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SYNTHESIS OF ALKALOID DERIVATIVES WITH POTENTIAL BIOLOGICAL ACTIVITY

PhD Theses

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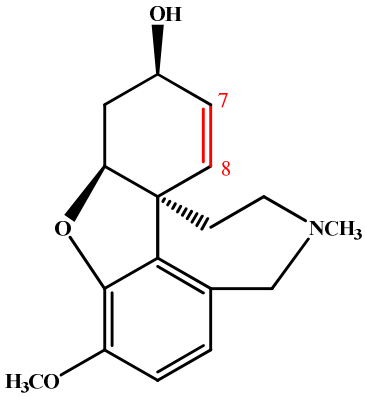
1. INTRODUCTION

The synthetic investigation of biologically active natural compounds serves three main purposes: (*i*) the isolation of organic compound from the given plant, (*ii*) the total synthesis of alkaloids and their analogues, (*iii*) modification of the structures for producing more effective, more selective, or less toxic derivatives.

In our research group we worked in two fields, in one of them, there were researches with galanthamine (**1**) being used in treating Alzheimer's disease, in the other one, researches in connection with vinblastine (**4**) and vincristine (**5**) which have antitumor effect. My task was to produce new derivatives with potential biological activity by the chemical modification of these natural compounds.



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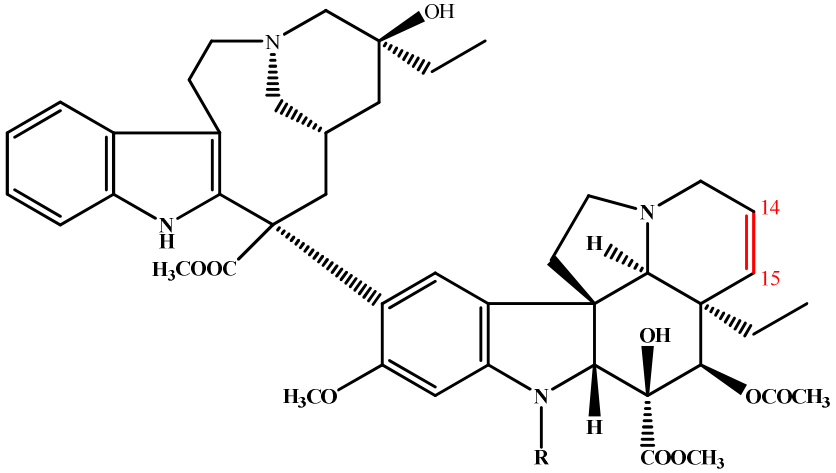


4 R = CH_3
5 R = CHO

It had been observed that saturating the C(14)=C(15) carbon carbon double-bond of vinblastine (**4**) with catalytic hydrogenation, the antitumor activity decreased about two orders[1]. Since this little alteration in this big molecule caused such a drastic change, we came to conclusion that this double-bond may have a key role in the biological effect. As these compounds showed themselves to be appropriate for cyclopropanation, the question arose, how the biological effect changes, if this double-bond is replaced by a cyclopropane ring. Therefore, the main purpose of my work was to produce vindoline and vinblastine derivatives containing a cyclopropane ring at position 14,15, as well as extending the cyclopropanation to galanthamine. So, we can hopefully achieve more effective, more selective, and less toxic derivatives.

[1] Noble R. L., Beer M. D. C. T., McIntyre, R. W.; *Cancer*, **1967**, *20*, 885-890.

2. LITERATURE BACKGROUND

In the nature, the cyclopropane skeleton can be found in numerous compounds in condensed and separated forms. Besides, the semi-synthetic modification of many natural organic compounds including cyclopropanation led to biologically active derivatives[2,3,4,5].

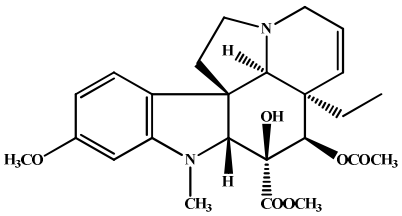
According to its unique structure, the cyclopropane ring has special properties. From the investigation of different cyclopropane derivatives by nuclear magnetic resonance spectroscopy, it was concluded that in the cyclopropane ring, the carbon-hydrogen bonds have more *s* character, than in other hydrocarbons. It follows that the carbon-carbon bonds must have more *p* character. It was pointed out that these types of carbon-carbon bonds have 17% of *s* character showing a real sp^5 hydridization supported by the measured carbon-carbon coupling constants[6]. Therefore, the cyclopropane ring cannot be characterized by the classical valency theories. This raises the question whether the cyclopropane ring may have any influence on the biological activity of a molecule.

Galanthamine (**1**) is a member of the *Amaryllidaceae* alkaloids, it is a selective acetylcholinesterase inhibitor. Galanthamine (**1**) earlier isolated from the Narcissus pseudonarcissus and the Caucasian snowdrop (Galanthus woronowii), but several total synthesis have been elaborated to produce it by now. It is mainly used for the treatment of Alzheimer's disease. My experiments on the cyclopropanation of galanthamine (**1**) are only a small part of my PhD work. The topic in connection with the derivatives of galanthamine is summarised well by an earlier publication of our research group[7].

Vindoline (**2**) and catharanthine (**3**) are alkaloids with indole skeleton which coupled to each other form vinblastine (**4**) and vincristine (**5**). The difference between vinblastine (**4**) and vincristine (**5**) is that the former has a methyl, while the latter a formyl group on the indole nitrogen of the vindoline skeleton. These compounds belong to *Vinca* alkaloids and they were first isolated from the Madagaskar periwinkle plant (*Catharantus roseus*) in the 1950s. These dimeric alkaloids have cytotoxic activity which are widely used in antitumor therapy. In the course of cell proliferation they act as inhibitors during the metaphase of the cell cycle and by binding to the microtubules inhibit the development of the mitotic spindle. In tumor cells

[2] Faust R.; *Angew. Chem. Int. Ed.*, **2001**, *40*, 2251-2253.
[3] Law J. H.; *Acc. Chem. Res.*, **1971**, *4*, 199-203.
[4] Wessjohann L. A., Brandt W.; *Chem. Rev.*, **2003**, *103*, 1625-1647.
[5] Taylor R. E., Engelhardt F. C., Schmitt M. J.; *Tetrahedron*, **2003**, *59*, 5623-5634.
[6] Weigert F. J., Roberts J. D.; *J. Am. Chem. Soc.*, **1967**, *89*, 5962-5963.
[7] Herke K., Gorka-Kereskényi Á., Hazai L.; *Magyar Kémiai Folyóirat*, **2010**, *116 (2)*, 72-76.

these agents inhibit the DNA repair and the RNA synthesis mechanisms, blocking the DNA-dependent RNA polymerase. Their main uses are in leukemia and lymphomas.



