RESEARCH ARTICLE

Vinca Hybrids with Antiproliferative Effect

Authors
Szabolcs Mayer, Péter Keglevich* and László Hazai

Affiliation
Department of Organic Chemistry and Technology, Budapest University of Technology and Economics, H-1111 Budapest, Gellért tér 4., Hungary

Corresponding author
Péter Keglevich
E-mail: keglevich.peter@vbk.bme.hu

Abstract:
Vinca alkaloids used in anticancer therapy are the well-known vinblastine and vincristine as classical antitumor agents. These dimeric molecules consist of two monomers, vindoline and catharanthine, which have no particular activity on their own. The goal in our research work was to obtain derivatives of one of these, vindoline, resulted in molecules with important antiproliferative effect. This type of derivatives were hybrids; vindoline was conjugated with several pharmacophores with and/or without linkers. Pharmacophores were amino acid esters, steroids, triazoles, and flavones. In several cases, the synthesized compounds showed on some cell lines an even better effect than vinblastine.

Keywords: vinblastine, vindoline, hybrids, linkers, pharmacophores, antiproliferative activity

Introduction

Vinblastine (**1**) and vincristine (**2**) used in the anticancer therapy as classic tubulin inhibitors. They are *Vinca* alkaloids isolated from the periwinkle *Catharanthus roseus*, which contains in addition their building blocks, *i.e.* the monomers vindoline (**3**) and catharanthine (**4**); the former (**3**) is present in largest quantities in the plant (Fig. 1).

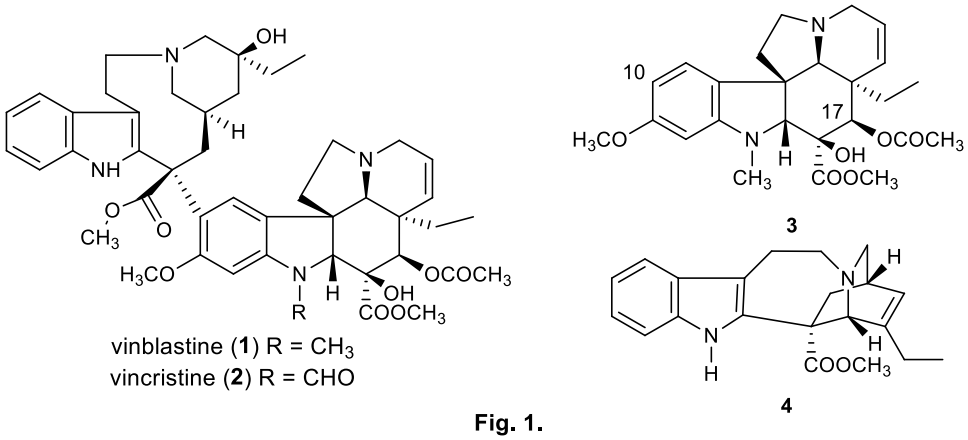


Fig. 1.

Although a number of efforts have been made to prepare new and less toxic molecules [1, 2], apart from some new derivatives as the fluorine substituted vinflunine, the ring-contracted vinorelbine, or the amide vindesine [2, *and references cited therein*], in the oncology no real breakthrough has happened yet.

Combination therapy, as cocktails of drugs, *e.g*. vinblastine (**1**), is a known and widespread option in the clinical practice. The concept of molecular hybridization, which started two decades ago, was thoroughly and comprehensively reviewed in all aspects [3, 4]. In short, the most common forms of hybrids are two distinct pharmacophores coupled covalently with or without spacers/linkers; the former allow a non-rigid connection between the parts of the structure. Hybrid molecules may provide opportunities for beneficial change in bioavailability, membrane penetration, drug-drug interactions, or drug resistance, *etc*.

Typical examples were presented in the area of tubulin polymerization inhibitors by Passarella and in Ref [5]. The different indole alkaloids vindoline (**3**), anhydrovinblastine and vinorelbine were coupled with the pharmacophores *N*-desacetylthiocolchicine (**a**), podophyllotoxin (**b**) and baccatin III (**c**) through two types of spacers (Fig. 2) resulting in hybrids **5a-c**, **6a-c**, and **7a, 7b**.

