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Syntheses of new azaheterocycles with special properties

ABSTRACT

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The doctoral thesis entitled "Syntheses of new azaheterocycles with special properties" comprises 216 pages, appendices (they contain the published scientific articles) and has the following structure:

Table of contents

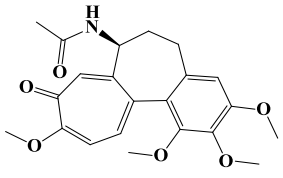
The work is accompanied by 176 bibliographic references, and the summary includes a succinct form of personal research, general conclusions and part of the bibliographic references. The original numbering of the chapters, figures, reaction schemes and tables from the doctoral thesis is preserved.

Introduction

Cancer is currently a major cause of death worldwide. Antitumor agents used in the treatment of cancer generally have cytostatic or cytotoxic activity by interfering with the mechanisms responsible for cell division. Even though huge efforts have been made to find a cure, cancer remains one of the most difficult diseases to treat, as most patients only achieve a longer survival. Every year, the number of new cases of cancer increases, which is the main reason why researchers turn their attention to the synthesis of new compounds with antitumor action, with the aim of curing where possible or prolonging and improving the quality of life of patients.

According to the World Health Organization1, cancer is the second leading cause of death worldwide, after cardiovascular diseases, causing approximately 10 million deaths in the last year. In 2020[1], the most common types of cancer were breast cancer, lung cancer, colon cancer, prostate cancer, skin cancer (non-melanoma) and stomach cancer. Lung cancer, colon cancer, liver cancer, stomach cancer and breast cancer were the most common causes of cancer death in the same year. It is estimated that between 30-50% of cancer cases are currently preventable by avoiding risk factors and that the burden of cancer can be reduced by early detection, appropriate treatment and patient care.

Currently, oncology research has as its main strategy the targeting of certain enzymes, such as tubulin, farnesyltransferase, kinase and topoisomerase, which have an important role in cell division. Thus, the synthesis of compounds with potential biological activity, especially anticancer, is achieved by using the studies and structures of compounds known as antitumor agents. The new generation of antitumor compounds is often combined with classical cytotoxic agents to achieve therapeutic effects. To avoid the resistance mechanisms developed by many tumors to antitubulin agents, it is necessary to identify new compounds with different mechanisms of action and improved pharmacodynamic properties.



1

Figure I.1. Structure of colchicine.

An example of a compound with antitumor activity is colchicine[2] (compound **1**, **Figure I.1**), an alkaloid found in the autumn sorrel and used as a cytostatic (prevents cell division) only in the final stages of the disease, with no other alternative.

Starting with the structure of colchicine, analogues with antitumor action were synthesized, for example combrestatin A-4[3] (CA-4), isocombrestatin[3] and fenstatin[3] (**Figure I.2**).

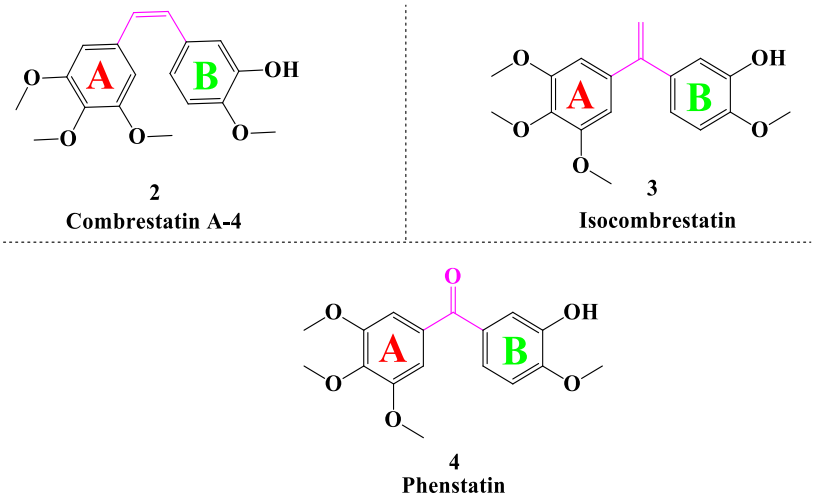


Figure I.2. Structures of colchicine analogues.

Tubulin plays an important role in a variety of essential cellular processes, such as intracellular transport, the formation and maintenance of cell shape, thus, by inhibiting its polymerization, cancer cells can no longer grow, drugs have either a cytostatic effect (they stop the growth of cells cancerous), or cytotoxic effect (destroy cancer cells).

An inhibitor of tubulin polymerization is CA-4 which has as structural elements two rings A and B of the 3,4,5-trimethoxyphenyl and 3-hydroxy-4-methoxyphenyl type, respectively, connected by a double bond. There are two forms of CA-4, *cis* and *trans*, but only the cis form exhibits antitumor activity. For this reason, modulations were made on the structure, obtaining analogs like isocombrestatin and fenstatin.[3,4]

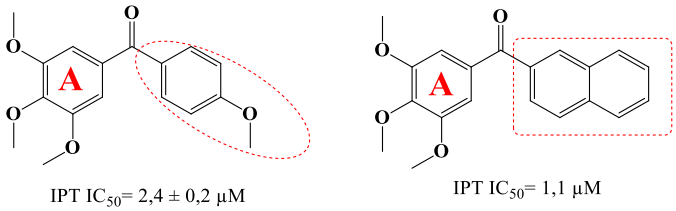
Phenstatin is a benzophenone-type CA-4 analog, synthesized by Pettit et al.[3], with a carbonyl-type connector between the aromatic rings. It is equivalent to Combrestatin A-4 in its interactions with tubulin, currently in clinical trials to evaluate antitumor activity.

The carbon atom belonging to the carbonyl group in phenstatin, sp^2 hybridized constrains the *cis* orientation and often improves chemical stability and water solubility. This has led to the synthesis of novel microtubule inhibitors exhibiting improved pharmacological profiles (with antiproliferative activity at nanomolar concentrations against a panel of human cancer cell lines).[4]

Phenstatin and its derivatives are potential anticancer candidates due to their inhibitory properties on tubulin polymerization, cell growth, and antivascular activity.

Over time, various modulations have been made on the structure of Phenstatin, with the aim of obtaining new compounds with biological activity. The A and B rings were alternately

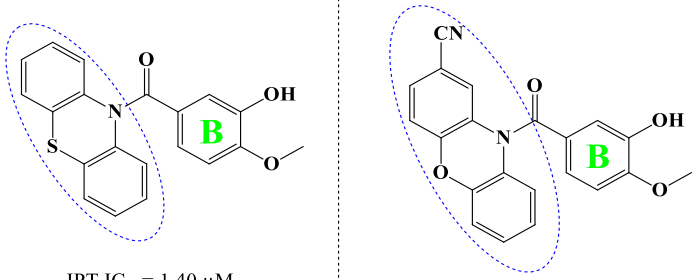
replaced, and the resulting compounds were tested for their anticancer activity. **Figure I.4** shows some examples of phenstatin analogs, with modified B cycle, which show activity on the inhibition of tubulin polymerization.[7,8]



IPT IC_{50}= 2,4 ± 0,2 µM — **6**

IPT IC_{50}= 1,1 µM — **7**

Figure I.4. Phenstatin analogs with B ring modified.



IPT IC_{50}= 1,40 µM — **8**

IPT IC_{50}= 0,52 µM — **9**

Figure I.5. Phenstatin analogs with A ring modified.

Replacing ring A and preserving ring B represented another modulation that led to the synthesis of some compounds with activity on the inhibition of tubulin polymerization (compounds **8** and **9**, **Figure I.5**).[9]

In this doctoral thesis, we proposed the synthesis and analysis of the biological properties of new compounds that will be evaluated for the ability to inhibit enzymes such as tubulin and/or farnesyltransferase. Thus, starting from the structure of Phenstatin, through different modulations, it is desired to introduce nitrogen heterocycles into the structure, because, as presented below, azaheterocycles represent a class of compounds of great importance in medical chemistry.

The main objectives

The aim of this doctoral thesis was the synthesis of new compounds, analogues of phenstatin, which contain in their structure heterocycles with one or more nitrogen atoms, because, as highlighted in the literature part of this work, nitrogen compounds arouse interest scientists presenting numerous biological activities. Starting from the structure of fenstatin, we made different modulations to synthesize new series of compounds and study their biological activity.

The first step in the design of the new compounds was to preserve the ketone-type connector and replace the B ring in the phenstatin structure with the pyrrole ring. The A ring was varied by replacing it, either with differently substituted phenyl cores or with other heterocycles. Another modulation was to modify the ketone-type linker so that it is alternately replaced by heterocycles such as pyrazole and pyridine, and the A and B rings are varied, being replaced by other differently substituted phenyl-type structures or other heterocycles (**Figure II.1**).

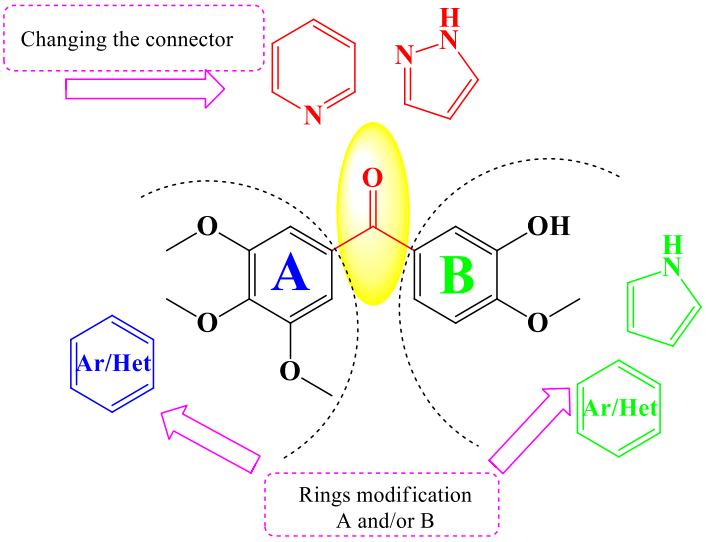


Figure II.1. Pharmacomodulations made on the structure of fenstatin.

The molecular design was designed to obtain three series of new compounds, whose structure contains the three azaheterocycles studied in the first chapter:

- Series I: analogues of phenstatin in the structure of which the ring B is replaced by the pyrrolic ring;
- Series II: phenstatin analogues in which the linker is of the pyrazole type;
- Series III: phenstatin analogues in which the linker is of the pyridinic type.

I.1. Design, synthesis and biological evaluation of some pyrrole derivatives - Series I

Specialized studies have shown that the presence of a pyrrolic nucleus in the structure can increase the biological potential of the synthesized structures. Thus, starting from the structure of fenstatin, through different modulations, we synthesized a series of pyrrolic derivatives, in order to study the biological activity. A first modulation was the introduction of the substituted pyrrolic nucleus with cyano (-CN) and amino (-NH_2) groups instead of the B ring in phenstatin and its preservation in the structures of all the synthesized compounds. We then introduced the ring (zone) C linked directly or through the methylene bridge (–CH_2-) to the ring B.

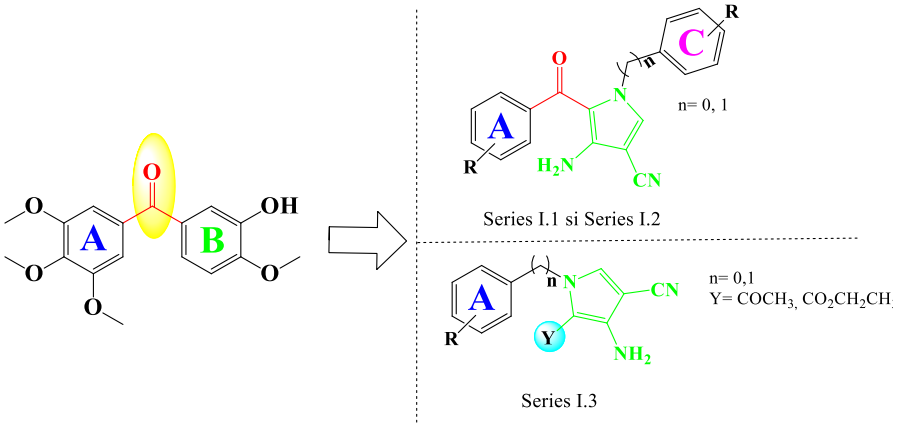
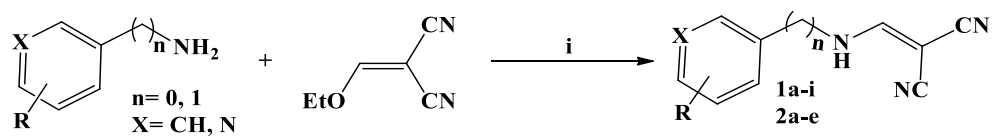


Figure II.3. General structures of synthesized pyrrolic derivatives.

Two series of compounds were obtained (**Figure II.3**):

- *Series I.1*: The fixed, pyrrole B ring and varying the A ring by replacing it with a different substituted phenyl core and introducing the C ring, directly linked to the B ring.
- *Series I.2*: Ring B, fixed pyrrole type, varying ring A by replacing it with a different substituted phenyl core and introducing ring C, linked to ring B by a methylene linker.

The first step in obtaining the target compounds was the synthesis of *β*-dicyano-enamine intermediates by means of the reaction between 2-ethoxymethylene-malononitrile and differently substituted anilines respectively differently substituted benzylamines (**Scheme II.2**).[128,129]



Scheme II.2. Synthesis reaction of *β*-dicyano-enamine type intermediates. **Reagents and conditions:** (i) EtOH, reflux, 6–8 h.

