„ALEXANDRU IOAN CUZA" UNIVERSITY OF IAŞI

FACULTY OF CHEMISTRY

DOCTORAL SCHOOL OF CHEMISTRY

Syntheses of new chalcone, indolizine and pyridine derivatives with biological properties

ABSTRACT

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The doctoral thesis titled " *Syntheses of new chalcone, indolizine and pyridine derivatives with biological properties*" has 201 pages, appendices (published scientific articles) and follows the structure outlined below:

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The abstract contains a short presentation of the results of the research undertaken in this doctoral thesis, the final conclusions and the selective bibliography, keeping the numbering of chapters, figures, schemes, tables and chemical structures.

Introduction

Diseases are part of the history of human civilization. People make constant efforts to combat them, in the hope of survival and improving the quality of life. Over time, the diseases evolved and influenced human existence, the most well-known being the diseases that led to the death of a large number of people, for example through infection: smallpox, influenza, plague, tuberculosis.[1] In our century, cancer has become just as well known.

Cancer is a genetic disease characterized by the uncontrolled multiplication of cells and their spread in the surrounding tissues. A form of cancer can develop from any part of the human body. Normally, normal cells grow and divide to form new cells required by the body, which will undergo the process of apoptosis when they age or can no longer perform their specific functions. Unlike normal cells, abnormal or defective cells do not perform specific functions and are able to ignore the body's signals such as stopping division or starting cell apoptosis.[3] These cells proliferate and multiply uncontrollably. They can form cancerous (malignant) or non-cancerous (benign) tumors.

Malignant tumors spread to nearby tissues and can also migrate to other distant areas of the body, forming new tumors, a process called metastasis (**Figure 2**).[5] In most types of cancer, tumors are solid and sometimes they can be removed through surgical procedures, but in leukemias this is not possible. Benign tumors do not spread, and when removed, generally do not recur.

Cancer develops as a result of genetic mutations. In general, it is not possible to determine exactly why cancer occurs in a particular person, but based on studies, certain risk factors have been established that can increase the likelihood of developing the disease.[6]

There are several types of cancer treatements, but the body’s response to a therapy is different depending on the body and the form of the disease. In some cases, a single treatment can be used (such as surgery), but mostly the treatments are combined (for example: surgery with chemotherapy, radiotherapy, hyperthermia). Hyperthermia, also known as thermotherapy, involves heating the cells to a temperature of 45 °C with the aim of destroying the cancer cells without harming the normal cells.[7,8] Chemotherapy is the treatement in which cytostatic and cytotoxic drugs are used.

There are more than 100 forms of cancer, generally the name is assigned according to the affected organ or tissue or the type of cell from which the cancer starts: colon, lung, ovarian, prostate, breast, leukemia, carcinoma, sarcoma.[3] In 2018, IARC estimated that 18.1 million new cases of cancer will occur globally and 9.6 million deaths will be caused by cancer, [9] the most

widespread forms being: prostate, lung, colon, stomach and breast cancer.[10] In 2020, about a quarter of new cancer cases (4.4 million) and 1/5 of deaths (1.9 million) occurred in Europe, although it represents only 9% of the global population.[11]

As a result, at a global level, the aim is to mobilize the scientific research community in cancer towards developing new approaches to identify and understand anticancer targets (proteins, enzymes or processes involved in transduction and multiplication) and implicitly the development of new inhibitory agents.

Due to the very good bioavailability of chalcones (compound **1**, **Figure 3**), research on them and their derivatives has acquired a special interest in the design of new anticancer agents. Currently, a significant number of compounds containing chalcone units are in clinical trials.[12]

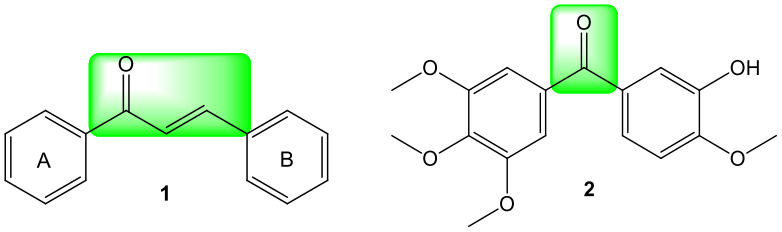


Figure 3. Structures of chalcone **1** and phenstatin **2**.

The present paper is comprised of two main parts, structured into three chapters: literature review, personal contributions and experimental section. Personal contributions are based on the design, synthesis and biological evaluation of new chalcone-type compounds (analogues of phenstatin, compound **2**, **Figure 3**) and aza-heterocyclic compounds with five and six atoms. Also, we are seeking to improve some synthesis methods, to enhance the efficiency of the process in terms of time, yield or toxicity, respectively the implications on the environment, which represents for us an attractive direction of research. In terms of biological properties, the following activities were observed: anticancer, antimicrobial. In the design of compounds the anticancer targets were farnesyltransferase and tubulin.

I. Results and discussions

Objectives of this thesis

The research conducted during the doctoral years had the primary objective of acquiring novel compounds with biological properties., mainly anticancer and antimicrobial activity.

The research is based on the literature study summarized in the first part of the doctoral thesis and involves the following stages:

- design of target compounds (chalcone, indolizine and pyridine derivatives while considering the known structure-activity relationships up to this point,
- the synthesis of the compounds with an emphasis on process efficiency in terms of time, yield, atom economy, toxicity, and environmental implications (while adhering to principles of green chemistry),
- and the biological evaluation.

In the design of the compounds, we varied the three structural elements of chalcone, which is considered an analogue of phenstatin.

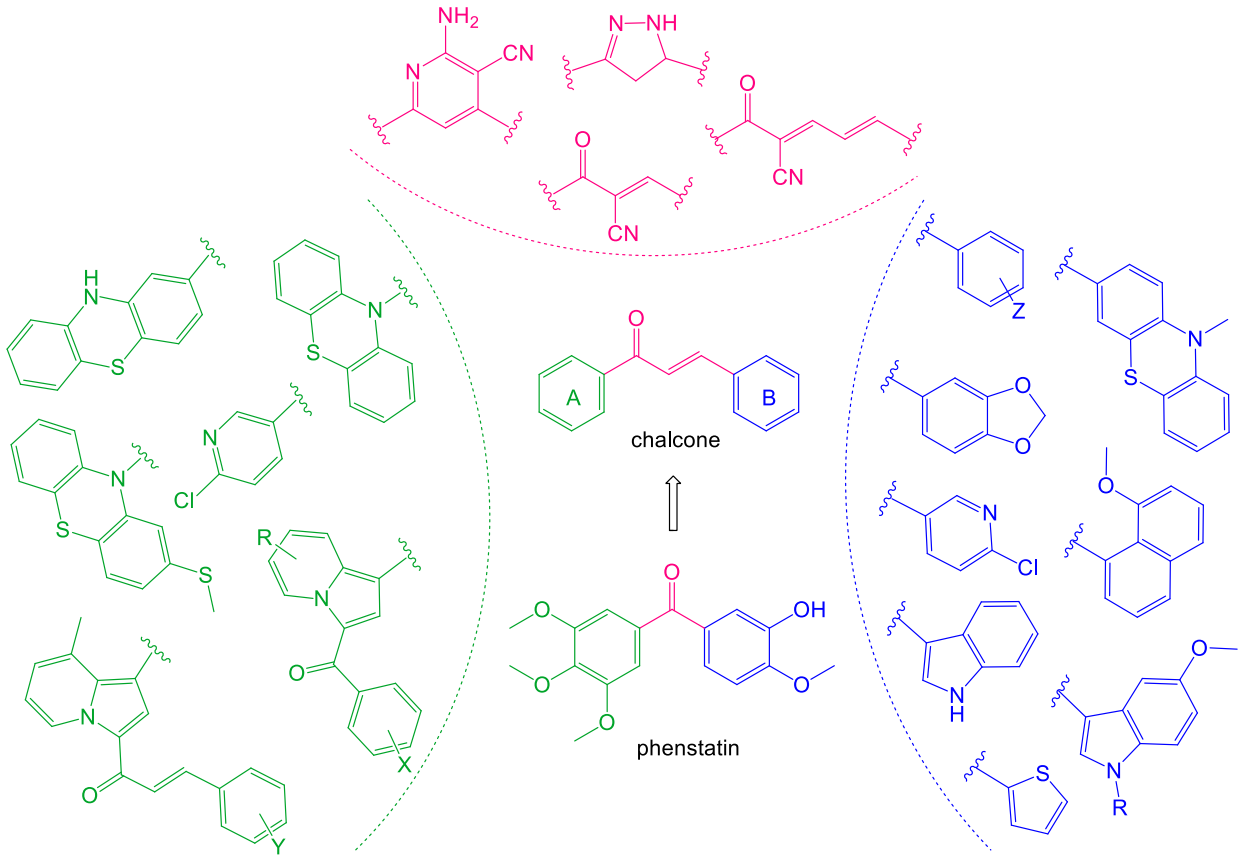


Figure 1. The main modulations proposed in the design of target compounds.

The modulations of the phenstatin structure have involved:

- replacing **aromatic ring A** with aza-heterocycle such as: phenothiazine, 2-methylthio-phenothiazine, 6-chloropyridine, differently substituted indolizine;
- replacing **aromatic ring B** with differently substituted phenyl (with methyl, methoxy, cyano, dimethylamino, halogeno), with 2-methoxynaphthalene, with aza-heterocycle (indole, pyridine, phenothiazine), oxygen heterocycles (1,3-benzodioxole) or sulfur heterocycle (thiophene);
- replacing **ketone connector** with chalcone bridge (the 3-carbon 2-propenone bridge); in terms of modulation on the chalcone connector, we followed the introduction of cyano group in the α position relative to the carbonyl group >C=O from the unsaturated chain of 3 carbon atoms, replacing ketone group with amide group, increasing the chain with one more ethylene group and changing the linker into an aza-heterocycle: pyridine, 4,5-dihydro-1H-pyrazole.

The evaluation of synthesized compounds for their biological potential:

- ✓ anticancer activity on a panel of 60 cancer cell lines by the NCI, SUA and for inhibition of FTase in collaboration with Junia, Lille, France and Institut de Chimie des Substances Naturelles, UPR2301 CNRS, Centre de Recherche de Gif, Gif-sur-Yvette Cedex, France;
- ✓ antifungal activity in collaboration with Junia, Lille and Medicine Faculty, University of Lille, Pôle Recherche (CNRS-UMR 8576, Inserm U1285, Glycobiologie structurale et fonctionnelle), F-59000 Lille, Lille, France.

I. 1. Synthesis strategies of chalcone derivatives containing nitrogen heterocycles (pyridine and phenothiazine) – SERIES A

In this study, we have set out to apply two different synthesis procedures of calcone derivatives by the Claisen-Schmidt condensation in order to identify the more effective method for obtaining the series of compounds of interest.

One of the objectives of this doctoral thesis was to replace the ring A in the structure of chalcone (**Figure 2**) with various nitrogen heterocycles. In order to carry out the comparative study, we applied the conventional procedure (classical stirring experiments) and ultrasounds-mediated condensation for three synthesis reactions of some chalcone derivatives that have the pyridine or phenothiazine unit in their structure. We chose pyridine because it was previously reported in the literature[149] in the synthesis of some chalcone derivatives by the ultrasound-assisted method. Some compounds (**C**, **D, Figura 2**) have anticancer activity due to FTase inhibition and by inhibiting the growth of some human cancer cell lines.

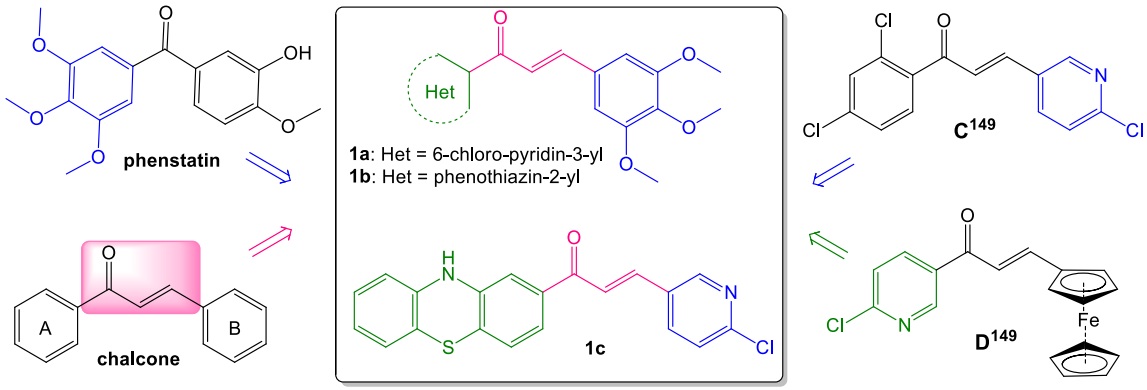


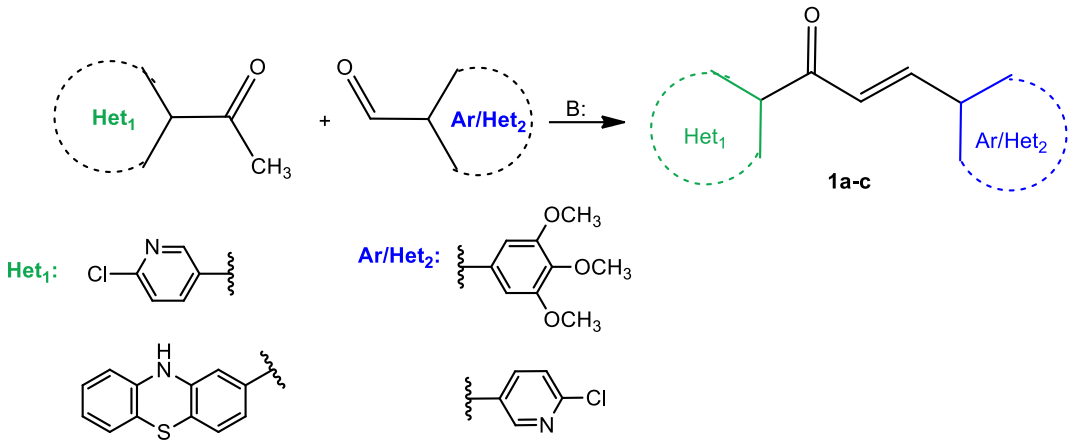
Figure 2. The structures of synthesized chalcone derivatives and of the reference compounds.

Obtaining families of compounds with biological activity is our main objective. Therefore, in the syntheses, we used 3,4,5-trimethoxyphenyl as an analogy with phenstatin (**Figure 2**) and 6-chloropyridin-3-yl by analogy with the compound **C** (**Figure 2**).

In order to determine the most efficient method for our synthesises we took into account a number of factors: time, yield, reaction processing, secondary reactions, solvent, catalyst, toxicity, which also supports a "green chemistry" strategy, considering global initiatives to reduce environmental pollution.

The three synthesis reactions of calcone derivatives involved the condensation of an aromatic aldehyde (3,4,5-trimethoxybenzaldehyde) or heteroaromatic aldehyde (6-chloropyridine-3-carboxaldehyde) with a heteroaromatic methyl ketone (3-acetyl-6-chloropyridine, 2-acetylphenothiazine) (**Scheme 1**). For the exemplification of the reaction,

we synthesized a known compound in the literature **1b**[152] and two new structures **1a**, **1c** (**Figure 3**).



Scheme 1. General synthesis of chalcone derivatives from methyl ketones and aldehydes.

In the case of the classical method (***method A***), target compounds **1a-c** have been synthesized according to the procedure described in the literature.[152] The reaction conditions and yields are given in **Table 1**.

Table 1. Conditions of Claisen-Schmidt reaction in the classical synthesis of chalcone derivaties **1a-c**.

No	Compd no.	Solvent	Quantities of reagent (equiv)	Catalyst	Time (h)	t (°C)	η (%)
1	**1a**	EtOH	1/1	LiOH	1	78	23
2	**1b**	MeOH	1/1	KOH	5	65	**35**
3	**1c**	MeOH	1/1	KOH	4	65	mixture (**1c** + **2c**)

aq – aqueous solution; t = temperature; η = yield.

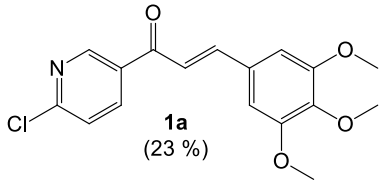
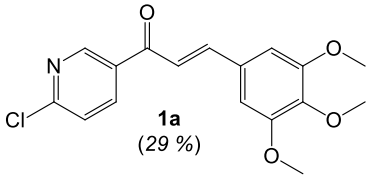
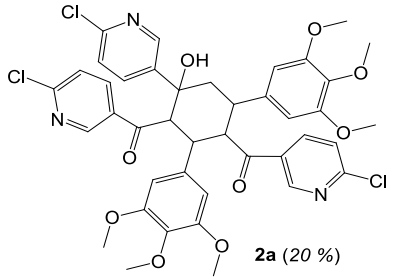
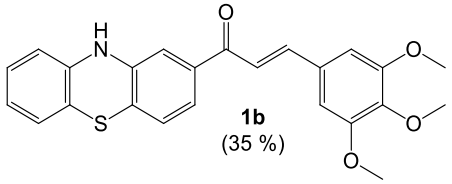
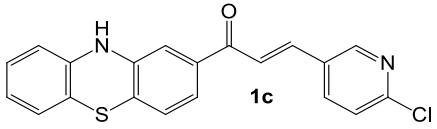
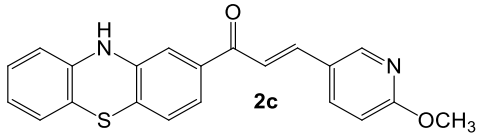
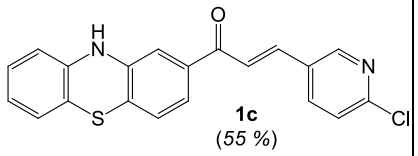
The reaction was performed using an ultrasonic QSONICA reactor. The amplitude was kept constant at 30%, while the reaction time, temperature and energy varied depending on aldehyde, methyl ketone and their solubility. Ultrasounds-mediated experiments (***metoda B***) were performed at JUNIA, Laboratoire Chimie durable et Santé, Lille, France.

Table 2. Conditions of Claisen-Schmidt ultrasounds-mediated reaction of chalcone derivatives **1a-c**.

Compd no.	Solvent (mL)	Quantities of reagent (equiv)	Cat.	Amp	Time (s)	$t_i - t_f$ (°C)	Energy (J)	Yield (%)	η_{sec} (%)
1a	50	1/1	LiOH	0.3	30	19-26	102	**29**	20
1b	50	1/1	LiOH	0.3	60	17-34	201	mixture	0
1c	50	1/1	LiOH	0.3	60	17-33	187	**55**	0

Cat. = catalyst; t_i = initial medium temperature; t_f = final medium temperature; η_{sec} = yield;

Table 3. Products obtained by the two Claisen-Schmidt condensation procedures.

