„ALEXANDRU IOAN CUZA" UNIVERSITY OF IAȘI
FACULTY OF CHEMISTRY
DOCTORAL SCHOOL OF CHEMISTRY AND LIFE AND EARTH SCIENCES

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# *FIVE AND SIX-MEMBERED NITROGEN-CONTAINING HETEROCYCLES. SYNTHESIS AND APPLICATIONS*

**PHD THESIS SUMMARY**

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**IAȘI**
**2015**

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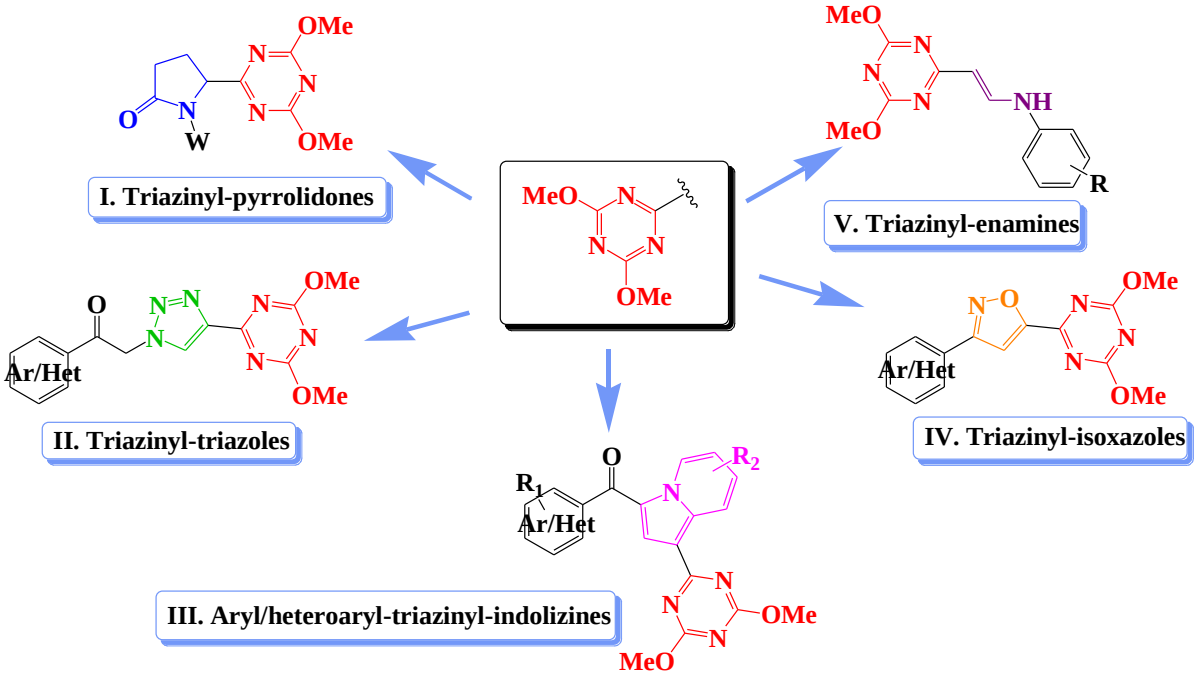
# OBJECTIVES

The heterocyclic combinations represent as far the biggest class of compounds in organic chemistry and have a great importance due the biological and industrial applications. Particularly, 1,3,5-triazine (or *s*-triazine) is the six-membered aromatic cycle with 3 nitrogen atoms, which represent the base of a well-known class of compounds, that continue to be the subject of important studies, due their numerous applications in different areas.

In the thesis we proposed the implementation of some structural modulation of 4,6-dimethoxy-1,3,5-triazinic cycle, which consist in binding in position 2 of some five or six-membered nitrogen-containing heterocycles, found in the structure of compounds with recognized pharmacological properties, in order to create a synergy of effects, that lead to improved biological properties.




***Scheme 1.*** *Research directions*

The established objectives of this thesis are following:

I. Synthesis, characterisation, study of reactivity and biological evaluation of some new triazin-pyrrolidones derivatives;

II. Synthesis, characterisation and biological evaluation of some new triazin-triazoles derivatives;

III. Synthesis, characterisation and biological evaluation of some new triazin-indolizines derivatives;

IV. Synthesis, characterisation and biological evaluation of some new triazin-isoxazoles derivatives;

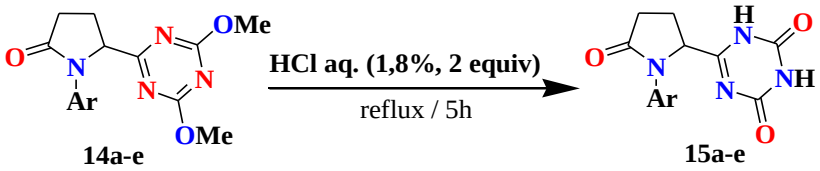
V. Study of the reactivity of 2-ethynyl-4,6-dimethoxy-1,3,5-triazine with amines.

# PERSONAL RESERCHES

## I. Synthesis, characterisation, study of reactivity and biological evaluation of some new triazin-pyrrolidones derivatives

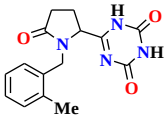
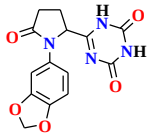
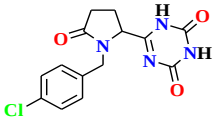
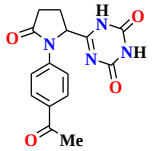
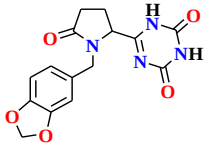
### *I.3.1. The total hydrolysis of methoxy groups*

By heating the dimethoxytriazines **14a-e** at reflux temperature in aqueous hydrochloric acid (1.8%) for five hours furnished a 47-85% yield of triazinediones **15a-e.** Interestingly, during these reactions with hydrochloric acid, partial hydrolysis or migration of a methyl group were not detected.



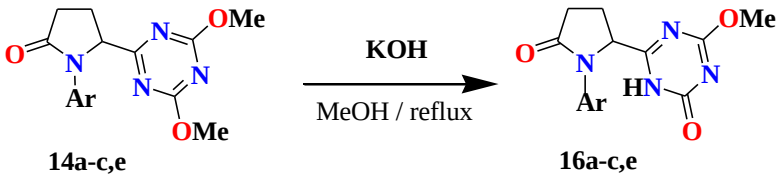

***Scheme 7.*** *The total hydrolysis of methoxy groups*

**Table 3.** The synthesized 2-substituted triazin-4,6-diones

| Compound | Structure | η % | Compound | Structure | η % |
|---|---|---|---|---|---|
| **15a** |  | 71 | **15d** |  | 81 |
| **15b** |  | 83 | **15e** |  | 47 |
| **15c** |  | 85 | | | |

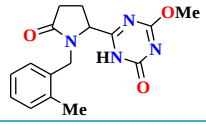
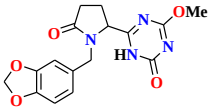
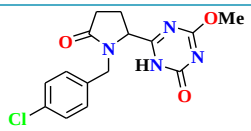
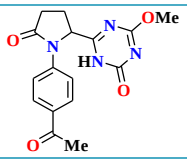
### *I.3.2. The partial hydrolysis of methoxy groups*

By heating dimethoxytriazines **14a**–**c,e** at reflux temperature in the presence of potassium hydroxide (1–2 equiv) in methanol for 20–28 hours to give 60–87% yields (Table 4) of 2-methoxy-1,3,5-triazin-6-ones **16a**–**c,e**. No by-products were isolated from these reactions.