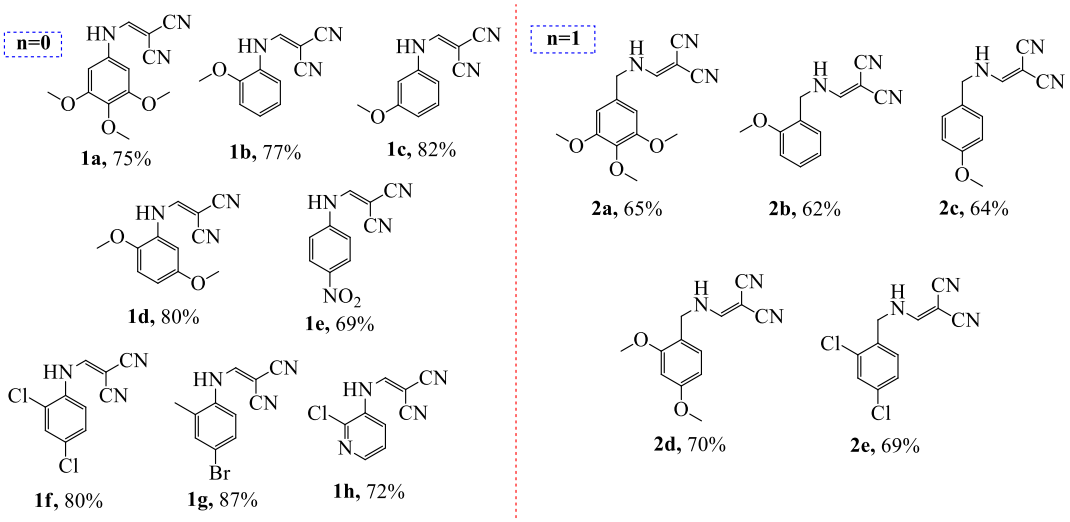
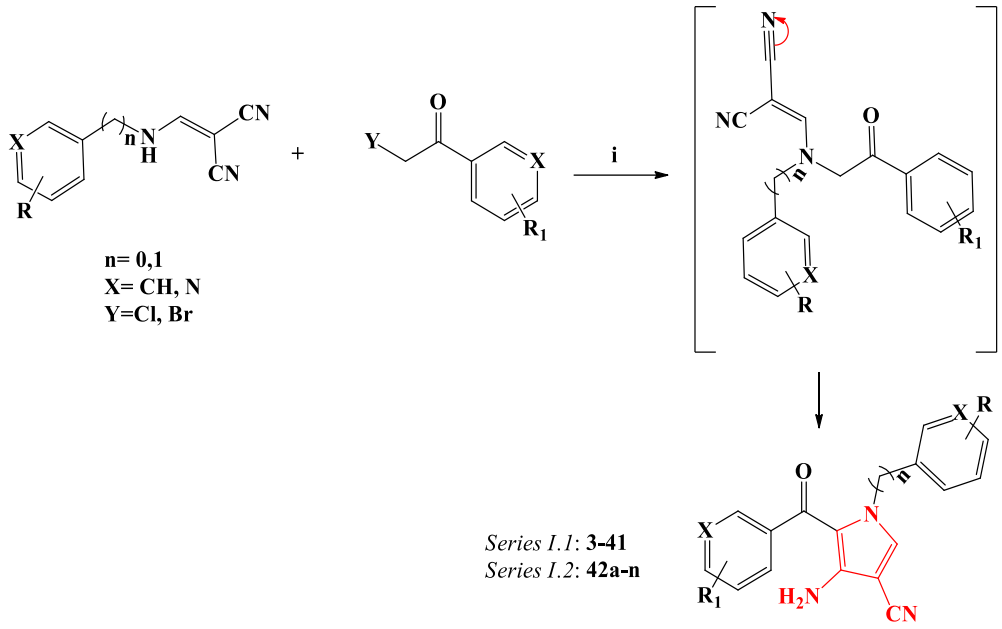


Figure II.6. Structures of compounds **1a-h** and **2a-e**.

To synthesize the pyrrolic derivatives of interest, the method described in the literature was used[128,129], the closing of the pyrrolic cycle is carried out by means of the reaction between the intermediates of the *β*-dicyano-enamine type (series **1a-h** and series **2a-e**) synthesized previously and different α-halogeno-ketones, in a basic environment (**Scheme II.3**).



Scheme II.3. Synthesis reaction of pyrrole derivatives. **Reagents and conditions**: (i) 3 equiv. TEA, DMF, reflux, 12-24 hours.

Thus, 39 compounds were synthesized in the structure of which both the substituents of the ring A and the substituents of the ring C were varied either with halogens (chlorine, bromine or fluorine) or with other groups of the methoxy or methyl type.

In order to be able to compare the influence of the methylene-type connector between the B and C rings, *Series I.2* of pyrroles was synthesized, using the same reaction (**Scheme II.3**), obtaining 14 compounds whose structure is presented in **Figure II.13**.

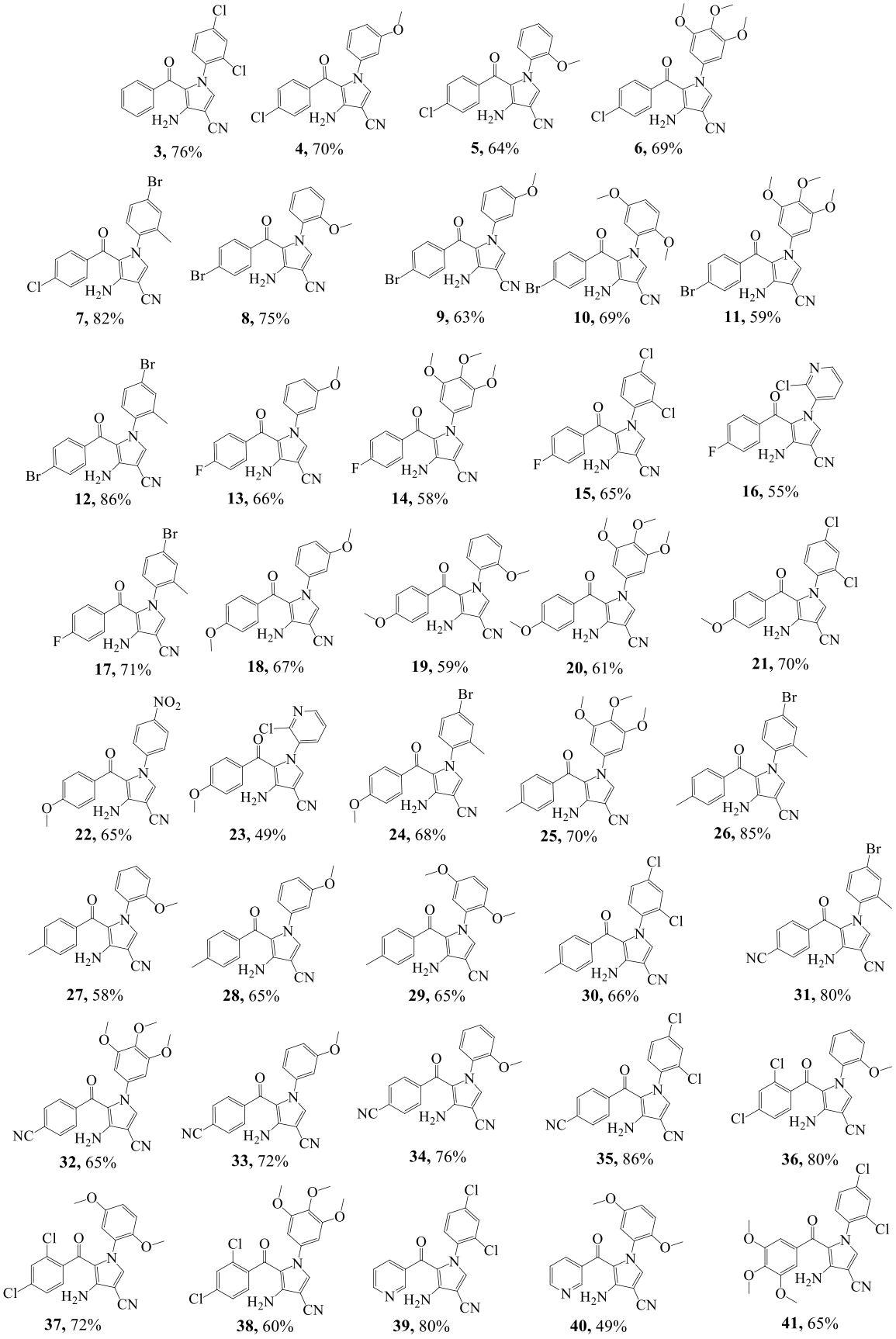


Figure II.7. The structures and yields of the compounds of *Series I.1*.

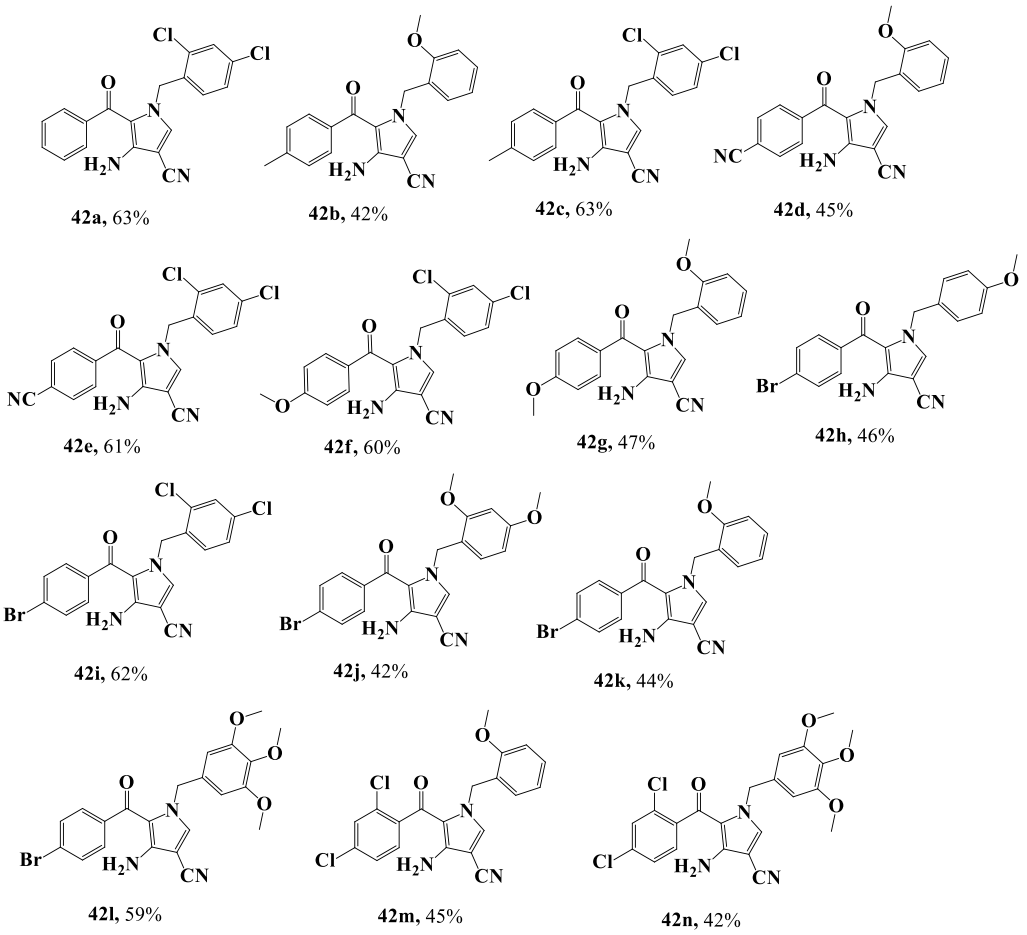
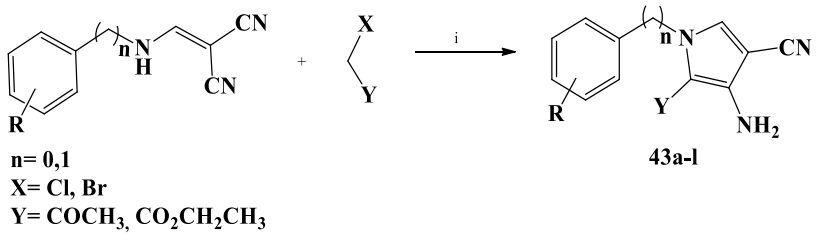


Figure II.13. The structures and yields of the pyrrole-type compounds of *Series I.2* with two connectors, one of the ketone type and one of the methylene type.

The last series of pyrrolic derivatives synthesized (*Series I.3*) according to **Scheme II.4**, is made up of compounds whose structure consists of two nuclei: ring A of different phenyl type substituted, either with halogens or with methoxy groups, and ring B of pyrrole type.



Scheme II.4. Synthesis reaction of pyrrole derivatives with two nuclei, *Series I.3*. **Reagents and conditions:** (i) 3 equiv. TEA, DMF, reflux, 12-24 hours.

To check whether the biological activity is influenced, the B ring is substituted differently in the 5-position, either with the acetyl group or with the ethyl ester group. Another modulation made was the presence of the connector between the two nuclei, thus in the structure of compounds **43a-g** the rings A and B are linked directly, and in the structure of compounds

43h-l the two rings are linked by the methylene type connector. The structures of the synthesized compounds are shown in **Figure II.17**.

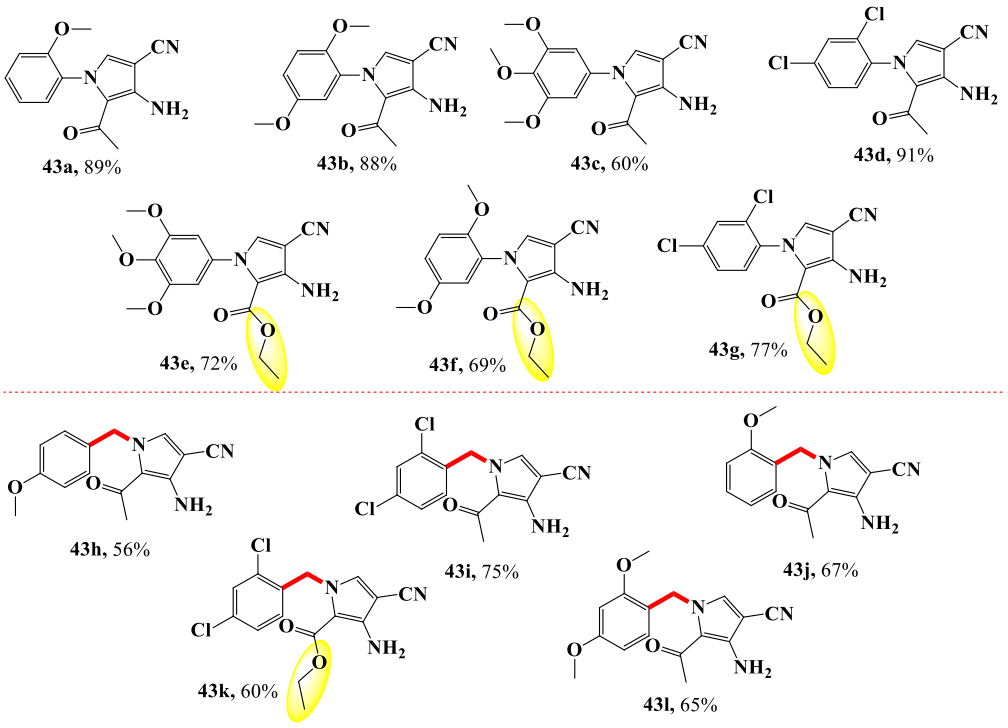


Figure II.17. Structures and yields of pyrrole-type compounds in *Series I.3.*

The three series of pyrrole type compounds (*Series I.1*, *Series I.2* and *Series I.3*) were proposed at the National Cancer Institute (NCI) to be studied for their anticancer activity through the ability to inhibit cell growth on 60 cancer cell lines.

