-f** produced similar spectra with little differences. The position and splitting of the aromatic protons remains similar to the benzamide aromatic protons, but the downfielded thioamide NH shifted well towards higher ppm values (~12 ppm).

Generally, the ^{1}H-NMR spectra of the thiazolo-phenothiazine derivatives **10a-f** are similar (*Figure 17*).

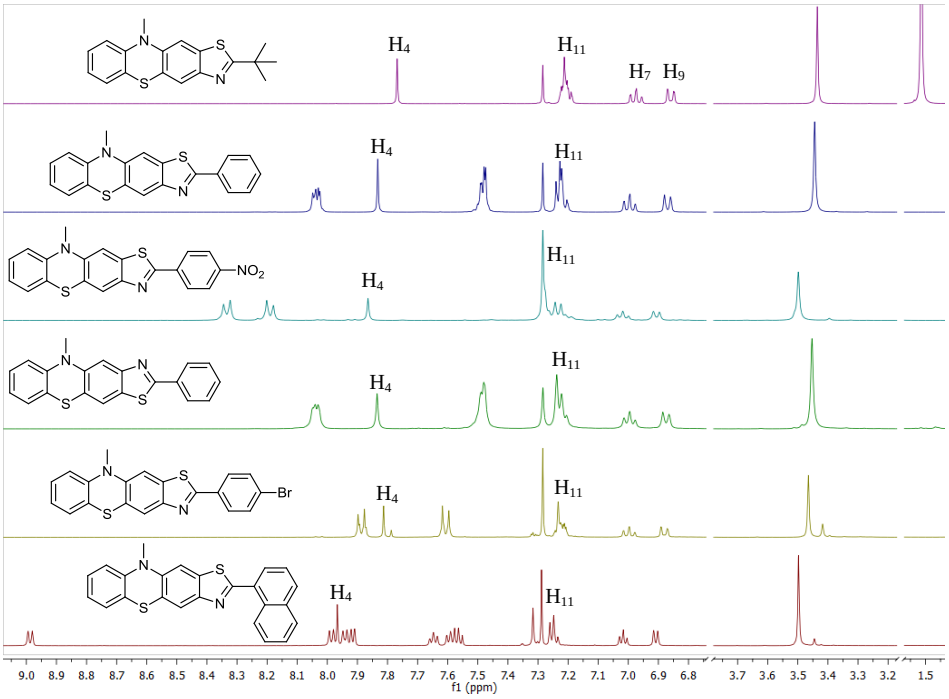


*Figure 17. ^{1}H-NMR of the thiazolo-phenothiazine derivatives **10a-f** at 400 MHz in $CDCl_3$*

3.2.3. In vitro evaluation of phenothiazines inhibitory capacity against leukemia cells proliferation

Cell cultures: HL-60 human promyelocytic- and THP-1 human monocytic leukemia cell lines were treated with the compounds **9a**, **9b**, **9c**, **9e**, **10a**, **10b**, **10c** and **9d** at a dilution series of 1000 μM to 1.25 μM, final concentration in cell suspension.

Cytotoxicity

Among phenothiazine thiones **9a**, **9d** and **9e** displayed cytotoxicity, and within the phenothiazine-thiazole group **10f** showed inhibitory activity against HL-60, but especially against THP-1 cell lines. **9a**, **9d** and **10f** were more effective against THP-1 cell line (lower IC_{50} value), **9e** is somewhat more cytotoxic against HL-60 cells. Some of the studied compounds (**9b**, **9c**, **10b**, **10c**, **10e**) does not exhibit significant growth inhibition nor against tumour, neither against normal cells, while **10a** was active only against HL-60 cells in vitro.

Cellular death mechanisms

The four most efficient compounds were tested for their capacity to trigger the programmed cell death in the leukemia cells. A remarkable apoptotic effect was observed for phenothiazine-thiazole **10f** in THP-1 and HL-60 cell lines. Phenothiazine thiones **9a** and **9d**

were also effective against both cell lines. The parallel measurement of necrotic cells stained with Propidium Iodide (PI) displayed a large amount of necrotic cells in **10a**-treated populations, and **9a** caused necrosis in THP-1 cells. The apoptosis induction capacity of **9d** and **9e**, although inferior to that of **10f** in THP-1 cells (one-way Anova, Bonferroni post-test, $p<0.001$) and at the 8-hour time point even in HL-60 cells, is well balanced by the low proportion of necrotic cells, showing a prevalence to programmed cell death induction.

Metabolic function monitoring

The four most efficient compounds were tested for their capacity to trigger the programmed cell death in the leukemia cells. The THP-1 cells reducing capacity diminished significantly after **9a**, **9d**, **9e**, **10a**, **10e**, and **10f** treatment, in HL-60 population **9e**, **9d**, **10a**, **10b**, and **10f** has the same activity. None of the compound has the capacity to influence the normal leukocytes metabolism, this phenomenon being correlated with no significant reduction of PBMC viability.

4. Porphyrin derivatives

4.1. Porphyrins

Porphyrins are biochemically important, medically useful, and synthetically interesting compounds, can be synthesized but it is important to mention that they already exist in nature with several metals in complex form.

The porphyrin molecules and their metal complexes in different forms are currently utilized in a variety of applications such as medicine (photodynamic therapy)[171,172], nonlinear optics [173,174], nanofabrication[175,176,177], in the coordination chemistry[178], photovoltaics[179,180], and catalysis[181,182].

The structure of porphyrins

The porphyrin macrocycle consists of four pyrrole rings joined by four interpyrrolic methine bridges to give a highly conjugated macrocycle. The simplest porphyrin is called porphine (*Figure 18*) with the chemical formula $C_{20}H_{14}N_4$[186].

