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Adriana Ignat-PhD Thesis Abstract

NEW DERIVATIVES OF THIAZOLE AND SELENAZOL WITH POTENTIAL BIOLOGICAL

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~2010~

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KEYWORDS:

- 2-hydrazino-thiazoles
- 2-hydrazino-selenezoles
- hydrazine-bis-thiazoles
- hantzsch condensation
- anti-inflammatory activity
- analgesic activity
- antiproliferative activity
- antimicrobial activity
- antifungal activity
- anti-tumor activity
- kinetic study
- reaction in microwave field
- phenothiazines
- p-toluenesulfonyl-hydrazinothiazoles
- prostate DU145, hepatocarcinoma HepG2 cell lines.

INTRODUCTION

Compounds with 2-hydrazino-thiazoles structure have been sytudied for over 50 years. However, these compounds are still of interest in organic chemistry because of the many applications in medicine (antitumor activity,[1] cytotoxic,[2] antimicrobial,[3] anti-inflammatory,[4] mitodepressive,[5] hypotensive,[6] anti-HIV,[7] hypoallergenic,[8] tuberculosis[9]) and agriculture (pesticide action[10]). Researches on this class of organic compounds have led to the obtention of the original compounds, which have been structurally characterized by spectral methods of analysis and evaluated in terms of biological potential.

Paper entitled "NEW DERIVATIVES OF THIAZOLE AND SELENAZOL WITH BIOLOGICAL POTENTIAL" is composed of two chapters:

Chapter I Literature review: presents the most significant and recent data on synthesis and reaction of 2-hydrazino-thiazoles compounds. The main objectives were to classify as logically and rogorously as possible the obtention methods and the chemical properties, and to establish relationships between structure and biological activity of these compounds.

Chapter II Personal contributions: presents the results of original studies in the synthesis of 2-hydrazino-selenazoles and 2-hydrazino-thiazoles derivatives, confirming the structures by spectral methods and their biological activity evaluation. This chapter is divided in three subsections:

The first subsection describes classical and microwave synthesis of new derivatives of p-toluensulfonyl-hydrazino-thiazoles and hydrazino-bis-thiazoles, given the

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fact that the most simple and effective in their synthesis is the Hantzsch condensation reaction. In the study we proposed to evaluate the anti-inflammatory, analgesic and anti-tumor activity, present as well in other compounds that contain the hydrazine-thiazole group.

Given the similarity of the chemical properties between sulfur and selenium, as well as heterocyclic compounds of selenium with biological importance,[11] in the second subsection we present the researches on the synthesis of hydrazino-selenazoles as well as their biological potential evaluation. Thus, we synthesized two series of hydrazino-selenazoles: ariliden-hydrazino-selenazoles and aroil-hydrazino-selenazoles, and then tested them from the point of view of antimicrobial, antifungal and antitumor activity.

In the third subsection, the objective of our research was the synthesis of compounds containing the phenothiazine nucleus linked to the thiazolic/selenazolic nucleus by a hydrazine bridge and test the biological properties of new compounds. Phenothiazine and its derivatives cover a wide spectrum of biological application by its actions: anti-tumor effect by inducing apoptosis or endotoxin neutralizing effect,[12] neuroleptics,[13] antiparkinsonian, antimicrobial,[14] etc.

The present thesis contains original results obtained through research in the Laboratory of Organic Chemistry at the "Iuliu Haţieganu" University of Medicine and Pharmacy, and the Laboratory of Organic Chemistry from the Faculty of Chemistry and Chemical Engineering of the "Babeş-Bolyai" University in Cluj-Napoca .

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5.1 p-TOLUENSULFONYL-HYDRAZINO-THIAZOLE AND HYDRAZINO-BIS - THIAZOLE

5.1 Synthesis of p-toluensulfonyl-hydrazino-thiazoles

The experimental research of pharmacological and pharmaceutical chemistry has elucidated the biological potential of compounds with the sulphonylhydrazinic group. The thiazolyl group is also of great importance in treating biological systems. Hence, the compounds of this functional group shows the antimicrobial,[15] antitumoural,[16] analgesic, anti-inflammatory and antipyretic[17] properties. Some synthetic thiazoles have exhibited a range of biological activities, such as antitumor, antifilarial, antibiotic, antibacterial, antifungal, and anti-inflammatory.[18] Recent studies have shown the synthesis of some new thiazole candidates as antimicrobial and anticancer agents.[19] In the previous work, acid hydrazides were very important compounds, for its high reactivity usefulness in heterorganic synthesis, as key starting materials to form various classes of biologically and pharmacologically active candidates.[8] Holla et al. synthesized a series of aryliden-hydrazinothiazoles and 2-furanyliden-hydrazinothiazoles with antimicrobial and anti-inflammatory activities.[14] This result encouraged us to study several hydrazinothiazoles with antimicrobial properties and also aroyl-hydrazinothiazoles that has been used as intermediate in the synthesis of thiazolo[2,3-c][1,2,4]triazoles[20] derivatives, has led us to collect structural information (sulfonyl-hydrazinic group and the thiazolic nucleus) on the new molecule and evaluation of the biological activities.

For the synthesis of these organic compounds, we used the Hantzsch type condensation reaction with a p-toluenesulfonylthiosemicarbazide **1** as reagents, which was obtained by acylation of thiosemicarbazide with the p-toluenesulfochloride. The reaction was done in both the presence of NaOH and pyridine. The best result was obtained using the NaOH method.

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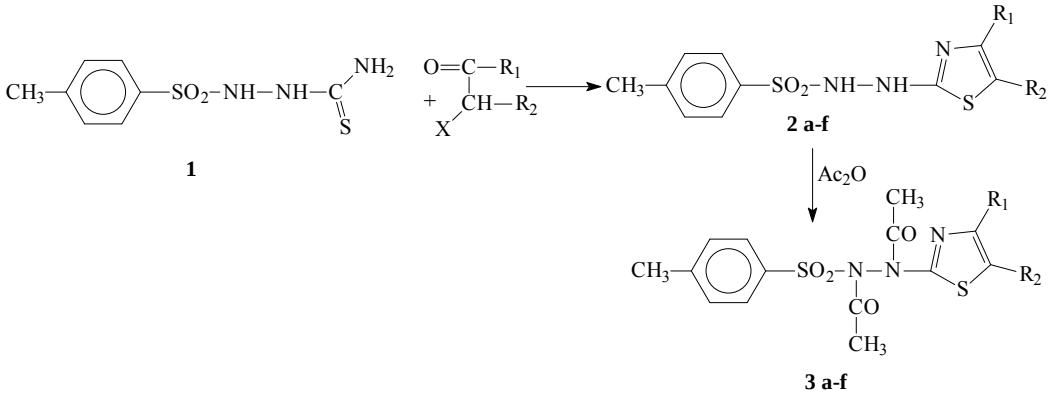
[20] A. Ignat, V. Zaharia, C.Mogoşan, N. Palibroda, C. Cristea, L. Silaghi-Dumitrescu, *Farmacia*, **2010**, Vol.58, 3 290-302

The derivatives of p-toluenesulfonyl-hydrazinothiazole **2a-f** were synthesized with the condensation reaction of p-toluenesulfonylthiosemicarbazide **1** and a series of α-halogenocarbonyls (chloroacetone; 1,3-dichloroacetone; α-bromoacetophenone; 3-chloroacetylacetone; ethyl α-bromoacetoacetate and ethyl γ-bromoacetoacetate), in acetone or DMF/acetone, mixture (**Scheme 1**). The compounds **3a-f** were obtained by the acylation reaction in the presence of acetic anhydrid.



	2a, 3a	**2b, 3b**	**2c, 3c**	**2d, 3d**	**2e, 3e**	**2f, 3f**
R_1	CH_3	CH_2Cl	C_6H_5	CH_3	CH_3	$CH_2COOC_2H_5$
R_2	H	H	H	$COCH_3$	$COOC_2H_5$	H

Scheme 1. Synthesis of compounds **3a-f**

5.2 Microwave assisted organic syntheses

On the other hand, according to literature data, almost all conventionally heated reactions, (including synthesis and functionalization of heterocyclic compounds) were performed using microwave assisted technique.[21] The advantages of microwave assisted organic synthesis (*MAOS)* on laboratory scale is based on particularly efficient heating processes which afford shorter reaction times, thus offering the possibility of rapid optimization of the reaction conditions. Although we already reported the synthesis of the target *p*-toluenesulfonyl-hydrazinothiazoles derivatives under classical conditions, another aim of this work was to optimize the microwave assisted Hantzsch condensation reaction.

