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# Design, synthesis, and structure–activity relationships of pyrimido[4,5-*b*]indole-4-amines as microtubule depolymerizing agents that are effective against multidrug resistant cells

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ABSTRACT

To identify the structural features of 9*H*-pyrimido[4,5-*b*]indoles as microtubule depolymerizers, pyrimido[4,5-*b*]indoles **2**–**8** with varied substituents at the 2-, 4- and 5-positions were designed and synthesized. Nucleophilic displacement of 2,5-substituted-4-chloro-pyrimido[4,5-*b*]indoles with appropriate arylamines was the final step employed in the synthesis of target compounds **2**–**8**. Compounds **2** and **6** had two-digit nanomolar potency ($IC_{50}$) against MDA-MB-435, SK-OV-3 and HeLa cancer cells in vitro. Compounds **2** and **6** also depolymerized microtubules comparable to the lead compound **1**. Compounds **2**, **3**, **6** and **8** were effective in cells expressing P-glycoprotein or the βIII isotype of tubulin, mechanisms that are associated with clinical drug resistance to microtubule targeting drugs. Proton NMR and molecular modeling studies were employed to identify the structural basis for the microtubule depolymerizing activity of pyrimido[4,5-*b*]indoles.



Microtubules are key components of the cytoskeleton for all eukaryotes and are involved in critical cellular processes including cell division, trafficking, signaling and migration.[1,2] These functions depend on the dynamic nature of microtubules, which arises from complex growth and shortening events related to GTP hydrolysis that is facilitated by microtubule associated proteins.[3] Microtubule targeting agents (MTAs) disrupt microtubule dynamics by altering αβ tubulin heterodimer addition and loss, which in turn disrupts microtubule-dependent events. In vitro, microtuble disruption initiates mitotic arrest and cell death, but evidence suggests that additional mechanisms are involved in the clinical activity of microtubule targeting drugs.[2,4] These drugs are some of the most successful anticancer agents used clinically.[1] In addition, MTAs are the only class of cytotoxic anticancer agents effective against p53-mutant cell lines, which constitute 39 of the 58 cell lines in the National Cancer Institute (NCI) 60-cancer cell line panel.[5,6]

MTAs are classified as microtubule stabilizing agents, which promote microtubule polymerization, or as microtubule depolymerizing agents, which inhibit microtubule polymerization. Microtubule depolymerizers can be further divided into three classes based on their tubulin binding site: the vinca domain, the maytansine site and the colchicine site. The vinca alkaloids, including vincristine, vinblastine and vindesine (Fig. 1), bind competitively within the vinca site. The vinca alkaloids are indicated for both adult and pediatric cancers.[7] Eribulin (Fig. 1), a simplified analog of halichondrin B,[8] binds within the vinca domain, and has unique effects on microtubule dynamics.[8,9] Structurally diverse natural products including rhizoxin and maytansine (Fig. 1) bind to a different site on β-tubulin, referred to as the maytansine site.[10] The occupancy of this site by maytansine causes microtubule depolymerization by inhibiting longitudinal tubulin interactions.[10] Eribulin and maytansine both have clinical utility, maytansine is the

*Abbreviations:* NCI, National Cancer Institute; MTAs, microtubule targeting agents; CA-4, combretastatin A-4; DMSO, dimethyl sulfoxide; PDB, Protein Data Bank; Pgp, P-glycoprotein; Rr, relative resistance; NMR, nuclear magnetic resonance.

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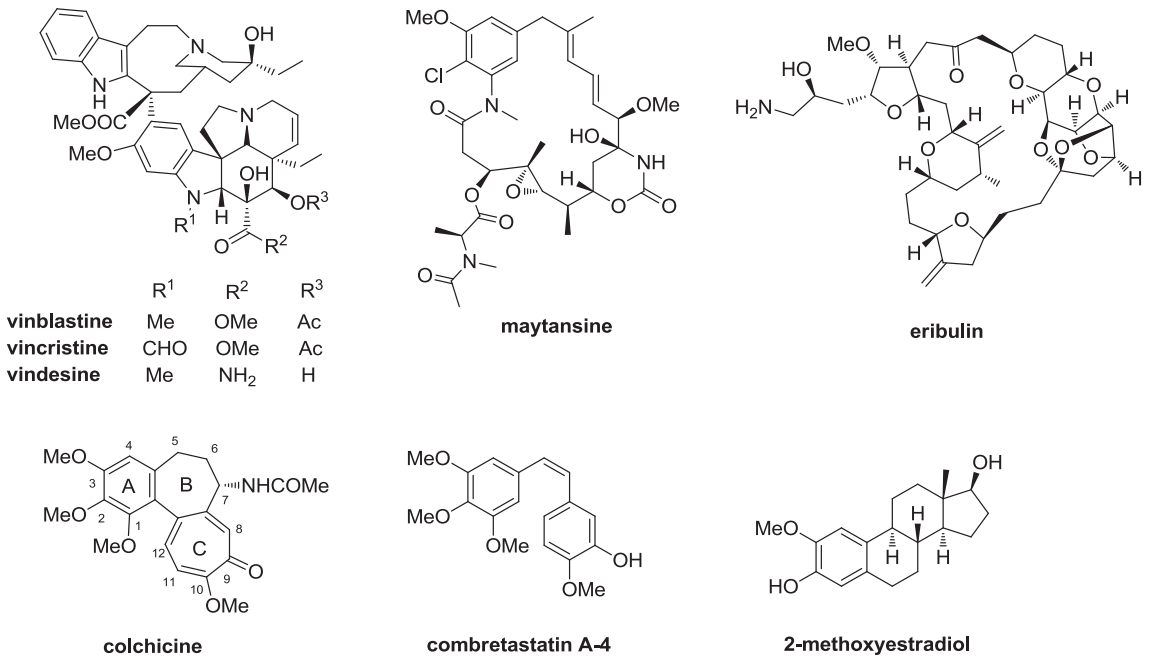


