Chapter 2.

Aims and Objective of the Present Work

2 AIMS AND OBJECTIVE OF THE PRESENT WORK

Increasing resistance of malaria parasites, in particular *P. falciparum,* demands a serious search for novel targets. Among potential new targets for chemotherapy are the proteases that degrade hemoglobin, a principal source of amino acids for the parasite. *P. falciparum,* the most virulent human malaria parasite, engulfs hemoglobin within an acidic food vacuole, where the protein is hydrolysed with the help of these proteases. These enzymes include aspartic, cysteine and metalloproteases[100]. Enzymes of each of these classes are potential chemotherapeutic targets. Malarial cysteine protease enzyme falcipain-2 provides an important target to design chemotherapeutic agents for the treatment of malaria. In fact, a few cysteine protease inhibitors[115, 116] have been shown to block hemoglobin degradation, indicating that the enzyme plays a key role in this process. Furthermore, it has been shown that cultured malarial parasites failed to develop when incubated with cysteine protease inhibitors.

Most of the inhibitors of the cysteine proteases reported in the literature depend on the chemical interaction of an electrophilic "warhead" present in the inhibitor with cysteine thiolate anion of the active site and, in principle; the enzyme

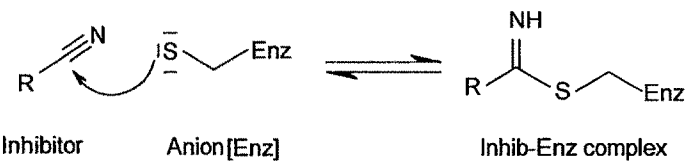
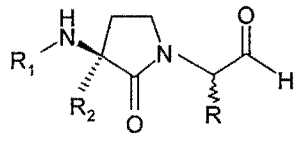


Figure 10: Inhibition of cysteine proteases by peptidyl nitriles

can be inactivated either irreversibly or reversibly by such inhibitors. Peptide aldehydes, diamino ketones, and peptide nitriles are the examples of inhibitors that form hemithioacetals, thioketals, and thioimidates, respectively, with the thiol of the active site cysteine residue causing reversible enzyme inhibition. A representative mechanism is shown for peptidyl nitrile inhibitors (Figure 10). Epoxysuccinyl

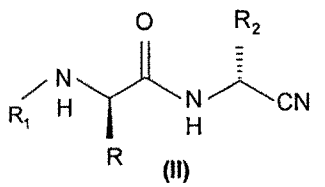
derivatives, peptide Michael acceptors, acyloxymethyl ketones and halomethyl ketones are examples of irreversible inhibitors, which form a hydrolytically stable covalent bond with the thiol of the active site cysteine.

Lactams (5-7 membered) have been shown to be useful new type of conformational constraints in peptides. Information may be obtained about the bioactive conformation of a peptide, and biological potency may be increased by incorporation of a lactam. Not only does this restriction fix the *trans* peptide bond but it also introduces constraints, which limits conformations by noncovalent interactions[128]. The γ-lactam or pyrrolidinone isosters have been reported as inhibitors of various classes of proteases. Conformationally constrained pyrrolidinone ring compounds attached with electron withdrawing group **(I)** like



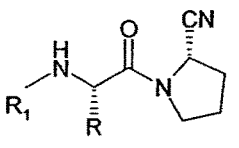
(I)

aldehydes have been reported as falcipain inhibitors[118]. Nitriles are known to be inhibitors of cysteine proteases and were first reported by Hanzlik[74] as inhibitors of plant protease, papain. Some simple dipeptidyl nitrile of the type **(II)** have been

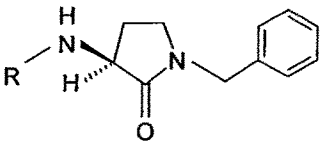


(II)

reported as cysteine protease inhibitors. These peptide nitriles were shown by NMR to form covalent bonds with the active site of cysteine, and this bond formation was shown to be reversible[75] (Figure 10). In light of the above observations, it was planned to synthesize some simple dipeptidyl nitriles of the type **(III)** as cysteine protease inhibitors, which could act as potential antimalarial drugs. It was also



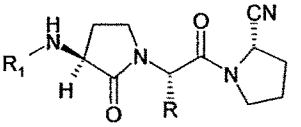
(III)



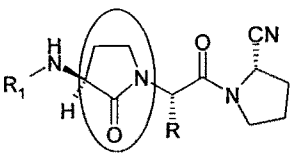
(IV)

planned to synthesize some conformationally constrained pyrrolidinone inhibitors attached with α, β-unsaturated keto acids and sulfonamides of the type **(IV)**.

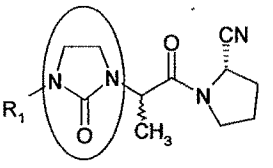
The structural features of the above-described molecules (Type **III** and **IV**) were fused in one structure to afford a hybrid structure **(V)** comprising of conformationally constrained pyrrolidinone moiety, which could help in fixing the conformation and nitrile group, would react with the cysteine thiolate anion of the active site. It was also planned to synthesize compounds in which the pyrrolidinone



(V)



(V)



(VI)

moiety (structure **V**) would be replaced with imidazolidinone moiety (structure **VI**). It was also planned to evaluate the synthesized compounds against falcipain enzyme by performing enzyme binding studies. These compounds would also be evaluated against Cathepsin-B and L, analogous cysteine proteases to determine the selectivity and specificity of inhibition for falcipain inhibition over Cathepsin-B and L inhibition.