The percentage values of cell growth inhibition for the 12 active compounds are shown in **Table II.3**.

The most active compounds, among those tested for antiproliferative activity on 60 cancer cell lines, are **18** and **36**. They belong to *Series I.1* of pyrrole derivatives, the C ring being directly linked to the pyrrole B ring (n= 0, **Figure II.4**). Another structural similarity is represented by the fact that the C rings of the two compounds is substituted with a methoxy group (*meta*-methoxy for compound **18** and *ortho*-methoxy for compound **36**).

In the case of compound **18**, ring A is also substituted with a methoxy group in the *para* position, and in the case of compound **36**, ring A is substituted with two chlorine atoms in the *ortho* and *para* positions. Compound **18** showed cytotoxicity on 6 cancer cell lines, and compound **36** on **9** cancer lines. The types of cancer on which the two compounds showed cytotoxic action are: cancer of the central nervous system (cell lines SF-539 and SNB-75), melanoma (line MDA-MB-435) and renal cancer (line A498).

Table II.3. Results of *in vitro* growth inhibition of human cancer cells for the compounds **5**, **8**, **13**, **18**, **19**, **27**, **36**, **37**, **42h**, **42j**, **42m** and **43k**.

Cell type	***Compound***	***5***	***8***	***13***	***18***	***19***	***27***	***36***	***37***	***42h***	***42j***	***42m***	***43k***
	Cell line	Cell Growth Inhibition, GI%[a,b] 10 μM											
Leukemia	CCRF-CEM	23	31	0	**81**	**80**	14	**92**	**88**	11	**55**	0	15
	HL-60(TB)	**63**	**64**	13	**96**	**100**[d,j]	43	**98**	**97**	**50**	**79**	11	39
	K-562	**79**	**79**	**60**	**85**	**86**	**71**	**87**	**85**	**75**	**84**	11	**75**
	MOLT-4	35	38	0	**70**	**69**	16	**79**	**70**	31	**79**	0	34
	RPMI-8226	11	0	0	**77**	**64**	0	**85**	**60**	0	41	0	16
	SR	**76**	**59**	**53**	**72**	**83**	**50**	**88**	**86**	**65**	**85**	11	**73**
Non-Small Cell Lung Cancer	A549/ATCC	0	21	0	**57**	46	10	**58**	48	0	**53**	15	23
	EKVX	13	14	11	**61**	**51**	10	**70**	**57**	0	41	15	18
	HOP-62	12	36	0	**69**	43	22	**61**	**50**	0	**59**	34	31
	HOP-92	0	n.d.[c]	22	**78**	47	n.d.[c]	**55**	0	35	44	30	26
	NCI-H23	0	12	0	**50**	**67**	10	**84**	**82**	20	45	12	15
	NCI-H460	12	20	0	**72**	**82**	0	**87**	**67**	13	**58**	25	18
	NCI-H522	**77**	**51**	33	**96**	**100**[d,k]	36	**100**[d,n]	**92**	0	**100**[d,a']	22	32
Colon Cancer	COLO 205	13	23	0	**74**	**61**	0	**80**	**67**	0	**69**	0	0
	HTC-116	36	41	19	**83**	**77**	23	**63**	**70**	23	**75**	20	35
	HCT-15	**63**	**64**	31	**80**	**73**	**52**	**75**	**78**	41	**73**	10	**51**
	HT29	47	**55**	16	**92**	**93**	26	**100**[d,o]	**95**	12	**95**	14	19
	KM12	41	**60**	24	**76**	**72**	40	**77**	**72**	14	**59**	12	39
	SW-620	**59**	**61**	0	**90**	**79**	47	**75**	**73**	23	**68**	0	**55**
CNS cancer	SF-295	21	**54**	16	**100**[d,e]	**91**	25	**80**	**71**	23	**60**	22	19
	SF-539	0	17	0	**100**[d,f]	**87**	0	**100**[d,p]	**74**	21	**52**	36	26
	SNB-19	30	28	0	**73**	**70**	22	**69**	**54**	16	44	**63**	31
	SNB-75	37	25	17	**100**[d]	**70**	17	**100**[d,q]	**100**[d,x]	0	49	35	**52**
	U251	0	33	10	**66**	**65**	20	**83**	**75**	0	**73**	47	35

Melanoma	LOX IMVI	32	33	0	**62**	**76**	19	**60**	**51**	20	**50**	16	21
	MALME-3M	40	**57**	0	**59**	**58**	40	**62**	49	15	**57**	37	14
	M14	n.d.[c]	n.d.[c]	16	**89**	n.d.[c]	n.d.[c]	**79**	**76**	21	**66**	12	27
	MDA-MB-435	**100**[d.m]	**96**	**61**	**100**[d.g]	**100**[d.l]	**94**	**100**[d,r]	**100**[d,y]	**78**	**99**	12	**100**[d,c']
	SK-MEL-2	21	29	0	**71**	**54**	20	**100**[d,s]	**100**[d,w]	0	**95**	23	0
	SK-MEL-28	25	30	0	**54**	40	26	42	40	14	45	0	0
	SK-MEL-5	39	22	0	**64**	**93**	0	**82**	45	0	**73**	0	22
	UACC-257	12	27	12	**52**	33	21	47	36	0	44	14	0
	UACC-62	**53**	**54**	34	**81**	**76**	**77**	**73**	**69**	19	**70**	24	40
Ovarian Cancer	IGROV1	22	32	0	**68**	66	15	**61**	46	0	**51**	11	36
	OVCAR-3	36	26	0	**69**	**85**	0	**82**	**71**	0	44	0	n.d.[c]
	OVCAR-8	0	0	0	**66**	44	0	**65**	31	0	**66**	44	0
	NCI/ADR-RES	**59**	**57**	21	**90**	**86**	39	**83**	**69**	0	**76**	35	38
Renal cancer	786-0	0	0	0	**62**	**56**	0	47	36	10	36	32	23
	A498	42	39	13	**100**[d,h]	**92**	25	**100**[d,t]	**71**	0	13	0	26
	ACHN	0	0	0	**61**	**53**	0	**51**	46	0	15	40	12
	CAKI-1	n.d.[c]	31	32	**69**	n.d.[c]	21	**66**	**63**	0	**54**	35	37
	RXF 393	n.d.[c]	14	15	**82**	n.d.[c]	23	**100**[d,u]	44	0	**58**	42	36
	SN12C	0	17	0	**67**	**54**	0	**65**	**51**	0	35	23	18
	UO-31	22	27	21	**66**	**52**	10	**57**	43	24	**65**	20	40
Renal cancer	PC-3	42	36	0	**66**	**63**	25	**81**	**62**	0	34	33	28
	DU-145	0	0	0	49	47	0	**64**	34	0	20	0	0
Breast cancer	MCF7	**67**	**66**	18	**82**	**86**	48	**83**	**83**	27	**72**	0	40
	MDA-MB 231/ATCC	31	20	0	**60**	**51**	0	**53**	35	0	39	18	**56**
	HS 578T	25	21	0	**88**	**86**	14	**100**[d,v]	**61**	0	**54**	32	36
	BT-549	0	11	0	**58**	35	16	**68**	**51**	0	**100**[d,b']	0	18

T-47D	11	39	12	**84**	**67**	17	**71**	**57**	0	**64**	0	21
MDA-MB-468	48	**68**	**62**	**100[d,i]**	**68**	**53**	**84**	**80**	39	**97**	19	**54**

[a] Data from NCI's *in vitro* screening of human tumor cells for a single dose (10 μM concentration). [b] Percent inhibition of cell growth. [c] Not determined. [d] A value of -x means x% cancer cells lethality of preexisting cells (cytotoxic effect).

[e] Percent inhibition of cell growth: -25%. [f] Percent inhibition of cell growth: -24%. [g] Percent inhibition of cell growth: -38%. [h] Percent inhibition of cell growth: -2%. [i] Percent inhibition of cell growth: -4%. [j] Percent inhibition of cell growth: -7%. [k] Percent inhibition of cell growth: -1%. [l] Percent inhibition of cell growth: -27%. [m] Percent inhibition of cell growth: -16%. [n] Percent inhibition of cell growth: -55%. [o] Percent inhibition of cell growth: -14%. [p] Percent inhibition of cell growth: -1%. [q] Percent inhibition of cell growth: -22%. [r] Percent inhibition of cell growth: -38%. [s] Percent inhibition of cell growth: -51%. [t] Percent inhibition of cell growth: -1%. [u] Percent inhibition of cell growth: -8%. [v] Percent inhibition of cell growth: -2%. [x] Percent inhibition of cell growth: -7%. [y] Percent inhibition of cell growth: -37%. [w] Percent inhibition of cell growth: -27%. [a'] Percent inhibition of cell growth: -29%. [b'] Percent inhibition of cell growth: -86%. [c'] Percent inhibition of cell growth: -7%.

The most active compounds of this step (testing at a single dose, 10 μM), **18** and **36**, were selected to proceed to the next step of testing, to determine the GI_{50} (the concentration at which cell growth is inhibited in proportion to 50%). The results are presented in **Table II.4.**

Table II.4. 5-Dose *in vitro* human cancer cell growth inhibition for compounds **18** and **36**.

Cell type	***Compound***	***18***	***36***	***Cell type***	***Compound***	***18***	***36***
	Cell line	GI_{50}[a,b] (μM)			**Cell line**	GI_{50}[a,b] (μM)	
Leukemia	CCRF-CEM	2.68	4.68	***Ovarian Cancer***	IGROV1	5.00	7.49
	HL-60(TB)	2.01	3.15		OVCAR-3	2.87	3.86
	K-562	**0.70**	3.63		OVCAR-4	18.0	8.96
	MOLT-4	3.65	4.84		OVCAR-5	7.36	9.71
	RPMI-8226	2.94	9.39		OVCAR-8	5.30	21.0
	SR	**0.77**	3.53		NCI/ADR-RES	2.35	3.55
Non-Small Cell Lung Cancer	A549/ATCC	5.68	7.39		SK-OV-3	4.09	3.36
	EKVX	4.67	8.24	***Renal cancer***	786-0	4.64	7.12
	HOP-62	4.12	6.40		A498	2.73	72.0
	HOP-92	9.53	16.8		ACHN	6.52	7.79
	NCI-H226	4.50	5.79		CAKI-1	3.20	4.70
	NCI-H23	5.61	7.55		RXF 393	2.05	4.37
	NCI-H460	3.41	4.66		SN12C	5.64	5.24
	NCI-H522	1.99	1.87		TK-10	6.14	52.9
Colon Cancer	COLO 205	3.01	5.70		UO-31	5.42	7.87
	HCC-2998	4.98	12.4	***Prostate cancer***	PC-3	3.75	5.60
	HTC-116	3.50	4.02		DU-145	4.70	9.73
	HCT-15	2.49	3.62	***Breast cancer***	MCF7	3.07	3.94
	HT29	2.64	3.18		MDA-MB-231/ATCC	7.80	9.05
	KM12	4.17	3.90		HS 578T	2.42	3.02
	SW-620	2.85	3.98		BT-549	4.41	3.41
CNS cancer	SF-268	5.65	9.77		T-47D	3.72	4.53
	SF-295	2.90	3.72		MDA-MB-468	1.12	2.48
	SF-539	2.41	3.37				
	SNB-19	3.92	6.60				
	SNB-75	1.88	2.61				
	U251	3.45	4.45				
Melanoma	LOX IMVI	4.60	4.89				
	MALME-3M	N.D.[d]	4.20				
	M14	2.51	3.27				
	MDA-MB-435	**0.78**	1.92				
	SK-MEL-2	4.58	2.69				

SK-MEL-28	49.8	7.58
SK-MEL-5	3.19	4.83
UACC-257	9.48	6.31
UACC-62	3.22	3.67

[a] Data obtained from NCI's in vitro 60-cell 5-dose screen; [b] GI_{50} is the molar concentration of synthetic compound causing 50% growth inhibition of tumor cells; [c] Bold values designate the best growth inhibition activity (the lowest GI50 values) obtained for the tested molecules. [d] Not determined.

Following the results received for the tests performed at 5 different concentrations, compound **18** showed the best anticancer activity, having 3 cell lines with $GI_{50} < 1.00$ μM (for leukemia on the K-562, SR lines and melanoma on the MDA-MB-435 line).

II.2. Design, synthesis and biological evaluation of some hydrazone derivatives - Series II

Another modification made on the structure of phenstatin was the one in which the ketone connector between the two substituted phenyl-type rings was replaced by a pyrazole-type heterocycle, because the compounds that contain such a heterocycle in their structure are known in the literature as having a wide spectrum of biological activities (for example anticancer activity[139] or anti-inflammatory activity[140]) (**Figure II.23**).

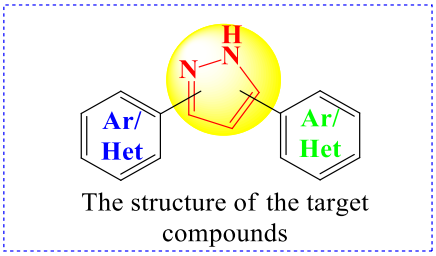
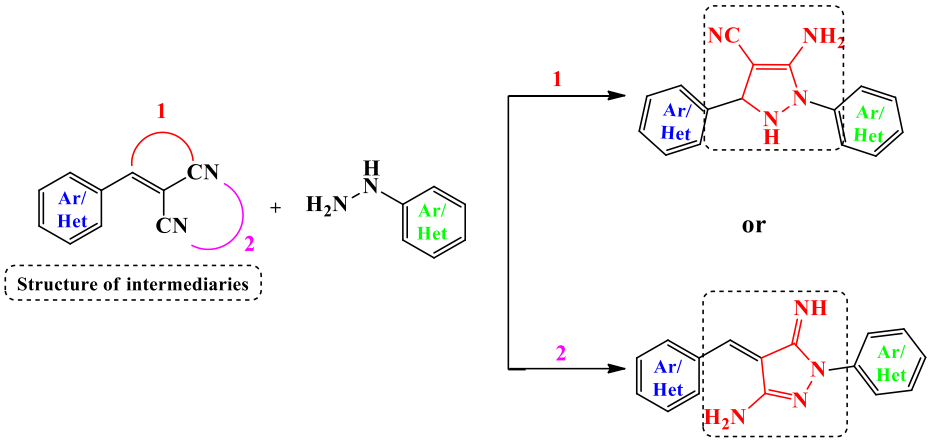


Figure II.23. The general structure of the series of pyrazole derivatives.

