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A Rationally Designed Anticancer Drug Targeting a Unique Binding Cavity of Tubulin

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Abstract—A novel mono-THF containing synthetic anticancer drug (WHI-261) was designed for targeting a previously unrecognized unique narrow binding cavity on the surface of tubulin. The anti-cancer activity of WHI-261 was confirmed using MTT assays. The structure-based design, synthesis, and biological activity of WHI-261 are reported.

Microtubules play a pivotal role in mitotic spindle assembly and cell division.[1–5] These cytoskeletal elements are formed by the self-association of the αβ tubulin heterodimers. Recently, the structure of the αβ tubulin dimer was resolved by electron crystallography of zinc-induced tubulin sheets.[6] According to the reported atomic model, each 46×40×65 Å tubulin monomer is made up of a 205 amino acid N-terminal GTP/GDP binding domain with a Rossman fold topology typical for nucleotide-binding proteins, a 180 amino acid intermediate drug-binding domain comprised of a mixed β sheet and five helices that contains the binding sites for both taxol and vinc alkaloids, and a predominantly helical C-terminal domain implicated in the binding of microtubule-associated protein (MAP) and motor proteins.[2,5]

In a systematic search for novel drug binding pockets within the intermediate domain of tubulin, we discovered a previously unidentified region with a remarkable abundance of leucine residues (viz, 7 leucine and 2 isoleucine residues) which could provide a highly hydrophobic binding environment for small molecule organic compounds. Notably, this unique region, which is located between the GDP/GTP binding site and the taxol binding site, contains a narrow cavity with elongated dimensions that could accommodate a fully stretched aliphatic chain with a length of up to twelve carbon atoms (Fig. 1). The enclosure of this putative binding cavity in α tubulin (but not β tubulin) is provided in part by an eight amino acid insertion loop (residues 361–368). A comprehensive structure search of our organic compound files led to the identification of the recently reported chiral THF-epoxide[7] as a potential molecular template for the rational synthesis of novel anticancer drugs containing structural elements capable of hydrophobic binding interactions with this leucine-rich binding cavity of tubulin. Herein, we report the modeling design, synthesis, and bioactivity of a new tubulin depolymerizing agent targeting this unique binding cavity.

The synthesis of the first enantiomerically pure prototype compound targeting this unique binding cavity was accomplished in an efficient two-step procedure using the THF epoxide **1**[7] as a template as outlined in Scheme 1. After the first step of epoxide opening in compound **1** by undecylmagnesium bromide, the debenzylation during the second and final step resulted in the formation of compound WHI-261 with an overall yield of 82%. The methyl-containing control compound WHI-260 lacking a long aliphatic chain was prepared by catalytic hydrogenation of compound **1**, whereas the benzyl-protected control compound WHI-262 was synthesized in one step by using methyl phenyl sulfone as the nucleophile for the epoxide opening of compound **1**. The hydroxymethyl-containing control compound WHI-259 was synthesized by treating compound **2**, a synthetic precursor of **1**, with potassium carbonate in methanol followed by hydrogenation to remove its hydroxy protecting group.

WHI-261 was docked into the putative binding pocket near the taxol binding site on β tubulin and the same region on α tubulin using the Affinity module within the

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INSIGHTII program. The binding region has approximate dimensions of 6×22×7 Å (Fig. 1). The long aliphatic chain of WHI-261 can interact with the leucine (or isoleucine) residues 209, 212, 217, 219, 234, 231, 230, 268, and 276 (Fig. 2). Additionally, the THF rings of WHI-261 can form favorable interactions with tubulin via hydrogen bonds with residue Asn226 on α tubulin. The results of our molecular modeling and docking

