Chapter 5.

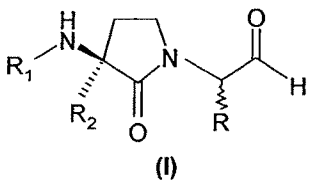
Summary

5. SUMMARY

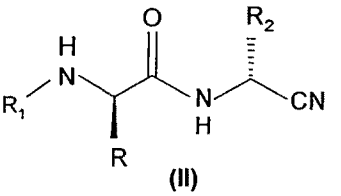
Malaria is one of the most important infectious diseases in the world infecting millions of people worldwide. A major factor in the persistence of widespread malaria is the increasing resistance of malaria parasites, in particular, *P. falciparum,* to available drugs. Malaria control efforts include attempts to eradicate mosquito vectors and develop an effective vaccine or new drugs. Efforts in the direction of vaccine development and control/eradication of the mosquito vectors have either failed or met with limited success. The limitations of antimalarial chemotherapy using the currently available drugs underscores the need for development of new drugs, ideally directed against new 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 to an acidic food vacuole, where the protein is hydrolyzed with the help of these proteases. These enzymes include aspartic and cysteine proteases. 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.

Most of the inhibitors of the cysteine proteases reported in the literature depend on the chemical interaction of an electrophilic "warhead" with cysteine thiolate anion of the active site. Peptide aldehydes, diamino ketones, and peptide nitriles are the examples of reversible inhibitors. Epoxysuccinyl derivatives, peptide Michael acceptors, acyloxy-methyl ketones and halomethyl ketones are examples of irreversible inhibitors. The γ-lactam or pyrrolidinone isosters attached with electron withdrawing group **(I)** like aldehydes have been reported as falcipain inhibitors[118]. Nitriles were first reported by Hanzlik[74] as inhibitors of plant protease, papain.

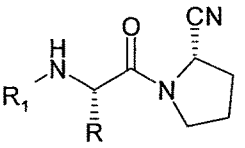


(I)

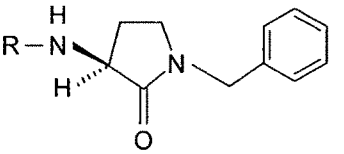


(II)

Some simple dipeptidyl nitriles of the type **(II)** have been reported as cysteine protease inhibitors. In light of these above observations, we have synthesized some



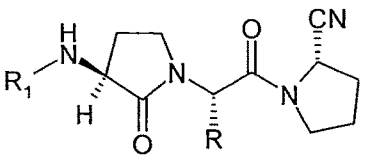
(III)



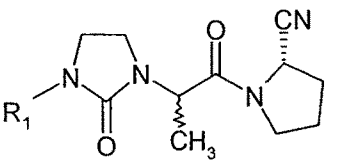
(IV)

simple dipeptidyl nitriles of the type **(III) (Scheme-II)** and some conformationally constrained pyrrolidinone inhibitors attached with α, β-unsaturated keto acids and sulfonamides of the type **(IV) (Scheme-III).**

The structural features of the above-described molecules (Type III and IV) were fused in one structure to afford a hybrid structure **(V) (Scheme-IV and V)**