Method A		***Method B***	
Main product	**Byproduct**	**Main product**	**Byproduct**
 **1a** (23 %)	-	 **1a** (29 %)	 **2a** (20 %)
 **1b** (35 %)	-	Inseparable mixture	-
 **1c** Mixed with the compound **2c**	 **2c** Spectrally identified	 **1c** (55 %)	-

I. 2. Design, synthesis and biological evaluation of phenothiazin-10-yl-calcone analogues – SERIES B

I. 2. 1. Design of phenothiazin-10-yl-calcone analogues

A heterocycle known for its pharmacological potential is phenothiazine. By analyzing the promising biological results of the reported *N*-benzoyl-phenthiazine derivatives, reported in the literature[58, 155, 156], we decided to introduce this heterocycle into the development of a new series of phenothiazin-10-yl-calcone analogues. Our aim was to enhance biological properties of compounds and to obtain dual anticancer inhibitors of tubulin polymerization and FTase.

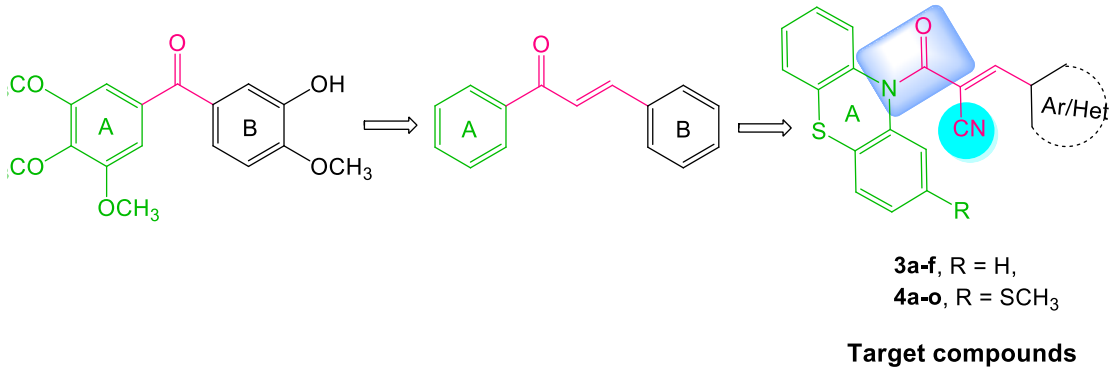


Figure 9. Structures of target compounds (**3a-f**, **4a-o**) and structures of reference compounds.

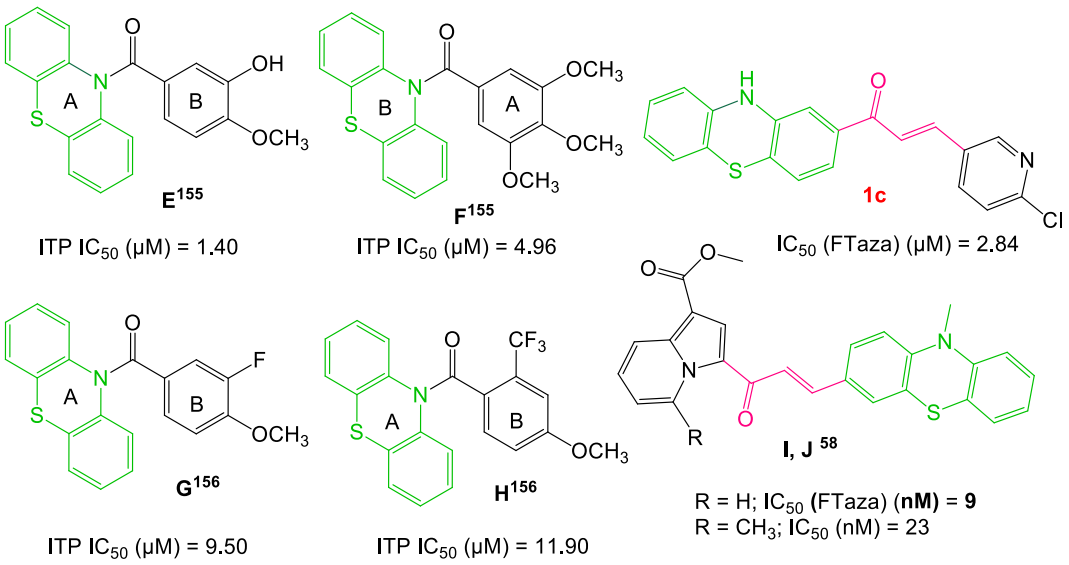


Figure 10. Analogues of phenstatin with phenothiazine ring.

To analyze also the influence of classic carbonyl connector on antitumor activity, we have followed its modulation. In addition, chalcone derivative **1c** (**Figure 10**) which have phenothiazine as ring A showed anticancer potential by inhibiting FTase at IC_{50} = 2.84 μM. Based on these encouraging results, we replaced the carbonyl group with prop-2-en-1-one and

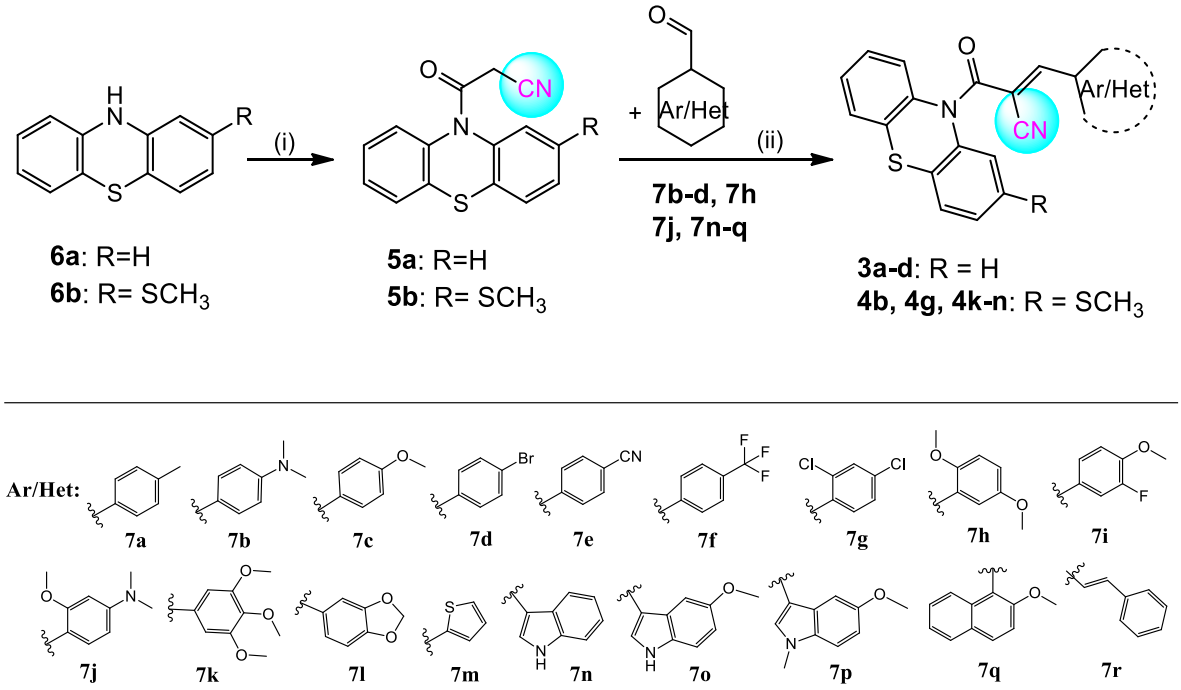
introduced the nitrile group in the α position of the carbonyl group in the unsaturated chain of 3 carbon atoms.

In order to have a broad vision on structure-activity relationships, we used phenothiazine and phenothiazine substituted with the methylthio group (analogy with thioridazine) as ring A.

I. 2. 2. Synthesis of phenothiazin-10-yl-chalcone analogues

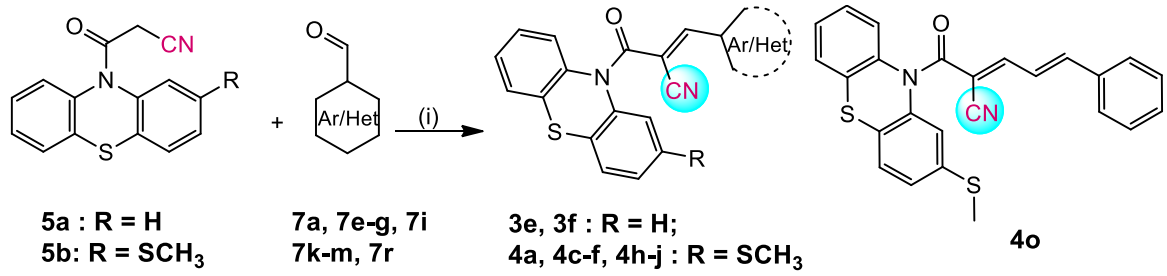
The general synthesis methode of the 21 new phenothiazin-10-yl-chalcone analogues **3a-f** and **4a-o** (**Figure 13**) is the Claisen-Schmidt condensation of an aromatic or heteroaromatic aldehyde with a methylene component, in the structure of witch, the amide group >N-CO-CH_2 is found. Intermediates used as methylene components *N*-cyanoacetyl-phenothiazines and *N*-cyanoacetyl-2-methylthio-phenothiazine were synthesized by nitrogen acylation, according to the literature[157].

To obtain chalcone analogues **3a-d**, **4b**, **4g**, **4k-n (Scheme 2)** classical condensation was applied (***method A***). By this method, 10 new compounds with yields above 55% were obtained (**Scheme 2**).



Scheme 2. Reagents and conditions: (i) 1.0 equiv **6a** or **6b**, 2.0 equiv cyanoacetic acid, 2.0 equiv acetic anhydride, 50 to 100 °C, 1h; (ii) 1.0 equiv **5a** or **5b**, 1.2 equiv aldehyde, piperidine (drops), EtOH or CH_3CN, reflux, 3-24 h.

Method B was performed using an ultrasonic QSONICA reactor. The amplitude was kept constant at 30%. The reaction conditions and yields are given in **Table 4**.



Scheme 3. Ultrasounds-mediated synthesis of phenothiazine-10-yl-chalcones. Reagents and conditions: (i) 0.7 equiv LiOH, ethanol, 45-120 s.

Table 3. Conditions of Claisen-Schmidt ultrasounds-mediated reaction of phenothiazin-10-yl-chalcone analogues **3d, 3f, 4a, 4c-f, 4h-j, 4o**.

Entry	Compd no.	EtOH (mL)	Quantities of reagent (mmol)	Quantities of aldehyde (mmol)	LiOH (equiv)	Time (s)	$t_i - t_f$ (°C)	Energy (J)	η (%)
1	**3e**	25	0.94	1.13	0.7	120	20-50	539	75
2	**3f**	30	1.87	2.26	0.7	120	19-52	575	74
3	**4a**	30	1.28	1.41	0.7	45	19-35	125	68
4	**4c**	30	1.28	1.60	0.7	60	18-41	169	78
5	**4d**	30	1.28	1.44	0.7	60	19-41	158	61
6	**4e**	30	1.28	1.53	0.7	60	19-45	160	74
7	**4f**	30	1.28	1.41	0.7	60	18-45	149	67
8	**4h**	25	1.08	1.08	0.7	90	20-59	282	67
9	**4i**	25	0.80	0.82	0.7	90	20-59	343	72
10	**4j**	30	1.28	1.43	0.7	60	19-44	169	77
11	**4o**	30	1.28	1.41	0.7	60	19-44	166	61

t_i = initial medium temperature; t_f = final medium temperature; η = yield;

We considered two synthesis procedures, because we wanted to use the most efficient method for our synthesises in terms of time, yield, solvent, catalyst, toxicity, which also supports a "green chemistry" strategy, considering global initiatives to reduce environmental pollution.

After purification, compounds **3a-f** and **4a-o** were physico-chemically and structurally characterized, using IR și ^{1}H, ^{13}C, ^{19}F NMR spectra, as well as HMQC and HMBC.

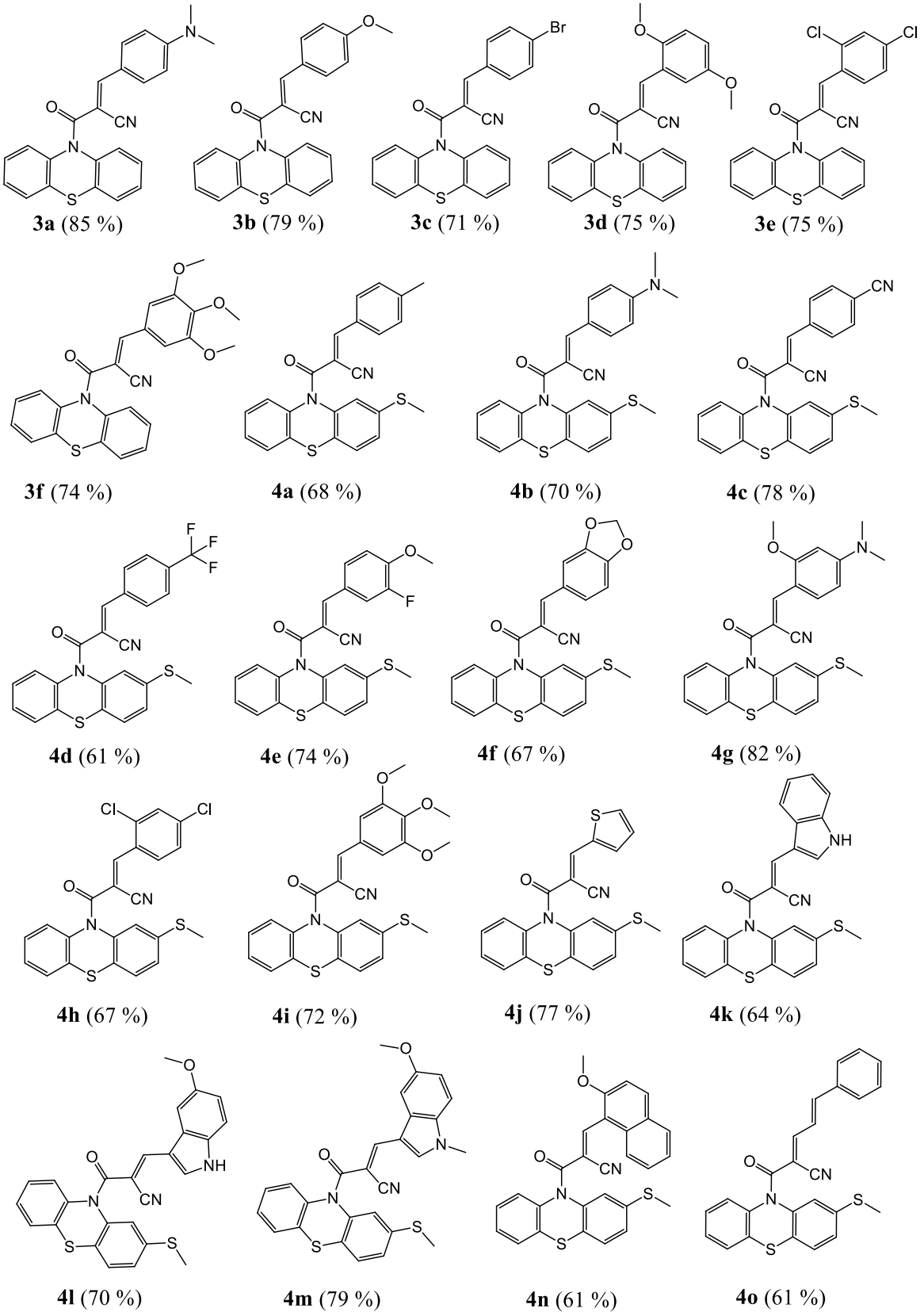


Figure 13. Phenothiazin-10-yl-calcone analogues synthesized in this study and their yields

To obtain a comprehensive understanding of structure-activity relationships and to determine the impact of methylthio group on yield, we have maintained cycle B in three

cases: 4-dimethylaminophenyl, **3a** (85%) and **4b** (70%), 2,4-dichlorophenyl, **3e (**75%) and **4h** (67%), 3,4,5-trimethoxyphenyl, **3f** (74%) and **4i** (72%).

I. 2. 3. Biological evaluation

Series B is part of an extensive study of our research group that involves obtaining compounds with dual anticancer activity, inhibitors of tubulin polymerisation and FTase.

Table 4. Inhibitory activities of compounds **3a-f**, **4h**, **4i** on human farnesyltransferase and tubulin polymerization *in vitro.*

Entry	Compound	% FTase[a,b]	IC_{50} (µM)[b]	R^2[c]	% TPI[d,b]	IC_{50} (µM)[b]	R^2[c]
1	**3a**	**65**	n. d.[e]	-	n. d.	-	-
2	**3b**	0	n. d.	-	n. d.	-	-
3	**3c**	48	n. d.	-	n. d.	-	-
4	**3d**	**76**	**7.268**	0.9335	5	-	-
5	**3e**	n. d	-	-	n. d.	-	-
6	**3f**	**68**	n. d.	-	n. d.	-	-
7	**4h**	**72**	30.510	0.9818	n. d.	-	-
8	**4i**	**58**	n. d.	-	n. d.	-	-
Phenstatin 3b		40	-	-	99	3.430	0.9378
Desoxypodophyllotoxin		-	-	-	100	1.760	0.9740
FTI-276		100	7	0.8369	-	-	-

[a] Inhibition of human farnesyltransferase at 100 µM concentration.

[b] IC_{50} values are indicated as the mean of two independent experiments

[c] Regression factor

[d] Inhibition of tubulin polymerization at a 100 µM concentration

[e] Not determined

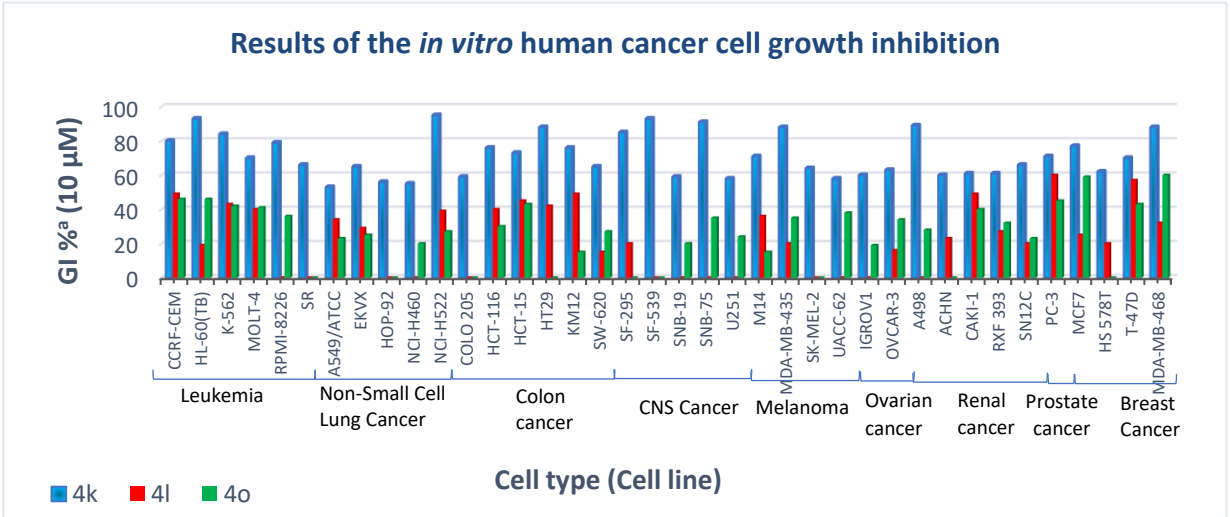


Figure 19. Results of the *in vitro* human cancer cell growth inhibition for selected compounds **4k**, **4l** și **4o**.

