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, cytotoxic, antimicrobial, anti-inflammatory, mitodepressive, hypotensive, anti-HIV, hypoallergenic, tuberculosis) and agriculture (pesticide action). 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 rigorously 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

1 B. S. Holla, European Journal of Medicinal Chemmistry, 2003, 38, 313.
Introduction

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 antitumor 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, 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, neuroleptics, antiparkinsonian, antimicrobial, etc.

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

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12 M. Koketsu, K. Kanoh and H. Ishiharab, *Heterocycles*, 2006, 68, 10, 2145
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, antitumoural, analgesic, anti-inflammatory and antipyretic properties. Some synthetic thiazoles have exhibited a range of biological activities, such as antitumor, antifilarial, antibiotic, antibacterial, antifungal, and anti-inflammatory. Recent studies have shown the synthesis of some new thiazole candidates as antimicrobial and anticancer agents. 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. Holla et al. synthesized a series of aryliden-hydrazinothiazoles and 2-furanyliden-hydrazinothiazoles with antimicrobial and anti-inflammatory activities. 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 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-toluensulfonylthiosemicarbazide 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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18 K. Bhat, B. Holla, Phosphorus, Sulfur and Silicon and the Related Elements **2004**, 179(6), 1019-1026
II.5 Personal contributions

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.

![Chemical structure](image)

<table>
<thead>
<tr>
<th></th>
<th>2a, 3a</th>
<th>2b, 3b</th>
<th>2c, 3c</th>
<th>2d, 3d</th>
<th>2e, 3e</th>
<th>2f, 3f</th>
</tr>
</thead>
<tbody>
<tr>
<td>R₁</td>
<td>CH₃</td>
<td>CH₂Cl</td>
<td>C₆H₅</td>
<td>CH₃</td>
<td>CH₃</td>
<td>CH₂COOC₂H₅</td>
</tr>
<tr>
<td>R₂</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>COCH₃</td>
<td>COOC₂H₅</td>
<td>H</td>
</tr>
</tbody>
</table>

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.

II.5 Personal contributions

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 1, when temperature and reaction time parameters were modified, show the possibility of obtaining enhanced reaction yields in reduced reaction times.

Table 1. Microwaves assisted Hantzsch condensation reaction parameters

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Temperature (°C)</th>
<th>Time (min)</th>
<th>Pressure (Bar)</th>
<th>Power W</th>
<th>Yields (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>25 (classical)</td>
<td>1440</td>
<td>1</td>
<td>-</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>120</td>
<td>1.7</td>
<td>200</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>120</td>
<td>1.7</td>
<td>200</td>
<td>82</td>
</tr>
<tr>
<td>2b</td>
<td>25 (classical)</td>
<td>1440</td>
<td>1</td>
<td>-</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>120</td>
<td>1.7</td>
<td>200</td>
<td>74</td>
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<td>120</td>
<td>1.7</td>
<td>200</td>
<td>92</td>
</tr>
<tr>
<td>2c</td>
<td>25 (classical)</td>
<td>1440</td>
<td>1</td>
<td>-</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>120</td>
<td>1.7</td>
<td>200</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>120</td>
<td>1.7</td>
<td>200</td>
<td>71</td>
</tr>
<tr>
<td>2d</td>
<td>25 (classical)</td>
<td>1440</td>
<td>1</td>
<td>-</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>120</td>
<td>1.7</td>
<td>200</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>120</td>
<td>1.7</td>
<td>200</td>
<td>81</td>
</tr>
<tr>
<td>2e</td>
<td>25 (classical)</td>
<td>1440</td>
<td>1</td>
<td>-</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>120</td>
<td>1.7</td>
<td>200</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>120</td>
<td>1.7</td>
<td>200</td>
<td>85</td>
</tr>
</tbody>
</table>
II.5 Personal contributions

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. 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).