[21] O. Kappe, Controlled Microwave Heating in Modern Organic Synthesis *Angew. Chem.* Int. Ed., **2004**, 43, 6250

The reaction mixture was subjected to microwave heating in sealed vessels using a Microwave instrument CEM Discover LabMate, equipped with a monomode reaction cavity. A microwave absorbing solvent (DMF) was employed to enhance the ability of the reaction mixture to efficiently absorb microwave energy taking advantage of the microwave dielectric heating phenomena such as dipolar polarization.

In order to optimise the reaction conditions, the MAOS experiments conducted at different temperatures (25ºC, 40ºC, 80ºC) and reaction times (0.5, 1, 2 hours) were applied in the condensation reaction of *p*-toluenesulfonyl-thiosemicarbazide **1** with α-halogenocarbonyls. (**scheme 1).**

A comparison between our previous results obtained in the syntheses of compounds **2a-f** performed at room temperature [(which required long reaction times (24 h) in order to achieve good reaction yields (56-73%)] with MAOS conditions shown in **table I,** when temperature and reaction time parameters were modified, show the possibility of obtaining enhanced reaction yields in reduced reaction times.

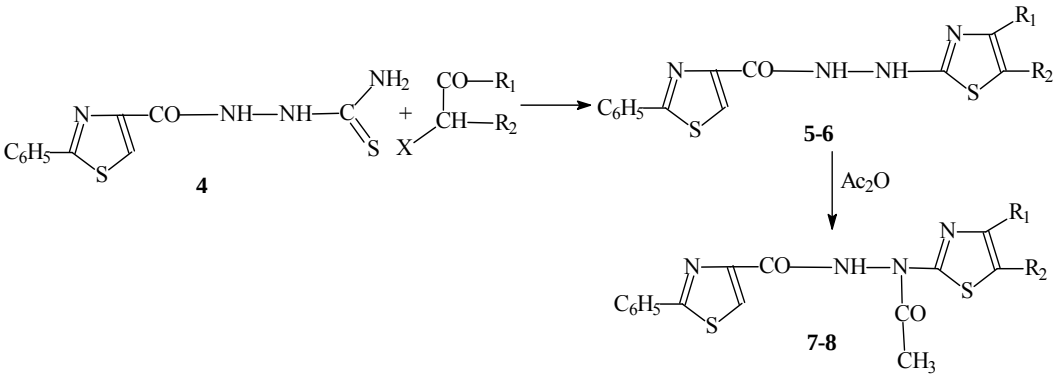
Table I. Microwaves assisted Hantzsch condensation reaction parameters

Compounds	Temperature (^{o}C)	Time (min)	Pressure (Bar)	**Power W**	**Yields (%)**
2a	25 (classical)	1440	1	-	60
	40	120	1.7	200	67
	80	120	1.7	200	82
2b	25 (classical)	1440	1	-	66
	40	120	1.7	200	74
	80	120	1.7	200	92
2c	25(classical)	1440	1	-	67
	40	120	1.7	200	55
	80	120	1.7	200	71
2d	25(classical)	1440	1	-	59
	40	120	1.7	200	64
	80	120	1.7	200	81
2e	25(classical)	1440	1	-	66
	40	120	1.7	200	75
	80	120	1.7	200	85

The results of this study show an important decrease in the reaction time and an increased reaction yield when condensation takes place under microwave irradiation at moderate or higher temperature. After 2 hours of microwave irradiation (power 200 W) at 40°C, the yields are comparable to those obtained in conventional reactions carried out at room temperature in 24 hours.[18] In all experiments performed, the highest reaction yields were obtained after microwave irradiation of the reaction mixture at 80°C for 2 hours.

5.3 Synthesis of hydrazino-bis-thiazoles

A series of compounds were also synthesized that are linked by two thiazole nucleus separated by the carbonylhydrazine (-CO-NH-NH-) group. For the synthesis of these organic compounds the 4-(2-phenyl-thiazol-4-carbonyl)-thiosemicarbazide **4** was condensed with 1,3-dichloroacetone and ethyl γ-bromoacetoacetate respectively (**Scheme 2**).



	5, 7	**6, 8**
R_1	CH_2Cl	$CH_2COOC_2H_5$
R_2	H	H

Scheme 2. Synthesis of compound **5-8** deriviatives

The thiazolic compounds reacted with acetic anhydride by the acylation reaction. In the synthesis of sulfonylhydrazinothiazole derivatives (**Scheme 1**), the hydrazinic group takes part in a double acylation with acetic anhydride, but in the case of thiazolyl-carbonylhydrazinothiazole, there was instead a monoacylation reaction with acetic anhyride.

There are no condensation and acylation reaction studies on synthesized *p*-toluenesulfonyl-hydrazinothiazole **2a-f, 3a-f and** thiazolyl-carbonylhydrazinothiazole **5-8** derivatives**,** reported in the literature. The adduct (**2a-f)** and acylated (**3a-f, 5-8)** products were isolated here for the first time via total synthesis by transformation of synthetics condensated **1** and **4** as a starting material.

5.4 Structural analysis

The structures of the synthesized compounds were assigned on the bases of its spectral data, mainly mass, elemental analysis, IR and ^{1}H-NMR.

5.5 Evaluation of biological potential

5.5.1 Anti-inflammatory activity

The synthesized *p*-toluenesulfonyl-hydrazinothiazole derivatives **2a-f** were tested on animal subjects for potential anti-inflammatory activity. The acute inflammation model was that of rat hind-paw edema induced with 10% kaolin, in order to evaluate the vascular phase of the inflammatory process. In the study were taken 15 lots of 8 white male rats *Wistar Bratislava*.

Substances were administrated intraperitoneal (i.p.) to the animals as follows: a control lot received the vehicle suspension, the second lot received a reference anti-inflammatory drug (phenylbutazone 50 mg/kg) and the other groups were treated i.p. with suspension of tested substances at doses of 50 mg/kg. At 30 minutes after the administration of tested substances, the volume of the left hind paw was measured using an Ugo Basile pletismometre and then in the paw was injected 0.1 ml suspension of kaolin 10% as edemogen agent. Evolution of inflammatory edema was monitored by measuring the volume of the swollen paw at 2 hours, 4 hours and respectively 24 hours after the administration of kaolin.

The results obtained in the experiments for the tested compounds, using a model of acute inflammation - the hind-paw edema induced with kaolin 10% in rat, are presented in **figure 1**.

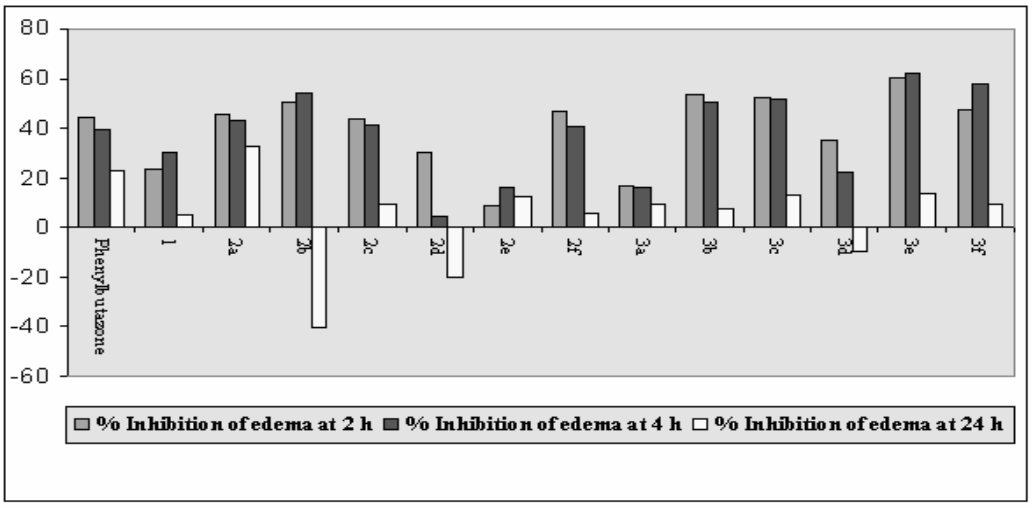


Figure 1: Percentage of inhibition for inflammatory edema in rats

In comparison with the standard anti-inflammatory phenylbutazone, after 2 hours from the induction of inflammation, the anti-inflammatory activity is better for compounds **2a, 2b, 2c, 2f, 3b, 3c, 3e** and **3f**. After 4 hours, the same compounds are more active than phenylbutazone. After 24 hours, the anti-inflammatory activity is still kept by the compound **2a**, the values being comparable with the anti-inflammatory potential of phenylbutazone.