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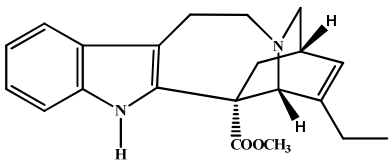

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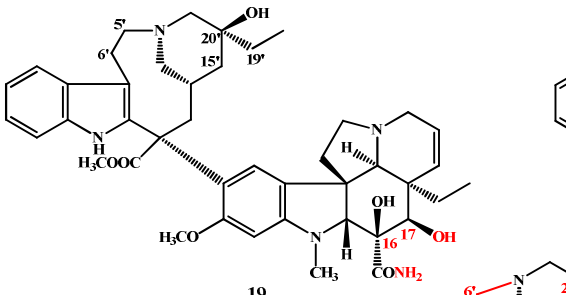
The chemical and biological characteristics of these dimeric alkaloids are presented in several reviews, of which only two publications are mentioned here[8,9]. The aim of our present work is to pruduce new vinblastine and vincristine derivatives, so our attention turned to the modifications on the basic skeleton of vinblastine and vincristine. Over the years a number of research groups have performed extensive and valuable work to synthesize new derivatives of vinblastine and vincristine. Modifications in the vindoline skeleton or in the catharanthine moiety resulted in a number of new antitumor agents with an enhanced selectivity or less toxic properties. The most important changes were in connection with the ring system of the catharanthine (**3**) and vindoline (**2**), the aromatic ring and the double-bond of catharanthine (**3)** at position 15',20' and the ester group of vindoline (**2**) at position 16. The natural vinblastine (**4**) and vincristine (**5**) as well as the semy-synthetic vinorelbine (**19**) and vindesine (**50**) have been used in antitumor therapy so far. Apart from them, vinflunine (**81**) is also significant, it is in phase III trials at present.



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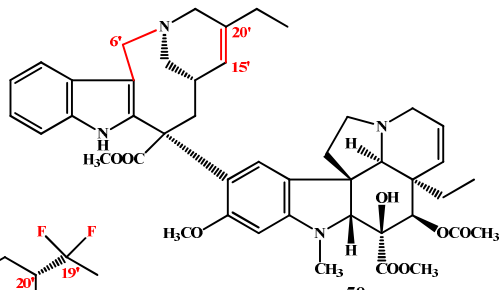

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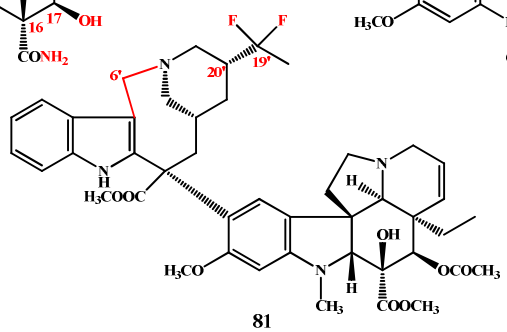

81

[8] Brossi A., Suffness M.; *The Alkaloids*, Academic Press Inc., New York, USA, **1990**, *37*, 1-240.

[9] Bölcskei H., Szabó L., Szántay Cs.; *Frontiers Nat. Prod. Chem*., **2005**, *1*, 43-49.

Vindesine (**19**) was synthesized from vinblastine (**4**) in reaction with refluxing ammonia which can be used among others for the treatment of melanoma in cancer therapy[10].

Coupling catharanthine (**3**) and vindoline (**2**), then forming 9-membered ring of the catharanthine part into an 8-membered ring by oxidative removal of the 5'-methylene group resulted in vinorelbin (**50**) which can mainly be used in the treatment of non-small cell lung cancer[11].

Vinflunine (**81**) can be prepared by fluorination of vinorelbine (**50**) in superacid media. This compound is excellent in the treatment of bladder cancer[12].

In the course of my PhD work, we have also compiled a review about the vinblastine and vincristine derivatives that can be found in the literature [2].

3. EXPERIMENTAL METHODS

During the synthesis of the compounds, the well established methods of preparative organic chemistry were used. The progress of reactions was followed by thin layer chromatography. The crude products were purified by preparative thin layer chromatograpy. Purity of the compounds was determined by thin layer chromatography, measuring melting points and optical rotations. Structures of the products were determined using ^{1}H- and ^{13}C-NMR, IR and MS spectroscopies.

4. RESULTS

Our aim was to prepare vindoline and vinblastine derivatives containing a cyclopropane ring at position 14,15 as well as extending the cyclopropanation to galanthamine.

Our first purpose was to form a cyclopropane ring on the double-bond at position 7,8 of galanthamine (**1**) and to produce the 7,8-cyclopropanogalanthamine (**6**). Reaction between galanthamine (**1**) and diazomethane generated from *N*-methyl-*N*-nitrosourea in dichloromethane solution in the presence of palladium(II) acetate at 0^{o}C resulted in a non-expected product, the cycloheptatriene derivative of galanthamine (**121**) which contains a 7-membered A-ring.

