N-alkylphenothiazines – synthesis, structure and application as ligands in metal complexes

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Abstract

Phenothiazines are a large group of heterocyclic, aromatic molecules with nitrogen and sulphur between two benzene rings. Their derivatives, *N*-alkylphenothiazines have substituent on heterocyclic nitrogen atom which gives them different properties. Also, a series of these molecules have substitution on carbon atom at place 2 of phenothiazine benzene ring. Alkylphenothiazines contain aminoalkyl substituent and their alkyl, acyl and sulphonil derivatives, as well as monocyclic and bicyclic heterocycles attached at thiazine nitrogen atom or directly linked to benzene ring. The *N*-alkylphenothiazines have been known as antipsychotic drugs, but they also possess antibacterial, antifungal, anticancer activity, and ability to react with macromolecules and to coordinate to the metals. Metal complexes with *N*-alkylphenothiazines are biological active compounds with different antimicrobial activities and cytotoxic effect against tumor cell lines. The large field of application of *N*-alkylphenothiazines is very attractive in terms of synthesis of new related derivatives, metal complexes, studying their properties and applications. This article presents a review of the literature and a contemporary view at *N*-alkylphenothiazines – their synthesis and application, as well as their metal complexes which have promising biological effects.

Keywords: *N*-alkylphenothiazines, antipsychotic drugs, anticancer, antibacterial.

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The *N*-alkylphenothiazines are heterocyclic aromatic compounds with nitrogen and sulphur atoms and substituents on aromatic ring and nitrogen. The central place of these molecules is a molecule of 10*H*-dibenzo-1,4-thiazine, phenothiazine (Fig. 1) known more than a hundred years [1].

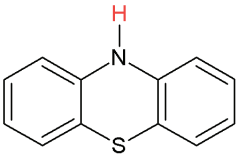

Figure 1. Structure of the 10-H-dibenzo-1,4-thiazine, phenothiazine.

The first phenothiazines, thionine and methylene blue (Fig. 2) were synthesized in reaction of *p*-phenylendiamine/*p*-aminodimethylaniline with sulphur in hydrochloric acid solution, in the middle of the 19th century [2]. In that time, these compounds with phenothiazine ring had an application in aniline dye industry.

After 20 years, the parent compound, 10*H*-dibenzo-1,4-thiazine was obtained in reaction of diphenylamine with sulphur by Bernthsen (Scheme 1). Until today, more than 5000 phenothiazine derivatives were synthesized with wide range of application [1].

Phenothiazines have many different applications, from industry to medicine. As biological active compounds, these molecules have antipsychotic, antiemetic, anthelmintic, antibacterial, antifungal and insecticidal properties, and they also represent promising anticancer drugs [1–8]. These activities represent the result of the interaction of phenothiazines with biological systems through their pharmacophoric substituent, the multicyclic ring system (π-π interaction, intercalation in DNA) or the lipophilic character allowing the penetration through the biological membranes. The activities were examined by using various biological systems such as cell lines, bacteria, viruses, parasites, laboratory mice, rats and rabbits, and monolayer and bilayer membranes [2]. The *N*-alkylphenothiazines as ligands are used in metal complexes, but biological properties of these complexes are investigated in recent years. This review shows current importance of the *N*-alkylphenothiazines and reveals their metal complexes as potent pharmacological group which can be a rich source of new compounds having desirable biological activities.

Structure of *N*-alkylphenothiazines

Most often, phenothiazines have substituted hydrogen atoms at position 2 (with some small molecule)

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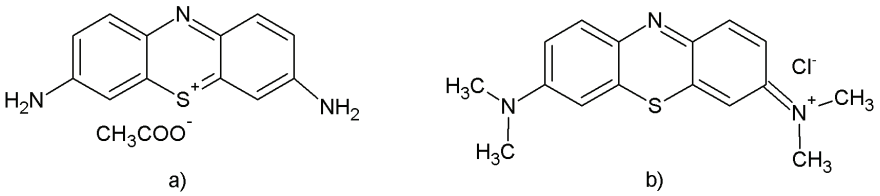
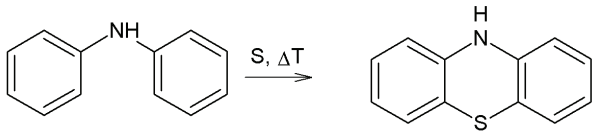


Figure 2. Structure of the 3,7-diamino-5-phenothiazinium acetate (thionine) and 3,7-bis(dimethylamino)phenazathionium chloride (methylene blue).



Scheme 1. Synthesis of 10-H-dibenzo-1,4-thiazine (phenothiazine).

and position 10 (with aminoalkyl groups). At this point, *N*-alkylphenothiazines (Figure 3) are classified into three groups depending on the type of the substitution on the nitrogen atom of phenothiazine ring [9,10]. First group contains phenothiazines with an aliphatic side chain, then the second group with the piperidine, and the third group contains piperazine derivatives (Table 1).

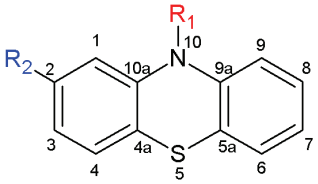


Figure 3. General structure of N-alkylphenothiazine.

Table 1. N-alkylphenothiazines classification depends on the type of substituent on N-10 and C-2 atoms

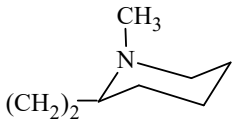
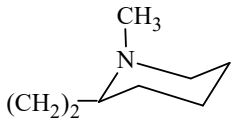
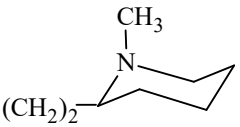
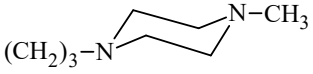
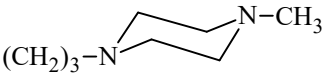
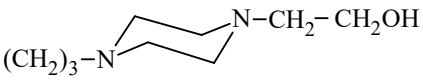
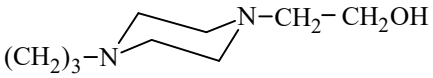
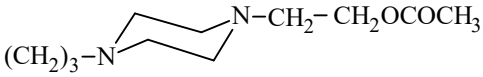
Compound	R_1	R_2
Phenothiazines with an aliphatic side chain		
Promazine (Pr)	$(CH_2)_3$–$N(CH_3)_2$	H
Chlorpromazine (Cpz)	$(CH_2)_3$–$N(CH_3)_2$	Cl
Triflupromazine (Tm)	$(CH_2)_3$–$N(CH_3)_2$	CF_3
Methotrimeprazine (Mtm)	CH_2–$CH(CH_3)$–$N(CH_3)_2$	OCH_3
Phenothiazines with a piperidine side chain		
Thioridazine (Tr)		$S(CH_3)$
Mesoridazine (Mr)		$SO(CH_3)$
Sulphoridazine (Sr)		$OSO(CH_3)$
Phenothiazines with a piperazine side chain		
Prochlorperazine (Pc)		Cl
Trifluoperazine (Tf)		CF_3
Perphenazine (Pz)		Cl

Table 1. Continued

Compound	R_1	R_2
	Phenothiazines with a piperazine side chain	
Fluphenazine (Fz)		CF_3
Thioprozapate (Tp)		Cl

The new derivatives of phenothiazines are synthesized from the parent phenothiazines in several directions: substitution of new groups with the thiazine nitrogen atom [11], an introduction of a new substituent at positions 1–4 and 6–9 aromatic rings, oxidation of the sulfide sulfur atom to a sulfoxide or sulfone group, as well as a substitution one or both benzene ring with homoaromatic or heteroaromatic rings [1].