<table>
<thead>
<tr>
<th>5, 7</th>
<th>6, 8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R_1</strong></td>
<td><strong>R_1</strong></td>
</tr>
<tr>
<td>CH_2Cl</td>
<td>CH_3COOC_2H_5</td>
</tr>
</tbody>
</table>

Scheme 2. Synthesis of compound 5-8 derivatives

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 thiazoyl-carbonylhydrazinothiazole, there was instead a monoacylation reaction with acetic anhydride.
II.5 Personal contributions

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 condensed 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 1H-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 plethysmometre 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.
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). 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 reevaluated 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](image)

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.
II.5 Personal contributions

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.
II.5 Personal contributions

Table 1. Activity of some of the compounds and doxorubicin on DU-145 and Hep G2 cancer cell lines

<table>
<thead>
<tr>
<th>Samples</th>
<th>DU145</th>
<th>HepG2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC50  (µg/ml)</td>
<td>Inhibition percentage (%) at 25 µg/ml</td>
</tr>
<tr>
<td>1</td>
<td>2.2</td>
<td>100.00 ± 0.00</td>
</tr>
<tr>
<td>2a</td>
<td>1.97</td>
<td>100.00 ± 0.00</td>
</tr>
<tr>
<td>2c</td>
<td>3.13</td>
<td>100.00 ± 0.00</td>
</tr>
<tr>
<td>2d</td>
<td>3.20</td>
<td>100.00 ± 0.00</td>
</tr>
<tr>
<td>2e</td>
<td>1.66</td>
<td>100.00 ± 0.00</td>
</tr>
<tr>
<td>3a</td>
<td>2.8</td>
<td>100.00 ± 0.00</td>
</tr>
<tr>
<td>3b</td>
<td>&gt;25</td>
<td>35.16 ± 3.12</td>
</tr>
<tr>
<td>3c</td>
<td>&gt;25</td>
<td>32.55 ± 2.11</td>
</tr>
<tr>
<td>3d</td>
<td>21.88</td>
<td>59.95 ± 3.98</td>
</tr>
<tr>
<td>3e</td>
<td>&gt;25</td>
<td>30.08 ± 1.97</td>
</tr>
<tr>
<td>3f</td>
<td>20.35</td>
<td>56.78 ± 4.03</td>
</tr>
<tr>
<td>5</td>
<td>17.23</td>
<td>57.28 ± 3.78</td>
</tr>
<tr>
<td>8</td>
<td>&gt;25</td>
<td>44.03 ± 2.09</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>&lt;1.6</td>
<td>100.00 ± 0.00</td>
</tr>
</tbody>
</table>

As doxorubicin, compounds 2a and 2e presented IC50 < 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

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II.6 Personal contributions

reducing certain inflammatory processes and in detoxification processes\textsuperscript{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\textsuperscript{27}, antibacterial (selenafurin),\textsuperscript{28} antiviral activity,\textsuperscript{29} inhibits the synthesis of nitric acid\textsuperscript{30} and they are antagonists for histamine H\textsubscript{2}\textsuperscript{31} receptors. 2-Dialkyl-amino-1,3-selenazole derivatives\textsuperscript{32} were employed in the synthesis of some dyes.\textsuperscript{33} N-acyl- derivatives of 2-amino-4-(isothiocyanatomethyl)-1,3-selenazole show antitumor activity.\textsuperscript{34} Recently was reported the inhibitory activity of 2-piperidin- and 4-phenyl-2-piperidin-1,3-selenazole upon anion-superoxidase.\textsuperscript{35}

Literature 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\textsuperscript{36}, \textsuperscript{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 Hantsch type cyclization reaction between these precursors and \(\alpha\)-halogeno-carbonyl derivatives.

\begin{footnotesize}
\begin{enumerate}
\item A. Wessjohann, A. Schneider, M. Brandt – Biol. Chem. 2007, 388, 997-1006
\item D. Keil, H. Hartmann - Dyes and Pigments 2000, 44, 149.
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\end{enumerate}
\end{footnotesize}
II.6 Personal contributions

New aryliden-hydrazinoselenazoles 10a-h and aryl-hydrazinoselenazoles 13a-d were prepared by Hantsch condensation reaction between a selenamide and a series of \( \alpha \)-halogenocarbonyl derivatives.