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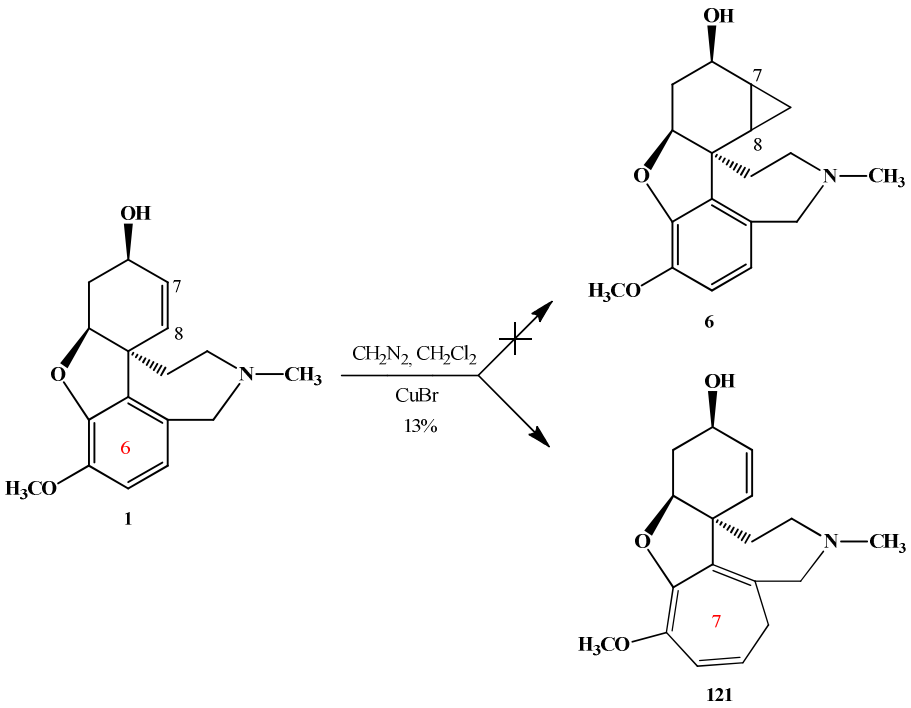
[10] Eli Lilly Company; DE Patent 22415980, 1974; [*Chem. Abstr.*, **1974**, *82*, 579967b].

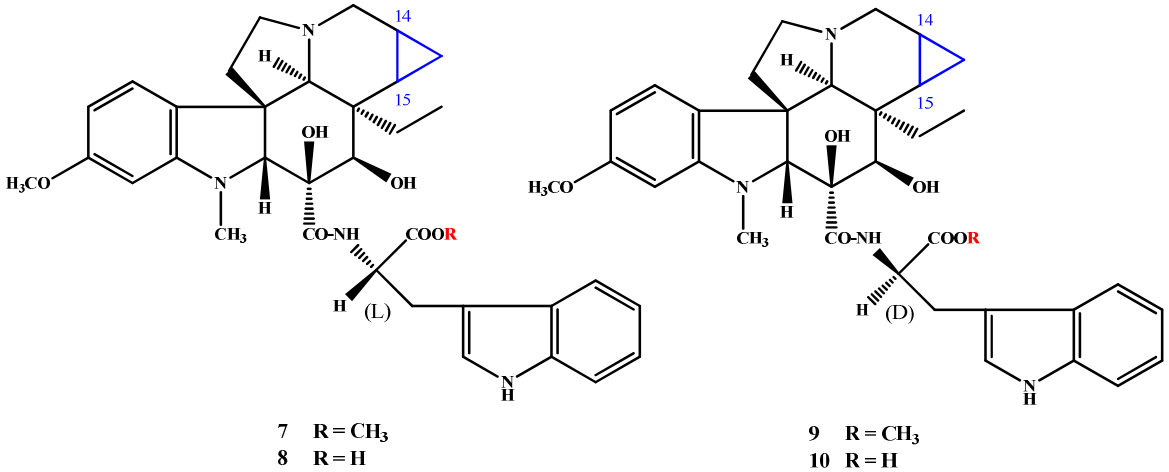
[11] Ngo Q. A., Roussi F., Cormier A., Thoret S., Knossow M., Guénard D., Guéritte F.; *J. Med. Chem.*, **2009**, *52*, 134-142.

[12] Jacquesy J.-C., Berrier C., Jouannetaud M.-P., Zunino F., Fahy J., Duflos A., Ribet J.-P.; *J. of Fluorine Chem.*, **2002,** *114,* 139-142.

In order to increase the low yield we optimized the reaction. We reached the best yield when copper(I) bromide was the catalyst, diazomethane was generated by potassium hidroxide base and the reaction was performed at room temperature. In this way, we succeeded in reaching 13% yield improving one order.



In our research group, a number of vindoline derivatives conjugated with L- and D-tryptophan methyl ester were produced before which had promising antitumor effect, so the synthesis of their analogues containing a cyclopropane ring at position 14,15 (**7** and **8**) was among our plans in order to investigate the structure-activity relationship. Hydrolysing the methoxycarbonil group of the obtained compounds we can get carboxylic acid derivatives (**9** and **10**) which can be conjugated to carrier peptids (octaarginine) and so the antitumor molecule can function getting directly into the cell decreasing the harmful side effects.