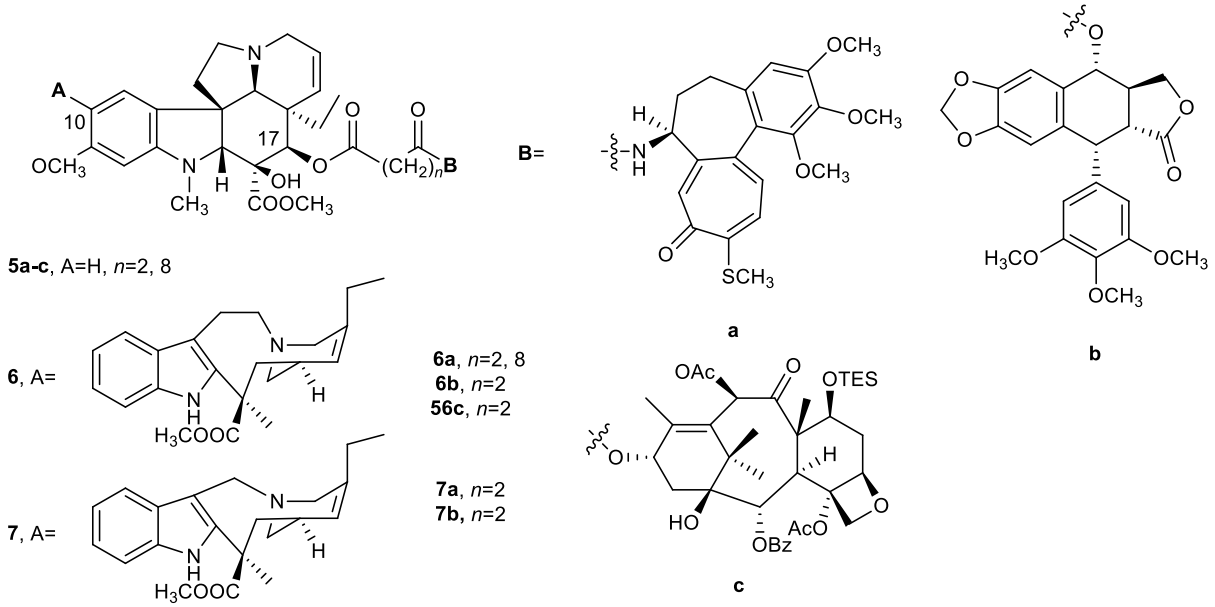
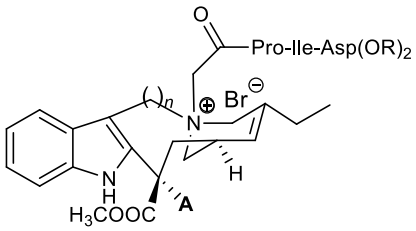


Fig. 2.

Although all three molecules (**a-c**) alone have anticancer effects, *N*-desacetylthiocolchicine (**a**) has several other biological effects, podophyllotoxin (**b**) presents antimitotic activity with strong toxicity, baccatin III **(c)** is closest to the real anticancer effect, as the precursor of paclitaxel. From the compounds synthesized **5a** (*n*=2) and **5c** (*n*=8) vindoline hybrids proved to be more potent than the controls during the antiproliferative studies investigated on human cancer cell line A549.

As a well-known example for hybrids without linker, was the phomopsin-*Vinca* conjugates. The tripeptide side chain (Pro-Ile-Asp) of antimitotic phomopsin A was built directly in the catharanthine nitrogen atom of anhydrovinblastine and vinorelbine (Fig. 3) as a quaternary salt (**8**), however, the cytotoxicity of the new derivatives against KB cell lines did not reach the values of control vinblastine (**1**) or anhydrovinblastine [6].

Substituted the vindoline ring with rather simple vindoline-like structures (**9a-e**) none of these compounds was active in the term of tubulin polymerization inhibition [7].



8, A=vindoline, *n=1,2*; R=H, benzyl

9, A=vindoline-like structures, **a-e,** *n=1,* 2; R=H, benzyl

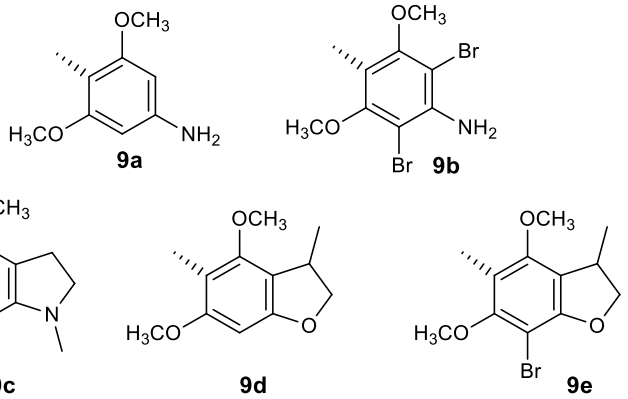
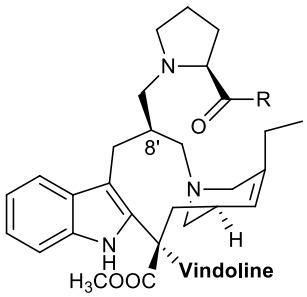
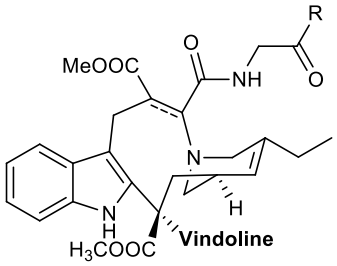


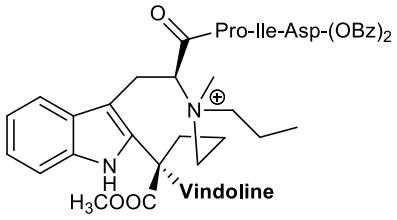
Fig. 3.



