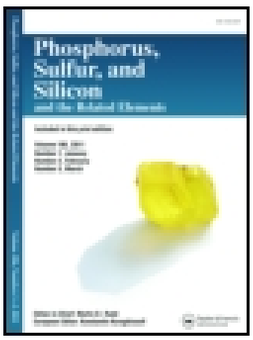

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Synthesis of vinca alkaloid–triphenylphosphine derivatives having potential antitumor effect

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ABSTRACT

The successful therapy of cancer is still unresolved. For that reason enormous efforts are being made to increase the effectiveness of anticancer drugs and reduce their side effects at least. An important approach to the development of selective molecules is the synthesis of drug conjugates, also called as "hybrids". Taking this into consideration two Vinca alkaloid derivatives conjugated with triphenylphosphine have been synthetized. The incorporation of the mentioned two pharmacophores in the same molecule could amplify the evolving cumulative antineoplastic impact and/or counterbalance the side effects. Thus a development of hybrid anticancer molecules has been started by synthesis of Vinca alkaloid–triphenylphosphine conjugates, which could have higher efficacies and safety profiles than the present drugs available in market.

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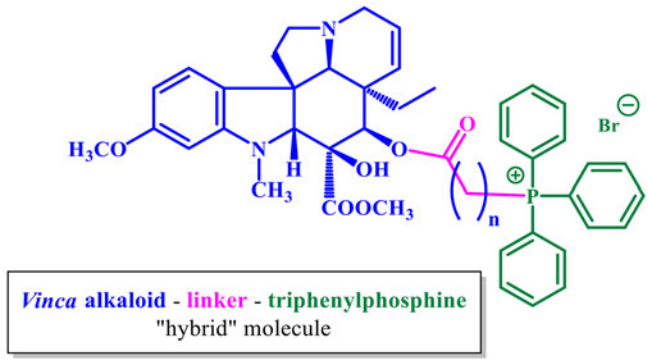
Anticancer drugs; triphenylphosphine; 17-desacetylvindoline; dimer alkaloids; hybrid molecules

GRAPHICAL ABSTRACT



Introduction

The dimer alkaloids vinblastine (vincaleukoblastine, VLB, **1**) and vincristine (VCR, **2**) (Figure 1), isolated from the Madagascar periwinkle *Catharanthus roseus* are remarkable representatives of the family called as "bisindole" alkaloids. Their structure consists of two monomers with an indole skeleton: derivatives catharanthine (**3**) and vindoline (**4**). VLB (**1**), VCR (**2**) and some of their semi-synthetic analogs have been playing a considerable part in cancer chemotherapy for decades[1,2] especially in the field of lymphomas and leukemia.

In order to improve the activity, the structures of the mentioned alkaloids have been modified using different methods.[3–5]

Another important approach in the development of selective anticancer drugs is the synthesis of special molecules called "hybrids". These derivatives are basically designed to react with multiple targets or to increase the effectiveness and/or decrease the coexisting side effects through the united molecule built by two or more beneficial pharmacophore parts. Building hybrid molecules is a well-known and widespread method in modern drug investigation, especially in the field of oncology.[6] Natural or originally natural molecules conjugated with (semi)synthetic compounds possessing at least two pharmacophores in one single derivative should be meant by "hybrids".

The main purpose of the project in question was to make and analyze some of these hybrids which may have reasonable safety profile and significant efficacy at the same time. Attempts have been made to get *Vinca* alkaloid conjugates coupled with synthetic pharmacophore compounds, first of all with triphenylphosphine (TPP), a generally known pharmacophore unit.

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Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/gpss.