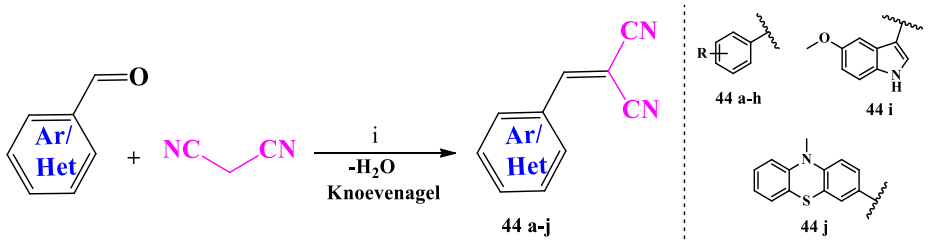
To obtain this class of compounds, a synthesis method described in the literature[141] was used, by means of compounds of the 2-(Aryl(Het)methylene)-malononitrile type and a series of differently substituted hydrazines. According to literature data, the closure of the pyrazole cycle can take place in two ways:

- With the help of a cyano group (CN) and the double bond, present in the structure of derivatives of type 2-(Aryl(Het)methylene)-malononitrile (path 1);
- via the two cyano groups (CN), (path 2), according to **Scheme II.5**, obtaining substituted dihydropyrazoles.



Scheme II.5. General scheme for the synthesis of dihydropyrazole derivatives.

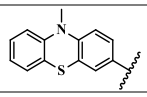
The first step in the design of the new structures was the synthesis of intermediates of the benzylidenemalononitrile type, respectively Het-methylenemalononitrile by means of the Knoevenagel type reaction, with the base, between different aldehydes and malononitrile (**Scheme II.6**).[141] The yields and substituents of each compound in the **44a-j** series are presented in **Table II.5**.



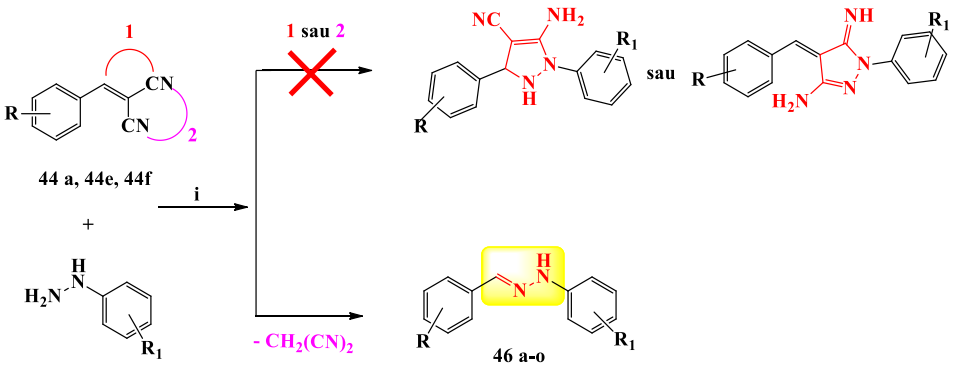
Scheme II.6. The reaction to obtain intermediates of the Aryl(Het)-methylene malononitrile type. **Reagents and conditions**: (i) piperidine, EtOH, reflux, 6-8 hours.

Table II.5. Substituents and yields of Aryl(Het)-methylenemalononitrile intermediates.

Compound	R	Yields (%)
44a[142]	3,4,5-triOCH_3	90
44b[143]	2,5-diOCH_3	85
44c[144]	3-OCH_3	87
44d[145]	4-OCH_3	80
44e[146]	4-Br	79
44f[147]	4-NO_2	87
44g[148]	4-$N(CH_3)_2$	85
44h[149]	4-OCH_3-3-NO_2	85
44i	-	86

44j[150]		-	72

The strategy for the synthesis of the target compounds was the reaction between the previously synthesized intermediates **44a–j** and various hydrazines. We performed the first reactions starting from 3,4,5-trimethoxybenzylidenemalononitrile and various hydrazines, in order to keep the A ring from phenstatin and to modify both the B ring and the ketone-type connector, according to the general synthesis scheme presented previously, (**Scheme II.5**) and to obtain the target compounds from **Figure II.19**. The compounds resulting from this reaction, after purification, were not the expected ones because the pyrazole-type heterocycle was not closed, eliminating the malononitrile according to **Scheme II.8**, thus obtaining hydrazone-type compounds (**Figure II.25**).



Scheme II.8. Scheme for obtaining hydrazone-type compounds. **Reagents and conditions**: (i) EtOH, reflux, 4-8 hours.

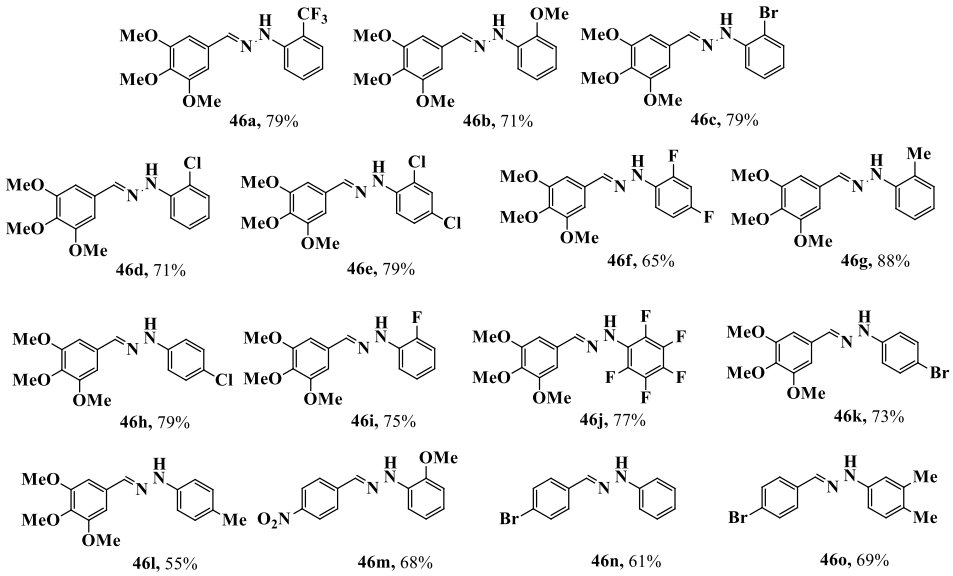


Figure II.25. Structures and yields of hydrazone-type compounds.

Compounds **46e** and **46i** were also analyzed by the single crystal X-ray diffraction method, thus confirming the hydrazone-type structure.

a b

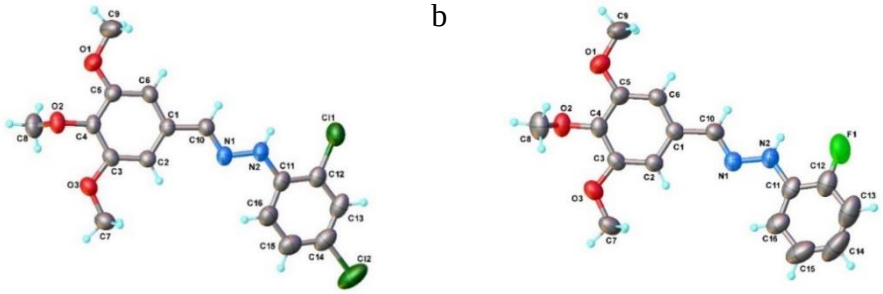
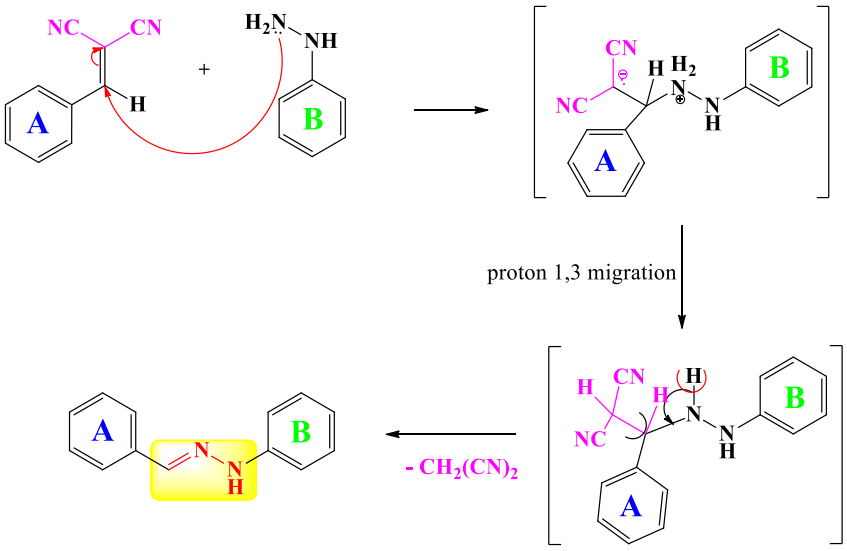


Figure II.29. Structures of compounds **46e** (a) and **46i** (b) obtained by X-ray diffraction.

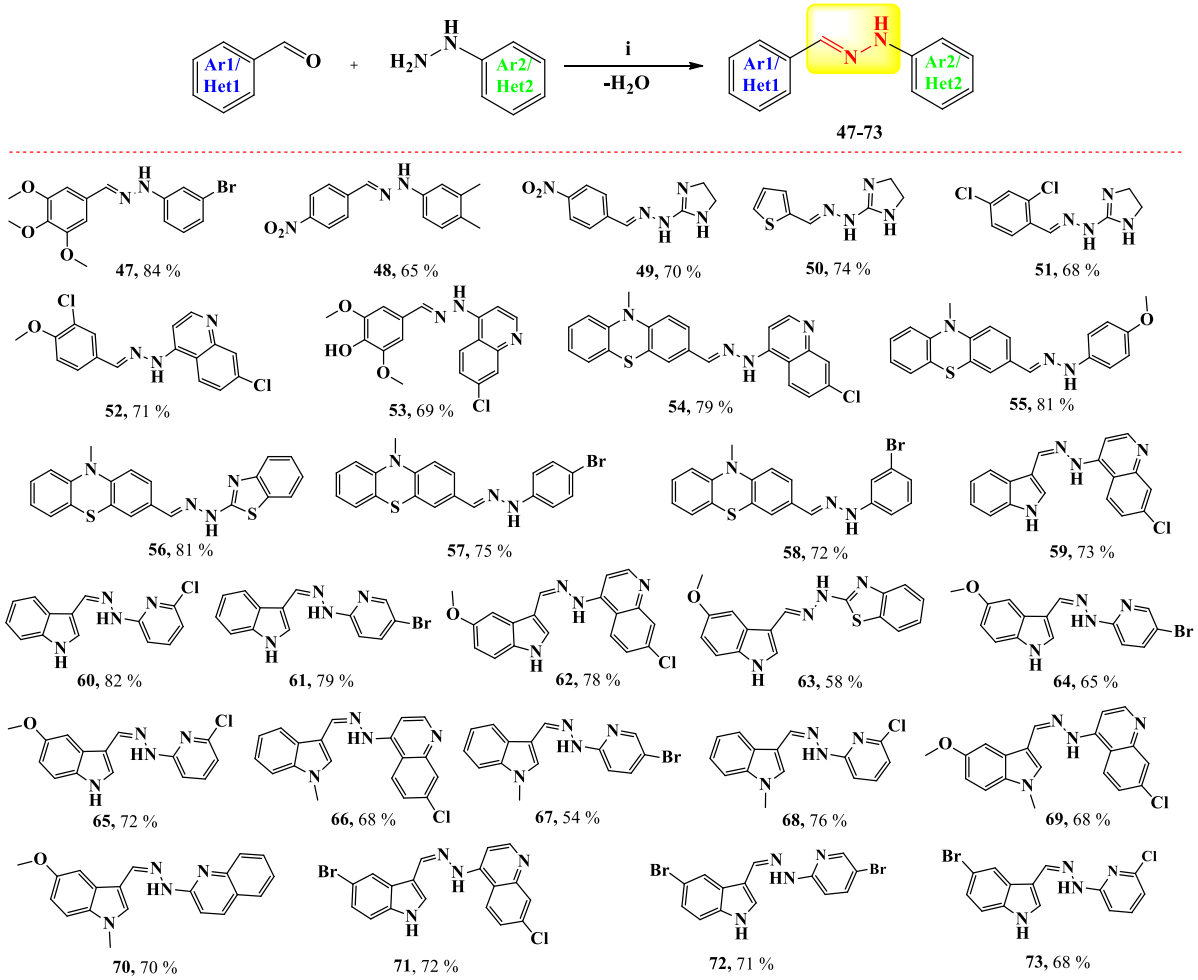


Scheme II.9. The proposed mechanism for obtaining hydrazone-type structures **46a-o**.

The reaction mechanism proposed to explain the formation of hydrazone-type compounds **46a-o** (**Scheme II.9**) involves the following steps:

- the first step is represented by the classical nucleophilic attack of the marginal nitrogen of hydrazine on the ethylene carbon of 2-cyano-3-aryl-acrylonitrile;
- the intermediate formed underwent a proton 1,3 migration and this allowed elimination of the malononitrile and the formation of hydrazones.

Starting from the fact that hydrazones present a varied range of biological activities (such as antibacterial[155] or anticancer[156]) and to be able to complete the series of hydrazone-type compounds obtained (**Scheme II.7**), the direct reaction between different aromatic aldehydes was also carried out as well as heteroaromatic with different aromatic or heteroaromatic hydrazines (**Scheme II.9**).



Scheme II.10. Reaction to obtain hydrazones **47-73**. **Reagents and conditions**: (i) EtOH, reflux, 4–8 hours.

From the direct condensation reaction between aldehydes and hydrazines (nucleophilic addition and elimination of water) a series of 27 hydrazone-type compounds were obtained which were characterized from a physico-chemical point of view and biologically evaluated.

The series of compounds **46a-o** was biologically tested for antifungal activity against 8 types of fungi: *Candida albicans* SC5314, *Candida dubliniensis*, *Candida glabrata*, *Candida parapsilosis*, *Candida albicans* from cystic fibrosis patients (*C. albicans* (mucoviscidosis)), *Candida albicans* resistant to echinocandins (*C. albicans (R echinocandins)*) and azole-resistant *Candida glabrata* (*C. glabrata* (*R azoles*)).