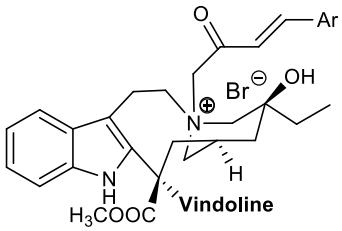
10, R=OMe (**a**), Ile-OMe (**b**), Ile-Asp-(OBn)$_2$ (**c**)



11, R=Pro-OMe (**a**), Pro-Ile-OMe (**b**), Pro-Ile-Asp-(OBn)$_2$ (**c**)



12



13, Ar=Cl-, OH-, MeO-phenyl, naphthyl

Fig. 4.

Gherbovet *et al.* [8, 9] tried to design the phomopsin A peptide in other vinblastine derivatives (Fig. 4), *e.g.* 7'-homoanhydro-vinblastine in different positions and with different spacers (**10**, **11**). Compounds **10a,b** showed similar activity as vinblastine on tubulin inhibition, saturated derivatives **11a** and **11b** were more active than enamines and have shown comparable effect with control vinblastine (**1**). The **12** ring-opened derivative and its some intermediates did not presented significant microtubule inhibitory activity on KB, MCF7 and MCF7R cell lines [10]. Some of compounds **13** presented selectively strong cytotoxic activity on human tumor cell lines KB, Hep-G2, LU-1 and MCF7, comparable with vinblastine even better than anhydrovinblastine [11].

Moreover, several vinblastine derivatives were evaluated containing, *e.g.* an octapeptide chain coupled in position 17 [12] with very strong activity against prostate cancer or vintafolide [see 2 *and reference cited therein*] conjugated through a spacer with folate coupled with a pentapeptide showing important anticancer effect in ovarian cancer treatment, however,

this big molecules really cannot be considered hybrids.

Results and Discussion

The aim of our work was to find molecular hybrids with one component of a *Vinca* alkaloid coupled with known and often used pharmacophores. So thus, the anticancer vinblastine and one of its building blocks, the inactive vindoline were selected.

The first vinblastine derivatives, which can be considered as hybrid molecules contained an amino acid, especially tryptophan, were conjugated with an oligoarginine cell-penetrating peptide chain [13]. Vinblastine was coupled with tryptophan methyl ester by the method of Bushana Rao [14] at the position 16, while the 17-OH was desacetylated. After hydrolysis of the tryptophan ester group the molecule was coupled with octaarginine chain (Fig. 5) resulting in the two epimers **14**(*R*) and **15**(*S*) separated.

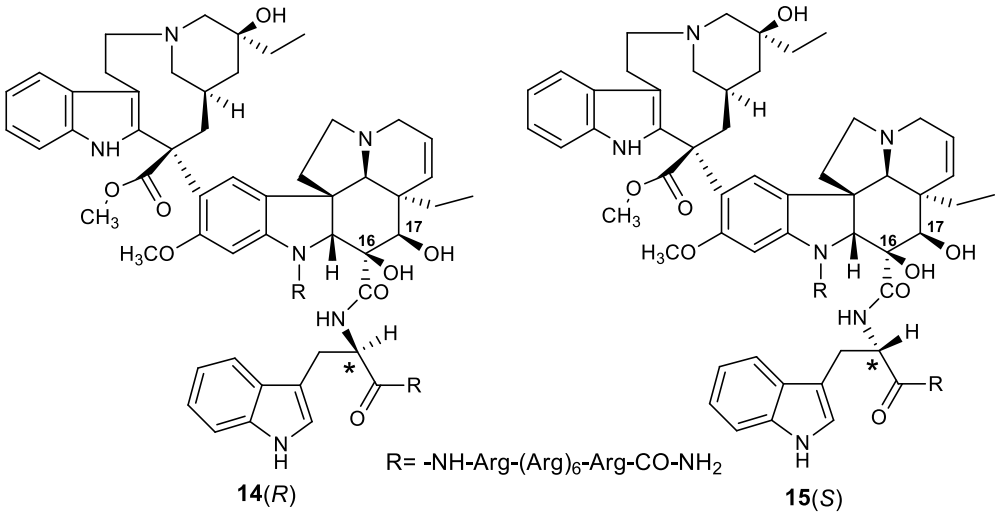


Fig. 5.