Synthesis of *N*-alkylphenothiazines

Promazine, *N*,*N*-dimethyl-3-(10*H*-phenothiazin-10--yl)-propan-1-amine, is synthesized by the alkylation of phenothiazines with 3-dimethylaminopropylchloride in the presence of sodium amide (Scheme 2) [12].

Chlorpromazine, 3-(2-chloro-10*H*-phenothiazin-10--yl)-*N*,*N*-dimethyl-propan-1-amine is prepared following two steps: in the first step, 3-chlorodiphenylamine with sulphur gives as product 2-chloro-10*H*-phenothiazine, while in the second step, this compound reacts with 3-dimethylaminopropylchloride, similar to the synthesis of promazine (Scheme 3) [12].

Trifluopromazine, *N*,*N*-dimethyl-3-[2-(trifluoromethyl)-10*H*-phenothiazin-10-yl]propan-1-amine (Fig. 4) is also prepared by alkylation of 2-trifluoromethyl--10*H*-phenothiazine in the presence of sodium amide [12].

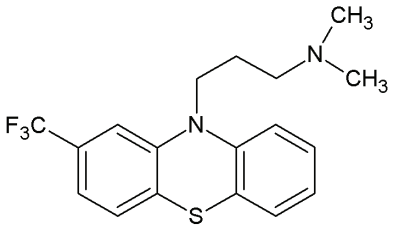
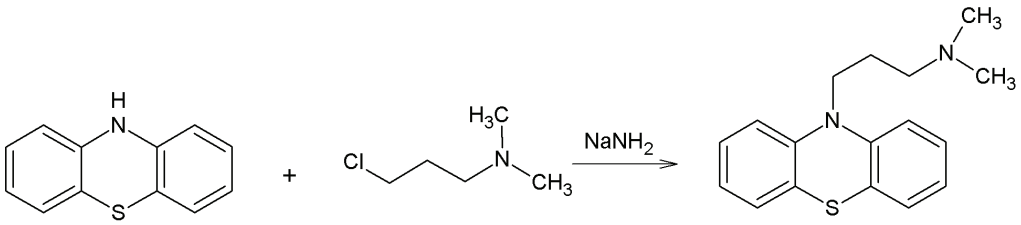


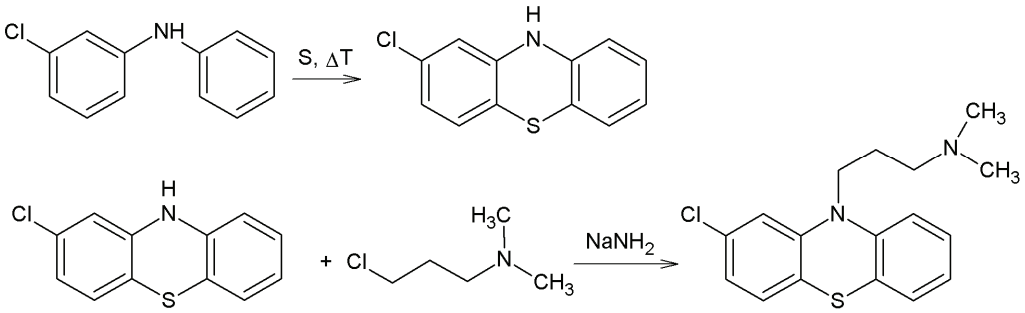
Figure 4. Structure of the N,N-dimethyl-3-[2-(trifluoromethyl)--10H-phenothiazin-10-yl]propan-1-amine, (trifluopromazine).

A series of molecules has substitution on carbon atom at position 2 of phenothiazine benzene ring of promazine as methopromazine (possess methoxy group) and acetopromazine (with aceto group on C-2 atom) which are synthesized in similar way.

Phenothiazines with a piperidine side chain, thioridazine 10-[2-(1-methyl-2-piperidyl)ethyl]-2-(methylthio)-10*H*-phenothiazine and mesoridazine 10-[2-(1--methyl-2-piperidyl)ethyl]-2-(methylsulfinyl)-10*H*-phenothiazine are also synthesized by alkylation of methylsulphonil-10*H*-phenothiazine/2-methylthio-10*H*-pheno-



Scheme 2. Synthesis of N,N-dimethyl-3-(10H-phenothiazin-10-yl)-propan-1-amine(promazine).

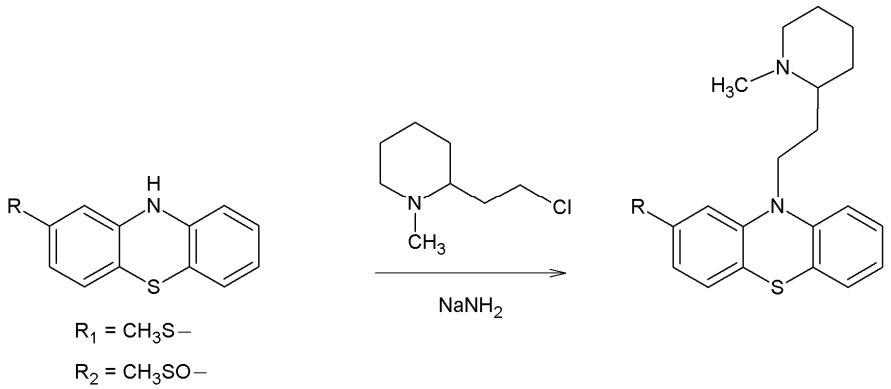


Scheme 3. Synthesis of 3-(2-chloro-10H-phenothiazin-10-yl)-N,N-dimethyl-propan-1-amine (chlorpromazine).