Sixteen of the new phenothiazin-10-yl-chalcone analogues synthesized, **3e**, **4a-o**, have been tested by NCI, USA for their ability to inhibit the proliferation on a panel of 60 human cancer cell lines. The test to evaluate the anticancer potential was conducted at a concentration of 10 μM on 60 cell lines. To highlight the antiproliferative action, we have made a selection of the best results, which are presented in **Table 6** and illustrated in **Figure 19**.

Table 5. Results of the in vitro human cancer cell growth inhibition for selected compounds **4k**, **4l**, **4m** and **4o**.

Cell type	Compound	4k	4l	4m	4o
	Cell line	GI%[a, b] (10 μM)			
Leukemia	CCRF-CEM	**80**	**49**	0	**46**
	HL-60(TB)	**93**	19	0	**46**
	K-562	**84**	43	0	42
	MOLT-4	**70**	40	33	41
	RPMI-8226	**79**	0	0	36
	SR	**66**	0	0	0
Non-Small Cell Lung Cancer	A549/ATCC	**53**	34	0	23
	EKVX	**65**	29	23	25
	HOP-92	**56**	0	29	0
	NCI-H226	**50**	35	11	0
	NCI-H460	**55**	0	0	20
	NCI-H522	**95**	39	0	27
Colon cancer	COLO 205	**59**	0	0	0
	HCT-116	**76**	40	11	30
	HCT-15	**73**	45	0	43
	HT29	**88**	42	0	0
	KM12	**76**	**49**	0	15
	SW-620	**65**	15	12	27
CNS Cancer	SF-295	**85**	20	0	0
	SF-539	**93**	0	0	0
	SNB-19	**59**	0	0	20
	SNB-75	**91**	0	22	35
	U251	**58**	0	0	24
Melanoma	M14	**71**	36	0	15
	MDA-MB-435	**88**	20	0	35
	SK-MEL-2	**64**	0	0	0
	UACC-62	**58**	0	0	38
Ovarian cancer	IGROV1	**60**	0	21	19
	OVCAR-3	**63**	16	0	34
Renal cancer	786-0	**50**	13	0	17
	A498	**89**	0	28	28
	ACHN	**60**	23	0	0
	CAKI-1	**61**	**49**	26	40
	RXF 393	**61**	27	15	32
	SN12C	**66**	20	0	23
Prostate Cancer	PC-3	**71**	**60**	0	45
Breast Cancer	MCF7	**77**	25	0	**59**
	HS 578T	**62**	20	16	0
	T-47D	**70**	**57**	10	43
	MDA-MB-468	**88**	32	15	**60**

[a] Data obtained from NCI's in vitro 60-cell one dose screen at 10 μM concentration.

[b] GI% is the percentage of growth inhibition of tumor cells.

I. 3. Design, synthesis and biological evaluation of indolizine derivatives – SERIES C

I. 3. 1. Design of indolizine derivatives

Indolizine (pyrrolo[1,2-a]pyridine) is a structural isomer of indole. Although drugs containing indole in their structure are available on the market, to our knowledge, a drug containing indolizine is not known. At the same time, numerous studies on the promising antitumor properties of indolizine derivatives are reported in the literature.[151, 156, 160-163]

Since phenothiazin-10-yl-chalcone analogues (**series B**) have demonstrated anticancer potential, we wanted to see if by using the indolizine ring and maintaining the chalcone bridge, we would obtain more active structures, both anticancer and with other biological properties.

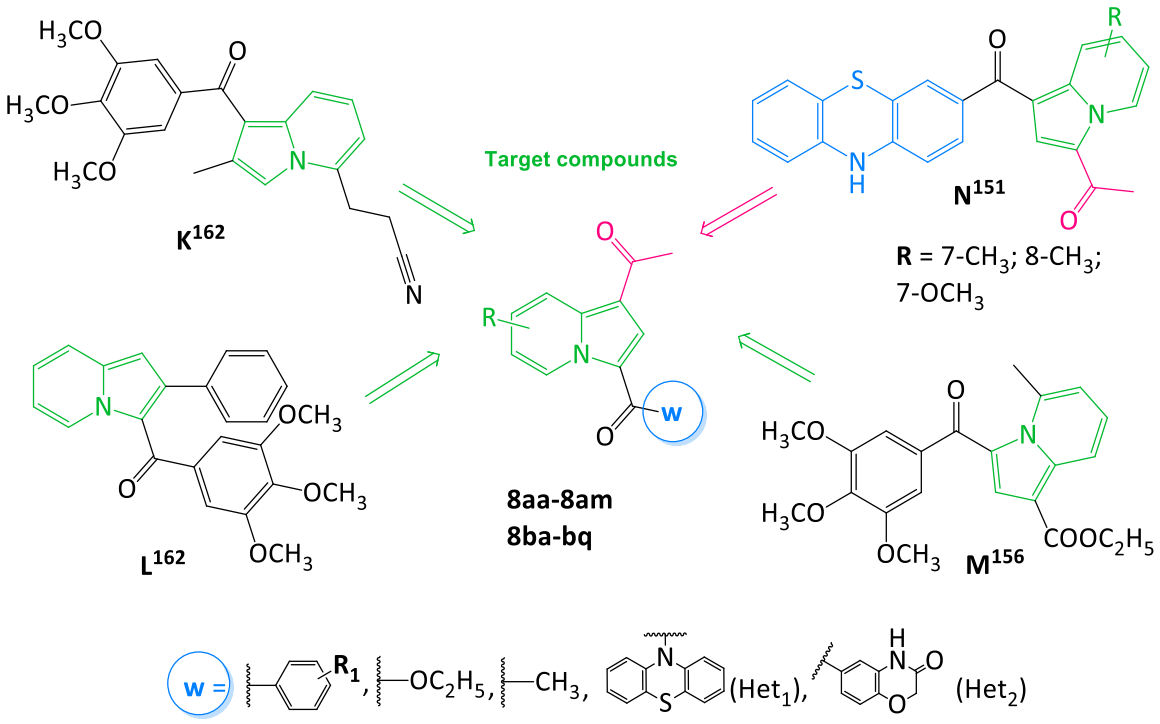


Figure 20. Structure of previously described indolizine derivatives (**K**-**N**) and structure of target compounds (**8aa-am**, **8ba-bq**)

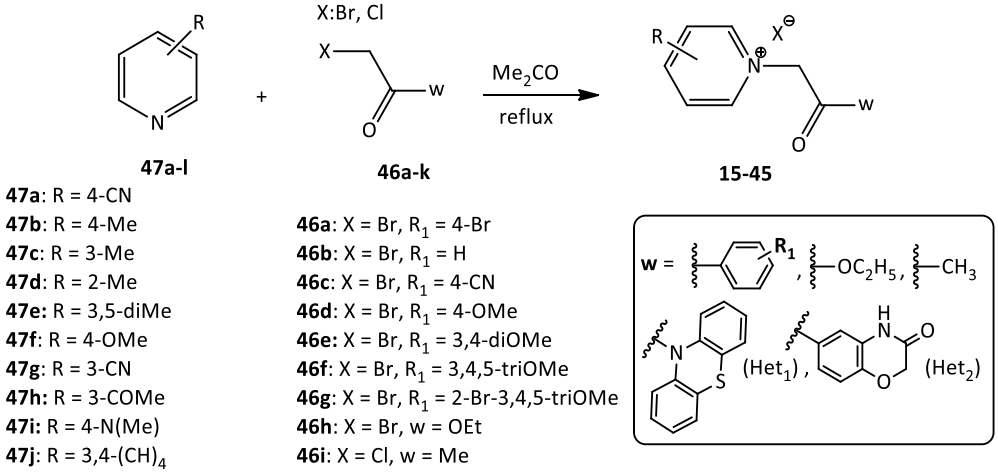
In this context, the series of indolizine derivatives was conceived, which we considered both analogues of phenstatin from the perspective of biological properties, as well as intermediates in synthesis. We have used indolizine derivatives as intermediates in obtaining new series of compounds (indolizine-chacone hybrids that consists of three centers A, B and C, connected by a carbonyl bridge, and 2-propenone moiety - **series D** - and other heterocyclic derivatives, indolizine-pyridine hybrids - **series E**-). To further explore the structure-activity relationships, we also modified the ring A (3,4,5-trimethoxyphenyl) by substituting it with a

simple or substituted phenyl group and heterocycle such as 2*H*-benzo[b][1,4]oxazin-3(4*H*)-one or phenothiazin-10-yl.

Another significant modulation in the design on this family of compounds involved the introduction of an acetyl group in the 1st position of the indolizine ring. This modulation was really important for both structure-activity relationships exploration and the creation of a reactive center for obtaining new series of compounds with biological properties (**series D** and **series E**).

I. 3. 2. Synthesis of indolizine derivatives

This series of compounds was obtained through a two-step synthesis, previously used in our research group. Pyridines were treated with (bromo)chloromethylketones and ethyl bromoacetate in acetone at reflux to easily obtain cycloimmonium salts **15-45** (**Scheme 4**) in good yields. Pyridinium, quinolinium and isoquinolinium salts were obtained as described previously in the literature[58, 156, 161, 165-170]. 2-bromo-3',4',5'-trimethoxyacetophenone was synthesized according to the procedure previously reported in the literature.[156]



Scheme 4. Synthesis of pyridinium, quinolinium and isoquinolinium salts

Table 6. Pyridinium, quinolinium and isoquinolinium salts **15-45**

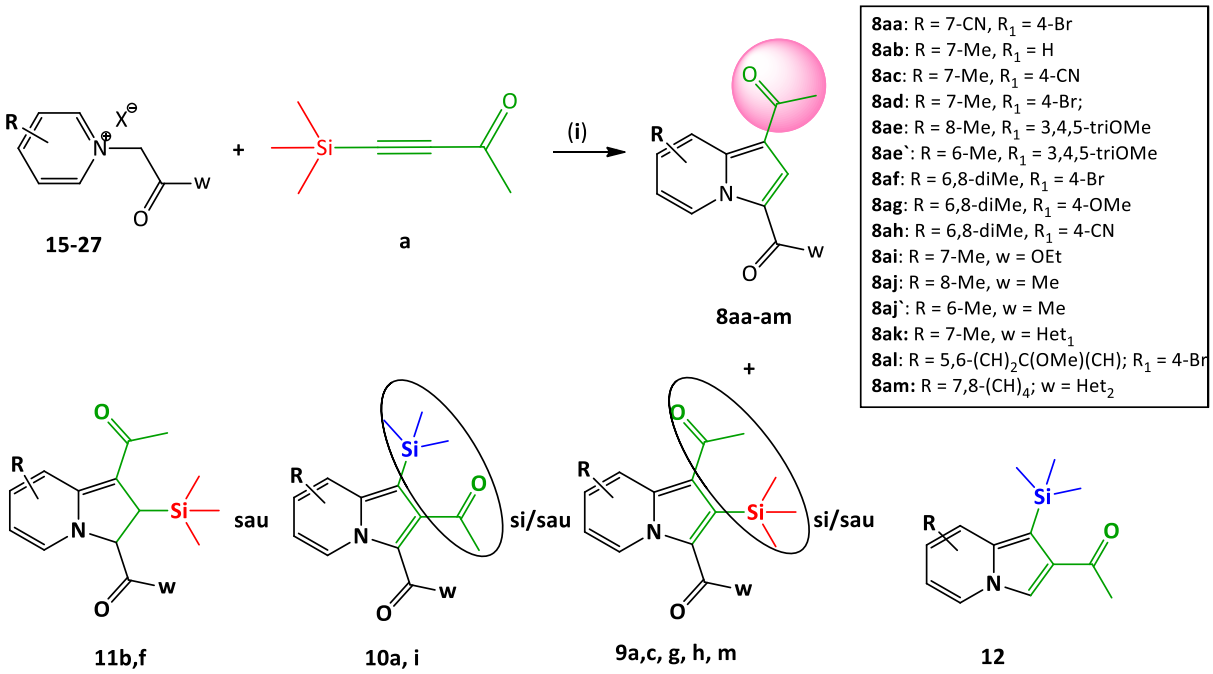
Salt No	X	R	R1 sau w
15	Br	4-CN	4-Br
16	Br	4-Me	H
17	Br	4-Me	4-CN
18	Br	4-Me	4-Br
19	Br	3-Me	3,4,5-triOMe

20	Br	3,5-diMe	4-Br
21	Br	3,5-diMe	4-OMe
22	Br	3,5-diMe	4-CN
23	Br	4-Me	w = OEt
24	Cl	3-Me	w = Me
25	Cl	4-Me	w = Het_1
26	Br	2,3-(CH)C(OMe)$(CH)_2$	4-Br
27	Cl	4,5-$(CH)_4$	w = Het_2
28	Br	2-Me	H
29	Br	2-Me	4-Br
30	Br	4-Me	4-OCH_3
31	Br	4-OMe	4-Br
32	Br	3-CN	4-Br
33	Br	2-Me	3,4,5-triOMe
34	Br	4-Me	3,4,5-triOMe
35	Br	3-$COCH_3$	OMe
36	Br	4-$N(Me)_2$	3,4-diOMe
37	Br	3,5-diMe	H
38	Br	3,5-diMe	3,4,5-triOMe
39	Cl	2-Me	**w** = Het_1
40	Br	2,3-$(CH)_4$	H
41	Br	3,4-$(CH)_4$	H
42	Br	3,4-$(CH)_4$	3,4,5-triOMe
43	Cl	2,3-$(CH)_4$	**w** = Het_1
44	Cl	3,4-$(CH)_4$	**w** = Het_1
45	Br	2-Me	2-Br-3,4,5-triOMe

In step two, the [3+2] dipolar cycloaddition of all salts with an alkyne (dipolarophile) furnished target compounds, 1-acetyl-lindolizines.[164] We used two dipolarophiles: 4-(trimethylsilyl)-3-butyn-2-one (**Scheme 5**) and 3-butyn-2-one (**Scheme 7**).

The dipolarophile 4-(trimethylsilyl)-3-butyn-2-one has lower toxicity and is more financially advantageous, but the yields of target products are smaller, as a results of obtaining various unexpected by-products (1-acetyl-2-trimethylsilylindolizines and 1-trimethylsilyl-2-acetyl-indolizines).

In this way, we obtained 22 new indolizine derivatives (**8ac-8am, 9a, 9c, 9g, 9h, 9m, 10a,i, 11b, 11f, 12**), of which 10 trimethylsilylindolizines (**9-12**) and 3 compounds known in literature (**8aa, 8ab**, **8aj'**)[165-167] (**Figure 21**).



Scheme 5. Synthesis of acetyl-indolizines derivatives with 4-(trimethylsilyl)-3-butyn-2-one. Reaction conditions: (i) 1.3 equiv TEA, 1.3 equiv 4-(trimethylsilyl)-3-butyn-2-one, DMF:DMSO (5:1, v:v), 80 ˚C, 24 - 30 h.

The dipolarophile 4-(trimethylsilyl)-3-butyn-2-one is also interesting since, to the best of our knowledge, it has been used for the first time in reaction with cycloimmonium salts and the synthesis of 2-acetyl-3-benzoylindolizines has not been described in the literature.

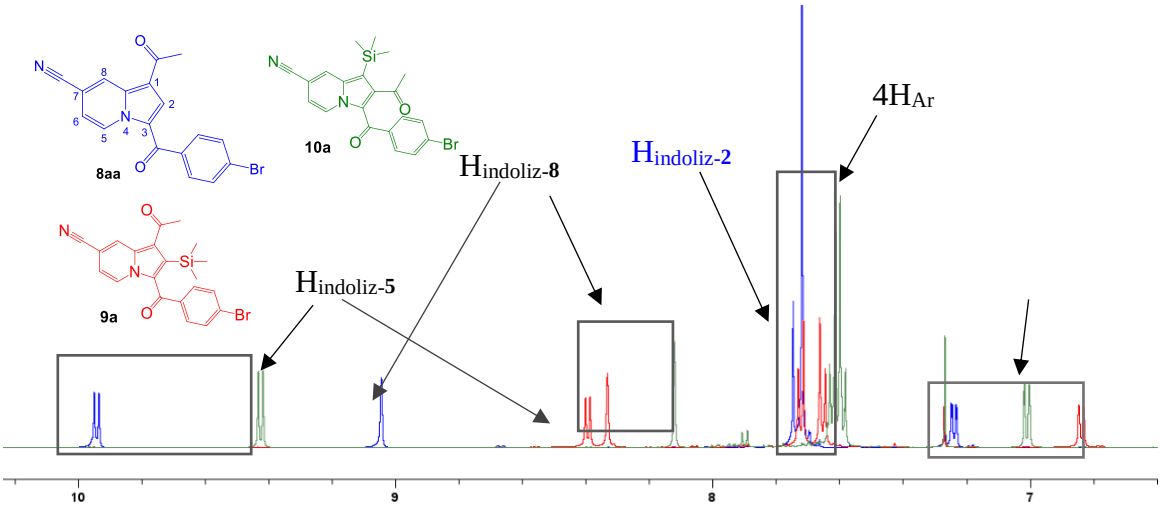


Figure 23. Superimposition of ^{1}H NMR spectra (aromatic region) of compounds **8aa** (blue), **9a** (red) and **10a** (green).