The antiproliferative activity of the compounds was investigated on HL-60 sensitive and resistant leukemia cells and on HeLa cells, too. The activities of conjugates **14** and **15** (IC_{50}: 0.97-1.26 μ*M*) were comparable with those of vinblastine sulfate control (IC_{50}: 1.26 μ*M*), however, conjugates were effective also on the resistant cells. Remarkable result is that compound **14**(*R*) preferentially destroys the mitotic spindle in the case of cancerous HeLa cells resulting the inhibition of cell cycle.

In the following, it was obvious to examine how the effect would change after the removal of one of the structural parts. For the rather simple chemistry and the reduced costs the vindoline monomer, which occurs most extensively in the plant, was investigated. The synthesis was similar to the previous method [15]. Although the antitumor effect of the obtained compounds **16**(*R*) and **17**(*S*) (Fig. 6) was significant, they did not reach the activity of the derivatives shown in Figure 5 above. On cell lines HL-60, MDA-MB-231 (IC_{50}: 10-15.1 μ*M*) and MCF-7 (IC_{50}: 5.1-6.4 μ*M*) the effect of the two epimers was almost the same, on

C26 and P388 tumor cell lines the epimer **17**(*S*) proved to be more effective.

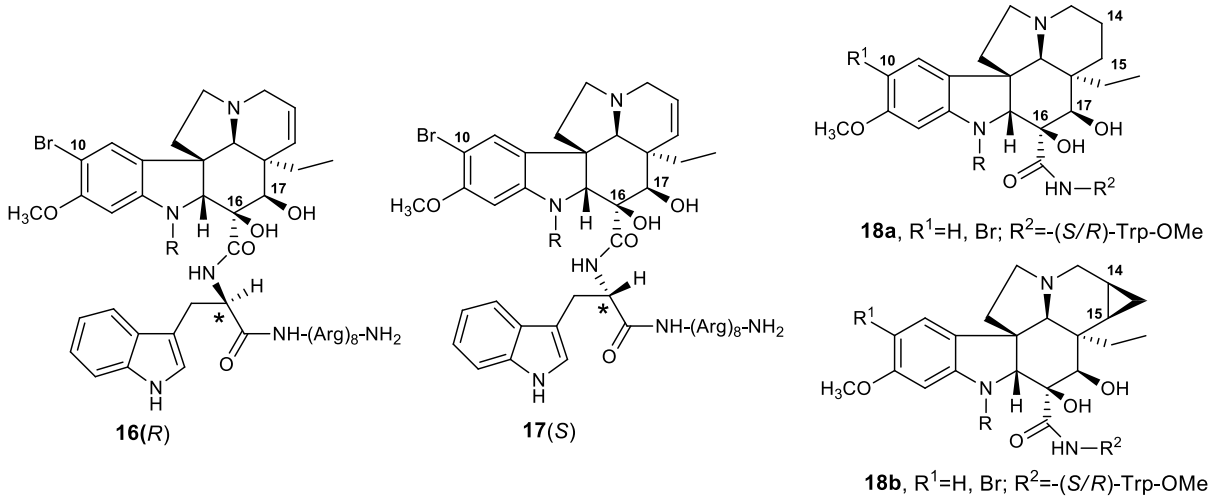


Fig. 6.

The effect of conjugates on the tumor growth was studied *in vivo* by using two tumor models. P388 and C26 cells were injected subcutaneously into mice. Conjugate **17**(*S*) exhibited a tendency to inhibit the tumor growth, while **16**(*R*) had essentially no inhibitory effect. It is interesting to mention that **17**(*S*) - under these conditions - was slightly, but not significantly more influential than vinblastine. In contrast, in the treatment of C26 colon carcinoma with the two isomers essentially no change in the tumor volume was observed.

Several modified vindoline structures were also synthesized [16], saturated derivatives at positions 14,15 obtained by catalytic reduction (**18a**) and 14,15-cyclopropano derivatives (**18b**) prepared with Simmons-Smith reaction. Among these only **18a** (R^1=H, R^2= -(*S*)-Trp-OMe) saturated compound showed moderate activity (IC_{50}: 27.5 μ*M*) on HL-60 cells, so thus the carrier peptide side chain was not built in the molecule.

New vindoline amino acid hybrids were synthesized containing spacer between the two pharmacophores [17]. The spacer was built into the positions 10 and 17 of vindoline skeleton. So thus, 10-aminovindoline by acylation with succinic anhydride followed amidation with (L)- and (D)-tryptophan methyl ester resulted in hybrids **19** and **20** (Fig. 7).