**Fig. 1.** Structurally diverse microtubule depolymerizing agents.

cytotoxin in the antibody-drug conjugate trastuzumab emtansine used for HER2-positive breast cancer[11] and eribulin is approved for the treatment of metastatic or locally advanced breast cancer[12] and liposarcoma.[13] The colchicine site is a non-overlapping binding site located on β-tubulin at its interface with α-tubulin.[14,15] While colchicine (Fig. 1) is too toxic for use in cancer therapy, multiple colchicine-site binding agents including combretastatin A-4 phosphate (CA-4P, fosbretabulin),[16–19] combretastatin A-1 diphosphate (CA-1P, OXi4503),[20] and 2-methoxyestradiol[21,22] (Fig. 1) were evaluated in early phase clinical trials. The microtubule stabilizers that increase the density of microtubules in cells and disrupt microtubule dynamics include the taxanes, laulimalide, peloruside A, epothilones, zampanolide and taccalonolides.[2,23,24] However, only paclitaxel, docetaxel and an epothilone B derivative ixabepilone are used clinically. Paclitaxel and docetaxel are widely used in the treatment of solid tumors such as breast, prostate, gastric, and lung cancers among others, ixabepilone is used in the US for the treatment of refractory metastatic breast cancer.[23]

Multidrug resistance is a major factor in the failure of cancer chemotherapy.[25] Expression of the ABC transporter, P-glycoprotein (Pgp) or the expression of βIII-tubulin are two major mechanisms of tumor resistance to taxanes and vinca alkaloids.[26,27] Development of MTAs that circumvent Pgp and/or βIII-tubulin-mediated resistance[28] could have advantages in patients who fail to respond to current MTAs. Most of the colchicine site agents circumvent Pgp and βIII-tubulin mediated resistance[28,29] and could be beneficial. However, thus far, no colchicine site agent has been approved as an anticancer agent. Hence, this site provides new opportunities for drug discovery.

Gangjee et al.[29] reported pyrimido[4,5-*b*]indole **1** (Fig. 2) as a potent microtubule depolymerizer ($EC_{50}$ of 133 nM) with potent in vitro cytotoxic activity in MDA-MB-435 cells ($IC_{50}$ of 14.7 nM). Compound **1** is a colchicine site agent and it also circumvents clinically relevant Pgp and βIII-tubulin mediated resistance. Compounds **2**–**8** (Fig. 2) were designed to identify key structural features of **1** responsible for microtubule depolymerizing activities and binding within the colchicine site.

Compounds **2** and **3** (Fig. 2) with 2-Me and 2-H substitutions, respectively, were designed to evaluate the importance of the 2-$NH_2$ group for microtubule depolymerizing activity. Similar substitutions were incorporated in the cyclopenta[*d*]pyrimidine series[30] and resulted in improved potency in some cases. Compounds **4** and **5**, which have different substitutions at the 5-position, were designed to evaluate the role of the electronics of the C-ring and/or hydrophobicity on microtubule depolymerization. Compounds **4** and **5**, in addition, conformationally restrict the rotation around the N-C4 bond (a) in **1** (Fig. 2) as well as the N-C1′ bond (b) in **1** due to steric hindrance caused by the presence of a larger 5-Me or 5-Cl moiety respectively, instead of the 5-H in **1**. Compounds **6** and **7** were designed as conformationally restricted analogs by incorporating the bicyclic 6-methoxytetrahydroquinoline moiety onto the 4-position of the pyrimido[4,5-*b*]indole. Compared to the *N*-methylanilines, **1**–**5**, the tetrahydroquinoline moiety of **6** and **7** eliminates rotation around the (b) bond, thereby restricting the conformation of the phenyl ring. The restricted conformation of the phenyl group in **6** and **7** results in a much more rigid structure than **1** and **4**, respectively, but still maintains the phenyl and alkyl substitutions on the $N^4$-position. Compound **8** was designed as a bioisostere of **1** by replacement of the 4′-OMe group with a 4′-SMe moiety to determine the bulk tolerance (O vs S) as well as the importance of hydrogen bonding.

Synthesis of target compounds **2** and **3** is shown in Scheme 1. Compounds **9**[31] and **10**[32] were synthesized using reported procedures. Treatment of **9** and **10** with $POCl_3$ afforded the 4-chloropyrimido[4,5-*b*]indoles **11** and **12**, respectively. Displacement of the 4-Cl of **11** and **12** with 4-methoxy-*N*-methyl aniline provided target compounds **2** and **3** in 78% and 38% yields, respectively.

The synthesis of target compounds **4** and **5** is shown in Scheme 2. Displacement of the 2-F of commercially available **13** with ethyl cyanoacetate anion provided **14** in 82% yield. Reduction of the nitro group of **14** followed by cyclization furnished the indole **15**. Cyclocondensation of **15** with carbamimidic chloride hydrochloride afforded the 2-amino-4-oxo-pyrimido[4,5-*b*]indole **16**. Pivaloyl protection of the 2-$NH_2$ of **16** gave **17**, which, upon chlorination at the 4-position, provided **18** in 86% yield. Nucleophilic displacement of the 4-Cl of **18** and **19**[33] with 4-methoxy-*N*-methyl aniline provided target compounds **4** and **5**, respectively.