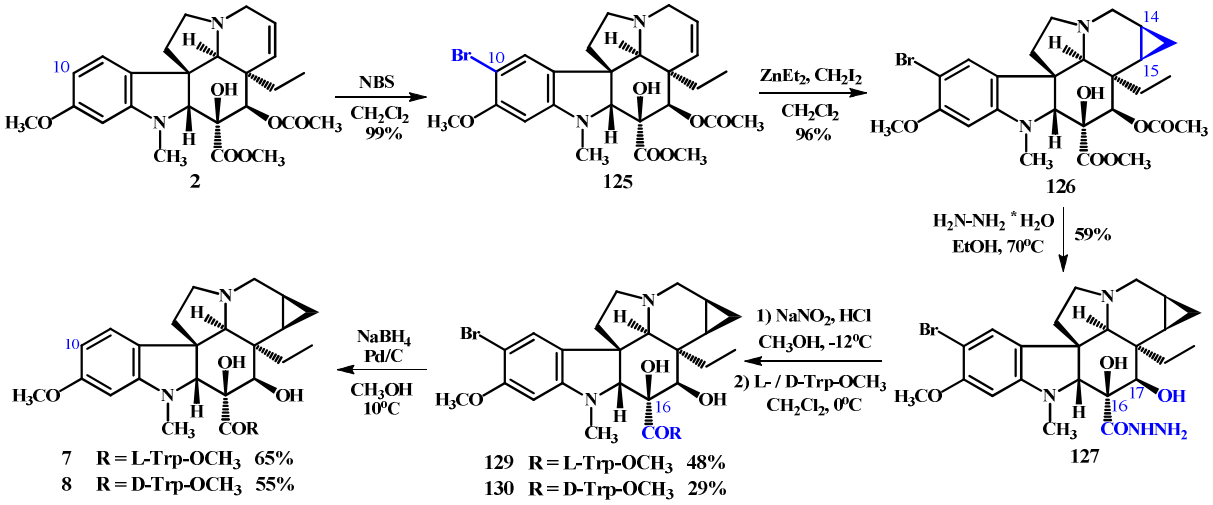


7 R = CH_3
8 R = H

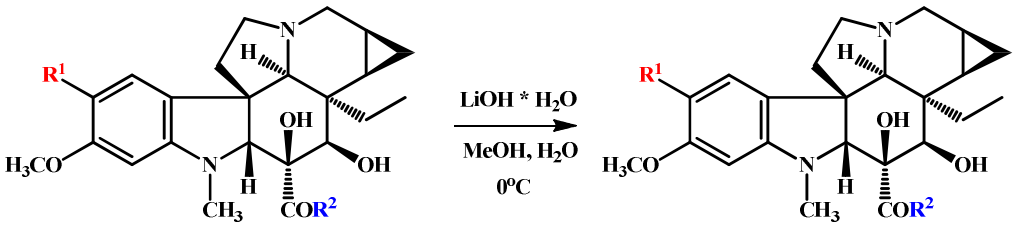
9 R = CH_3
10 R = H

The first step to synthesize the target compounds was the establishment of a cyclopropane ring on the double-bond of vindoline (**2**) at position 14,15. We reacted vindoline (**2**) with diethylzinc and diiodomethane in the classical Simmons-Smith reaction and we managed to form the cyclopropane ring at position 14,15, but a coupled dimer at position 10 (**124**) was obtained. In order to avoid the formation of the dimer, we built in a bromo atom into the vindoline (**2**) at position 10 with *N*-bromosuccinimide, then we performed the cyclopropanation reaction with the obtained 10-bromovindoline (**125**) and so we succeeded in getting the required 10-bromo-14,15-cyclopropanovindoline (**126**).

The tryptophan unit was coupled with vindoline derivative **126** following the reaction steps previously elaborated in our research group. The first step was to prepare hydrazide **127** with hydrazine hydrate. In the second step, we conjugated L- and D- tryptophan methyl ester to the compound **127** using the azide-coupling methode. In the last step, we performed removing the bromo atom from position 10 with sodium borohydride and palladium on charcoal.



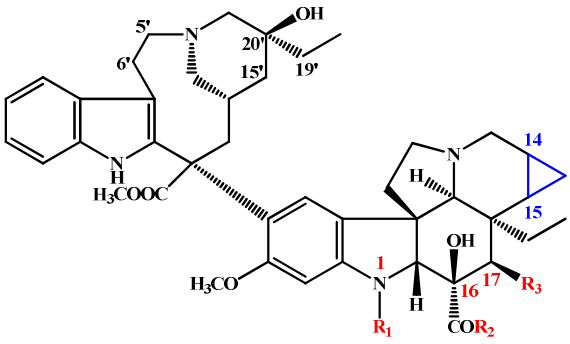
Our next purpose was to obtain carboxylic acid derivatives (**9**, **10**, **131** and **132**) by hydrolysis of the methoxycarbonyl group which can be found on the tryptophan part of the coupled compounds. We performed the reactions using lithium hydroxide monohydrate.



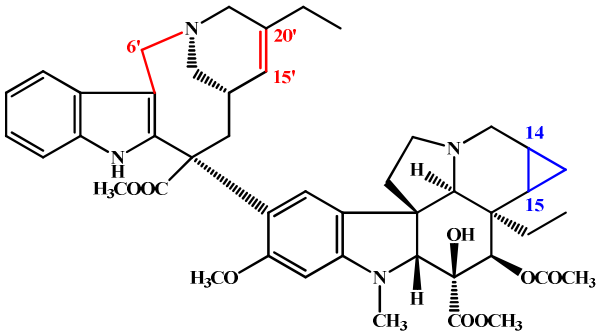
7 $R^1 = H, R^2 =$ L-Trp-OCH_3
8 $R^1 = H, R^2 =$ D-Trp-OCH_3
129 $R^1 = Br, R^2 =$ L-Trp-OCH_3
130 $R^1 = Br, R^2 =$ D-Trp-OCH_3

9 $R^1 = H, R^2 =$ L-Trp-OH 96%
10 $R^1 = H, R^2 =$ D-Trp-OH 94%
131 $R^1 = Br, R^2 =$ L-Trp-OH 58%
132 $R^1 = Br, R^2 =$ D-Trp-OH 74%

Among our further aims was to extend the cyclopropanation to the dimeric *Vinca* alkaloids, too. Therefore, we decided to synthesize the 14,15-cyclopropane derivatives of vinblastine (**15**), vincristine (**16**), vindesine (**17**) and vinorelbine (**18**).