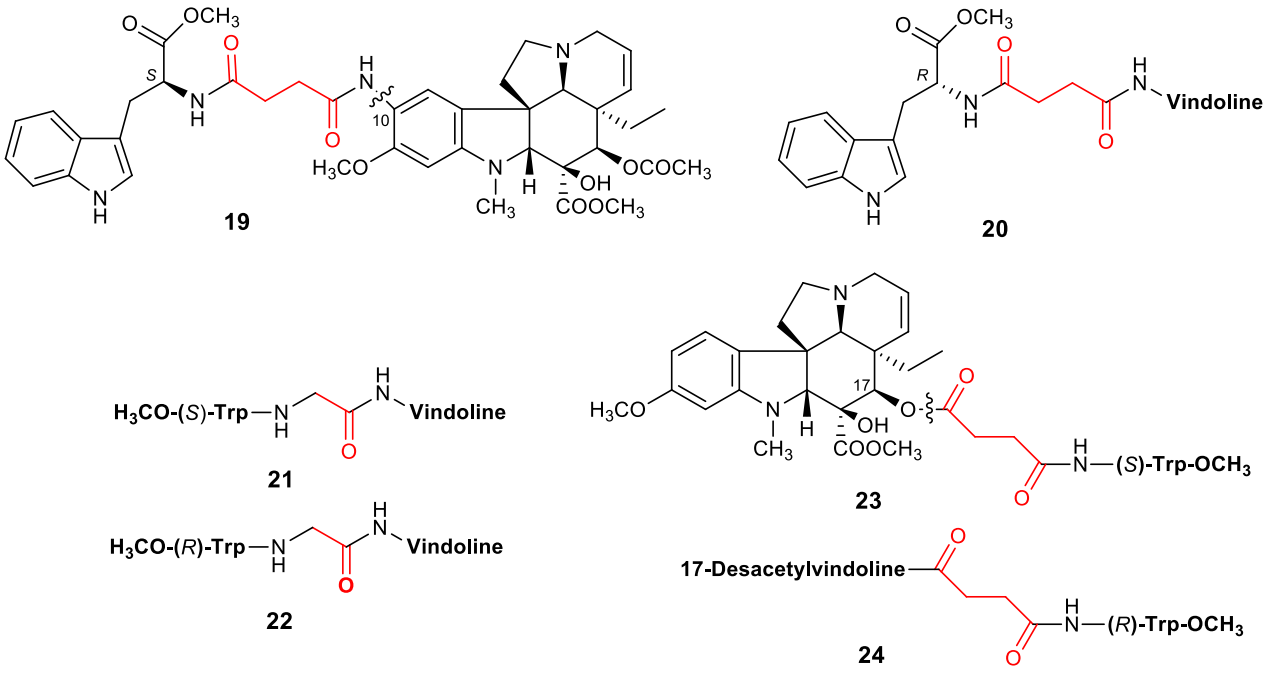


Fig. 7.

Similarly, **21** and **22** were prepared by chloroacetylation of 10-aminovindoline and after alkylation of (L)- and (D)-tryptophan methyl esters of the 10-chloroacetamido-vindoline, hybrids **21** and **22** were obtained containing a shorter and less flexible spacer. Position 17 of vindoline was coupled also by the same procedure mentioned above, from 17-desacetylvindoline and epimers **23**, **24** were synthesized also. The antiproliferative activity of compounds obtained was investigated on human gynecological cancer cells: HeLa, SiHa cervical, and MCF-7, MDA-MB-231 breast cell lines. On SiHa cells the control vinblastine was rather resistant (IC_{50}: 14.42 μ*M*), however, compound **23** proved to be more effective than vinblastine (IC_{50}: 6.01 μ*M*). Compound **24** showed moderate activity on all of the investigated cells. The vindolines coupled on position 10 proved to be inactive.

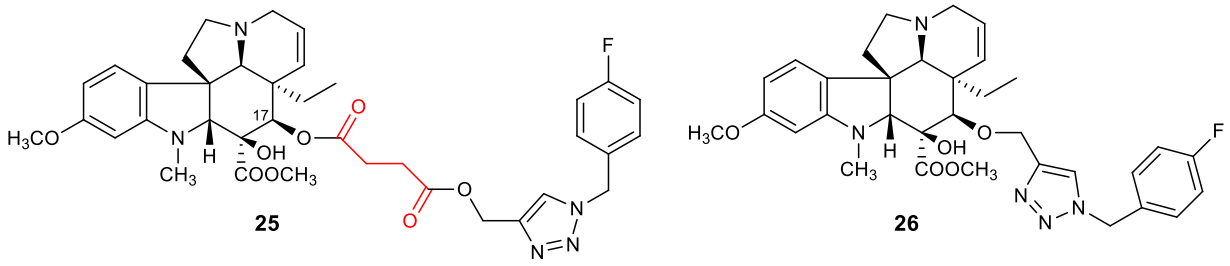


Fig. 8.