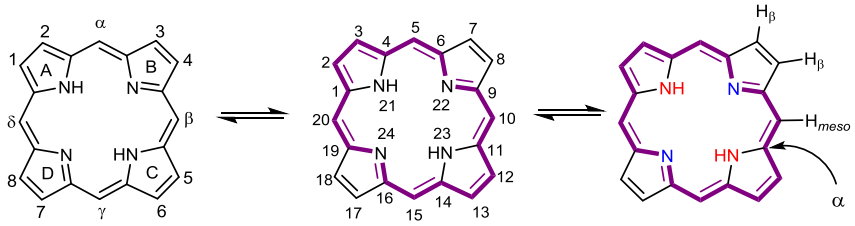


Figure 18. Molecular structure of porphine and its nomenclature

The entire ring system is considered to be planar but with complexation with different metals can be moved out of the plain. Another reason could be related to the substitution of the macrocycle at *β* or *meso* positions by bulky groups. The *β*-substituted porphyrins are very similar to the naturally occurring porphyrins, the *meso*-substituted porphyrins are not found in nature but have wide applications as biomimetic models and as useful components in chemistry.

UV-Vis spectra of the porphyrins

The name porphyrin comes from the Greek word *porphura*, which means violet, as a result of their intense colour. Porphyrins and their derivatives are highly coloured, they presents an interesting and characteristic visible spectrum due to two specific different types of bands (*Figure 19*). For free base porphyrins, we can observe four *Q* bands. While variations of the peripheral substituents on the porphyrin ring often cause minor changes to the intensity and wavelength of the absorption features, protonation of two of the inner nitrogen atoms or the insertion/change of metal atoms into the macrocycle usually strongly change the visible absorption spectrum.

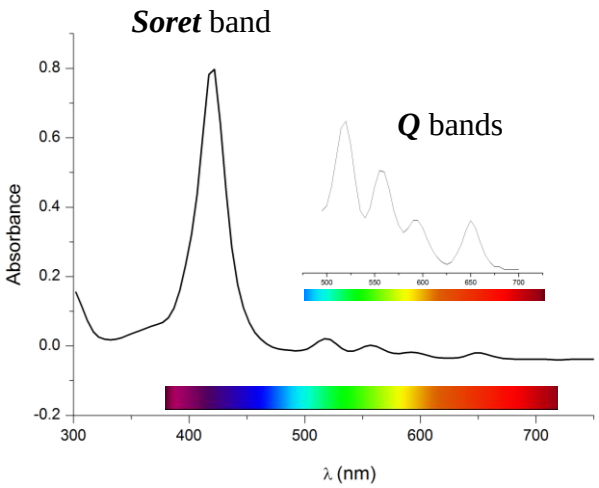


Figure 19. Typical UV Vis absorption spectra of meso substituted porphyrins

Fluorescence spectra of the porphyrins

The fluorescence excitation for free-base porphyrins (*Figure 21*) are close to the absorption maxima, both in the *Soret* and the longer wavelength regions. Usually, the fluorescence emission spectrum shows a distinct major peak at 615 nm and another at approximately 675 nm.

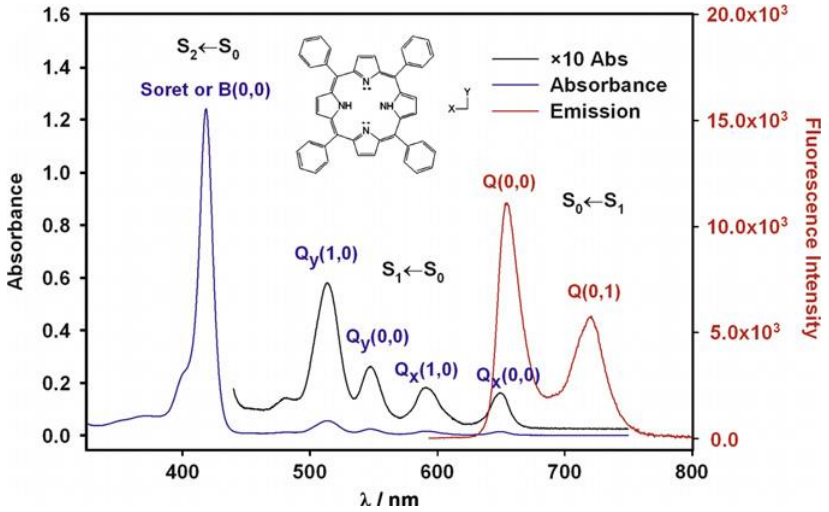


Figure 21. Absorbance and fluorescence emission spectra of the free-base tetraphenylporphyrin

Metal ion chelation affects porphyrin fluorescence dramatically. The heavy metals increase the radiation less decay rate for the inter system crossing to the excited triplet state resulting in a decrease in the fluorescence quantum yield.

NMR study of the porphyrins

The NMR spectrum of the aromatic tetrapyrrole shows anisotropic effects[189]. The ring current generated by the applied field induces a local magnetic field similar to that in benzene (*Figure 22*). The NH protons, inside the porphyrin ring system are therefore shifted upfield around -3 ppm in porphyrins whereas the deshielded *meso* protons appears at very low field (δ~ 10 ppm)[194]. Although the aromaticity of these porphyrin systems makes their NMR spectra a challenge to assign, the tendency toward aggregation in some cases makes for even more complicated spectra[195].