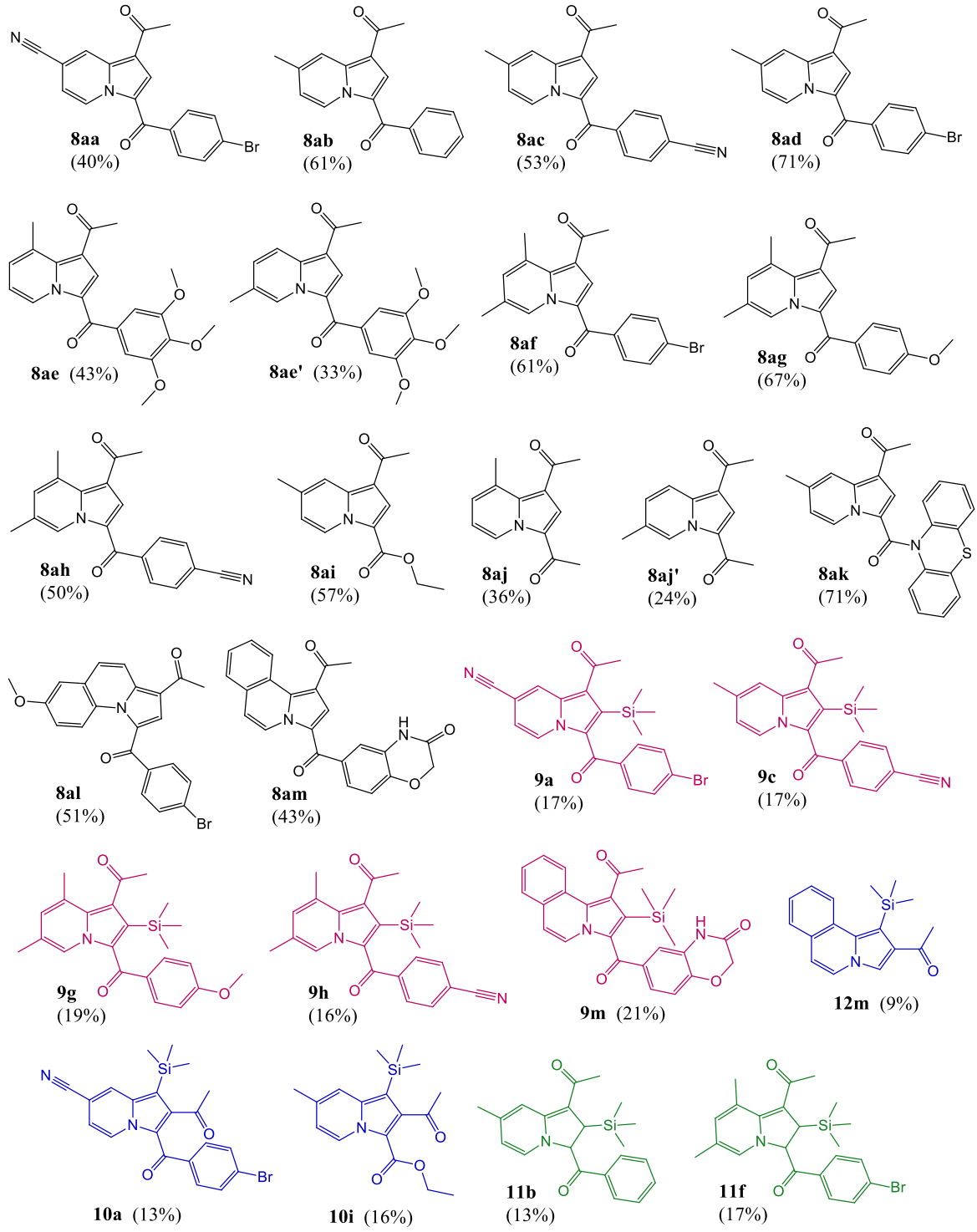


Figure 21. Structures of synthesized compounds with 4-(trimethylsilyl)-3-butyn-2-one.

The structure of the two regioisomers (**9a** and **10a**, **Figure 21**, **Table 8**) was secured by X-ray crystallography. In some cases, the partially unsaturated 2,3-dihydroindolizines were also detected in the crude as traces and only two were isolated in very low yields: **11b** (13%) and **11f** (17%), respectively (**Figure 8**).

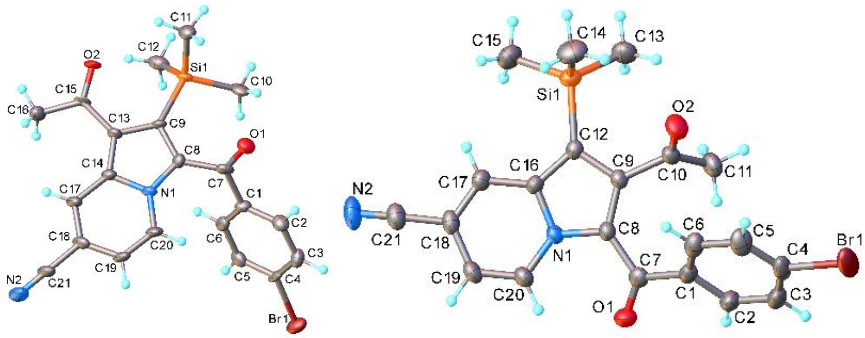
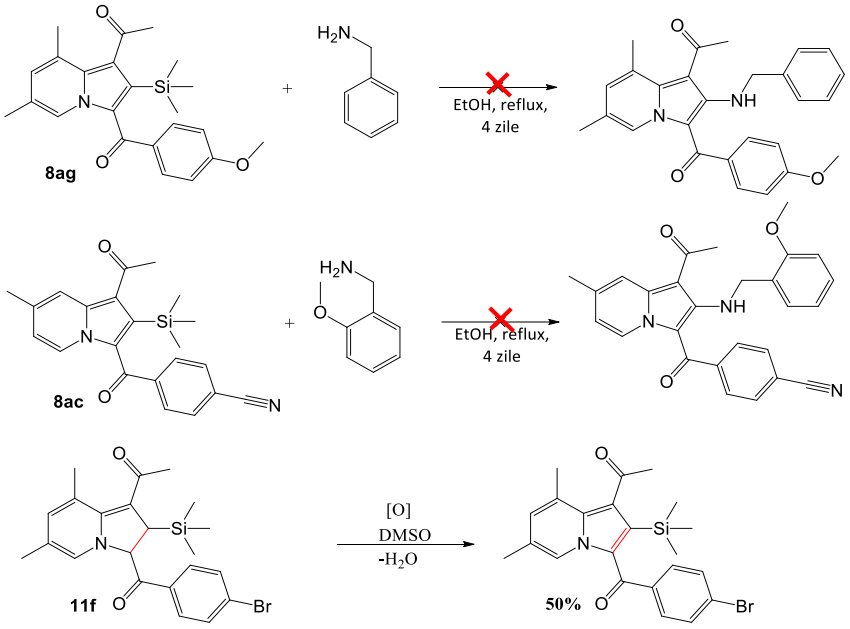


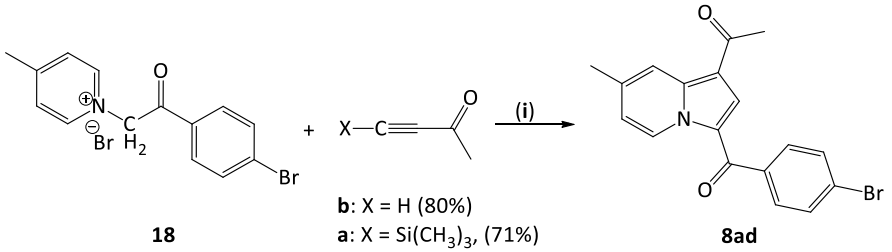
Figure 22. X-Ray molecular structures of regioisomers **9a** and **10a**

The reactivity of trimethylsilyl group in this type of compounds was next tested by refluxing in ethanol with the selected nucleophiles. (**Scheme 6**)



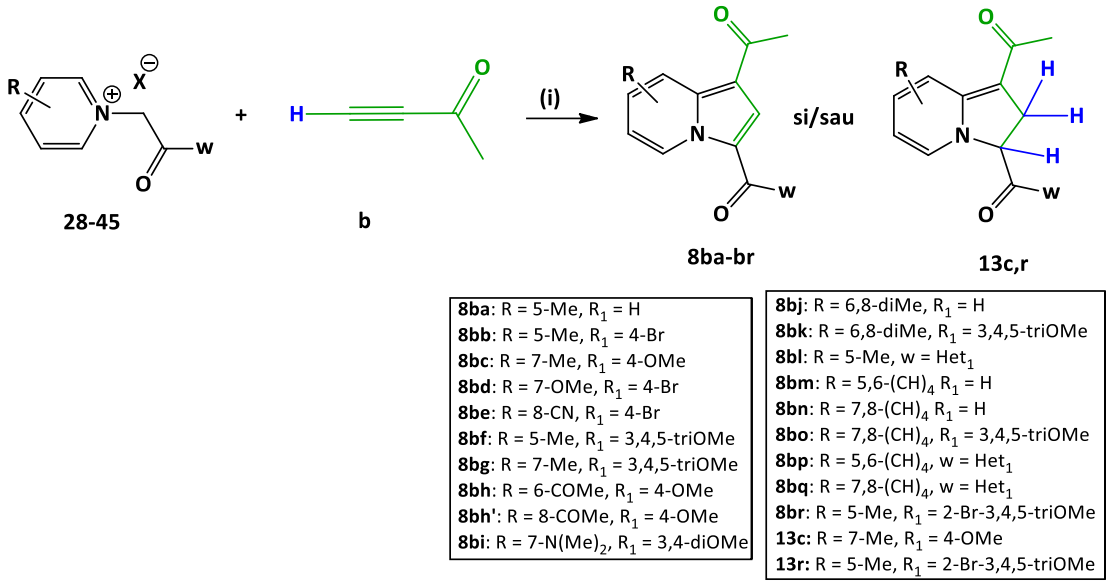
Scheme 6. Reactions of trimethylsilyl-indolizine derivatives.

To determine the influence of dipolarophile on the cycloaddition reaction, we carried out the synthesis of compound **8ad** with the two dipolarophiles under the same reaction conditions (**Scheme 7**).



Scheme 7. Reaction conditions: (i) 1.3 equiv TEA, 1.3 equiv dipolarophile, DMF:DMSO (5:1 v:v), 80 ˚C, 24 h.

In the synthesis of other indolizine derivatives we used 3-butin-2-one as dipolarophile (**Scheme 8**). With 3-butyn-2-one we obtained 20 new indolizine derivatives (**8ba-bl**, **8bo-br**, **14, Figure 28**), of which 2 partially aromatized compounds (**13c**, **13r, Figure 28**), and 2 known compounds (**8bm, 8bn**)[171] (**Figure 28)**.



Scheme 8. Synthesis of acetyl-indolizine derivative with 3-butyn-2-one.

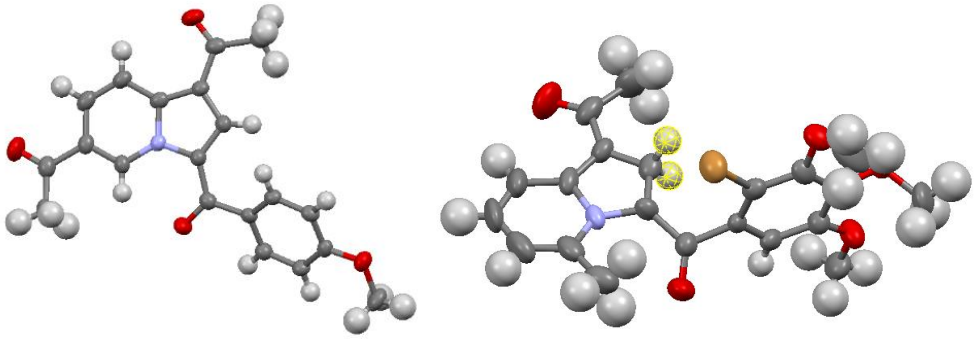


Figure 29. X-Ray structures of expacted isomer **8bh** and partially unsaturated 2,3-dihydroindolizines **13r**.

Following the reactions carried out with 3-butyn-2-one we found that its purity determines the reaction pathway. When we used a reactant stored in appropriate conditions, the [3+2] cycloaddition reaction proceeded in the expected mode, thus obtaining the compounds **8ba-8br** (**Scheme 8**).

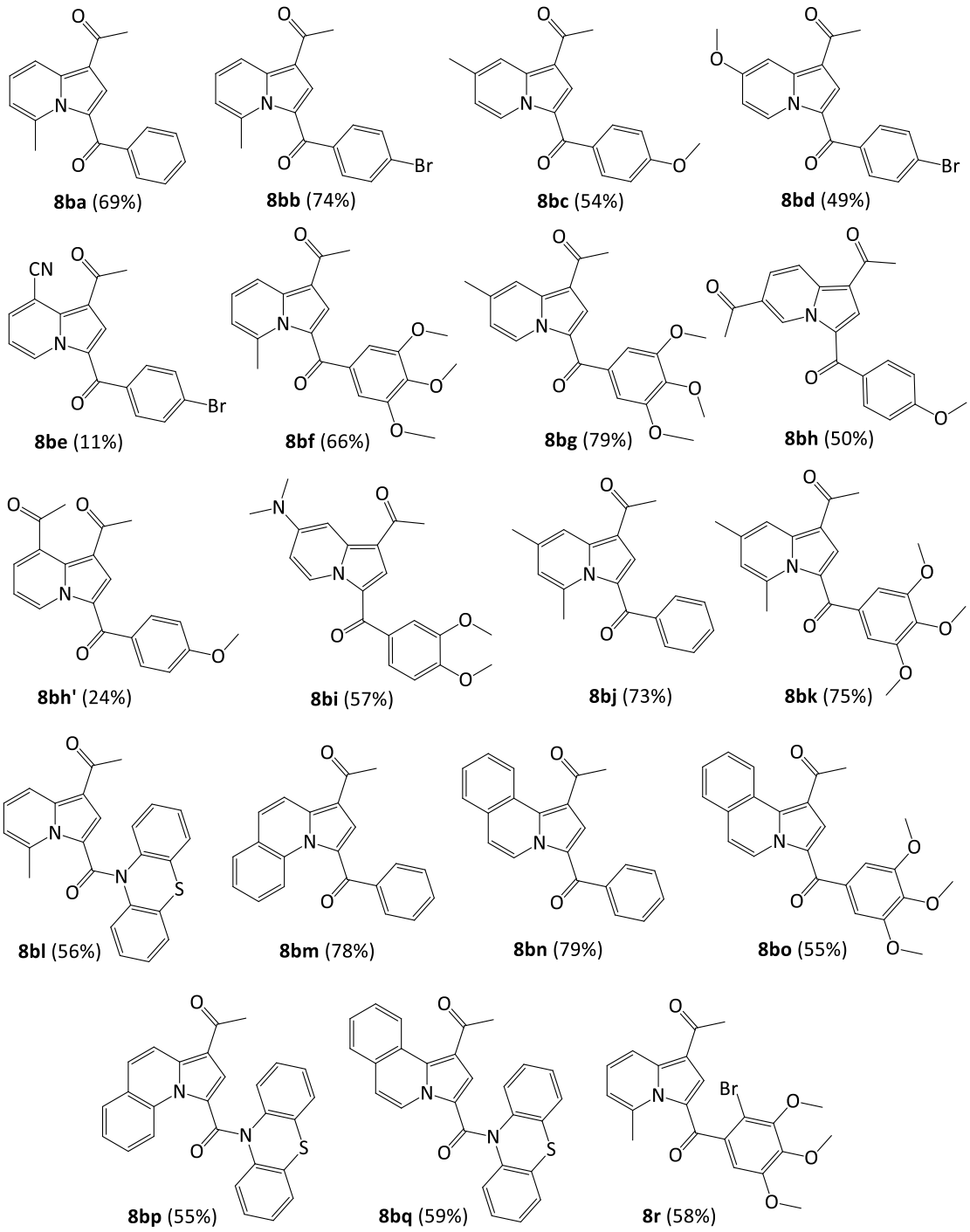
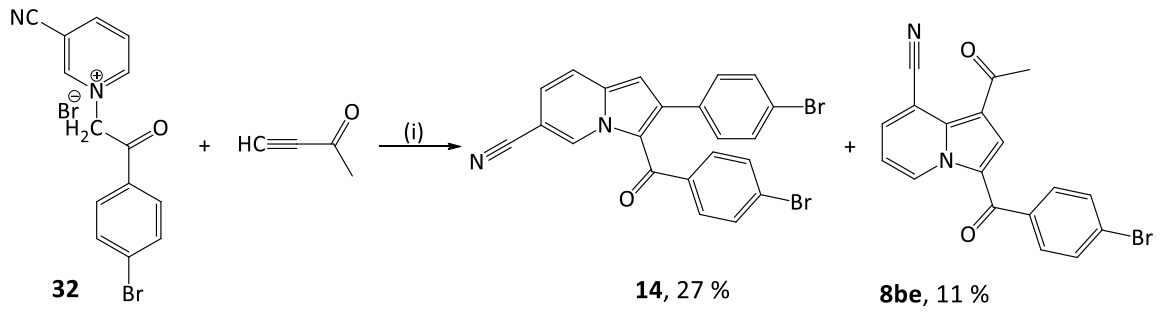
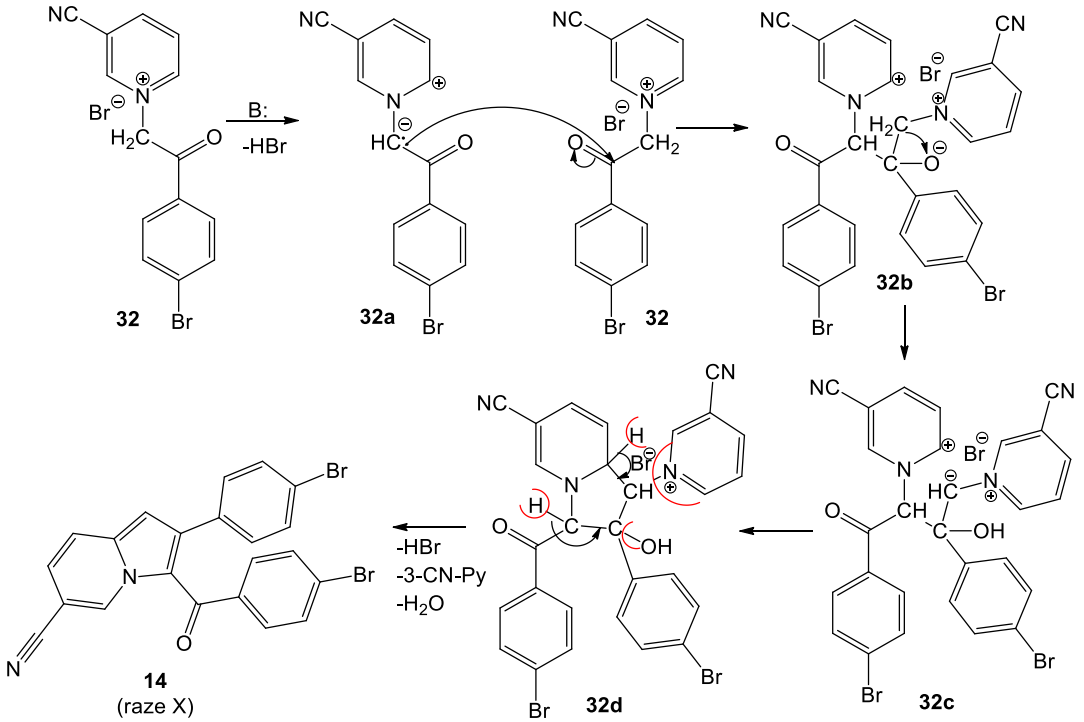


Figure 28. Structures of synthesized compounds with 3-butyn-2-one and their yields.