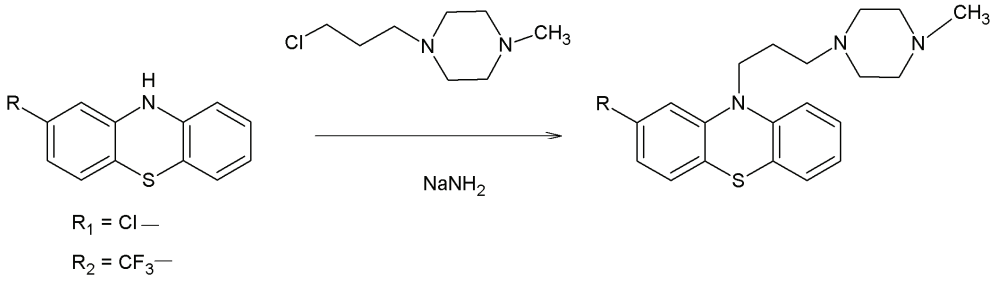
thiazine using 2-(2-chloroethyl)-1-methylpiperidine in the presence of sodium amide (Scheme 4) [12].

At last, phenothiazines with piperazines chain, prochlorperazine, 2-chloro-10-[3-(4-methyl-1-piperazinyl)-propyl]-10*H*-phenothiazine and trifluoperazine, 10-[3--(4-methylpiperazin-1-yl)propyl]-2-(trifluoromethyl)--10*H*-phenothiazine are prepared by alkylation of 2-chloro-10*H*-phenothiazine/2-trifluoromethyl-10*H*-phenothiazine using 4-methyl-1-piperazinylpropylchloride in presence of sodium amide (Scheme 5) [12].

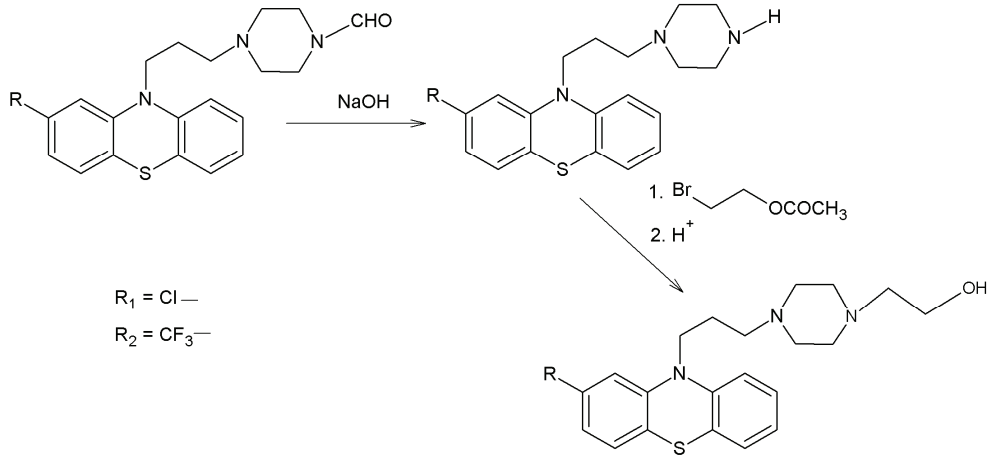
Perphenazine, 2-[4-[3-(2-chloro-10*H*-phenothiazin--10-yl)propyl]piperazin-1-yl]ethanol, and fluphenazine, 2-[4-[3-(2-(trifluoromethyl)-10*H*-phenothiazin-10-yl)propyl]piperazin-1-yl]ethanol, are synthesized in a similar way as previously described. In the first step, 2-chloro--10*H*-phenothiazine/2-trifluoromethyl-10*H*-phenothiazine is alkylated using 4-formyl-1-piperazinylpropylchloride in the presence of sodium amide and then *N*-formyl group was removed by alkaline hydrolysis giving the intermediate product (Scheme 6). The final step is alkylation of intermediate compound by 2-bro-



Scheme 4. Synthesis of 10-[2-(1-methyl-2-piperidyl)ethyl]-2-(methylthio)-10H-phenothiazine (thioridazine, R_1) and 10-[2-(1-methyl-2--piperidyl)ethyl]-2-(methylsulfinyl)-10H-phenothiazine (mesoridazine, R_2).



Scheme 5. Synthesis of 2-chloro-10-[3-(4-methyl-1-piperazinyl)propyl]-10H-phenothiazine (prochlorperazine, R_1) and 10-[3-(4-methyl-piperazin-1-yl)propyl]-2-(trifluoromethyl)-10H-phenothiazine (trifluoperazine, R_2).



Scheme 6. Synthesis of 2-[4-[3-(2-chloro-10H-phenothiazin-10-yl)propyl]piperazin-1-yl]ethanol (perphenazine, R_1) and 2-[4-[3-[2-(trifluoromethyl)-10H-phenothiazin-10-yl]propyl]piperazin-1-yl]ethanol (fluphenazine, R_2).

methanol-1-acetate by acid hydrolysis which removes acetyl group [12]. Also, these molecules contain an alcohol group which provides an opportunity for derivatization in order to obtain new compounds with pharmacological using.

Use and application

The phenothiazine group of compounds has the great spectrum of application from industry to medicine for more than a century due to their diverse syntheses. Substituted phenothiazines have been used for the preparation of the polymers [13], the solar cells [14,15], biological stains [16], as well as medicaments [12]. They possess antimicrobial [6,21–24], anthelmintic [7], antiemetic [8], antihistaminic [8] anticancer properties [2], and they are used in clinical practice more than 50 years as antipsychotics [12,17].

In many researches, it was shown that *N*-alkylphenothiazines possess antimicrobial activity [18–20]. Kristiansen *et al*. [18] demonstrated that chlorpromazine and thioridazine reduce the susceptibility of methicillin-resistant *Staphylococcus aureus* (MRSA) while Martins *et al*. [19] in their research classified a few *N*-alkylphenothiazines as non-antibiotics (helper compounds) in the treatment of multidrug resistant Gram-negative infections. As anthelmintics, phenothiazines reduced faecal eggs of resistant strains of three nematodes*, Haemonchus contortus, Trichostrongylus colubriformis* and *Ostertagia spp.*, but without significant effect on worm number [7].

Phenothiazines have been shown to inhibit *in vitro* growth of multi drug resistance strain (resistant to rifampin and isoniazid) *Mycobacterium tuberculosis*. The killing of intracellular bacteria promoted by phenothiazine can inhibit many transport processes of potassium and calcium pump activation. The increased permeability ensures phenothiazine molecules to reach the DNA, intercalate between the bases, inhibit the entire DNA and hence inhibit transcription and translation processes [21]. The ability of chlorpromazine to cure tuberculosis resulted in a series of *in vitro* studies that showed that chlorpromazine was more effective antimycobacterial compound than other *N*-alkylphenothiazines [21–24]. Because chronic administration of chlorpromazine is known to produce a wide range of mild-to-severe side effects, the use of this compound in tuberculosis therapy was not seriously considered. Thioridazine can more effectively accelerate the recovery of infected mice from *Mycobacterium tuberculosis,* as shown in preliminary study [24], providing strong direct evidence that thioridazine, a neuroleptic that is milder and less toxic than chlorpromazine, kills intracellular *Mycobacterium tuberculosis* isolates that are resistant to two or more antibiotics [22]. These investigations indicate that phenothiazines have been considered as an adjuvant for the treatment of *Mycobacterium tuberculosis.*

The blockade of histamine receptors in brain gives sedative effect to the most of *N*-alkylphenothiazines. Prochlorperazine, promethazine and perphenazine are known as antipsychotic drugs which block postsynaptic dopaminergic receptors in the brain and have antiemetic effects by their antagonic actions in the dopamine receptors (D2) predominantly, but also may possess antagonist actions at histamine 1, cholinergic M1 and α_1-adrenergic receptors in the vomiting center to reduce nausea and vomiting [8]. Promethazine hydrochloride is in pharmacological use as Phenergan cream, topical antihistaminic preparation for systemic eczematous dermatitis.