Further pharmacophores were also investigated [17]. Vindoline (**3**) was coupled with the well-known anticancer pharmacophore *N*-benzyltriazole derivative (Fig. 8), with a longer

and with a shorter spacer, resulting **25** and **26**, respectively. The synthesis was carried out in accordance with the mentioned before and in the literature presented, *i.e*. propargylation and then the correspondent click reaction. The obtained derivatives **25** and **26**, however, did not show significant activities.

A special group of hybrids were steroid derivatives, containing *e.g.* 5α-dihydrotestosterone and 19-nortestosterone which have anticancer activity [18]. The compounds were prepared with the known methods presented above. Vindoline (**3**) was coupled into the position 10 *via* an amino group (**27, 28**) and into the position 17 (**29**, **30**) by acylation of the 10-amino and the desacetyleted 17-hydroxyl group, respectively. Acylation took place with succinic anhydride developing the spacer (Fig. 9).

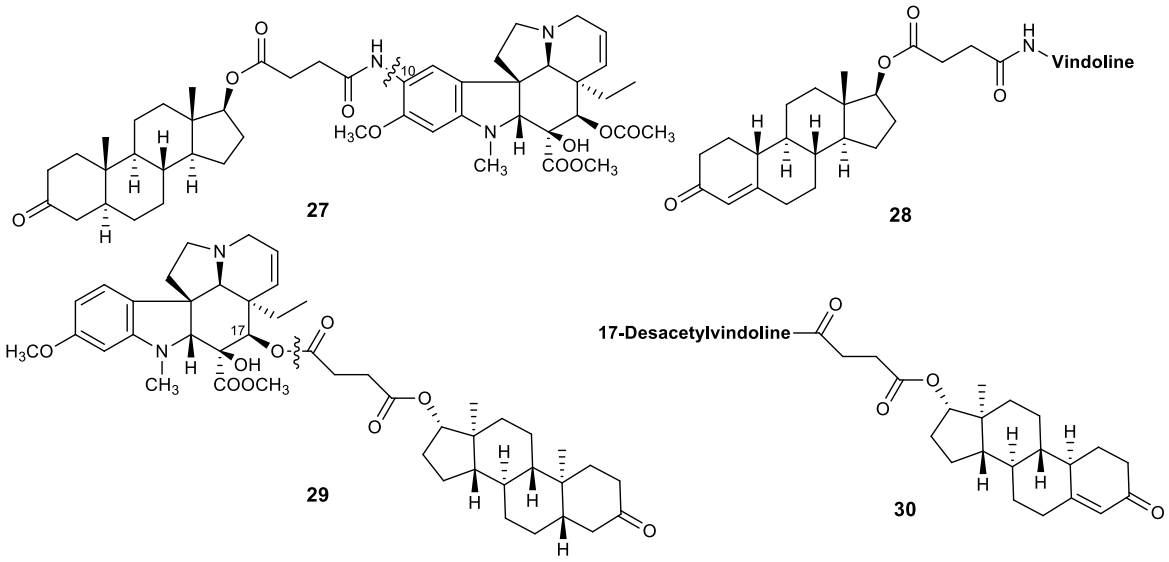
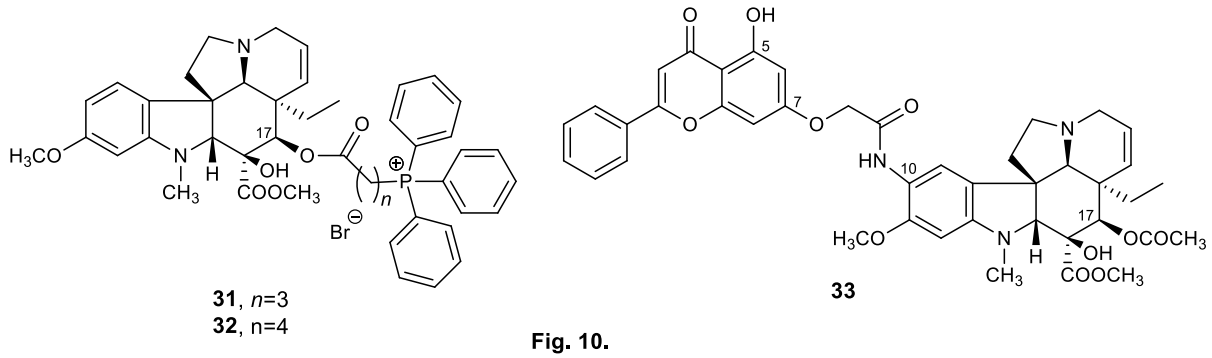


Fig. 9.