15 R_1=CH_3, R_2=OCH_3, R_3=$OCOCH_3$
16 R_1=CHO, R_2=OCH_3, R_3=$OCOCH_3$
17 R_1=CH_3, R_2=NH_2, R_3=OH



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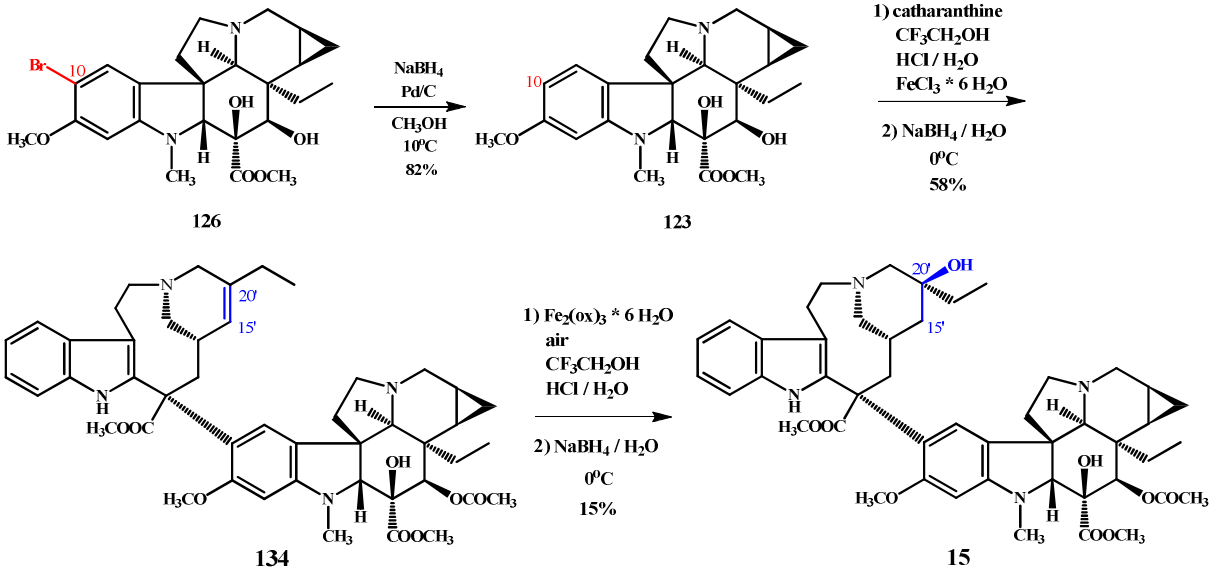
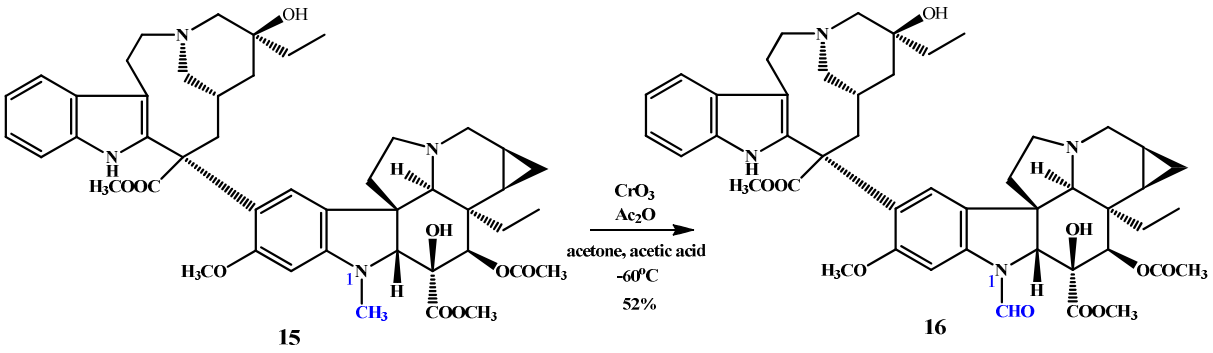
14,15-Cyclopropanovinblastine (**15**) was synthesized from 10-bromo-14,15-cyclopropanovindoline (**126**). In the first step, we removed the bromo atom from position 10 with sodium borohydride and palladium on charcoal, then the 14,15-cyclopropanovindoline (**123**) obtained was coupled with catharanthine (**3**) resulting in the formation of 14,15-cyclopropano-anhydrovinblastine (**134**). The final step was the oxidation of 14,15-cyclopropano-anhydrovinblastine (**134**). In this way, we successfully prepared the required 14,15-cyclopropanovinblastine (**15**).



We prepared the 14,15-cyclopropanovincristine (**16**) by the oxidation of the methyl group belonging to the nitrogen atom of 14,15-cyclopropanovinblastine (**15**) at position 1 to a formyl group. The reaction was performed with chromium trioxide.



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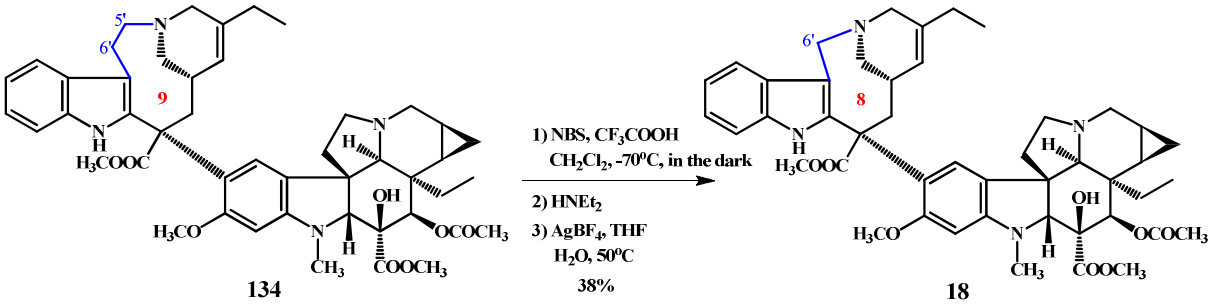
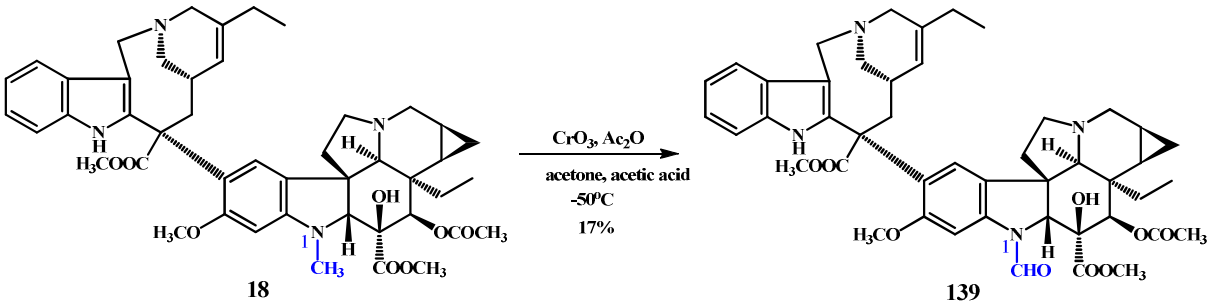
Our next aim was to synthesize the 14,15-cyclopropanovinorelbine (**18**) which was prepared from 14,15-cyclopropano-anhydrovinblastine (**134**) using silver tetrafluoroborate in a ring contraction reaction.



134 18

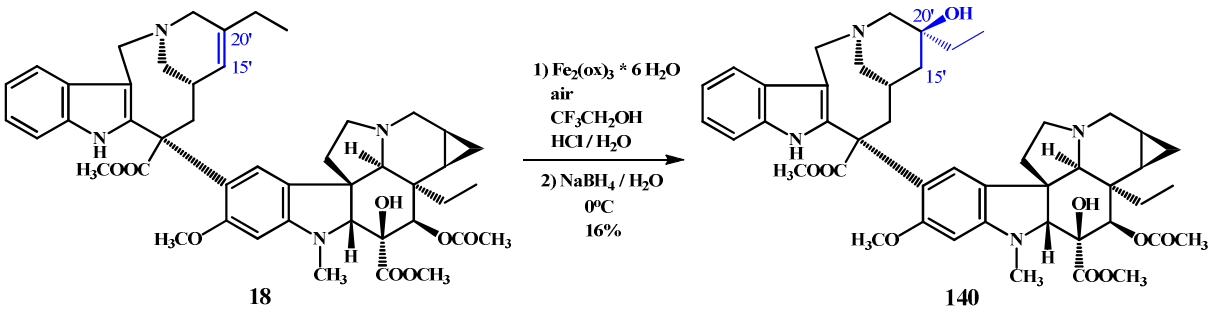
The preparation of the 14,15-cyclopropanovinorelbine (**18**) made possible the synthesis of two other cyclopropane derivatives, the 1-*N*-formyl-14,15-cyclopropanovinorelbine (**139**) and the nor-5'-14,15-cyclopropanovinblastine (**140**).