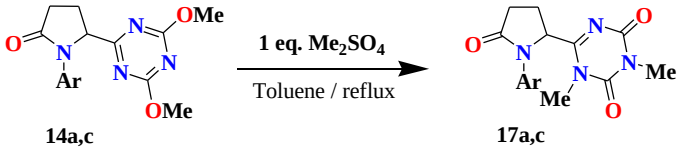

***Scheme 8.*** *The partial hydrolysis of methoxy groups*

**Tabelul 4.** The synthesized 2-substituted 2-methoxy-1,3,5-triazin-6-ones

| Compound | Structure | η % | Compound | Structure | η % |
|---|---|---|---|---|---|
| **16a** |  | 80 | **16c** |  | 73 |
| **16b** |  | 87 | **16e** |  | 62 |

### *I.3.3. Hilbert-Johnson migration*

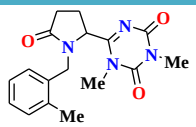
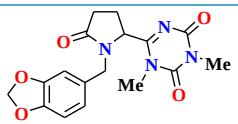
The dimethoxytriazines **14a** and **14c** were heated at reflux temperature in toluene in the presence of dimethyl sulfate for 24–28 hours to give 1,3-dimethyl-1,3,5-triazines **17a** and **17c** (Scheme 9); again, no by-products were isolated from these reactions.




**14a,c** **17a,c**

***Scheme 9.*** *Hilbert-Johnson migration*

**Table 5.** The synthesized 2-substituted 3,5-dimethyl-triazin-4,6-diones

| Compound | Structure | η % |
|---|---|---|
| **17a** |  | 52 |
| **17c** |  | 46 |

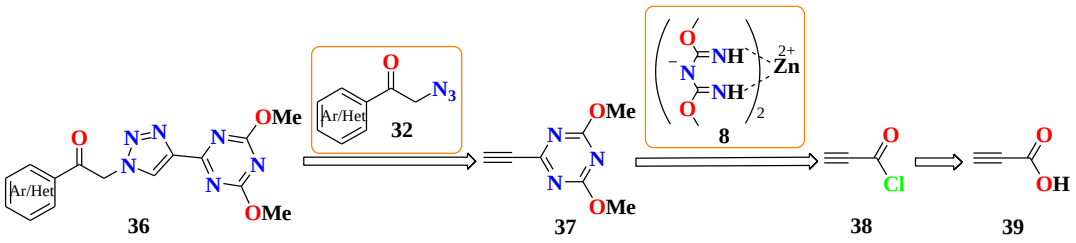
Products **15a,c** and **16b**,**c** were selected by the National Cancer Institute (NCI) for a biological screening on a 60-cell line panel, but the results were modests. The antifungal properties of these compounds will be reported in due course.

The results of this study represent the subject of a scientific publication[157]: *Studies on Pyrrolidinones: Chemistry of Dimethoxytriazines*, **Liliana Lucescu**, Philippe Gautret, Souhila Oudir, Benoît Rigo, Dalila Belei, Elena Bîcu, Alina Ghinet, *Synthesis*.

# II. Synthesis, characterisation and biological evaluation of some new triazin-triazoles derivatives

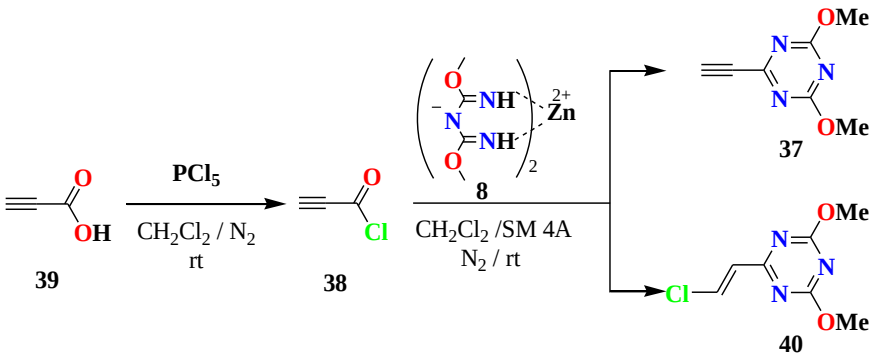
## II.4. Synthesis strategies

The synthetic way adopted for obtaining the target hybrid compounds starts with 1,3,5-triazine cyclization, followed by 1,2,3-triazole ring closing, as it described in scheme 14.



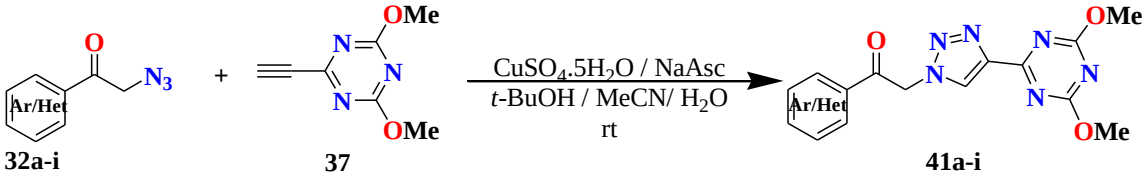

***Scheme 14.*** *The second synthetic strategie adopted for the access to triazinil-triazoles derivatives*

The synthetic pathway started from propiolic acid **39**, which after activation as acid chloride **38**, by treatement with phosphorus pentachloride,[178] reacted with salt **8** and furnished the target acetylenic derivative **37** (Scheme 15)



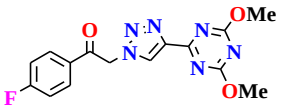
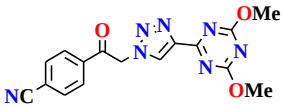
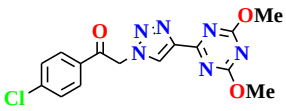
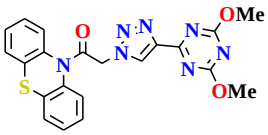
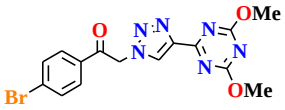
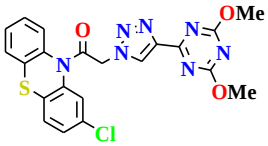
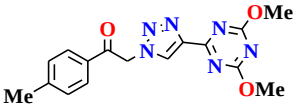
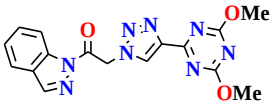
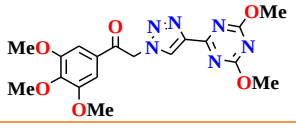

***Scheme 15.*** *The synthetic pathway for 2-ethynyl-4,6-dimehtoxy-1,3,5-triazine*

The last step of our synthesis was represented by the catalytic ring-closing of 1,2,3-triazole unit, using a *click chemistry* reaction with acetylenic derivative **37** and azides **32a-i** (Scheme 17).




