Synthesis, Structure-Activity Relationships, and Mechanism of Action of Novel Indirubin Derivatives as Potent Anti-proliferative Agents with CDK2 Inhibitory Activities

Yong-Chul Kim[a], Jung-Hoon Yoon[b], Sang-Gun Ahn[b], Si Wouk Kim[c], and Sang Kook Lee[d]

[a]Department of Life Science, Gwangju Institute of Science and Technology
[b]Department of Oral Pathology, College of Dentistry, Chosun University
[c]Department of Environmental Engineering, Chosun University
[d]College of Pharmacy, Ewha Womans University

Oncogenic alterations of cyclins, cyclin dependent kinases (CDKs) and other upstream regulators of retinoblastoma (Rb) protein as the important components of cell cycle, occur in a variety of human tumors. Recently, CDK2 or cyclin E1/E2 deficient cells were reported to proliferate normally without centrosome duplication defect, which evoke strong encouragement for the therapeutic intervention to develop CDK inhibitors as potential pharmacological agents to treat proliferative diseases such as cancer. Indirubin, an active ingredient of a traditional Chinese recipe Danggui Longhui Wan for the treatment of chronic myelocytic leukemia, has been known as a CDK inhibitor competing with ATP for binding to the catalytic site of cyclin-dependent kinases (CDKs). A series of novel indirubin analogs were synthesized and evaluated for antiproliferative and CDK2 inhibitory activities. Among the indirubin derivatives tested in the growth inhibitions against several human cancer cell lines, 5-nitro, halide, and bulky group containing acylamino substituted analogs showed high anti-proliferative effects. Selected analogs showing potent anti-proliferative activities were evaluated in CDK2 enzyme assay resulted in different profile of the inhibitory activities, which could be explained by the analysis through molecular docking study of each indirubin analog with CDK2 crystal structure. Additional studies for the mechanism of anti-proliferative activity of a potent indirubin analog against human lung cancer cells showed that the analog arrested cell cycle progression at the G_2/M phase and induced apoptosis via p53- and mitochondria-dependent pathway. Further study on the anti-cancer effects of the indirubin analogs in an animal model, where k-ras over-expressed RK3E-k-ras stable cell line was implanted, displayed remarkable suppression of tumor growth.