The 1-*N*-formyl-14,15-cyclopropanovinorelbine (**139**) was prepared from 14,15-cyclopropanovinorelbine (**18**) with chromium trioxide.



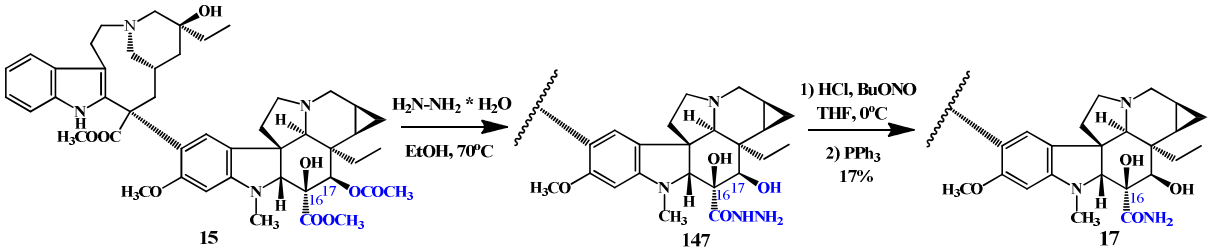
18 139

We synthesized the nor-5'-14,15-cyclopropanovinblastine (**140**) by the oxidation of the double-bond at position 15',20' from 14,15-cyclopropanovinorelbine (**18**).



18 140

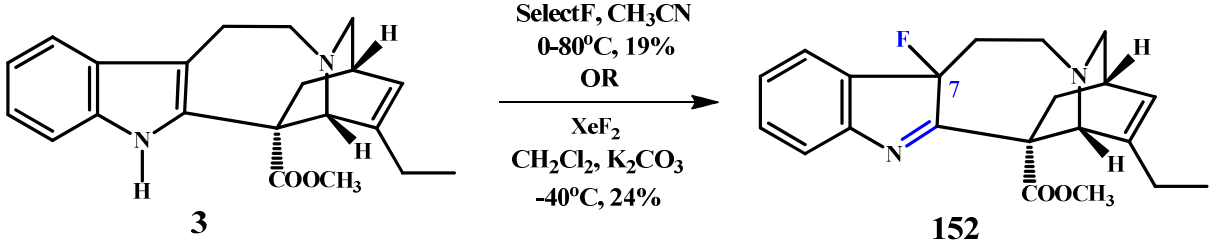
Then, we tried to prepare the 14,15-cyclopropanovindesine (**17**). We started with 14,15-cyclopropanovinblastine (**15**), reacting it with hydrazine hydrate and we obtained compound **147**. The next step was a modified Staudinger reaction, during which we synthesized the 14,15-cyclopropanovindesine (**17**) from compound **147** with triphenylphosphine through the azide analogue. This is a new result, so we have not been able to publish the synthesis of 14,15-cyclopropanovindesine (**17**) yet.



15 147 17

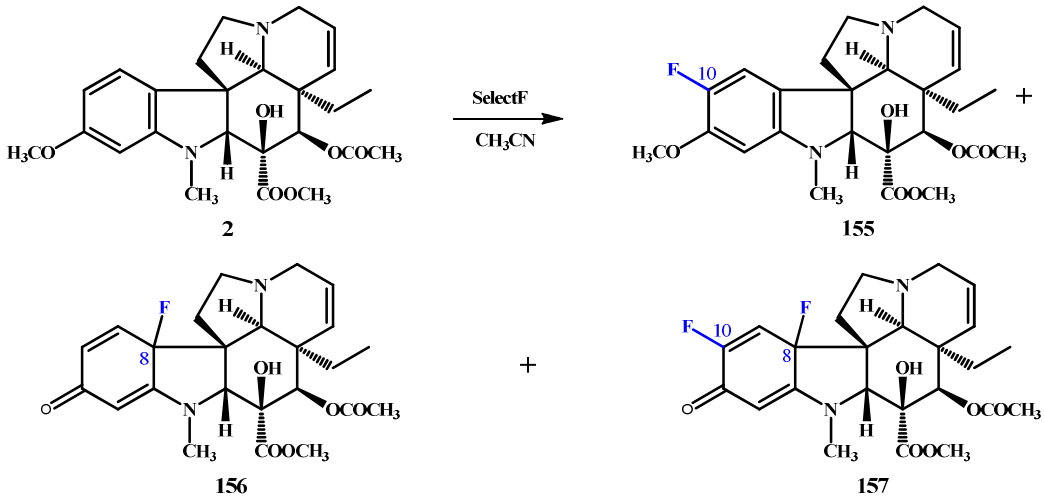
Our next aim was to prepare vindoline and vinblastine derivatives containing halogen atom and a cyclopropane ring. First of all, we wanted to synthesize analogues containing fluoro atom, since the lipophilicity of the molecule affected by fluor grows which promotes better agent linkage and improves the transport qualities. The following reactions have not been published yet.

First, we investigated the fluorination of catharanthine (**3**) and vinblastine (**4**). When we fluorinated catharanthine (**3**) with xenon difluoride or Selectfluor we obtained **152**, in which the fluoro atom coupled to the bridgehead carbon atom at position 7. Fluorinating vinblastine (**4**), we could not isolate any product.