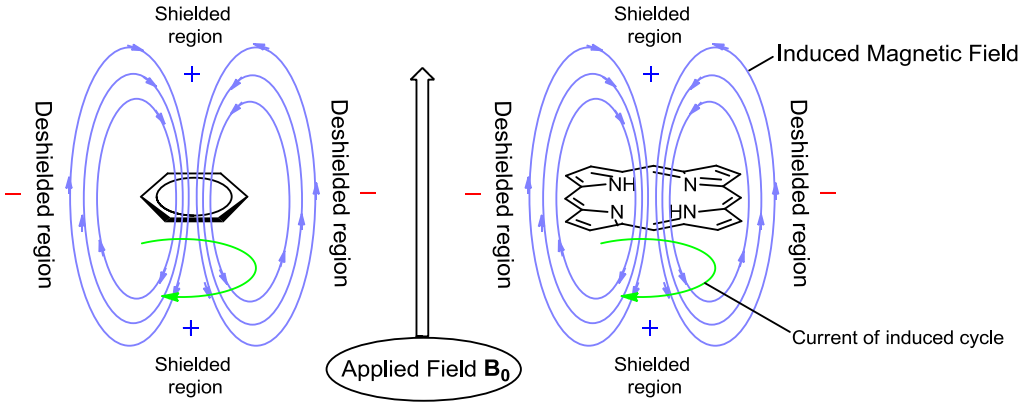


Figure 22. Anisotropi of the porphyrins

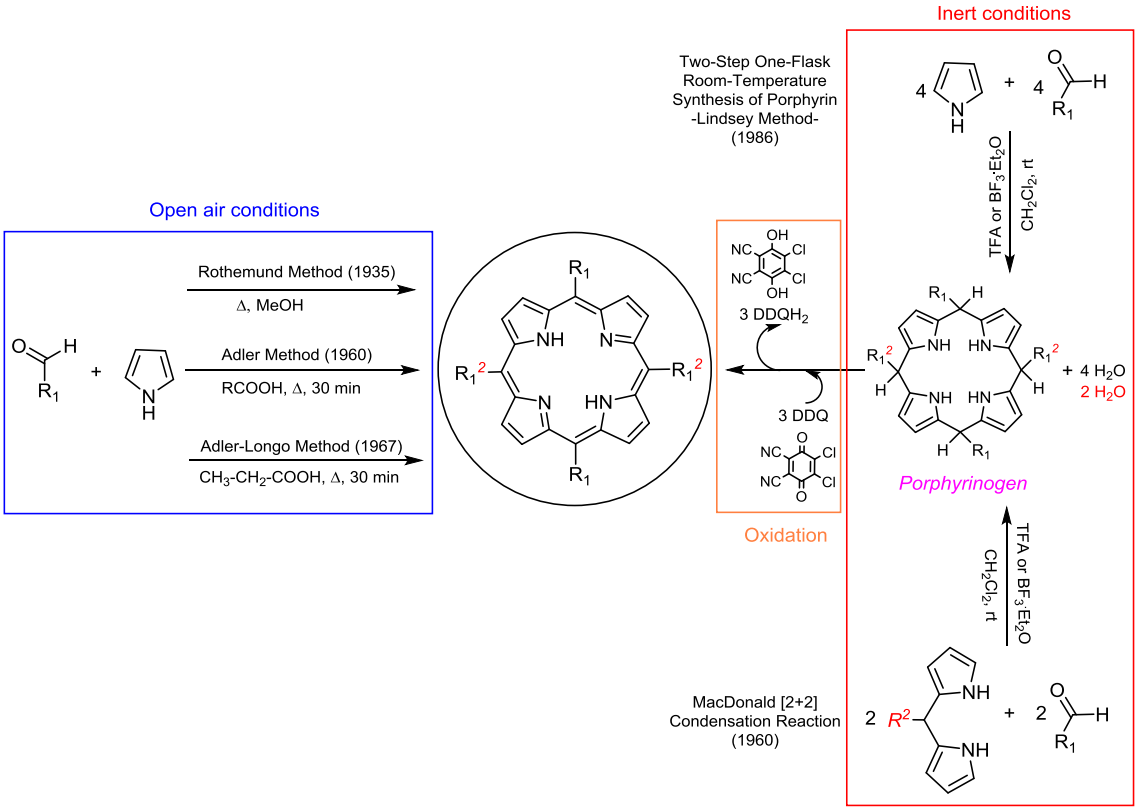
In case of metalated porphyrins, the first thing observed in the NMR spectrum is the absence of the signal at high field (-3 ppm).

The ^{13}C-NMR of a porphyrin could be divided into three different zones: *α* pyrrolic, *β* pyrrolic and *meso* carbons. In case of *α* and *β* pyrrolic carbons there is a problem to detect the corresponding peaks due to NH tautomerism[196]. These signals are clearer at low temperature since NH tautomerism is slow as temperature decreases.

Synthesis of *meso*-substituted porphyrins

Many methods have been reported for porphyrin synthesis (*Scheme 42*). These methods gave porphyrins substituted at *β* and/or *meso* positions with different yields. Every method has its advantages and disadvantages.

After a short reflux of the higher concentrations of aldehydes and pyrrole in propionic acid, porphyrin crystals could be isolated upon cooling of the solution. This synthesis is known as the Adler-Longo method[202].

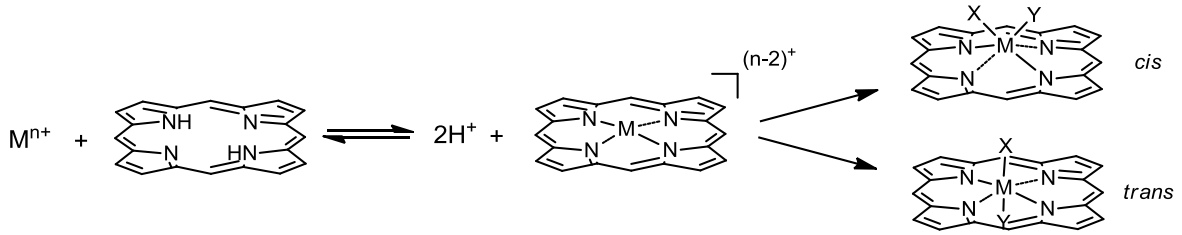


Scheme 42. Synthetic methods to obtain porphyrin macrocycles

4.2. Metallo complexes of porphyrins

Introduction and background

Transition metals play critical roles in biological processes, the majority of the metals from equatorial complexes with porphyrins, and many of this metallocomplexes are capable of binding additional axial ligands (*Scheme 44*). Ion size decreases across the periodic table. This has a propound effect on metalloporphyrin geometry and chemistry: early transition metals tend to be too large to fit in the central cavity so are placed to one side and have additional ligands placed *cis* while late metals tend to fit reasonably well into the plane and so have *trans* axial coordination chemistry. Ion size increases down the periodic table so the lower metals tend to be too large to fit into the cavity and also to give relatively unstable metal complexes.



Scheme 44. Equatorial coordination of the metal, X and Y are ligands.

4.3. Original contributions: Porphyrin derivatives functionalized with Phenothiazine units

The *meso*-phenothiazinyl porphyrins (MPP) described in this work are new chromophore architectures based on arrays of phenothiazine and (hetero)aromatic units directly linked to the porphyrin core at the *meso* position.

Suitable candidates for photoinduced energy transfer systems and fluorescence-emitting materials were obtained by attaching various substituents to the peripheral positions of the porphyrin core. Star shaped *meso*-tetraarylporphyrin with neutral phenothiazine arms extending the linear π-conjugation, showed emission of intense red light with fluorescent quantum yields higher than in the most reported porphyrins.