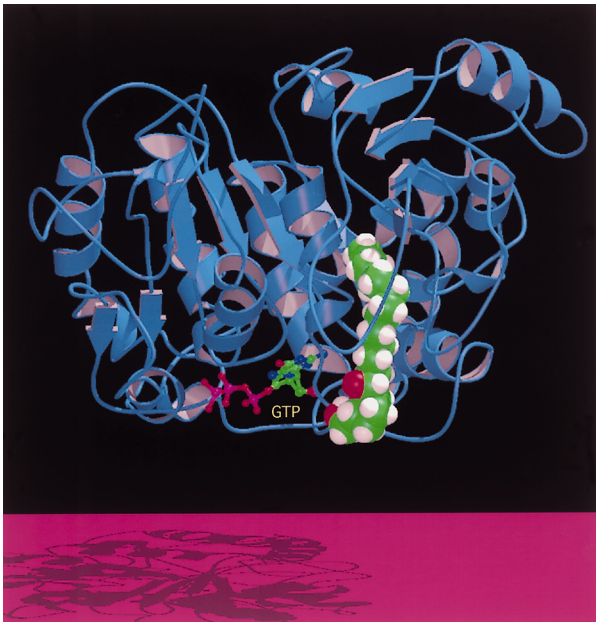


Figure 1. A ribbon representation of β tubulin structure and a space-filling model of the compound WHI-261 which was docked into the tubulin binding site, prepared by Molscript and Raster3D[9–11] based on the electron crystallographic structure (Nogales, et al., *Nature,* **1998**, *391*, 199). The compound WHI-261 forms extensive interactions with the leucine-rich region located in and extending beyond the taxol binding site on β tubulin, and also with the same location of α tubulin. The leucine residues are shown in white spheres and red bonds. Most of the residues in the binding site are identical for α and β tubulin. The binding site on α tubulin has an eight amino acid insertion (361–368) which provides additional hydrophobic contact and constitutes the major difference from the binding site on β tubulin.

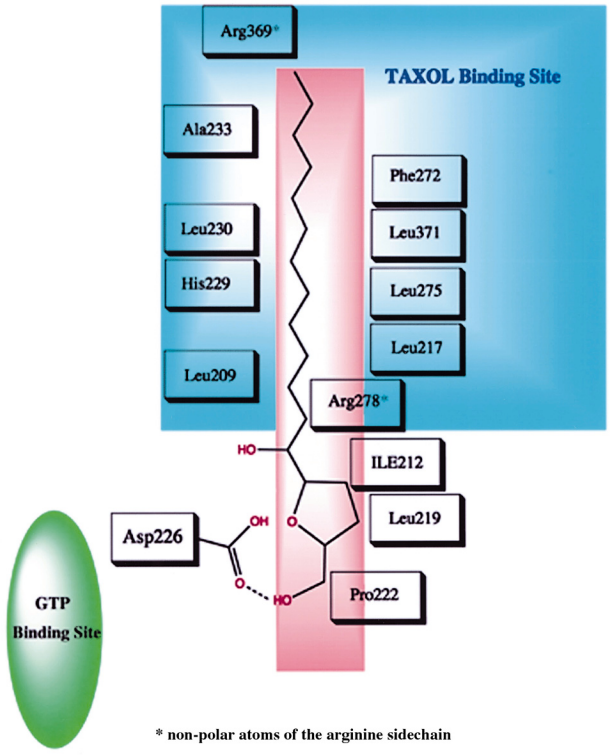
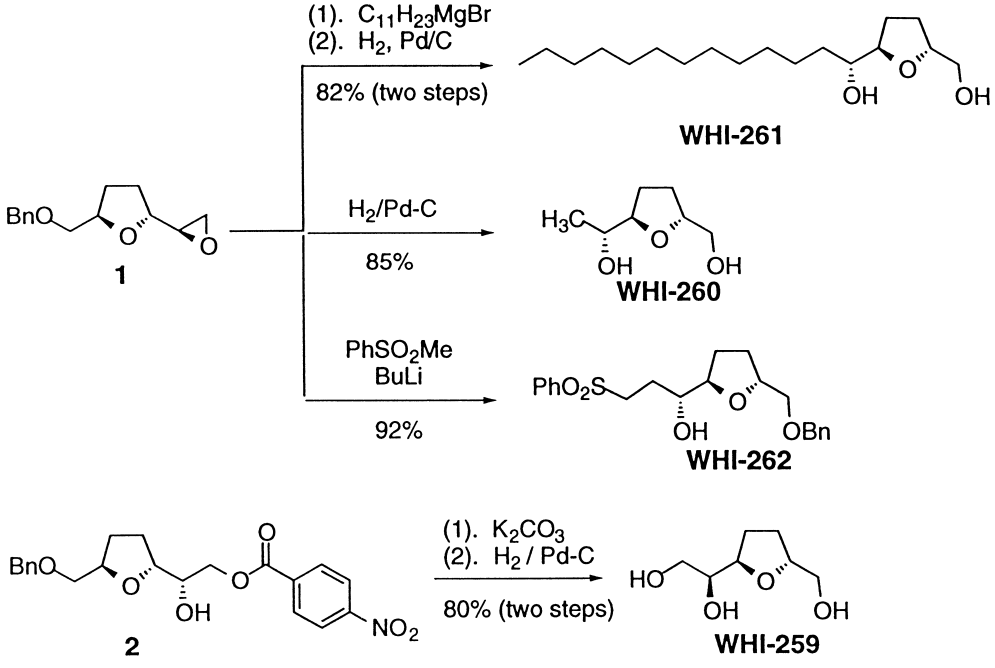