Preliminary testing of cell growth inhibition at a single concentration of 32 µg/mL was performed in triplicate. The Minimum Inhibitory Concentration (MIC) was thus determined. The antifungal action was studied in collaboration with the Institut Pôle de Biologie Pathologie Génétique, Center Hospitalier Universitaire (CHU) in Lille, France. Five of the tested

hydrazones **46c**, **46d**, **46i**, **46k** and **46l** showed antifungal activity against *Candida species* with MIC values between 16 and 32 μg/mL.

The results obtained are presented in **Table II.7**, with Fluconazole as a reference. The other synthesized hydrazones were less active, with MIC values > 32 pg/mL (data not shown).

Tabel II.7. MIC values of active hydrazones **46c**, **46d**, **46i**, **46k** and **46l** on *Candida spp.*

Entry	Compound	MIC values (μg/mL) on Candida spp. [a,b,c,d]							
		C. albicans SC5314	*C. dubliniensis*	*C. glabrata*	*C. parapsilosis*	*C. tropicalis*	*C. albicans* (mucoviscidosis)	*C. albicans* (R echinocandins)	*C. glabrata* (R azoles)
1	**46c**	32	>32[e]	32	32	>32	>32	>32	32
2	**46d**	32	>32	16	>32	>32	>32	>32	32
3	**46i**	32	>32	32	32	>32	>32	>32	32
4	**46k**	32	>32	16	32	32	>32	>32	>32
5	**46l**	32	>32	16	32	32	>32	>32	32
6	Fluconazole	0.5	0.5	0.5	0.5	0.5	0.5	0.5	>32

[a] *In vitro* inhibition percentage of pathogens. [b] Values represent mean of three experiments. [c] Compounds were tested

Active hydrazones have as a common structural unit the ring A 3,4,5-trimethoxyphenyl and the ring B monosubstituted phenyl type. The best chemical modulation was the substitution of the phenyl ring B in the *ortho* position with a halogen atom (Br, Cl and F) in hydrazones **46c**, **46d** and **46i**. By comparison, compound **46d** showed the highest activity, especially on *C. glabrata* (MIC=16 μg/mL).

In addition to testing the antifungal activity against *Candida species*, the series of hydrazones **46a-o** and **55** were also biologically tested for the evaluation of farnesyltransferase inhibition activity, six compounds showing activity, and the results are presented in **Table II.8**.

Table II.8. *In vitro* FTase inhibitory activity for hydrazones **46b, 46i, 46l, 46h, 46m** and **55**.

Compound	**% FTI[a]**	**IC_{50} (μM)[b]**	**R^{2}[c]**
46b	94.9	20.93	0.8705
46i	22.7	-	-
46l	38.9	-	-
46h	28.7	-	-
46m	**98.8**	**0.27**	**0.8288**
55	76.1	4.89	0.9929

[a] Inhibition of farnesyltransferase at a concentration of 100 μM. [b] Values represent the mean of two experiments. [c] The regression factor.

Among all the tested compounds, only three showed very good farnesyltransferase inhibition activity (**46b**, **46m** and **55**). The most active is the hydrazone **46m**, which is structurally composed of a *para*-nitrophenyl nucleus and an *ortho*-methoxyphenyl nucleus, its inhibition percentage being 98.8% and the IC_{50} value of 0.27 μM. The presence in the structure of the 3,4,5-trimethoxyphenyl nucleus instead of the *para*-nitrophenyl nucleus led to a decrease in activity until its complete reduction.

All synthesized hydrazones (series **46a-o** and series **47-73**) were accepted at the National Cancer Institute (NCI-USA) to be tested for cell growth inhibition potential on 60 cancer cell lines. The first step was testing at a concentration of 10 μM on all cell lines of the experimental screening.

The hydrazones of the **46a-o** series showed moderate cell growth inhibition activity, the most active hydrazones being **46k**, **46g** and **46f**, and the results are presented in **Table II.9**.

Table II.9. *In vitro* human cancer cell growth inhibition results for hydrazones **46k**, **46g** and **46f**.

Cell type	**Compund**	**46k**	**46g**	**46f**
	Cell line	Cell Growth Inhibition, GI%[a,b] 10 μM		
Leukemia	CCRF-CEM	**68**	31	49
	HL-60(TB)	**67**	0	34
	K-562	**90**	**53**	**81**
	MOLT-4	**56**	20	39
	RPMI-8226	41	18	21
	SR	31	11	16
Non-Small Cell Lung Cancer	A549/ATCC	45	22	25
	NCI-H460	**68**	0	0
	NCI-H522	**60**	28	39
Colon Cancer	HCC-2998	**54**	0	0
	HCT-15	**81**	46	42
	HT29	**70**	0	30
	KM12	**83**	48	**53**
	SW-620	**53**	0	25
CNS cancer	SF-268	47	27	30
	SF-295	39	**60**	12
Melanoma	LOX IMVI	**86**	**51**	**60**
	MALME-3M	44	0	0
	M14	**81**	27	43
	MDA-MB-435	**60**	19	28
	UACC-62	**63**	**71**	**71**
Ovarian Cancer	IGROV1	42	19	29

	OVCAR-4	49	16	20
	OVCAR-8	45	13	29
	NCI/ADR-RES	42	0	15
Renal cancer	ACHN	**68**	21	42
	CAKI-1	**89**	**64**	**67**
	UO-31	**65**	38	35
Breast cancer	MCF7	49	21	15
	MDA-MB-231/ATCC	**53**	11	28
	T-47D	**69**	24	46
	MDA-MB-468	**50**	0	0

[a] Data from NCI's in vitro screening of human tumor cells for a single dose (10 μM concentration). [b] Percent inhibition of cell growth. [c] Not determined.

As can be seen in the table above, the most active structure is **46k** structurally composed of the 3,4,5-trimethoxyphenyl ring A and the *para*-bromophenyl ring B. The percentage of cell growth inhibition is over 50% on 21 human cell lines, with the best activity on the K-562 line, with a 90% percentage of cell growth inhibition. The structural changes on the B ring led to the decrease of the activity or its total loss.

In order to study the anticancer activity, an intermediate, **44j**, described in the literature but untested, was proposed to the NCI. In the first stage of testing, it exhibited good anticancer activity, with a percentage of GI% inhibition of 53 on the UACC-62 cell line (melanoma) and GI% inhibition of 62 on the OVCAR-5 cell line (ovarian cancer). It also showed cytotoxic activity on three cell lines: GI% inhibition of -32 on NCI-H226 (non-small cell lung cancer), GI% inhibition of -19 on A498 (renal cancer), and GI% inhibition of -24 on MDA-MB-468 (breast cancer).

Hydrazones from the **47-73** series were also tested at NCI on the 60 cell lines. The results for the most active compounds are presented in **Table II.10**.

Hydrazones **52**, **53**, **59**, **62**, **66**, **69** and **71** show cytotoxic activity on most cell lines. The structural similarity between the seven compounds is the presence in the structure instead of the B ring of the 7-chloroquinoline ring.

The most active structure with the average cytotoxic activity on the 60 cell lines at -80 is hydrazone **62**, which consists of a substituted indole heterocycle at position 5 with a methoxy group, and a substituted quinoline heterocycle at position 7 with a chlorine atom. By comparing the structures of the most active compounds, it is evident that the 7-chloroquinoline heterocycle plays an extremely important role in enhancing the antiproliferative activity. Therefore, all compounds containing such a nucleus in their structure exhibited cytotoxic effects.

Tabel II.10. *In vitro* single-dose human cancer cell growth inhibition results for hydrazones **47**, **52, 53, 55**, **58-66**, **68**, **69**, **71**.

Cell type	***Compound***	**47**	**52**	**53**	**55**	**58**	**59**	**60**	**61**	**62**	**63**	**64**	**65**	**66**	**68**	**69**	**71**
	Cell line	Cell Growth Inhibition, GI%[a,b] 10 μM															
Leukemia	CCRF-CEM	**53**	**-19**[d]	90	26	43	**-10**	**91**	**93**	**-42**	**52**	**88**	**90**	**-35**	**51**	**-29**	**-7**
	HL-60(TB)	**56**	**-37**[d]	**-44**	42	32	**-34**	**86**	**86**	**-56**	**60**	**94**	**76**	**-60**	43	**-52**	**-74**
	K-562	**84**	**-52**[d]	**-54**	**95**	**77**	**-22**	**86**	**88**	**-4**	**75**	**90**	**80**	**97**	**79**	**-48**	**-25**
	MOLT-4	**54**	**-25**[d]	**-13**	**61**	23	**-48**	**76**	**82**	**-48**	**64**	75	**85**	**-32**	**67**	**-49**	**-48**
	RPMI-8226	28	**-37**[d]	**-9**	21	0	**-25**	**95**	**97**	**-62**	**52**	**-11**	**-1**	**-41**	**52**	**-42**	**-42**
	SR	**76**	**-9**[d]	**-23**	**57**	**66**	87	**86**	**97**	**-27**	**54**	**96**	**82**	**-25**	**72**	**-22**	**-45**
Non-Small Cell Lung Cancer	A549/ATCC	31	**-41**	**-82**	42	0	**-24**	47	**62**	**-49**	47	**77**	30	**-38**	24	**-69**	**98**
	EKVX	15	**87**	**-71**	34	0	**-79**	**67**	**75**	**-96**	**50**	**77**	**55**	**-85**	42	**-81**	**-92**
	HOP-62	0	**-42**	**-78**	33	0	**-75**	**53**	**68**	**-56**	N.D.[c]	**54**	40	**-63**	24	**-81**	**-59**
	HOP-96	29	**-57**	**-74**	32	0	**-88**	**-10**	**-28**	**-91**	47	**-24**	**73**	**-68**	**57**	**-81**	**-69**
	NCI-H226	28	**30**	**0**	**-68**	15	**-52**	49	**64**	**-78**	24	**60**	**51**	**-67**	41	**-52**	**-64**
	NCI-H23	29	**-11**	**-41**	23	0	**-88**	42	**58**	**-91**	32	**63**	35	**-84**	40	**-80**	**-85**
	NCI-H322M	38	**-64**	**-45**	22	17	**-89**	34	**52**	**-100**	20	**56**	28	**-99**	10	**-95**	**-96**
	NCI-H460	48	**-78**	**-63**	**71**	0	**-87**	**71**	**91**	**-78**	**67**	**83**	**52**	**-67**	36	**-79**	**90**
	NCI-H522	**63**	**-73**	**-73**	**53**	**58**	**-77**	**83**	**75**	**-62**	**71**	**61**	**72**	**-68**	25	**-65**	**-66**
Colon Cancer	COLO 205	0	**-82**	**28**	0	0	**-85**	**62**	**51**	**-56**	19	**62**	42	**-59**	32	**-86**	**-44**
	HCC-2998	48	**-39**	**-81**	15	0	**-92**	**69**	**-1**	**-89**	**58**	**89**	**60**	**-87**	**51**	**-86**	**-92**
	HTC-116	35	**-89**	**-68**	46	33	**-91**	**76**	**93**	**-50**	**53**	**91**	**62**	**-46**	**66**	**-82**	**-10**
	HCT-15	47	**-20**	**-58**	15	28	**-82**	**81**	**91**	**-88**	**77**	**93**	**70**	**-92**	**71**	**-85**	**-80**
	HT29	49	**-55**	**91**	0	0	**-72**	**69**	**86**	**-42**	40	**76**	39	**-40**	**55**	**-44**	**-3**
	KM12	**66**	**-88**	**-53**	**83**	47	**-77**	**90**	**99**	**-77**	**86**	**-1**	**78**	**-94**	**66**	**-78**	**-81**
	SW-620	**66**	**-88**	**-30**	**72**	42	**-74**	**47**	**79**	**-71**	25	**80**	20	**-62**	34	**-76**	**97**
CNS cancer	SF-268	44	**-64**	**-37**	46	19	**-58**	**80**	**78**	**-60**	44	**79**	**67**	**-73**	44	**-67**	**-35**
	SF-295	40	**-53**	**-80**	**93**	0	**-85**	**50**	**74**	**-100**	41	**82**	**52**	**-93**	26	**-84**	**-99**
	SF-539	31	**-90**	**-84**	42	0	**-86**	**93**	**89**	**-100**	29	**81**	**73**	**-92**	35	**-82**	**-87**
	SNB-19	0	**-79**	**-24**	19	0	**-92**	**56**	**56**	**-100**	20	48	38	**-79**	23	**-68**	**-85**
	SNB-75	13	**-88**	**-81**	24	11	**-96**	**84**	**73**	**-94**	33	**56**	38	**-85**	29	**-91**	**-91**
	U251	33	**-90**	**-87**	36	17	**-84**	**65**	**80**	**-83**	44	**79**	42	**-49**	43	**-80**	**-25**
Melanoma	LOX IMVI	**71**	**-35**	**-96**	**71**	**50**	**-92**	**82**	**-2**	**-92**	**69**	**92**	**71**	**-83**	49	**-78**	**-77**
	MALME-3M	23	**-78**	**-88**	0	0	**-95**	49	35	**-97**	0	36	23	**-82**	29	**-77**	**-83**
	M14	48	**-84**	**-90**	**59**	0	**-89**	**56**	**85**	**-79**	48	**91**	**50**	**-66**	44	**-71**	**-46**
	MDA-MB-435	**53**	**-84**	**-85**	**82**	37	**-92**	**80**	**69**	**-90**	33	**86**	**55**	**-83**	**89**	**-92**	**-77**
	SK-MEL-2	0	**-88**	0	0	0	**-82**	26	26	**-83**	19	26	0	**72**	12	**-80**	**-93**
	SK-MEL-28	19	**-92**	0	0	0	**-92**	20	36	**-100**	16	38	0	**-89**	0	**-85**	**-98**
	SK-MEL-5	14	**-93**	**-92**	23	0	**-98**	**58**	48	**-100**	16	**78**	35	**-98**	28	**-95**	**-100**
	UACC-257	13	**-84**	0	0	0	**-91**	32	29	**-84**	18	47	15	**-71**	11	**-84**	**-83**

