Recent Development of Vinca-domain Anticancer Drugs Binding to Tubulin

Lalita Das

Department of Chemistry, Surendranath College, 24/2 M.G. Road, Kolkata-700009[1].

Date of Submission: 1st August, 2014 Date of Acceptance: 27th August, 2014

Abstract

Studies on vinca domain binding drugs were done in great details as they are recognized as a potential target for anticancer drug development. Their structures, properties, mode of action, success and failures have been discussed in short detail in this review. The toxicity level of these drugs towards the host cells and the extent of efflux of drugs by the P-glycoprotein mediated pump are also discussed.

Key words: Tubulin, Microtubules, Anticancer Drugs, Vinblastine, Vincristine, Hemiasterline, Halichondrin-B, Dolastatin 10, Cryptophycin.

1. Introduction

The vital role played by tubulin in eukaryotic cells are highlighted by the fact that tubulin is the proposed target for clinically used anticancer drugs. These antimicrotubuler drugs may be broadly categorized as either microtubule stabilizer or microtubule destabilizers.[1-5] Microtubules play an important role in eukaryotic cells. Alpha- and beta-tubulin, the main components of microtubules, have gained considerable interest because of their functions and biophysical properties and has become the subject of intense study. The addition of tubulin ligands can affect microtubule stability and function, including mitosis, cell motion and intracellular organelle transport. Tubulin binding molecules have generated significant interest after the introduction of the taxanes into clinical oncology and the general use of the

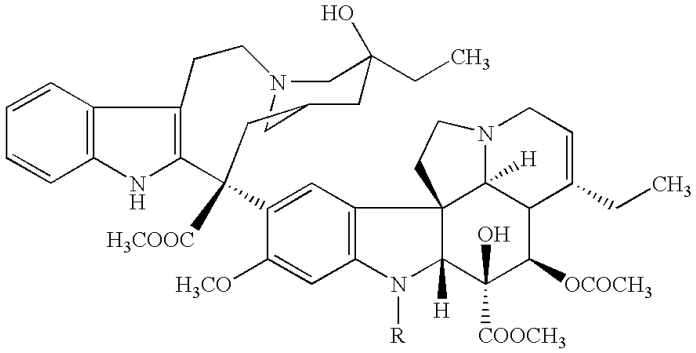
Email: das.lalita1@gmail.com

vinca alkaloids. These compounds inhibit cell mitosis by binding to the protein, tubulin in the mitotic spindle and preventing polymerization or depolymerization into the microtubules. This mode of action is also shared with another natural agent called, colchicine. Apart from vinblastine and paclitaxel, extensive research has been going on to find out more new drugs to overcome the limitations associated with acquired multidrug resistance due to expression of pgp pump, mutation in the tubulin gene, occurrence of various tubulin isotypes and side effects like neurotoxicity. Though success has been achieved in many cases, particularly, in case of combination therapy, still there is room for improvement as high mortality rate due to cancer is a concern and a big challenge for modern scientists.

2. Vinca Domain Binding Drugs of Plant and Fungal Origin

Vinblastine (VLB) and Vincristine

From the clinical point of view, vinca alkaloids, especially vinblastine and vincristine are the most important antimitotic drugs. Vinblastine derived from *Catharanthus (vinca) roseus* (alkaloid family) and vincristine derived from plant vinca *rosea Linn* (Figure 1). This effect is mediated by the interaction of these drugs with tubulin/microtubule system.



Vinblastine: R = CH_3
Vincristine: R = CHO

Figure 1. Structure of Vinblastine and Vincristine

They diminish the microtubule dynamics by suppressing dynamic instability at both ends of microtubules.[6] Both of them favor the formation of spirals, tubules and paracrystals at high concentrations.[7, 8] In all cases, the normal microtubule function is hampered. The