The selected *meso*-phenothiazinyl porphyrins were evaluated as photosensitizers for photodynamic therapy (PDT), described below, by in vitro irradiation of two human skin cell lines (HaCaT and A431) using red and blue light.

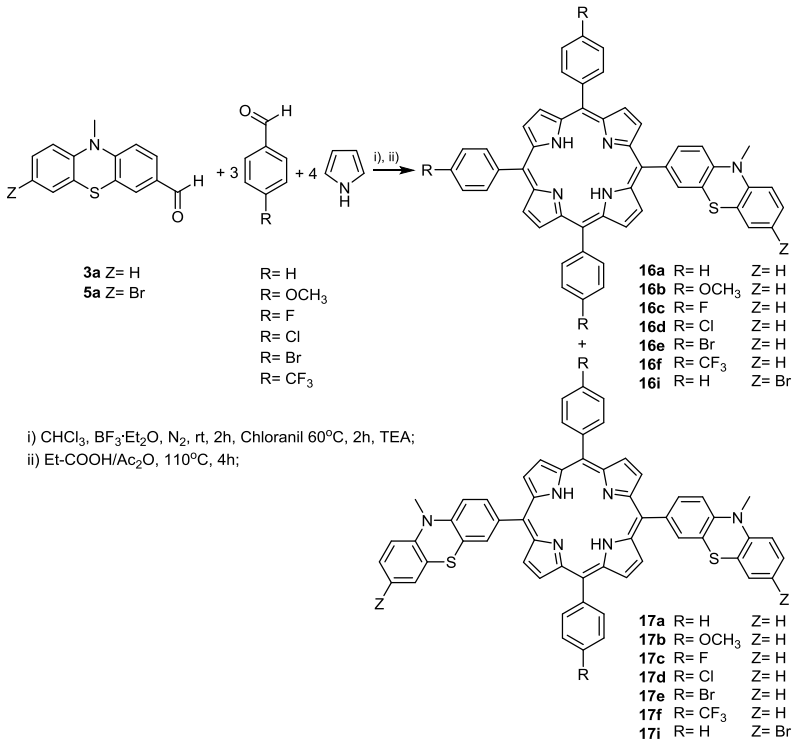
The structures were assigned based on HRMS and NMR spectra. Their optical properties were evaluated according UV-Vis spectroscopic data, which located the absorption maxima around 420 nm (*Soret* band) with molar absorptivities 10^5 $M^{-1}cm^{-1}$ and emission characterized in solution by large *Stokes* shifts (3800-4500 cm^{-1}) and fluorescence quantum yields (Φ_F) ranging from 0.01 to 0.06.

4.3.1. *Meso*-phenothiazinyl-porphyrins (MPP)

Our goal was to develop a convenient procedure for the condensation of 10-alkyl-phenothiazine-3-carbaldehyde with pyrrole. The benefits induced by the variation of solvent (chloroform, acetic or propionic acid), catalyst ($BF_3 \cdot Et_2O$ or TFA) and oxidizing agent (p-chloranil, DDQ, air, or nitrobenzene) were explored by applying the Lindsay[225], Adler[226] or Gonsalves[227] procedures.

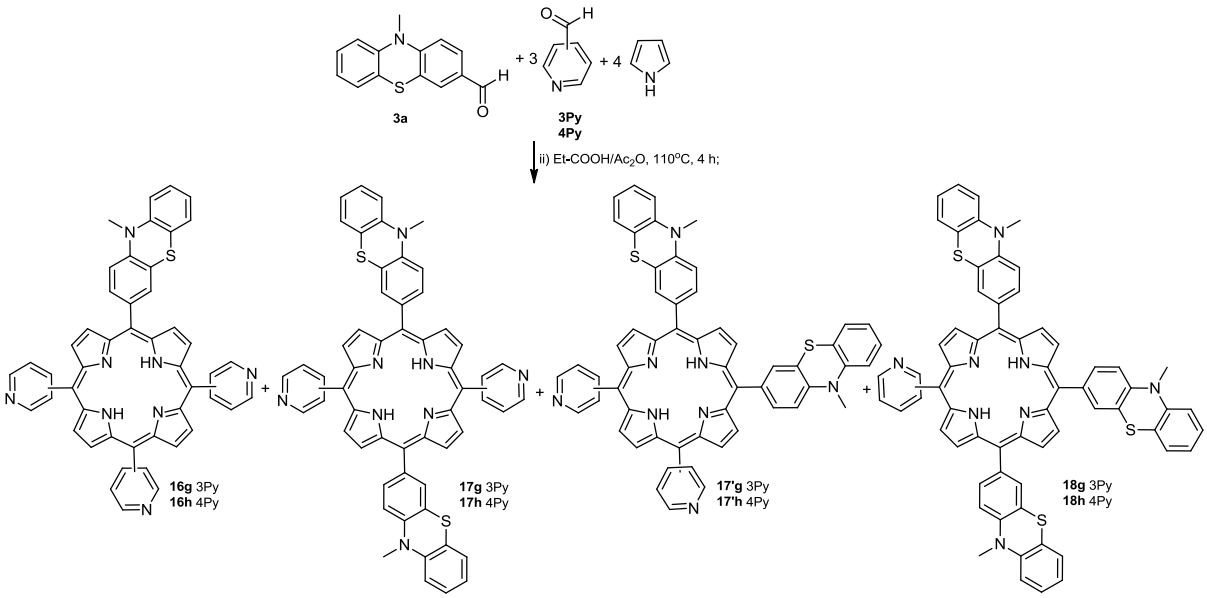
Both the one pot and two step synthetic protocols proved to be successful for the mixed condensation of 10-methyl-phenothiazine-3-carbaldehyde and different *para*-

substituted benzaldehydes with pyrrole (*Scheme 46*, *i, ii*). In each case A_3B **16a-i** and A_2B_2 **17a-i** type porphyrins were obtained as quantitatively isolable products in raw 2:1 M ratio from the obtained porphyrin mixture after repeated column chromatography.



Scheme 46. Synthesis of aryl-MPP by mixed condensation of the aldehydes.