	UACC-62	**59**	**-86**	0	**81**	20	**-61**	44	45	**-98**	26	48	38	**-66**	32	**-57**	**-85**
Ovarian Cancer	IGROV1	27	**59**	**-83**	44	15	**-87**	**-4**	**98**	**-94**	**70**	**89**	**94**	**-72**	**53**	**-82**	**-41**
	OVCAR-3	N.D.[c]	**-92**	N.D.[c]	22	N.D.[c]	**-66**	**-12**	**80**	**-82**	N.D.[c]	**69**	**82**	**-89**	36	**-80**	**-82**
	OVCAR-4	43	**-60**	**-50**	31	32	**-61**	**58**	**58**	**-100**	32	46	40	**-76**	39	**-76**	**-78**
	OVCAR-5	34	**-71**	**-5**	46	17	**-83**	47	**54**	**-100**	26	44	22	**-90**	0	**-87**	**-66**
	OVCAR-8	47	**-57**	**-55**	34	34	**-67**	**59**	**55**	**-70**	32	**66**	42	**-52**	19	**-66**	**-64**
	NCI/ADR-RES	46	**99**	**97**	20	25	**-71**	**-4**	**94**	**-60**	33	**92**	**77**	**-79**	**63**	**-71**	**-77**
	SK-OV-3	0	0	0	12	0	**-85**	36	28	**-95**	34	34	0	**-71**	0	**-89**	**-84**
Renal cancer	786-0	33	**-94**	**-87**	11	16	**-89**	**86**	**99**	**-92**	47	**-4**	**74**	**-65**	36	**-81**	**-54**
	A498	29	**63**	0	**82**	0	**-93**	24	**58**	**-98**	36	49	0	**-80**	0	**-89**	**-77**
	ACHN	**77**	**-99**	**-98**	48	37	**-98**	55	**72**	**-100**	36	**73**	**52**	**-92**	28	**-97**	**-90**
	CAKI-1	**73**	**-83**	**-92**	**73**	53	**-97**	**64**	**73**	**-99**	**58**	**69**	**53**	**-85**	33	**-92**	**-94**
	RXF 393	29	**-58**	**-86**	0	0	**-87**	N.D.[c]	N.D.[c]	**-100**	39	N.D.[c]	N.D.[c]	N.D.[c]	N. D.[c]	**-81**	N.D.[c]
	SN12C	25	**-70**	**-64**	20	0	**-77**	**83**	**84**	**-100**	30	**76**	**61**	**-59**	30	**-81**	**-56**
	TK-10	0	**-81**	**-69**	0	0	**-91**	28	40	**-96**	0	46	0	**-72**	0	**-79**	**-67**
	UO-31	**58**	**-89**	**-96**	**57**	24	**-95**	**89**	**97**	**-100**	**62**	**89**	**66**	**-90**	**59**	**-89**	**-95**
Prostate cancer	PC-3	**57**	**-24**	**-61**	**56**	**51**	**-81**	**74**	**79**	**-86**	**66**	**87**	**64**	**-51**	41	**-83**	**-52**
	DU-145	22	**-57**	**-24**	41	0	**-90**	**51**	**62**	**-98**	39	**69**	35	**-92**	27	**-93**	**-79**
Breast cancer	MCF7	49	**-12**	**-72**	**67**	28	**-71**	**82**	**90**	**-69**	**77**	**96**	**68**	**-78**	**64**	**-80**	**-56**
	MDA-MB231/ATCC	43	**-71**	**-74**	15	19	**-83**	**-1**	**-25**	**-93**	32	**93**	**96**	**-90**	48	**-82**	**-90**
	HS 578T	12	**-53**	**-38**	0	0	**-47**	**71**	48	**-49**	39	**74**	**50**	**-63**	30	**-53**	**-58**
	BT-549	0	**-79**	0	0	0	**-89**	**96**	**-2**	**-91**	41	**-3**	**87**	**-64**	**56**	**-76**	**-79**
	T-47D	**62**	**-61**	**-56**	48	44	**-54**	**72**	**74**	**-53**	**97**	**73**	**68**	**-60**	**52**	**-64**	**-54**
	MDA-MB-468	44	**-71**	**-72**	N.D.[c]	20	**-75**	**-10**	**-35**	**-80**	**59**	**-40**	**-13**	**-83**	**-1**	**-87**	**-94**

[a] Data from NCI's *in vitro* screening of human tumor cells for a single dose (10 μM concentration). [b] Percent inhibition of cell growth. [c] Not determinated. [d] Cytotoxic effect: cell growth percentage <0; total inhibition of cell prolifer

The compounds in this series showed a strong antiproliferative activity. Hydrazones **52**, **53**, **59**, **60**, **61**, **62**, **64**, **66**, **69**, **71** had an anticancer effect (cytostatic or cytotoxic) on almost all cell lines tested, thus fulfilling the conditions to move to the next stage and to be tested to determine the GI_{50} (concentration at which cell growth is inhibited by 50%). GI_{50} values obtained from the NCI *in vitro* assay on 60 cell lines are shown in **Table II.11**.

Table II.11. GI_{50} values from the NCI in vitro assay on 60 cell lines for the compounds **52**, **53**, **59-62**, **64**, **66**, **69**, **71**.

Cell type	Compound	52	53	59	60	61	62	64	66	69	71
	Cell line	GI_{50}[a,b] (μM)									
Leukemia	CCRF-CEM	2.14	N.D.[f]	**0.32**	**0.30**	**0.83**	**0.31**	**0.46**	**0.28**	**0.25**	**0.20**
	HL-60(TB)	1.96	1.89	**0.24**	1.28	2.81	**0.26**	2.54	**0.20**	**0.25**	**0.46**
	K-562	2.49	1.63	**0.24**	1.94	4.05	**0.30**	2.94	**0.20**	**0.19**	**0.20**
	MOLT-4	2.20	2.36	1.05	1.55	2.67	**0.26**	2.85	**0.34**	**0.23**	**0.26**
	RPMI-8226	2.20	2.18	1.18	**0.83**	3.07	**0.27**	1.61	**0.30**	**0.24**	**0.22**
	SR	**0.70**	1.90	**0.25**	2.93	2.28	**0.12**	4.80	**0.25**	**0.15**	**0.28**
Non-Small Cell Lung Cancer	A549/ATCC	1.77	1.74	1.45	13.2	12.6	1.48	8.12	1.48	**0.18**	1.58
	EKVX	1.73	1.74	1.45	1.97	4.44	1.69	2.71	1.26	1.43	1.16
	HOP-62	1.66	1.69	1.67	10.0	11.0	1.91	10.1	1.52	1.45	1.58
	HOP-92	1.38	1.44	**0.53**	5.32	6.66	1.32	2.22	1.42	**0.50**	1.33
	NCI-H226	1.80	1.79	1.89	1.65	3.78	1.86	2.02	1.50	1.93	1.47
	NCI-H322M	1.72	1.72	1.73	11.4	>100	1.44	13.4	1.56	**0.30**	1.57
	NCI-H460	2.04	2.05	1.72	11.5	14.1	2.02	7.68	**0.50**	**0.19**	**0.37**
	NCI-H522	1.68	1.66	1.77	1.03	2.53	1.34	3.11	1.16	**0.22**	1.79
Colon Cancer	COLO 205	1.82	1.86	1.79	13.8	>100	**0.25**	15.7	1.21	**0.35**	**0.35**
	HCC-2998	1.75	1.73	1.78	12.0	9.35	1.62	5.74	**0.71**	**0.26**	**0.76**
	HTC-116	1.69	1.92	**0.33**	12.1	13.2	**0.19**	5.97	**0.19**	**0.16**	**0.19**
	HCT-15	1.62	1.78	**0.37**	8.10	9.19	**0.18**	4.46	**0.29**	**0.17**	**0.18**
	HT29	1.53	1.81	**0.35**	15.0	29.4	**0.21**	8.77	**0.18**	**0.17**	**0.27**
	KM12	2.08	2.39	1.79	9.78	3.68	1.65	2.71	1.65	**0.18**	1.80
	SW-620	1.90	1.69	**0.79**	13.3	76.0	**0.18**	15.7	**0.24**	**0.18**	**0.36**
CNS cancer	SF-268	1.90	2.00	1.76	2.99	4.15	1.86	5.23	1.72	1.55	1.70
	SF-295	1.79	1.79	1.82	11.4	19.1	1.32	9.13	1.36	1.43	1.61
	SF-539	1.80	1.75	1.72	1.47	4.70	**0.18**	2.65	**0.18**	**0.21**	1.66
	SNB-19	1.93	1.97	1.67	12.7	11.4	1.17	20.7	1.65	**0.36**	1.54
	SNB-75	1.55	1.67	1.38	**0.14**	**0.38**	1.58	**0.28**	**0.36**	1.23	1.07
	U251	1.74	1.81	**0.83**	10.7	21.0	**0.75**	15.9	**0.25**	**0.17**	**0.27**
Melanoma	LOX IMVI	2.02	2.15	**0.23**	3.32	6.47	**0.21**	3.40	**0.17**	**0.18**	**0.17**
	MALME-3M	1.90	1.87	1.76	7.33	>100	1.80	82.7	1.62	1.40	1.60
	M14	1.80	1.76	1.73	11.9	78.5	1.54	7.27	1.57	**0.57**	1.69
	MDA-MB-435	1.79	1.69	1.75	2.46	79.4	1.61	6.73	**0.60**	**0.20**	1.69
	SK-MEL-2	1.79	16.3	1.83	12.9	>100	1.83	21.4	1.60	1.74	1.79
	SK-MEL-28	1.75	1.79	1.81	14.5	>100	1.69	29.1	1.59	1.84	1.59
	SK-MEL-5	1.77	1.77	1.75	8.61	25.6	1.65	7.41	1.29	1.76	1.50
	UACC-257	1.69	1.77	1.65	11.6	>100	1.82	25.1	1.52	1.82	1.58
	UACC-62	1.76	1.71	1.77	10.3	37.3	1.63	8.45	1.50	1.75	1.66
Ovarian Cancer	IGROV1	1.91	1.85	1.54	1.37	1.63	**0.30**	**0.63**	1.64	**0.17**	1.65
	OVCAR-3	1.88	2.41	N.D.[f]	3.85	8.63	1.58	11.6	**0.17**	N.D.[f]	1.66
	OVCAR-4	1.83	1.85	1.83	10.5	77.9	1.36	43.2	1.29	**0.28**	1.77
	OVCAR-5	1.85	1.67	1.63	11.0	>100	1.17	4.17	1.59	**0.19**	1.53
	OVCAR-8	2.09	2.02	1.68	8.12	26.3	**0.40**	20.8	1.63	**0.20**	1.80
	NCI/ADR-RES	1.98	2.13	1.93	2.98	7.03	**0.58**	5.33	1.04	**0.24**	1.41
	SK-OV-3	1.82	1.72	1.67	2.40	56.2	1.86	6.19	1.56	1.54	1.57
Renal cancer	786-0	1.55	1.89	1.41	11.1	13.2	**0.31**	12.6	**0.47**	**0.17**	1.44
	A498	1.72	18.9	1.51	18.7	35.2	1.49	20.5	2.07	1.73	1.87
	ACHN	1.95	1.83	1.87	10.5	11.6	**0.34**	7.76	1.59	**0.28**	1.66
	CAKI-1	1.68	1.66	1.68	2.23	4.35	1.54	3.01	1.30	1.58	1.46
	RXF 393	1.75	1.75	1.47	2.84	2.50	1.40	2.09	1.48	1.54	1.42
	SN12C	1.75	1.80	1.56	2.28	2.28	**0.65**	2.38	1.73	**0.19**	1.75

	TK-10	1.74	1.81	2.09	19.5	48.0	1.80	18.6	2.61	2.81	2.27
	UO-31	1.68	1.69	1.32	1.21	1.46	1.47	1.09	1.43	1.53	1.44
Prostate cancer	PC-3	1.64	1.66	1.55	2.98	4.83	**0.53**	2.55	1.15	**0.24**	1.31
	DU-145	1.95	1.90	1.53	13.0	20.5	**0.46**	13.3	1.30	**0.19**	**0.92**
Breast cancer	MCF7	1.63	1.58	1.25	2.67	2.43	**0.18**	1.70	**0.28**	**0.16**	**0.95**
	MDA-MB231/ATCC	1.93	2.05	1.65	1.37	2.20	**0.25**	1.93	**0.19**	**0.17**	1.24
	HS 578T	1.99	2.03	1.73	4.17	7.45	2.00	6.91	1.32	1.70	1.52
	BT-549	1.54	1.75	1.55	**0.24**	0.17	1.76	**0.17**	1.62	1.95	1.48
	T-47D	1.83	1.80	1.55	4.04	3.97	1.28	3.22	**0.33**	**0.16**	1.61
	MDA-MB-468	1.76	1.65	1.01	1.89	1.88	**0.80**	1.74	**0.27**	**0.22**	1.24

[a] Data from NCI *in vitro* assay on 60 cell lines for 5 doses. [b] The GI_{50} represents the molar concentration of the compound that causes 50% of tumor cell growth inhibition. [c] Bold values represent the best GI_{50} values obtained for the structures tested (GI_{50} < 1.00 μM).

The obtained results show that hydrazone **69** is the most active, having 38 cell lines with GI_{50} < 1.00 μM. Structurally, this compound consists of an indole-type ring substituted in the 5-position with the methoxy group and at the nitrogen atom with the methyl group and the 7-chloroquinoline-type ring.

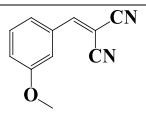
Some of the tested hydrazones (**59, 60, 62, 64, 66, 69, 71**) show selective activity on several cell lines: CCRF-CEM and HL-60(TB) for Leukemia, SNB-75 and U251 for nervous system cancer central and LOX IMVI for melanoma.

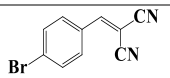
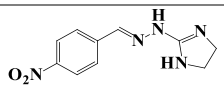
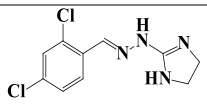
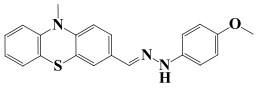
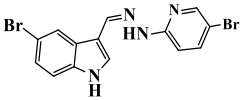
Hydrazones 47-73 and 2-(Aryl(Het)methylene)-malononitrile intermediates **44a–j** were tested at the Université de Brest, France, for their activity against rare prion diseases. The term "prion" comes from "proteinaceous infectious particle" and refers to transmissible, abnormal, acellular pathogens that are capable of producing errors in the process of forming specific proteins, called prion proteins, which are found especially in brain tissue. [164]

The main feature of prion diseases is rapidly progressive dementia. Considering the fact that, until now, there are no therapies that lead to the reversal of the transformation process of the modified protein into the normal protein or to limit the process of accumulation of these proteins in the human body, the synthesis of new compounds with activity against the disease is desired prion.[166]

The results obtained from the tests showed that 3 intermediates and 4 hydrazones showed activity against prion disease, these being mirrored in **Table II.12**, compounds with high activity are marked with "+" and those with "+/-" are those with moderate action.

Table II.12. Test results against prion diseases.

Compound	**Structure**	**[*PSI*+]**
44d		**+/-**

44e		+
48		+
51		+
55		+
72		+

According to the results obtained from the tests, 2 differently substituted benzylidenemalononitrile intermediates (**44d**, **44e**) and 4 hydrazone-type structures (**48, 51, 55, 72**) showed activity against prion disease. The *meta*-methoxy substituted intermediate (**44d**) showed moderate activity, whereas the *para*-bromo substituted intermediate (**44e**) had high activity. Regarding the activity of hydrazones, it was elevated in all 4 cases.

