Chapter 3.

Results and Discussion

3 RESULTS AND DISCUSSION

The work carried out towards achieving the proposed plan has been discussed under the following two main headings

1. Chemical studies and
2. Biological evaluation

3.1 CHEMICAL STUDIES

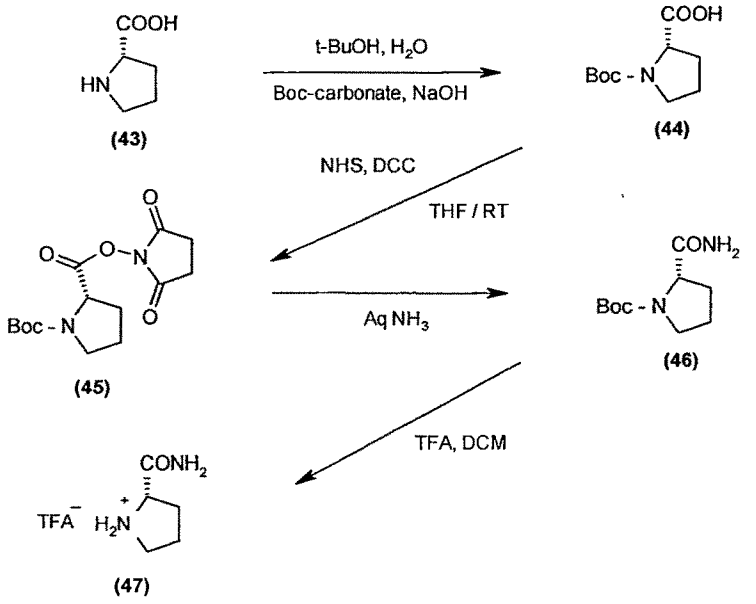
The chemical work carried out has been divided into following different categories: *L*-Prolinamide TFA salt; *N*-Substituted (*S*)1-[(*S*)2-Cyanopyrrolidine-1-carbonyl]-3-methylbutylamines; Alkyl/arylsubstituted 3-acrylamido/ sulfonamido-2-oxo-1-benzylpyrrolidines; *N*-{(*S*)1-[(*S*)1-((*S*)2-Cyanopyrrolidine-1-carbonyl)-3-methylbutyl]-2-oxo-3-pyrrolidino}sulfonamides/carboxamides; *N*-{(*S*)1-[(*S*)1-Benzyl-((*S*)2-cyano-1-pyrrolidino)-2-oxoethyl]-2-oxo-3-pyrrolidino} sulfonamides/carboxamides; (*S*)1-{2-[3-(Substituted)-2-oxo-1-imidazolidinyl] pyrrolidine-2-carbonitriles.

Compounds belonging to individual categories have been synthesized following six major schemes as described below:

3.1.1 *L*-Prolinamide TFA salt (47)

L-Prolinamide required for the synthesis of some of the target compounds, was synthesized as its TFA salt **(47)** as outlined in Scheme-I. The intermediate Boc-*L*-prolinamide **(46)** was prepared starting from *L*-Proline **(43)**. *L*-Proline **(43)** was treated with Boc-carbonate in presence of t-butanol and aq solution of sodium hydroxide for 15 h at room temperature. The reaction mixture was acidified with citric acid to liberate the acid, Boc-*L*-proline **(44)** as a white crystalline solid. Compound **(44)** was treated with NHS and DCC in dichloromethane. The reaction mixture was filtered to remove DCU and concentrated under vacuum to get N-hydroxysuccinimide ester **(45)** as a white solid. The ester **(45)** without further characterization was reacted with aq ammonia (30 %) in THF, to give Boc-*L*-prolinamide **(46)**. The presence of

characteristic peaks at 3381 and 3201 (NH_2 stretching), 1706 (carbamate C=O stretching) and 1684 cm^{-1} (amide C=O stretching) in the IR spectrum confirmed



Scheme-I

the formation of compound **(46)**. The amide **(46)** was treated with trifluroacetic acid in dichloromethane at lower temperatures to get a yellow sticky mass of TFA salt of *L*-prolinamide **(47)** in almost quantitative yield. The salt **(47)** was used as such in all further reactions without any more purification and characterization.

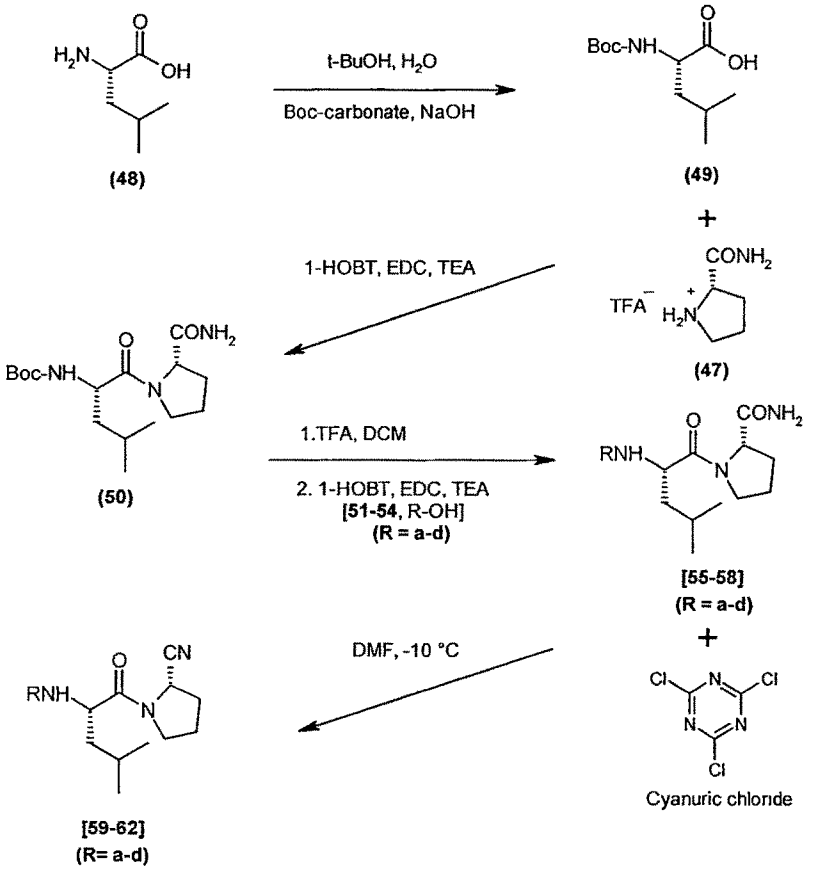
3.1.2 *N*-Substituted (*S*)1-[(*S*)2-Cyanopyrrolidine-1-carbonyl]-3-methylbutyl-amines (59-62)

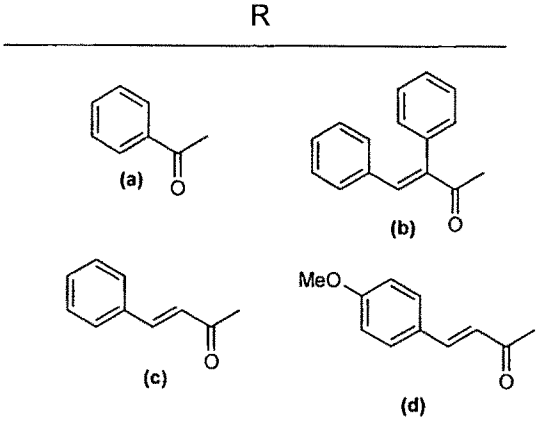
Simple dipeptidyl nitrile derivatives have been reported as inhibitors of papain and a variety of other cysteine proteases like cathepsins S and B[76, 77]. By varying the amino acid sequence at P_1, P_2 and P_3 (Figure 3) attached with electrophilic warhead like nitrile, selectivity could be achieved towards a particular protease. Reports[115, 116] in the literature showed that for falcipain

inhibitors leucine is a preferred amino acid at P_2 position for binding to the enzyme. In light of this observation we decided to adopt this strategy for preparing compounds with better selectivity towards falcipains. Accordingly, we planned to synthesize some simple dipeptidyl nitrile derivatives **(59-62)** as outlined in Scheme-II. Thus, the designed molecules have leucine at P_2 position for binding to the enzyme and proline nitrile at P_3 position, which was expected to react with cysteine residue, the active site present in the enzyme. Moreover, compounds **(60-62)** also possess active sites in the form of α,β-unsaturated systems where Michael-type of addition reaction could take place with the enzyme.