***Scheme 17.*** *Triazinyl-triazoles **41a-i** synthesis*

**Table 8.** The synthetized triazinyl-triazoles

| Compound | Structure | η % | Compound | Structure | η % |
|---|---|---|---|---|---|
| **41a** |  | 52 | **41f** |  | 43 |
| **41b** |  | 65 | **41g** |  | 61 |
| **41c** |  | 58 | **41h** |  | 83 |
| **41d** |  | 38 | **41i** |  | 26 |
| **41e** |  | 79 | | | |

## II.5. Biological evaluation

The activity of newly triazin–triazoles **41a-i** was evaluated on *farnesyltransferase* (FTase) as a potential zinc chelating moiety. The best result of the study were obtained for *p*-bromophenyl derivative **41c** and *p*-chlorophenyl derivative **41b.**

**Table 9.** Results of the human *farnesyltransferase* assay

| Compound | FTase%[a,b] | $IC_{50}$ (μM) | Compound | FTase% | $IC_{50}$ (μM) |
|---|---|---|---|---|---|
| **41a** | 19 | -[c] | **41f** | 37 | - |
| **41b** | 59 | 72,05±6,9 | **41g** | 0 | - |
| **41c** | 73 | 38,62±1,7 | **41h** | 35 | - |
| **41d** | 48 | - | **41i** | 24 | - |
| **41e** | 0 | - | | | |

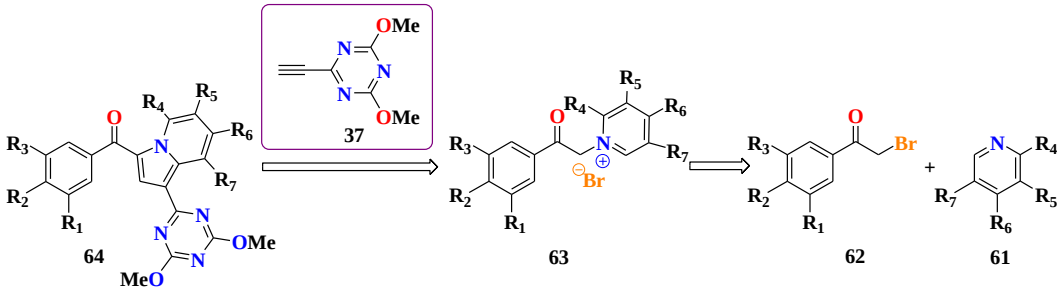
[a] Inhibition ratio of protein *farnesyltransferase* at a 100 μM concentration
[b] Values represent mean of two experiments
[c] Not determined.

The results of this study represent the subject of a scientific publication:[184] *Synthesis and biological evaluation of a new class of triazin-triazoles as potential inhibitors of human farnesyltransferase*, **Liliana Lucescu**, Elena Bîcu, Dalila Belei, Sergiu Shova, Benoît Rigo, Philippe Gautret, Joëlle Dubois, Alina Ghinet, *Research on Chemical Intermediates*.

# III. Synthesis, characterisation and biological evaluation of some new triazin-indolizines derivatives

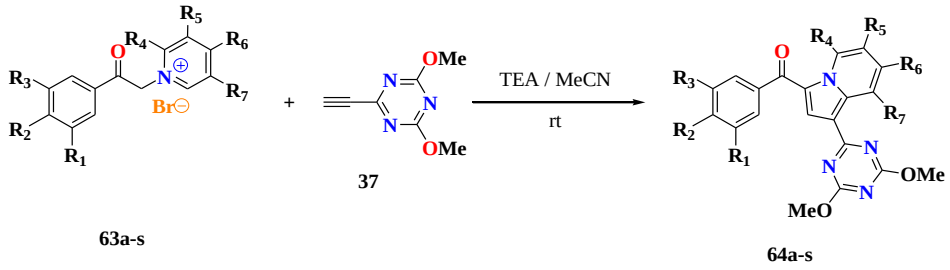
## IIIA. Benzoyl-triazin-indolizines derivatives

The target compounds were obtained in two steps, as described in scheme 18. First of all we synthesized the pyridinium salts, which next participated to a 1,3-dipolar cycloaddition reaction with 2-ethynyl-4,6-dimehtoxy-1,3,5-triazine **37**, previously synthesized.



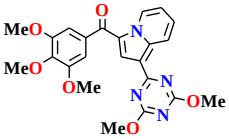
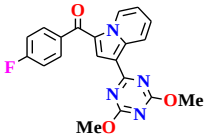
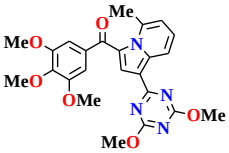
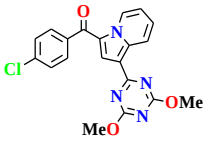

***Scheme 18.*** *The synthetic pathway for target benzoyl-triazin-indolizines*

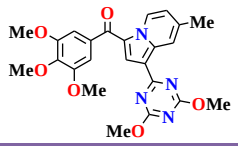
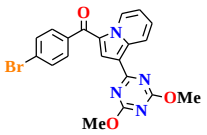
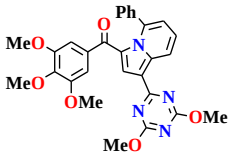
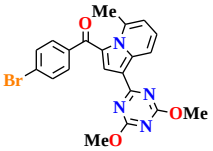
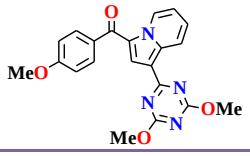
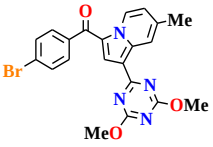
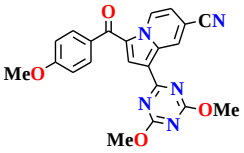
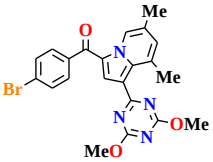
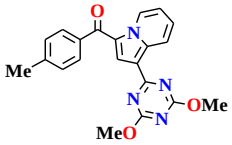
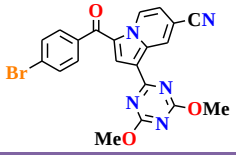
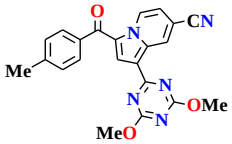
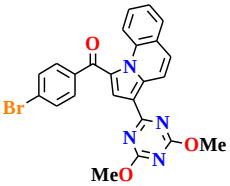
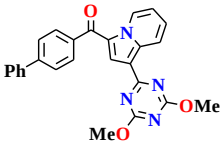
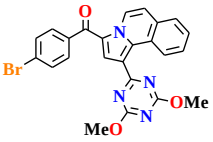
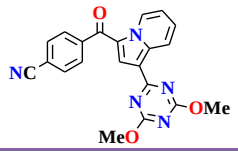
The construction of the indolizine unit in products **64a-s** achieved by reaction of the corresponding ylide, generated *in situ* by base treatment of pyridinium salts **63a-s**, with 2-ethynyl-4,6-dimehtoxy-1,3,5-triazine **37** (Scheme 20).




