ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most lethal cancers and causes high rate of mortality worldwide. The clinical outcomes of HCC treatment remains limited due to severe adverse effects and chemotherapeutic resistance. Inspired by the well-documented tumor protecting ability of paullones, a new paullone-like scaffold, indole fused benzo and pyrido heteroazepines were designed via rational approach of drug designing. To design the molecule, a molecular docking, ADMET profiling and MD simulation studies were carried out on various cancer-related inflammatory (IL-2, IL-6, COX-2) and apoptotic (Caspase-3 and Caspase-8) targets. Molecular docking study displayed considerable interaction energies and crucial hydrogen and π bond interactions, with notable ADMET profiling and ligand-protein stability on inflammation-associated targets. Further, a one-pot efficient synthetic route was employed to synthesize forty derivatives of the titled compounds. The synthesized compounds were characterized by fourier-transform infrared (FTIR), proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectroscopy and mass (MS) spectrometry.

All the compounds were subjected to the preliminary *in vitro* analysis against HCC specific Hep-G2 cancer cell line using SRB assay. A total of nine compounds (**6a**, **10a**, **13a**, **14a**, **15a**, **7c**, **10c**, **13c** and **14c)** effectively controlled the growth of cancerous cells with GI_{50}<10μg/mL. Preliminary structure–activity relationship (SAR) among the tested compounds produced an assumption that the electronegative substituents at phenyl ring attached with indole fused heteroazepine, are instrumental for the activity. Among the nine active compounds, three compounds (**6a**, **10a** and **15a**) from indole fused benzooxazepines (IFBOs) series were of remarkable efficacy on the basis of *in vitro* analysis and docking study and thus, selected for further *in vivo* pharmacological screening.

Prior to *in vivo* study, an acute toxicity study was performed where an oral administration of 10 mg/kg in albino Wistar rats was considered safe. Secondly, we performed a pharmacokinetic study using reversed phase high-performance liquid chromatographic method for the determination of **6a**, **10a** and **15a** (IFBOs) in rat plasma. The study suggests that IFBOs have a lower rate of absorption, higher volume of

distribution and lower clearance rate, indicating good therapeutic response for *in vivo* activity.

Further, these three IFBOs (**6a**, **10a** and **15a**) were subjected to *in vivo* study using the N-nitrosodiethyl amine (NDEA)-induced HCC rat model. Treatment with IFBOs at 10 mg/kg body weight showed remarkable attenuation of cellular proliferation, as evidenced through a decrease in the number of nodules, restoration of body weight, oxidative stress parameters, liver marker enzymes and histological architecture. Later, gene and protein expression analysis showed that these IFBOs inhibit HCC through the blockade of IL-6 mediated JAK2/STAT3 oncogenic signaling pathway. Interestingly, using ^{1}H NMR-based serum metabolomics approach we further discovered that IFBOs can restore the perturbed metabolites linked to the HCC condition. Particularly, the efficacy of **6a** for an anti-HCC response was probably better than the marketed chemotherapeutic drug, 5-fluorouracil. Altogether, these remarkable findings open up possibilities of developing IFBOs as newer lead molecules for HCC treatment.