Similar one-pot procedure was used for preparing pyridinyl-phenothiazinyl-porphyrins through refluxing 10-methylphenothiazine-3-carbaldehyde (**3a**), pyridine-3(4)-carbaldehyde (**3Py**, **4Py**) and pyrrole with *in situ* dried propionic acid (*Scheme 47*, *i*). Tris-pyridyl-MPP **16g**, **16h**, bis-pyridyl-MPP **17g**, **17h**, **17'g**, **17'h** and mono-pyridyl-MPP **18g**, **18h** were found in 1(*tris*):2(*trans*):1(*cis*):4(*mono*) M ratio after column chromatography separation of the resulting mixture of porphyrins. All four porphyrins could be separated on a single silica gel column.



Scheme 47. Synthesis of pyridyl-MPP by mixed condensation of the heteroarylaldehydes.

Efficient and convenient conditions for the preparation of *trans*-A_2B_2-porphyrins bearing two phenothiazinyl moieties, via dipyrromethane, using the MacDonald [2+2] condensation reaction from benzaldehyde and heteroaryl-dipyrromethane was achieved. Phenothiazinyl-dipyrromethane **11h** was subjected to the condensation with benzaldehyde in the presence of catalytic amounts of trifluoroacetic acid. The synthesis selectively afforded *trans*-A_2B_2-*meso*-phenothiazinyl-phenyl-porphyrin **17a**, albeit in low overall yield (12%).

Optical properties of MPP

A slight bathochromic shift (9-12 nm) of the *Soret* band (ε= 0.9-3·10^5) responsible for the dark purple colour of all compounds, is observable in the series **16g** (417 nm) **17g** (420 nm) **18g**(424 nm) **19a** (429 nm) illustrated in *Figure 24*, as well as in the series **16a** (420 nm), **17a** (421 nm), **19a** (429 nm), or **16g** (417 nm) **17g** (420 nm) **18g**(424 nm) **19a** (429 nm), respectively and can be directly correlated with the increasing number of electron-donating phenothiazine units in the MPP.

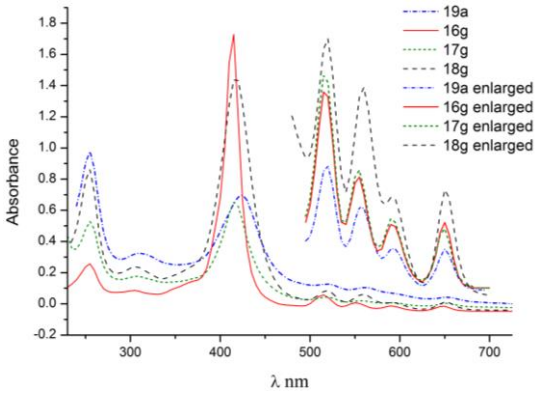


*Figure 24. UV-Vis absorption spectra of MPP **19a**, **16g**, **17g**, **18g** (10^{-4} M in CH_2Cl_2).*

The *para* substitution of the phenyl rings in the MPP **16b-f** and **17b-f** with halogen, electron donor metoxy-, or electron withdrawing trifluoromethyl groups does not produce noticeable effects upon the absorption properties of MPPs.

Quantum yield

By definition, the fluorescence quantum yield (Φ_F) is the ratio of photons emitted to photons absorbed through fluorescence. The most reliable method for recording Φ_F is the comparative method of Williams *et al*[229].

In case of free-base porphyrin systems, the fluorescence quantum yield calibration standard is the *meso*-tetraphenylporphyrin (TPP). The TPP possesses fluorescence quantum yield of 0.12[230] in degassed toluene solution, 0.13[231] in benzene and 0.13[232] in CH_2Cl_2 solution.

A simple ratio of the integrated fluorescence intensities measured under identical conditions of the two solutions will yield the ratio of the quantum yield values. Since Φ_F for the standard sample is known, then it is easy to calculate the Φ_F for the test sample.

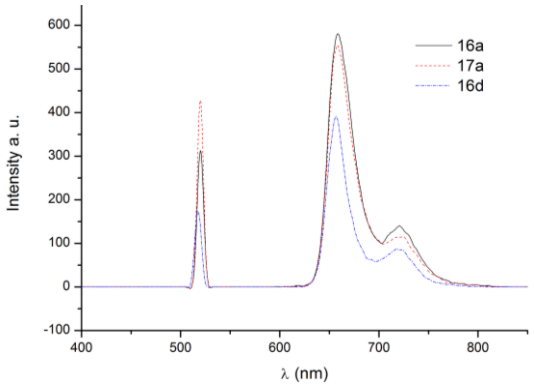


*Figure 25. Excitation and emission spectra of MPP, **16a**, **17a** and **16d** (10^{-5} M in CH_2Cl_2).*

Figure 25 illustrates the emission spectra of MPP **16a**, **17a** and **16d** which displayed the highest emission in solution. The presence of *para* -F, -OCH_3 or -CF_3 substituent in the

phenyl rings diminishes the MPP emission properties, as well as the increasing number of pyridinyl substituents in the MPP fluorophore.

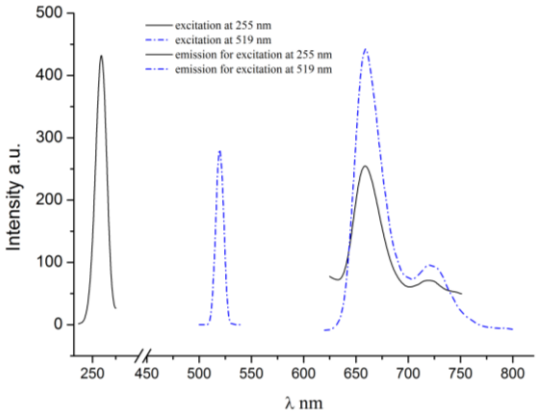
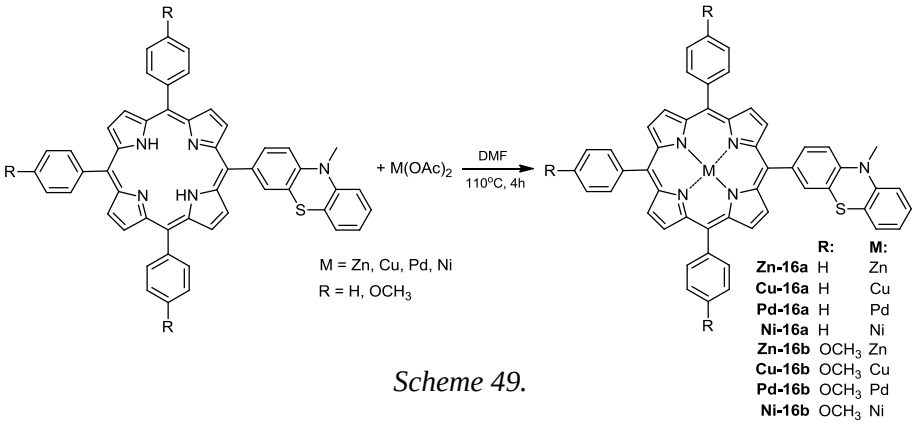