***Scheme 20.*** *The construction of indolizine unit*

**Table 12.** The synthesized 1-(4,6-dimethoxy-1,3,5-triazinyl)-indolizines **64a-s**

| Compound | Structure | η % | Compound | Structure | η % |
|---|---|---|---|---|---|
| **64a** |  | 58 | **64k** |  | 47 |
| **64b** |  | 51 | **64l** |  | 52 |

| | | | | | |
|---|---|---|---|---|---|
| **64c** |  | 59 | **64m** |  | 44 |
| **64d** |  | 55 | **64n** |  | 49 |
| **64e** |  | 45 | **64o** |  | 58 |
| **64f** |  | 52 | **64p** |  | 63 |
| **64g** |  | 53 | **64q** |  | 55 |
| **64h** |  | 44 | **64r** |  | 55 |
| **64i** |  | 40 | **64s** |  | 52 |
| **64j** |  | 52 | | | |

28 of synthesized indolizines, were selected by the National Cancer Institute (NCI) for a biological screening on the NCI-60 cell lines panel and the results are summarized in tables 14 and 15. These results showed that the presence of dimethoxytriazine cycle is esential for bioactivity. The most promising candidate of the study was **64m**, with a $GI_{50}$ value of 870 nM on SNB-75 CNS cancer cells and of 920 nM on MDA-MB-231/ATCC breast cancer.

By using the program NCI COMPARE for the most active derivative of our study **64m**, led to interesting results, which showed a correlation of 0,942 with an inhibitor of PLK1 (polo-like kinase 1).

**Table 14.** *In vitro* percentage of growth inhibition caused by the compounds **64a-h,l** at 10 μM concentration

| | **Compound** | **64a** | **64b** | **64c** | **64d** | **64e** | **64f** | **64g** | **64h** | **64l** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Panel** | Cell line | | | | | | | | | |
| **Leukemia** | CCRF-CEM | -[a] | 10 | 26 | **62** | - | - | 13 | - | 11 |
| | HL-60(TB) | - | 33 | **60** | - | - | - | - | - | - |
| | K-562 | 10 | 45 | **56** | 29 | - | - | - | - | - |
| | RPMI-8226 | - | 10 | 30 | **52** | - | - | 26 | - | 13 |
| | SR | 20 | **67** | **74** | 29 | 19 | 13 | 22 | - | 26 |
| **Non-small cell lung cancer** | A549/ATCC | - | 17 | 36 | 28 | 21 | - | - | 11 | 21 |
| | HOP-62 | - | 34 | 28 | - | **97** | 25 | - | **60** | **77** |
| | NCI-H460 | - | - | 16 | 14 | - | - | - | - | 20 |
| | NCI-H522 | - | **56** | 43 | 11 | **60** | - | - | 21 | 47 |
| **Colon cancer** | HCT-116 | - | 15 | 27 | - | 36 | - | 12 | - | 26 |
| **CNS cancer** | SF-295 | - | 15 | - | 40 | 47 | - | - | 47 | 33 |
| | SNB-75 | - | **67** | **58** | 23 | **-17** | 46 | 18 | **-14** | **-3** |
| | U251 | - | - | 15 | - | **56** | - | - | 6 | 44 |
| **Melanoma** | MALME-3M | 11 | 37 | **61** | - | - | - | - | 17 | 30 |
| | MDA-MB-435 | 35 | **86** | **100** | - | - | - | - | - | - |
| | SK-MEL-5 | - | 41 | **63** | **67** | - | - | 19 | - | - |
| | UACC-62 | - | 30 | **63** | 9 | 11 | - | - | 14 | 14 |
| **Ovarian cancer** | OVCAR-3 | - | 19 | **75** | 28 | - | - | - | - | - |
| | OVCAR-8 | - | 12 | 15 | 41 | 16 | - | - | 14 | 24 |
| | NCI/ADR-RES | - | 15 | 24 | 38 | 9 | - | - | | - |
| | SK-OV-3 | - | 8 | 29 | - | **54** | - | - | 42 | **55** |
| **Renal cancer** | 786-0 | - | - | 20 | - | 27 | - | - | 40 | 31 |
| | ACHN | - | 30 | 47 | 0 | 4 | - | - | 15 | **56** |
| | RXF 393 | - | 31 | 41 | 15 | 9 | 14 | - | **52** | 37 |
| **Prostate cancer** | PC-3 | - | 19 | 36 | 37 | 8 | - | 13 | - | 19 |
| **Breast cancer** | MCF7 | - | **55** | **73** | 22 | 13 | - | 10 | - | 19 |
| | MDA-MB-231/ATCC | - | 17 | 25 | 23 | - | 18 | 35 | 39 | 43 |
| | HS 578T | - | 17 | 35 | 14 | 25 | 16 | - | 42 | 46 |

[a]GI%<10%

**Tabelul 15.** *In vitro* percentage of growth inhibition caused by **64m-o,q-s** at 10 µM concentration

| | **Compus** | **64m** | **64n** | **64o** | **64q** | **64r** | **64s** |
|---|---|---|---|---|---|---|---|
| **Panel** | Cell line | | | | | | |
| **Leukemia** | K-562 | 22 | -[a] | - | - | - | - |
| | RPMI-8226 | 26 | - | - | - | - | - |
| | SR | 36 | - | - | - | - | - |
| **Non-small cell lung cancer** | A549/ATCC | **62** | - | - | - | 49 | **55** |
| | HOP-62 | **96** | - | - | - | **79** | - |
| | HOP-92 | **59** | - | - | - | - | - |
| | NCI-H226 | **75** | - | - | - | 35 | 28 |
| | NCI-H460 | **70** | - | - | - | **68** | **71** |
| **Colon cancer** | HCT-116 | **65** | - | - | - | - | - |
| **CNS cancer** | SF-295 | **87** | 22 | 20 | - | **59** | **76** |
| | SF-539 | **76** | - | - | - | - | - |
| | SNB-75 | **-5** | **75** | - | 27 | **69** | **75** |
| | U251 | **61** | - | - | - | - | - |
| **Melanoma** | MALME-3M | 37 | 18 | **73** | - | **58** | **67** |
| | SK-MEL-28 | **63** | 14 | 30 | - | 43 | **52** |
| | UACC-257 | **62** | - | 31 | 11 | 30 | 32 |
| | UACC-62 | 30 | - | - | - | - | - |
| **Ovarian cancer** | OVCAR-3 | 44 | 15 | - | - | 31 | **51** |
| | OVCAR-4 | **88** | 18 | **95** | - | 30 | 33 |
| | OVCAR-8 | **52** | 29 | - | - | **50** | **50** |
| | NCI/ADR-RES | **50** | 14 | - | - | 43 | 46 |
| | SK-OV-3 | **-11** | 20 | - | - | - | - |
| **Renal cancer** | 786-0 | **59** | 14 | - | - | 45 | **81** |
| | ACHN | **70** | 30 | - | - | 22 | 29 |
| | CAKI-1 | **56** | - | - | - | 33 | 44 |
| | RXF 393 | **75** | 36 | | 14 | **61** | **81** |
| | TK-10 | **78** | - | - | - | - | - |
| **Prostate cancer** | PC-3 | **55** | - | - | - | 37 | **58** |
| **Breast cancer** | MCF7 | 18 | 24 | 38 | - | 44 | **60** |
| | MDA-MB-231/ATCC | 48 | 27 | - | 11 | **80** | **96** |
| | HS 578T | **96** | 22 | - | - | **56** | **65** |
| | T-47D | **52** | 19 | - | - | 31 | 38 |