Vinca alkaloids bind to the β-subunit of tubulin dimers at a distinct region. They bind to tubulin rapidly, and this binding is reversible and independent of temperature (between 0°C and 37°C). Vinca alkaloids bind to the microtubule directly. They do not first form a complex with the soluble tubulin nor do they copolymerize to form the microtubule, however, they are capable of bringing about a conformational change in tubulin in connection with tubulin self-association.[9] Vinca alkaloids bind to the tubulin with high affinity at the microtubule ends but with low affinity at the tubulin sites present along the sides of the microtubule cylinder. The binding of these drugs at the high affinity sites results in strong kinetic suppression of tubulin exchange even at low drug concentration, while their binding to the low affinity sites in relatively high drug concentration depolymerizes microtubules.[10]

Attempts to localize the binding site for VLB to either α or β subunit of tubulin is achieved with limited success. From the cross-linking studies with bifunctional cross-linking agents (N, N′-ethylene) bis-iodoacetamide, it is observed that Cys β 239-Cys β 354 cross-linking is enhanced by VLB but Cys β 12-Cys β 201 or Cys β 211 is inhibited. Recently, the X-ray crystal structure of vinblastine bound to tubulin in a complex with the RB3 protein stathmin-like domain (RB3-SLD) complex has been determined at 4.1 Å resolution (Figure 2).[11]

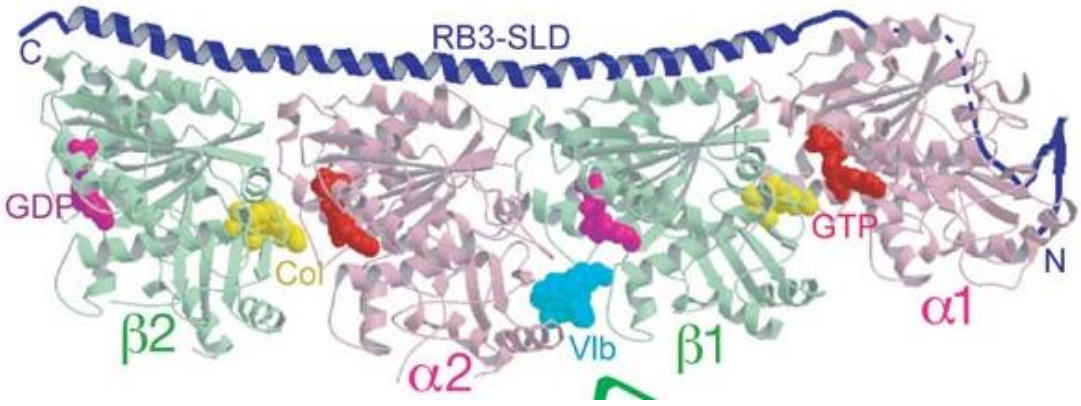


Figure 2. Vinblastine-tubulin crystal struc-ture [adapted from Nature. 2005; 435, 519-22.]

3. **Vinca Domain Binding Drugs of Marine Sponge Origin**

Hemiasterline

It has been found that naturally occurring small peptides of marine origin (Cymbastela *sp*.) have very good potential for inhibition of microtubule assembly (Figure 3).[12] Many of them are active against human xenograft tumors in immunodefficient mice.[13,14] Hemiasterline like dolastatin 10 and cryptophycin protects the colchicine binding activity of tubulin. Their binding sites are different from vinca-alkaloid binding site and inhibit vinblastine binding in a noncompetitive fashion (K_i=2.0 μM).[15] They impede nucleotide exchange on tubulin resulting oligopolymer formation. In contrast, all drugs without oligomer formation capacity, e.g., maytansine, halichondrin-B, are devoid of such colchicines-site stabilizing activity.[16-18]