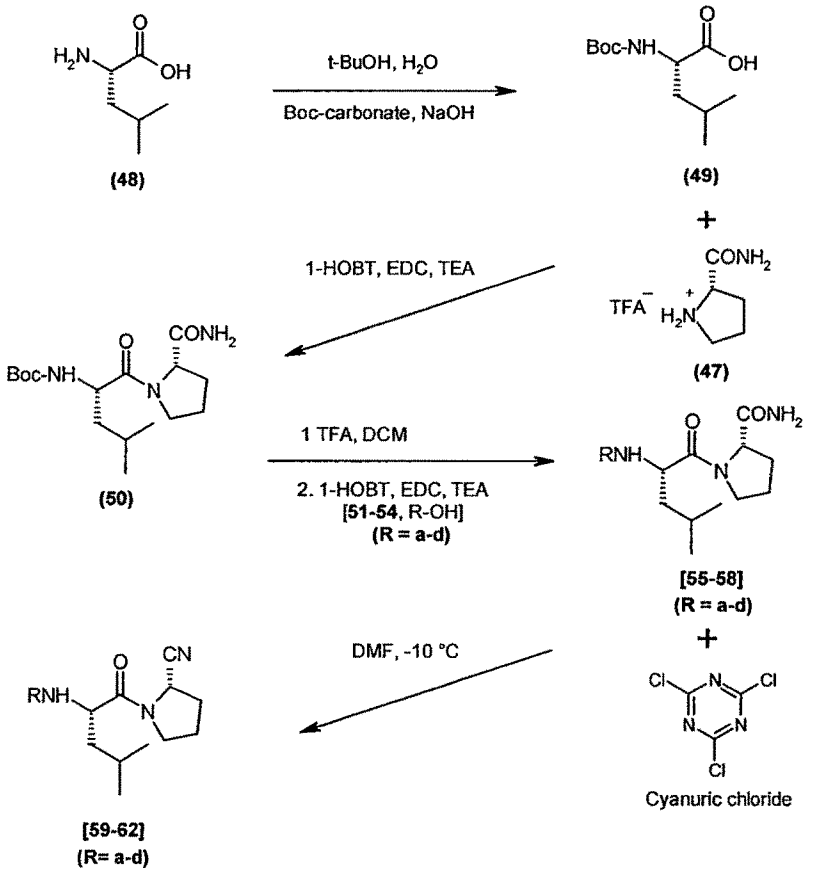


(V)

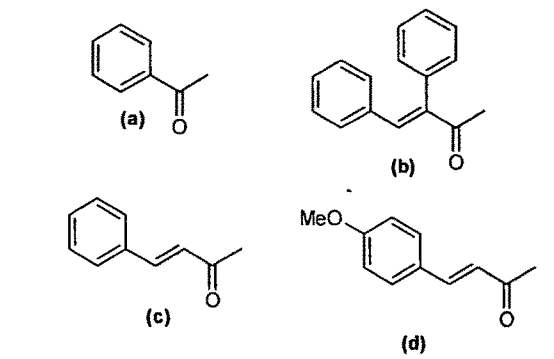


(VI)

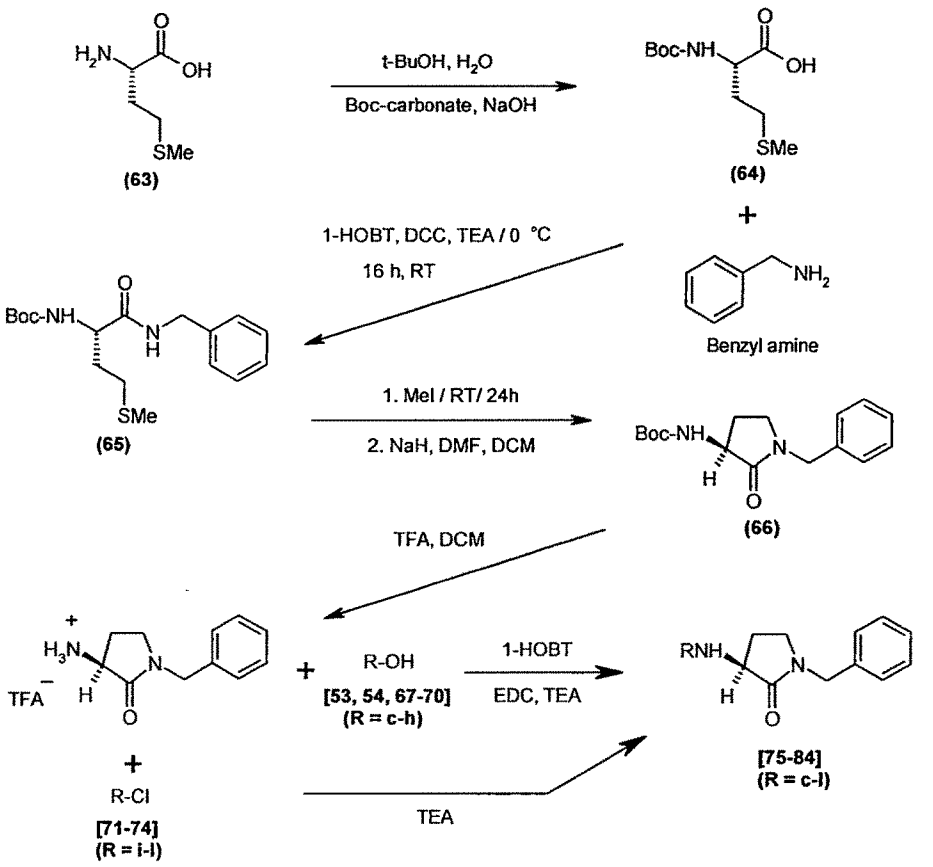
comprising of conformationally constrained pyrrolidinone moiety, which will help in fixing the conformation, and nitrile group, which will react with the cysteine thiolate anion of the active site. Isosteric replacement of pyrrolidinone ring was carried out in structure **(V)** to afford imidazolidinone type of compounds as shown in structure **(VI) (Scheme-VI).**