Figure 26. Fluorescence spectra of MPP 16a (10^{-5} M in CH_2Cl_2 solution) excited at 255 nm (black) and 520 nm (blue).

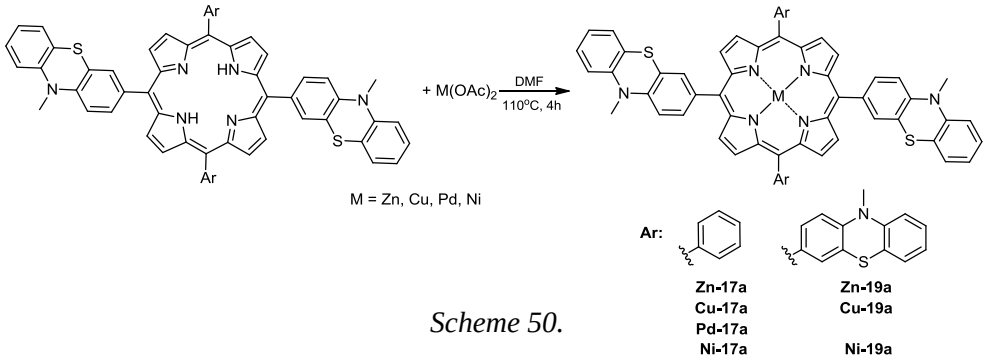
Similar emission maxima were recorded upon excitation with the λ_{max} corresponding to the electronic excitation of the phenothiazine chromophore (255 nm), or the Q_4 absorption band of the porphyrin chromophore (520 nm) thus supporting a large conjugation between the two chromophore units. *Figure 26* shows the emission spectra of **16a** upon excitation at two different wavelengths.

4.3.2. Metallo complexes of *Meso*-Phenothiazinyl-Porphyrins

Incorporation of different metal ions such as Zn(II), Ni(II), Cu(II) or Pd(II) into the central cavity of *meso*-Phenothiazinyl-Porphyrins (MPP) was accomplished by the treatment of the previously reported MPP free base with the appropriate metal(II) acetate in *N*,*N*-dimethylformamide (DMF) solution by adapting a previously reported procedure of Adler and Longo[234]. Thus, metallo-5-phenothiazinyl-10,15,20-*tris*-phenyl-porphyrins **M-16a**, their *p*-methoxy-phenyl analogues **M-16b** (*Scheme 49*), metallo-5,15-diphenothiazinyl-10,20-diphenyl-phenothiazinyl-porphyrins **M-17a** and metallo-5,10,15,20-*tetra*-phenothiazinyl-porphyrins **M-19a** (*Scheme 50*) were obtained in moderate yields and characterized by MS and NMR spectroscopy.



Scheme 49.



Scheme 50.

In the case of zinc, and palladium the formation of metallo MPP was easily confirmed in ^{1}H-NMR analysis by the disappearance of the NH proton signals situated in the range -2.72 to -2.8 ppm illustrated in *Figure 28*. In the aliphatic part the nature of the peaks doesn't change but in the aromatic region, depending on the central metal atom, we can observe little deviation from the free base porphyrin.

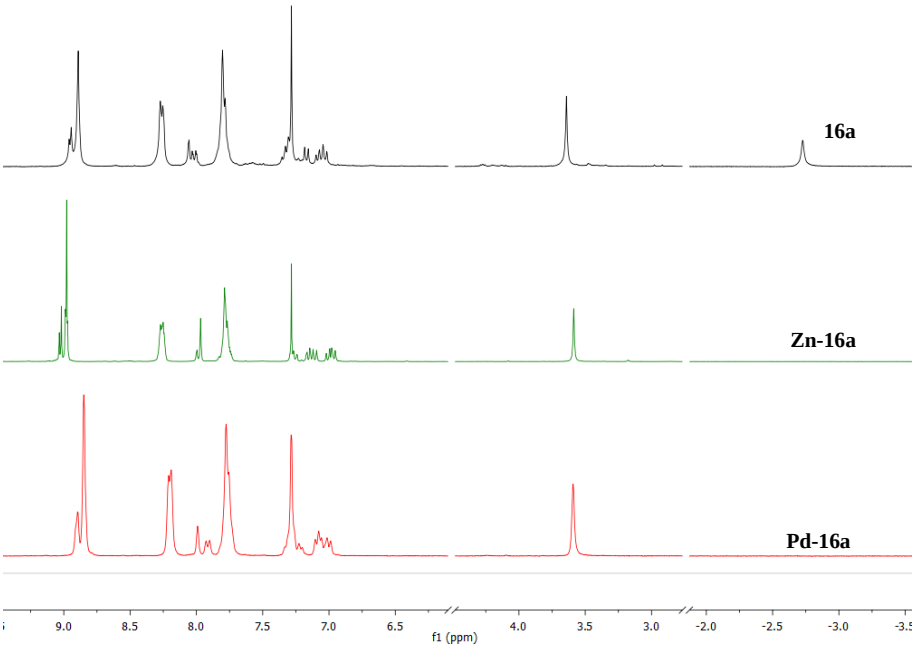


*Figure 28. ^{1}H-NMR segments of **16a**, **Zn-16a** and **Pd-16a** in $CDCl_3$*

The structure of the paramagnetic **Ni-MPPs** and **Cu-MPP**s were identified by HRMS (ESI^+) spectrometry.