5.5.2 Analgesic activity

The analgesic properties of the target compounds were tested using a model of central analgesia where the painful stimulus is represented by a hot plate to 56° C (Hot/cold plate Ugo Basile)[4]. In the study were taken 14 lots of 8 male white mice, each one with weight 25-35 g. The experiment consisted in measuring the reaction to pain as the time (in seconds) between the moment when the animal was placed on the hot plate and the moment when it begins to lick its back paws in response to painful stimulus.

Substances taken in the study were administered i.p. The control lot received the suspension vehicle; the second control lot received an analgesic (heroin 5 mg/kg). Lots 3-14 received the tested compounds as suspension in doses of 50 mg/kg. The time response of the animal to painful stimulus was revaluated at 30, 60, 90 and 120 minutes interval after

the administration of tested substances. The recorded results were used to calculate for each lot of animals the average response time to painful stimulus and the standard error. Statistical analysis was performed by Student test ($p < 0.05$).

The results obtained by the study of analgesic activity of compounds **2a-f** using a model of pain induced by thermal stimulus (Hot Plate) are presented in **figure 2**.

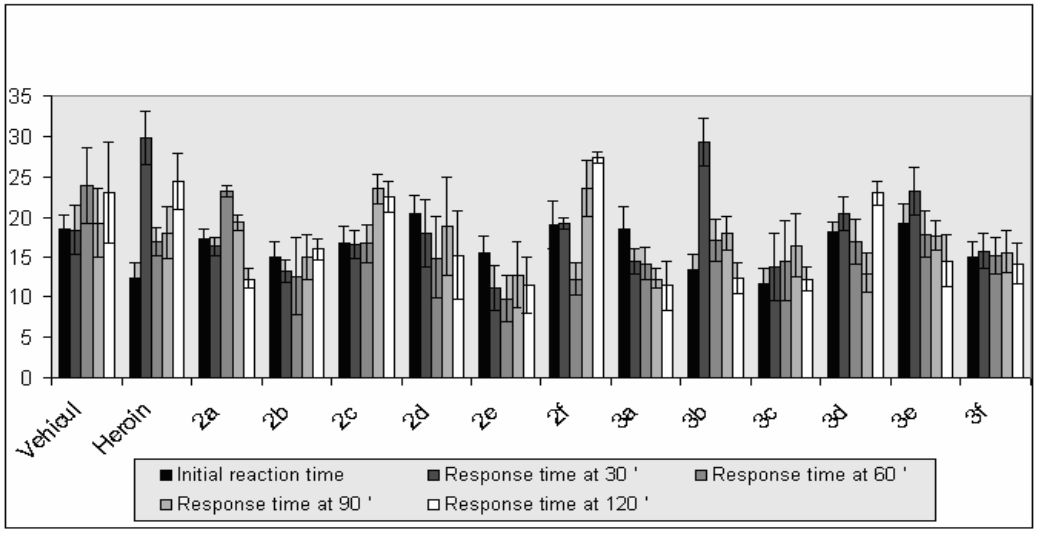


Figure 2: Time response to painful stimulus

The results of this study, based on a model of central analgesia tested by heated plate test (56°C), indicate that a dose of 5 mg/kg heroin induces a strong analgesic effect significantly prolonging the reaction time of the animals after 30 minutes from administration, a fact which corresponds to the well known analgesic profile of this compound which was chosen as a reference.

A significant increase of the response time (as compared to initial response time) was produced by compounds **2a, 2c, 2f, 3b, 3d,** and **3e**. After 30 minutes, the compound **3b** show a response time similar with that of the standard analgesic heroin. At 90 minutes after administration, compounds **2c** and **2f** produced a similar analgesic effect with that of standard analgesic heroin (according to approximately equal values of reaction time). At 120 minutes after administration, compound **2f** presented the best analgesic activity.

Among the tested compounds, **2f** and **3b** are recommended for potential analgesic activity, **3b** with a profile similar to heroin, and **2f** with a different profile (the analgesic effect being set up more slowly as compared to heroin).

5.5.3 Antiproliferative activity

From the anticancer activity obtained with the reference antineoplastic compound (doxorubicin) on both prostate DU145 and hepatocarcinoma HepG2 cancer cell lines, some of the compounds can be considered as promising anticancer candidate.