[a]GI%<10%

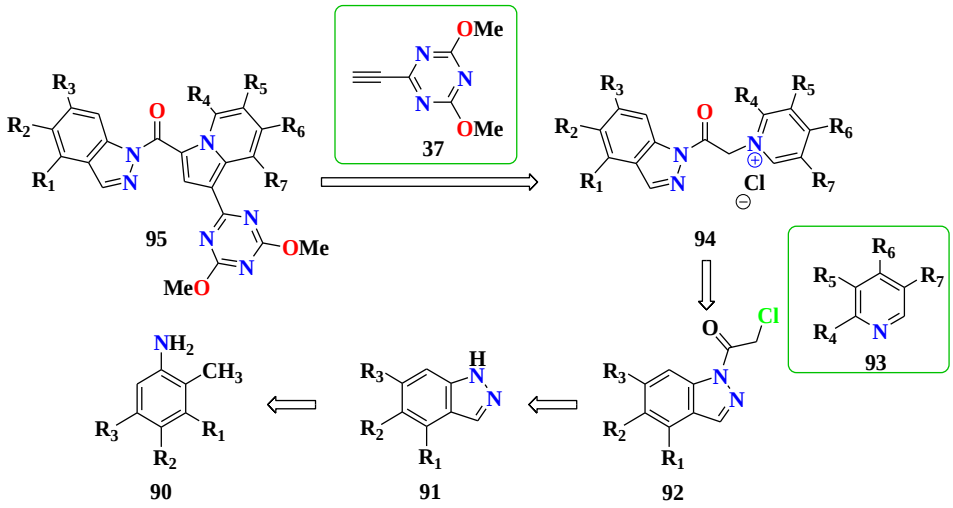
The results of this study represent the subject of two scientific papers,[209,210] a published one and a submitted one:

1. *Discovery of indolizines containing triazine moiety as new leads for the development of antitumoral agents targeting mitotic events*, Liliana Lucescu, Alina Ghinet, Dalila Belei, Benoît Rigo, Joëlle Dubois, Elena Bîcu, *Bioorganic & Medicinal Chemistry Letters*.

1. *Synthesis and biological evaluation of some new indolizine derivatives as antitumoral agents*, Liliana Lucescu, Elena Bîcu, Dalila Belei, Joëlle Dubois, Alina Ghinet, *Letters in Drug Design and Discovery*.

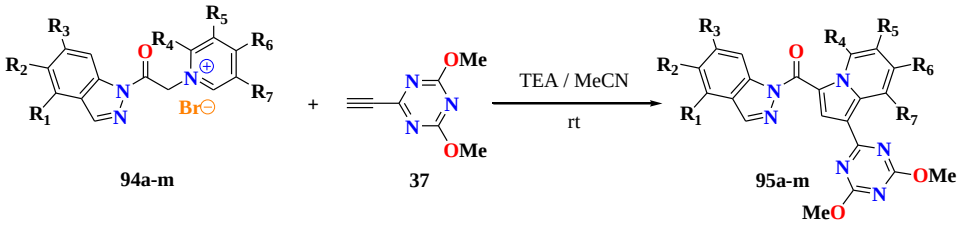
## IIIB. Indazoyl-triazin-indolizines

The synthesis of target hybrids indazoyl-triazin-indolizines was achieved in four steps. The first step consisted in indazol cyclization **91**, using *o*-toluidines **90** as starting material. The resulting indazoles participated to an acylation reaction with chloroacetyl chloride. The pyridinium salts **94** were obtained by reacting the acylated derivatives **92** with different pyridines. In the last stage, the construction of indolizine unit was accomplished.



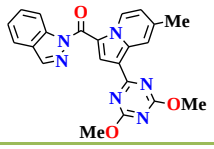
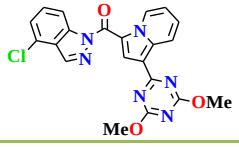
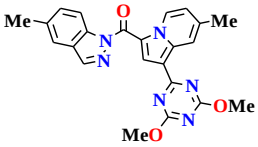
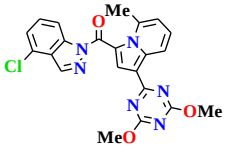
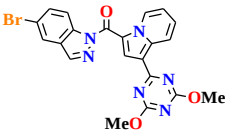
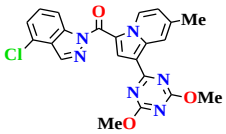
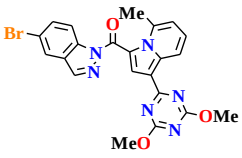
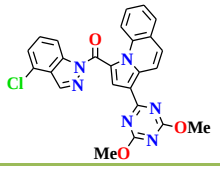
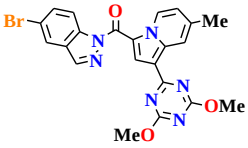
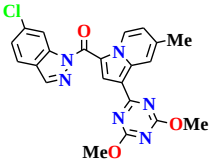
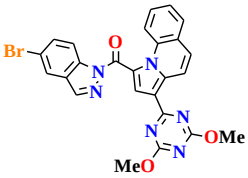
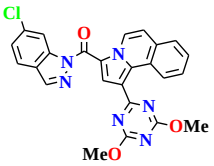
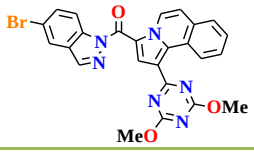

***Scheme 27.*** *The synthetic pathway for target indazoyl-triazin-indolizines derivatives* ***95***

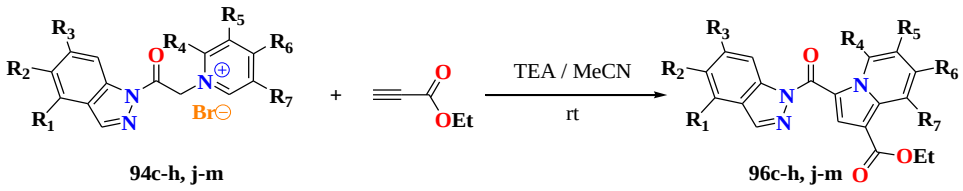
Target indolizines **95a-m** and **96c-h,j-m** were obtained by [3+2] cycloaddition reaction of the corresponding ylide, generated *in situ* by triethylamine treatment of pyridinium salts **94a-m**, with 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **37** (Scheme 30) or ethyl propiolate (Scheme 31).




***Schema 30.*** *The construction of indolizine unit with 2-ethynyl-4,6-dimethoxy-1,3,5-triazine* ***37***

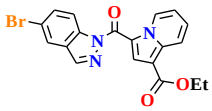
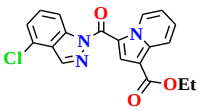
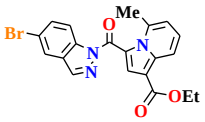
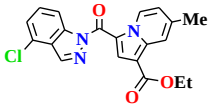
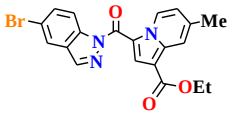
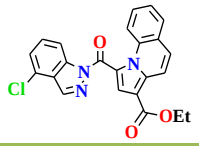
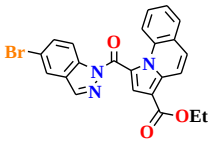
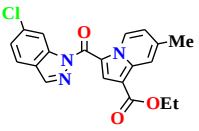
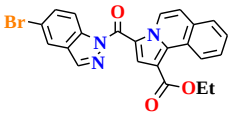
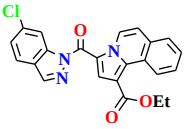
**Table 21.** The synthesized indazoyl-triazinyl-indolizines hybrids **95a-m**

| Compound | Structure | η % | Compound | Structure | η % |
|---|---|---|---|---|---|
| **95a** |  | 23 | **95h** |  | 50 |
| **95b** |  | 42 | **95i** |  | 44 |
| **95c** |  | 36 | **95j** |  | 58 |
| **95d** |  | 37 | **95k** |  | 50 |
| **95e** |  | 26 | **95l** |  | 48 |
| **95f** |  | 59 | **95m** |  | 37 |
| **95g** |  | 39 | | | |




