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Structure activity relationships (SAR) study to design and synthesize new tubulin inhibitors with enhanced anti-tubulin activity: *In silico* and *in vitro* analysis

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ABSTRACT

Tubulysin family of natural products is a class of highly cytotoxic tetrapeptides that induce apoptosis by arresting the mitosis of a cell. In this article, we have analysed 49 reported tubulysin derivatives with known IC_{50} values to develop two and three-dimensional quantitative structure-activity relationships (2D and 3D QSAR) to explore the critical structural framework required for anti-tubulin activity. In the 2D technique, hologram QSAR (HQSAR models were generated by examining different combinations of the field parameters, number of components, and fragment sizes. Reliability of the best HQSAR model was validated by obtaining optimum values for statistical parameters such as cross-validated ($q^2 = 0.92$) and correlation ($r^2 = 0.93$) coefficients. Further, 3D-QSAR models were generated via CoMFA and CoMSIA methods. In CoMFA, using the Gast-Huck charge assigning method, the best correlation values for q^2 and r^2coefficients were found to be 0.59 and 0.93, respectively. Whereas in CoMSIA, by considering field contribution parameters such as steric, electrostatic, hydrophobic interactions, and hydrogen bond acceptor ability, a fine model was generated with best cross-validated ($q^2 = 0.58$) and correlation ($r^2 = 0.94$) coefficients values. Tubulysin M is one of the most potent anticancer agents known with an excellent IC_{50} of 0.02 nM to kill cancer cells. In the present study, tubulysin M co-crystallised with tubulin protein (PDB ID: 4ZOL) was used for molecular docking studies to analyze the drug-protein interactions. The results of QSAR and molecular docking studies were compiled and through *in silico* studies, structure activity relationships (SAR) were established. The *in silico* SAR results were utilized to design novel third generation tubulysin derivatives, which are easy to synthesize when compared to complex natural tubulysin derivatives. One of the third generation derivatives, Tub_01, was chemically synthesized and found to have an excellent anticancer activity against cervical cancer cell line (HeLa) with IC_{50} of 9.4 nM and 88.6 nM for different incubation time.

1. Introduction

Tubulin protein 3-D structure was discovered in 1998 using electron diffraction studies and shown to play an essential role in intracellular transportation and cell division [1]. Tubulin protein polymerizes into long chains or filaments to form microtubules or hollow fibers. These fibers serve as a skeletal system for living cells and are the most sort target for anticancer drugs [2]. Inhibition of microtubule formation by targeting tubulin protein induces cell death by apoptosis [3]. Drugs such as taxanes, vinca alkaloids, colchicine etc., bind at various sites of the tubulin protein within microtubule threads and consequently affect the dynamics of microtubule assembly [4].

The idea of targeting various sites on tubulin by natural products has turned out to be a potential treatment strategy for both hematological and solid tumors [5]. In this category, tubulysins are a class of natural and highly cytotoxic tetrapeptides that bind at the vinca domain and inhibit tubulin polymerization. These natural products were first isolated from *myxobacteria* by Hoffle and co-workers in the year 2000 [6]. Experiments have validated that tubulysins have potent antitumor activity against various cancer

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