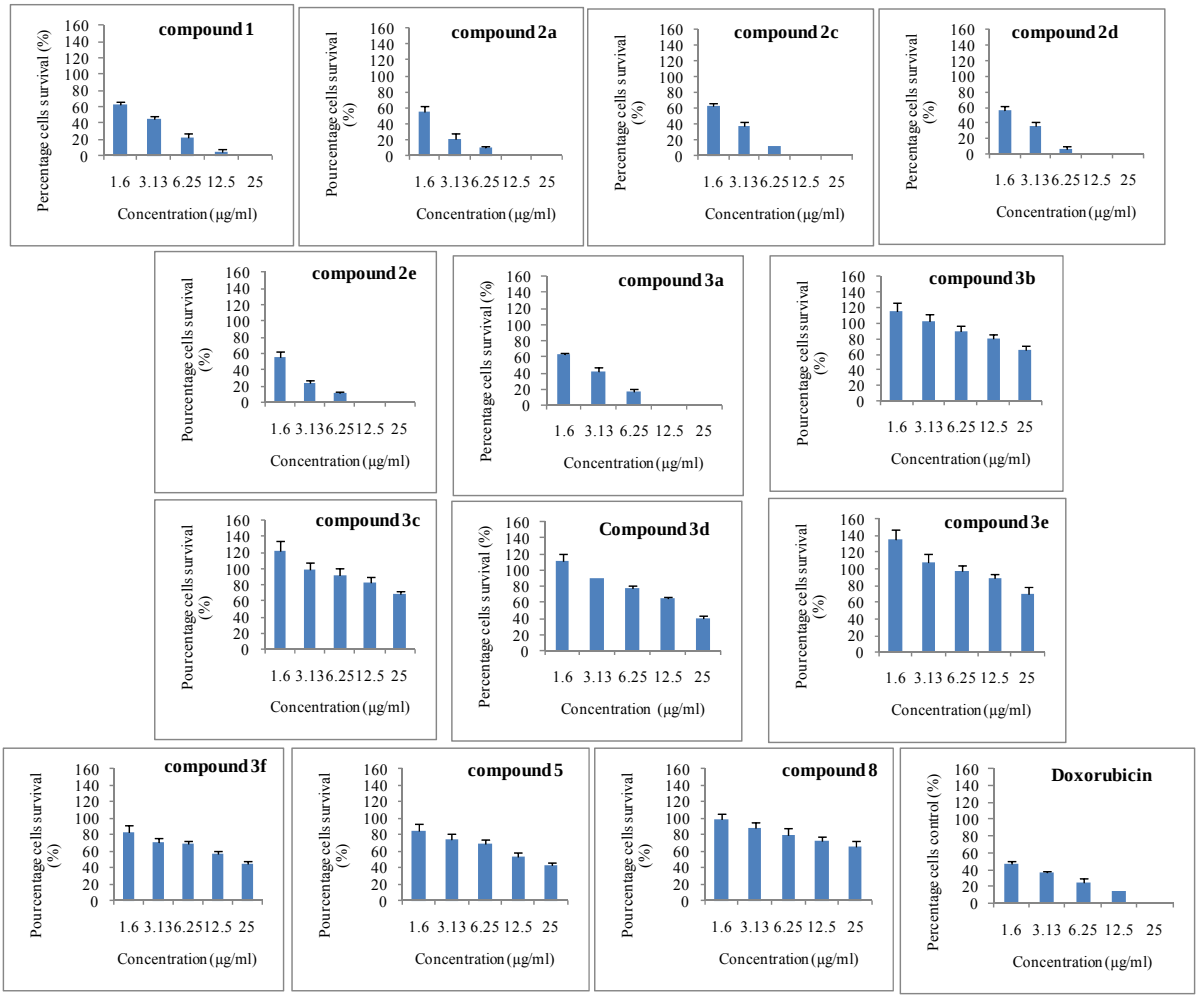


Figure 3. Effects of the compounds on DU-145

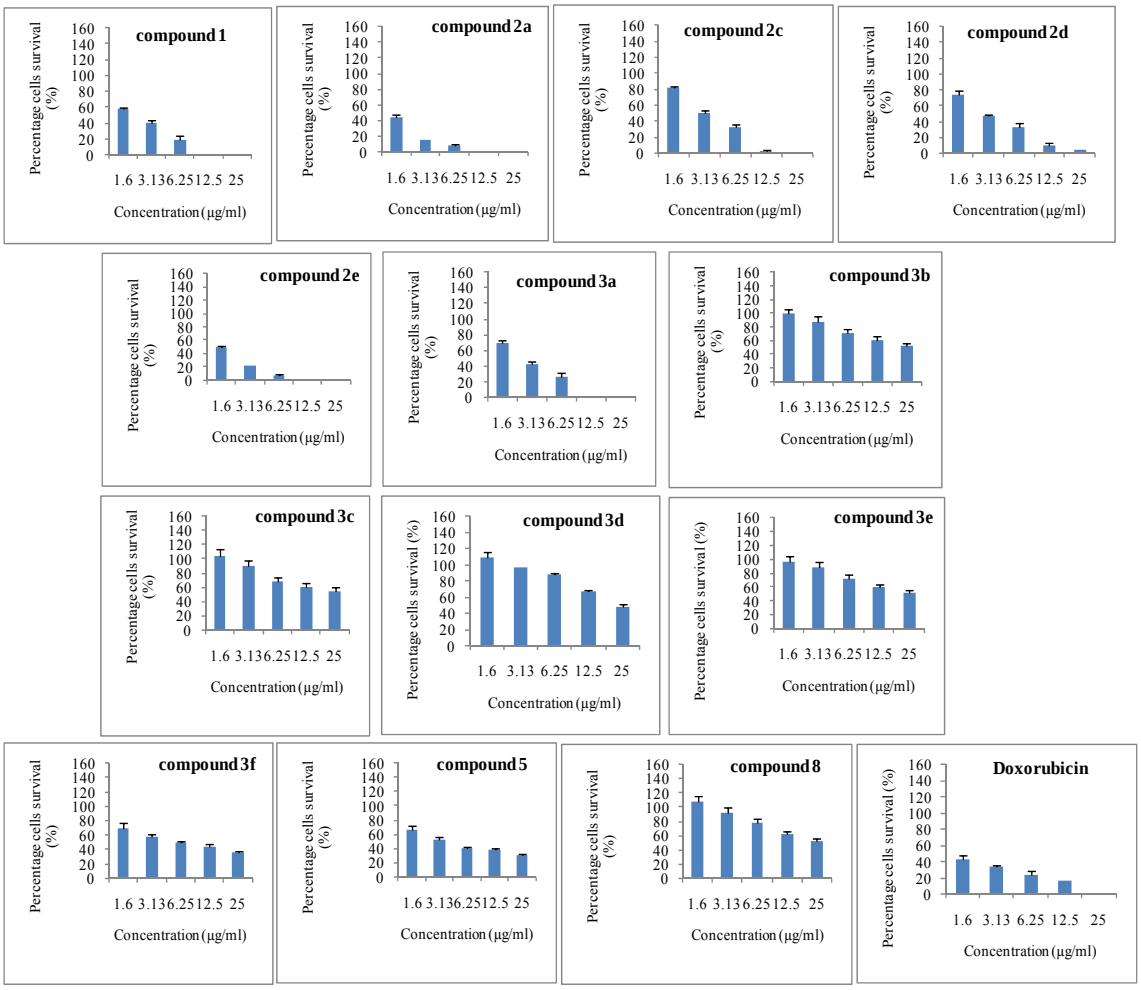


Figure 4. Effects of the compounds on HepG2 cells survivals

Compounds **1**, **2a**, **2c**, **2d**, **2e** and **3a** showed 100% inhibition of DU145 proliferation at 25 µg/ml (**Figure 3**). Total inhibition (at 25 µg/ml) of HepG2 proliferation was also recorded with **1**, **2a**, **2c**, **2e** and **3a** (**Figure 4**). Though compound **1** (the substrate) was active on both DU145 and HepG2, it should be noted that some of the synthesized derivatives were more active, as they presented lower IC_{50} values. This includes compound **2a** and **2e** on the two cancer cell lines.

Table1. Activity of the some of the compounds and doxorubicin on DU-145 and Hep G2 cancer cell lines

Samples	DU145		HepG2	
	IC50 (μg/ml)	Inhibition percentage (%) at 25 μg/ml	IC50 (μg/ml)	Inhibition percentage (%) at 25 μg/ml
1	2.2	100.00 ± 0.00	2.2	100.00 ± 0.00
2a	1.97	100.00 ± 0.00	<1.6	100.00 ± 0.00
2c	3.13	100.00 ± 0.00	3.16	100.00 ± 0.00
2d	3.20	100.00 ± 0.00	2.85	95.89 ± 0.24
2e	1.66	100.00 ± 0.00	<1.6	100.00 ± 0.00
3a	2.8	100.00 ± 0.00	2.7	100.00 ± 0.00
3b	>25	35.16 ± 3.12	>25	47.03 ± 2.32
3c	>25	32.55 ± 2.11	>25	44.98 ± 1.78
3d	21.88	59.95 ± 3.98	23.48	53.33 ± 2.56
3e	>25	30.08 ± 1.97	>25	49.17 ± 2.16
3f	20.35	56.78 ± 4.03	4.81	63.89 ± 5.19
5	17.23	57.28 ± 3.78	3.93	68.92 ± 4.76
8	>25	44.03 ± 2.09	>25	46.77 ± 2.05
Doxorubicin	<1.6	100.00 ± 0.00	<1.6	100.00 ± 0.00

As doxorubicin, compounds **2a** and **2e** presented IC_{50} < 1.6 μg/ml), highlighting their interesting anticancer potencies (**Table 1**). When regarding the structure-activity relationship, it can mainly be deduced from the results obtained with compounds from series 2 (Compounds **2a**, **2c**, **2d**, **2e**, **2f**) and **3** (Compounds **3a**, **3c**, **3d**, **3e**, **3f)** that acetylation significantly reduced the anticancer activity. This hypothesis is verified when analysing the results of compounds **5** and **8** on the two cancer cell lines.

6. HYDRAZINO-SELENAZOLE

6.1. Synthesis of aryliden-hydrazino-selenazoles

Selenium used to be considered as an essential nutrient constituent of selenoproteins involved in self-defense mechanism against oxidative stress, [22, 23, 24] but it was also identified as causing livestock poisoning through selenium-accumulating plants grown in seleniferous soil.[25] Selenium appears to be also involved in

[22] O. Lopez, S. Maza, V. Ulgar, I. Maya, J.G. Fernandez-Bolanos - Tetrahedron, **2009**, 65, 2556-2566
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reducing certain inflammatory processes and in detoxification processes[26]. Based on these benefits associated to the presence of selenium, the synthesis of many organoselenium derivatives characterized by a large variety of biological activities was developed.