L-Leucine **(48)** was reacted with Boc-carbonate in basic medium at room temperature to obtain Boc-*L*-leucine monohydrate **(49).** IR spectrum of the compound **(49)** showed characteristic peaks at 3463 (OH), 3338 (NH), 1716 (acid C=O) and at 1677 cm^{-1} (carbamate C=O). Compound **(49)** was treated with 1-HOBT and EDC in dichloromethane and the resulting reactive species was reacted with compound **(47)** in presence of base, TEA. Compound **(50)** so formed was precipitated out in the acidic medium and purified by column chromatography. It offered characteristic peaks at 3381 and 3201 (NH_2 stretching), 1706 (ester C=O stretching) and 1684 cm^{-1} (amide C=O stretching) in the IR spectrum. The Boc-protected amide **(50)** was treated with trifluroacetic acid to deprotect the amino group resulting into formation of the TFA salt of the amine. The amine salt was used as such without further purification and characterization. Different acids **(51-54)** were reacted with the resulting amine salt leading to the formation of various amides **(55-58).**

Acids **(52, 54)** required for the synthesis of final compounds **(60, 62)** were prepared following the reported procedure[129]. A mixture of benzaldehyde, phenylacetic acid, acetic anhydride and TEA was refluxed and the mixture so obtained was steam distilled. The residue obtained was acidified to afford compound **(52; R = b).** A mixture of anisaldehyde, malonic acid, pyridine and catalytic amount of piperidine was heated at 60 °C. The reaction mixture on


t-BuOH, H_2O
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)

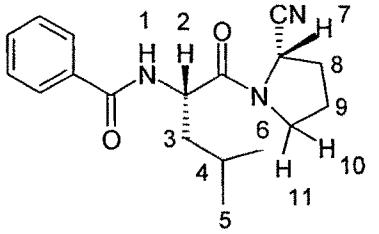

R
(a)
(b)
(c)
(d)
MeO

Scheme-II

acidification afforded compound (**54; R = d**). Benzoic acid (**51; R = a**) was activated with 1-HOBT and EDC in dichloromethane and reacted with the TFA salt of compound (**50**) to obtain the diamide (**55; R = a**). Its IR spectrum showed peaks at 3500 and 3258 (NH stretching), 1684, 1669 and 1635 cm^{-1} (for the three amide C=O stretching).

Compounds (**56-58**) were synthesized in the same manner as described above for compound (**55**). For compound (**56; R = b**), α-phenylcinnamic acid (**52; R = b**) was used instead of benzoic acid. Its IR spectrum showed characteristic peaks at 3408 and 3300 (NH_2 stretching), 1686 ($CONH_2$), 1646 (amide C=O directly attached to the pyrrolidine ring) and at 1615 cm^{-1} (amide C=O of the conjugated system). *Trans*-cinnamic acid (**53; R = c**) afforded amide (**57; R = c**). Its IR spectrum showed characteristic peaks at 3381 and 3258 (NH_2 stretching), 3176 (NH), 1686 ($CONH_2$), 1666 (amide C=O stretching) and 1632 cm^{-1} (C=O of the conjugated system). Compound (**58; R = c**) was synthesized in a similar fashion using p-methoxycinnamic acid (**54; R = d**) to yield amide (**58**). The IR spectrum showed characteristic peaks at 3463 and 3368 for NH_2 stretching, 3171 for NH, 1686 for $CONH_2$, 1663 for amide C=O and 1632 cm^{-1} for C=O of the conjugated system, in accordance with compounds (**56** and **57**).

In order to obtain the targeted nitrile derivatives (**59-62**), it was planned to perform dehydration reaction on primary amide functionality of the amides (**55-58**) using some mild dehydrating agent. Cyanuric chloride proved an efficient



(59)

dehydrant for this purpose. Dehydration of terminal amide group in compound (**55**) with the cyanuric chloride in dry DMF at -10 °C offered the desired dipeptide nitrile (**59; R = a**) which was purified by column chromatography.

Peaks in its IR spectrum were observed at 3268 for NH, 2245 for CN and 1673 and 1635 cm^{-1} (for the C=O of two amides). PMR spectra of this series of compounds **(59-62)** offered interesting observations. Aromatic protons for compound **(59)** appeared at δ 7.78 (d, 2H; o-Ar*H*) and δ 7.49 (m, 3H). The N*H* proton got split into a doublet due to coupling with proton (2-C*H*) and appeared at δ 6.75-6.78. Other signals appeared at δ 4.95-5.02 (m, 1H; 2-C*H*), 4.77-4.81 (m, 1H; 7-C*H*), 2.17-2.32 (m, 4H; 8-CH_2 and 9-CH_2), 1.70-1.81 (m, 2H; 3-CH_2) and 1.61-1.65 (m, 1H; 4-C*H*). Interestingly, both the methylene protons of the pyrrolidine ring (10-C*H* and 11-C*H*) got split into two separate multiplets appearing at δ 3.68-3.74 and 3.85-3.92. Similarly, the methyl protons of isopropyl group (5-CH_3 and 6-CH_3) appeared as separate doublets at δ 1.03-1.05 and δ 0.98-1.00 in the PMR spectrum. The compound **(59)** gave quasi molecular ion peak at 314 in its mass spectrum. Other major fragments were obtained at 336 (M+Na), 218, 190 and 105 (Figure 11).