t-BuOH, H2O
Boc-carbonate, NaOH
(48)
(49)
1-HOBT, EDC, TEA
(47)
(50)
1 TFA, DCM
2. 1-HOBT, EDC, TEA
[51-54, R-OH]
(R = a-d)
[55-58]
(R = a-d)
DMF, -10 °C
Cyanuric chloride
[59-62]
(R= a-d)

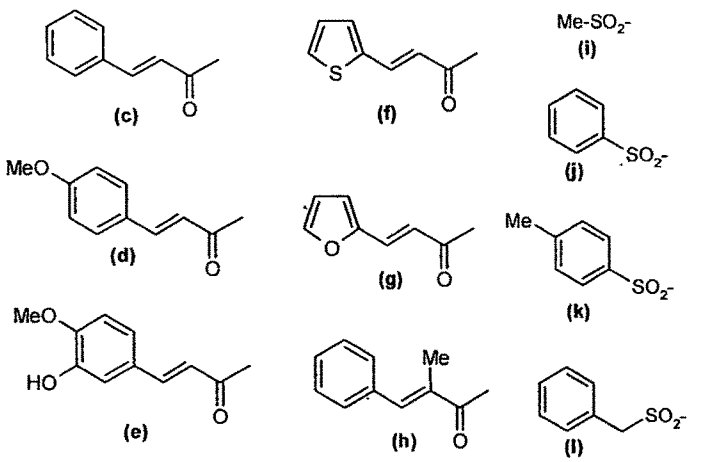
R


(a)
(b)
(c)
(d)
MeO

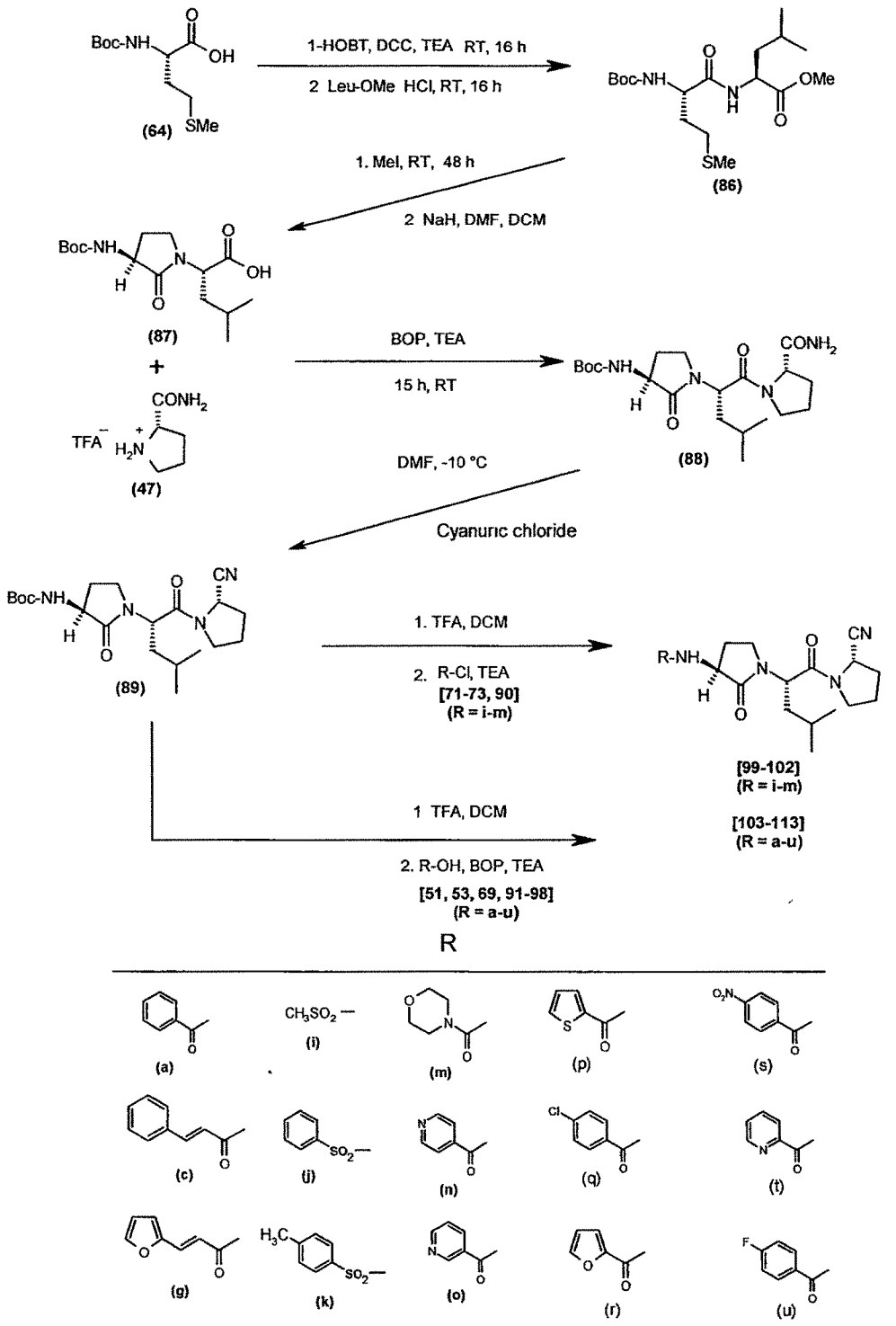
Scheme-II


t-BuOH, H2O
Boc-carbonate, NaOH
(63)
(64)
1-HOBT, DCC, TEA / 0 °C
16 h, RT
Benzyl amine
(65)
1. MeI / RT/ 24h
2. NaH, DMF, DCM
(66)
TFA, DCM
R-OH
[53, 54, 67-70]
(R = c-h)
1-HOBT
EDC, TEA
[75-84]
(R = c-l)
R-Cl
[71-74]
(R = i-l)
TEA