When the experiment was performed with an old batch of 3-butyn-2-one, visually observable and confirmed by ^{1}H NMR spectra, the reaction followed a new path, obtaining the expected 1-acetylindolizine **8be** and an unexpected product **14** (**Scheme 9**). The exact structure of indolizine **14** was secured by X-ray crystallography. For this reaction, a reaction mechanism was proposed (**Scheme 10**).



Scheme 9. Reaction conditions: (i) 1.3 equiv TEA, 1.3 equiv dipolarophile, DMF:DMSO (5:1), 80 ˚C, 24 h..



Scheme 10. Proposed mechanism for the formation of indolizine 10n'.

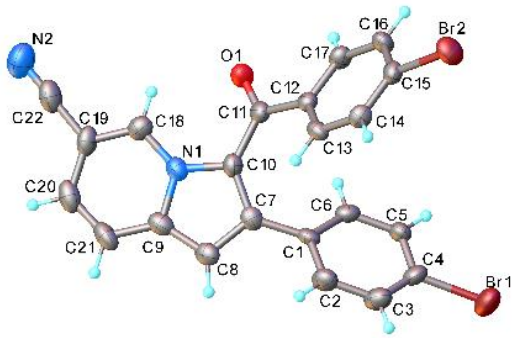
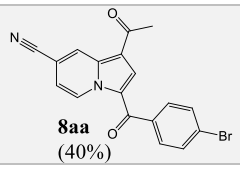
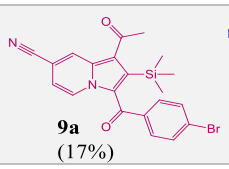
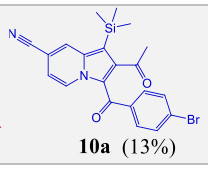
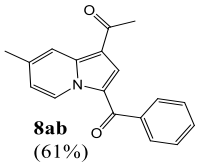
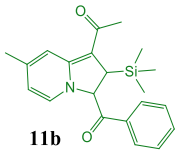
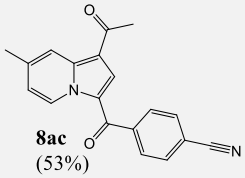
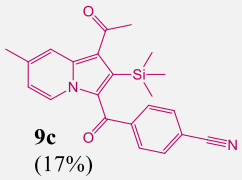
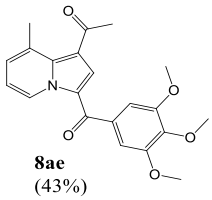
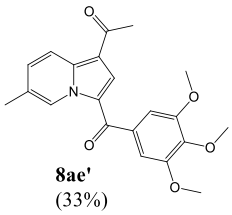
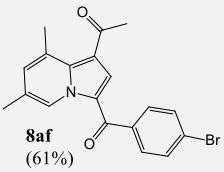
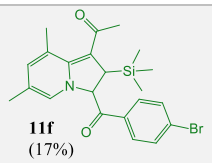
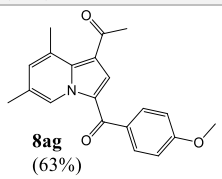
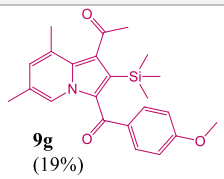
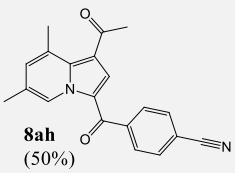
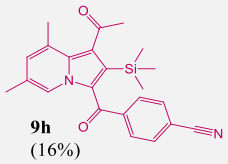
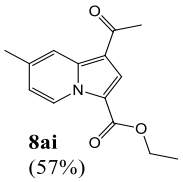
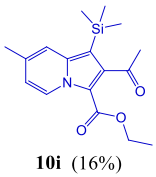
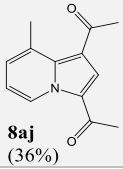
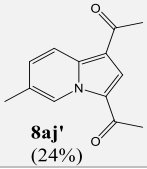
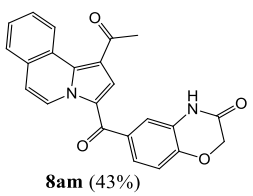
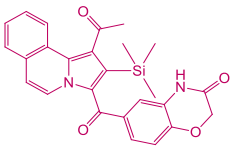
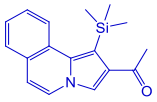
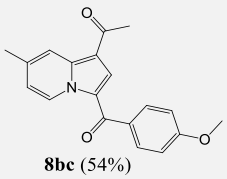
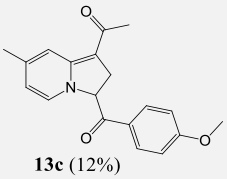
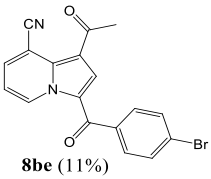
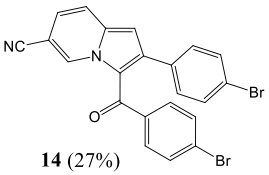
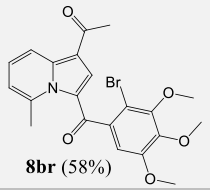
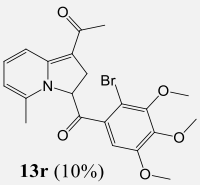


Figure 30. X-Ray molecular structures of unexpected indolizine **14**.

Series C contains a number of **78 synthesized compounds**, of which **48 new**. Some reactions led to two or more products, that are given in **Table 8**.

Table 7. Target compounds and their by-products.

Entry	*Isolated target compound*	*Isolated by-product*
1.	 **8aa** (40%)	 **9a** (17%)  **10a** (13%)
2.	 **8ab** (61%)	 **11b** (13%)
3.	 **8ac** (53%)	 **9c** (17%)
4.	 **8ae** (43%)	 **8ae'** (33%)
5.	 **8af** (61%)	 **11f** (17%)
6.	 **8ag** (63%)	 **9g** (19%)
7.	 **8ah** (50%)	 **9h** (16%)
8.	 **8ai** (57%)	 **10i** (16%)
9.	 **8aj** (36%)	 **8aj'** (24%)

10.	 **8am** (43%)	 **9m** (21%)  **12** (9%)
11.	 **8bc** (54%)	 **13c** (12%)
12.	 **8be** (11%)	 **14** (27%)
13.	 **8br** (58%)	 **13r** (10%)

I. 3. 3. Biological evaluation

Forty indolizines (seven with trimethylsylil group) were selected by the NCI for biological evaluation on a panel of 60 cancer cell lines. Compounds were tested at 10 μM concentration. The best results are presented in **Table 9**.

Table 8. Results of the *in vitro* human cancer cell growth inhibition for selected compounds **8ae, 8ae', 8ag, 8ak, 8al, 8am, 12, 8bf, 8bi, 8bl, 8bo, 8bp, 8bq**.

	Compus	**8ae**	**8ae'**	**8ag**	**8ak**	**8al**	**8am**	**12**	**8bl**	**8bf**	**8bi**	**8bo**	**8bp**	**8bq**
Tip de cancer	**Linie celulară**	**GI%[a, b] (10 μM)**												
Leukemia	CCRF-CEM	4	**53**	0	**75**	0	17	18	**0**	**55**	23	51	**64**	**72**
	HL-60(TB)	11	***91***	22	**67**	0	5	0	36	**89**	27	**67**	**86**	***90***
	K-562	5	**81**	14	**81**	18	16	37	32	**84**	18	**76**	**76**	**80**
	MOLT-4	8	**72**	18	**77**	0	33	40	40	**61**	21	**65**	45	**51**
	RPMI-8226	5	45	32	**80**	0	37	35	42	49	14	48	**52**	**60**
	SR	3	**73**	**68**	***93***	22	37	31	44	**83**	**55**	**83**	**89**	**83**
Non-Small Cell Lung Cancer	A549/ATCC	0	**53**	**55**	43	17	**70**	11	12	**50**	6	38	41	**50**
	EKVX	0	**53**	**51**	47	19	25	10	11	46	4	-[d]	21	39
	HOP-62	0	48	**65**	41	**60**	26	0	7	**63**	18	44	44	**58**
	HOP-92	0	3	**53**	**62**	19	0	7	-	**66**	0	**73**	15	**55**
	NCI-H226	10	34	**56**	**60**	36	38	12	8	48	19	33	40	**55**
	NCI-H23	0	**51**	**52**	**58**	27	42	23	8	40	4	36	21	37
	NCI-H460	0	**84**	**56**	**88**	22	36	0	0	**84**	32	**55**	**62**	**70**
	NCI-H522	10	**62**	***98***	**50**	35	28	9	22	**81**	25	48	**100[c]**	**100[c]**
Colon Cancer	COLO 205	0	**60**	7	32	14	8	0	0	**89**	11	**61**	**60**	**64**
	HCC-2998	0	38	24	**67**	14	12	9	0	39	0	37	22	26
	HCT-116	5	**82**	**80**	***93***	27	**55**	31	39	**85**	38	**58**	**74**	**76**

	HCT-15	11	**81**	34	***90***	9	28	28	27	**82**	**59**	**71**	**58**	**71**
	HT29	0	**84**	28	**67**	19	43	43	18	**91**	0	**60**	**70**	**85**
	KM12	3	**71**	29	**72**	0	49	18	4	**70**	5	**56**	**55**	**68**
	SW-620	0	**68**	18	**74**	19	7	9	6	**74**	3	**55**	**58**	**74**
CNS Cancer	SF-268	0	39	**77**	**51**	26	21	6	8	46	18	26	32	**56**
	SF-295	0	***92***	**69**	**64**	45	0	0	8	**82**	0	45	**59**	**81**
	SF-539	2	**65**	**76**	**67**	34	**68**	7	11	**55**	15	25	31	**70**
	SNB-19	21	**60**	**75**	**50**	38	30	7	19	**60**	7	36	42	**51**
	SNB-75	35	***94***	***98***	17	**55**	0	0	-	***95***	22	**55**	**61**	**65**
	U251	3	**66**	**68**	**64**	28	**73**	12	5	**69**	24	26	**52**	**62**
Melanoma	LOX IMVI	3	**66**	45	**100**	18	43	6	8	**60**	20	42	41	**59**
	MALME-3M	11	**56**	28	46	23	**100**	0	0	**54**	8	35	38	**50**
	M14	4	**84**	15	**64**	0	35	5	17	***94***	0	41	59	**57**
	MDA-MB-435	3	**100**	**51**	**72**	27	19	0	3	**100**	3	***95***	***97***	***99***
	SK-MEL-2	0	**62**	29	28	0	0	0	0	**100**	0	49	**62**	**100**
	SK-MEL-28	0	42	27	**51**	20	12	0	0	38	10	19	38	37
	SK-MEL-5	3	**63**	**76**	**100**	14	42	19	14	**66**	34	**73**	**56**	**63**
	UACC-257	0	33	21	**68**	0	14	6	6	44	0	24	48	43
	UACC-62	30	**83**	37	**76**	29	5	13	37	**87**	25	**62**	**66**	**79**
Ovarian Cancer	IGROV1	0	**58**	19	**63**	7	4	6	16	**56**	8	47	47	**50**
	OVCAR-3	0	**72**	**56**	42	7	0	5	0	26	8	34	34	-
	OVCAR-4	10	20	**50**	48	40	**52**	29	13	34	3	31	22	26
	OVCAR-8	0	49	20	**54**	0	**50**	6	0	49	3	37	28	30
	NCI/ADR-RES	0	**82**	**65**	**82**	33	31	0	7	**88**	8	**72**	**64**	**87**
	SK-OV-3	0	23	**71**	0	17	0	21	19	**53**	13	44	40	**51**
Renal Cancer	786-0	9	49	**55**	**56**	30	48	0	16	**53**	0	30	39	**62**
	A498	**100**	**62**	14	15	0	3	9	0	**78**	22	**66**	**70**	**87**
	ACHN	0	49	44	**71**	33	**50**	0	15	49	19	28	**50**	**58**
	CAKI-1	16	**66**	**61**	**65**	38	45	0	40	**67**	18	**70**	**55**	**60**
	RXF 393	19	41	***92***	**58**	5	14	12	0	**53**	0	**51**	33	**68**
	SN12C	19	**58**	45	**58**	32	22	14	8	**55**	16	26	13	34
	UO-31	24	**56**	32	**65**	10	41	17	39	**55**	28	**57**	35	**57**
Prostate Cancer	PC-3	10	44	17	**72**	12	20	12	40	44	31	**52**	**54**	**62**
Breast Cancer	MCF7	17	**85**	**65**	**74**	23	36	31	-	**80**	20	**56**	**69**	**82**
	MDA-MB-231/ATCC	24	45	25	**54**	29	**70**	3	22	**63**	21	43	0	23
	HS 578T	9	41	**57**	**46**	26	37	7	17	**56**	21	**59**	45	**84**
	BT-549	0	47	**75**	**84**	7	22	6	26	**74**	6	41	47	**63**
	T-47D	18	**62**	**51**	**79**	16	25	**70**	39	**80**	11	**75**	24	**72**
	MDA-MB-468	18	**100**	40	***97***	13	**50**	18	25	**85**	14	**56**	49	**57**

[a] Data obtained from NCI's in vitro 60-cell one dose screen at 10 μM concentration.

[b] GI% is the percentage of growth inhibition of tumor cells.

[c] Cytotoxic effect; a value of -x means x% cancer cells lethality of preexisting cells: **-10%** on the A498 cell line by **8ae**, **-13%** on MDA-MB-435 cell line and **-3%** on MDA-MB-468 cell line by **8ae'**, **-40%** on LOX IMVI cell line and **-36%** on SK-MEL-5 cell line by **8ak**, **-6%** on MALME-3M cell line by **8am**, **-30%** on MDA-MB-435 cell line and **-12%** on SK-MEL-2 cell line by **8bf**, **-1%** on NCI-H522 cell line by **8bp**, **-38%** on NCI-H522 cell line and **-78%** on SK-MEL-2 cell line by **8bq**.

[d] Not determined.

Nine of the tested compounds completely inhibit cell growth (100%) on some cancer cell lines, showing a cytotoxic effect between -1 and -78, suggesting up to 78% cancer cell death. The most active compounds in terms of cytostatic effect (GI% over 50) and cytotoxic effect (cell death) are **8ae**, **8ae'**, **8ag**, **8ak**, **8am**, **8bf**, **8bo**, **8bp**, **8bq**.

I. 4. Design, synthesis and biological evaluation of indolizine-1-yl-chalcone hybrids - SERIES D

I. 4. 1. Design of indolizine-1-yl-chalcone hybrids

The anticancer results of synthesized compounds in the previous series are promising, so in the design of this new series of compounds, we proposed to enrich the acquired structure-activity relatioship. The newly compounds have tree rings, A, B and C, joined by two connectors and are classified into two subseries: indolizine-1-yl-chacone hybrids and bis-chalcones (**Figure 31**).

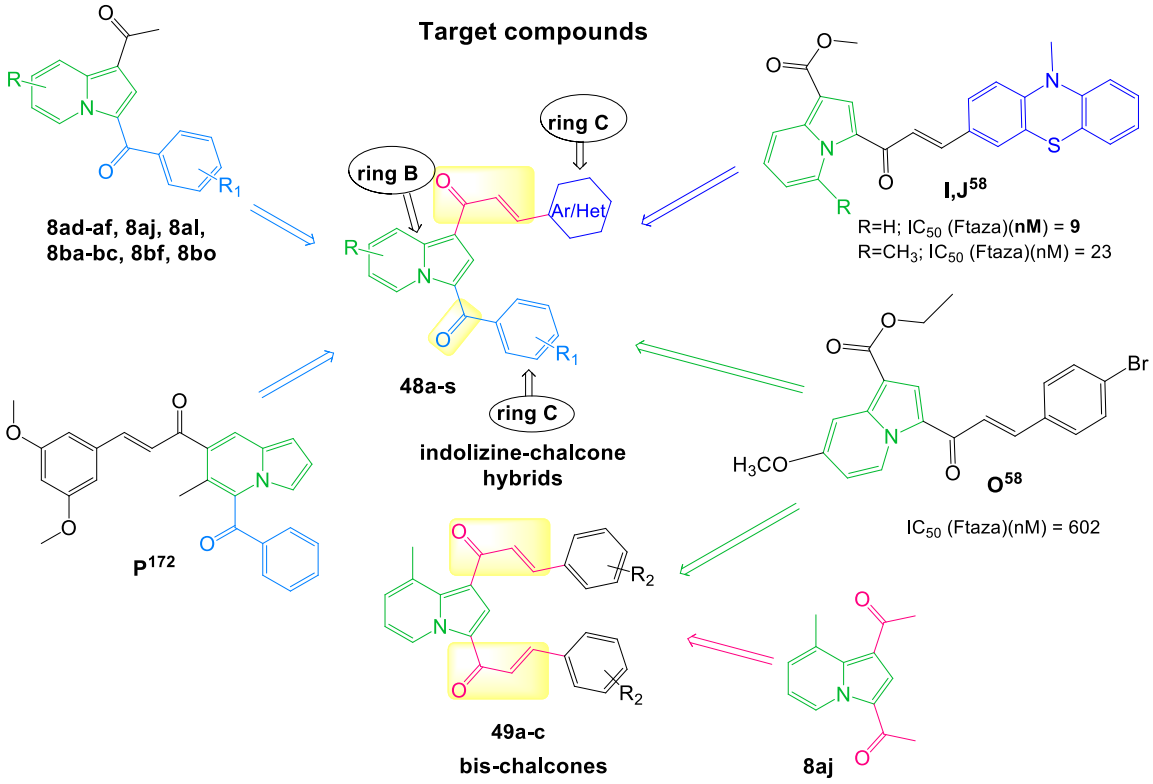


Figure 31. Structures of target compounds (**48a-s**, **49a-c**) and structures of previously reported indolizine derivatives.