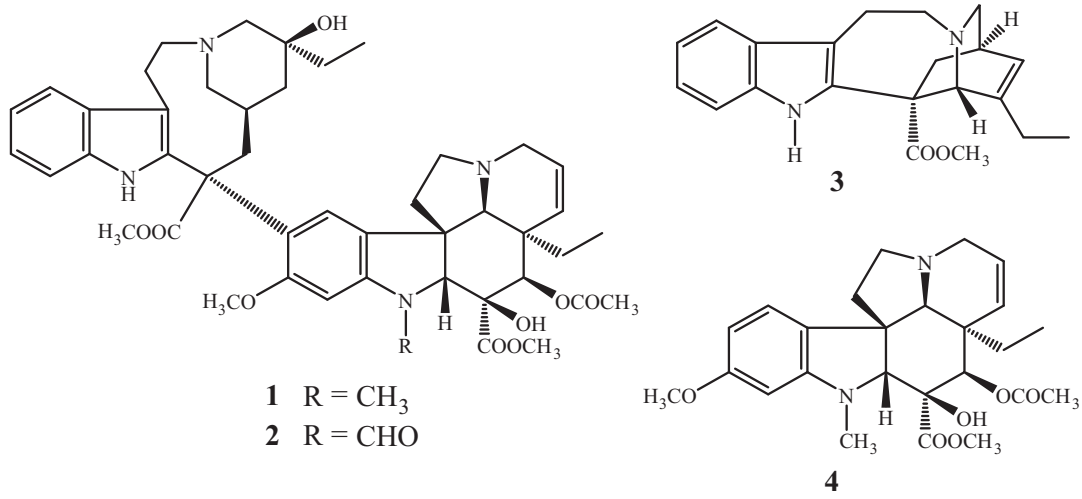


Figure 1. The alkaloids vinblastine (1), vincristine (2), catharanthine (3) and vindoline (4).

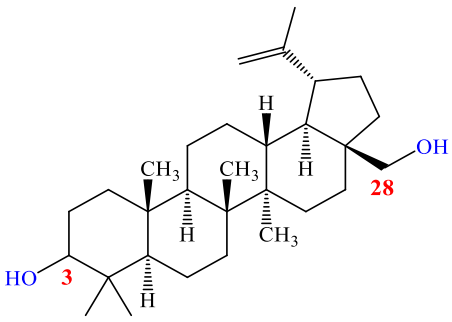


Figure 2. The structure of the triterpenoid betulin (5).

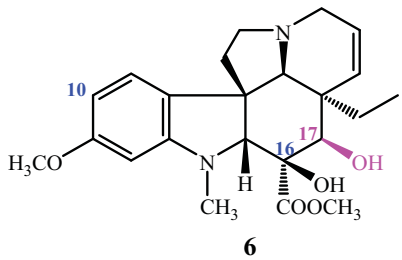


Figure 3. The structure of the 17-desacetylvindoline (6) with possible coupling positions.

A series of new TPP derivatives of the triterpenoid betulin (**5**) (Figure 2) were synthesized and evaluated for cytotoxic effects by Tsepaeva *et al.*[7]

In their experiments the TPP moiety was applied as a carrier group through an acyl linker to betulin (**5**) promoting cellular and mitochondrial accumulation of the compound. An enhanced antiproliferative activity toward vinblastine-resistant MCF-7[1] cells was demonstrated. Betulin (**5**) is a naturally occurring triterpenoid which can be isolated from the bark of birch trees.[8] This molecule has a pentacyclic ring structure and two hydroxyl groups in positions C3 and C28. It is famous for its biological activity and several examinations have shown the effectiveness of the derivatives of betulin (**5**) against variety of tumors *in vitro* and *in vivo*.[9]

We were interested in not only the TPP moiety which might be built into the *Vinca* alkaloids but in acyl linkers with different length of the chain. The length of the linker compound may affect the cytotoxic activity of the conjugated product. Noble *et al.* reported that minor modifications in the bisindole structure can result in major changes in the biological activity of the whole molecule.[10]

It is important to mention that vindoline (**4**) could be connected indirectly with linkers in several positions such as 10, 16, and 17. In this project the starting point was 17-desacetylvindoline (17-DV, **6**) (Figure 3). This material can be obtained by a simple hydrolysis of vindoline (**4**) in position 17.[11]

Table 1. Growth percent rates of the investigated molecules at NIH (9[15] and 11[16]) against variety of cell lines of different tumors.