***Scheme 31.*** *The construction of indolizine unit with ethyl propiolate*

**Table 22.** The synthesized 1-carboxyethyl substituted indolizines **96c-h,j-m**

| Compound | Structure | η % | Compound | Structure | η % |
|---|---|---|---|---|---|
| **96c** |  | 57 | **96h** |  | 59 |
| **96d** |  | 26 | **96j** |  | 34 |
| **96e** |  | 44 | **96k** |  | 43 |
| **96f** |  | 57 | **96l** |  | 44 |
| **96g** |  | 45 | **96m** |  | 48 |

Our previous results concerning the ability of indolizine derivatives as *farnesyltransferase* inhibitors,[224] encouraged us to test the new indolizinyl-indazole derivatives on this enzyme. The best results for this assay were obtained in the 1-(4,6-dimethoxy-1,3,5-triazinyl)-indolizines serie.

The results from the NCI biological assay revealed a strong antiproliferative activity and a cytotoxic activity against the cell line OVCAR-4, for compound **96c**.

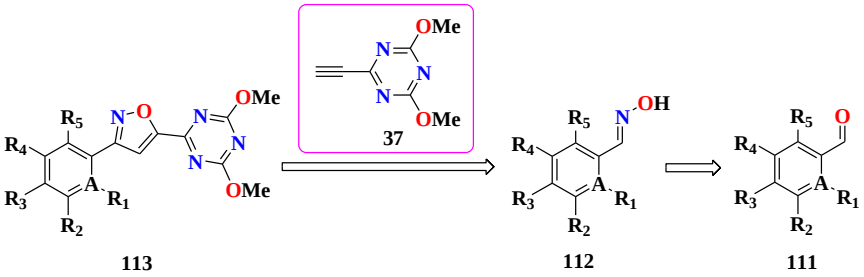
**Table 23.** Results of the human *farnesyltransferase* assay

| Compound | %Ftase[a,b] | $IC_{50}$ FTase (μM) | Compound | %Ftase[a,b] | $IC_{50}$ FTase (μM) |
|---|---|---|---|---|---|
| **95a** | 68,31 | 52,50±16,07 | **95j** | 39,88 | nd |
| **95b** | 12,09 | nd[c] | **95k** | 86,45 | nd |
| **95c** | 58,46 | nd | **95l** | 60,19 | nd |
| **95d** | 70,37 | 58,60±16,79 | **95m** | 51,37 | nd |
| **95e** | 82,36 | 27,08±4,93 | **96f** | 24,82 | nd |
| **95f** | 5,53 | nd | **96g** | 37,70 | nd |
| **95h** | 67,03 | nd | **96h** | 11,14 | nd |
| **95i** | 55,84 | nd | **96k** | 55,80 | nd |

[a]Inhibition ratio of protein *farnesyltransferase* at a 100 μM concentration
[b]Values represent mean of two experiments
[c]Not determined.

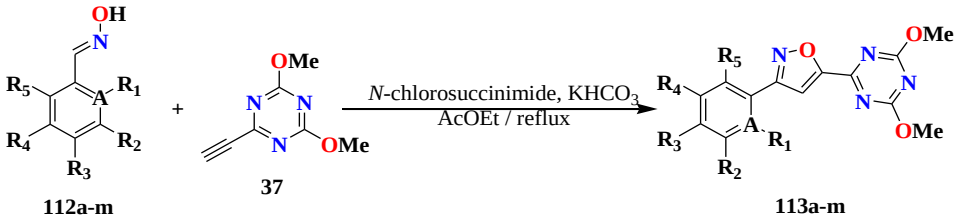
## IV. Synthesis, characterisation and biological evaluation of some new triazin-isoxazoles derivatives

The synthesis of target isoxazole derivatives **113a-m** was achieved in two steps. We started with oximes **112** preparation, using suitable substituted benzaldehyde **111** and hydroxylamine hydrochloride. The last step consisted in the construction of isoxazole unit.



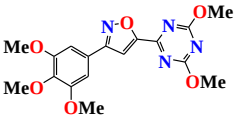
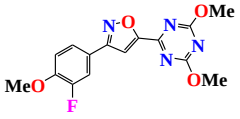
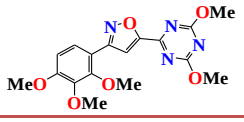
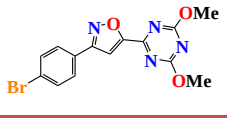
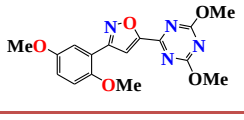
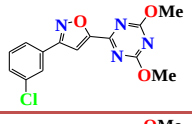
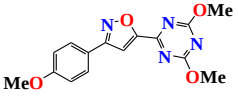
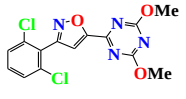

***Scheme 32.*** *The synthetic pathway for target triazinyl-isoxazole derivatives* ***113***

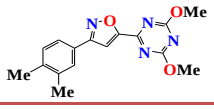
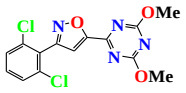
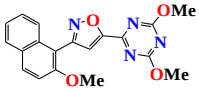
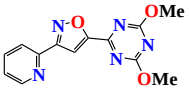
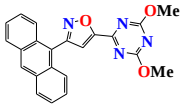
For the preparation of isoxazole unit in compounds **113a-m** and **114c** we utilized a 1,3-dipolar cycloaddition, realised *one-pot* with oximes **112a-m**, 2-ethynyl-4,6-dimethoxy-1,3,5-triazine **37** or ethyl propiolate as dipolarophile.[236]




***Scheme 34.*** *One-pote synthesis of triazinyl-isoxazole derivatives* ***113a-m***

**Table 26.** The synthesized triazinyl-isoxazole derivatives **113a-m** and **114c**

| Compound | Structure | η % | Compound | Structure | η % |
|---|---|---|---|---|---|
| **113a** |  | 27 | **113h** |  | 56 |
| **113b** |  | 67 | **113i** |  | 21 |
| **113c** |  | 42 | **113j** |  | 31 |
| **113d** |  | 27 | **113k** |  | 71 |

| | | | | | |
|---|---|---|---|---|---|
| **113e** |  | 25 | **113l** |  | 5 |
| **113f** |  | 23 | **113m** |  | 42 |
| **113g** |  | 64 | **114c** |  | |

The reaction proved to be a regioselective one, independent on electronic effects of dipolarophile substituents, leading each time to 3,5-disubstituted-isomer. The confirmation of reaction regioselectivity was achieved through RX diffraction.