Optical properties of the complexes

UV-Vis absorption

As it can be observed from *Figure 31* which presents the UV-Vis absorption spectra of metallo-5-phenothiazinyl-10,15,20-*tris*-*p*-methoxy-phenyl-porphyrins **M-16a,** a more intense and bathochromic shift of the *Q* band occurred for **Zn-16a** and a week *Soret* band was formed in the case of **Pd-16a**.

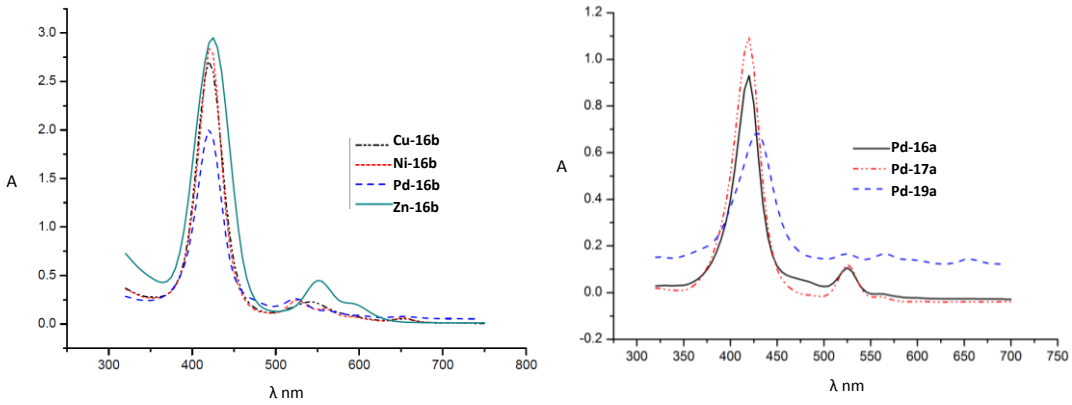


*Figure 31. UV-Vis absorption spectra of metalloMPP 5µM in CH_2Cl_2 **M-16b** (left), b) Pd mono-, di-, tetrakis-phenothiazinyl-phenyl-porphyrins (right)*

In the metalloMPP series containing the same central metal ion the electronic spectra exhibit red shifted maxima for *tetra*-phenothiazinyl or *tris*-*p*-methoxyphenyl substituents attached to the porphyrine core as a consequence of substituents electron donor character. In right figure (*Figure 31*) are depicted the UV-Vis absorption patterns for Pd(II)MPP containing one, two and four phenothiazine substituents respectively attached to the macrocyclic ligand.

Emission in solution and in solid state

Among the prepared metallo MPP, only **Zn-16a**, and **Zn-16b** showed in solution a red-orange day light fluorescence similar to the corresponding free bases, with emissions which occurred upon irradiation with any of the near UV, *Soret*, or *Q* absorption bands respectively as shown in *Figure 32*.

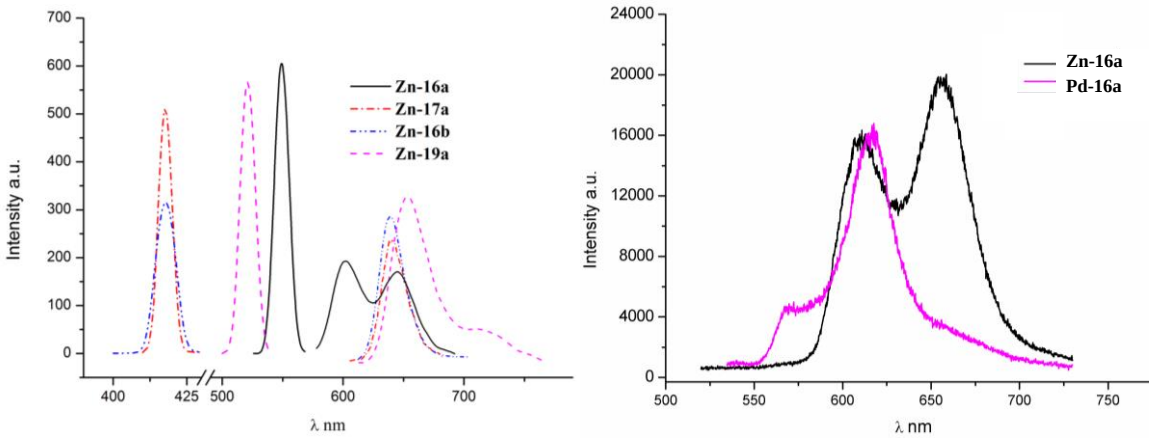


Figure 32. (left) UV-Vis excitation and emission spectra of Zn-MPPs in DCM solution (5μM), (right) Representative one photon excited fluorescence emission spectra of Zn- and Pd-16a in solid-state, λ_{exc} *= 405 nm.*

UV-Vis emission in solid state

The emission spectra of **Zn-16a**, **Zn-17a**, **Zn-19a**, **Pd-16a**, **Cu-16b** in solid-state, crystallites deposited on microscope glass slides, were collected with a confocal *microscope MicroTime 200* upon excitation at 405 nm with a pulsed diode laser. A comparison of the spectra presented in *Figure 32* (left and right) for **Zn-16a** emphasize a similar pattern of the emission spectrum in both solution and powder state, featuring two emission bands located around 610 nm and 658 nm, respectively.

Nonlinear optical properties

Two-photon excited (TPE) fluorescence emission were observed for **Zn-16a**, **Zn-17a**, **Zn-19a**, **Pd-16a**, **Cu-16b** microcrystals deposited on glass substrate when excited with titanium-sapphire (Ti:Sa) femtosecond laser operating at 810 nm, 80 MHz repetition rate and average power of 10 mW. In this work it was found that the investigated metalloMPP microcrystallites are two-photon emissive in the 573-681 nm spectral range under excitation at 810 nm. However, while for **Zn-17a** and **Pd-16a** the same band remains dominant in both *1PE* and *TPE* spectra, in the case of **Zn-16a** we observed a decrease of the 624 nm band compared to 650 nm band when passing from *1PE* to *TPE*. Additionally, we can observe that the *TPE* spectra of **Zn-19a** and **Cu-16b** are significantly blue-shifted compared to *1PE* spectra.