The structural similarity between the 4 active hydrazones is the presence of a heterocycle, thus, hydrazones **48** and **51** have instead of the B ring, the 4,5-dihydro-1*H*-imidazole-type ring, compound **55** has the phenothiazine-type ring A, and compound **72** has ring A of the 5-bromoindole type and ring B of the 5-bromopyridine type.

II.3. Design, synthesis and biological evaluation of some pyridine derivatives - Series III

In the last sub-chapter of this doctoral thesis, a new series of pyridine-type azaderivatives were synthesized by introducing the pyridine ring instead of the ketone-type connector in phenstatin. Also, the two rings A and B were varied by replacing them, either with other rings of a different substituted phenyl type, or with heterocycles that can increase the biological activity of the final structures. The modulations made are shown in **Figure II.40**.

The first step in the design of the new structures was to preserve the A, 3,4,5-trimethoxyphenyl ring of phenstatin and replace the ketone-type linker with pyridine substituted in the 2-position with the amino group and in the 3-position with the cyano group, and the B

ring was replaced with 3,4-dimethoxyphenyl. The next step was to vary the rings A and B, either by replacing them with either differently substituted phenyl or heterocycles.

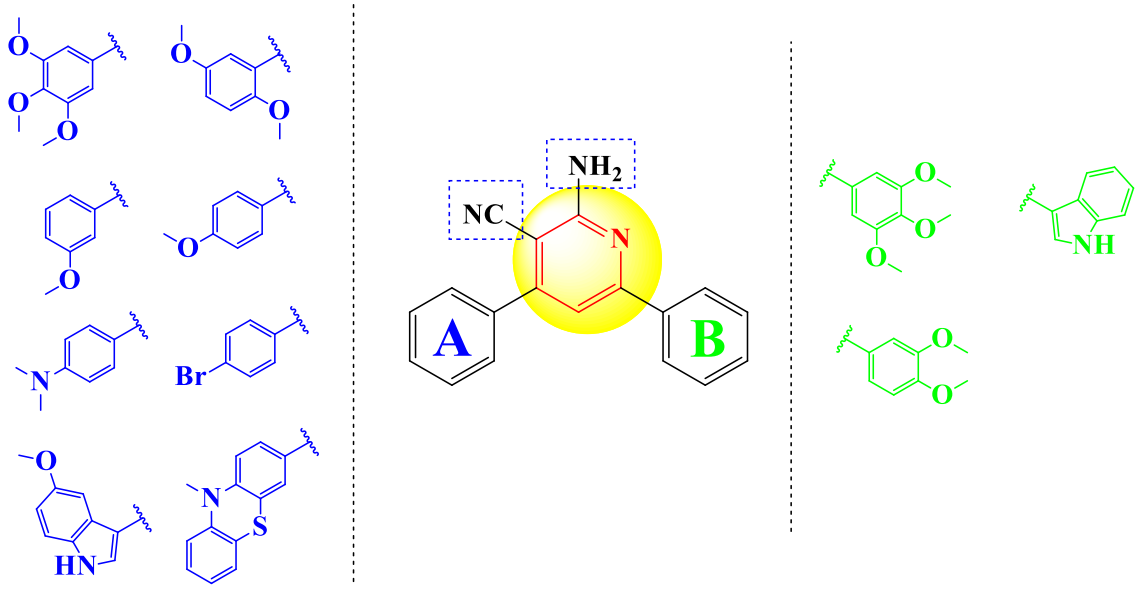
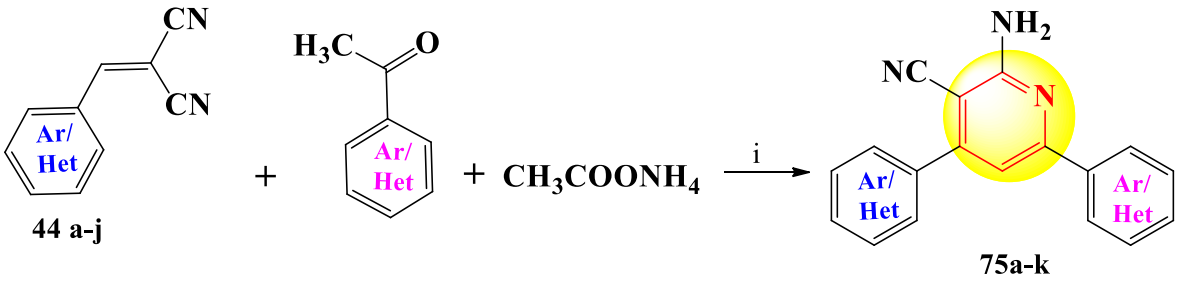


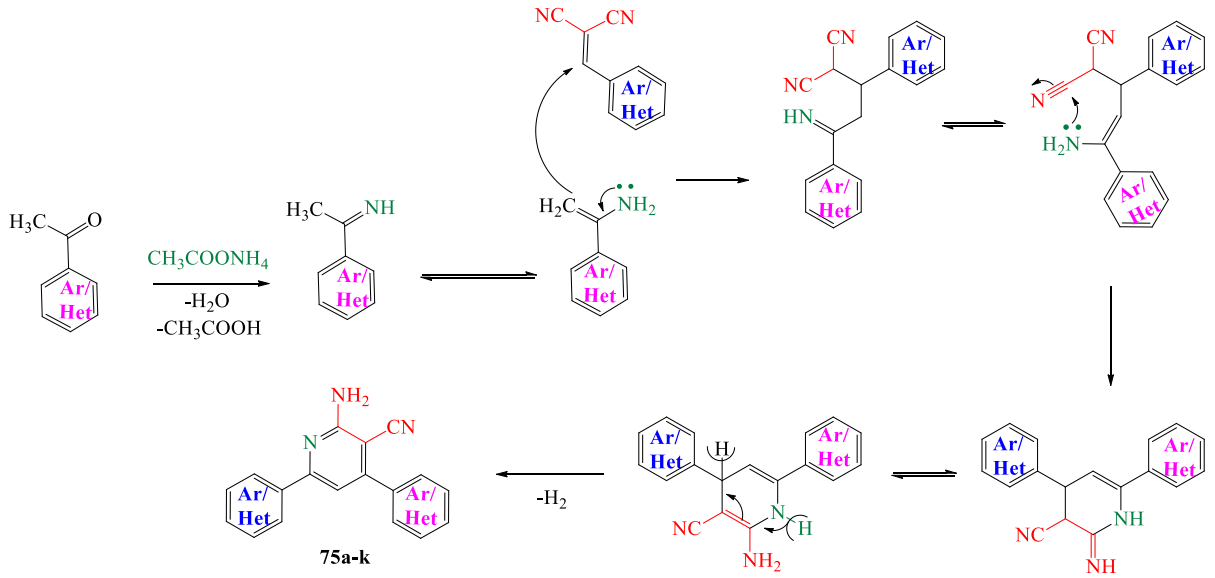
Figure II.40. Modulations of phenstatin to obtain series of pyridine derivatives.

To synthesize the desired compounds, the series of previously synthesized intermediates **44a-j** was used (**Scheme II.6**, Chapter II.2). The thus synthesized intermediates reacted with various commercially available ketones, closing the pyridinic ring and obtaining the desired compound.[173,174]



Scheme II.13. Synthesis reaction of pyridine derivatives. **Reagents and conditions**: (i) EtOH, reflux, 7-12 h.

The reaction mechanism and the formation mode of the target pyridine-type compounds is presented in **Scheme II.14**.[173]



Scheme II.14. Mechanism of formation of pyridine-type compounds.

By means of this reaction, a new series of 11 compounds, pyridine derivatives, whose structure is presented in **Figure II.43**.

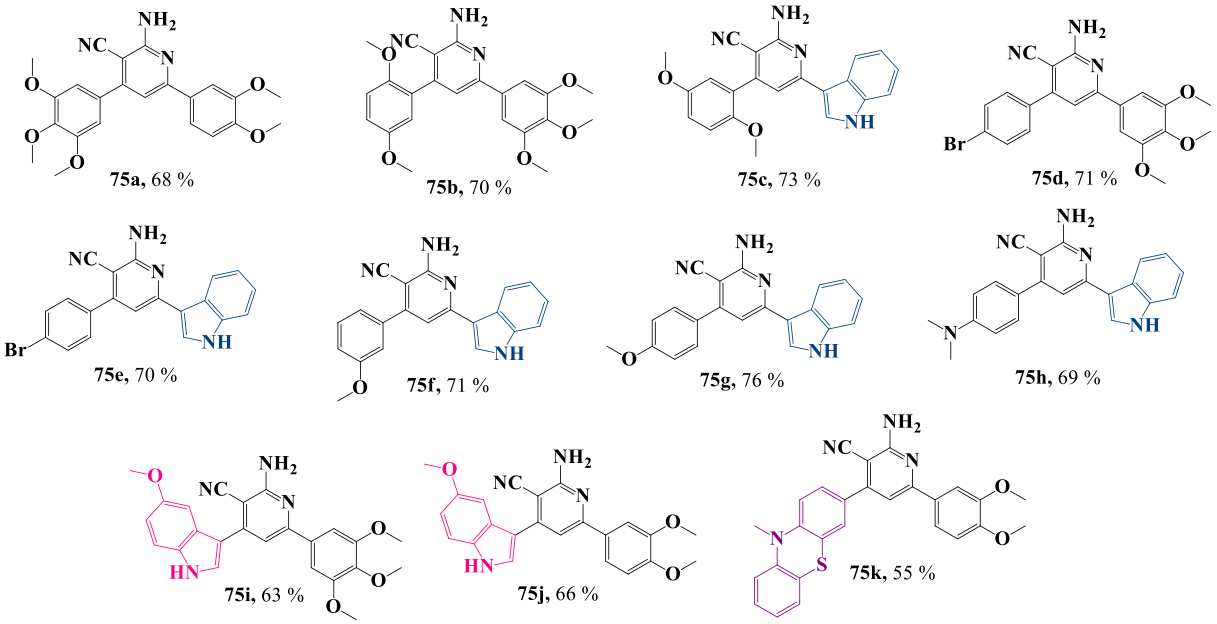


Figure II.43. Structures and yields of compounds from *Series III* **, 75a-k**.

All synthesized compounds were proposed, accepted and tested *in vitro* at the National Cancer Institute (NCI) to study their anticancer activity on 60 cancer cell lines, the first step being testing at a concentration of 10 μM. **Table II.13** shows the cell growth inhibition percentages for the most active 6 of the tested structures.

Table II.13. *In vitro* results of human cancer cell growth inhibition for pyridines **75c, 75e-i**.

Cell type	***Compound***	***75c***	***75e***	***75f***	***75g***	***75h***	***75i***
	Cell line	Cell Growth Inhibition, GI%[a,b] 10 μM					
Leukemia	CCRF-CEM	**91**	**65**	**67**	**58**	**70**	27
	HL-60(TB)	**100**[d,h]	**71**	**63**	**51**	**79**	39
	K-562	**91**	**81**	**77**	**76**	**82**	**69**
	MOLT-4	**92**	**58**	**68**	**55**	**64**	27
	RPMI-8226	**93**	**65**	**60**	**64**	**95**	0
	SR	**91**	**74**	**83**	**81**	**90**	**69**
Non-Small Cell Lung Cancer	A549/ATCC	**78**	**57**	**57**	**56**	**74**	28
	EKVX	**86**	48	N.D.[c]	34	38	0
	HOP-62	**90**	**57**	37	25	**64**	31
	HOP-92	**100**[d,i]	**56**	38	31	**72**	36
	NCI-226	**86**	39	24	20	37	0
	NCI-H23	**100**[d,j]	48	22	17	45	15
	NCI-H322M	**75**	32	0	20	30	0
	NCI-H460	**100**[d,k]	**83**	**76**	**79**	**92**	35
	NCI-H522	**100**[d,l]	**89**	**65**	**64**	**97**	27
Colon Cancer	COLO 205	**100**[d,m]	48	37	0	**77**	18
	HCC-2998	**100**[d,n]	39	37	23	**50**	24
	HTC-116	**100**[d,o]	**72**	**67**	**59**	**85**	40
	HCT-15	**100**	**83**	**82**	**76**	**88**	**68**
	HT29	**100**[d,p]	**72**	**60**	**55**	**88**	49
	KM12	**100**[d,q]	**71**	**63**	**73**	**81**	41
	SW-620	**93**	**72**	**52**	**52**	**78**	42
CNS cancer	SF-268	**90**	43	39	41	47	10
	SF-295	**100**[d,r]	47	45	**50**	**73**	31
	SF-539	**100**[d,s]	**56**	51	**56**	**62**	12
	SNB-19	**88**	33	26	19	**59**	19
	SNB-75	**100**[d,t]	**62**	48	26	**78**	14
	U251	**100**[d,u]	47	**59**	**61**	**73**	20
Melanoma	LOX IMVI	**100**[d,v]	**71**	**70**	**61**	**88**	22
	MALME-3M	**100**[d,w]	26	0	30	35	11
	M14	**100**[d,x]	**55**	48	46	**100**[d,e]	26
	MDA-MB-435	**100**[d,y]	**100**	**72**	**66**	**86**	**88**
	SK-MEL-2	**100**[d,z]	**60**	29	0	**100**[d,f]	30
	SK-MEL-28	**95**	40	27	24	40	10
	SK-MEL-5	**100**[d,a']	41	40	50	**70**	13
	UACC-257	**77**	0	21	0	40	0
	UACC-62	**100**[d,b']	35	24	25	**58**	25

Ovarian Cancer	IGROV1	**84**	**65**	45	38	**57**	21
	OVCAR-3	**100**[d,c']	32	31	27	42	N.D.[c]
	OVCAR-4	**77**	25	33	19	30	0
	OVCAR-5	**84**	36	0	29	**51**	0
	OVCAR-8	**93**	33	44	36	**74**	10
	NCI/ADR-RES	**100**[d,d']	**75**	**65**	49	**85**	41
	SK-OV-3	**65**	47	31	0	**69**	13
Renal cancer	786-0	**100**[d,e']	20	48	**59**	**82**	11
	A498	**97**	36	**53**	**60**	**100**[d,g]	21
	ACHN	**91**	42	26	29	**52**	0
	CAKI-1	**92**	**69**	**53**	**55**	**75**	46
	RXF 393	N.D.[c]	**74**	**52**	49	**97**	31
	SN12C	**97**	33	23	28	**56**	0
	TK-10	**62**	0	21	0	27	0
	UO-31	**100**[d,f']	**56**	40	45	**55**	19
Prostate cancer	PC-3	**86**	44	48	32	**62**	23
	DU-145	**87**	30	34	**50**	**50**	0
Breast cancer	MCF7	**96**	**81**	48	47	**72**	**52**
	MDA-MB 231/ATCC	**87**	**54**	36	**59**	**64**	22
	HS 578T	**100**[d,g']	41	31	40	**64**	0
	BT-549	**100**[d,h']	**67**	23	21	**72**	20
	T-47D	**100**	**70**	34	39	**60**	41
	MDA-MB-468	**100**[d,i']	**92**	**50**	**70**	**60**	**53**