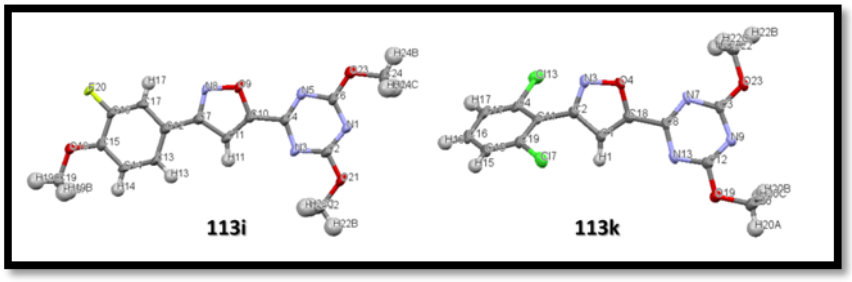



***Figure 26.*** *The structures of compounds* ***113i*** *and* ***113k*** *determined by RX diffraction*

In order to identify the biological targets of the synthesized compounds, they were tested for the inhibitory capacity against human *farneziltrasferase*. In general, the compounds showed a modest activity, except the derivatives **114c** and **113l**.

**Table 27.** Results of the human *farnesyltransferase* assay

| Compound | %Ftase[a,b] | $IC_{50}$ FTase (μM) | Compound | %Ftase | $IC_{50}$ FTase (μM) |
|---|---|---|---|---|---|
| **113a** | 28,15 | n.d.[c] | **113h** | 33,22 | n.d. |
| **113b** | 17,05 | n.d. | **113i** | 31,47 | n.d. |
| **113c** | 0 | n.d. | **113j** | 34,52 | n.d. |
| **113d** | 0 | n.d. | **113k** | 52,78 | n.d. |
| **113e** | 0 | n.d. | **113l** | 86,16 | 37,31±4,18 |
| **113f** | 13,84 | n.d. | **113m** | 24,64 | n.d. |
| **113g** | 0 | n.d. | **114c** | 87,47 | 37,22±12,75 |

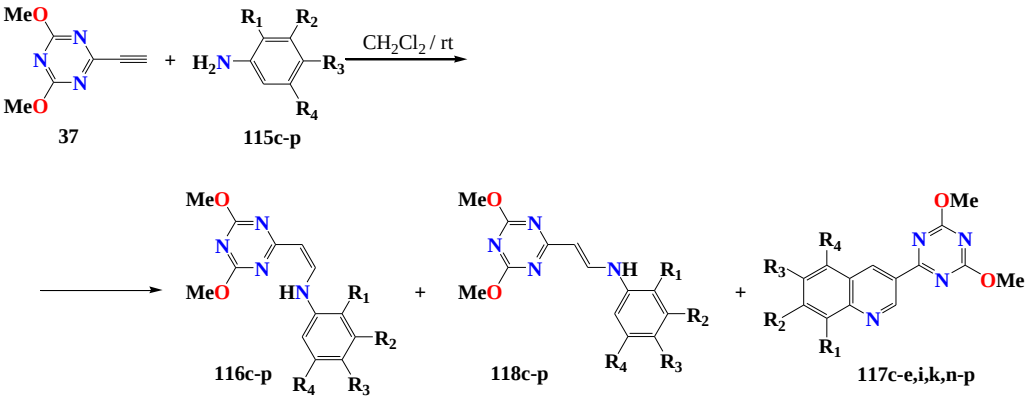
[a]Inhibition ratio of protein *farnesyltransferase* at a 100 μM concentration
[b]Values represent mean of two experiments
[c]Not determined.

# V. Study of the reactivity of 2-ethynyl-4,6-dimethoxy-1,3,5-triazine with various amines

## V.2. The reaction of 2-ethynyl-4,6-dimethoxy-1,3,5-triazine with anilines

After the reactions of acethylenic derivative **37** with secondary amines were identified two products, corresponding to *cis* and *trans* isomers of expected enamine. Particularly, after the reaction with anilines **115c-e,i,k,n-p**, a quinoline derivative was also observed (Scheme 41).

Opposite theory, the *Z*-isomer was more stable, while *E*-isomer is converting in time in corresponding *Z*-isomer. This is explained by the intramolecular hydrogen bond formed whitin NH group and one nitrogen atom from trazine cycle.




***Scheme 41.*** *The reaction of 2-ethynyl-4,6-dimethoxy-1,3,5-triazine with various anilines*

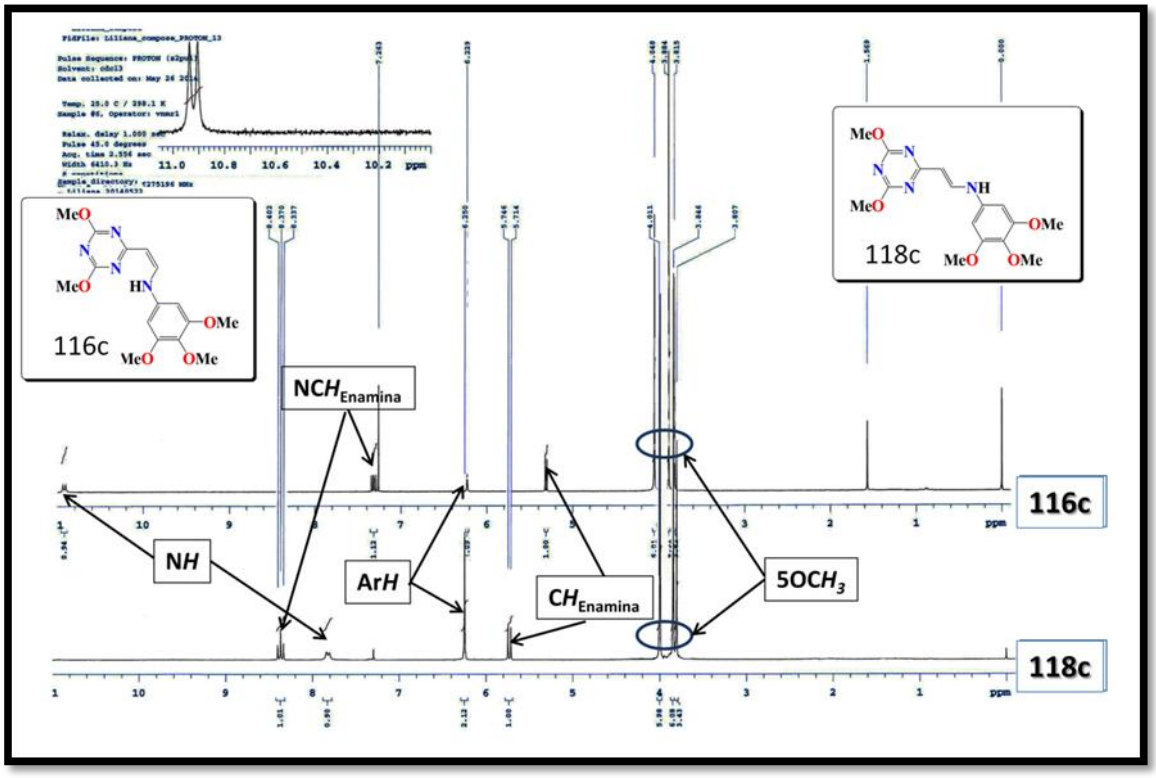
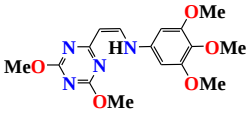
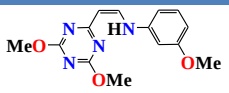
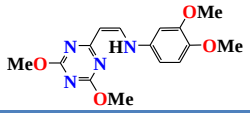
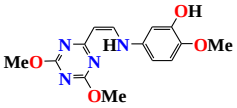
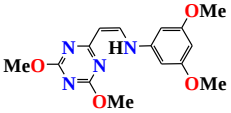
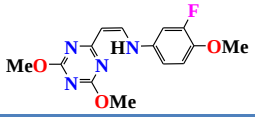
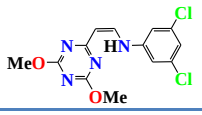
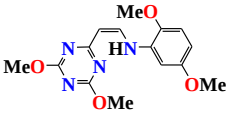
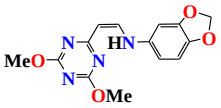
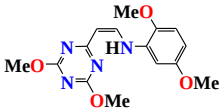
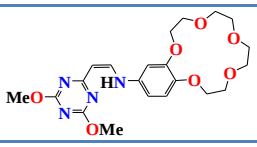
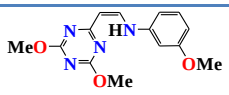
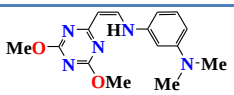


