Estradiol Dimer Inhibits Tubulin Polymerization and Microtubule Dynamics

Microtubule dynamics is one of the major targets for new chemotherapeutic agents. Here, we present the synthesis and biological profiling of steroidal dimers based on estradiol, testosterone and pregnenolone bridged by 2,6-bis(azidomethyl)pyridine between D rings. The biological profiling revealed unique properties of the estradiol dimer including cytotoxic activities on a panel of 11 human cell lines, ability to arrest in the G2/M phase of the cell cycle accompanied with the attenuation of DNA/RNA synthesis. Thorough investigation precluded a genomic mechanism of action and revealed that the estradiol dimer acts at the cytoskeletal level by inhibiting tubulin polymerization. Further studies showed that estradiol dimer, but none of the other structurally related dimeric steroids, inhibited assembly of purified tubulin (IC50, 3.6 M). The estradiol dimer was more potent than 2-methoxyestradiol, an endogenous metabolite of 17-estradiol and well-studied microtubule polymerization inhibitor with antitumor effects that was evaluated in clinical trials. Further, it was equipotent to nocodazole (IC50, 1.5 ), an antimitotic small molecule of natural origin. Both estradiol dimer and nocodazole completely and reversibly depolymerized microtubules in interphase U2OS cells at 2.5 M concentration. At lower concentrations (50 nM), estradiol dimer decreased the microtubule dynamics and growth life-time and produced comparable effect to nocodazole on the microtubule dynamicity. *In silico* modeling predicted that estradiol dimer binds to the colchicine-binding site in the tubulin dimer. Finally, dimerization of the steroids abolished their ability to induce transactivation by estrogen receptor  and androgen receptors. Although other steroids were reported to interact with microtubules, the estradiol dimer represents a new structural type of steroid inhibitor of tubulin polymerization and microtubule dynamics, bearing antimitotic and cytotoxic activity in cancer cell lines.