[a] Data from NCI's in vitro screening of human tumor cells for a single dose (10 μM concentration). [b] Percent inhibition of cell growth. [c] Not determinated. [d] Cytotoxic effect: cell growth percentage <0; total inhibition of cell proliferation and cell death. [e] Percent inhibition of cell growth: -27%. [f] Percent inhibition of cell growth: -1%. [g] Percent inhibition of cell growth: -1%. [h] Percent inhibition of cell growth: -33%. [i] Percent inhibition of cell growth: -37%. [j] Percent inhibition of cell growth: -10%. [k] Percent inhibition of cell growth: -43%. [l] Percent inhibition of cell growth: -6%. [m] Percent inhibition of cell growth: - 24%. [n] Percent inhibition of cell growth: -38%. [o] Percent inhibition of cell growth: -46%. [p] Percent inhibition of cell growth: -13%. [q] Percent inhibition of cell growth: -28%. [r] Percent inhibition of cell growth: -70%. [s] Percent inhibition of cell growth: -61%. [t] Percent inhibition of cell growth: -9%. [u] Percent inhibition of cell growth: -2%. [v] Percent inhibition of cell growth: -66%. [w] Percent inhibition of cell growth: -4%. [x] Percent inhibition of cell growth: -9%. [y] Percent inhibition of cell growth: -6%. [z] Percent inhibition of cell growth: -19%. [a'] Percent inhibition of cell growth: -85%. [b'] Percent inhibition of cell growth: -9%. [c'] Percent inhibition of cell growth: -24%. [d'] Percent inhibition of cell growth: -35%. [e'] Percent inhibition of cell growth: -53%. [f'] Percent inhibition of cell growth: -16%. [g'] Percent inhibition of cell growth: -17%. [h'] Percent inhibition of cell growth:-26%. [i'] Percent inhibition of cell growth: -10%.

The most active compounds of the first stage of testing are **75c** and **75h**. These were selected to go to the next stage and to be tested at 5 different doses, determining the GI_{50} (the concentration at which cell growth is inhibited by 50%). The results of the second stage of testing the two structures, **75c** and **75h**, at 5 different concentrations, are presented in **Table II.14**.

Table II.14. GI_{50} values from the NCI *in vitro* assay on 60 cell lines for the compounds **75c** and **75h**.

Cell type	***Compound***	***75c***	***75h***	***Cell type***	***Compound***	***75c***	***75h***
	Cell line	GI_{50}[a,b] (μM)			**Cell line**	GI_{50}[a,b] (μM)	
Leukemia	CCRF-CEM	2.40	1.54	***Ovarian Cancer***	IGROV1	5.59	2.35
	HL-60(TB)	2.42	1.79		OVCAR-3	2.94	N.D.[d]
	K-562	**0.88**	**0.65**		OVCAR-4	5.42	4.78
	MOLT-4	3.84	1.89		OVCAR-5	5.94	3.17
	RPMI-8226	1.95	1.95		OVCAR-8	4.94	2.78
	SR	**0.53**	**0.41**		NCI/ADR-RES	1.22	1.82
Non-Small Cell Lung Cancer	A549/ATCC	3.74	1.92		SK-OV-3	3.63	2.05
	EKVX	1.57	3.38	***Renal cancer***	786-0	2.61	1.93
	HOP-62	2.88	1.83		A498	8.86	1.78
	HOP-92	1.30	2.14		ACHN	4.92	2.79
	NCI-H226	5.81	4.23		CAKI-1	1.51	1.67
	NCI-H23	2.82	3.13		RXF 393	1.77	1.30
	NCI-H460	1.58	1.06		SN12C	5.06	2.87
	NCI-H522	**0.73**	**0.44**		TK-10	11.1	4.10
Colon Cancer	COLO 205	2.81	2.11		UO-31	3.90	1.88
	HCC-2998	2.98	2.15	***Prostate cancer***	PC-3	1.93	2.79
	HTC-116	2.42	1.60		DU-145	8.20	3.45
	HCT-15	**0.90**	**0.48**	***Breast cancer***	MCF7	**0.13**	1.51
	HT29	2.74	1.72		MDA-MB-231/ATCC	2.06	2.16
	KM12	2.71	1.85		HS 578T	2.17	2.08
	SW-620	2.90	2.16		BT-549	1.96	2.22
CNS cancer	SF-268	3.63	3.89		T-47D	2.18	2.32
	SF-295	3.43	1.86		MDA-MB-468	**0.06**	1.97
	SF-539	2.90	1.33				
	SNB-19	5.47	2.97				
	SNB-75	1.30	1.84				
	U251	3.20	1.53				
Melanoma	LOX IMVI	2.95	1.52				
	MALME-3M	3.25	1.72				
	M14	1.60	1.70				
	MDA-MB-435	**0.39**	**0.46**				
	SK-MEL-28	3.46	1.79				
	SK-MEL-5	**0.80**	1.84				
	UACC-257	9.47	1.89				
	UACC-62	2.67	2.05				

[a] Data from NCI *in vitro* assay on 60 cell lines for 5 doses. [b] The GI_{50} represents the molar concentration of the compound that causes 50% of tumor cell growth inhibition. [c] Bold values represent the best GI50 values obtained for the tested compounds (GI_{50} < 1.00 μM). [d] Not determinated.

Following the results received for the second test stage, it turned out that structure **75c** is the most active, having GI_{50} value < 1.00 μM on 8 cell lines, and **75h** on 5 cell lines. Both structures also showed selective activity on certain cell lines: K-562 and SR for leukemia, NCI-H522 and HCT-15 for colon cancer, and MDA-MB-435 for melanoma.

II.4. General conclusions and perspectives

The main objective of this PhD thesis was the design and synthesis of new compounds with biological activity, especially anticancer.

The starting point of this study was the structure of phenstatin, on which various modulations were made, thus obtaining **121 new compounds**, not described in the specialized literature, grouped into three series and characterized with the help of IR, ^{1}H-NMR spectra , ^{13}C-NMR and in some cases X-ray.

The modulations made on the structure of phenstatin consisted in replacing either the ketone connector or the two aromatic rings, A and B. The compounds obtained were grouped into three series, as follows:

***Series I*:** 65 new pyrrolic compounds, grouped into three series, according to structural features:

- *Series I.1*: 39 compounds whose structure consists of 3 cycles, of which cycle B is pyrrolic. It is directly related to the nucleus;
- *Series I.2*: 14 pyrrole derivatives whose structure consists of three rings, and ring B, of pyrrole type, is linked to ring C through a methylene type connector;
- *Series I.3*: 12 compounds structurally composed of two rings (one of pyrrole type), the two being linked directly or through a methylene type connector.

All the synthesized compounds were tested at the National Cancer Institute-USA, and the results showed that the series showed good anticancer activity, two compounds being selected to go to the next stage of testing, to determine the GI_{50} (the concentration at which cell growth is inhibited by 50%). The most active compound presented GI_{50} < 1.00 μM on three cancer cell lines (leukemia on the K-562, SR lines and melanoma on the MDA-MB-435 line).

***Series II*:** composed of 42 hydrazones divided into two miniseries (**46a-o** and **47-73**), depending on the method of synthesis:

1. By the reaction of derivatives of the type 2-(Aryl(Het)methylene)-malononitrile and various hydrazines;
2. Through the direct condensation reaction between aldehydes and hydrazines.

The compounds were obtained by performing the following structural modulations:

- ❖ The ketone connector in phenstatin has been replaced by the hydrazone type;
- ❖ The A ring has been replaced with either a differently substituted phenyl ring or heterocycles such as thiophene, 10-methylphenothiazine and differently substituted indole;
- ❖ The B ring has been replaced with either differently substituted phenyl or nitrogen heterocycles such as quinoline, differently substituted pyridine, benzothiazole and 4,5-dihydro-1*H*-imidazole.

The biological activity of the synthesized hydrazones varies, thus:

- ➢ The hydrazones **46a-o**, obtained by the first synthesis method, showed good antifungal activity, the most active structure (especially on *C. glabrata*, with MIC=16 μg/mL) is the one in which B ring is *ortho*-chlorophenyl. The anticancer activity of this series was moderate (testing was performed at the National Cancer Institute-NCI-USA).
- ➢ The hydrazones **47-73** showed excellent anticancer activity (National Cancer Institute-NCI-USA). In the first stage of testing, at a single dose (10 μM), 10 compounds were active on all cell lines, 7 of them showing cytotoxic activity on all tested cell lines. The most active was the hydrazone **62** with the indole A ring substituted in the 5-position with the methoxy group. It showed cytotoxic activity on all cell lines tested, with an average activity of -80. The 10 active hydrazones went to the second stage, on 5 doses, the most active was hydrazone **69**, having 38 cell lines with GI_{50} < 1.00 μM, with the indole-type ring A substituted both in position 5 with the methoxy group and to the nitrogen atom with the methyl group.
- ➢ 2 differently substituted benzylidenemalononitrile intermediates (**44d**, **44e**) and 4 hydrazone-type structures (**48**, **51**, **55**, **72**) showed activity against prion disease. and intermediate **44e** and all 4 hydrazones showed high activity.

***Series III*:** the last series of this PhD work consists of 11 new pyridine-type compounds, substituted in the 2-position with the amino group (NH2) and in the 3-position with the cyano

group (CN). The A and B rings in phenstatin have been replaced, either with differently substituted phenyl or with heterocycles (phenothiazine and indole) that have the ability to increase the biological activity of the final structures.

Anticancer activity was studied at NCI-USA (National Cancer Institute) on 60 cell lines for the ability to inhibit the growth of cancer cells.

Of the synthesized pyridine derivatives, six showed good anticancer activity, five of them having selective activity on several cancer cell lines, the inhibition percentage being over 50%. The two most active compounds were selected to go to the next stage (testing at 5 doses) and for which the GI_{50} was calculated. The most active anticancer pyridine derivative had a $GI_{50} < 1.00$ μM on 8 cancer cell lines.

The anticancer activity, *in vitro*, of all synthesized compounds was studied at the National Cancer Institute-USA by analyzing the ability to inhibit cell growth on 60 cancer cell lines. In the case of the first stage, testing at a single dose (10 μM), the most active compounds were hydrazones **52, 53, 59, 62, 66, 69, 71,** which had cytotoxic activity on all cancer cell lines, and pyridine **75c**, which had cytotoxic activity on 28 cell lines. In the second test stage, at 5 doses, hydrazones **62, 66, 69** and **71** show very good anticancer activity, compound **69** having 38 lines with GI_{50} value < 1.00 μM.

Antifungal activity was tested, in collaboration with the Institut Pôle de Biologie Pathologie Génétique, Center Hospitalier Universitaire (CHU) in Lille, France, for the series of hydrazones **46a-o**, and the most active compound was **46d** (especially on C. glabrata , with MIC=16 μg/mL).

Activity against prion diseases (rare diseases) was tested in collaboration with the Université de Brest in France, and the most active were found to be hydrazones **48**, **51**, **55**, **72.**

Some of the obtained results were disseminated by presenting them at various **conferences**:

Negru, G., Ghinet, A., Belei, D., Bîcu, E., Synthesis and characterization of some arylidenmalononitriles, Sesiunea de comunicări științifice a studenților, masteranzilor și doctoranzilor, Iași, România, 20-21 Iunie ***2019.***

Negru, G., Ghinet, A., Shova, S., Bîcu, E.,Synthesis and characterization of some of analogues of phenstatin, IasiChem 2019, Faculty of Chemistry Conference, Iași, România, 31 Octombrie-01 Noiembrie ***2019****.*

Negru, G., Ghinet, A., Bîcu, E., Design,synthesis,chemical and biological characterization of new compounds, pyrrole derivatives, Sesiunea de comunicări științifice a studenților, masteranzilor și doctoranzilor, Iași, România, 29-30 Octombrie ***2020****.*

Negru, G., Kamus, L., Bîcu, E., Shova, S., Sendid, B., Dubar, F., Ghinet, A., A new series of hydrazones with antifungal activity against candida species, Sesiunea de comunicări științifice a studenților, masteranzilor și doctoranzilor, Iași, România, 11-12 Noiembrie ***2021****.*

Negru, G.,Zubaș, A., Ghinet, A., Bîcu, E., A new series of pyridine derivatives as anticancer agents: design, synthesis and biological evaluation, Sesiunea de comunicări științifice a studenților, masteranzilor și doctoranzilor, Iași, România, 28 Octombrie ***2022****.*

By publishing articles:

Negru, G., Kamus, L., Bîcu, E., Shova, S., Sendid, B., Dubar, F., Ghinet, A., Attempts to access a series of pyrazoles lead to new hydrazones with antifungal potential against Candida species including azole-resistant strains, Molecules, 26(19), 5861, ***2021****. DOI: https://doi.org/10.3390/molecules26195861, Factor de impact* ***4.412.***

Negru (Apostol), G.; Ghinet, A.; Bîcu, E. 7-Chloroquinolinehydrazones as First-in-Class Anticancer Experimental Drugs in the NCI-60 Screen among Different Investigated Series of Aryl, Quinoline, Pyridine, Benzothiazole and Imidazolehydrazones[176]*, Pharmaceuticals* ***2023****, 16(5), 691. DOI: https://doi.org/10.3390/ph16050691. Factor de impact:* ***5.215.***

During my doctoral internship, I was a member of two research projects: **POCU/380/6/13/123623** (Doctoral students and postdoctoral researchers prepared for the labor market 2019-2020) and **PN-III-P4-ID-PCE-2020-0818/ 195/2021** (P2X7R Ligands as Potential Therapeutic Tools for the Treatment of Inflammatory Bowel Disease and Related Cancers 2021-2023).

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development and innovation through the development of the institutional capacity of the "Alexandru Ioan Cuza" University in Iasi) and the project **PN-III-P4-ID-PCE-2020-0818/195/2021** (P2X7R Ligands as Potential Therapeutic Tools for the Treatment of Inflammatory Bowel Disease and Related Cancers 2021-2023).

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