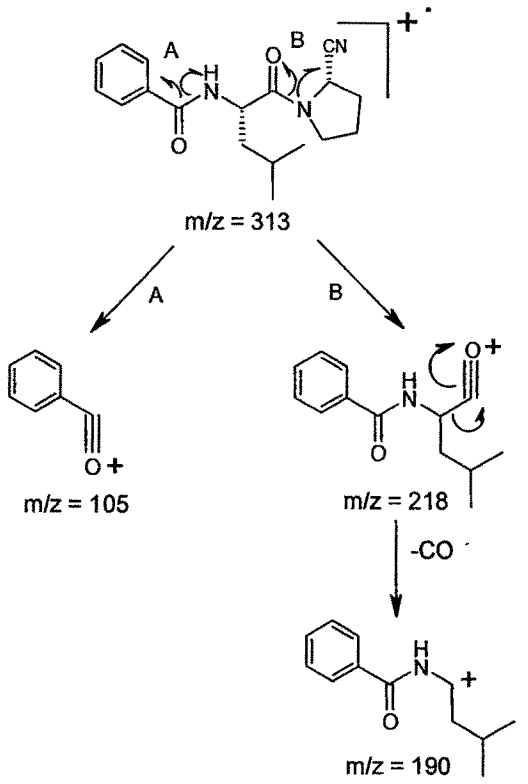
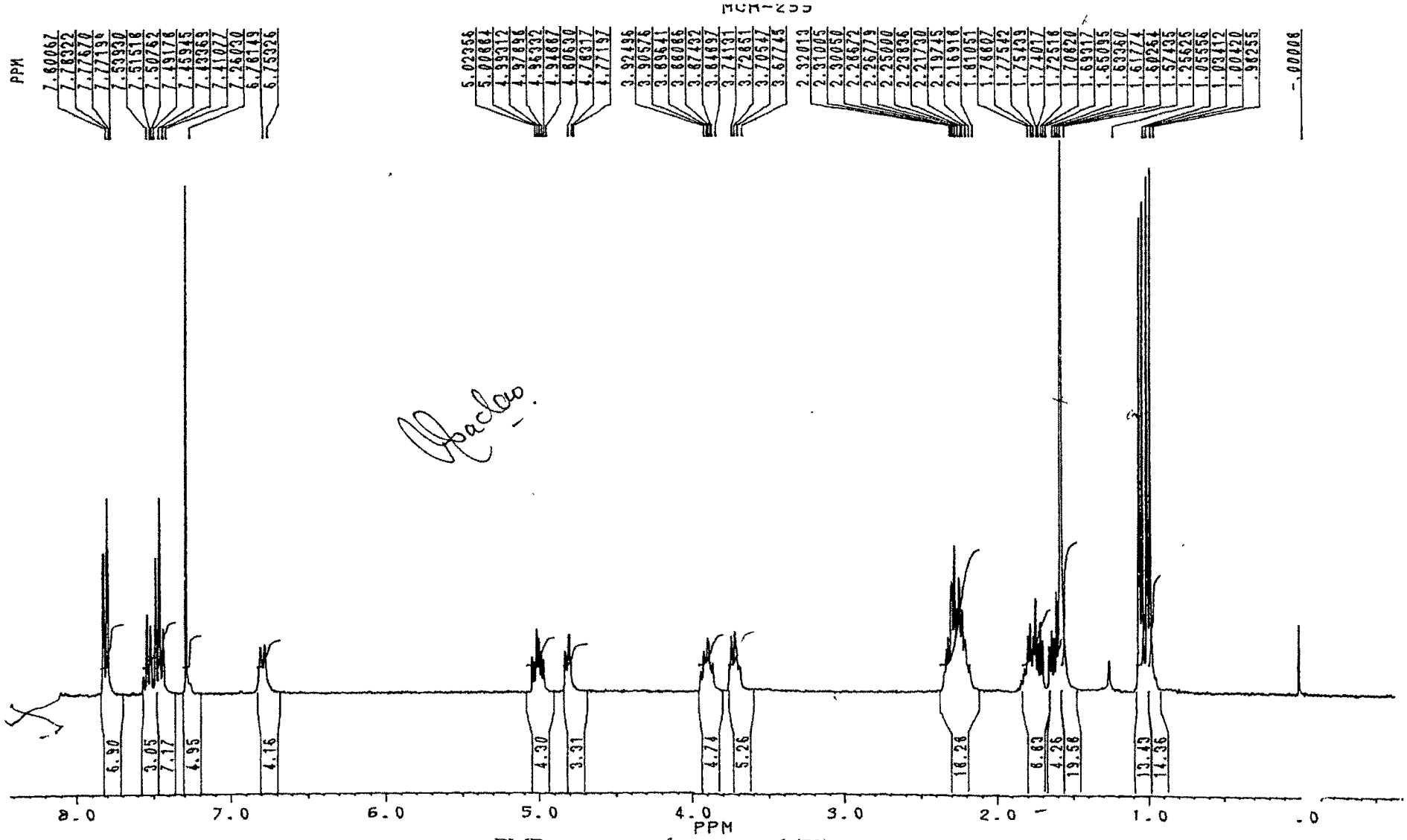
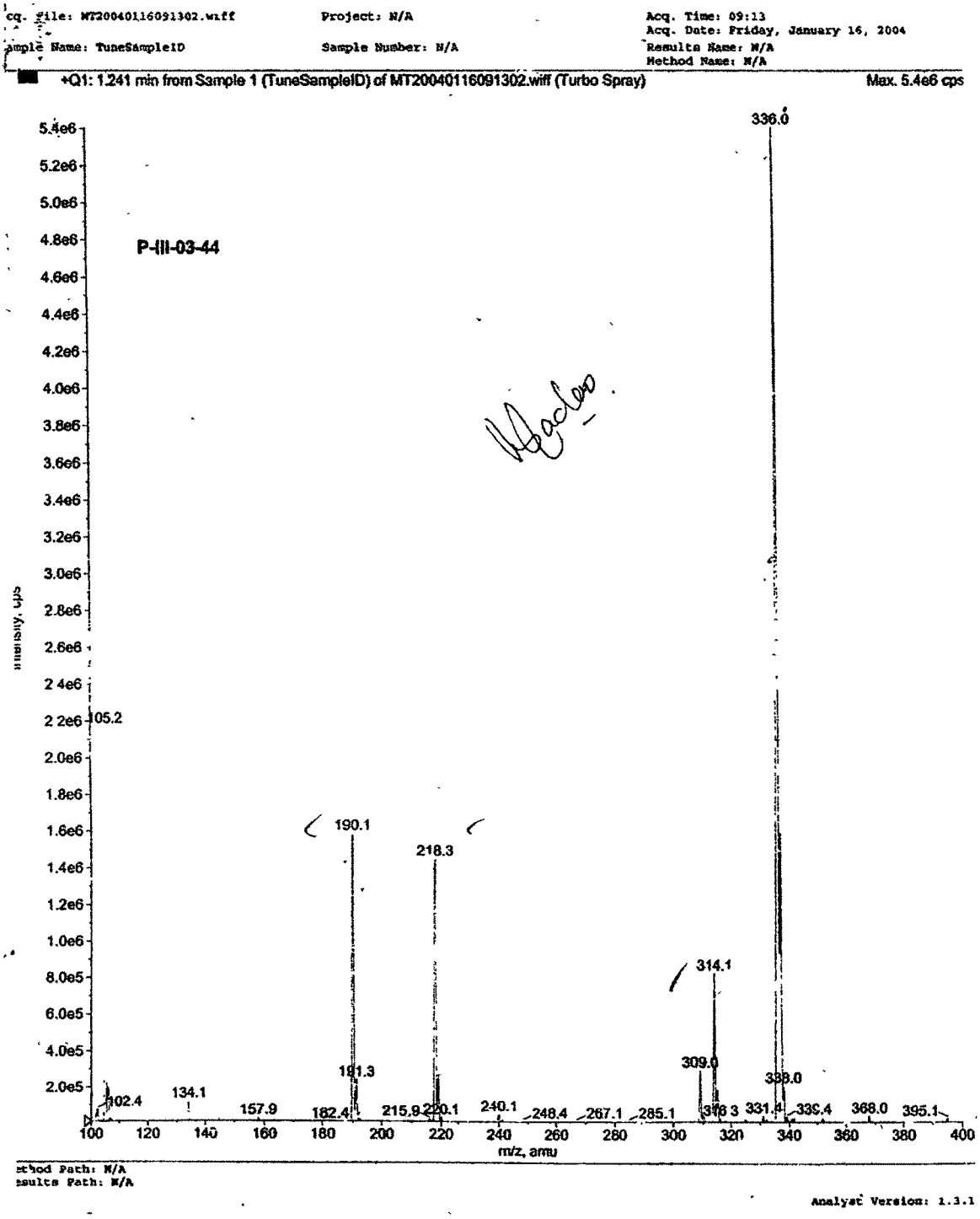


Figure 11: Mass spectral fragmentation pattern of compound **(59)**

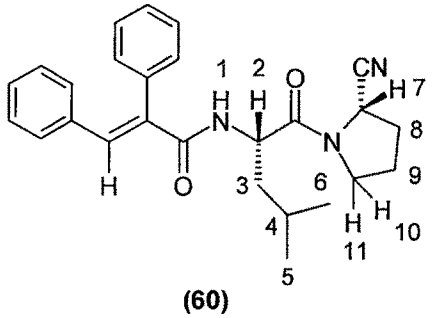


PMR spectrum of compound **(59)**



Mass spectra of compound (59)

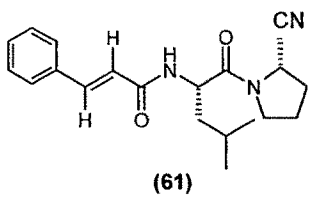
Compounds **(60-62)** were synthesized as described above. Compound **(60)** offered characteristic peaks at 3422 (NH), 2232 (CN), 1666 and 1652 cm^{-1} (for two amide C=O stretching) in its IR spectrum. In the PMR spectrum of the compound **(60)** the vinylic proton appeared as a singlet at δ 7.80 and the aromatic protons



(60)

appeared at δ 7.43-7.49 (m, 3H), 7.24-7.27 (m, 2H), 7.10-7.20 (m, 3H) and 6.96-6.99 (m, 2H). Other signals were at δ 6.00-6.04 (d, N*H*), 4.81-4.88 (m, 1H; 7-C*H*), 4.75-4.78 (m, 1H; 2-C*H*), 3.84-3.92 (m, 1H; 10-C*H*), 3.45-3.68 (m, 1H; 11-C*H*), 2.19-3.00 (m, 4H; 8-CH_2 and 9-CH_2), 1.52-1.65 (m, 2H; 3-CH_2), 1.25-1.48 (m, 1H; 4-C*H*), 0.97-0.98 (d, 3H; 5-CH_3) and 0.90-0.92 (d, 3H; 6-CH_3).