Figure 2. A schematic drawing of WHI-261 interaction with the protein residues in the vicinity of binding site on β tubulin. The interaction pattern is very similar in the same site α tubulin with a few different residues such as Leu368 substituting for Arg369, Ile234 for Ala233, Ala278 for Arg278 and Asn226 for Asp226.



Scheme 1. Synthesis of WHI-261.[14]

Table 1. Anti-proliferative activity of WHI-261 against human tumor cell lines

Compounds	MDA-MB-231 Breast cancer	PC-3 Prostate cancer	IC_{50}[MTT] (μM) SQ20B Head/neck cancer	U87 glioblastoma	NALM-6 leukemia
WHI-259	>250	>250	>250	>250	>250
WHI-260	>250	>250	>250	>250	>250
WHI-261	77	171	140	107	49
WHI-262	>250	>250	>250	>250	>250

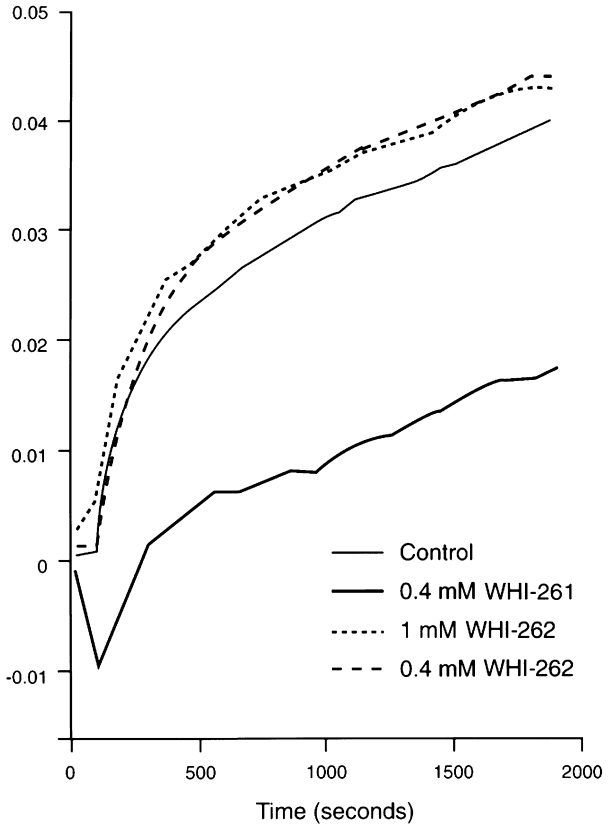


Figure 3. Turbidity measurements. Bovine brain tubulin (Sigma, St. Louis, MO) was used in standard turbidity assays to test the effects of WHI-261 and WHI-262 on GTP-induced tubulin polymerization. Compounds (in 1% DMSO) were added to tubulin (1 mg/mL, 0.1 M MES, 1 mM EGTA, 0.5 mM MgCl2, 0.1 mM EDTA, 2.5 M glycerol, 1 μg/mL leupeptin, 1 μg/mL aprotinin, pH 6.5) followed by stimulation of polymerization with 1 mM GTP at 2 mm and 1 mM taxol at 30 min. Optical density was measured using a Becton Dickinson UV spectrophotometer (350 nm) using a thermostated cuvette holder to keep the reaction at 37 °C. Readings obtained from the spectrophotometer were standardized by subtracting the background absorbance of the compound in water from the sample reading following drug addition.

studies indicated that WHI-261 would fit much better the binding cavity on α tubulin than the corresponding region on β tubulin. The reason for this selectivity involves an enclosure on the target binding cavity which is provided in part by an 8-amino acid insertion loop in α tubulin (residues 361–368), which is not present in β tubulin. WHI-261 has a total molecular surface area of 350 $Å^2$ (defined by Connolly[8]), approximately 256 $Å^2$ of which is in contact with the binding pocket on α tubulin based on our calculations.