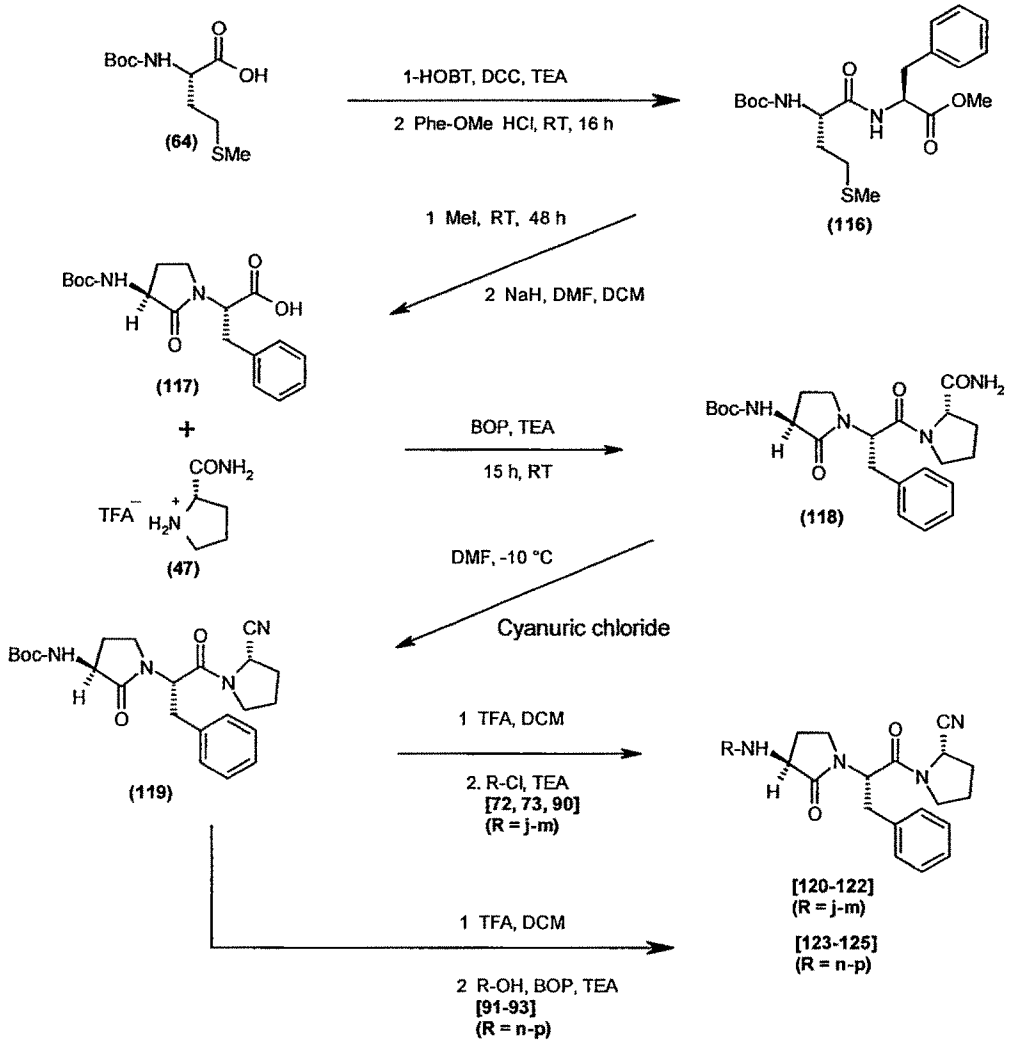
R


(c)
(f)
Me-SO2-
(i)
(j)
(d)
(g)
(k)
(e)
(h)
(l)