Trans-cinnamic acid **(53; R = c)** was used instead of benzoic acid to afford nitrile **(61; R = c)**. Its IR spectrum showed characteristic peaks at 3280 for NH,

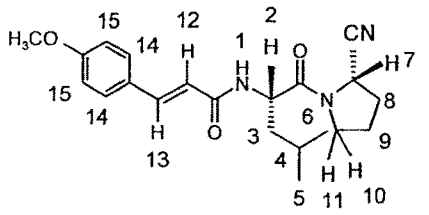


(61)

2246 for CN, 1652 for keto directly attached to pyrrolidine ring and 1628 cm^{-1} for keto amide.

Compound **(62; R = d)** was synthesized as described above for compound **(59)** with the difference that p-methoxycinnamic acid **(54; R = d)** was used instead of benzoic acid to get the amide **(58)** which was dehydrated using cyanuric chloride to obtain compound **(62)**. Peaks were observed at 3272 (NH), 2245 (CN), 1659 (C=O attached to pyrrolidine ring) and at 1625 cm^{-1} (amide C=O stretching)

in its IR spectrum. In the PMR spectrum vinylic protons appeared as two doublets at δ 7.51- 7.56 (13-C*H*; *J*= 15.6 Hz) and 6.26-6.41 (12-C*H*; *J*= 15.6 Hz)



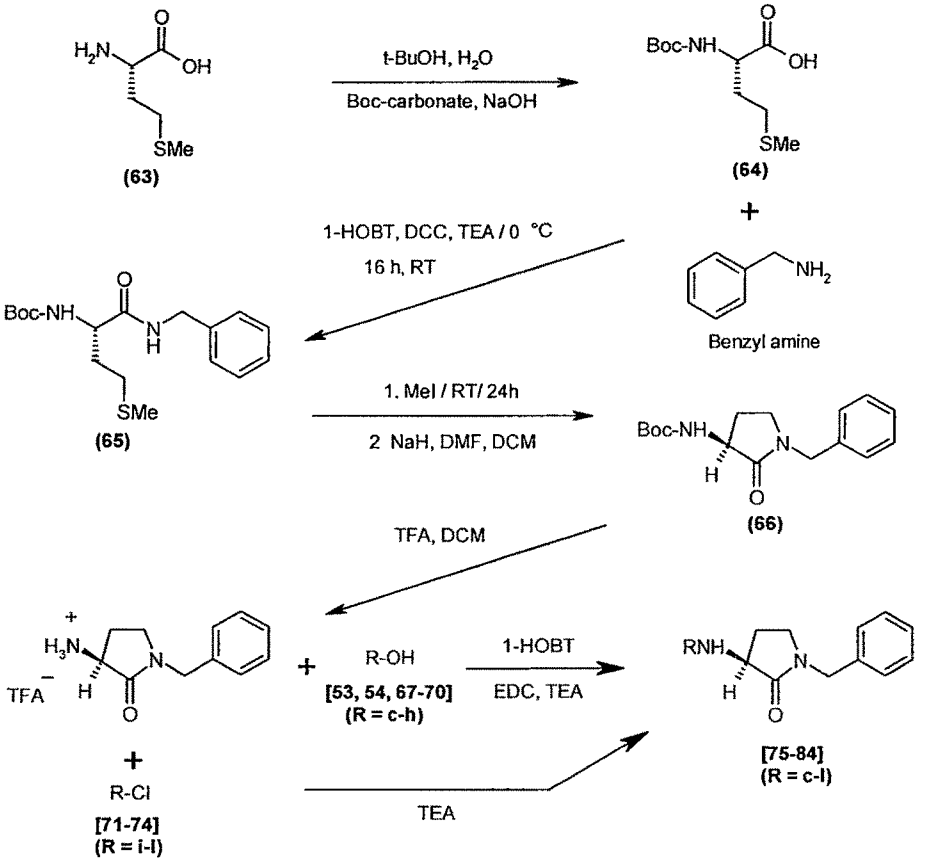
(62)

and similarly, four aromatic protons appeared as two doublets at δ 7.39-7.43 (2H, 14-C*H*; *J*= 11.5 Hz) and 6.86-6.89 (2H, 15-C*H*; *J*= 11.5 Hz). Other signals in the spectrum appeared at δ 6.38-6.41 (d, 1H; 1-N*H*), 4.86-4.94 (m, 1H; 2-C*H*), 4.78-4.80 (m, 1H; 7-C*H*), 3.85-3.93 (m, 1H; 10-C*H*), 3.83 (s, 3H; -OCH_3), 3.66-3.71 (m, 1H; 11-C*H*), 2.18-2.31 (m, 4H; 8-CH_2 and 9-CH_2), 1.26-1.81 (m, 3H; 3-CH_2 and 4-C*H*), 1.01-1.03 (d, 3H; 5-CH_3) and 0.97-0.99 (d, 3H; 6-CH_3).

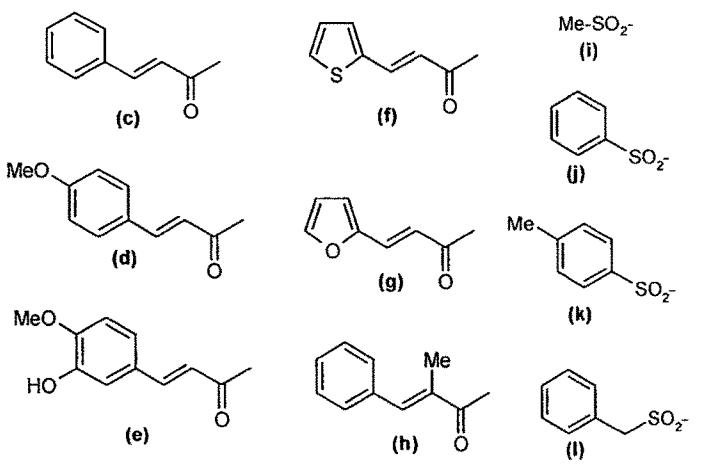
It was interesting to note the splitting of N*H* protons (*J*~ 8 Hz), existence of difference in chemical shift of the two protons in the pyrrolidine ring (10-C*H* and 11-C*H*) and appearing of both the methyl groups (5-CH_3 and 6-CH_3) as two separate doublets at two δ values, in the PMR spectra of all these final compounds **(59-62)**.

3.1.3 Alkyl/arylsubstituted 3-acrylamido/sulfonamido-2-oxo-1-benzyl-pyrrolidines (75-84)

Peptides and unconstrained peptide inhibitors can exist in a number of conformations in solution. Limiting the number of conformations of a drug can improve its binding affinity to the effector by lowering the entropic contribution of a particular favourable conformation. Different approaches have been used in the designing of peptides and peptidomimetic compounds to incorporate a conformational constraint. One such approach is the replacement of an amide bond with γ-lactam or pyrrolidinone, which would have a restricted rotation[128].