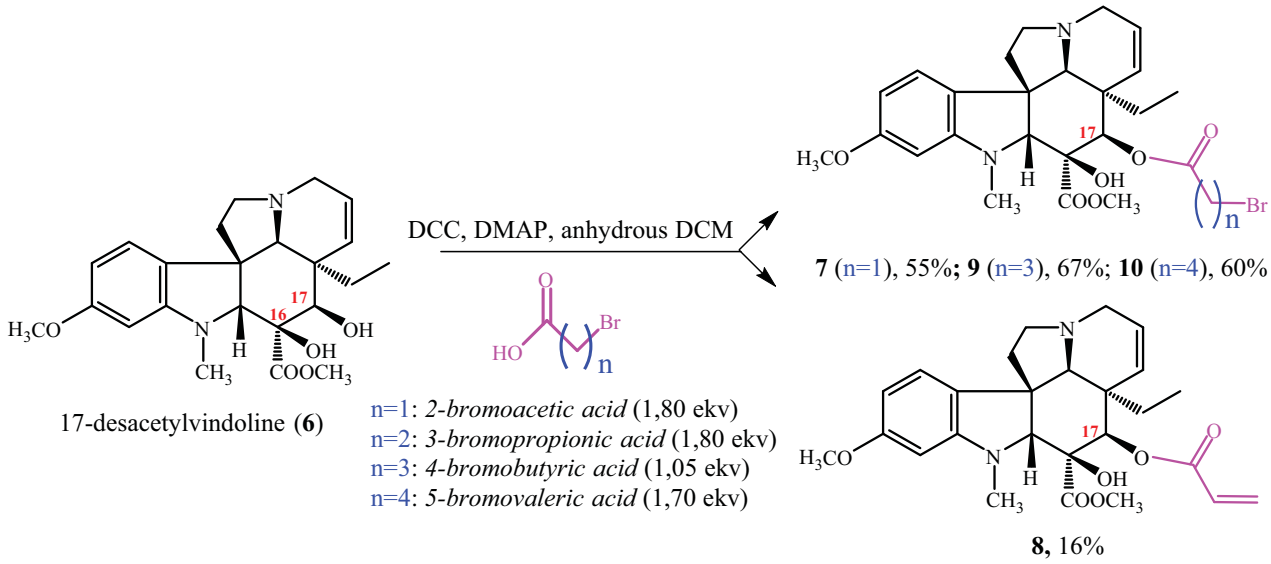
	9[15]	11[16]
Leukemia		
HL-60(TB)	90.48	**−53.10**
Non-small cell lung cancer		
HOP-92	77.57	**−40.05**
NCI-H522	79.00	**−35.39**
Colon cancer		
COLO 205	101.01	**−87.91**
Melanoma		
SK-MEL-2	103.22	**−65.31**
SK-MEL-5	84.26	**−91.47**
UACC-62	83.77	**−59.27**
Breast cancer		
MDA-MB-468	95.74	**−75.59**

9: 17-desacetylvindoline linked with 4-bromobutiric acid.
11: 17-desacetylvindoline–4-bromobutiric acid–triphenylphosphine hybrid.

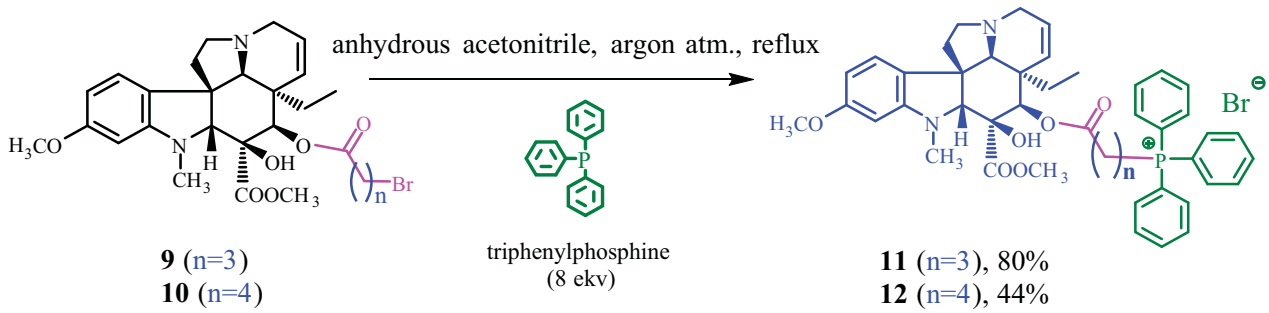
Results and discussion

17-DV (**6**) is a monomer which does not possess any biological activity but it is stable and relatively easy to work with. In contrast, VLB (**1**) or VCR (**2**) have antitumor activity, but also suffer from degradability. Therefore a hypothesis has been made predicting that not just dimeric *Vinca* alkaloids, but also an ineffective monomer—in our case 17-DV (**6**)—could be cytotoxic as part of a hybrid molecule.

[1]**MCF-7:** breast cancer cell line. It is the acronym of Michigan Cancer Foundation-7.



Scheme 1. Esterification of 2-bromoacetic acid, 3-bromopropionic acid, 4-bromobutyric acid and 5-bromovaleric acid.



Scheme 2. Synthesis of the *Vinca* alkaloid–TPP hybrids (11[16] and 12[17]).