The occupation of the binding pocket by WHI-261 was predicted to interfere with the formation of the α/β tubulin dimer and induce tubulin depolymerization. These predictions were experimentally confirmed in tubulin turbidity assays.[12] WHI-261 caused partial depolymerization of tubulin and inhibited its polymerization in the presence of GTP (Fig. 3). The antiproliferative activity of WHI-261 and the control mono-THF containing compounds WHI-259, WHI-260 and WHI-262 against a panel of 5 different human tumor cell lines was examined using standard MTF assays.[13] All cell lines were inhibited by WHI-261 (but not by any other compound) in a concentration-dependent fashion (Table 1).

In summary, we used a three-dimensional computer model of tubulin constructed based upon its recently resolved electron crystallographic structure for rational design of a novel mono-THF containing synthetic anticancer drug targeting a previously unrecognized unique narrow binding cavity on the surface of tubulin. This unique binding pocket has elongated dimensions and was predicted to favorably interact with the aliphatic side chains of WHI-261. Our modeling studies also indicated that WHI-261 is capable of favorable interactions with tubulin via hydrogen bonding with the Asn226 residue. The anti-cancer activity of WHI-261 was confirmed using MTT assays. The THF binding region extended from the hydrophobic cavity is relatively exposed and thus is more forgiving in accommodating different substituents on the THF ring at the opposite side of the long chain (Fig. 2). This binding region is also more hydrophilic and is compatible with the hydroxyl group and the oxygen atom of the THF-ring. These observed geometric features of the WHI-261 binding site may provide the structural basis for the future development of more potent compounds targeting this binding region.

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14. Characterization data of **WHI-261**: $[\alpha]_D^{22}$ 49.0 (*c* 2.45, $CHC1_3$); ^1H NMR (300 MHz, $CDC1_3$) δ 4.08 (m, 1H), 3.81 (m, 1H), 3.57 (m, 1H), 3.49 (m, 1H), 3.39 (m, 1H), 2.39 (d, $J = 6.0$ Hz, 1H), 2.03–1.93 (m, 3H), 1.68 (m, 2H), 1.49–1.23 (m, 22H), 0.86 (t, $J = 6.5$ Hz, 3H); ^{13}C NMR (75 MHz, $CDC1_3$) δ 82.90, 79.63, 74.15, 64.76, 33.41, 31.94, 29.68, 29.38, 28.54, 27.82, 25.62, 22.71, 14.16; IR (neat) 3406, 2924, *2854,* 1466, 1068, 758 cm^{-1}.