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

\[
\text{Ar} - \text{CH}=\text{O} + \text{H}_2\text{N} - \text{NH} - \text{C}-\text{NH}_2 \rightarrow \text{Ar} - \text{C}=\text{N} - \text{NH} - \text{C}-\text{NH}_2
\]

<table>
<thead>
<tr>
<th>Ar</th>
<th>a</th>
<th>b</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(_6)H(_5)</td>
<td>C(_6)H(_4)Cl((4))</td>
<td>C(_6)H(_4)OCH(_3)((4))</td>
<td></td>
</tr>
</tbody>
</table>

Scheme 3

Selenosemicarbazones 9a-d were further subjected to condensation reaction with different \( \alpha \)-halogenocarbonyl derivatives such as monochloroacetone, 1,3-dichloroacetone, \( \alpha \)-bromoacetophenone, 3-chloro-acetylacetone and \( \alpha \)-bromoacetylacetic ester, in DMF/acetone solution. Hydrazinoselenazoles 10a-h thus obtained were further acetylated using acetic anhydride, as presented in scheme 4.
II.6 Personal contributions

![Chemical diagram showing the synthesis of aroyl-hydrazino-selenazoles](image)

<table>
<thead>
<tr>
<th>10, 11</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
<th>g</th>
<th>h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ar</td>
<td>C₆H₄Cl(4)</td>
<td>C₆H₄Cl(4)</td>
<td>C₆H₄Cl(4)</td>
<td>C₆H₄Cl(4)</td>
<td>C₆H₄OCH₃(4)</td>
<td>C₆H₄OCH₃(4)</td>
<td>Th</td>
<td>Th</td>
</tr>
<tr>
<td>R₁</td>
<td>CH₃</td>
<td>CH₂Cl</td>
<td>C₆H₅</td>
<td>CH₃</td>
<td>C₆H₅</td>
<td>CH₃Cl</td>
<td>CH₃</td>
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</tr>
<tr>
<td>R₂</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>COOC₂H₅</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>COCH₃</td>
</tr>
</tbody>
</table>

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.

![Chemical diagram showing the synthesis of aroyl-selenosemicarbazides](image)

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)
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

<table>
<thead>
<tr>
<th>Cpd</th>
<th>Heating technique</th>
<th>Temp. (°C)</th>
<th>Time (min)</th>
<th>Pressure (Bar)</th>
<th>Power W</th>
<th>Yields (%)</th>
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<tbody>
<tr>
<td>10a</td>
<td>convective</td>
<td>25</td>
<td>1440</td>
<td>1</td>
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<td>52</td>
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<tr>
<td></td>
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<td>40</td>
<td>60</td>
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<td>94</td>
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<tr>
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<td>25</td>
<td>1440</td>
<td>1</td>
<td>-</td>
<td>51</td>
</tr>
<tr>
<td></td>
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<td>60</td>
<td>1.7</td>
<td>100</td>
<td>91</td>
</tr>
<tr>
<td>10c</td>
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<td>1440</td>
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<td>-</td>
<td>56</td>
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<td>60</td>
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<td>100</td>
<td>92</td>
</tr>
</tbody>
</table>
II.6 Personal contributions