Preparation of linker containing 17-desacetylvindoline (6) derivatives

The first step of our current work has been the investigation of the reactions of 17-DV (**6**) and the mentioned acyl linkers. α-, β-, γ- and δ bromocarboxylic acids have been used as conjunctives. First the 2-bromoacetic acid was investigated in coupling reaction with 17-DV (**6**) in the presence of *N,N′*-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)-pyridine (DMAP). The appropriate product (**7**) was successfully obtained. Then our work has been continued with the coupling reaction of 3-bromopropionic acid, however, we could not obtain the expected product, only its analog (**8**, 16%) after elimination of hydrogen bromide. In the case of 4-bromobutyric acid and 5-bromovaleric acid the expected products (**9**[15], 67% and **10**, 60%) were obtained. In addition, **10**, delta-valerolactone was also obtained as a byproduct. The above-mentioned reactions can be seen in Scheme 1.

The question arises why only the OH group in position 17 was acylated. This regioselectivity is the result of steric hindrance proceeded by the tertiary character of OH group in position 16.

Synthesis of 17-desacetylvindoline (6)–triphenylphosphine (TPP) hybrids

After the corresponding conjugates (**7**, **9**[15] and **10**) were produced with in-built linkers, the next step was to couple them with TPP. First an acetonitrile solution of product **7** was treated with TPP at room temperature then under reflux for a few hours. However, a pure product could not be obtained from the reaction mixture. As a continuation the remaining experiments were completed with the rest of the conjugates (**9**[15] and **10**, Scheme 2). The expected hybrid molecules were successfully obtained (**11**[16], 80% and **12**[17], 44%). The order of the addition of the reactants was important, the linkers were always coupled first with 17-DV (**6**) and only after with TPP to avoid working with salts.

Biology

The biological activity of two synthesized compounds (**9**[15] and **11**[16]) were investigated at the National Institutes of Health (NIH), US. The cytotoxic effect of the mentioned derivatives were measured on 60 different tumor cell lines of 9 often occurring cancer types *in vitro*. It was found that compound **9**[15] was ineffective, however, hybrid molecule **11**[16] showed increased antiproliferative activity. This fact proved our hypothesis that although *Vinca* alkaloid monomers do not show any biological activity on their own but TPP moiety can promote the anticancer efficacy when a monomer will be converted to a hybrid molecule. Compound **11**[16] has had especially significant inhibiting

effect in colon cancer (COLO 205), melanoma (SK-MEL-2 and SK-MEL-5) and breast cancer (MDA-MB-468). There was no cytotoxicity study regarding compound **10**, however, the analysis of hybrid molecule **12**[17] is currently in progress.

The NCI screening procedures have been already described[12] as well as the origins and processing of the cell lines.[12–14] In Table 1 the percentages of growth are listed for compounds **9**[15] and **11**[16] at the concentration of 10^{-5} M. The larger negative numbers show a more significant decrease of cell numbers and the smaller positive numbers mean greater growth inhibition. The results are summarized in Table 1. Only cell lines were represented where a minimum of one of the two investigated derivatives (**9**[15] and **11**[16]) reached at least -30% growth percent.

Conclusions

A production of *Vinca* alkaloid conjugates was proposed in conformity with the new trend of building "hybrids" in modern drug research.[6] A demonstrable antitumor effect was presumed by connecting a *Vinca* alkaloid monomer with triphenylphosphine (TPP). Coupling reactions of 17-desacetylvindoline (**6**) with TPP resulted in the expected hybrid molecules (**11**[16] and **12**[17]) originated by two previously produced conjugates (**9**[15] and **10**) linked with bromocarboxylic acids. The anticancer investigation of hybrid molecule **11**[16] showed that it had been effective in different tumor cell lines. Its promising result confirmed the expected positive role of TPP group which can facilitate *Vinca* alkaloid monomers to be potent antitumor drugs.

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[15] **Appearance:** white, crystalline. **Mp** 70–74 °C. **TLC** (dichloromethane-methanol =20:1); R_f = 0.60. **IR** (KB_r): 2963, 1738, 1616, 1598, 1502, 1435, 1247, 1225, 1168, 1121, 1028 cm^{-1}.

[16] **Appearance:** pale pink, crystalline. **Mp** 157–161 °C. **TLC** (dichloromethane-methanol =20:1); R_f = 0.14. **IR** (KBr): 3397, 2875, 1735, 1438, 1249, 1113, 742, 508 cm^{-1}.

[17] **Appearance:** pale pink, crystalline. **Mp** 112–116 °C. **TLC** (dichloromethane-methanol =20:1); R_f = 0.09. **IR** (KBr): 3391, 2932, 1735, 1615, 1501, 1438, 1249, 1112, 691, 531 cm^{-1}.