Heterocyclic compounds containing selenium presents a large spectrum of biological activities. 1,3-Selenazole derivatives show anticancer,[27] antibacterial (selenafurin),[28] antiviral activity,[29] inhibits the synthesis of nitric acid[30] and they are antagonists for histamine H_2[31] receptors. 2-Dialkyl-amino-1,3-selenazole derivatives[32] were employed in the synthesis of some dyes.[33] N-acyl- derivatives of 2-amino-4-(isothiocyanatomethyl)-1,3-selenazole show antitumor activity.[34] Recently was reported the inhibitory activity of 2-piperidin- and 4-phenyl-2-piperidin-1,3-selenazole upon anion-superoxidase.[35]

Litterature data regarding biological effects of selenazole derivatives and the similarity between the chemical properties of selenium and sulfur, also completed with our experience regarding the chemical synthesis and evaluation of antimicrobial and antitumoral effects of hydrazino-thiazole[36, 37] derivatives, encouraged us to synthesize new hydrazinoselenazols derivatives in order to study their chemical and biological behaviour. The selected precursors were selenosemicarbazones prepared by the condensation of selenosemicarbazides with (hetero)aromatic carbaldehydes, or aroyl-selenosemicarbazide derivatives prepared by the acylation of selenosemicarbazide respectively. The selenazole derivatives were obtained by Hantszch type cyclization reaction between these precursors and α-halogeno-carbonyl derivatives.

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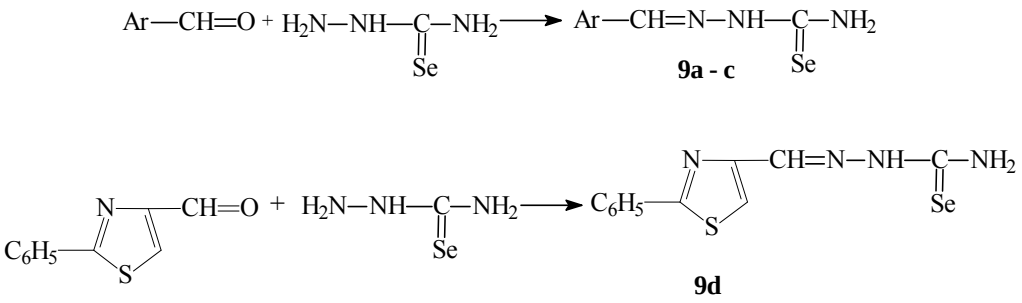
New aryliden-hydrazinoselenazoles **10a-h** and aroyl-hydrazinoselenazoles **13a-d** were prepared by Hantszch condensation reaction between a selenamide and a series of α-halogenocarbonyl derivatives.

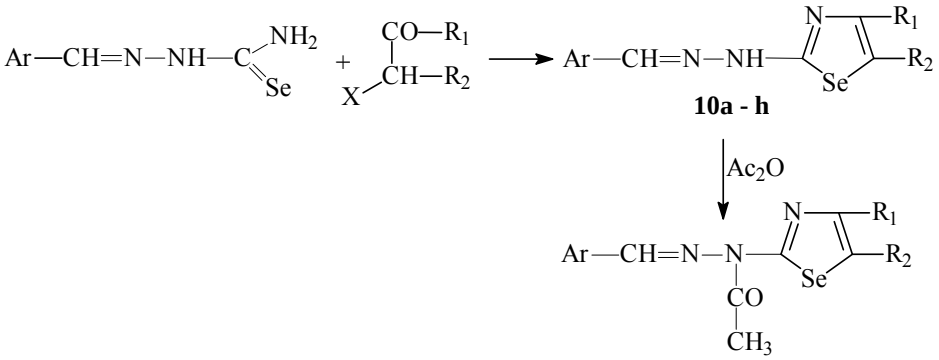
Aryliden-selenosemicarbazones **9a-d** were obtained by the reaction of several aromatic carbaldehydes such as benzaldehyde, *p*-chlorobenzaldehyde, *p*-methoxy-benzaldehyde and 2-phenyl-thiazol-4-carbaldehyde respectively, with selenosemicarbazide (**scheme 3**).



	a	b	c
Ar	C_6H_5	$C_6H_4Cl(4)$	$C_6H_4OCH_3(4)$

Scheme 3

Selenosemicarbazones **9a-d** were further subjected to condensation reaction with different α-halogenocarbonyl derivatives such as monochloroacetone, 1,3-dichloroacetone, α-bromoacetophenone, 3-chloro-acetylacetone and α-bromoacetylacetic ester, in DMF/acetone solution. Hydrazinoselenazoles **10a-h** thus obtained were further acetylated using acetic anhydride, as presented in **scheme 4.**



10a - h

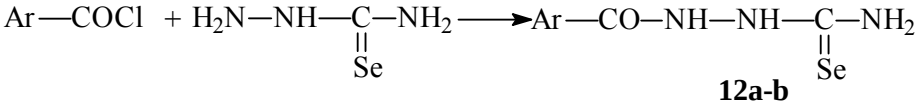
11a - h

10, 11	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**
Ar	$C_6H_4Cl(4)$	$C_6H_4Cl(4)$	$C_6H_4Cl(4)$	$C_6H_4Cl(4)$	$C_6H_4OCH_3(4)$	$C_6H_4OCH_3(4)$	Th	Th
R_1	CH_3	CH_2Cl	C_6H_5	CH_3	CH_3	C_6H_5	CH_2Cl	CH_3
R_2	H	H	H	$COOC_2H_5$	H	H	H	$COCH_3$

Scheme 4

6.2 Synthesis of aroyl-hydrazino-selenazoles

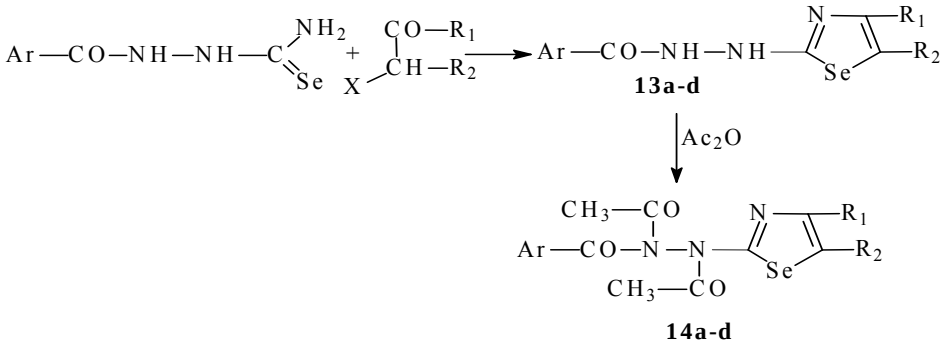
Aroyl-selenosemicarbazides **12a-b**, were obtained by the reaction of acid chlorides (benzoylchloride and *p*-chlorobenzoylchloride respectively) with selenosemicarbazide as shown in **scheme 5.**



12a-b

Scheme 5

Intermediates **12a-b** were subjected to condensation with monochloroacetone and *α*-bromoacetophenone respectively and thus aroyl-hydrazino-selenazoles **13a-d** were obtained in good yields. Further acetylation with acetic anhydride generated diacetyl derivatives **14a-d** (**scheme 6**)



13,14	a	b	c	d
Ar	C_6H_5	C_6H_5	C_6H_5Cl (4)	C_6H_5Cl (4)
R_1	CH_3	C_6H_5	CH_3	C_6H_5
R_2	H	H	H	H

Scheme 6

6.3 Synthesis of aryliden-hydrazino-selenazoles by microwave activation

An alternative technique applied for the synthesis of selenazole derivatives **10a-h** and **13a-d** is based on the dielectric heating induced by microwaves assisted conditions. Different reaction temperatures (40°C, 60°C or 80°C) and times (30', 60'and 90' respectively) were applied in order to optimize the reaction conditions in the presence of DMF solvent. Temperatures above 80 °C produced the decomposition of the reaction product. The best yields were obtained after 60 minutes of microwave irradiation which maintained a temperature of 40°C inside the reaction vessel.