Phenothiazines as lipophylic molecules are interacting with various macromolecules and possess anticancer activity. Chlorpromazine, promethazine, thioridazine and trifluoperazine, in clinically relevant plasma concentrations 2–36 μM, markedly decreased the viability of leukemic cells without any toxic effects on normal lymphocytes [25]. Phenothiazines show antiproliferative activity against breast, ovarian, lung, CNS, prostate and melanoma cell lines. Trifluoperazine, chlorpromazine and thioridazine in concentration up to 20 μM expressed a selective cytotoxicity, as well as antiproliferative activity and induced apoptosis [4]. Also, *N*-alkylphenothiazines have the capacity to increase the lethal effect at low doses of the therapeutic drugs [26]. Cytotoxicity of these compounds could be attributed to oxidative stress, *via* glutathione oxidation and lipid peroxidation.

The most common application of phenothiazines is in psychiatry [10,17]. Modern psychopharmacology cannot be imagined without chlorpromazine. Chlorpromazine was synthesized in 1951 by Paul Charpentier, and became available on prescription in France in 1952, under the proprietary name of Largactil as pharmaceutical drug [17]. In medical practice, chlorpromazine is used in the treatment of patients with schizophrenia, neurosis, manic-depressive condition and chronic paranoids, alcoholic psychoses as well as for stress-sampled neuroses, insomnia and fears. Promazine and triflupromazine have similar application. Trifluoperazine is one of the most active antipsychotic drugs with common synonyms in pharmacy as stelazine, triftazin and others. This drug has strong anticonvulsant activity and it is used in treatment for schizophrenia and other mental diseases. Mesoridazine has application for behavior problems, schizophrenia, chronic alcoholism and other. Phenothiazines with aliphatic side chain and piperidine substituent have more pronounced sedative effect than piperazine derivatives. These molecules are non-selective and competitive D_1 and D_2 antagonists that block dopamine activity at corresponding receptor

site. Also, their activity could be expressed by blocking α-adenoreceptors, serotonine, cholinergic, nicotine and muscarinic receptors [12].

In the aim of finding new applications of these compounds, they are used as ligands in coordination chemistry. Until today many complexes containing *N*-alkylphenotiazines are synthesized and characterized. Gowda and his group [27,28] prepared Ir(III) and Pd(II) complexes with *N*-alkylphenothazines and proposed heterocyclic S atom coordination. Keshavan *et al*. synthesized many complexes of lanthanide with *N*-alkylphenothiazines indicating that the tertiary nitrogen, as well as phenothiazine's nitrogen, represents the coordination site of ligand [29,30]. Also, complexes of lanthanide showed moderately antibacterial activity against some Gram-positive and Gram-negative bacteria. The structure of lanthanide complexes with phenothiazine was determined by X-ray single crystal diffraction analysis (Fig. 5) displaying lantanide- nitrogen bond [31].

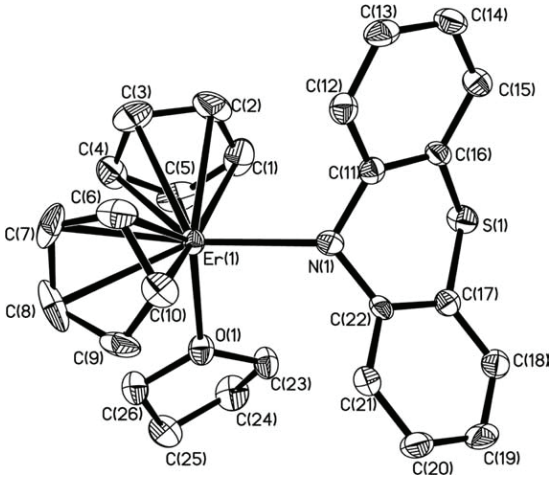


Figure 5. ORTEP diagram of complex (C_5H_5)2ErPtz(THF) [22].

The complex $HgCl_2(ptz)]_2{\cdot}HgCl_2$ was synthesized by reaction of $HgCl_2$ and phenothiazine in benzene as dark-blue powder [32]. The final products were characterized by X-ray diffraction which confirmed S-coordination of phenothiazine (Fig. 6).

A series of conjugated polymers with bipyridine groups were synthesized successfully *via* Suzuki coupling polycondensation using carbazole and phenothiazine as the main chain moieties. Through a one-pot post-functionalization reaction, ruthenium complexes were introduced into the polymer through a flexible spacer in high yields, which lead to a new simplified strategy to synthesize this special type of conjugated polymer with metal complex linked to pendant groups through a flexible chain. Furthermore, the obtained Ru-containing polymers PM1PT-x-Ru exhibited broad absorption in the visible region, and possessed a narrow electrochemical band gap (approximately 1.36–1.42 eV) and low HOMO energy level (about 5.47–5.34 eV), indicating that they are suitable candidates as photosensitive materials for solar cells [33].

Platinum complexes with chlorpromazine and trifluoperazine were prepared from $K_2[PtCl_6]$ in water (Scheme 7). Complexes were characterized by spectral (IR, ^1H, ^{13}C, 2D ^1H–^{13}C heteronuclear correlation spectra, ^{195}Pt NMR and MS) analysis. Outer-coordination sphere was proposed for ((TFH·HCl)$[PtCl_5H_2O]$), while in ((CP·H)$[Pt(CP{\cdot}HCl)Cl_5]{\cdot}H_2O$), the ligand was coordinated to the metal *via* heterocyclic sulphur. The complexes exhibit antibacterial effect on strains of *Bacillus subtilis, Bacillus cereus, Bacillus pumilus* and methicillin-resistant *Staphylococci* as Gram-positive bacteria, and an *Escherichia coli* as Gram-negative bacteria, as well as the reference strains [34].