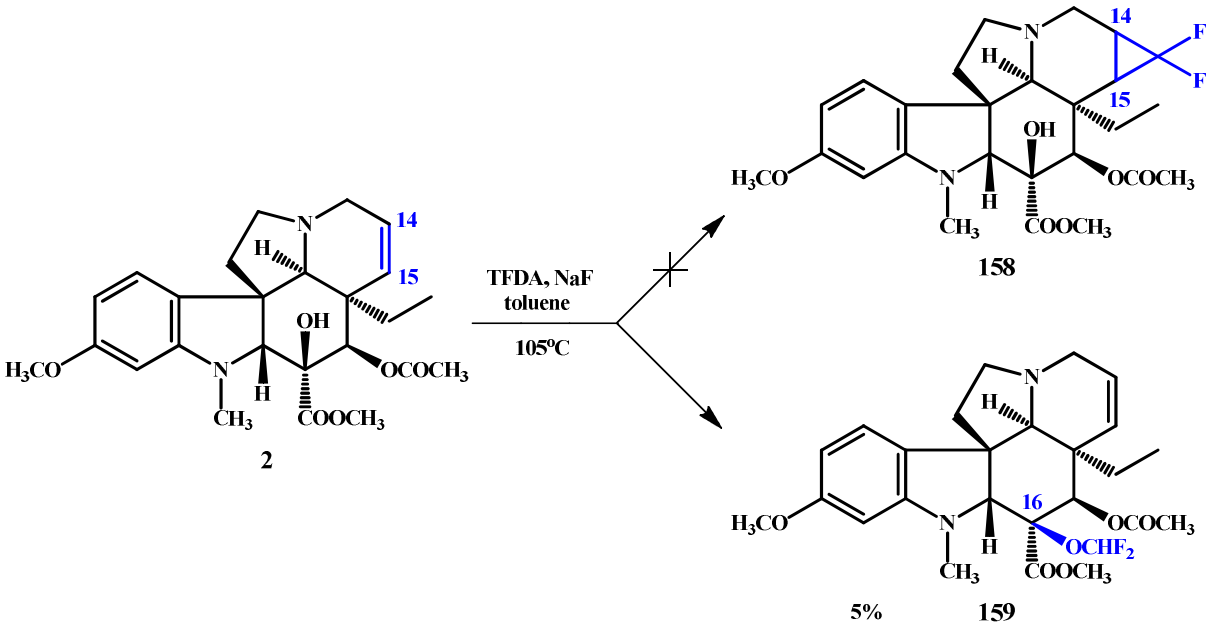
3 152

After this, we made attempts to fluorinate the vindoline (**2**), resulted in the mixture of three products containing fluoro-substituent (**155**, **156** and **157**) in the reaction performed with Selectfluor.

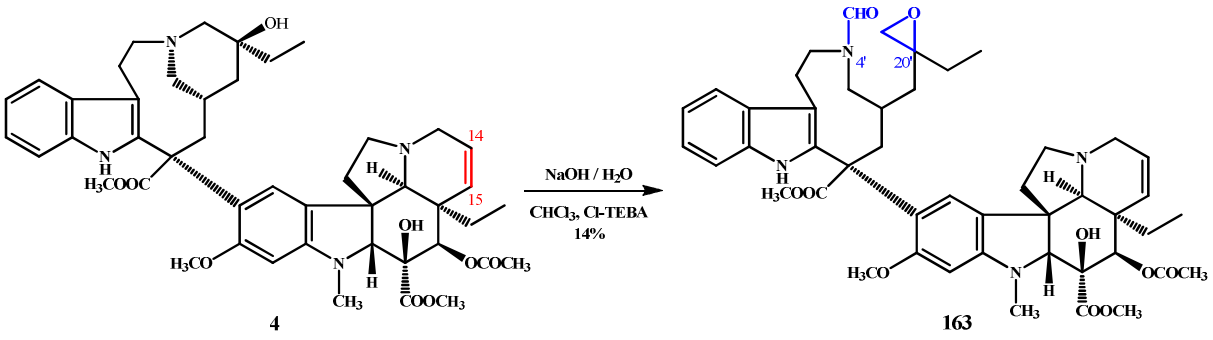


Performing the fluorination with xenon difluoride, the quinoidal structure **156** was obtained, in which the fluoro atom was coupled to the bridgehead carbon atom at position 8.

Finally the question arose how the biological effect would change, if we formed a difluoro- (**158**), and dichlorocyclopropane ring (**160**) instead cyclopropane ring of vindoline at position 14,15, so we planned to synthesize these two compounds. We tried to form the difluorocyclopropane ring with TFDA which is an efficient difluorocarbene precursor, but instead of the expected product (**158**) a vindoline derivative containing a difluoromethyl ether group at position 16 (**159**) was obtained.



After that, we examined the building of the dichlorocyclopropane ring. Performing the classical dichlorocarbene reaction (chloroform, sodium hydroxide, ClTEBA) with vindoline (**2**), 10-formylvindoline (**161**) was obtained known in the literature. Performing the reaction with vinblastine (**4**) no dichlorocyclopropanation took place either, instead, we could isolate compound **163**, in which the piperidine ring of the catharanthine part opened and an oxirane ring was formed.



4 **163**

5. THESES

1. The reaction between galantamine and diazomethane in the presence of palladium(II) acetate did not result in the expected 7,8-cyclopropanogalanthamine, but a methylene unit was inserted into the aromatic ring, when the product was the cycloheptatriene derivative of galanthamine. [1]

2. Using diethylzinc and diiodomethane in Simmons-Smith reaction we built a cyclopropane ring on the double-bond at position 14,15 of 10-bromovindoline, then we synthesized the 14,15-cyclopropanovindoline by removal of the bromo atom at position 10 in hydrogenolytic reaction, and through the hydrazide derivative using the azide-coupling methode we prepared its derivatives conjugated with L- and D-tryptophan. [3, 4, 5]

3. Coupling 14,15-cyclopropanovindoline and catharanthine, we synthesized the 14,15-cyclopropano-anhydrovinblastine, whose oxidation resulted in the 14,15-cyclopropanovinblastine. [3, 5]

4. We synthesized the 14,15-cyclopropane derivative of vincristine by oxidating 14,15-cyclopropanovinblastine with chromium trioxide. [3, 5]

5. The ring contraction reaction of 14,15-cyclopropano-anhydrovinblastine led to the 14,15-cyclopropane derivative of vinorelbine. [3, 6]

6. Oxidation of the 14,15-cyclopropane derivative of vinorelbine with chromium trioxide led to the 1-*N*-formyl-14,15-cyclopropanovinorelbine which is a vincristine derivative while its hydration afforded the nor-5'-14,15-cyclopropanovinblastine which is a vinblastine derivative. [3, 6]