Table II. Experimental conditions applied in the synthesis of selenazole derivatives

Cpd	Heating technique	Temp. (°C)	Time (min)	Pressure (Bar)	**Power W**	**Yields (%)**
10a	convective	25	1440	1	-	52
	dielectric	40	60	1.7	100	94
10b	convective	25	1440	1	-	51
	dielectric	40	60	1.7	100	91
10c	convective	25	1440	1	-	56
	dielectric	40	60	1.7	100	92

10d	convective	25	1440	1	-	56
	dielectric	40	60	1.7	100	92
10e	convective	25	1440	1	-	56
	dielectric	40	60	1.7	100	91
10f	convective	25	1440	1	-	53
	dielectric	40	60	1.7	100	93
10g	convective	25	1440	1	-	52
	dielectric	40	60	1.7	100	92
10h	convective	25	1440	1	-	57
	dielectric	40	60	1.7	100	94
13a	convective	25	1440	1	-	63
	dielectric	40	60	1.7	100	91
13b	convective	25	1440	1	-	67
	dielectric	40	60	1.7	100	87
13c	convective	25	1440	1	-	63
	dielectric	40	60	1.7	100	96
13d	convective	25	1440	1	-	59
	dielectric	40	60	1.7	100	98

Table II summarizes the experimental conditions applied and show the results obtained in the synthesis of the new selenazole derivatives. A comparison between the two alternative techniques namely the classical convective heating and the microwave irradiation, emphasize the advantages of microwaves assisted synthesis which affords almost quantitave reaction yields in much shorter reaction times.

7.Phenothiazinyl-hydrazino-thiazoles and phenothiazinyl-hydrazino-selenazoles

7.1 Synthesis of phenothiazinyl-hydrazino-thiazoles

Phenothiazines are 3-ring heterocyclic compounds with widespread therapeutic applications.[38, 39] The biologic effect of several phenothiazine-derived agents was

[38] S. C. Mitchell, Curr. Drug Targets, 7(9), (2006), 1181-1189

investigated and a large number of compounds have proven to possess cytotoxic potential. Phenothiazine derivates are effective against ovarian cancer cells,[40] human cervical cancer cells,[41] lymphomas and COLO 320 cells,[42] they are able to induce apoptosis in neuroblastoma, glioma[43] and lung fibroblast cell lines.[44] Moreover, they induce antiproliferative effects on multidrug- resistant cancer cells and exhibit a synergic effect in combination with some antineoplastic drugs.

Microwave-assisted synthesis was applied in the preparation of various heterocycles, including phenothiazine.[45, 46] Here we wish to emphasize the advantages of microwave irradiation over classical heating technique, in the synthesis of new phenothiazinyl-thiazolyl-hydrazine derivatives.

The strategy applied for the chemical synthesis of the target heterocyclic hydrazine derivatives **16a-g** containing thiazole and phenothiazine units in the same molecular structure, is based on two reaction steps.

The first one is the codensation of 10-alkyl-10*H*-phenothiazine-3-carbaldehyde with thiosemicarbazide, which generates 1-((10-alkyl-10H-phenothiazin-3-yl)-methylidene)-thiosemicarbazide **15** as presented in **scheme 7**. The second reaction step is the Hantzsch thiazole synthesis using intermediate **15** and several α-haloketones as presented in **scheme 8**. In order to find the most suitable reaction conditions, the syntheses were performed under both classical convective and microwave assisted heating techniques.

Condensation of 10-ethyl-10*H*-phenothiazine-3-carbaldehyde with thiosemicarbazide in ethanol under reflux required a long reaction time (4h) in order to obtain good yields (87%) of **15.**

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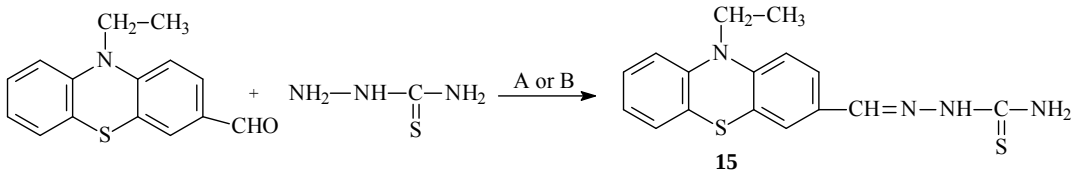


15

Scheme 7. Condensation of 10-ethyl-10*H*-phenothiazine-3-carbaldehyde with thiosemicarbazide Reaction conditions: A) abs. ethanol, ~80 ºC, convective heating; B) abs. ethanol or ethanol-water mixture, ~100 ºC, MW irradiation

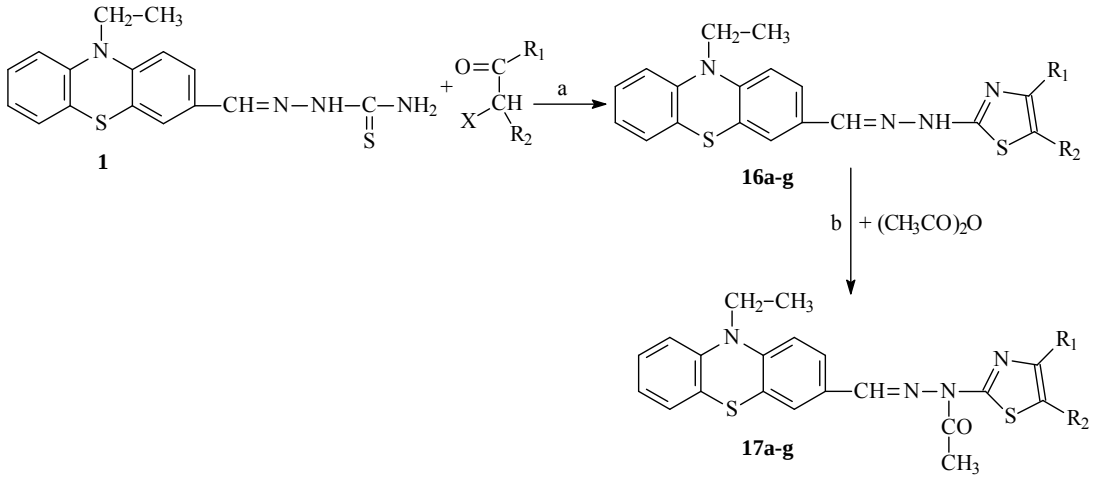
In **table III** are listed the alternative conditions applied for the optimization of the microwave assisted synthesis. Good to excellent yields of **15** were obtained in the presence of ethanol solvent in much shorter reaction times. Attempts to replace the organic solvent with water, indicate the possibility of using it only as a co solvent in the microwave assisted condensation (table III, entry 5, 6).

Table III Microwave[a] assisted synthesis of thiosemicarbazone **1**5

Entry	Solvent	Reaction time (min.)	Temperature (ºC)	Yield (%)
1	Ethanol	30	100	95
2	Ethanol	20	100	65
3	Ethanol	10	100	48
4	Ethanol	10	80	61
5	Ethanol: Water	20	100	51
6	Water	20	100	5

[a] Power P= 200 W

Several *α*-halogeno-carbonyl derivatives (chloroacetone, 1,3-dichloroacetone, *α*-bromoacetophenone, 3-chloroacetylacetone, ethyl-*α*-bromoacetylacetate, ethyl-*γ*-bromoacetylacetate, ethyl-bromopyruvate) were employed in the Hantzsch cyclization of thiosemicarbazone **15** as shown in **scheme 8**. Compounds **16a-g** were further subjected to acylation reaction with acetic anhydride and acetyl derivatives **17a-h** were obtained in high yields (**Scheme 8**).



	a	**b**	**c**	**d**	**e**	**f**	**g**
R_1	-CH_3	-CH_2Cl	-C_6H_5	-CH_3	-CH_3	-CH_2-COO-C_2H_5	-COO-C_2H_5
R_2	-H	-H	-H	-CO-CH_3	-COO-C_2H_5	H	H

Scheme 8. Synthesis of phenothiazyl-thiazolyl-hydrazine derivatives **16a-g** and **17a-g.** (a) Hantzsch cyclization, DMF, ethanol, reaction conditions are located in Table 2. b) acetic anhydride, pyridine (catalytic amount), reflux 15 minutes.

7.2 Synthesis of phenothiazinyl-hydrazino-thiazoles/selenazoles by microwave activation

The target phenothiazyl-thiazolyl-hydrazine derivatives **16a-g** were obtained in moderate yields by classical procedure at room temperature, but excelent yields were observed when microwave irradiation was applied (**Table IV)**.