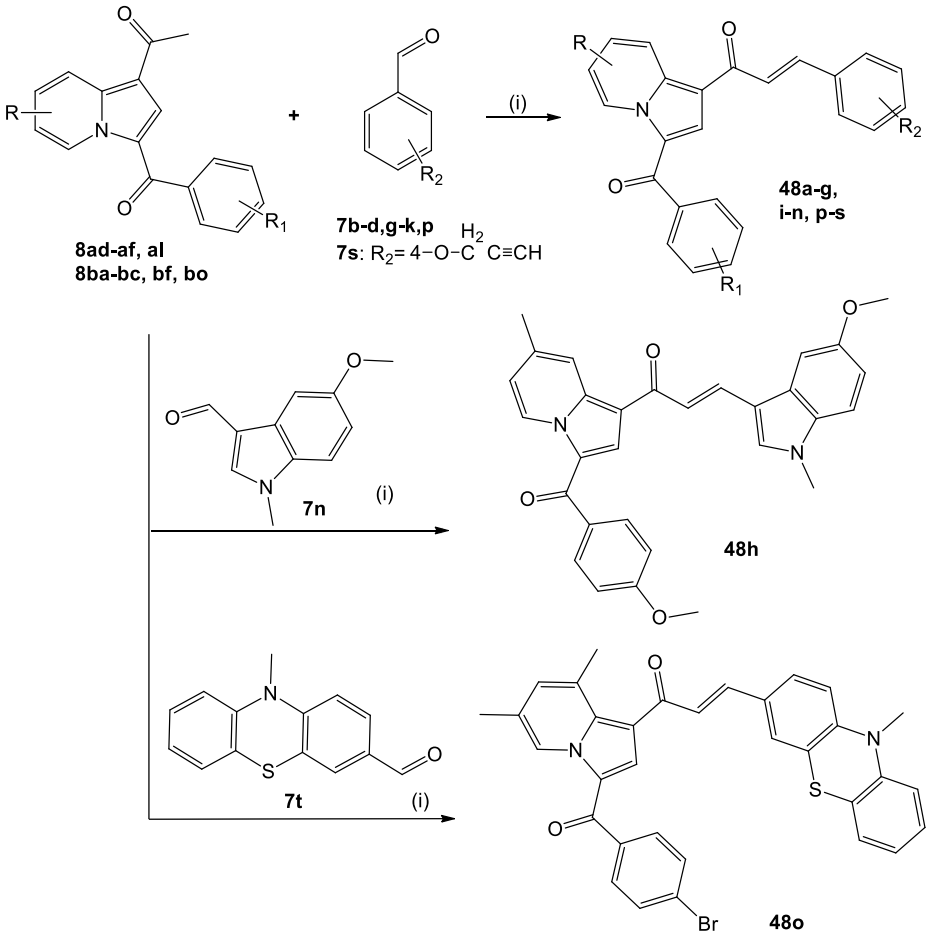
In the subseries of indolizine-1-yl-chalcone hybrids, cycles A and B are joined by a carbonyl bridge, and cycles B and C by a 2-propenone bridge.

In the bis-chalcones subseries, the three rings are joined by the 2-propenone connector. In both subseries, ring B is indolizine.

I. 4. 2. Synthesis of indolizine-1-yl-chalcone hybrids – series D1

The target compounds were synthesized by Claisen-Schmidt reaction of an aromatic or heteroaromatic aldehyde with an indolizine derivative, with the role of a methylene component, containing the acetyl group in the 1st position of indolizine (**8ad-af, 8al, 8ba-bc, 8bf, 8bo**), (**Scheme 11**). 3-Formyl-10-methylphenothiazine was synthesized in the laboratory by Vilsmeier–Haack reaction. [173]

In this series, we obtained 19 new indolizine-1-yl-chalcone hybrids (**48a-s**) with good yields, above 73%, which were physico-chemical characterized and biologically evaluated.



Scheme 11. Reaction conditions: (i) NaOH, EtOH, reflux.

To obtain target hybrids, we tried to perform the experiments by US, but we failed to isolate them (**4t**, **4u**) from the mixture with the starting indolizine derivatives. Thus, we decided to make classic syntheses.

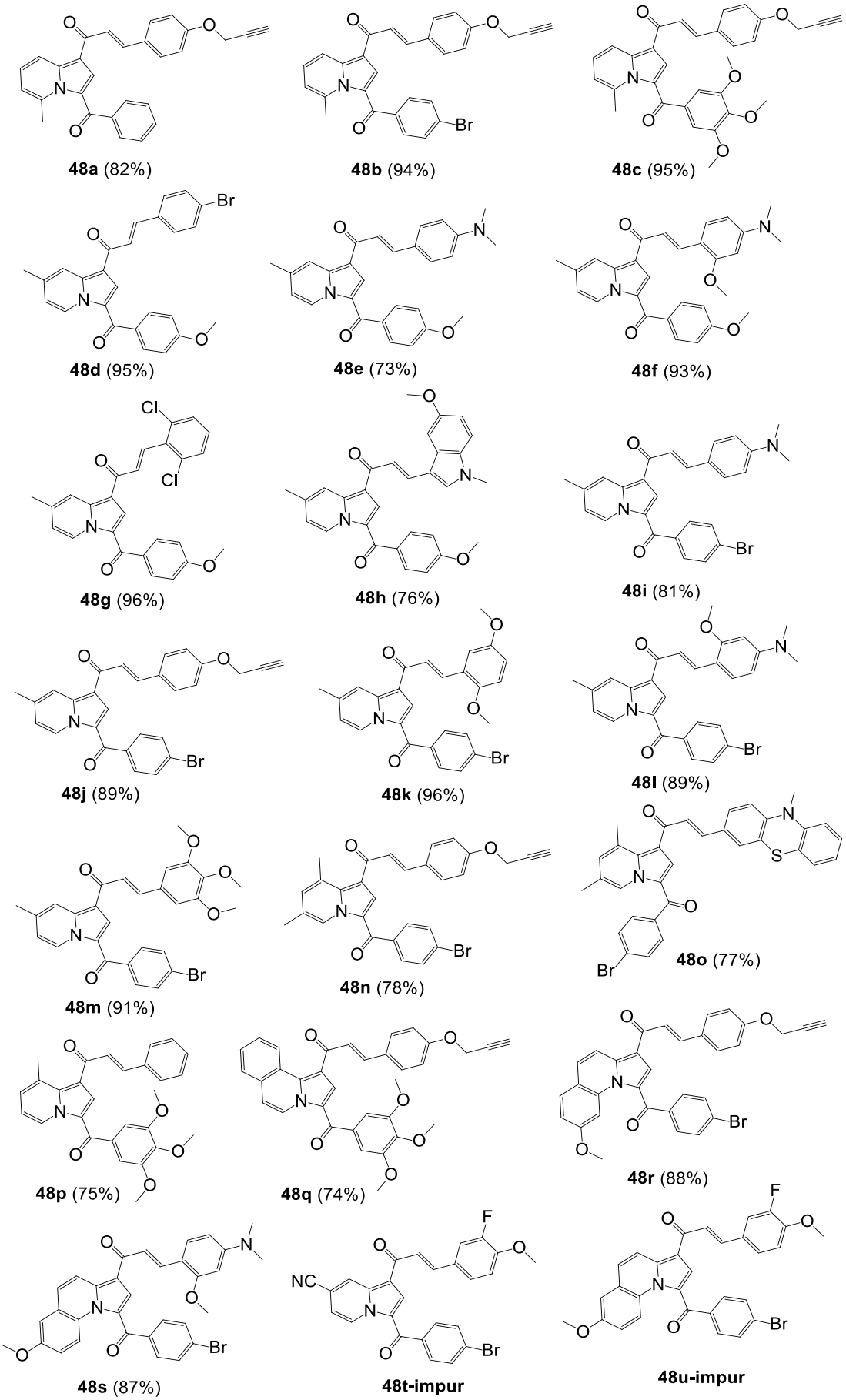
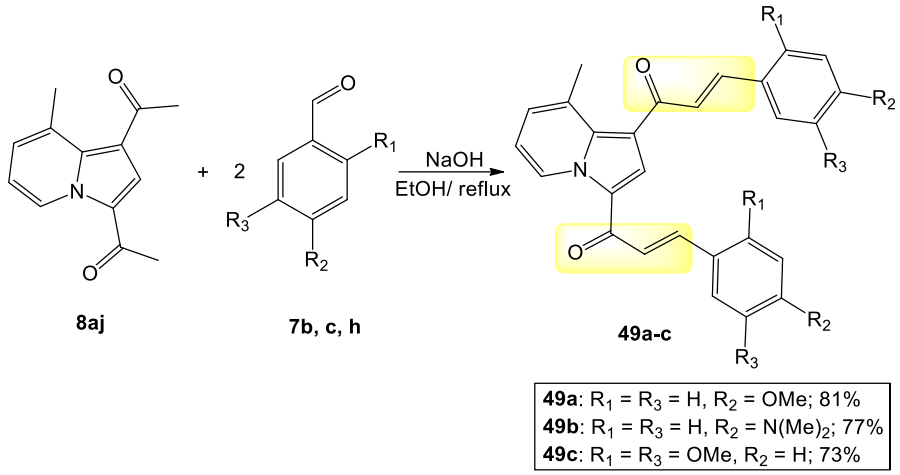


Figure 32. Synthesized indolizine-1-yl-chalcone hybrids **48a-u**.

I. 4. 3. Synthesis of bis-chalcones – series D2

To continue exploring the structure-activity relationships, we decided to synthesize bis-chalcone derivatives, through a one-step reaction (**Scheme 12**).



Scheme 12. Synthesis of bis-chalcones **49a-c**.

With ^{1}H NMR and ^{13}C NMR spectra it was confirmed that the condensation takes place in both acetyl groups of indolizine ring.

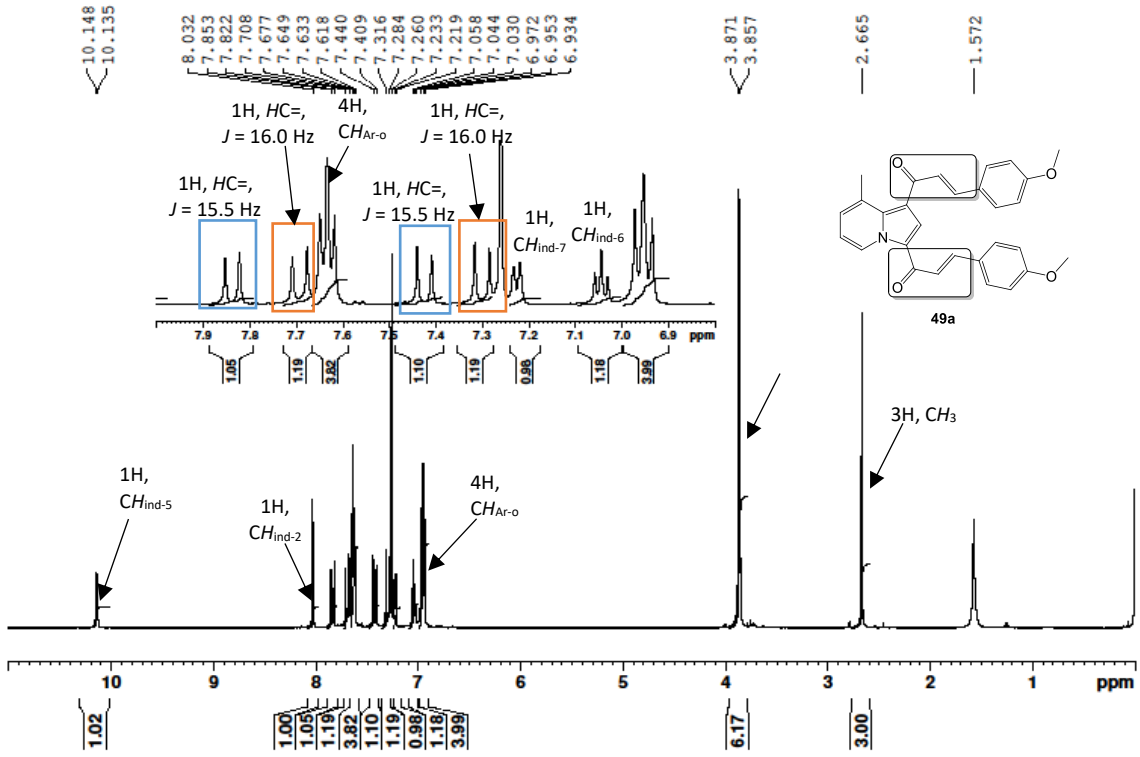
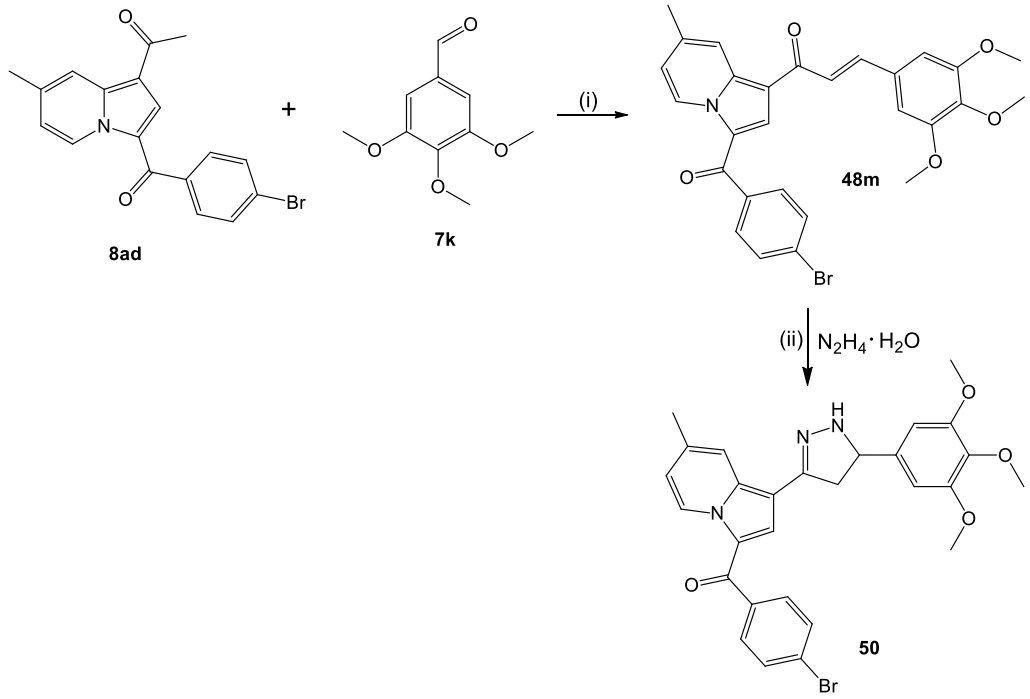


Figure 35. ^{1}H RMN ($CDCl_3$) spectra of bis-chalcone **49a**.

I. 4. 4. Reactions of indolizine-1-yl-chalcone hybrids – cyclic compounds

To demonstrate the possibility of using this series of hybrids in a new direction, we synthesized cyclic derivative **50** (**Scheme 13**).



Scheme 13. Reaction conditions: (i) NaOH, EtOH, reflux; (ii) EtOH, reflux.

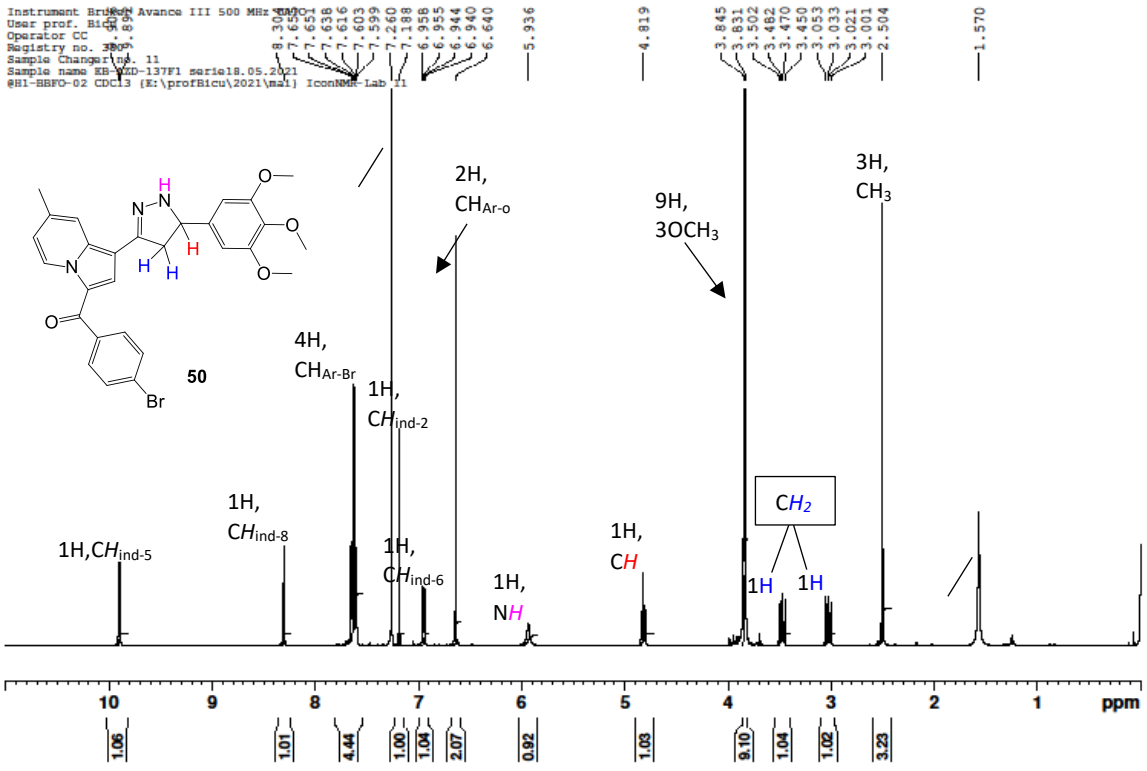


Figura 37. ^{1}H RMN ($CDCl_3$) spectra of compound **50**.

In the ^{1}H NMR spectra (**Figure 37**), the signals belonging to protons of dihydropyrazole are highlighted. Signals corresponding to protons belonging to 4,5-dihydro-1H-pyrazole have been identified using HMQC.

I. 4. 5. Biological evaluation

In terms of biological activity, 22 new compounds (**series D**) have been tested for anticancer and antifungal potential.

The **22 new compounds** were selected by the NCI for biological evaluation on a panel of 60 cancer cell lines. Compounds were tested for their antitcancer potential at a 10 µM concentration on cancer cell growth.

Table 9. Results of the *in vitro* human cancer cell growth inhibition for selected compounds **48a, 48b, 48k, 48m, 48p, 48r**.