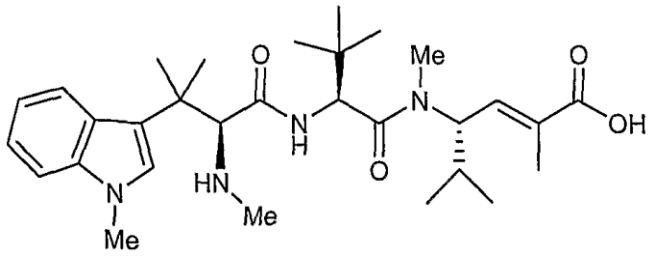


Figure 3. Structure of Hemiasterline

Halichondrin-B

This polyether macrolide (Figure 4) of marine origin was isolated from the marine sponge *Halichondria okadai* and subsequently from a more unrelated sponge, *Axinella* species.[19] Homohalichondrin-B, a less potent derivative of halichondrin, was also isolated, but for their reduced toxicity and very low availability in comparison to halichondrin-B, detailed studies were not done with this derivative. This compound (halichondrin-B) inhibits microtubule assembly *in vitro* and *in vivo* and inhibits the binding of vinblastine to tubulin in a noncompetitive manner (apparent K_i = 5.0 μM). It does not stabilize colchicine binding to tubulin like other noncompetitive inhibitors of peptide origin (Dolastatin 10).[20]

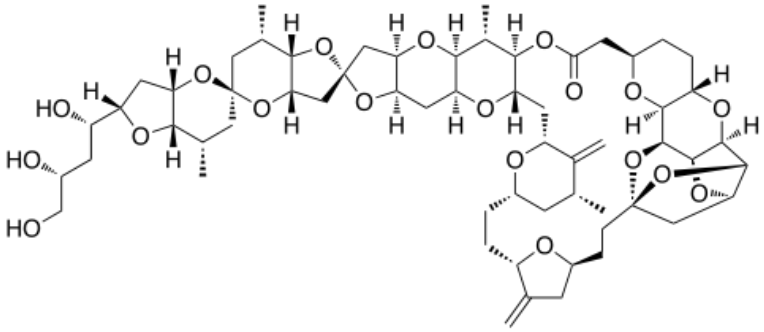


Figure 4. Structure of Halichondrin-B

Halichondrin-B inhibits tubulin dependent GTP hydrolysis, interferes with the exchange of GTP on β-tubulin in a highly temperature dependent fashion, being more extensive at low temperatures. Halichondrin-B and homohalichondrin-B inhibit the formation of intra-chain cross links between two sulfhydryl groups in β-tubulin like dolastatin 10 and vinblastine but are inactive in alkylation by iodoacetamide.[21] Another important effect of this drug is that, it enhances the exposure of hydrophobic areas of Tubulin, whereas homo derivative of this drug, like vinblastine and dolastatin 10, is inactive. Therefore, in spite of binding to the same "vinca-domain", these groups of drugs also have differences in their behaviors towards tubulin structure.

Recently, the discovery of synthetic route for halichondrin-B indicates that the antimitotic activity lies in the macrocyclic lactone C_1-C_{38} moiety. This important Information helps to develop synthetic analogues ER-076349 and ER-086526, which retain the antimitotic activity of parent compound. ER-076349 and ER-086526 differs in their C_{35} alcohol and amine substituents respectively, but the second is more potent than the first. The reason behind this observation is that ER-076349 is metabolized more readily than the other compound and binding of ER-086526 is less reversible compared to the other. This property makes them more effective for in vivo experiments than vinblastine and paclitaxel when they are used as internal standards.[22]

Dolastatin 10

Dolastatin 10 (Figure 5) is a pseudo peptide containing four unusual amino acids and isolated from the marine shell less mollusk *Dolabela auricularia* but subsequently it is synthesized by Pettit *et al.*[23]