Hybrid **30** showed the highest cell number decreasing activity especially on colon cancer (COLO 205) and melanoma (SK-MEL-2, SK-MEL-5), displaying significant tumor cell inhibiting effect. The other compounds exhibited only low or moderate antitumor activity.

Triphenylphosphine (TPP) containing hybrids represent an interesting area [19, 20], *i.e*. the TPP moiety may act as a carrier group promoting the cellular and mitochondrial accumulation of the molecule in question. So thus, TPP was coupled to vindoline into the position 17 through an acyl linker with different chain lengths (Fig. 10). The antiproliferative activity of compounds **31** and **32** was quite similar. The 50% growth inhibition (GI_{50}/μ*M*) data show that the compounds are very effective on several cell lines, including colon, melanoma, ovarian and breast cancer (GI_{50}<0.5 μ*M*), however, compound **31** shows an outstanding value (GI_{50}=0.066 μ*M*) on cell line HOP-92 (non-small cell lung cancer).



31, *n*=3
32, n=4

33

Fig. 10.

An important group of hybrids formed with flavones; this type of compounds can be similar to flavone alkaloids [21]. Vindoline and chrysin were coupled with different positions, to the 10 and/or 17-position of vindoline substituting the 7-hydroxy group of chrysin with or without spacer. In addition, 5,7-Disubstituted chrysin containing two vindoline skeleton was also prepared. Nevertheless, only one of the compounds prepared proved to be effective. In the case of **33** the value of GI_{50} was below 1.5 μM on cell lines of non-small cell lung cancer (HOP-92), CNS cancer (SNB-75), melanoma (LOX IMVI), renal cancer (A498 and CAKI-1), prostate cancer (PC-3) and breast cancer (MCF7).

Conclusion

Vindoline was coupled with several types of synthetic and natural pharmacophores directly or through a spacer resulted in new type of antitumor hybrids containing *Vinca* alkaloids. The antiproliferative activity of some compounds proved to be comparable to the effect of vinblastine, even exceeded of that. Experience from previous research has shown that it is worthwhile to continue the synthesis of *Vinca* hybrids, especially by conjugating the vinblastine molecule itself instead of the vindoline, which can be rather considered as a model. In addition, the study of the toxicity of the most prominent molecules may decide the further trends in the research of anticancer hybrids. The use of further synthetic and/or semisynthetic *Vinca* alkaloids in building up the hybrid structures would be the scope of this anticancer project.

References

1. Keglevich P, Hazai L, Kalaus G, Szántay C. Modifications on the Basic Skeletons of Vinblastine and Vincristine. *Molecules*. 2012; 17(5):5893-5914. https://doi.org/10.3390/molecules17055893.
2. Mayer S, Keglevich P, Keglevich A, Hazai L. New Anticancer Vinca Alkaloids in the Last Decade - A Mini-Review. *Curr Org Chem*. 2021; 25(10):1224-1234. https://doi.org/10.2174/1385272825666210216123256.
3. Nepali K, Sharma S, Sharma M, Bedi PMS, Dhar KL. Rational approaches, design strategies, structure activity relationship and mechanistic insights for anticancer hybrids. *Eur J Med Chem*. 2014; 77:422-487. https://doi.org/10.1016/j.ejmech.2014.03.018.
4. Choudhary S, Singh PK, Verma H, Singh H, Silkari O. Success stories of natural product-based hybrid molecules for multifactorial diseases. *Eur J Med Chem*. 2018; *151*:62-97. https://doi.org/10.1016/j.ejmech.2018.03.057.
5. Passarella D, Giardini A, Peretto B, et al. Inhibitors of tubulin polymerization: Synthesis and biological evaluation of hybrids of vindoline, anhydrovinblastine and vinorelbine with thiocolchicine, podophyllotoxin and baccatin III. *Bioorg Med Chem*. 2008; 16(11):6269-6285. https://doi.org/10.1016/j.bmc.2008.04.025.
6. Ngo QA, Roussi F, Cormier A, et al. Synthesis and Biological Evaluation of Vinca Alkaloids and Phomopsin Hybrids. *J Med Chem*. 2009; 52(1):134-142. https://doi.org/10.1021/jm801064y.
7. Ngo QA, Roussi F, Thoret S, Guéritte F. Elaboration of Simplified Vinca Alkaloids and Phomopsin Hybrids. *Chem Biol Drug Des*. 2010; 75(3):284-294. https://doi.org/10.1111/j.1747-0285.2009.00922.x.
8. Gherbovet O, Coderch C, Alvarez MCG, et al. One-Pot Synthesis of Vinca Alkaloids-Phomopsin Hybrids. *J Med Chem*. 2014; 57(12):5470-5476. https://doi.org/10.1021/jm500530v.
9. Gherbovet O, Sanchez-Murcia PA, Alvarez MCG, et al. Synthesis and evaluation of hybrid molecules targeting the vinca domain of tubulin. *Org Biomol Chem*. 2015; 13(10):3144-3154. https://doi.org/10.1039/C4OB02114B.
10. Rannoux C, Roussi F, Martin M-T, Guéritte F. Elaboration of vinblastine hybrids using a reactive *in situ* generated *N*-carboxyanhydride. *Org Biomol Chem*. 2011; 9(13):4873-4881. https://doi.org/10.1039/C0OB01065K.
11. Ngo QA, Nguyen LA, Vo NB, et al. Synthesis and antiproliferativeactivity of new vinca alkaloids containing an α,β-unsaturated aromatic side chain. *Bioorg Med Chem Lett*. 2015; 25(13):5597-5600. https://doi.org/10.1016/j.bmcl.2015.10.040.
12. Brady SF, Pawluczyk JM, Lumma PK, et al. Design and Synthesis of a Pro-Drug of Vinblastine Targeted at Treatment of Prostate Cancer with Enhanced Efficacy and Reduced Systemic Toxicity. *J Med Chem*. 2002; 45(21):4706-4715. https://doi.org/10.1021/jm020139f.