7. From the 14,15-cyclopropanovinblastine with hydrazine hydrate we synthesized the hydrazide derivative out of which through the azide analogue in a modified Staudinger reaction using triphenylphosphine we prepared the 14,15-cyclopropanovindesine. [3]

6. POSSIBLE APPLICATIONS

Among the compounds prepared and sent to biological investigation the vindoline derivatives condensed with a cyclopropane ring and conjugated with tryptophan methyl ester showed promising antitumor effect on leukemia cells. In the case of dimer alkaloids containing a cyclopropane ring the cytostatic activity of 14,15-cyclopropano-vinblastine and –vincristine is slightly different from the effect of vinblastine and vincristine being used in anticancer therapy. The analogues containing a cyclopropane ring inhibit cell proliferation better or destroy the tumor cells on most of the investigated cell lines. The 14,15-cyclopropanovinblastine used in the case of leukemia, non-small cell lung cancer, colon cancer, melanoma and breast cancer, while the 14,15-cyclopropanovincristine used in the case of colon cancer, melanoma, ovarian cancer and prostate cancer shows outstanding antitumor effect. Among the vinorelbine derivatives the 14,15-cyclopropanovinorelbine has the most important effect, causing significant cell death in the case of non-small cell lung cancer, colon cancer, CNS cancer, melanoma and breast cancer. The 1-*N*-formyl-14,15-cyclopropanovinorelbine has excellent selectivity on one of the cell line of colon cancer. After having these compounds patented, they may play a role in the process of becoming a drug and being applied in anticancer therapy.

7. PUBLICATIONS

7.1. Publications related to the PhD thesis

1. **Keglevich P.**, Kovács P., Hazai L., Sánta Zs., Dubrovay Zs., Háda V., Szántay Cs. Jr., Kalaus Gy., Szántay Cs.: A new derivate of galanthamine: methylene insertion into the aromatic ring in place of cyclopropanation. *Heterocycles*, **2012**, *84 (2)*, 1171–1178. [IF: 1,077]

2. **Keglevich P.**, Hazai L., Kalaus Gy., Szántay Cs.: Modifications on the Basic Skeletons of Vinblastine and Vincristine. *Molecules*, **2012**, *17*, 5893-5914. [IF: 2,428]

3. Szántay Cs., Hazai L., Kalaus Gy., **Keglevich P.**: New bis-indole alkaloids as anticancer drugs. **2013** (Hungarian patent application, P1300349)

4. **Keglevich P.**, Hazai L., Gorka-Kereskényi Á., Péter L., Gyenese J., Lengyel Zs., Kalaus Gy., Dubrovay Zs., Dékány M., Orbán E., Bánóczi Z., Ifj. Szántay Cs., Szántay Cs.: Synthesis and in vitro antitumor effect of new vindoline derivatives coupled with amino acid esters. *Heterocycles*, **2013**, *87 (11)*, 2299–2317. [IF: 1,077]

5. **Keglevich P.**, Hazai L., Dubrovay Zs., Dékány M., Ifj. Szántay Cs., Kalaus Gy., Szántay Cs.: Bisindole Alkaloids Condensed with a Cyclopropane Ring, Part 1. 14,15-Cyclopropano-vinblastine and -vincristine. *Heterocycles*, **2014**, *89 (3)*, 653-668. [IF: 1,077]

6. **Keglevich P.**, Hazai L., Dubrovay Zs., Sánta Zs., Dékány M., Ifj. Szántay Cs., Kalaus Gy., Szántay Cs.: Bisindole Alkaloids Condensed with a Cyclopropane Ring, Part 2. Cyclopropano-vinorelbine and its derivatives. *Heterocycles*, **2014**, *90 (1)*, DOI: 10.3987/COM-14-S(K)20 (accepted for publication) [IF: 1,077]

7.2. Other publications

1. Makó A., Bakó P., Szöllősy Á., Bakó T., Peltz Cs., **Keglevich P.**: Synthesis of chiral pyridino-15-crown-5 type ligands containing α-D-hexapyranoside unit and their application in asymmetric synthesis. ARKIVOC, **2009**, *vii*, 165-179. [IF: 1,057]

2. Nyitrai G., Kékesi O., Pál I., **Keglevich P.**, Csíki Zs., Fügedi P., Simon Á., Fitos I., Németh K., Visy J., Tárkányi G., Kardos J.: Assessing toxicity of polyamidoamine dendrimers by neuronal signaling functions. NANOTOXICOLOGY, **2012**, *6 (6)*, 576-586. [IF: 7,844]

7.3. Presentations related to the topics of the PhD thesis

1. **Keglevich P.**, Hazai L., Kalaus Gy., Dubrovay Zs., Háda V., ifj. Szántay Cs., Szántay Cs.: Új vindolinszármazékok szintézise. MTA Alkaloidkémiai munkabizottság ülése, 2011. május 16-17., Balatonalmádi

2. **Keglevich P.**, Hazai L., Kalaus Gy., Dubrovay Zs., Háda V., ifj. Szántay Cs., Szántay Cs.: Új, ciklopropángyűrűt tartalmazó, vegyületek szintézise. MTA Alkaloid- és Flavonoidkémiai munkabizottság ülése, 2012. május 14-15., Balatonalmádi

3. **Keglevich P.**, Hazai L., Kalaus Gy., Dubrovay Zs., Háda V., ifj. Szántay Cs., Szántay Cs.: Új, daganatellenes hatású, ciklopropángyűrűt tartalmazó vindolin- és vinblasztinszármazékok előállítása. Oláh György Doktori Iskola X. Konferenciája, 2013. február 7., Budapest

4. **Keglevich P.**, Hazai L., Kalaus Gy., Dubrovay Zs., Háda V., ifj. Szántay Cs., Szántay Cs.: Új, daganatellenes hatású, ciklopropángyűrűt tartalmazó vindolin- és vinblasztinszármazékok előállítása. MTA Alkaloid- és Flavonoidkémiai munkabizottság ülése, 2013. május 13-14., Balatonalmádi

5. **Keglevich P.**, Hazai L., Kalaus Gy., Dubrovay Zs., Dékány M.., ifj. Szántay Cs., Szántay Cs.: Ciklopropángyűrűvel kondenzált daganatellenes hatású dimer alkaloidok szintézise. MTA Alkaloid- és Flavonoidkémiai munkabizottság ülése, 2014. május 12-13., Balatonalmádi