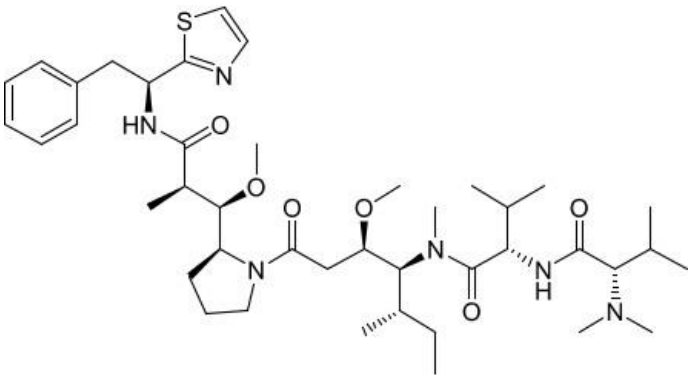


Figure 5. Structure of Dolastatin 10

The apparent target of this drug is the protein, tubulin. This peptide inhibits microtubules assembly (IC_{50}=1.2 μM) and arrests cells at the metaphase stage of cell division. It is a noncompetitive inhibitor of vinblastine binding and it also inhibits GTP exchange.[19] The binding stoichiometry of dolastatin 10 with tubulin is not known very clearly. Attempts to isolate drug tubulin complex is met with a limited success. At low drug concentration, the distinct species obtained are complex containing two drug molecules per two molecules of tubulin dimers. At higher drug concentrations, massive polymer of aberrant morphology (tightly coiled spirals) are obtained.[24] Dolastatin 10 is a potent inhibitor of a number of cancer cell lines and is active against human sarcoma, melanoma and ovarian xenographt in nude mice. This drug leads to apoptosis by bcl-2 phosphorylation.[25, 26]

Dolastatin 10 has much more cytotoxic effect than vinblastine because of its 15-19 times more retention power within the cell. Vinblastine within the cell is subjected to rapid efflux than dolastatin 10 in absence of ATP indicating that the entry of this drug is mediated through a active transport process. Dolastatin 10 is accumulated within the cell through passive diffusion and no detectable efflux is observed for this drug for at least two hour in a drug free medium. The potency of this drug is due to the better retention of this drug and tighter binding to tubulin. Like paclitaxel-estramustine combination therapy this drug also produces synergistic effect when applied in combination with bryostatin.[27, 28]

Dolastatin 10 has undergone phase II clinical trial.[29] This drug produces bone marrow toxicity and a set of local reactions. Peripheral neurotoxicity is another problem associated with this drug. Microtubules are essential components for axonal transport in neurons and this drug inhibits the function of Schwann cells, which limits this drug from repeated applications. The failure of this drug despite showing antimitotic action in the clinical study

is due to the lack of a balance between efficacy and toxicity. So they have poor therapeutic index.

4. Vinca Domain Binding Drugs of Microbial Origin

Cryptophycin

Cryptophycin is an antimitotic compound isolated from the cyanobacterium (*Nostoc* species) (Figure 6). The main target of the cryptophycin-action is the tubulin/microtubule system. MT assembled in the presence of paclitaxel is stable upon treatment with cryptophycin suggesting cryptophycin binds to unpolymerized tubulin. Several characteristics of cryptophycin

binding to microtubule system are as follows: Cryptophycin binds to tubulin dimer instantaneously to form tubulin-cryptophycin complex. Binding is temperature independent and irreversible. Two types of binding sites are observed for cryptophycin on tubulin,[30, 31] one high

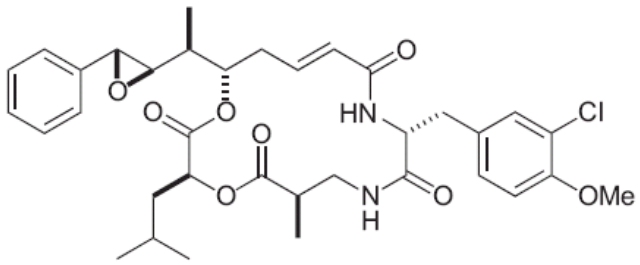


Figure 6. Structure of Cryptophycin

affinity-binding site along with a number of lower affinity binding sites. It inhibits the binding of vinblastine to tubulin in a noncompetitive fashion.[18] Similarly, reversal of this phenomenon, that is the inhibition of cryptophycin binding on tubulin, is also observed by vinblastine. In electron microscopy, ring type of structures is visible during tubulin-cryptophycin complex, which is in contrast with the effect of vinblastine binding to tubulin, which form spirals at high concentration. It binds to the end of the microtubule but not to its surface like paclitaxel, which is known from the study of Panda.[32] It forms poorly reversible complex with tubulin and the binding stoichiometry is 5 molecules of the drug per microtubule end. At this dose it alters the polymer mass by only 15% but suppressed the dynamicity by 50%. This explains the reason why this drug is so potent and capable of

exerting its antimitotic action at very low concentration. This lower drug concentration is not only sufficient to stabilize microtubule dynamics but also not producing too much cytotoxicity for the host cell. It increases the time spent by the microtubules in the resting stage by decreasing the shortening rate of the microtubules. Cryptophycin is more potent compared to VLB. It blocks paracystal formation initiated by 5 μM VLB. More importantly, it is able to circumvent a common form of multiple drug resistance i.e. P-glycoprotein mediated efflux of anticancer drugs. The apparent irreversibility of the effects of cryptophycin on microtubules makes this drug useful both *in vitro* and in intact cells for structural studies.

