BIOLOGICAL EVALUATION OF KINASE INHIBITORS AS POTENTIAL ANTI CANCER AGENTS

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Abstract

Drug discovery in the last forty years has largely focused on agents to prevent or to treat cancer as a result of increased incidence and prevalence of cancer due to life style changes and people living longer as a result of improved healthcare. As a result of recent understanding of key cellular signaling pathways involved in cellular growth, proliferation and carcinogenesis small molecule protein kinase inhibitors are being developed as potential drugs in cancer chemotherapy. In the current study, our screening programme based on cellular potency, CDK 9 inhibitory activity and caspase 3 activity resulted in identification and biological evaluation of several kinase inhibitors. The first of these inhibitors was novel compound CDKI-77. Detailed cellular mode of action studies of this compound compared to flavopiridol, a clinical CDK inhibitor is reported here. The compound showed potent inhibitory activity against CDK 9. In a panel of human tumour cell lines, CDKI-77 showed diverse anti-proliferative activity. In A2780 cells, the compound down-regulated anti-apoptotic proteins Mcl-1, HDM2 and XIAP consistent with transcriptional inhibition by the compound via CDK 9 inhibition. Concentrations showing down-regulation of apoptotic proteins also elicited significant apoptosis in A2780 cells corroborating the role of anti-apoptotic proteins` down-regulation in the apoptosis-inducing activity of the compound. Similar effects were observed in CDK 9 knockdown cells confirming that effects seen in compound treated cells were largely due to CDK 9 inhibition and that CDK 9

inhibition is enough to drive cells into apoptosis and is therefore a valid target in cancer chemotherapy.

The second compound class evaluated comprised benzo imidazole derivatives. Improved cellular potency of the compounds (HMK-38, 76 and 87) compared with the lead compound DRB was observed in colon and breast cancer cell lines. However, based on CDK 9 inhibitory activity evaluation, none of the compounds showed improved potency against CDK 9 compared with DRB as a result of chemical optimisation. HMK-38, the most promising among the compounds, showed cellular CDK 9 inhibitory activity and down-regulation of anti-apoptotic proteins Mcl-1 and HDM2 consistent with transcription inhibition in HCT-116 cells,resulting in apoptosis.

The next compound studied was a pan-CDK inhibitor CDKI-73; its activity was compared to that of flavopiridol in p53 isogenic cell lines. Potent inhibitory activity against both transcriptional (CDK 9) and cell cycle (CDK 1 and 2) CDKs was observed by this compound. The compound also showed wide spectrum anti-proliferative activity in various human cancer cell lines and was comparable to clinical CDK inhibitor flavopiridol. In HCT-116 p53 isogenic cells, the compound showed a p53 independent anti-proliferative mechanism of action involving down-regulation of anti-apoptotic proteins, G2/M arrest, p21 induction and DNA damage induction.

The most selective CDK 9 inhibitors synthesised by the group (DF-030263, HS3-47, HS3-72, HKMII-77, HMKII-58 and HMK-122) were

then evaluated in patient derived CLL cells. In the evaluation, the compounds have shown outstanding cytotoxicity that was independent of p53 status in five patient-derived CLL cell lines. Further evaluation by Western blot has indicated that the cytotoxicity of these compounds might be as a result of down-regulation of Mcl-1, the survival anti-apoptotic protein of CLL cells. Evidence of enhanced apoptosis was also confirmed by PARP cleavage and p53 stabilisation.

Finally, the anti-proliferative potential of some styrylbenzylsulfones was investigated in wide variety of human cancer cell lines. The compounds (Oc-20, Oc-23 and Af1) showed remarkable anti-proliferative activity in a panel of human tumour cell lines. Cellular inhibitory activity against PLK 1 was confirmed with reduced phosphorylation of Cdc-25 Ser198 in cells after treatment with the compounds. The investigation has also demonstrated that the compounds caused a polo-arrest (G2/M block) at concentrations causing reduced phosphorylation of Cdc-25 indicating that the polo-arrest might be a consequence of PLK 1 inhibition.

The implications of these findings to cancer chemotherapy have been discussed in this thesis.