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

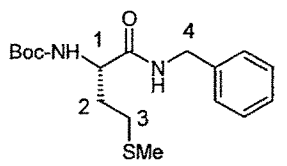
R


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

Scheme-III

The pyrrolidinone isoster has been shown to be an effective conformational constraint for certain classes of protease inhibitors[118]. Keeping the above fact in mind it was decided to synthesize compounds **(75-84)** as outlined in Scheme-III. Some of these compounds **(75-80)** could provide a site for Michael addition reaction by a nucleophile present in the enzyme, alongwith general physical binding affinities, in the form of α, β-unsaturated system.

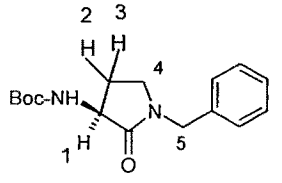
L-Methionine **(63)** was treated with Boc-carbonate in a mixture of t-butanol and aq sodium hydroxide to provide Boc-*L*-methionine **(64)** as a sticky mass. Protection of amino group in compound **(64)** was confirmed by its IR spectrum. Presence of peaks at 3450 (OH), 3326 (NH), 1710 (acid C=O) and 1686 cm^{-1} (carbamate C=O) confirms the formation of **64**. Compound **(64)** was treated



(65)

with benzyl amine in presence of DCC, 1-HOBT and TEA to give compound **(65)**. Characteristic peaks at 3337 and 3317 for NH, 1680 for carbamate C=O and at 1656 for amide C=O were observed in its IR spectrum. The PMR spectrum showed multiplet at δ 7.27-7.54 for five aromatic protons, broad singlets at δ 6.61 and 5.21 for two amide CON*H*, doublet at δ 4.46-4.47 for (4-CH_2, *J*= 8 Hz), multiplet at δ 4.29-4.30 for 1-C*H*, multiplet at δ 2.49-2.63 for 3-CH_2, multiplet at δ 2.11-2.18 and δ 1.92-1.98 for 2-CH_2, a sharp singlet at δ 2.09 for three protons of (-SCH_3) and a singlet for nine protons at δ 1.44 for ((CH_3)$_3$). Compound **(65)** on treatment with methyl iodide offered methyl sulfenium iodide salt of compound **(65)**, which was cyclized to γ-lactam **(66)** on sodium hydride treatment117. IR spectrum of the compound **(66)** gave peaks at 3287 for NH, 1710 for lactam C=O, and 1671 cm^{-1} for carbamate C=O. The PMR spectrum showed peaks at δ 7.28-7.36 and at δ 7.20-7.23 for five aromatic protons, broad singlet at δ 5.14 for (N*H*), double doublet at δ 4.46-4.53 for 5-CH_2, multiplet at 4.20-4.22 for 1-C*H*,

double doublet at δ 3.17-3.22 for 4-CH_2, multiplet at δ 2.57-2.64 and 1.75-1.89 for

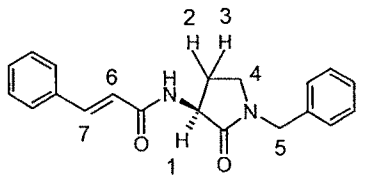


(66)

2-C*H* and 3-C*H* and a sharp singlet at δ 1.45 for nine protons of ((CH_3)$_3$). Mass spectrum of the compound **(66)** showed quasi molecular ion peak at 291.3.

Acids **(67-69)** required for the synthesis of final compounds **(77-79)** were prepared following the reported procedure[129]. Ferulic acid **(67; R = e)** was synthesized as described above for p-methoxycinnamic acid **(54; R = d)** except that vanillin was used instead of p-anisaldehyde. 2-Thiopheneacrylic acid **(68; R = f)** and 2-furylacrylic acid **(69; R = g)** were obtained from thiophene-2-carboxaldehyde and furfural, respectively.

The protecting group in amide **(66)** was removed by treating the compound with trifluroacetic acid to afford TFA salt of compound **(66)**. The TFA salt was not characterized and used as such for the next step. It was treated with *trans*-cinnamic acid **(53; R = c)** under standard peptide coupling conditions using EDC and 1-HOBT in presence of TEA to afford **(75; R = c)**. It exhibited peaks at 3279 (NH), 1683 (lactam C=O) and 1646 cm^{-1} (amide C=O) in the IR spectrum.

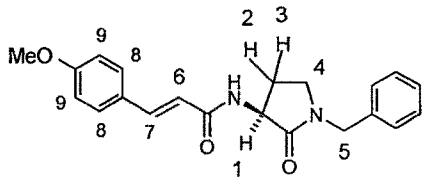


(75)

The PMR spectrum showed two doublets at δ 7.62-7.67 (7-C*H*) and 6.45-6.51 (6-C*H*), multiplets at δ 7.48-7.52 and 7.24-7.42 for ten aromatic protons, and a broad singlet at 6.44 for N*H* proton. Multiplet for three protons at δ 4.46-4.58 (1-C*H* and 5-CH_2), multiplet at δ 3.23-3.34 for two methylene protons (4-CH_2), two multiplets

equivalent to one proton each at δ 2.77-2.86 and at δ 1.91-1.95 for 2-C*H* and 3-C*H* were also observed. It offered quasi molecular ion peak at 321.3 in its mass spectrum.

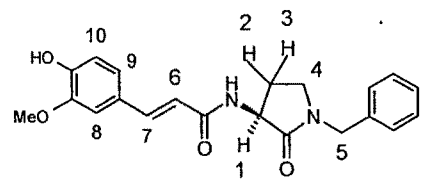
Other carboxamides **(76-80)** were synthesized in similar way as described above. Compound **(76; R = d)** was synthesized by using p-methoxycinnamic acid **(54; R = d)** instead of cinnamic acid. Its IR spectrum offered peaks at 3269 (NH), 1700 (lactam C=O) and at 1647 cm^{-1} (amide C=O). PMR spectrum of compound **(76)** showed peaks at δ 7.57-7.62 (d, 1H; 7-C*H*), 7.44-7.47 (d, 2H; 8-ArC*H*), 7.24-7.39 (m, 5H; Ar-*H*), 6.88-6.91 (d, 2H; 9-ArC*H*), 6.32-6.37 (d, 1H; 6-C*H*), a broad



(76)

singlet at 6.32 (N*H*), 4.46-4.58 (m, 3H; 1-C*H* and 5-CH_2), 3.82 (s, 3H; OCH_3), 3.25-3.32 (m, 2H; 4-CH_2), 2.76-2.85 (m, 1H; 2-C*H*) and 1.82-1.95 (m, 1H; 2-C*H*). The compound **(76)** gave quasi molecular ion peak at 351.3 in its mass spectrum.

Ferulic acid **(67; R = e)** was coupled with the TFA salt of compound **(66)** to afford the carboxamide **(77; R = e)**. Absorption bands at 3261, 3080, 1686 and 1664 cm^{-1} were observed in its IR spectrum for NH, OH, lactam C=O and amide C=O

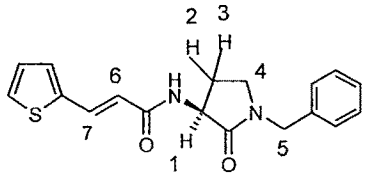


(77)

groups, respectively. The PMR spectrum of compound **(77)** showed signals for vinylic protons at δ 7.50-7.55 (d, 1H; 7-C*H*; *J* = 15.6 Hz) and 6.26-6.34 (d, 1H; 6-C*H*; *J* = 15.6 Hz), and aromatic protons at δ 7.23-7.38 (m, 5H), 7.00-7.04 (d, 1H; 9-ArC*H*), 6.97 (s, 1H; 8-ArC*H*) and 6.87-6.91 (d, 1H; 10-ArC*H*). Other signals were

at δ 6.54-6.56 (d, 1H; N*H*), 6.02 (s, 1H; O*H*), 4.51-4.59 (m, 3H; 5-CH_2 and 1-C*H*), 3.92 (s, 3H; OCH_3), 3.25-3.30 (m, 2H; 4-CH_2), 2.72-2.82 (m, 1H; 2-C*H*) and 1.81-1.93 (m, 1H; 3-C*H*). It offered quasi molecular ion peak at 367.3 in its mass spectrum.