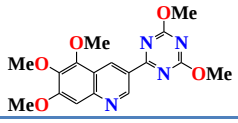
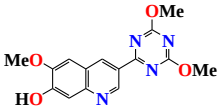
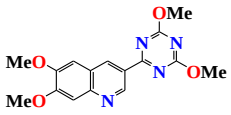
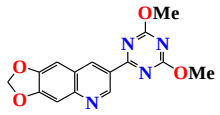
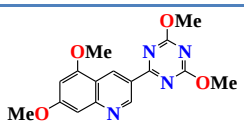
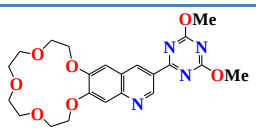
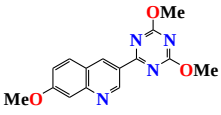
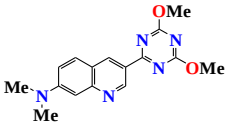

***Figure 31.*** *$^1$H NMR spectra of isomers **116c** and **118c***

**Table 28.** The synthesized *Z*-enamines derivatives **116c-p**

| Compound | Structure | η % | Compound | Structure | η % |
|---|---|---|---|---|---|
| **116c** |  | 25 | **116j** |  | 44 |
| **116d** |  | 6* | **116k** |  | 56 |
| **116e** |  | 28 | **116l** |  | 45 |
| **116f** |  | 46 | **116m** |  | 40 |
| **116g** |  | 56 | **116n** |  | 38 |
| **116h** |  | 59 | **116o** |  | 49 |
| **116i** |  | 28* | **116p** |  | * |

*Observed in the $^{1}$H NMR spectrum of the crude reaction, not isolated.

**Table 29**. The synthesized 3-(4,6-dimethoxy-1,3,5-triazinyl)-quinolines **117c-e,i,k,n-p**

| Compus | Structură | η % | η RMN% | Compus | Structură | η % | η RMN% |
|---|---|---|---|---|---|---|---|
| **117c** |  | 58 | 58 | **117k** |  | 27 | 50 |
| **117d** |  | 29 | 29 | **117n** |  | 12 | 25 |
| **117e** |  | 60 | 85 | **117o** |  | 72 | 85 |
| **117i** |  | <5 | 5 | **117p** |  | 5 | 45 |

Some of the synthesized compounds were biological evaluated for inhibitory ability on human *farnesyltransferase* and for anti-tubulin activity and the results are listed in table 32. The results obtained from the bioassays were promising, highlighting the high potential of these compounds capable to interact with both biological targets.

**Table 32.** Inhibitory activities on tubulin polymerization and *farnesyltransferaze*

| Compound | %TPI[a] | $IC_{50}$ Tubuline (μM) | %FTase[b] | $IC_{50}$ Ftase (μM) |
|---|---|---|---|---|
| **116a** | 14 | n.d.[c] | 54 | n.d. |
| **116b** | 53 | n.d. | 53 | n.d. |
| **116c** | 65,60 | 44,23±1.97 | 101,41 | 11,59±1,98 |
| **116e** | 47,34 | n.d. | n.d. | n.d. |
| **116f** | 23,64 | n.d. | n.d. | n.d. |
| **116g** | 70,65 | 25,17±2,64 | 101,95 | 9,56±0,68 |
| **116h** | 68,56 | 42,28±2,40 | 110,76 | 7,03±0,5 |
| **116j** | 74,34 | 33,93±2,56 | 47 | n.d. |
| **116k** | 67,74 | 96,01±9,83 | n.d. | n.d. |
| **116m** | 27,11 | n.d. | n.d. | n.d. |
| **116n** | 39,20 | n.d. | n.d. | n.d. |
| **116o** | 70,90 | 84,48±12,06 | n.d. | n.d. |
| **117c** | 23,56 | n.d | 0 | n.d. |
| **117d** | 22,5 | n.d. | 103,66 | 3,01±0,42 |
| **117e** | 0 | n.d. | n.d. | n.d. |
| **117k** | 0 | n.d. | n.d. | n.d. |
| **117p** | 11,41 | n.d. | n.d. | n.d. |

[a]Inhibition ratio of tubulin polymerization at a 100 μM concentration.
[b]Inhibition ratio of protein *farnesyltransferase* at a 100 μM concentration.
[c]Not determined.

# GENERAL CONCLUSIONS

- The various biological properties of triazines, along with their promising anti-tumor potential, influenced us to direct our investigations to this area.

- During the three years of study, different structural modulation were performed at 1,3,5-triazine cycle, aiming to generate new compounds with improved biological properties. Thus, we synthesized a total number of 216 compounds, of which **157 new, not mentioned in the literature**.

- The resulting compounds were purified and then characterized by IR, $^{1}$H NMR, $^{13}$C NMR, $^{19}$F NMR, MS spectra, by elemental analysis and by RX diffraction. The results of spectra analysis are summarized in the chapter entitle „Experimental section".

- The main research directions addressed are the following:
  1. Synthesis, characterisation, study of reactivity and biological evaluation of some new triazin-pyrrolidones derivatives;
  2. Synthesis, characterisation and biological evaluation of some new triazin-triazoles derivatives
  3. Synthesis, characterisation and biological evaluation of some new triazin-indolizines derivatives;
  4. Synthesis, characterisation and biological evaluation of some new triazin-isoxazoles derivatives;
  5. Study of the reactivity of 2-ethynyl-4,6-dimethoxy-1,3,5-triazine with amines

- Out of the synthesized compounds in this study, a total of 54 participated in the biological screening developed by the NCI, 11 compounds showed inhibitory activity against *farneziltrasferase* enzyme and 6 compounds have shown promising ability to inhibit tubulin polymerization.

- The information obtained after all the investigations carried out, allowed us to establish a series of structure-biological activity relationships, that can represent the starting point for the design of novel triazine potential biological actives.

- A part of the results of the research carried out during PhD studies represent the subject of four ISI scientific publications, three published and one submitted for publication and some oral communications and posters presented at some national and international conferences.

***Scientific publications:***

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- *Synthesis and biological evaluation of a new class of triazin-triazoles as potential inhibitors of human farnesyltransferase*, **Liliana Lucescu**, Elena Bîcu, Dalila Belei, Sergiu Shova, Benoît Rigo, Philippe Gautret, Joëlle Dubois, Alina Ghinet, *Research on Chemical Intermediates* **2015**, doi 10.1007/s11164-015-2131-1.

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