Ru(II) complexes with *N*-alkylphenothiazines

Three new complexes of the general formula $L[RuCl_3(DMSO)_3]$ (**1**–**3**), where L is chlorpromazine hydrochloride, trifluoperazine dihydrochloride or thioridazine hydrochloride, were prepared by the reaction of the starting complex $[RuCl_2(DMSO)_4]$ and the corresponding ligands in a mole ratio of 1:1.6 in absolute ethanol [35]. The complexes were characterized by elemental analysis and spectroscopic methods. The crystal structure of the complex (TF.H2)$[RuCl_3(DMSO)_3]Cl{\cdot}C_2H_5OH$ contains trifluoperazine dihydrochloride in outsphere position, but its chloride atom had substituted one of DMSO molecules in starting complex and coordinated to the Ru(II) atom (Fig. 7).

The investigation of antitumor properties of complexes **1**–**3** in four human cell lines (MCF-7, MDA-MB-453, SW-480 and IM9) showed dose-dependence and

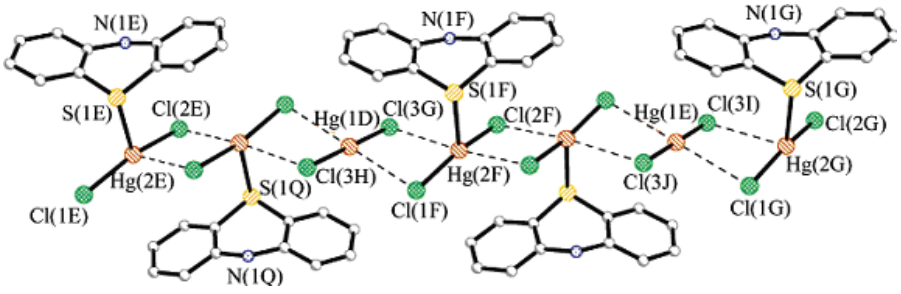
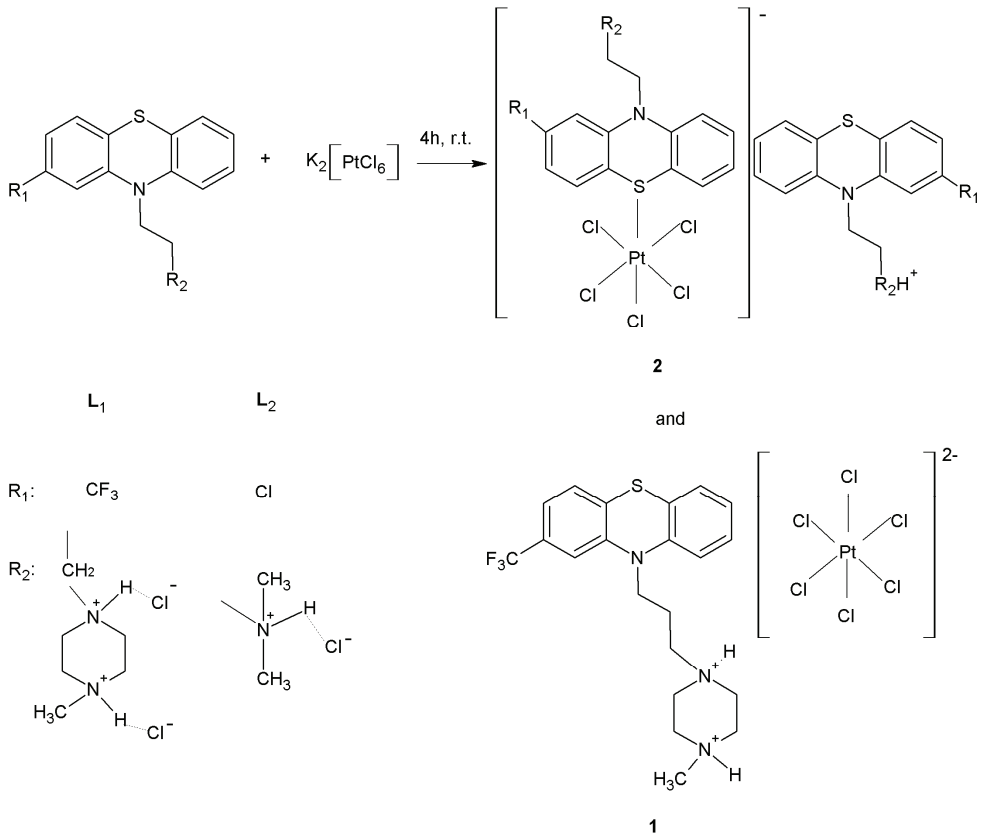


Figure 6. ORTEP diagram of complex $[HgCl_2(ptz)]_2{\cdot}HgCl_2$ [23].



Scheme 7. Reaction scheme for synthesis Pt(II) complexes with chlorpromazine hydrochloride and trifluoperazine dihydrochloride.

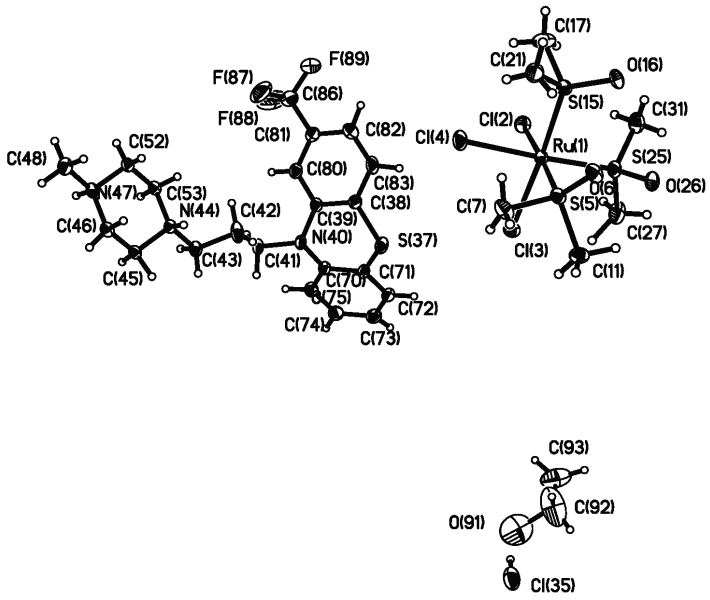


Figure 7. Asymmetric unit of (TF.H2)[$RuCl_3(DMSO)_3$]Cl·C_2H_5OH.

