ABSTRACT

Microtubules are long, filamentous tube-shaped polymers of α and β tubulin that are essential in all eukaryotic cells. They are crucial in the development and maintenance of cell shape, in the transport of vesicles, mitochondria and other components throughout the cells, in cell signaling, and in cell division or mitosis. Their importance in mitosis and cell division make microtubules an obvious and important target for anticancer drugs. The dynamic process of assembly and disassembly of microtubules to tubulin is blocked by various agents that bind to distinct sites such as binding sites for colchicines, taxol, and vinca alkaloids, finally leading to arrest in mitosis and cell death by apoptosis or necrosis. Several microtubule targeting drugs, - namely, Paclitaxel, Epothilones, and Dolastatin are in clinical use or in various stages of clinical development (Hadfield et al., 2003).

The design of novel anticancer agents based on the combretastatins, a group of antimitotic agents isolated from the bark of the South African willow tree Combretum caffrum, is of considerable contemporary interest. Chemically, they are stilbene derivatives having two phenyl rings separated by a C-C double bond. Ring-A has three methoxy groups in 3,4,5-positions while in ring B one hydroxy group is at the C-3 position and one methoxy group at the C-4 position (Srivastava et al., 2005). Combretastatin A-4, the most active compound in the group, due to its unique dual features of antitubulin and antivascular properties, has drawn significant attention of medicinal chemists for the design of analogues as novel antitumor agents. The disodium phosphate salt of CA4, CA4P is currently in Phase II and III at various stages of clinical trials. A family of 2,3-diaryl-4/5-hydroxy-cyclopent-2-en-1-one analogues replacing the cis-double bond by 4/5-hydroxy cyclopentenone moieties was designed and synthesized to study their potential cytotoxicity.

In Chapter-1 of this thesis, 56 (Fifty six) CA4 novel derivatives with modifications in ring B belonging to class of 2,3-Diaryl-4/5-hydroxy-cyclopent-2-en-1-one were screened for anti-cancer activity based on cytotoxicity to human tumor cell lines and endothelial cell line (primary screening). Out of these, 2 (two) derivatives viz. compound 11 and compound 42 (from the class of 5-hydroxy-cyclopent-2-en-1-one) were selected from the primary screening and tested in an extended panel of human cancer cell lines. In the
secondary screening these two derivatives were found to be cytotoxic and more sensitive to colon, breast, oral, ovary, prostate, skin fibroblast, pancreas, leukemia, kidney and lung cancers. Further, the above derivatives were also tested for their ability to inhibit tubulin polymerization. Of the two derivatives tested, the most potent one (compound 42) was established for its in vivo anti-tumor activity in ovary, prostate and colon human tumor xenografts.

In Chapter-2 of this thesis, the in vitro anti-angiogenic activity of combretastatin A-4 and its potent derivatives compound 11 and 42 short-listed from chapter-1 have been demonstrated for their effect on stimulated human umbilical vein endothelial cells (HUVEC). Both the derivatives were found to inhibit vital processes of angiogenesis i.e. stimulated endothelial cell proliferation, migration and capillary tube formation. Further, compound 42 with its more potent anti angiogenic (in vitro) activity was assessed for inhibition of vasculature in vivo and showed significant inhibition at 25mg/kg dose.

In Chapter-3 of this thesis, combretastatin A-4 and its derivatives compound 11 and 42 were tested for endothelial apoptosis (ECV304) and growth factor stimulated endothelial primary culture (HUVEC). Both compound 11 and compound 42 led to cell cycle arrest in proliferating endothelial cells (ECV304). At the concentration of 1 μM, the analogues displayed better activity than the parent Combretastatin A-4. The analogues also induced apoptosis in ECV304 cells as shown by DNA fragmentation and DAPI staining of micronuclei of proliferating ECV304 cells, thereby indicating that the analogues may have similar apoptotic activity as that of combretastatin A-4. Whereas surprisingly, when FGF-2 stimulated HUVECs were incubated with CA-4, compound 11 and compound 42, neither did they induce endothelial cell death through apoptosis nor it is driven by caspase – 3 enzyme. A detailed investigation of the apoptotic markers will further unravel its mechanism of action at both molecular and genetic level.

Our studies have demonstrated the potential of developing compound 42 as an anticancer agent with promising anti angiogenic activity. Compound 42 exhibits drug-like physicochemical properties. Appropriate formulation studies need to be carried out to deliver the molecule systemically and preferably in a targeted fashion. While pre clinical
safety studies have demonstrated an acceptable safety index, a more detailed toxicology study needs to be performed to evaluate the safety of the compound upon long-term systematic administration.