Chapter 1

Introduction

1.1 Background

Microtubules (MTs) are polarized hollow cylinders composed of α-tubulin and β-tubulin heterodimers that have (-)-ends that are nucleated at the microtubule-organizing center (MTOC) and (+)-ends that extend outward. It is one of the three primary cytoskeletal elements, which are known to regulate directed cell migration, vesicle and organelle trafficking, and mitosis (Logan & Menko, 2019). The MTOC includes anchoring and adapter proteins for MT attachment, as well as γ-tubulin, an MT nucleator. Heterodimers interact with the γ-tubulin ring complex (γ-TuRC) at the MTOC, nucleating and anchoring MTs with their (-)-ends (Hohmann & Dehghani, 2019). The γ-TuRC, composed of γ-tubulin, five paralogous γ-tubulin complex proteins (GCP2-6), actin, and the tiny mitotic spindle organizing proteins Mzt1 and Mzt2, is responsible for nucleating MTs in vertebrates. Meanwhile, exposed γ-tubulin molecules recruit α/β-tubulin heterodimers, highlighting the role of γ-tubulin complexes (γ-TuCs) as structural templates for MT nucleation (Zheng et al., 2025).

Given their significance in mitosis, MTs can be effectively targeted in the treatment of solid tumors and hematological malignancies. Drugs that destabilize MT polymers, for instance, can prevent the formation of mitotic spindle MTs and potentially disrupt them, stopping cell division in metaphase. Microtubule-targeting agents (MTAs) are therefore used to modulate the MT dynamics, resulting in mitotic arrest and cell death (Wang et al., 2023; Yeo & Loong, 2014). While traditional MTAs such as taxanes and vinca alkaloids can achieve clinical efficacy in cancers by disrupting MTs' dynamic instability, their use is often limited by significant systemic toxicities, notably dose-limiting peripheral neuropathy and myelosuppression, stemming from their nonselective impact on the MT cytoskeleton that is present everywhere (Huang, Liu, et al., 2022).

Breast cancer (BC) is one of the most common malignant tumors in the world, accounting for 10.4% of all malignancies and the main cause of death among women aged 20 to 50 years (Iacoviello et al., 2020). Interestingly, BC significantly overexpresses γ-tubulin compared to normal breast tissue, which can be observed in BC subclasses (Niu et al., 2009). Furthermore, when γ-tubulin and retinoblastoma tumor suppressor (RB1) are absent, E2Fs' unchecked transcriptional activity upregulates apoptotic genes, resulting in cell death (Alvarado-Kristensson, 2018). This compound is particularly useful for the treatment of triple-negative breast cancer (TNBC), which frequently loses its RB1 (Jones et al., 2016).

Previously, no compound targeting specifically γ-tubulin was available, thus pushing researchers to investigate β-tubulin-binding compounds due to the 34% structural similarity between β- and γ-tubulin. The screening for colchicine-site binders led to the identification of glaziovianin A (AG1), a naturally occurring isoflavone that weakly interacts with γ-tubulin. Importantly, O7-benzylation of the AG1 scaffold produced gatastatin, the first γ-tubulin-selective inhibitor, which blocks γ-tubulin functions but does not interfere with α/β-tubulin polymerization (Chinen et al., 2015). Gatastatin and its stronger version, gatastatin G2 (G2), stopped GTP from binding to γ-tubulin, hence disrupting the γ-tubulin-dependent nucleation activity of centrosomes, causing mitotic spindle defects and cell cycle arrest (Shintani et al., 2020).

A crucial point that remains unanswered is whether gatastatin and its derivatives are indeed selective for γ-tubulin compared to the significantly more abundant α/β-tubulin. Vottero et al. (2023) reported that computational data did not reproduce the reported γ-tubulin specificity for gatastatin. For all drugs analyzed, the estimated binding affinities for α/β-tubulin were always better than for γ-tubulin. This observation is relevant as α/β-tubulin comprises about 2–2.5% of total cellular protein in all cells, while γ-tubulin is less than 1% even when overexpressed in cancer. If a chemical binds more to α/β-tubulin than to γ-tubulin, it would compromise the therapeutic rationale of γ-tubulin as a cancer-selective target. This suggests additional derivatization, guided by computational predictions, to

discover a molecule that exhibits real selectivity for γ-tubulin. Furthermore, G2, along with other derivatives, is also predicted to induce cardiotoxicity due to its inhibiting hERG property (Vottero et al., 2023).

Concerns about safety and effectiveness still hamper the development of γ-tubulin-targeted therapies. As such, in silico techniques offer an effective way to evaluate potential drugs at an early stage by predicting their binding affinity, selectivity, and pharmacokinetic characteristics. Thus, in silico screening of gatastatin and selected derivatives against γ-tubulin, α/β-tubulin, and the hERG protein, including drug-likeness and ADME assessment, was performed to identify candidate compounds with favorable predicted selectivity, binding affinity, and safety profiles for further experimental development as γ-tubulin-targeted anticancer agents.

1.2 Objective

This study aims to in silico screen gatastatin and its derivatives against the GTP-binding site of γ-tubulin (3CB2), the chalcone site of α/β-tubulin (5JVD), and the hERG channel (8ZYO) with ADME profiling, drug-likeness assessment, and molecular docking to identify derivatives with better predicted γ-tubulin binding affinity, selectivity over α/β-tubulin, and reduced cardiotoxic effects.

1.3 Hypothesis

This study is expected to yield at least one gatastatin derivative with a higher predicted binding affinity for the γ-tubulin GTP-binding site than the α/β-tubulin chalcone site; having lower binding affinity to hERG; satisfying Lipinski's Rule of Five and Veber's Rule; and having an acceptable pharmacokinetic profile compared to gatastatin.