Optimal reaction conditions were established after several experiments performed at different reaction temperatures (40°C or 60°C) and times (30, 60or 90 min.) in the presence of dimethylformamide as solvent. Very high yields of each compound **16a-g** were obtained by microwave assisted synthesis after 90 minutes at 60 °C.

Table IV. Experimental conditions applied in the synthesis of phenothiazinyl-thiazolyl-hydrazine derivatives **16a-g**.

Compound	Method	Temperature (° C)	Reaction Time (min)	Pressure (Bar)	Power (W)	Yield (%)
16a	A	25	1440	1	-	57
	B	60	90	1.7	100	98
16b	A	25	1440	1	-	43
	B	60	90	1.7	100	92
16c	A	25	1440	1	-	62
	B	60	90	1.7	100	99
16d	A	25	1440	1	-	63
	B	60	90	1.7	100	89
16e	A	25	1440	1	-	60
	B	60	90	1.7	100	92
16f	A	25	1440	1	-	68
	B	60	90	1.7	100	94
16g	A	25	1440	1	-	59
	B	60	90	1.7	100	91

A Classical Hantzsch condensation

B Microwave assisted synthesis

The comparison between classical and microwave assisted reaction conditions presented in table 2 show that Hantzsch cyclization in classical conditions afforded 43-68% yields of target compounds **16a-b** after a very long reaction time at room temperature, while important improvements of the reactions yields (91-98%) were observed when microwave irradiation was applied for much shorter reaction times (1.5 hours).

7.5 Antiproliferative activity

The mathematic parameter used to quantify the compounds antiproliferative effect against the tumor cells was the half maximal inhibitory concentration (IC50), the concentration which inhibits 50% of the cell growth in the treated cell population. These data were obtained by generating dose-response curves for every compound using the

biostatistics software. For both HepG2 and CC531S cell lines we obtained the IC50 values for each compound (Table V and VI).

Table V. *In vitro* chemosensitivity of HepG2 tumor hepatic cells treated with phenotiazine derivates expressed as IC50 values.

Compound	**15**	**16a**	**16b**	**16c**	**16d**	**16e**	**16f**	**17b**	**17c**	**17d**	**17e**	**17g**	Cisplatin
IC50 µg/ml (Exp.1)	5.646	2.408	12.72	29.87	24.95	4.255	2.782	1.835	3.849	7.922	14.78	16.35	2.390
IC50 µg/ml (Exp.2)	3.428	4.223	8.641	37.39	19.58	4.747	2.217	2.604	4.933	14.14	11.65	13.34	2.124
IC50 µg/ml (Exp.3)	2.985	3.674	8.047	31.75	14.72	6.775	3.261	2.579	4.779	13.49	10.28	13.19	2.656

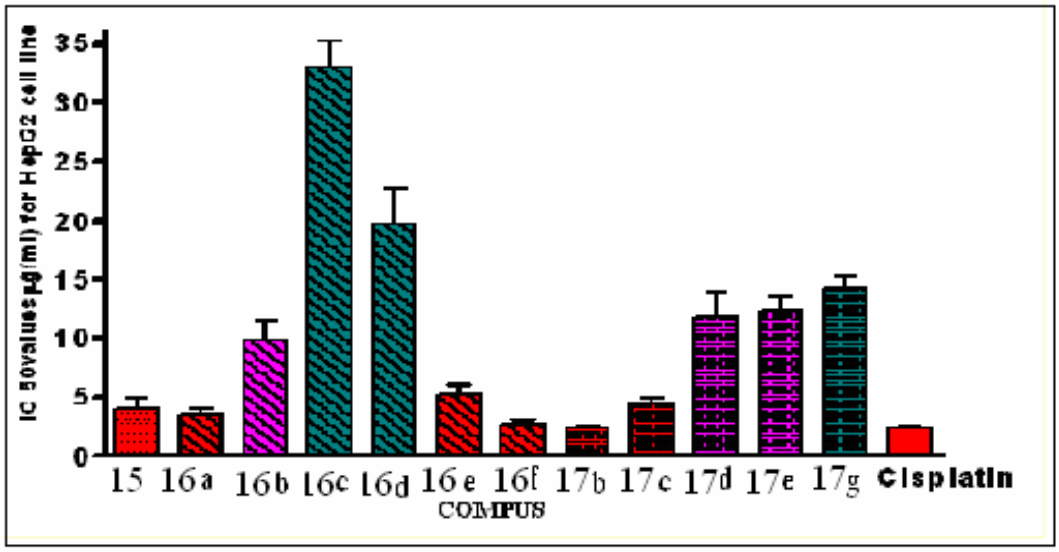


Figure. 5. Biologic activity against HepG2 cell line expressed as IC50 values.

The hepatic HepG2 tumor cell line growth was inhibited by all the 12 studied compounds (Figure 5). We compared the treated cells proliferation with the untreated, control tumor cells. As positive reference values we determined the IC50 values of the cisplatin, a commonly used chemotherapy drug, which showed effectiveness in tumor cell inhibition, and is proven to be active against HepG2 and CC531S cells. Cytotoxicity of cells treated with compounds **15-÷17g** was significant in each case; GraphPad Prism column statistics two-tailed t-test indicates us significant inhibition (95% Cl of discrepancy, two-tailed P value < 0.0395 for every compound). ANOVA one-way analysis of variance and Dunnett's multiple comparison test indicates that action of

compounds **15, 16a, 16e, 16f, 17b** and **17c** is comparable with the cisplatin, while activity of **16b, 16c, 16d, 17d, 17e** and **17g** is significantly lower as cisplatin cytotoxicity (P<0.05, P value extremely significant).

Table VI. *In vitro* antiproliferative activity of phenotiazine derivates against CC531S colorectal tumor cells expressed as IC50 values.

Compound	**1**	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**	**3b**	**3c**	**3d**	**3e**	**3g**	Cisplatin
IC50 µg/ml (Exp.1)	4.219	4.362	7.033	3.610	9.881	10.94	8.182	16.84	6.018	9.008	13.28	9.868	2.604
IC50 µg/ml (Exp.2)	6.108	7.011	9.152	4.126	7.185	16.61	9.421	20.12	13.49	9.357	9.820	9.273	1.638
IC50 µg/ml (Exp.3)	8.456	5.003	10.80	6.777	14.75	12.44	10.27	13.34	8.185	11.62	21.15	5.500	1.878

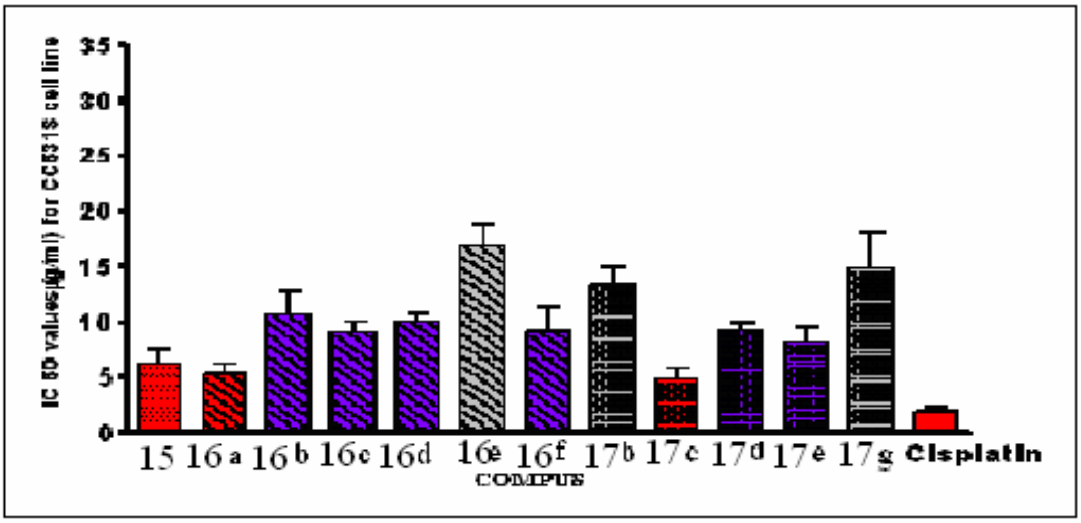


Figure 6. Phenothiazine activity quantified by IC50 values for CC531S cell line

The colon carcinoma CC531S cells proliferation was inhibited by the **15÷17g** phenothiazines (**Figure 6**). According to the two-tailed t-test and the ANOVA analysis the cytotoxicity of studied compounds is in every case significant relatively to the untreated cells (95% confidence interval, two-tailed P value not higher as 0.043). Biologic effect of compounds **15, 16a, 17c** is similar to cisplatin, while the other compounds activity shows significantly differences (for P<0.05). Within this group, we have two categories: **16c, 17d, 16f** and **16d** cytotoxicity differ slightly from the chemotherapy drug (Dunnett's multiple comparison test, P value summary significant),

while **16b**, **17b**, **16e** and **17g** have significantly lower activity (P value summary extremely significant).