variation by cell types. The most active compound is complex (CP.H)[$RuCl_3(DMSO)_3$]·C_2H_5OH, which demonstrates a higher activity against MCF-7 and IM9, but it is less effective against other cell lines and did not cause complete cell death. Complex:
(TF.H2)[$RuCl_3(DMSO)_3$]Cl·C_2H_5OH is the most sensitive against human breast cancer cell line (MDA-MB-453) and human colon adenocarcinoma cell line (SW-480), while other two complexes were active even in low concentrations, and induced almost total cell death at 25 μM during 48 h of treatment. Moreover, complex (TF.H2)[$RuCl_3(DMSO)_3$]Cl·C_2H_5OH is

the only compound in our investigations displaying cytotoxic activities against all tumour cell lines. The selective cytotoxicity of these complexes, especially complex (TF.H2)[$RuCl_3(DMSO)_3$]Cl·C_2H_5OH, against the cancer cells suggests their great potential for development as anticancer drugs. It is also investigated the effect of this complex on antioxidative enzymes in rat blood. Biological assays provide the clear evidence that complex (TF.H2)[$RuCl_3(DMSO)_3$]Cl·C_2H_5OH under physiological conditions can act as a scavenger of $NO^{\cdot}$ radicals in lower doses of 0.4 and 4.5 μM/kg bw, and as a scavenger of free radicals, such as superoxide anion and hydroxyl radicals (Fig. 8). This compound also influences the SOD and CAT activities, even in dose of 90.4 μM/kg bw. As non-toxic compound under physiological conditions, this complex could provide potential therapeutic benefits in lower doses (0.4 and 4.5 μM/kg bw) for disorders where these reactive species are involved.

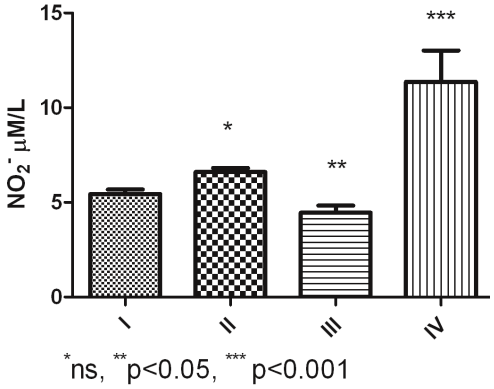


Figure 8. Content of $NO_2^{\cdot}$: I – control group; I – rats treated i.p. with complex (TF.H2)[$RuCl_3(DMSO)_3$]Cl·C_2H_5OH at dose of 0.4 μM/kg bw; III-rats treated i.p. with complex (TF.H2)[$RuCl_3(DMSO)_3$]Cl·C_2H_5OH at dose of 4.5 μM/kg bw; IV – rats treated i.p. with complex (TF.H2)[$RuCl_3(DMSO)_3$]Cl·C_2H_5OH at dose of 90.4 μM/kg bw.

The thermal decomposition pattern of the starting complex [$RuCl_2(DMSO)_4$] and complexes L[$RuCl_3(DMSO)_3$], where L is protonated chlorpromazine hydrochloride, trifluoperazine dihydrochloride or thioridazine hydrochloride, does not depend on the atmosphere: the thermal curves are almost identical in nitrogen and air atmospheres [36]. Also, decomposition of these complexes is not completed at 700, but near 1000 °C as residue is elemental ruthenium. This property of complexes is in agreement to biological potential and provides an opportunity for these compounds to adapt to different ambient conditions.

In reaction of *N*-alkylphenothiazines with [$RuCl_2(\eta^6$-*p*-cymene)]$_2$ in 2-propanol, after about 3 h, orange precipitate was formed. As in previously research, complex with trifluoperazine gave single crystal for X-ray analysis (Fig. 9). The crystal structure of complex 2 contains the Ru center in a pseudo-octahedral "piano-stool" geometry, with *p*-cymene and three chloride ions in the coordination sphere, and *N*-alkylphenothiazine in the outer-sphere. *In vitro* cytotoxic activities of complexes were assayed in four human carcinoma cell lines MCF-7, MDA-MB-453, SW-480 and IM9. The highest cytotoxicity (12.1 ≤ *IC50* ≤ 17.3 μM) and induced a total (SW-480) or almost total cell death (MCF-7, MDA-MB-453) at 25 μM in 48 h of treatment were observed for complex (TF.H2)[$RuCl_3(\eta^6$-*p*-cymene)] Cl·C_3H_7OH [37].

Biological assays provide clear evidence that this compound demonstrates a positive effect on the heart muscle, as suggested by unchanged LDH1 levels and a lower level of LDH2. Complex (TF.H2)[$RuCl_3(\eta^6$-*p*-cymene)] Cl·C_3H_7OH inhibited SOD activity in all doses, although influence on CAT activity was not observed. Results of this study suggest the cardio protective potential of complex (TF.H2)[$RuCl_3(\eta^6$-*p*-cymene)] Cl·C_3H_7OH, since the decreased activity of LDH2 was revealed after its oral administration. This investigation illustrates the potential of Ru(II)-cymene complexes with pharmacologically active substances as novel and promising bioinorganic drugs.

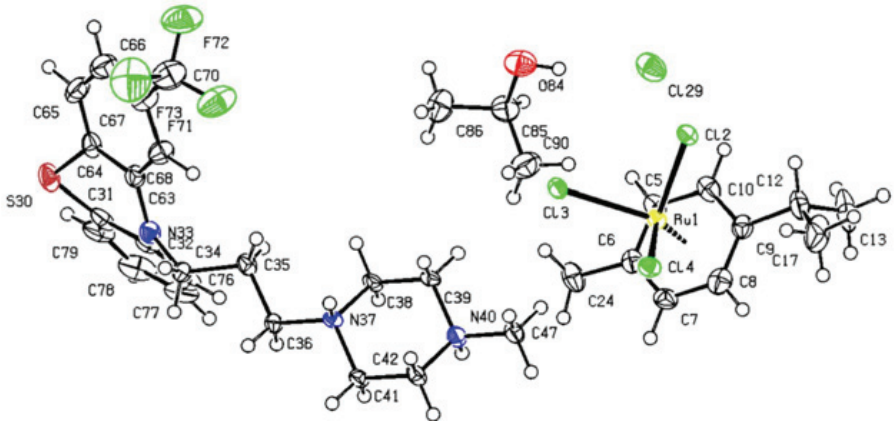


Figure 9. Asymmetric unit of (TF.H2)[$RuCl_3(\eta^6$-p-cymene)] Cl·C_3H_7OH.

Thermal analysis of these complexes were shown a great thermal stability of starting complex $[RuCl_2(\eta^6\text{-}p\text{-cymene})]_2$, while thermal decomposition of chlorpromazine's and trifluoperazine's Ru-complex began at low temperature [38].

CONCLUSIONS

The phenothiazine belongs to the group of heterocyclic aromatic compounds with nitrogen and sulphur atoms. The *N*-alkylphenothiazines are formed in the reaction of substitution on the nitrogen atom of phenothiazines ring with aliphatic side chain, piperidine, piperazine or with some new group of molecule. The other substituent is at carbon atoms in position 2 of benzene ring, but new generation of *N*-alkylphenothiazines introduces substituent at position 1-4 and 6-9 at the benzene ring. These molecules possess many applications, but the most important implementation is in medicine. They are medicinal drugs with the major effect on central nervous system almost irreplaceable in treatment of mental diseases. Many of them have shown cytotoxic effect against different cancer cell lines or in small doses supported the therapeutic drugs to lead apoptosis of cancer cells.