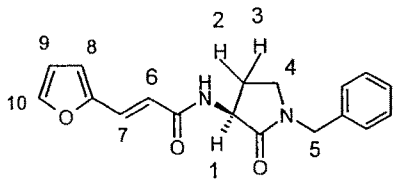
The TFA salt of compound **(66)** was treated with 2-thiopheneacrylic acid **(68; R = f)** following the procedure as described for compound **(75)** to afford compound **(78; R = f)**. Peaks in IR spectrum were observed at 3279, 1680 and 1648 cm^{-1}, respectively for NH, lactam C=O and amide C=O stretching vibrations. The PMR spectrum showed doublet at δ 7.71-7.76 for (7-C*H*; *J* = 15.4 Hz), multiplet at δ 7.01-7.38 for eight aromatic protons, doublet at δ 6.40-6.42 for N*H*, doublet at



(78)

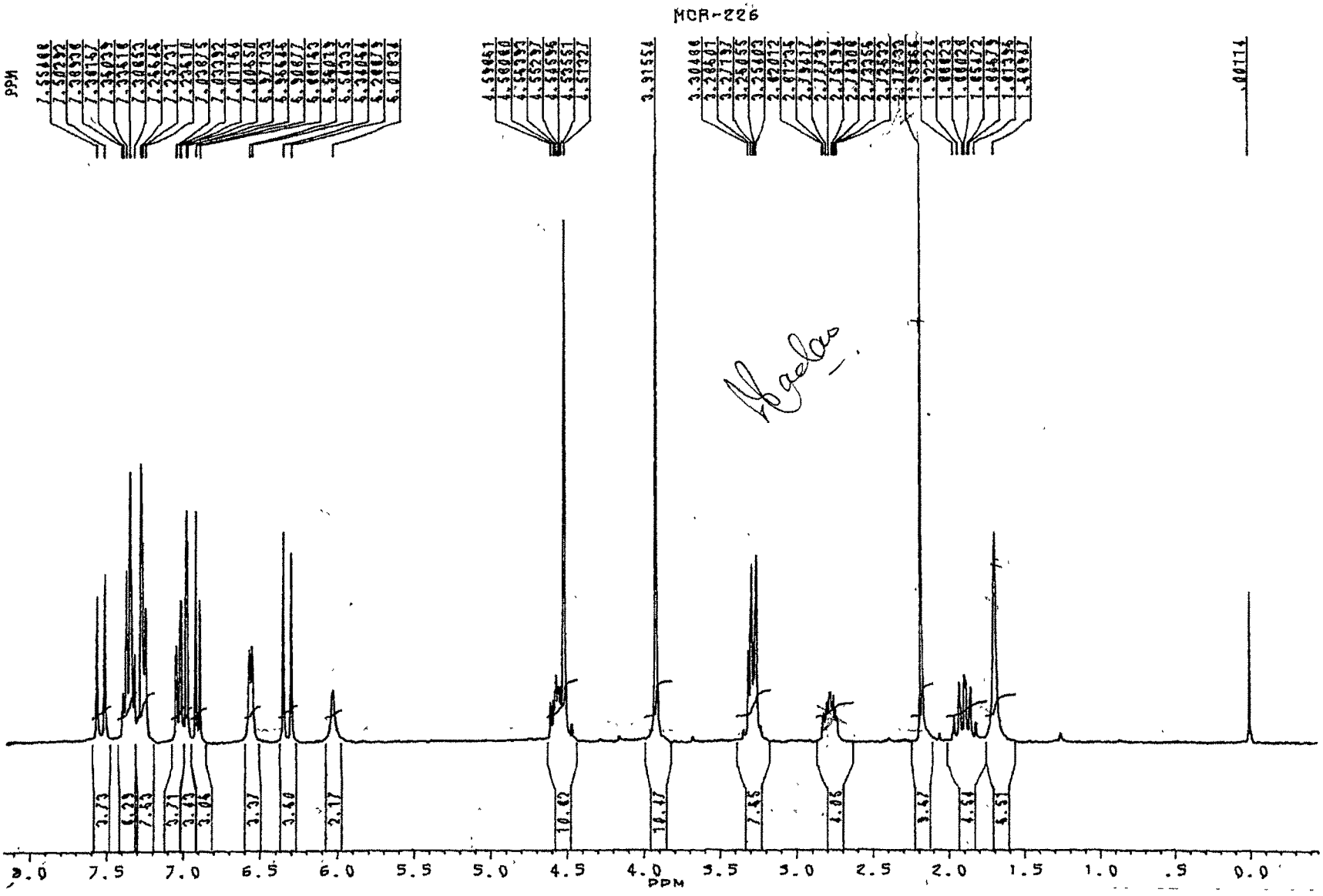
6.26-6.31 (6-C*H*; *J* = 15.4 Hz), multiplet at 4.44-4.56 (1-C*H* and 5-CH_2), multiplet at 3.24-3.29 (4-CH_2) and multiplet at 2.73-2.82 and 1.78-1.92 (2-C*H* and 3-C*H*). The quasi molecular ion peak was observed at 327.1 in its mass spectrum.

Similarly, reaction between TFA salt of compound **(66)** and 2-furylacrylic acid **(69; R = g)** as described above for compound **(75)** gave compound **(79; R =**

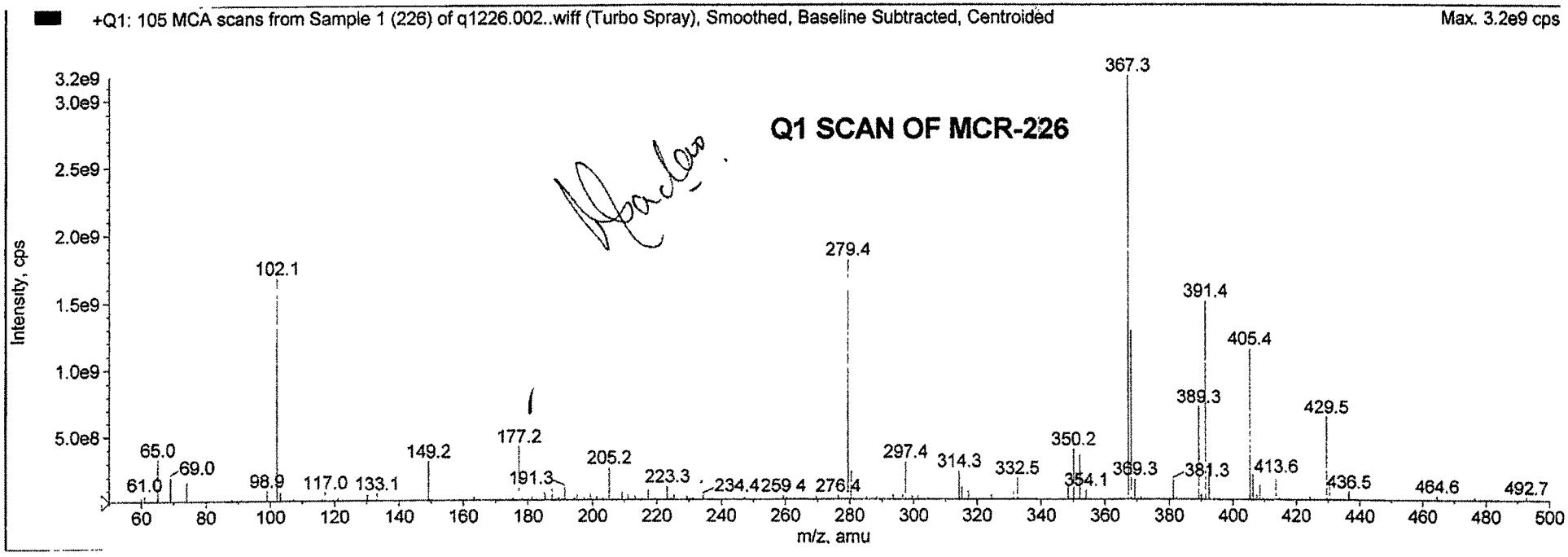


(79)

g). Peaks at 3300, 1696 and 1652 cm^{-1}, for NH, lactam C=O and amide C=O respectively, were observed in the IR spectrum of compound **(79)**. PMR spectrum offered signals at δ 7.23-7.69 (m, 8H; 7-C*H*, 8-ArC*H*, 10-ArC*H* and phenyl protons), 6.55-6.56 (d, 1H; N*H*), 6.44-6.45 (m, 1H; 9-ArC*H*), 6.35-6.40 (d,



