the important protease inhibitor which is currently used in the HIV-treatment-regimen. Mutation combination L90M/C95F is observed to confer resistance against this drug. Both are non-active site mutations.

To study the structural effects of these mutations, mutant clones L90M/C95F and C95F of tethered HIV-1 protease, where two sub-units of protease are covalently linked by a five residue linker, were prepared in our laboratory using site directed mutagenesis technique. Mutant proteins were expressed, purified and co-crystallized with SQV. X-ray diffraction data were collected on the FIP-beamline at European synchrotron research facility (ESRF). Crystal structures were solved by difference Fourier method using native coordinates. Comparisons of SQV bound mutant structures in the present study with that of native enzyme have shown reshaping of the active site cavity in the mutant structures. This is the result of altered packing in the core of the enzyme. SQV and in fact most of the other anti HIV-1 protease drugs are designed as competitive inhibitors so that they bind to the enzyme more tightly than its natural substrate. Hence, any change in the shape of active site cavity may affect the binding of these drugs more than that of natural substrates. Small changes in the conformation of SQV were also seen in response to those in the active site. Conversely, flexible substrates could adapt to these changes comparatively easily. Presence of these mutations may limit the structural flexibility of the active site loop which is required to accommodate the incoming inhibitor. These mutants have lower dime stability as compared to wild type enzyme as shown by reduction in the transition temperatures (T_m) as determined by using circular dichroism spectroscopy. This can be another cause of drug resistance.

Keywords: HIV-1 protease, saquinavir, drug resistance

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Toluene-sulfonic acid derivatives of 6-deoxyclitoriacetal, Biological Properties

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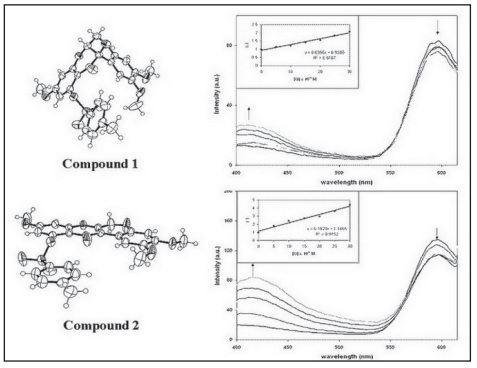

The compounds of toluene-4-sulfonic acid derivatives of 6-deoxyclitoriacetal have been synthesized and characterized. The crystal structures are different. One is in the bent shape (compound **1**) and another one is planar (compound **2**). In addition, the cytotoxic activities of both compounds are evaluated with KB cell line (epidermoid carcinoma of oral cavity), MCF-7 cell line (breast adenocarcinoma), and NCL-H187 (small cell lung carcinoma). Interestingly, the bent shaped (compound **1**) exhibits the potent significant cytotoxicity against KB (0.033 μM) and NCL-H187 (0.035 μM) but not active to MCF-7, while the planar shaped (compound **2**) is inactive for all tested cell lines. The molecular shape might effect on the cytotoxic activities of these tested cell lines. Therefore, the binding affinity with calf thymus DNA (CT-DNA) has been calculated by using UV-Vis and fluorescence spectroscopy. Moreover, the melting temperature (T_m) of CT-DNA when added with compound **1** and **2** are determined.

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Keywords: 6-deoxyclitoriacetal, binding affinity, cytotoxic activities

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Structural basis for inhibition of human and bacterial uridine phosphorylases by 2.2'-anhydrouridine, a modulator of 5-fluorouracil activity

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Uridine phosphorylase (Uph) is a key enzyme in the metabolism of pyrimidine based drugs. These drugs, primarily 5-fluorouracil (5-FU), remain the major components of many therapeutic regimens, particularly, in gastrointestinal malignancies. The inhibitors of Uph can potentiate the efficacy of 5-FU. Furthermore, Uph inhibition is lethal for some pathogenic parasites. Design of new clinical Uph antagonists requires a detailed investigation into the spacious organization of complexes of human and bacterial Uph at the atomic resolution. We used X-ray analysis to resolve for the first time the structure of Salmonella typhimurium Uph in a non-liganded state as well as in complexes with physiological ligands such as the phosphate anion and the potassium cation. We also determined the structure of complexes of Uph with 2.2'-anhydrouridine (ANU), 5-FU and their combinations at the atomic resolution. The structure of human Uph-ANU and Uph-phosphate ion complexes was determined by molecular modeling. Our experiments and literature data demonstrated that ANU is a reversible and competitive Uph inhibitor. Using molecular dynamics we revealed a role for K+ in stabilization of the structure of S.typhimurium Uph. The amino acid residues in the L9 loop of the active center and in the initial portion of the H8 helix were the most flexible. In the presence of K+ the intersubunit contacts in dimers became more dense. Molecular dynamics simulations showed that the L9 loop changed its position and conformation. Importantly, these data allowed us to perform an in silico design of high affinity Uph inhibitors based on ANU. These drug candidates were 5- or 6-substituted ANU derivatives containing an aliphatic saturated chain ended with an aromatic group. For 5-substituted ANU derivatives the pyridine cycle and the imidazole ring in the terminal aromatic moiety are preferred for inhibiting S.typhymurium and human Uph, respectively. This difference is attributed to variabilities in amino acid residues that form active centers of human and bacterial enzymes. For 6-substituted ANU derivatives the length of the carbohydrate chain is the critical prerequisite for the optimal inhibitor. Together, our data provide the ultrastructural basis for design of selective and efficient antitumor and anti-infective drugs.

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Keywords: biomacromolecule, cancer, drug