	Compound	**48a**	**48b**	**48k**	**48m**	**48p**	**48r**
Cell type	**Cell line**	**GI%[a, b] (10 µM)**					
Leukemia	CCRF-CEM	27	16	28	19	**81**	21
	K-562	**66**	**69**	9	34	**53**	43
	MOLT-4	29	0	14	11	**62**	20
	RPMI-8226	29	15	-[d]	11	**83**	0
	SR	**100**[c]	**51**	10	37	**78**	**67**
Non-Small Cell Lung Cancer	HOP-62	35	22	26	11	**50**	5
	HOP-92	13	13	**70**	35	**67**	0
	NCI-H460	**58**	24	**56**	0	35	31
	NCI-H522	42	**67**	**53**	26	49	27
Colon Cancer	HCT-116	3	3	27	19	**84**	0
	HCT-15	**54**	**54**	13	41	**63**	36
	KM12	20	20	15	36	**51**	35
CNS Cancer Melanoma	SNB-19	29	21	**56**	9	36	34
	SF-295	**66**	41				**91**
	SNB-75	-	-	-	38	**82**	-
Melanom Ovarian Cancer	MDA-MB-435	**84**	**93**	12	**75**	41	**52**
	LOX IMVI	**68**	26				**56**
	MALME-3M						**75**
	UACC-62	47	38	16	27	**54**	22
Cancer ovarian	OVCAR-3	7	5	31	11	**67**	0
	OVCAR-4	21	4	0	8	**54**	**62**
	OVCAR 8						**65**
	NCI/ADR-RES	-	-	27	30	**61**	-
	SK-OV-3	14	0	30	-	**62**	0
Renal Cancer	786-0	24	25	**51**	0	21	**57**
	ACHN	32	22	41	40	**56**	8
	CAKI-1	**51**	**51**	**72**	30	**70**	15
	RXF 393	43	18	**73**	0	**62**	19
	UO-31	12	17	15	13	**58**	17
Breast Cancer	MCF7	**55**	35	12	33	**84**	48
	MDA-MB 231/ATCC	28	23	30	16	**54**	24
	HS578T	**50**	44	**75**	0	34	**51**
	BT-549	**52**	**54**	39	7	**51**	**98**
	T-47D	42	25	27	22	**56**	21
	MDA-MB-468	**51**	33	49	37	**84**	**51**

[a] Data obtained from NCI's *in vitro* 60-cell one dose screen at 10 µM concentration.

[b] GI% is the percentage of growth inhibition of tumor cells.

[c] Cytotoxic effect: growth percent of **-22%** on SR cells induced by hybrid **48a**.

[d] Not determined.

Compounds of **series D** stand out for their antifungal activity against *C. albicans*, so the absence of cytotoxicity is an advantage in the context of obtaining antifungal drugs.

In terms of antifungal activity, it was evaluated *in vitro* against *C. albicans* for all hybrids and *in vivo* for the most effective compounds according to MIC (the minimum inhibitory concentration). By determining their MIC, it was found that 5 compounds have MIC below 32 μg/mL. This value is set by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and indicates the potential of an antimicrobial for industrial development. The most active compounds for antifungal activity are highlighted in **Table 11**.

Table 10. Results of the *in vitro* antifungal inhibition - MIC obtained for selected hybrids **48a-s** and **49a-c**.

Compound	*Final concentration (μg/mL)*	*MIC[a] (μg/mL)*
48d	5.00E-04	237.000
48f	5.00E-04	234.270
48g	5.00E-05	23.2170
48h	5.00E-05	23.9300
48m	5.00E-05	26.7200
48o	5.00E-04	296.765
48p	5.00E-05	22.7850
48s	5.00E-05	29.1700
49c	5.00E-04	255.785

[a] MIC – the minimum inhibitory concentration – the lowest concentration when *in vitro* growth of fungi *C. albicans* is stopped;

The *in vivo* test of the best antifungal agents was performed at two different concentrations, 10^{-4} μg/mL și $5 \cdot 10^{-5}$ μg/mL, on *Caenorhabditis elegans* nematode, which was previously infected with *C. albicans.* In the first stage of the tests, no nematodes survived and further investigations are to be carried out.

I. 5. Design, synthesis and biological evaluation of indolizine-pyridine hybrids– SERIES E

I. 5. 1. Design of indolizine-pyridine hybrids

Another modulation through which we proposed to enrich the structure-activity relationships acquired so far, is the modification of chalcone bridge. Studying the literature, we saw that the 2-propenone bridge can be change into a five-membered[175-179] or six-membered[180, 181] heterocycle, depending on the other reagent. A pharmacologically interesting six-membered heterocycle is pyridine[183-186].

In this series, we focused our attention on the design of new indolizine-pyridine hybrids (**Figure 41**). The new synthesized compounds (**series E**) contain in their structures four rings A, B, C and D. Cycles A and B are joined by the ketone bridge, and B and C by the cycle D. Ring D can also be considered a bridge and it was constantly 2-amino-3-cyanopyridine-4,6-disubstited.

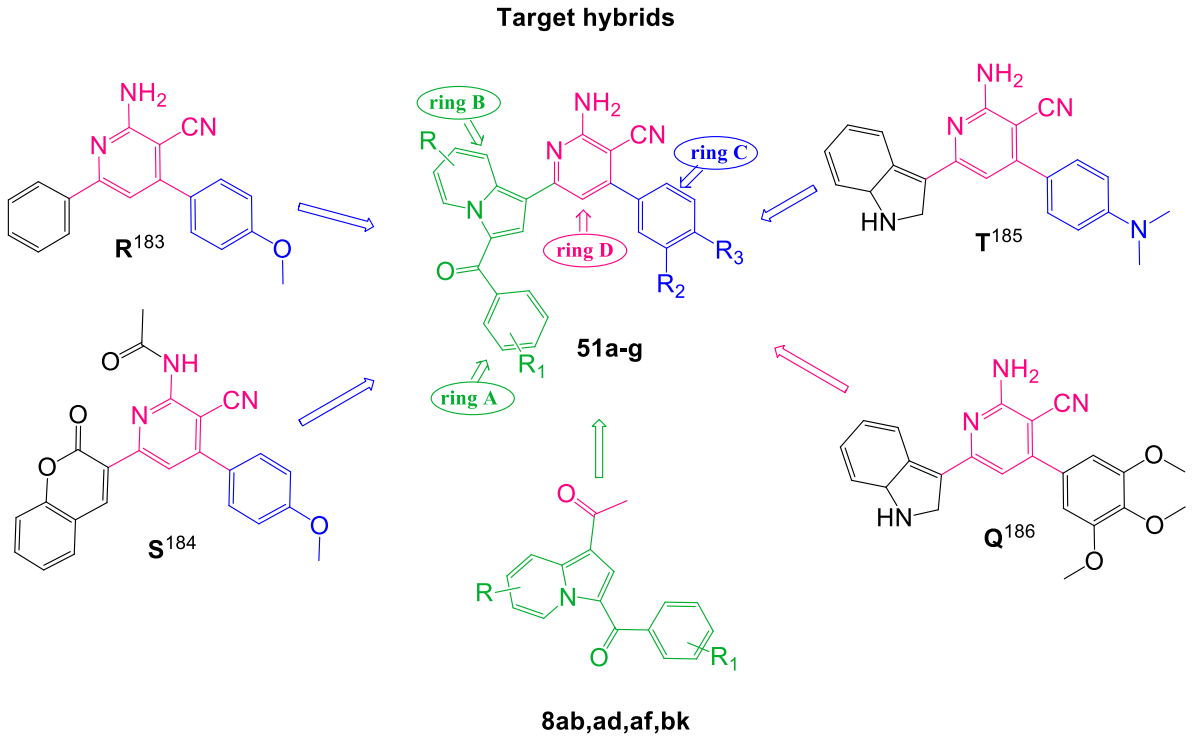


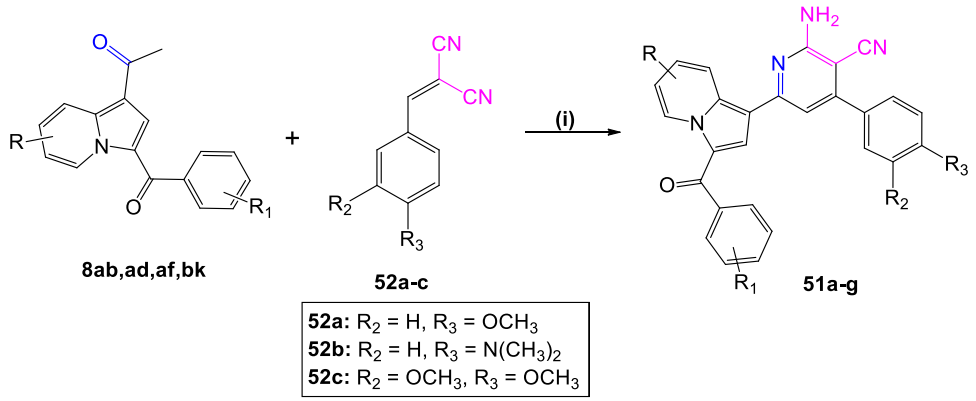
Figure 41. Structure of previously described 2-amino-3-cyanopyridine derivatives as anticancer compounds (**R**, **S**, **T**, **Q**) and structure of target compounds **51a-g**.

I. 5. 2. Synthesis of indolizine-pyridine hybrids

The compounds of this series were synthesized by the reaction of the 1-acetylindolizine derivatives (**8ab**, **ad**, **af**, **8bk**), as methyl-ketone, with benzylidenemalononitrile derivatives (**52a-c**) and ammonium acetate (**Scheme 14**), according to the literature[189].

Up to this point, **7 new** indolizine-pyridine **hybrids** (**51a-g**, **Table 12**, **Figure 41**) have been synthesized.

The obtained compounds were purified by column chromatography and/or recrystallization and characterized by ^{1}H, ^{13}C NMR spectra, HMQC, HMBC (2D experiments) and IR.



Scheme 14. Reaction conditions: (i) CH_3COONH_4, EtOH, reflux, 14-20h;

Table 11. Synthesized indolizine-pyridine hybrids **51a-g**

Compound	R	R_1	R_2	R_3	Yield (%)
51a	7-Me	H	H	OMe	63
51b	7-Me	H	H	$N(Me)_2$	64
51c	7-Me	Br	H	OMe	68
51d	7-Me	Br	H	$N(Me)_2$	69
51e	6,8-diMe	Br	H	$N(Me)_2$	59
51f	6,8-diMe	Br	OMe	OMe	65
51g	6,8-diMe	3,4,5-triOMe	H	$N(Me)_2$	74

I. 5. 3. Biological evaluation

Seven hybrids were selected by the NCI for biological evaluation *in vitro* on a panel of 60 human cancer cell lines. Compounds were tested for their antitcancer potential at a 10 µM concentration on cancer cell growth.

The best results for these compounds are presented in **Table 13**.

Tabel 12. Results of the *in vitro* human cancer cell growth inhibition for compounds **51a-d**.

Cell type	Compound	51a	51b	51c	51d
	Cell line	**GI%[a, b] (10 µM)**			
Leukemia	RPMI-8226	21	18	13	16
	SR	13	24	21	36
Non-Small Cell Lung Cancer	A549/ATCC	20	**54**	7	30
	EKVX	19	12	10	32
	HOP-62	**70**	43	5	21
	HOP-92	**100[c]**	**76**	13	**69**
	NCI-H226	**64**	**83**	40	39

	NCI-H23	45	**54**	37	**72**
	NCI-H460	**55**	44	15	**59**
	NCI-H522	29	21	10	46
Colon Cancer	COLO 205	27	34	33	**55**
	HCC-2998	19	8	0	35
	HCT-116	42	37	26	**64**
	HCT-15	22	27	10	**60**
	HT29	25	37	23	**65**
	KM12	15	27	18	38
	SW-620	21	30	8	**59**
CNS Cancer	SF-268	**59**	39	6	32
	SF-295	**55**	31	11	32
	SF-539	**92**	**100**	**95**	**76**
	SNB-19	**55**	39	9	32
	SNB-75	8	22	0	0
	U251	**55**	**68**	35	**80**
Melanoma	LOX IMVI	49	**88**	46	**69**
	MALME-3M	49	36	26	**100**
	M14	20	19	35	43
	MDA-MB-435	21	21	7	42
	SK-MEL-2	15	19	0	35
	SK-MEL-28	12	26	30	49
	SK-MEL-5	32	42	13	47
	UACC-257	16	14	12	20
	UACC-62	34	35	19	23
Ovarian Cancer	IGROV1	21	14	10	23
	OVCAR-3	40	42	0	**50**
	OVCAR-4	**76**	**56**	14	37
	OVCAR-8	**80**	**85**	34	34
	NCI/ADR-RES	**56**	49	18	**62**
	SK-OV-3	6	35	0	6
Renal Cancer	786-0	**86**	**79**	43	**59**
	A498	**0**	0	0	8
	ACHN	44	**69**	18	29
	CAKI-1	30	47	32	33
	SN12C	30	38	7	23
	UO-31	35	35	36	37
Prostate Cancer	PC-3	37	22	11	35
Breast Cancer	MCF7	47	46	21	**54**
	MDA-MB-231/ATCC	**66**	**74**	40	**60**
	HS 578T	**91**	**67**	9	30
	BT-549	**88**	42	0	38
	T-47D	35	36	17	49
	MDA-MB-468	**51**	49	**76**	**100**

[a] Date obținute de la NCI – screening monodoză *in vitro* la o concetrație de 10 µM.

[b] GI% is the percentage of growth inhibition of tumor cells.

[c] Cytotoxic effect; a value of -x means x% cancer cells lethality of preexisting cells: **-7%** on the HOP-92 cell line induced by **51a**; **-62%** on the SF-539 cell line by **51b**; **-2%** on the MALME-3M cell line and **-15%** on MDA-MB-468 cell line induced by **51d**;

The most active indolizine-pyridine hybrids from the perspective of antiproliferative and cytotoxic effect are **51a-d**. Three of tested compounds (**51a, 51b, 51d**) also exhibits cytotoxic effect on several different cell lines.

By comparing the antiproliferative effect for compounds **51a** with **51b (Figure 45** and **Table 13)** and **51c** with **51d**, respectively **(Figure 46** and **Table 13)**, we can deduce that introduction of 4-dimethylaminophenyl as ring C (**51b, 51d**) causes better anticancer activity than 4-methoxyphenyl (**51a, 51c**).

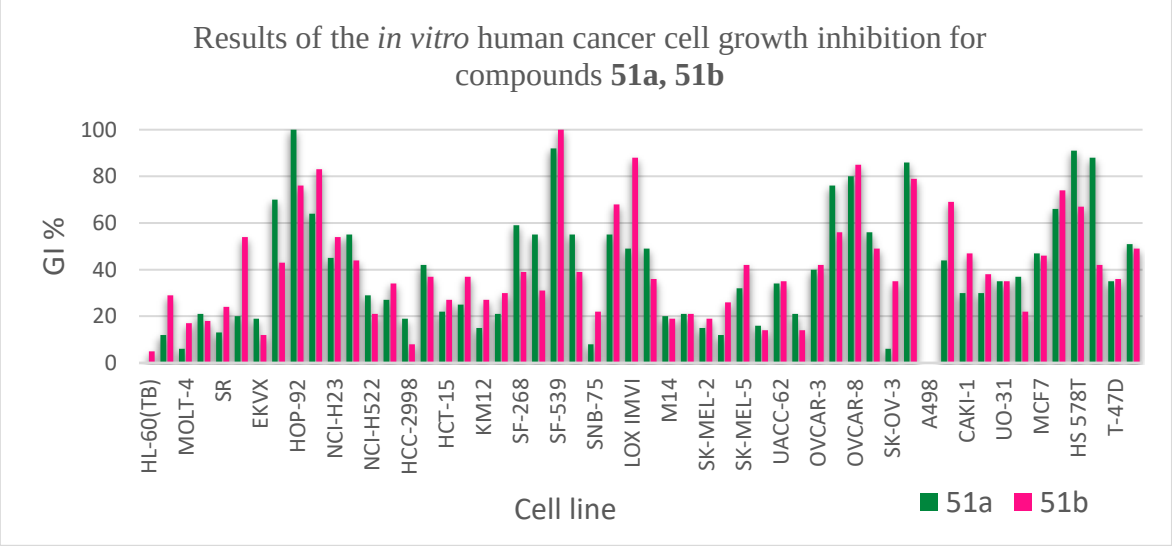


Figure 45. Comparison of *in vitro* GI% results for hybrids **51a** and **51b**.

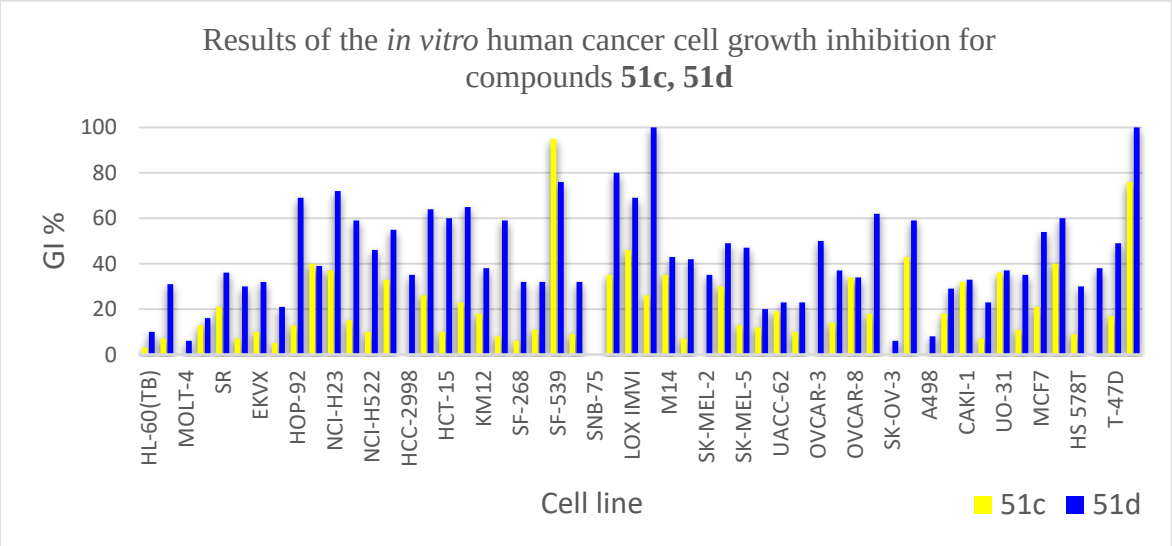


Figure 46. Comparison of *in vitro* GI% results for hybrids **51c** and **51d**.

I. 6. Conclusions and perspectives

The research carried out within this doctoral thesis was focused on the design of new compounds with **biological properties**, mainly with anticancer or antimicrobial action, following a **"green chemistry"** strategy in the syntheses carried out. In this doctoral thesis are described the design, synthesis and biological properties of **103 new compounds not described previously in the literature**. The compounds were characterized with NMR spectra (^{1}H, ^{13}C, ^{19}F) and HMQC, HMBC, COSSY (two-dimensional experiments), IR and in particular cases MS or X-Ray diffraction.

The design of target compounds was based on the structure-activity relationships known within our research group, taking into consideration the structure of chalcone, a compound with multiple pharmacological actions. In the compound design, modifications were made to the three structural components of chalcone, which were considered analogues of phenstatin. These components include ring A, ring B, and the bridge.

At global level, the aim is to mobilize the scientific cancer research community towards the development of new ways to identify and understand anticancer targets and, implicitly, the development of new inhibitors of them. Thus, one of our objectives was to design new antitumor agents that are farnesyltransferase inhibitors and/or compounds with cytostatic/cytotoxic activity. At the same time, we followed the synthesis through the most "green" methods. In this context, the first two series of compounds were designed and obtained: **series A** and **series B**.