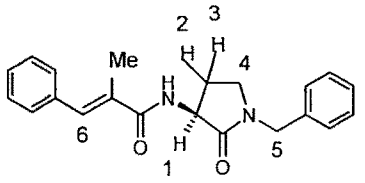
PMR spectrum of compound (77)



Mass spectrum of compound (77)

1H; 6-C*H*), 4.45-4.57 (m, 3H; 1-C*H* and 5-CH_2), 3.21-3.32 (m, 2H; 4-CH_2), 2.73-2.83 (m. 1H; 2-C*H*) and 1.78-1.95 (m, 1H, 3-C*H*).

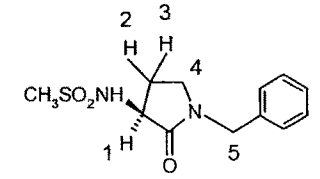
The TFA salt of compound **(66)** was treated with α-methylcinnamic acid **(70; R = h)** following the procedure as described for compound **(75)** to afford compound **(80; R = h)**. Characteristic absorption bands at 3279, 1683 and 1646 cm^{-1} appeared in the IR spectrum of compound **(80)** for NH, lactam C=O and



(80)

amide C=O, respectively. PMR spectrum showed a doublet at δ 7.41-7.44 for vinylic proton (6-C*H*), multiplet at δ 7.24-7.35 (m, 10H; Ar*H*) and a doublet at 6.80-6.81 for N*H* proton. Other signals in the PMR appeared at δ 4.48-4.56 (m, 3H; 1-C*H* and 5-CH_2), 3.26-3.31 (m, 2H; 4-CH_2), 2.73-2.83 (m, 1H; 2-C*H*), 2.13 (s, 3H; CH_3) and 1.81-1.95 (m, 1H; 3-C*H*). The quasi molecular ion peak was observed at 335.5 in its mass spectrum.

Sulfonamide derivatives **(81-84)** were also synthesized using the deprotected amine of compound **(66)**. The Boc-protected amide **(66)** was treated with trifluroacetic acid to afford TFA salt of amine as described above. Treatment

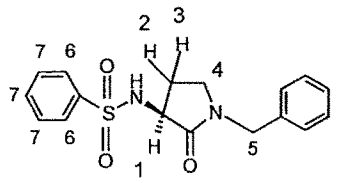


(81)

of this salt with mesyl chloride **(71; R = i)** in the presence of TEA offered sulfonamide **(81; R = i)**. This compound showed bands at 3163 and 1680 cm^{-1} for NH and lactam C=O, respectively in the IR spectrum and two strong bands at 1320 and 1151 cm^{-1} for the SO_2 stretching vibrations. In PMR, signals appeared at

δ 7.26-7.37 (m, 3H, aromatic protons), 7.19-7.22 (m, 2H, aromatic protons), 5.28-5.30 (d, 1H; N*H*), 4.46 (s, 2H; 5-CH_2), 4.14-4.22 (m, 1H; 1-C*H*), 3.20-3.25 (m, 2H; 4-CH_2), a sharp singlet at 3.15 (CH_3), 2.51-2.61 (m, 1H; 2-C*H*) and 1.89-2.00 (m, 1H; 3-C*H*). It showed M+H peak at 269.5 in its mass spectrum.

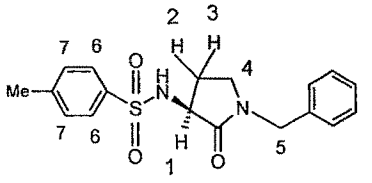
Compounds **(82-84)** were synthesized as described above. Benzenesulfonyl chloride **(72; R = j)** was used instead of mesyl chloride to afford sulfonamide **(82; R = j)**. Characteristic absorption band appeared at 3166, 1675,



(82)

1329 and 1162 cm^{-1} in the IR spectrum. PMR spectrum of compound **(82)** showed aromatic protons at δ 7.90-7.94 (d, 2H; 6-ArC*H*), 7.54-7.58 (m, 3H; 7-ArC*H*) and 7.15-7.32 (m, 5H; Phenyl). Other signals in the spectrum were at δ 5.33 (s, 1H; N*H*), 4.36-4.48 (dd, 2H; 5-CH_2), 3.69-3.76 (m, 1H; 1-C*H*), 3.11-3.24 (m, 2H; 4-CH_2), 2.49-2.59 (m, 1H; 2-C*H*) and 1.96-2.10 (m, 1H; 3-C*H*). It showed M+H peak at 331.4 in its mass spectrum.

Reaction between TFA salt of compound **(66)** and tosyl chloride **(73; R = k)** as described above for compound **(81)** yielded compound **(83; R = k)**. It

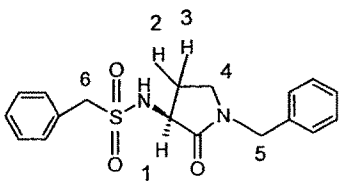


(83)

showed peaks at 3171, 1675, 1330 and 1160 cm^{-1} in its IR spectrum. PMR of compound **(83)** showed a doublet at δ 7.77-7.81 (6-ArC*H*), multiplet at 7.26-7.35 for five aromatic protons and a doublet at 7.15-7.18 (7-ArC*H*). Other signals were present at δ 5.27 (s, 1H; N*H*), 4.36-4.48 (dd, 2H; 5-CH_2), 3.65-3.72 (m, 1H; 1-C*H*),

3.11-3.23 (m, 2H; 4-C*H*$_2$), 2.48-2.58 (m, 1H; 2-C*H*), 2.43 (s, 3H; C*H*$_3$) and 1.96-2.10 (m, 1H; 3-C*H*). It showed quasi molecular ion peak at 345.6.

Similarly, compound **(84; R = l)** was prepared using α-toluene sulfonyl chloride **(74; R = l)** as described above for compound **(81)**. It showed characteristic absorption bands at 3215 (NH), 1684 (lactam C=O), 1320 and 1155 cm^{-1} for sulfonyl symmetric and asymmetric vibrations, respectively. PMR