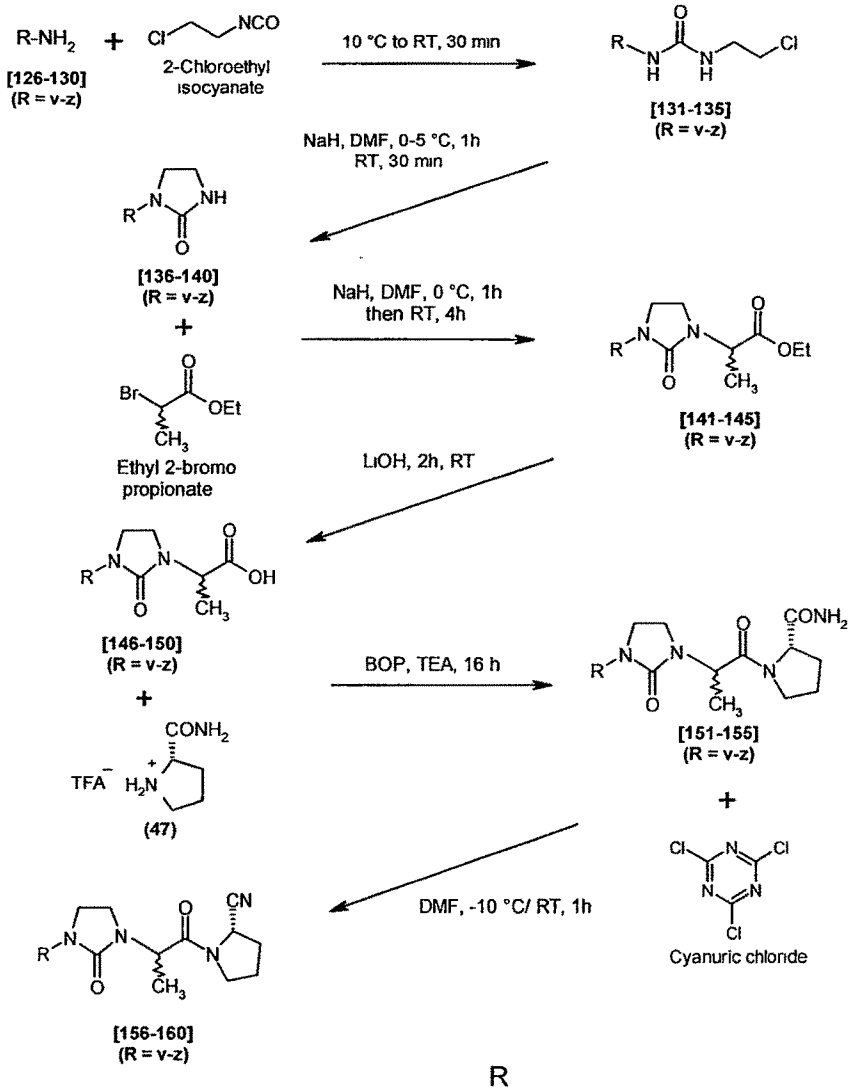
Scheme-III


Boc-NH
OH
SMe
(64)
1-HOBT, DCC, TEA RT, 16 h
2 Leu-OMe HCl, RT, 16 h
OMe
(86)
1. MeI, RT, 48 h
2 NaH, DMF, DCM
(87)
BOP, TEA
15 h, RT
CONH2
TFA
(47)
(88)
DMF, -10 °C
Cyanuric chloride
CN
(89)
1. TFA, DCM
2. R-Cl, TEA
[71-73, 90]
(R = i-m)
R-NH
[99-102]
(R = i-m)
[103-113]
(R = a-u)
1 TFA, DCM
2. R-OH, BOP, TEA
[51, 53, 69, 91-98]
(R = a-u)
R
(a)
(i)
(m)
(p)
(s)
(c)
(j)
(n)
(q)
(t)
(g)
(k)
(o)
(r)
(u)