13. Bánóczi Z, Gorka-Kereskényi Á, Reményi J, et al. Synthesis and in Vitro Antitumor Effect of Vinblastine Derivative-Oligoarginine Conjugates. *Bioconjug Chem*. 2010; 21(11):1948-1955. https://doi.org/10.1021/bc100028z.
14. Bhushana Rao KSP, Collard MPM, Dejonghe JPC, Atassi G, Hannart JA, Trouet A. Vinblastin-23-oyl amino derivatives: chemistry, physicochemical data, toxicity, and antitumor activities against P388 and L1210 leukemias. *J Med Chem*. 1985; 28(8):1079-1088. https://doi.org/10.1021/jm00146a017.
15. Bánóczi Z, Keglevich A, Szabó I, et al. The effect of conjugation on antitumor activity of vindoline derivatives with octaarginine, a cell-penetrating peptide. *J Pept Sci*. 2018; 24(10):e3118. https://doi.org/10.1002/psc.3118.
16. Keglevich P, Hazai L, Gorka-Kereskényi Á, et al. Synthesis and *in vitro* Antitumor Effect of New Vindoline Derivatives Coupled with Amino Acid Esters. *Heterocycles*. 2013; 87(11):2299-2317. https://doi.org/10.3987/COM-13-12827.
17. Keglevich A, Dányi L, Rieder A, et al. Synthesis and Cytotoxic Activity of New Vindoline Derivatives Coupled to Natural and Synthetic Pharmacophores. *Molecules*. 2020; 25(4), 1010-1028. https://doi.org/10.3390/molecules25041010.
18. Keglevich A, Zsiros V, Keglevich P, et al. Synthesis and In Vitro Antitumor Effect of New Vindoline-steroid Hybrids. *Curr Org Chem*. 2019; 23(8):959-967. https://doi.org/10.2174/1385272823666190614113218.
19. Keglevich A, Szigetvári Á, Dékány M, Szántay C Jr, Keglevich P, Hazai L. Synthesis of vinca alkaloid–triphenylphosphine derivatives having potential antitumor effect. *Phosphorus Sulfur Silicon Relat. Elem.* 2019; 194(4-6):606-609. https://doi.org/10.1080/10426507.2018.1550780.
20. Keglevich A, Szigetvári Á, Dékány M, Szántay C Jr, Keglevich P, Hazai L. Synthesis and in vitro Antitumor Effect of New Vindoline Derivatives Coupled with Triphenylphosphine. *Curr Org Chem*. 2019; 23(7):852-858. https://doi.org/10.2174/1385272823666190524083236.
21. Mayer S, Keglevich A, Sepsey Für C, et al. Results in Chemistry of Natural Organic Compounds. Synthesis of New Anticancer Vinca Alkaloids and Flavone Alkaloids. *Chemistry*. 2020; 2(3):714-726. https://doi.org/10.3390/chemistry2030046.
22. Mayer S, Nagy N, Keglevich P, et al. Synthesis of Novel Vindoline-Chrysin Hybrids. *Chem Biodivers*. 2021; 19:e202100725. https://doi.org/10.1002/cbdv.202100725.