4.3.3. *Meso*-phenothiazinyl-porphirins as sensitizers in Photodynamic therapy experiments

MPP as photosensitizers for photodynamic therapy

In vitro photosensitization experiments were performed on two human skin cell lines: normal keratinocyte HaCaT and epidermoid carcinoma A431 respectively. The cells seeded on well plates were treated with tris-aryl-MPP **16a**, **16c**, **16d**, **16e** and bis-aryl-MPP **17c** in serial dilutions. The cytotoxic effect of treatments was assessed using the spectrophotometric MTT method, which indicated that none of the tested MPP were dark toxic to the two cell lines. The exposure to visible light of the cells treated with MPP led to some cytotoxic effects.

The cells lines treated with MPP were irradiated for 30 min with red light and for 5 s with blue light respectively. Bis-aryl-MPP **17c** had a slight photodynamic effect on A431 cells when exposed to blue light, but this effect was not statistically significant. MPP **16d** and **17c** showed no photosensitizer properties upon irradiation with neither blue or red light. Compounds **16a**, **16c** and **16e** showed variable photodynamic effects in correlation with the concentrations, cell lines and irradiation wavelengths employed.

5. General conclusions

The present doctoral thesis deals with the synthesis, structure characteristics of 124 compounds, from various classes, including:

- ✓ phenothiazine carbaldehyde regioisomers and functionalized phenothiazine-3-carbaldehydes
- ✓ aryl- and phenothiazinyl-dipyrromethanes
- ✓ phenothiazinyl-dipyrrine containing homoleptic transitional metal complexes
- ✓ phenothiazinyl-1,9-diacyldipyrromethane containing tin complexes
- ✓ phenothiazinyl-bis(indolyl)methanes
- ✓ phenothiazinyl-amides, -thioamides , -benzothiazoles
- ✓ *meso*-phenothiazinyl-porphyrins (MPP) and their metal complexes (Metallo-MPP)

From the 124 compounds, 96 were obtained and characterized for the first time in this work. The synthesis of phenothiazine carbaldehyde regioisomers were optimized, **3g**, **3h**, **3j**, **4a**, **4j** were obtained in moderate to high yields by a two-steps procedure involving a phenothiazine-lithium intermediate further trapped by the treatment with dimethylformamide electrophile. Phenothiazine-3-carbaldehydes (**3a-f**) were easily available by Vilsmeier-Haack formylation of the N-alkyl-phenothiazines (**2a-f**). From **3a**, **3b** after halogenation with elemental bromine in acidic media unsymmetrically functionalized phenothiazines (**5a**, **5b**) were obtained in god yields.

(Aryl/phenothiazinyl)-dipyrromethanes (**11a-p**) were obtained in moderate yields, by condensation of excess pyrrole with the corresponding (aryl/phenothiazinyl)-carbaldehydes in dichloromethane under inert conditions to avoid any degradation of the products. Dipyrromethanes **3e-p** were synthesized and characterized for the first time.

The new phenothiazinyl-dipyrrin **12** was obtained in moderate yield by oxidation of **11h** with chloranil. Dipyrromethene **12** forms unique metal complexes with various metal acetates. A one-flask synthesis of bis(dipyrrinato)metal(II) complexes **13-Cu**, **13-Ni**, **13-Pd**, **13-Zn** were developed by oxidation of the dipyrromethane **11h** with p-chloranil in the presence of $M^{(II)}(OAc)$.

Two methods (Grignard, Vilsmeier) were examined for the direct 1,9-diacylation of the dipyrromethane **11h**. To facilitate isolation of phenothiazinyl-1,9-diacyldipyromethane (**14a**, **14b**), the crude acylation mixture were treated with triethylamine and dialkyltin dichloride (R'_2SnCl_2, R'= Me, Bu) to afford **14a-SnR'$_2$, 14b-SnR'$_2$** in moderate yields.

A new series of phenothiazinyl-bis(indolyl)methanes (**15a**, **15g**, **15h**, **15i**, **15j**, **17a**) have been synthesized by condensation of indole and regioisomer phenothiazinyl-aldehydes in acetonitrile containing catalytic amount of sodium bisulphate.

A new series of phenothiazinyl-thiobenzanilides **9a-f** were synthetized via thionation with Lawesson reagent from the corresponding phenothiazinyl-benzamides **8a-f**. The phenothiazinyl-benzamides were synthesized and characterized for the first time in this study. Cyclizations of phenothiazinyl-thiobenzanilides through C-H functionalization / intramolecular C-S bond formation process, afforded the new thiazolo[5,4]phenothiazine derivatives **10a-f**. A simple and efficient method was developed for the synthesis of new 2-phenothiazinyl-1,3-benzothiazoles (**6a-c**, **6g-j**) from the phenothiazinyl-carbaldehyde and 2-thioaniline in refluxing DMSO. HL-60 human promyelocytic- and THP-1 human monocytic leukemia cell lines were treated with the compounds **9a**, **9b**, **9c**, **9d**, **9e**, **10a**, **10b**, **10c**, **10e** and **10f**. Phenothiazine thiones **9a**, **9d** and **9e** and from the phenothiazine-thiazole series **10a** proved in vitro efficiency against leukemia cell lines, selectively, consequently they present prodrug potential in leukemia, and they have good perspectives to be applied in solid tumours too.

Convenient one-pot procedures were developed for the statistical synthesis of new *meso*-phenothiazinyl-porphyrins **16a-i** and **17a-i**. All MPPs showed red-orange daylight fluorescence. In vitro PDT experiments demonstrated that **16a** showed increased phototoxicity when activated by red light and **16e** had also phototoxic effects, but only when activated by blue light and it showed a higher selectivity against tumour cells.

Incorporation of transition metal ions (Zn, Ni, Cu, Pd) into the central cavity of *meso*-Phenothiazinyl-Porphyrins (MPP) was accomplished by the treatment of the free base porphyrine with the appropriate metal(II) acetate in DMF. The variable number of peripheral phenothiazine units exhibited one photon, two photons, Vis- absorption and fluorescence emission properties in the visible spectral range finely tunned by the *d*-shell occupancy of the central metal atom. **Pd-16a** and **Cu-16b** were found as promising candidates for efficient optical limiting at the wavelength 780 to 840 nm under excitation.

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