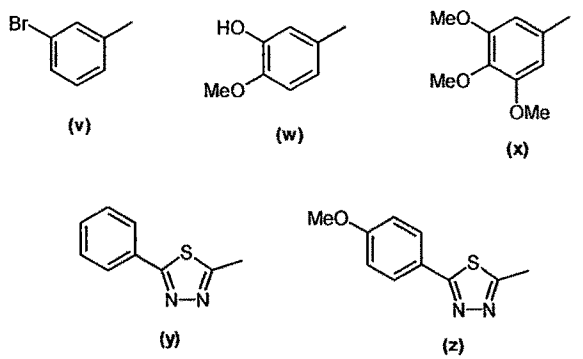
Scheme-IV


Boc-NH
OH
(64)
SMe
1-HOBT, DCC, TEA
2 Phe-OMe HCl, RT, 16 h
Boc-NH
OMe
SMe
(116)
1 MeI, RT, 48 h
2 NaH, DMF, DCM
Boc-NH
OH
(117)
+
TFA
CONH2
(47)
BOP, TEA
15 h, RT
Boc-NH
CONH2
(118)
DMF, -10 °C
Cyanuric chloride
Boc-NH
CN
(119)
1 TFA, DCM
2. R-Cl, TEA
[72, 73, 90]
(R = j-m)
R-NH
CN
[120-122]
(R = j-m)
1 TFA, DCM
2 R-OH, BOP, TEA
[91-93]
(R = n-p)
[123-125]
(R = n-p)
R
SO2
(j)
(m)
(o)
Me
SO2
(k)
(n)
S
(p)

Scheme-V


R-NH2
[126-130]
(R = v-z)
2-Chloroethyl isocyanate
10 °C to RT, 30 min
[131-135]
(R = v-z)
NaH, DMF, 0-5 °C, 1h
RT, 30 min
[136-140]
(R = v-z)
NaH, DMF, 0 °C, 1h
then RT, 4h
[141-145]
(R = v-z)
Ethyl 2-bromo propionate
LiOH, 2h, RT
[146-150]
(R = v-z)
BOP, TEA, 16 h
[151-155]
(R = v-z)
(47)
DMF, -10 °C/ RT, 1h
Cyanuric chloride
[156-160]
(R = v-z)

R


(v)
(w)
(x)
(y)
(z)

Scheme-VI

All the synthesized compounds were characterized by their physical, spectral and elemental data.

Biological studies:

As the aim of the current study was to prepare newer potential antimalarial agents, which would be active against the targets, not previously exploited. The preliminary screening of the compounds was done by *in vitro* method of falcipain inhibition[130]. IC_{50} values against recombinant Falcipain-2 and 3 were determined as described in the literature[132]. Equal amounts of recombinant enzyme were incubated for 30 min at room temperature in 100 mM sodium acetate, pH 5.5, 10 mM DTT with different concentrations of tested inhibitors. Inhibitor solutions were prepared from stock in DMSO (maximum concentration of DMSO in the assay was 1%). After 30 min incubation the substrate Z-Leu-Arg-AMC (Benzoxycarbonyl-Leu-Arg-7-amino-4-methyl-coumarin) in the same buffer was added to final concentration 25 M. Fluorescence was monitored for 15 min at room temperature in a Labsystems Fluoroskan Ascent sectroflurometer. IC_{50} values were determined from plots of percents of activity over the compound concentration using Graph pad Prism Software. All the screened compounds showed IC_{50} values above 50 μM (Table 2).

These compounds were also evaluated against Cathepsin-B and L, analogous cysteine proteases to determine the selectivity and specificity of inhibition of the synthesized compounds for falcipain inhibition over Cathepsin inhibition[131]. Some of them from Scheme-II and -III (Compound **60**(cat L)IC_{50} = 5.3 μM, Compound **83** (cat B)IC_{50} = 79 μM, Table 3) were found to be good inhibitors of Cathepsin.

Although, the falcipain inhibition of all these compounds is mild, a better molecule can be obtained through the structure activity study based on the observations that we have obtained over here. In fact, Scheme-VI is the positive step in this direction where the pyrazolidinone ring was isosterically replaced with imidazolidinone. Also, the compounds from Series-IV to VI are inactive against cathepsin. At the same time Schemes-II and -III can be exploited for

cathepsin inhibitory activity as some of the compounds from these series are potent inhibitors of cathepsin-L and -B.