Discovery of potential RAF-selective back pocket as a promising biological target for BRAF inhibitors in the treatment of resistant melanoma; design, synthesis, biological evaluation and *in silico* studies.

The mutated BRAF kinase (V600E) is considered the key component in the MAPK signaling pathway that has been reported to be significantly contributed to melanoma disease. Vemurafenib and dabrafenib are examples of drugs that have been approved by FDA to treat melanoma through inhibition of mutated BRAF kinase (V600E). However, drug resistance has been reported after 6 – 7 months of treatment using these drugs due to activation of this signaling pathway through another RAF kinase (CRAF). As a Drug Discovery research group, we were interested to study and identify the possible biological target to inhibit this resistant form of melanoma. We have run molecular modeling simulation to study the key features of the active sites of RAF kinases and we have taken vemurafenib as a starting point to design our compounds. We have identified a selective back pocket in the active site of RAF kinases that can be targeted to enhance the inhibition of these kinases to overcome the drug resistance in the resistant melanoma. We have designed a library of compounds based on imidazothiazole core scaffold decorated with different hydrophobic substituents to target the selectivity back pocket. Among the designed and synthesized series, KS16, that showed potent biological profile against RAF kinases compared to vemurafenib as a standard. In the cellular level, KS16 showed the same potencies against mutated BRAF-based melanoma cell line (A375) as that of vemurafenib. To evaluate the selectivity profile of KS16, kinase panel assay against 60 kinases has been investigated that showed high selectivity profile of KS16 (98 – 100% inhibition against ^{WT}BRAF, CRAF and ^{V600E}BRAF). In addition, KS16 was submitted to National Cancer Institute (NCI) to be tested against 60 human cancer cell lines in 5-point assay. It showed selective cytotoxic inhibition against melanoma cell lines compared to the other types of cancer cell lines. To evaluate the efficacy of KS16 against resistant melanoma, both KS16 and vemurafenib were tested against resistant melanoma cell line (A375R). The results revealed that KS16 showed the capability to inhibit the growth of resistant melanoma cell line in contrast to vemurafenib which have failed to exhibit the same inhibition profile. The drug-candidate cardiac safety profile of KS16 was emphasized in this current project. KS16 was tested against hERG using E4031 (IC_{50} = 0.025 μM) as positive control to set up the hERG channel binding assay. The results revealed that KS16 showed relatively weak binding to hERG (% inh = 64%) at 10 μM compared to that of the potent positive control (E4031, % inh = 97%) at the same molar concentration of KS16. The results revealed that KS16 is a potential drug candidate with minimal cardiotoxic effect. Further pharmacokinetic studies are being performed to KS16 to investigate and develop the ADME profile of KS16 to be a potential selected drug candidate in the early drug discovery against resistant melanoma disease.

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