N-alkyphenothiazines are also used in synthesis of metal complexes which showed antibacterial activity. Complexes of Ru(II) with *N*-alkylphenothoazines showed significant anticancer activity against four cancer lines. These complexes possess positive effect in small doses on antioxidant enzymes, as well as the complex (TF.H2)[$RuCl_3(\eta^6\text{-}p\text{-cymene})$] $Cl{\cdot}C_3H_7OH$ demonstrated cardio protective potential by decreased activity of LDH2 isoenzymes. Having into consideration all of the above, *N*-akylphenothiazines have promising potential in both bioinorganic chemistry and medicine in the future.

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REFERENCES

[1] K. Pluta, B. Morak-M1odawska, M. Jeleń, Recent progress in biological activities of synthesized phenothiazines, Eur. J. Med. Chem. **46** (2011) 3179–3189.

[2] S.C. Mitchell, Phenothiazines: The Parent Molecule, Curr. Drug Targets **7** (2006) 1181–1189.

[3] J.J. Aaron, M.D.G. Seye, S. Trajkovska, N. Motohashi, Bioactive Phenothiazines and Benzo[*a*]phenothiazines: Spectroscopic Studies, and Biological and Biomedical Properties and Applications, Top. Heterocycl. Chem. **16** (2009) 153–231.

[4] N. Motohashi, M. Kawase, K. Satoh, H. Sakagami, Cytotoxic Potential of Phenothiazines, Curr. Drug Targets **7** (2006) 1055–1066.

[5] Z. Zhelev, H. Ohba, R. Bakalova, V. Hadjimitova, M. Ishikawa, Y. Shinohara, Y. Baba, Phenothiazines suppress proliferation and induce apoptosis in cultured leukemic cells without any influence on the viability of normal lymphocytes, Cancer Chemother. Pharmacol. **53** (2004) 267–275.

[6] M. Martins, S.G. Dastidar, S. Fanning, J.E. Kristiansen, J. Molnar, J.-M. Pages, Z. Schelz, G. Spengler, M. Viveiros, L. Amaral, Potential role of non-antibiotics (helper compounds) in the treatment of multidrug-resistant Gram-negative infections: mechanisms for their direct and indirect activities, Int. J. Antimicrob. Agents **31** (2008) 198–208.

[7] J.D. Kelly, H.V. Whitlock, M. Gunawan, D. Griffin, C.J. Porter, I.C. Martin, Anthelmintic efficacy of low-dose phenothiazine against strains of sheep nematodes susceptible or resistant to thiabendazole, levamisole and morantel tartrate: effect on patent infections, Res. Vet. Sci. **30** (1981) 161–169.

[8] H.S. Smith, L.R. Cox, B.R. Smith, Dopamine receptor antagonists, Ann. Palliat. Med. **1** (2012) 137–142.

[9] K.H. Chen, C.E. Lin, W.S. Liao, W. Y. Lin, Y.Y. Hsiao, Separation and migration behavior of structurally related phenothiazines in cyclodextrin-modified capillary zone electrophoresis, J. Chromatogr., A **979** (2002) 399–408.

[10] A. Jaszczyszyn, K. Gsiorowski, P. Switaek, W. Malinka, K. Cieoelik-Boczula, J. Petrus, B. Czarnik-Matusewicz, Chemical structure of phenothiazines and their biological activity, Pharmacol. Rep. **64** (2012) 16–23.

[11] G.C. González-Muñoz, M.P. Arce, B. López, C. Pérez, A. Romero, L. Barrio, M.D.M. Saavedra, J. Egea, R. León, M. Villarroya, M.G. López, A.G. García, S Conde, M.I.R. Franco, *N*-acylaminophenothiazines: Neuroprotective agents displaying multifunctional activities for a potential treatment of Alzheimer's disease, Eur. J. Med. Chem. **46** (2011) 2224–2235.

[12] R.S. Vardanyan, V.J. Hruby, Synthesis of Essential Drugs, Elsevier, Amsterdam, 2006.

[13] S.S. Nikulin, V.M. Misin, V.M. Komarov, M.V. Misin, Synthesis and Properties of 2-Vinylphenothiazine Polymers, Russ. J. App. Chem. **76** (2003) 1327–1330.

[14] M.A. Reddy, B. Vinayak, T. Suresh, S. Niveditha, K. Bhanuprakas, S.P. Singh, A. Islam, L. Han, M. Chandrasekharam, Highly conjugated electron rich thiophene antennas on phenothiazineand phenoxazine-based sensitizers for dye sensitized solar cells, Synthetic Met. **195** (2014) 208–216.

[15] R.Y.Y. Lin, T.M. Chuang, F.L. Wu, P.Y. Chen, T.C. Chu, J.S. Ni, M.S. Fan, Y.H. Lo, K.C. Ho, J.T. Lin, Anthracene/Phenothiazine p-Conjugated Sensitizers for Dye-Sensitized Solar Cells using Redox Mediator in Organic and Water-based Solvents, Chem. Sus. Chem. **8** (2015) 105–113.

[16] M. Wainwright, The use of dyes in modern biomedicine, Biotech. Histochem. **78** (2003) 147–155.

[17] T.A. Ban, Fifty years chlorpromazine: a historical perspective, Neuropsych. Dis.Treat. **3** (2007) 495–500.

[18] M.M. Kristiansen, C. Leandro, D. Ordway, M. Martins, M. Viveiros, T. Pacheco, J.E. Kristiansen, L. Amaral, Phe-

nothiazines alter resistance of methicillin-resistant strains of *Staphylococcus aureus* (MRSA) to oxacillin in vitro, Int. J. Antimicrob. Agents **22** (2003) 250–253.

[19] M. Martins, S.G. Dastidar, S. Fanning, J.E. Kristiansen, J. Molnar, J.M. Pages, Z. Schelz, G. Spengler, M. Viveiros, L. Amaral, Potential role of non-antibiotics (helper compounds) in the treatment of multidrug-resistant Gram-negative infections: mechanisms for their direct and indirect activities, Int. J. Antimicrob. Agents **31** (2008) 198–208.

[20] M. Kolaczkowski, K. Michalak, N. Motohashi, Phenothiazines as potent modulators of yeast multidrug resistance, Int. J. Antimicrob. Agents **22** (2003) 279–283.

[21] K. Akilandeswari, K. Ruckmani, Studies on Anti microbial Potential of Non-antibiotics on Resistant Bacteria – A Review, J. Young Pharm. **7** (2015) 63–68.

[22] D. Ordway, M. Viveiros, C. Leandro, R. Bettencourt, J. Almeida, M. Martins, J.E. Kristiansen, J. Molnar, L. Amaral, Clinical Concentrations of Thioridazine Kill Intracellular Multidrug-Resistant *Mycobacterium tuberculosis*, Antimicrob. Agents Ch. **47** (2003) 917–922.