Series A consists of 3 chalcone derivatives containing nitrogen heterocycles and a polycyclic derivative. In the structure of chalcone derivatives, ring A is pyridine or phenothiazine, and ring B is 3,4,5-trimethoxyphenyl or pyridine, the bridge is keto-ethylene. The compounds were obtained by two methods: Claisen-Schmidt condensation - classical stirring experiments (***method A***) and **ultrasounds-mediated synthesis** (***method B***). The advantages of applying the unconventional method in our syntheses are: short reaction time (**30-60 seconds**), use of solvents and catalysts with low **toxicity**, much lower temperatures (20-35 °C) which it leads to a much lower consumption of resources (energy and water). The new compounds obtained in this series have been tested *in vitro* as Ftase inhibitors. The most active compound (**1c**) have phenothiazine as ring A and pyridine as ring B, and exhibits 90% inhibition of farnesyltransferase action with a concentration $\mathbf{IC_{50} = 2.84\ \mu M}$.

Series B, of phenothiazin-10-yl-chalcone analogues, includes modulations in all three points of the structure of chalcone: ring A - phenothiazine or 2-methylthiophenotiazine, ring B

- differently substituted phenyl or nitrogen or oxygen or heterocycles, and in the 2-propenone bridge, the -CN group was introduced in α position relative to the carbonyl group. In this series, **23 compounds** were synthesized: 21 phenothiazine-10-yl-chalcone analogues and 2 intermediates, 3-oxo-3-(10*H*-phenothiazin-10-yl)propanenitrile and 3-(2-(methylthio)-10*H*-phenothiazin-10-yl)-3-oxopropanenitrile. The **21 new chalcone analogues** were obtained by Claisen-Schmidt condensation, 10 compounds by conventional method and 11 compounds by US-mediated synthesis (1-2 minutes). In terms of biological activity, three 2-methylthio-phenothiazin-10-yl-chalcone have good antiproliferative potential on prostate cancer (PC-3 cell line) and breast cancer (MDA-MB-468, MCF7, T-47D cell lines). The most active compound, **4k**, with 2-methylthio-phenothiazine-10-yl (ring A) and 1*H*-indole-3-yl (ring B), has cytostatic effect on all types of cancer, the most pronounced antiproliferative activity being on the NCI-H522 cell line (Non-Small Cell Lung Cancer, **GI% ~ 95**). Some compounds of this series were tested *in vitro* as Ftase inhibitors, the most active analogue (**3d**) shows **IC_{50} = 7.268 µM**.

Since the phenothiazine-10-yl-chalcane analogues of **series B** demonstrated anticancer potential, we wanted to continue exploring the acquired structure-activity relationships and introduce another heterocycle (indolizine) into the new series of compounds (**series C**). At the same time, we followed to obtain a new series of compounds that we approached from two perspectives: phenstatin analogues following anticancer properties and intermediates in the synthesis of new series of compounds: indolizine-chalcone hybrids, **series D**, and indolizine-pyridine hybrids, **series E**.

Series C consists of acetyl-indolizine derivatives and involves the union of ring B (indolizine, pyrrolo[1,2-a]quinoline and pyrrolo[2,1-a]isoquinoline) with a classic ring A: trimethoxyphenyl, phenyl substituted with methoxy, bromo, cyano, or non-classic rings: phenothiazine, 2*H*-benzo[b][1,4]oxazine-3(4*H*)-one. At the same time, acetyl group was introduced into 1st position of indolizine to create a new reaction center. In this series, **78 compounds** were synthesized, of which 47 products and by-products and 31 intermediates**.** From obtained compounds, there **are 42 new indolizine derivatives** and **6 new pyridinium salts.** In the synthesis of indolizine derivatives, we used two dipolarophiles (activated alkyne). When dipolarophile trimethylsilyl-3-butyn-2-one was used, we obtained 25 compounds of which **22 new products**. Experiments with trimethylsilyl-3-butyn-2-one led to obtaining two or more reaction products: 1-acetyl-indolizine, 1-acetyl-2-trimethylsilyl-indolizine, 2-acetyl-1-trimethylsilyl-indolizine. When we used 3-butyn-2-one as dipolarophile, we obtained 22 compounds, of which **20 are new products**. When the cycloaddition reaction was performed with a 3-butyn-2-one of lower purity, the reaction followed a different path, obtaining an

unexpected cycloaddition product. **Nine compounds** of this series exhibit a good antiproliferative effect, and **seven** of them showed cytotoxic effect. The most active derivative is 1-(3-(10*H*-phenothiazine-10-carbonyl)pyrrolo[2,1-a]isoquinoline-1-yl)ethanone and exhibits **cytotoxic effect, GI = -78%**. The compounds of **series C** showed the best anticancer results on melanoma (**GI% = 100** on some cell lines). Compounds with phenothiazine ring also showed cytotoxicity on the NCI-H522 cell line corresponding to Non-Small Cell Lung Cancer. The compound with trimethoxyphenyl (ring A) and 1-acetyl-8-methylindolizine-3-yl (ring B) has selective cytotoxic effect on the A498 cell line (renal cancer).

Compounds from the chalcones family have been used, over time, due to their multiple biological activities, they possess. For this reason, we followed to obtain chalcone analogues, with multiple biological activities.

Series D consists of **23 new compounds**: 19 indolizine-1-yl-chalcones hybrids, 3 bis-chalcones and a derivative with the 2-propenone bridge converted intodihydropyrazole. The compounds of this series have 3 rings in their structure, A, B and C, which are joined by **two bridges**. In the case of indolizine-1-yl-chalcones hybrids, one bridge is ketone and the second one is keto-ethylene. In the case of bis-chalcones the two bridges are keto-ethylene, ring A and ring C being identical. Biological results confirmed the antifungal potential of this series against *C. albicans*: 5 compounds gave **very good MIC**, around **20 µg/mL** and 4 compounds have MIC with a lower biological significance (200-300 µg/mL). At the same time, the compounds were evaluated for anticancer activity. The most active compound (**48p**) is distinguished by a good cytostatic effect, which indicates its multiple biological action, being **at the same time cytostatic and antifungal**. The other compounds of this series have the ability to inhibit moderate cell growth, with the exception of hybrid **48a** which exhibits cytotoxic effect on a cell line. The absence of cytotoxicity is an advantage in the perspective of developing antifungal and drugs.

Series E consists of **7 new indolizine-pyridine hybrids** and was designed to evaluate the influence of 2-amino-3-cyanopyride bridge on biological activity. The structures of indolizine-pyridine hybrids have 4 cycles: ring A, differently substituted phenyl, joined by ring B, indolizine-3-yl, through the ketone bridge, ring C is 4-aminophenyl, 4-methoxyphenyl or 3,4-dimethoxyphenyl, and ring D is 2-amino-3-cyanopyridine-4,6-disubstituted. Four hybrids have a good cytostatic effect on several cancer cell lines (GI% > 50), and 3 of them (**51a**, **51b**, **51d**) exhibit **cytotoxic effect (GI% = 100)** on some cancer cell lines: HOP-92 (non-small cell lung cancer), SF-539 (CNS cancer), MALME-3M (melanoma) and MDA-MB-468 (breast cancer), with values **-2% ≤ GI% ≤ -62%**.

In terms of biological evaluation, the target compounds were selected by the NCI for biological evaluation *in vitro* on a panel of 60 human cancer cell lines. Compounds have been tested at 10 μM concentration. New compounds of **series A** and a part of **series B** have been tested anticancer *in vitro* for the ability to inhibit FTase action. The most active farnesyltransferase inhibitor, **1c**, (IC_{50} = **2.84 μM**) has phenothiazine-2-yl ring joined by 2-propenone bridge with 6-chloropyridine-3-yl ring. The compounds of **series D** were also tested *in vitro* and *in vivo* for antifungal activity. The most effective antifungal (**MIC = 22.785 μg/mL**) was also found to be a good cytostatic, having in its structure 3,4,5-trimethoxyphenyl (ring A) joined with 8-methylindolizine (ring B) by ketone bridge in position 3 and the phenyl joined by the chalcone bridge in 1st position of the indolizine ring.

Next, we aim to improve the ultrasound-mediated synthesis method of compounds, in order to broaden the area of reactions in which it can be applied. We also want to continue the synthesis of indolizine-pyridine hybrids, in order to obtain conclusive results in terms of structure-activity relationships, the compounds proving at this stage good antitumoral activity. Another perspective is to change the 2-propenone bridge from indolizine-chalcone hybrids into a five- or six-atom heterocycle (such as the dihydropyrazole derivative). We follow this in order to enrich the structure-activity relationships acquired so far and to develop stronger antitumor agents. At the same time, we intend to further investigate the best cycles for biological properties, in order to obtain dual anticancer inhibitors and dual antimicrobial and anti-inflammatory agents.

A part of the results obtained in this doctoral thesis has been disseminated through two **published articles**:

- **Zubaș, A.**; Ghineț, A.; Shova, S.; Bîcu, E. 1,3-Dipolar cycloaddition of cycloimmonium salts and 4-(trimethylsilyl)-3-butyn-2-one to access new functionalized indolizines with potential cytostatic activity. *New J. Chem*. **2023**, *47*, 3758-3772.[191] (I.F. = **3.925**)
- **Zubaș, A.**; Ghineț, A.; Farce, A.; Dubois, J.; Bîcu, E. Phenothiazine- and carbazole-cyanochalcones as dual inhibitors of tubulin polymerization and human farnesyltransferase. *Pharmaceuticals* **2023**, *16*, 888.[158] (I.F. = **5.215**)

and the participation at **conferences**:

- *Design and synthesis of new phenothiazine-chalcones with potential biological activities.* **Zubaș, A.**; Ghineț, A.; Dubois, J.; Bîcu, E. Scientific Communications Session of Undergraduate, Master's, and PhD Students, Iasi (Romania), 29-30 October **2020**. (poster)

- *A new electron-deficient alkyne as dipolarophile in the synthesis of new indolizine derivatives*. **Zubas, A.**; Ghinet, A.; Shova, S.; Bîcu, E. Scientific Communications Session of Undergraduate, Master's, and PhD Students, Iasi (Romania), 11-12 November **2021**. (poster)
- *New indolizine-1-yl-chalcone hybrids as antifungal and anti-inflammatory agents: synthesis and biological evaluation.* **Zubaș, A.**; Ghineț, A.; Jawhara, S.; Bîcu, E. Scientific Communications Session of Undergraduate, Master's, and PhD Students, Iasi (Romania), 28 October **2022**. (oral communication)
- *A new series of pyridine derivatives as anticancer agents: design, synthesis and biological evaluation.* Negru, G.; **Zubaș, A.**; Ghineț, A.; Bîcu, E., Scientific Communications Session of Undergraduate, Master's, and PhD Students, Iasi (Romania), 28 October **2022**. (oral communication).

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Bibliography

1. Cartwright, F.; Biddiss, M. *Bolile si istoria.* Editura All, **2008**.
3. *Understanding Cancer*. Accesat în iulie 2022, pe National Cancer Institute: https://www.cancer.gov/about-cancer/understanding/what-is-cancer.
5. *Metastatic Cancer: When Cancer Spreads*. Accesat în iulie 2022, pe National Cancer Institute: https://www.cancer.gov/types/metastatic-cancer
6. *Risk Factors for Cancer*. Accesat în iulie 2022, pe National Cancer Institute: https://www.cancer.gov/about-cancer/causes-prevention/risk
7. *Types of Cancer Treatment*, Accesat în august 2022, pe National Cancer Institute: https://www.cancer.gov/about-cancer/treatment/types
8. Behrouzkia, Z; Joveini, Z; Keshavarzi, B; Eyvazzadeh, N; Aghdam, R.-Z. *Oman Med J.* **2016**, *31*, 89-97.
9. Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.-L.; Torre, L.-A.; Jemal, A. *CA Cancer J Clin.* **2018**, *68*(6), 394-424.
10. *Cancer*. Accesat în august 2022, pe World Health Organization: https://www.who.int/news-room/fact-sheets/detail/cancer
11. Ferlay, J.; Colombet, M.; Soerjomataram, I.; Parkin, D.-M.; Pineros, M.; Znaor, A.; Bray, F. *Int. J. Cancer* **2021**, *149*, 778-789.
12. https://clinicaltrials.gov/, Accesat în August 2022.
58. Moise, I.-M.; Ghinet, A.; Belei, D.; Dubois, J.; Farce, A.; Bîcu, E. *Bioorg. Med. Chem. Lett.* **2016**, *26*(15), 3730-3734.
149. Homerin, G.; Nica, A.-S.; Farce, A.; Dubois, J.; Ghinet, A. *Bioorg. Med. Chem. Lett.* **2020**, *30*(11), 127149.
151. Moise, I.-M.; Bîcu, E.; Farce, A.; Dubois, J.; Ghinet, A. *Bioorg. Chem.* **2020**, *103*, 104184.
152. Gul, H.-I.; Yamali, C.; Gunesacar, G.; Sakagami, H.; Okudaira, N.; Uesawa, Y.; Kagaya, H. *Med Chem Res* **2018**, *27*, 2366–2378.
155. Prinz, H.; Chamasmani, B.; Vogel, K.; Bohm, K.-J.; Aicher, B.; Gerlach, M.; Gunther, E.-G.; Amon, P.; Ivanov, I.; Muller, K. *J. Med. Chem.* **2011**, *54*, 4247-4263.
156. Ghinet, A.; Abuhaie, C.-M.; Gautret, P.; Rigo, B.; Dubois, J.; Farce, A.; Belei, D.; Bîcu, *E. Eur. J. Med. Chem*. **2015**, 89, 115-127.
157. Fadda, A.-A.; Fekri, A.; Bayoumy, N.-M. *RSC Adv.* **2015**, *5*, 80844-80852.
158. **Zubaș, A.**; Ghineț, A.; Farce, A.; Dubois, J.; Bîcu, E. *Pharmaceuticals* **2023**, *16*, 888.

160. Dumea, C.; Belei, D.; Ghinet, A.; Dubois, J.; Farce, A.; Bicu, E. *Bioorg. Med. Chem. Lett.* **2014**, *24*(24), 5777-5781.
161. Lucescu, L.; Ghinet, A.; Belei, D.; Rigo, B.; Dubois, J.; Bîcu, E. *Bioorg. Med. Chem. Lett.* **2015**, *25*(18), 3975-3979.
162. Kim, N.-D.; Park, E.-S.; Kim, Y.-H.; Moon, S.-K.; Lee, S.-S.; Ahn, S.-K.; Yu, D.-Y.; No, K.-T.; Kim, K.-H. *Bioorg. Med. Chem.* **2010**, *18*, 7092-7100.
163. Lucescu, L.; Bîcu, E.; Belei, D.; Dubois, J.; Ghinet, A. *Lett. Drug Des. Discov.* **2016**, *13*, 479- 488.
164. Bâcu, E.; Samson-Belei, D.; Nowogrocki, G.; Couture, A.; Grandclaudon, P. *Org. Biomol. Chem.* **2003**, *1*, 2377-2382.
165. Dumitrascu, F.; Georgescu, E.; Georgescu, F.; Filip, P.; Miu, B.; Dumitrescu, D. G. *Rev. Roum. Chim*. **2008**, *53*, 589-594
166. Tamuea, Y.; Sumida, Y.; Haruki, S.; Ikeda, M. *J. Chem. Soc., Perkin Trans*. **1973**, *1*, 2091-2095.
167. Armarego, W. L. F. *J. Chem. Soc*. **1964**, 4226-4233.
168. Cheng, M.; Yang, X.; Li, J.; Chen, C.; Zhao, J.; Wang, Y.; Sun, L. *Chem. Eur. J*. **2012**, *18*, 16196-16202.
169. Abuhaie, C.-M.; Bicu, E.; Rigo, B.; Gautret, P.; Belei, D.; Farce, A.; Ghinet, A. *Bioorg. Med. Chem. Lett*. **2013**, *23*, 147-152.
170. Dascalu, A.-E.; Bîcu, E.; Shova, S.; Lipka, E.; Rigo, B.; Billamboz, M.; Ghinet, A. *ChemistrySelect* **2019**, *4*, 13724-13730.
171. Zhang, X.; Cao, W.; Wei,X.; Hu, H. *Synth. Commun.* **1997**, *27*(8), 1395-1403.
173. Xiao, H.; Xin, K.; Dou, H.; Yin, G.; Quan, Y.; Wang, R. *Chem. Commun*. **2015**, *51*, 1442-1445.
175. Kumari, P.; Mishra, V.-S.; Narayana, C.; Khanna, A.; Chakrabarty, A.; Sagar, R. *Sci Rep.* **2020**, *10*, 6660.
176. Manna, F.; Chimenti, F.; Fioravanti, R.; Bolasco, A.; Secci, D.; Chimenti, P.; Ferlini, C.; Scambia, G. *Bioorg. Med. Chem. Lett.* **2005**, *15*(20), 4632-5.
177. Venugopala, K.-N.; Habeebuddin, M.; Aldhubiab, B.-E.; Asif, A.-H. *Molecules* **2021**, *26*, 5235.
178. Tilekar, K.; Upadhyay, N.; Meyer-Almes, F.-J.; Loiodice, F.; Anisimova, N.-Y.; Spirina, T.-S.; Sokolova, D.-V.; Smirnova, G.-V.; Choe, J. et all. *Chem. Med. Chem.* **2020**, *15*(19), 1813-1825.

179. Khodadad, H.; Pourshamsian, K.; Hatamjafari, F.; Sadeghi, B. *Rev. Roum. Chim.* **2020**, *65(*2), 165-171.

180. Eissa, A.-A.-M.; Aljamal, K.-F.-M.; Ibrahim, H.-S.; Abdelrasheed Allam, H. *Bioorg. Chem.* **2021**, *116*, 105318.

181. Pasha, M.-A.; Nagashree, S. *Ultrason. Sonochem.* ***2014***, *21*(4), 1279-1283.

183. Mansour, S.-Y.; Sayed, G.-H.; Marzouk, M.-I.; Shaban, S.-S. *Synth. Commun.* **2021**, *51*(8), 1160-1170.

184. Fayed, E.-A.; Sabour, R.; Harras, M.-F.; Mehany, A.-B.-M. *Med Chem Res* **2019**, *28*, 1284–1297.

185. Negru, G. *Teză de doctorat*, Universitatea „Alexandru Ioan Cuza din Iasi", **2023**.

186. Zhang, F.; Zhao, Y.; Sun, L.; Ding, L.; Gu, Y.; Gong, P. *Eur. J. Med. Chem.* **2011**, *46*(7), 3149-57.

189. Belhadj, F.; Kibou, Z.; Cheikh, N.; Choukchou-Braham, N.; Villemin, D. *Tetrahedron Lett.* **2015**, *56*(44), 5999-6002.

191. **Zubaș, A.**; Ghineț, A.; Shova, S.; Bîcu, E. *New J. Chem*. **2023**, *47*, 3758-3772.