<p>| | | | | | |</p>
<table>
<thead>
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</thead>
<tbody>
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<td>1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>dielectric</td>
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<td>60</td>
<td>1.7</td>
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<td>1440</td>
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</tr>
<tr>
<td></td>
<td>dielectric</td>
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<td>60</td>
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<td>100</td>
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<tr>
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<td>1440</td>
<td>1</td>
<td>-</td>
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<tr>
<td></td>
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<td>60</td>
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<td>100</td>
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</tr>
<tr>
<td>10h</td>
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<tr>
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<td>60</td>
<td>1.7</td>
<td>100</td>
</tr>
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<td>1440</td>
<td>1</td>
<td>-</td>
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<td>60</td>
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<td>100</td>
</tr>
<tr>
<td>13b</td>
<td>convective</td>
<td>25</td>
<td>1440</td>
<td>1</td>
<td>-</td>
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<tr>
<td></td>
<td>dielectric</td>
<td>40</td>
<td>60</td>
<td>1.7</td>
<td>100</td>
</tr>
<tr>
<td>13c</td>
<td>convective</td>
<td>25</td>
<td>1440</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>dielectric</td>
<td>40</td>
<td>60</td>
<td>1.7</td>
<td>100</td>
</tr>
<tr>
<td>13d</td>
<td>convective</td>
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<td>1440</td>
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<td>-</td>
</tr>
<tr>
<td></td>
<td>dielectric</td>
<td>40</td>
<td>60</td>
<td>1.7</td>
<td>100</td>
</tr>
</tbody>
</table>

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 quantitative 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.\textsuperscript{38, 39} The biologic effect of several phenothiazine-derived agents was

\textsuperscript{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,\textsuperscript{40} human cervical cancer cells,\textsuperscript{41} lymphomas and COLO 320 cells,\textsuperscript{42} they are able to induce apoptosis in neuroblastoma, glioma\textsuperscript{43} and lung fibroblast cell lines.\textsuperscript{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.\textsuperscript{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 \textit{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-10H-phenothiazine-3-carbaldehyde with thiosemicarbazide, which generates 1-((10-alkyl-10H-phenothiazin-3-yl)-methylidene)-thiosemicarbazide \textit{15} as presented in \textit{scheme 7}. The second reaction step is the Hantzsch thiazole synthesis using intermediate \textit{15} and several $\alpha$-haloketones as presented in \textit{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-10H-phenothiazine-3-carbaldehyde with thiosemicarbazide in ethanol under reflux required a long reaction time (4h) in order to obtain good yields (87\%) of \textit{15}.

\textsuperscript{40} F. Barbieri, A. Alama, B. Tasso, V. Boido, F. Sparatore, Invest. New Drugs 21(4), (2003), 413-20.
Scheme 7. Condensation of 10-ethyl-10H-phenothiazine-3-carbaldehyde with thiosemicarbazide

Reaction conditions: A) abs. ethanol, ~80 °C, convective heating; B) abs. ethanol or ethanol-water mixture, ~100 °C, 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-assisted synthesis of thiosemicarbazone 15

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Reaction time (min.)</th>
<th>Temperature (°C)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanol</td>
<td>30</td>
<td>100</td>
<td>95</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol</td>
<td>20</td>
<td>100</td>
<td>65</td>
</tr>
<tr>
<td>3</td>
<td>Ethanol</td>
<td>10</td>
<td>100</td>
<td>48</td>
</tr>
<tr>
<td>4</td>
<td>Ethanol</td>
<td>10</td>
<td>80</td>
<td>61</td>
</tr>
<tr>
<td>5</td>
<td>Ethanol: Water</td>
<td>20</td>
<td>100</td>
<td>51</td>
</tr>
<tr>
<td>6</td>
<td>Water</td>
<td>20</td>
<td>100</td>
<td>5</td>
</tr>
</tbody>
</table>

*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).
II.7 Personal contributions

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 excellent 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, 60 or 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.
II.7 Personal contributions