[23] L. Amaral, M. Martins, M. Viveiros, Enhanced killing of intracellular multidrug-resistant Mycobacterium tuberculosis by compounds that affect the activity of efflux pumps, J. Antimicrob. Chemoth. **59** (2007), 1237–1246.

[24] L. Amaral, Z. Udwadia, E. Abbate, D. Soolingen, The added effect of thioridazine in the treatment of drug-resistant tuberculosis, Int. J. Tuberc. Lung D. **16** (2012) 1706–1712.

[25] Z. Zhelev, H. Ohba, R. Bakalova, V. Hadjimitova, M. Ishikawa, Y. Shinohara, Y. Baba, Phenothiazines suppress proliferation and induce apoptosis in cultured leukemic cells without any influence on the viability of normal lymphocytes, Cancer Chemother. Pharmacol. **53** (2004) 267–275.

[26] G. Sudeshna, K. Parimal, Multiple non-psychiatric effects of phenothiazines: A review, Eur. J. Pharmacol. **648** (2010) 6–14.

[27] N.M.M. Gowda, M.M. Kyi, L. Zhang, Synthesis and characterization of iridium chloride complex I Use of *N*-alkylphenothiazine drugs as ligands. Transition Met. Chem. **18** (1993) 518–522.

[28] N.M.M. Gowda, R.K. Vallabhaneni, I. Gajula, S. Ananda, Palladium(II)-phenothiazine complexes: synthesis and characterization, J. Mol. Struct. **407** (1997) 125–130.

[29] B. Keshavan, P.G. Chandrashekara, N.M.M. Gowda, Synthesis, characterization, and spectral studies of lanthanide(III) nitrate complexes of promethazine, J. Mol. Struct. **553** (2000) 193–197.

[30] B. Keshavan, K. Gowda, Dioxobridged complexes of molybdenum (IV) and tungsten (IV) with *N*-alkylphenothiazines and their interactions with L-cysteine and L-histidine Proc. Indian Acad. Sci. (Chem. Sci.) **113** (2001) 165–172.

[31] L. Ma, Z. Jie, C. Ruifang, C. Zhenxia, W. Linhong , Z. Xigeng, Synthesis and reactivity of organolanthanide complexes containing phenothiazine ligand toward carbodiimide and isothiocyanate, J. Organomet. Chem. **690** (2005) 4926–4932.

[32] X. Zhang, Y. Xie, W. Yu, Q. Zhao, M. Jiang, Y. Tian, Formation of A Novel 1D Supramolecule $[HgCl_2(ptz)]_2$ $HgCl_2$ (pt z= Phenothiazine): A New Precursor to Submicrometer Hg_2Cl_2 Rods, Inorg. Chem. **42** (2003) 3732–3737.

[33] Y. Jin, Y. Liu , W. Wu, Q. Wu , H. Gao , C. Wang , S. Xu, S. Cao, Synthesis of conjugated polymers bearing pendant bipyridine ruthenium complexes, React. Funct. Polym. **90** (2015) 7–14.

[34] J.M. Poljarević, M.P. Krstić, S. Grgurić-Šipka, S.P. Sovilj, D.R. Mišić, T.J. Sabo, Platinum(IV) complexes with *N*-alkylphenothiazines: synthesis, characterization, and antibacterial activity, J. Coord. Chem. **66** (2013) 3760––3769.

[35] M. Krstić, S.P. Sovilj, S. Grgurić-Šipka, I. Radosavljević Evans, S. Borozan, J.F. Santibanez, J. Kocić, New ruthenium(II) complexes with *N*-alkylphenothiazines: Synthesis, structure, *in vivo* activity as free radical scavengers and in vitro cytotoxicity, Eur. J. Med. Chem. **45** (2010) 3669–3676.

[36] B. Hóllo, M. Krstić, S.P. Sovilj, G. Pokol, K.M. Szécsényi, Thermal decomposition of new ruthenium(II) complexes containing *N*-alkylphenothiazines. J. Therm. Anal. Calorim. **105** (2011) 27–32.

[37] M. Krstić, S.P. Sovilj, S. Grgurić-Šipka, I. Radosavljević Evans, S. Borozan, J.F. Santibanez, Synthesis, structural and spectroscopic characterization, in vitro cytotoxicity and in vivo activity as free radical scavengers of chlorido(*p*-cymene) complexes of ruthenium(II) containing *N*-alkylphenothiazines, Eur. J. Med. Chem. **46** (2010) 4168–4177.

[38] B. Hóllo, M. Krstić, S.P. Sovilj, K.M. Szécsényi, Thermal decomposition of new chlorido(p-cymene) ruthenium(II) complexes containing *N*-alkylphenothiazines, J. Therm. Anal. Calorim. **111** (2013) 1927–1932.

IZVOD

N-ALKILFENOTIAZINI – SINTEZA, STRUKTURA I PRIMENA KAO LIGANADA U KOMPLEKSIMA METALA

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(Pregledni rad)

Fenotiazini pripadaju velikoj grupi heterocikličnih, aromatičnih molekula koji između dva benzenova prstena sadrže azot i sumpor. Njihovi derivati, *N*-alkilfenotiazini poseduju supstituent na heterocikličnom azotovom atomu koji molekulima daje različita svojstva. Takođe, serija ovih jedinjenja ima supstituent i na ugljenikovom atomu u položaju 2 benzenovog prstena fenotiazina. Alkilfenotiazini sadrže aminoalkil grupu kao supstituent i njegovi alkil, acil i sulfonil derivati, kao i mono- i biciklični heterocikli vezani su za tiazinski azotov atom ili direktno za benzenov prsten. *N*-alkilfenotiazini su poznati kao antipsihotici, ali poseduju i antibakterijsku, antifungalnu, antitumorsku aktivnost, kao i sposobnost da reaguju sa makromolekulima i da se koordinuju za jone metala. Kompleksi metala sa *N*-alkilfenotiazinima pokazala su se kao biološki aktivna jedinjenja sa različitom antimikrobnom aktivnošću i citotoksičnim efektom na različite tumorske ćelijske linije. Upravo zbog njihovog širokog polja primene *N*-alkilfenotiazini su veoma atraktivni u smislu sinteza novih srodnih derivata, komleksa metala, proučavanja njihovih svojstava i istraživanjima na polju njihove dalje primene. Ovaj rad predstavlja pregled literature i savremeni pogled na *N*-alkilfenotiazine – njihovu sintezu i primenu, kao i njihovih metalnih kompleksa koji poseduju značajna biološka svojstva.

Ključne reči: *N*-Alkilfenotiazini • Antipsihotici • Antitumorski efekat • Antibakterijski efekat