A brief detail of ***vinca group*** of drug is enlisted in Table 1.

Drugs Binding to the Vinca-domain of Tubulin

Name of the drug	**Origin**	**Nature**	**Therapeutic uses**	**Stage of clinical development**
Vinblastine	Plant, *Cantharanthus (vinca) roseus*	Alkaloid	Hodgkin's disease, testicular germ cell cancer	In clinical use; 22 combination trials in progress
Vincristine	Plant, vinca *rosea Linn*	Alkaloid	Leukaemia, lymphomas	In clinical use; 108 combination trials in progress
Hemiasterlin e	Marine sponge (*Auletta sp*. and *Siphonochalina spp*.)	Peptide	-	Phase I
Dolastatin 10	Marine shellless mollusk (*Dolabela auricularia*)	Pseudo peptide	Potential vascular-targeting agent	Phase I; Phase II completed
Halichondri n B	Marine sponge (*Halichondria okadai* and *Axinella sp.*)	Polyether macrolid e	-	Phase I
Cryptophyci n 52	Cyanobacterium (*Nostoc* species)	Cyclic depsipept ides	Solid tumours	Phase III finished

Table 1

5. Conclusion

Though vinca group of drugs comprise one important category of antimitotic drugs, they are not free from limitations such as, sensitivity towards P-glycoprotein mediated pump and poor therapeutic index. To minimize cytotoxicity towards host cells, they are used in combination therapy with other drugs. Development of several drugs (e.g. Dolastatin 10) cannot be progressed further because of the associated peripheral neurotoxicity in cytoskeletal tubulin function. Targeted delivery of vincristine is sometimes attempted with the help of liposomal formulations.[33] Another novel approach is to tag antimitotic agents with the antibody raised against a specific tumor antigen. Taxol group of drugs, though not covered in this review, also suffers from similar type of problems. Besides its low solubility and complex chemical structure difficult to modify, cells often develop resistance towards taxol due to the variations in the isotype content and due to the mutations in the beta tubulin gene. So new approaches such as generating inhibitors of important regulators of cell cycle and targeted drugs have been designed to combat the problem of uncontrolled cell division and serve as potential anticancer drug of future.

References:

1. M.A. Jordan, D. Thrower, L.Wilson. *Cancer Res* **51,** 2212 (1991).
2. M.A. Jordan, R.J. Toso, D. Thrower, L.Wilson. *Proc Natl Acad Sci U S A.* **90**, 9552 (1993).
3. D.M. Bollag, P.A. McQueney, J. Zhu, O. Hensens, L. Koupal, J. Liesch, M. Goetz, E. Lazarides, C.M. Woods. *Cancer Res***. 55**, 2325 (1995).
4. D.T. Hung, J. Chen, S.L. Schreiber. *Chem Biol***. 3**, 287 (1996).
5. S.L. Mooberry, G. Tien, A.H. Hernandez, A. Plubrukarn and B.S. Davidson. *Cancer Research* **59,** 653 (1999).
6. R.J. Toso, M.A. Jordan, K.W. Farrell, B. Matsumoto, L. Wilson. *Biochemistry* **32,** 1285 (1993).
7. R.K.N. Warfield, G.B. Bouck. *Science* **186**,1219 (1974).
8. L. Wilson, M.A. Jordan, A. Morse, R.L. Margolis. *J Mol Biol***. 159**:125 (1982).
9. M.A. Jordan, L. Wilson. *Nature Reviews Cancer* **4**, 253 (2004).