We analyzed the biologic activity of the N-ethyl-3-formil phenothiazine (**15**), thiazolyl phenothiazine derivates (**16a**, **16b**, **16c**, **16d**, **16e, 16f)** and their acetylated analogues (**17b**, **17c**, **17d**, **17e**, **17g**). For the HepG2 cell line the cytotoxicity of compounds **16b** and **17b** differ significantly (Wilcoxon matched pair test, two-tailed P value 0,0074, very significant), difference between **16c** and **17c** is extremely significant (P=0,0002), in the pair **16e-17e** is a significant difference between IC50 values (P=0,0105), while activity of **16d** and **17d** does not diverge significantly. The presence of the acetyl group causes the decrease of the IC50 value of the compounds, except **17e**, which exhibit a bigger IC50 value.

We compared the activity of the analogous compounds, and the statistic analysis showed us that against the CC531S cell line they are no notably differences between **16b** and **17b, 16d** and **17d** (Wilcoxon matched pair test, Spearman two-tailed P value summary not significant), while for the pairs **16c - 17c** and **16e - 17e** the IC50 values differ significantly ($P<0.05$). The presence of the acetyl group in the molecule improved significantly the antiproliferative potential in compounds **17c** and **17e**. The presence of acetyl group in **17e** gives a divergent outcome; the compound is more cytotoxic against colorectal tumor cells, but less toxic against hepatic cells as his non-acetylated counterpart.

We compared the new compounds activity against the hepatic and the colorectal cells; the studied phenothiazines cytotoxicity is higher versus colorectal cells (two-way ANOVA test, Bonferroni post-test, $P<0.0001$).

The two groups of acetylated (**17b**, **17c**, **17d**, **17e, 17g**) and non-acetylated (**16a**, **16b**, **16c**, **16d**, **16e**) analogs were analyzed using grouped statistics; the two-way ANOVA test indicates that IC50 values for non-acetylated compounds are significantly higher for the HepG2 liver cells (Bonferroni post test, $p<0.01$), while in the case of CC531S cell line the differences are irrelevant between the two groups ($p>0.05$).

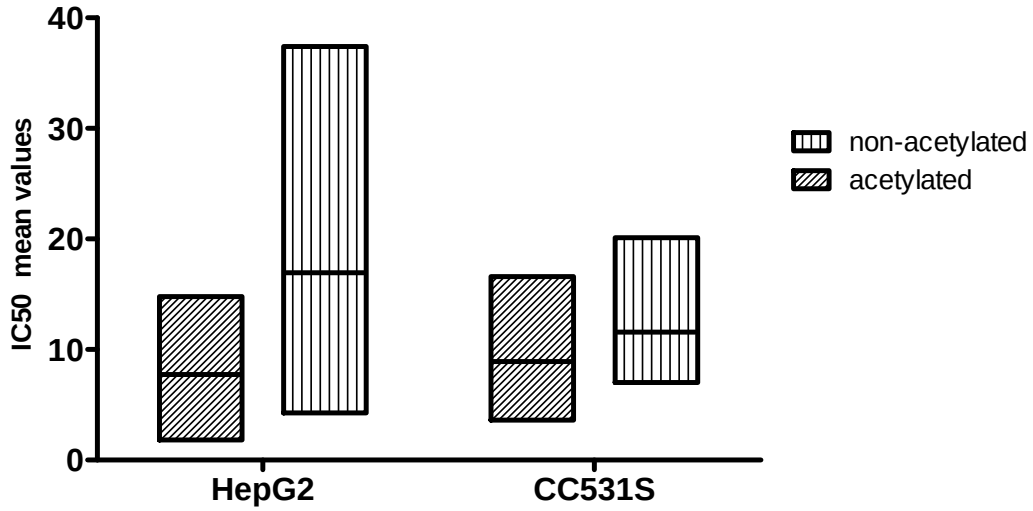


Figure 7. Comparison between acetylated and non-acetylated phenothiazines cytotoxicity against hepatic HepG2 and colon CC531S cell lines.

Compound **16a** which has the methyl moiety only, exhibit a very high cytotoxicity against both cell lines. Compound **16b** with the halogen in his structure and **16c** bearing the phenyl group exhibit lower toxicity against tumor cells, this effect is somehow enhanced for the acetylated analogue **17b** and **17c.** The molecule **16d** having already an acetyl group does not exhibit a notable antiproliferative activity among the studied series, and the presence of the second acetyl group in **17d** increase slightly the biologic effect. We studied the antitumoral properties of four molecules with acetyl acetic groups (**16e, 17e, 16f and 17g**). Among them, **16f** has a notable activity against both cell lines, while **17f** and **17g,** bearing two voluminous groups, have high steric hindrance which leads to a reduction in their activity.

8. GENERAL CONCLUSIONS

- This thesis presents the synthesis of 73 compounds, 69 of which being new compounds. We synthesised:
  - 13 compounds (**1, 2a-f, 3a-f**) from the hydrozin thiazole class, out of which 12 are new.
  - 5 compounds (**4-8**) from the hydrozino-bis-thiazole class, out of which 4 are new compounds.
  - 32 compounds (**9a-f, 10a-h, 11a-h, 12, 13, 14a-d, 15a-d**) from the hydrazine selenazole class, out of which 30 are new.
  - 15 compounds (**16, 17a-g, 18a-g**) from the phenothiazine hydrazin thiazole class, out of which 14 are new.
  - 8 compounds (**16', 17'a-g**) from the phenothiazine hydrazin selenazole class, all compouns being new.
- The Hantzsch condensation reaction conducted in a microwave field gives the advantage of shortening the reaction time and improving the chemical yield.
- The complete characterisation of the synthesized compounds was carried out using:
  - High resolution NMR (300, 400MHz), 1D: (^{1}H-RMN, ^{13}C-RMN) spectres and 2D: [(^{1}H-^{1}H) COSY, (^{1}H-^{13}C) HMQC şi (^{1}H-^{13}C) HMBC)] spectres.
  - Mass spectometry (EI)
  - Spectroscopy (IR)
  - Elemental analysis
- The kinetic study proved that the Hantzsch condensation reaction with the formation of the thiazolic nucleus takes place faster in polar aprotic solvents with high dielectric constant.
- We recorded short term anti-inflamatory action with **2a, 2b, 2f, 3b, 3c, 3e** and **3f** compounds. The **2a** compound has long term anti-inflamatory potential, even more efficient than the phenylbutazone.
- An analgesic profile similar to that of the heroine was found with the **3b** and **2f** compounds.

- We found antiproliferative action comparable to that of the doxorubicin in the **9b, 9f, 9d, 10g** şi **10f** compounds on the DU-145 cells as well as on DU-145 cells at the concentration of 25 μg/ml.
- We obtained efficient antimicrobian activity of the **9a, 9b, 9c** and **10e** compounds upon the Staphyloccocus aureus MSSA 25213 and Staphylococcus aureus MRSA 43300 strains. The activity disappears with the selanazols, therefore the introducing the selenium in the cycle is not favorable.
- The **16, 17a, 17e, 17f, 18b** and **18c** compounds ave antiproliferative action similar to that of the cisplatin upon the HepG2 cell line.
- The **16, 17a** şi **18c** compunds have antiproliferative potential similar to that of the cisplatin, at similar concentrations, on both cell lines.