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Method</th>
<th>Temperature (°C)</th>
<th>Reaction Time (min)</th>
<th>Pressure (Bar)</th>
<th>Power (W)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16a</td>
<td>A</td>
<td>25</td>
<td>1440</td>
<td>1</td>
<td>-</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>60</td>
<td>90</td>
<td>1.7</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>16b</td>
<td>A</td>
<td>25</td>
<td>1440</td>
<td>1</td>
<td>-</td>
<td>43</td>
</tr>
<tr>
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<td>60</td>
<td>90</td>
<td>1.7</td>
<td>100</td>
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</tr>
<tr>
<td>16c</td>
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<td>25</td>
<td>1440</td>
<td>1</td>
<td>-</td>
<td>62</td>
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<td>63</td>
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<td>60</td>
<td>90</td>
<td>1.7</td>
<td>100</td>
<td>92</td>
</tr>
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<td>16f</td>
<td>A</td>
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<td>1440</td>
<td>1</td>
<td>-</td>
<td>68</td>
</tr>
<tr>
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<td>90</td>
<td>1.7</td>
<td>100</td>
<td>94</td>
</tr>
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<td>16g</td>
<td>A</td>
<td>25</td>
<td>1440</td>
<td>1</td>
<td>-</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>60</td>
<td>90</td>
<td>1.7</td>
<td>100</td>
<td>91</td>
</tr>
</tbody>
</table>

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
II.7 Personal contributions

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 derivatives expressed as IC50 values.

<table>
<thead>
<tr>
<th>Compound</th>
<th>15</th>
<th>16a</th>
<th>16b</th>
<th>16c</th>
<th>16d</th>
<th>16e</th>
<th>16f</th>
<th>17b</th>
<th>17c</th>
<th>17d</th>
<th>17e</th>
<th>17g</th>
<th>Cisplatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC50 µg/ml (Exp.1)</td>
<td>5.646</td>
<td>2.408</td>
<td>12.72</td>
<td>29.87</td>
<td>24.95</td>
<td>4.255</td>
<td>2.782</td>
<td>1.835</td>
<td>3.849</td>
<td>7.922</td>
<td>14.78</td>
<td>16.35</td>
<td>2.390</td>
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<tr>
<td>IC50 µg/ml (Exp.3)</td>
<td>2.985</td>
<td>3.674</td>
<td>8.047</td>
<td>31.75</td>
<td>14.72</td>
<td>6.775</td>
<td>3.261</td>
<td>2.579</td>
<td>4.779</td>
<td>13.49</td>
<td>10.28</td>
<td>13.19</td>
<td>2.656</td>
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</tbody>
</table>

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% CI 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
II.7 Personal contributions

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 phenothiazine derivates against CC531S colorectal tumor cells expressed as IC50 values.

<table>
<thead>
<tr>
<th>Compound</th>
<th>1</th>
<th>2a</th>
<th>2b</th>
<th>2c</th>
<th>2d</th>
<th>2e</th>
<th>2f</th>
<th>3b</th>
<th>3c</th>
<th>3d</th>
<th>3e</th>
<th>3g</th>
<th>Cisplatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC50 µg/ml (Exp.3)</td>
<td>8.456</td>
<td>5.003</td>
<td>10.80</td>
<td>6.777</td>
<td>14.75</td>
<td>12.44</td>
<td>10.27</td>
<td>13.34</td>
<td>8.185</td>
<td>11.62</td>
<td>21.15</td>
<td>5.500</td>
<td>1.878</td>
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</tbody>
</table>

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),
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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 17e 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, 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).
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 hydrazin thiazole class, out of which 12 are new.
- 5 compounds (4-8) from the hydrazino-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 compounds 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: (1H-RMN, 13C-RMN) spectra and 2D: [(1H-1H) COSY, (1H-13C) HMQC şi (1H-13C) HMBC] spectra.
- Mass spectrometry (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-inflammatory action with 2a, 2b, 2f, 3b, 3c, 3e and 3f compounds. The 2a compound has long term anti-inflammatory potential, even more efficient than the phenylbutazone.

An analgesic profile similar to that of the heroine was found with the 3b and 2f compounds.
II.8 Personal contributions  

- We found antiproliferative action comparable to that of the doxorubicin in the 9b, 9f, 9d, 10g ş 10f compounds on the DU-145 cells as well as on DU-145 cells at the concentration of 25 µg/ml.
- We obtained efficient antimicrobial activity of the 9a, 9b, 9c and 10e compounds upon the S. aureus MSSA 25213 and S. 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 ş 18c compounds have antiproliferative potential similar to that of the cisplatin, at similar concentrations, on both cell lines.