10. M.A. Jordan. *Current Medicinal Chemistry-Anti-Cancer Agents* **2** (1), (2012).
11. B. Gigant, C. Wang, R.B. Ravelli, F. Roussi, M.O.Steinmetz, P.A. Curmi, A. Sobel, M. Knossow. *Nature.* **435**, 519 (2005).
12. E. Hamel. *Med Res Rev***. 16**, 207 (1996).
13. H.J. Anderson, J.E. Coleman, R.J. Andersen, M. Roberge. *Cancer Chemother Pharmacol* **39**, 223 (1997).
14. W.R. Gamble, N.A. Durso, R.W. Fuller, C.K. Westergaard, T.R. Johnson, D.L. Sackett, E. Hamel, J.H. Cardellina 2nd, M.R. Boyd. *Bioorg Med Chem* 1611-15 (1999).
15. R. Bai, N.A. Durso, D.L. Sackett, E. Hamel**.** *Biochemistry,* **38**,14302 (1999).
16. F. Mandelbaum-Shavit, M.K. Wolpert-DeFilippes, D.G. Johns. *Biochem Biophys Res Commun,* **72**, 47(1976).
17. B. Bhattacharyya, J. Wolff. *FEBS Lett.,*. **75**,159 (1977).
18. R. Bai, R.E. Schwartz, J.A. Kepler, G.R. Pettit, E. Hamel. *Cancer Res.*, **56,** 4398 (1996).
19. R. Bai, G.R. Pettit, E. Hamel. *Biochem Pharmacol.* **39**,1941 (1990).
20. R. Bai, K.D. Paull, C.L. Herald, L. Malspeis, G.R. Pettit, E. Hamel. *J. Biol. Chem*, **266**, 15882 (1991).
21. R.F. Luduena. *Mol Biol Cell.*, 445,. Review (1993).
22. M.J. Towle, K.A. Salvato, J. Budrow, B.F. Wels, G. Kuznetsov, K.K. Aalfs, S. Wesh, W. Zheng, B.M. Seletsk, M.H. Palme, G.J. Habgood, L.A. Singer, L.V. Dipietro, Y. Wang, J.J. Chen, D.A. Quincy, A. Davis, K. Yoshimatsu, Y. Kishi, M.J. Yu. *Cancer Res,* **61**,1013 (2001).
23. G.R. Pettit, Y. Kamano, C.L. Herald, A.A. Tuinman, F.E. Boettner, H. Kizn, J.M. Schmidt, R.J..Baczynskyj. *J. Am. Chem.Soc.* **109**, 6883 (1987).
24. R. Bai, G.F. Taylor, Z.A. Cichacz, C.L. Herald, J.A. Kepler, G.R. Pettit, E. Hamel. *Biochemistry,* **34**, 9714 (1995).
25. M.A. Ali, R. Rosati, G.R. Pettit, G.P. Kalemkarian. *Anticancer Res,* **18**,1021 (1998).
26. G.P. Kalemkarian, X. Ou, M.R. Adil, R. Rasoti, M.M. Khoulani, S.K. Madan, G.R. Pettit. *Cancer Chemother Pharmacol.*, **43**, 507(1999).
27. R.M. Mohammad, M. Varterasian, V.P. Almatchy, G.N. Hannoudi, G.R. Pettit, A.

Katib. *Clin. Cancer Res,* **4**,1337 (1998a).

28 R.M. Mohammad, G.R. Pettit, V.P. Almatchy, N. Wall, M. Varterasian, and A.Katib A. *Anticancer Drugs* **9**,149 (1998b).

29. J. Poncet. *Curr Pharm Des.* **5,**139 (1999).

30. G. Scatchard. *Ann. N. Y. Acad. Sci.* **51**, 660 (1949).

31. D.P. Panda, J.P. Singh, L. Wilson. *J. Biol. Chem.* **272**,7681 (1997).

32. D. Panda, V. Ananthnarayan, G Larson, C. Shih, M.A. Jordan, L. Wilson. *Biochemistry.* **39**,14121 (2000).

33. K.A. Gelmon, A. Tolcher, A.R. Diab, M.B. Bally, L. Embree, N. Hudon, C. Dedhar, D. Ayers, A. Eisen, B. Melosky, C. Burge, P. Logan, L.D. Mayer. *J Clin Oncol,* **17,** 697 (1999).