Targeting the Colchicine-binding Site of Tubulin with 4-(pyrimidin-2-yl)morpholines

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Microtubule (MT) dynamics play a crucial role in the regulation of cellular motility, maintenance of cell shape, secretion, intercellular transport, and are indispensable for spindle formation during mitosis.[1] Small molecules interfering with MT dynamics have been recognized as valuable therapies in cancer.[2, 3]

A systematic structure-activity relationship (SAR) study starting from morpholino-substituted biheteroaryls with moderate microtubule disrupting activities allowed for the optimization of biological activity, metabolic stability, and drug-like properties. In this study, we focused on compounds with pyrimidine cores substituted with small *N*-heterocyclic moieties and identified compounds that potently inhibit cellular microtubule polymerization with EC_{50} values of 20-120 nM. Cellular activity was confirmed by monitoring phosphorylation of Histone H3, nuclear DNA condensation and mitotic cell cycle arrest across multiple cell lines. The compounds were shown to be poor substrates for P-gp multi-drug resistance pumps, and consequently efficiently caused mitotic arrest and cell death in colchicine resistant cells.

The co-crystal structure of tubulin with selected compounds showed that 4-(pyrimidin-2-yl)morpholines bind to the colchicine-binding site located between the α and β subunits of the αβ-tubulin dimer. Relevant inhibitor contact residues include Lys352, Met259, Ala316, Leu248, Val238, Tyr202 and Cys241 of β-tubulin. Moreover, two water molecules link the morpholine oxygen to the α-tubulin bound GTP. Conformational changes induced by inhibitor binding suggest that free or plus end tubulin is targeted by this compound series.

Pre-clinical studies characterized a lead compound selection with excellent stability in human hepatocytes, and human, mouse and rat microsomes. Overall, these compounds qualify as a novel class of microtubule destabilizing agents that target the colchicine-binding site, and which warrant further development.

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