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Identification of Potential Antitubulin Agents with Anticancer Assets from a Series of Imidazo[1,2-*a*]quinoxaline Derivatives: In Silico and In Vitro Approaches

Kapil Kumar Goel [1,2], Afzal Hussain [3], Mohammad A. Altamimi [3,*], Satyendra Kumar Rajput [2], Prince Prashant Sharma [2], Rajeev Kharb [1,*], Wael A. Mahdi [3], Syed Sarim Imam [3], Sultan Alshehri [3], Osamah Abdulrahman Alnemer [3] and Anu Chaudhary [4]

[1] Amity Institute of Pharmacy, Amity University, Sector 125, Noida 201301, Uttar Pradesh, India
[2] Department of Pharmaceutical Sciences, Gurukul Kangri (Deemed to be University), Haridwar 249404, Uttrakhand, India
[3] Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia
[4] Department of Pharmaceutical Chemistry, Bhagwan College of Pharmacy, Bairagi 250611, Uttar Pradesh, India
* Correspondence: maltamimi@ksu.edu.sa (M.A.A.); rajeevkharb_2007@rediffmail.com (R.K.)

Abstract: Computer-aided drug design is a powerful and promising tool for drug design and development, with a reduced cost and time. In the current study, we rationally selected a library of 34 fused imidazo[1,2-*a*]quinoxaline derivatives and performed virtual screening, molecular docking, and molecular mechanics for a lead identification against tubulin as an anticancer molecule. The computational analysis and pharmacophoric features were represented as **1A2**; this was a potential lead against tubulin, with a maximized affinity and binding score at the colchicine-binding site of tubulin. The efficiency of this lead molecule was further identified using an in vitro assay on a tubulin enzyme and the anticancer potential was established using an MTT assay. Compound **1A2** (IC_{50} = 4.33–6.11 µM against MCF-7, MDA-MB-231, HCT-116, and A549 cell lines) displayed encouraging results similar to the standard drug colchicine in these in vitro studies, which further confirmed the effectiveness of CADD in new drug developments. Thus, we successfully applied the utility of in silico techniques to identify the best plausible leads from the fused azaheterocycles.

Keywords: anticancer; tubulin; azaheterocycles; molecular docking; molecular mechanics; virtual screening; in vitro screening

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1. Introduction

Tubulin is a highly overexpressed protein in almost all types of cancer and it is a validated anticancer drug target. Tubulin is composed of microtubules that exist as a heterodimer of α,β-tubulin [1]. These tubulin polymers exist in a dynamic equilibrium via polymerization and depolymerization. Thus, it provides a structural framework during the karyokinesis process of cellular division [2]. Despite cell division, tubulin plays a vital role in intracellular trafficking, cell migration, and angiogenesis. Tubulin also promotes cancer cell growth and prognosis [3]. Metabolically active cancer cells often evade programmed cell death and continue their growth and prognosis. In this context, drugs targeting tubulin or microtubules disrupt the tubulin–microtubule equilibrium and these allow apoptosis. To date, numerous approved or clinical candidates as tubulin inhibitors have been reported from both natural and synthetic origins that target the four major binding sites in tubulin protein, including the laulimalide, taxane, vinca alkaloid, and colchicine sites [4]. Among these, the colchicine-binding site (CBS) is one of the significant and most crucial pockets explored for developing potential tubulin polymerization destabilizers. The inhibitors exert their mechanism by disrupting the tubulin assembly and inhibiting microtubule formation. Two blockbuster drugs that act at the CBS are colchicine and combretastatin. These drugs

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