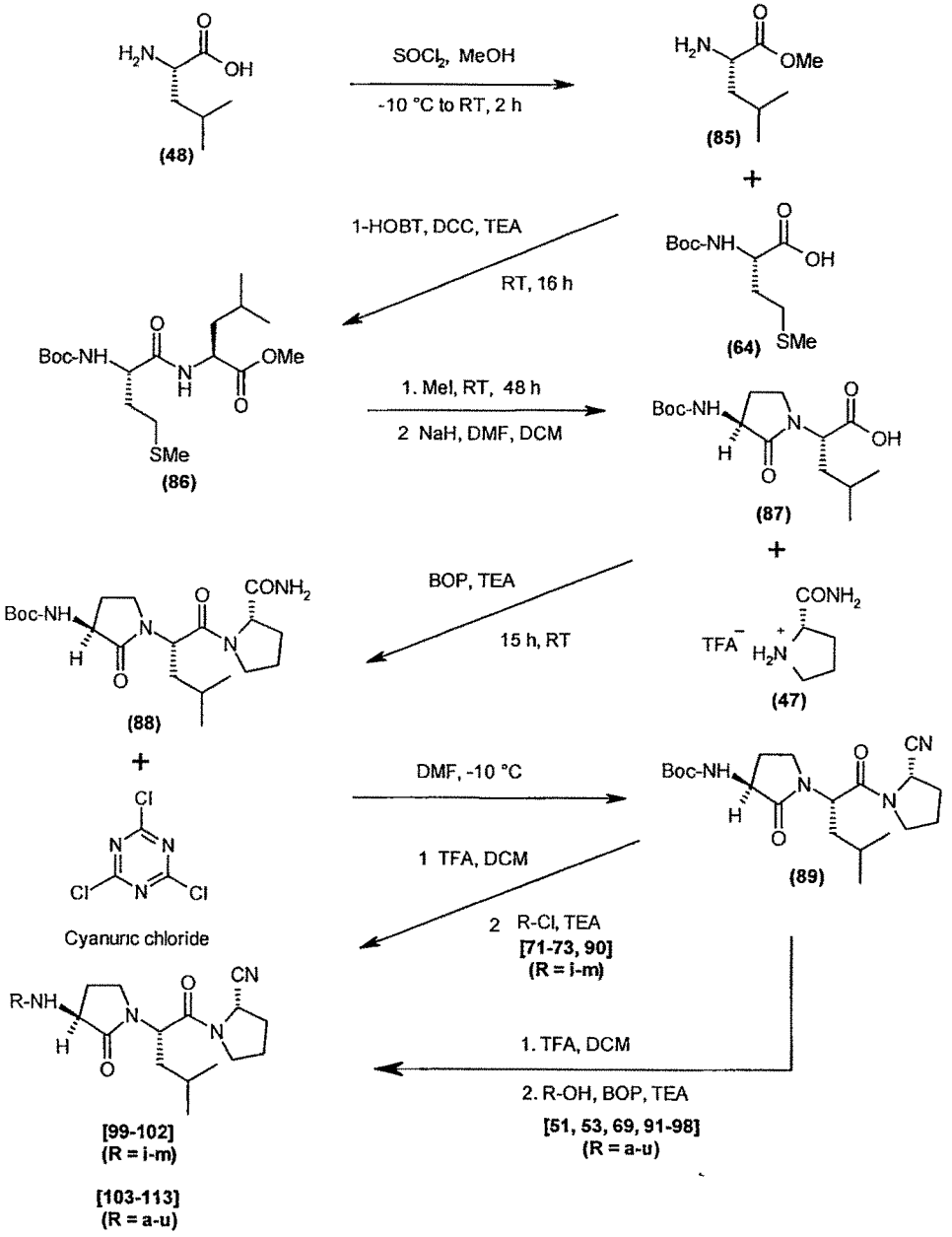


(84)

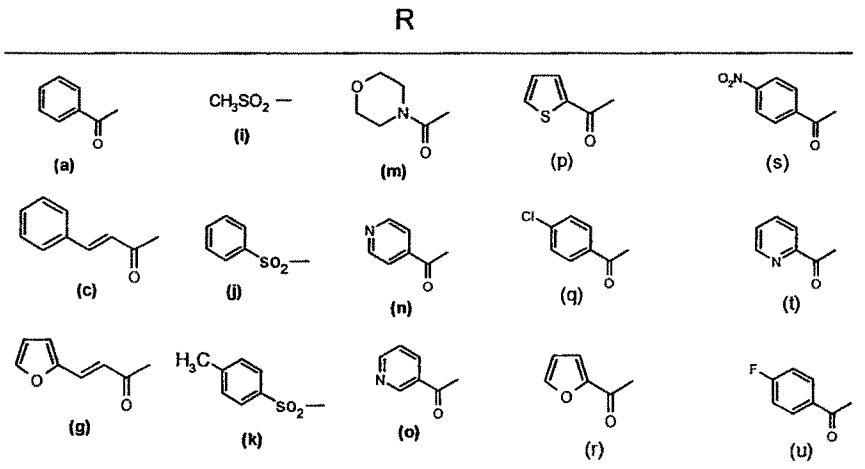
spectrum of compound **(84)** showed signals at 7.19-7.54 (m, 10H; Aromatic protons), 4.87-4.89 (d, 1H; N*H*), 4.41-4.52 (m, 4H; 5-C*H*$_2$ and 6-C*H*$_2$), 4.06-4.14 (m, 1H; 1-C*H*), 3.10-3.18 (m, 2H; 4-C*H*$_2$), 2.39-2.49 (m, 1H; 2-C*H*) and 1.70-1.87 (m, 1H; 3-C*H*).

3.1.4 *N*-{(*S*)1-[(*S*)1-((*S*)2-Cyanopyrrolidine-1-carbonyl)-3-methylbutyl]-2-oxo-3-pyrrolidino}sulfonamides/carboxamides (99-113)

After preparing the cyanopyrrolidines **(59-62)** and conformationally constrained systems **(75-84)** it was thought of combining both these systems into one single moiety. With this aim in mind it was thought of worthwhile to synthesize compounds of this type **(99-113)** using scheme-IV. Carboxylic group in *L*-Leucine **(48)** was protected by converting it into methyl ester **(85)**. *L*-leucine methyl ester hydrochloride **(85)** so obtained was treated with Boc-*L*-methionine **(64)** in presence of DCC, 1-HOBT and TEA to afford the dipeptide **(86)**. The characteristic peaks at 3337 (NH), 3300 (NH), 1758 (ester C=O stretching), 1682 (carbamate C=O stretching) and 1658 cm^{-1} (amide C=O stretching) were observed in the IR spectrum. PMR spectrum of compound **(86)** showed two broad doublets

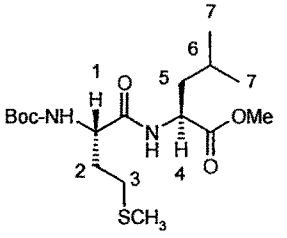

(48)
SOCl2, MeOH
-10 °C to RT, 2 h
(85)
+
1-HOBT, DCC, TEA
RT, 16 h
(64)
(86)
1. MeI, RT, 48 h
2 NaH, DMF, DCM
(87)
+
BOP, TEA
15 h, RT
(47)
(88)
+
DMF, -10 °C
1 TFA, DCM
2 R-Cl, TEA
[71-73, 90]
(R = i-m)
(89)
Cyanuric chloride
1. TFA, DCM
2. R-OH, BOP, TEA
[51, 53, 69, 91-98]
(R = a-u)
[99-102]
(R = i-m)
[103-113]
(R = a-u)

Contin..



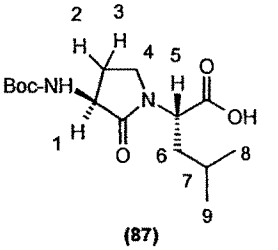
Scheme-IV

at δ 6.55-6.58 (Boc-N*H*) and 5.16-5.18 (CON*H*) and two multiplets at δ 4.55-4.62 (4-C*H*) and 4.27-4.29 (1-C*H*). Other signals in the spectrum were at δ 3.72 (s, 3H; OCH_3), 2.57-2.62 (t, 2H; 3-CH_2), 2.12 (s, 3H; SCH_3), 1.52-2.08 (m, 5H; 2-CH_2, 5-CH_2



(86)

and 6-C*H*), 1.44 (s, 9H; $(CH_3)_3$) and 0.92-0.94 (d, 6H; 7-$(CH_3)_2$). Synthesis of compound **(87)** was effected by an intramolecular alkylation route[117]. Boc-

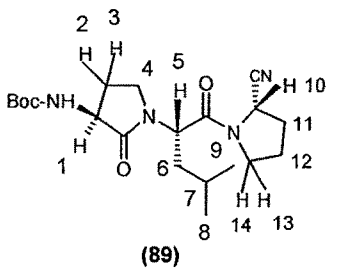


(87)

protected dipeptide **(86)** was dissolved in methyl iodide and stirred at room temperature to give methyl sulfenium salt. The salt was then cyclized to γ-lactam

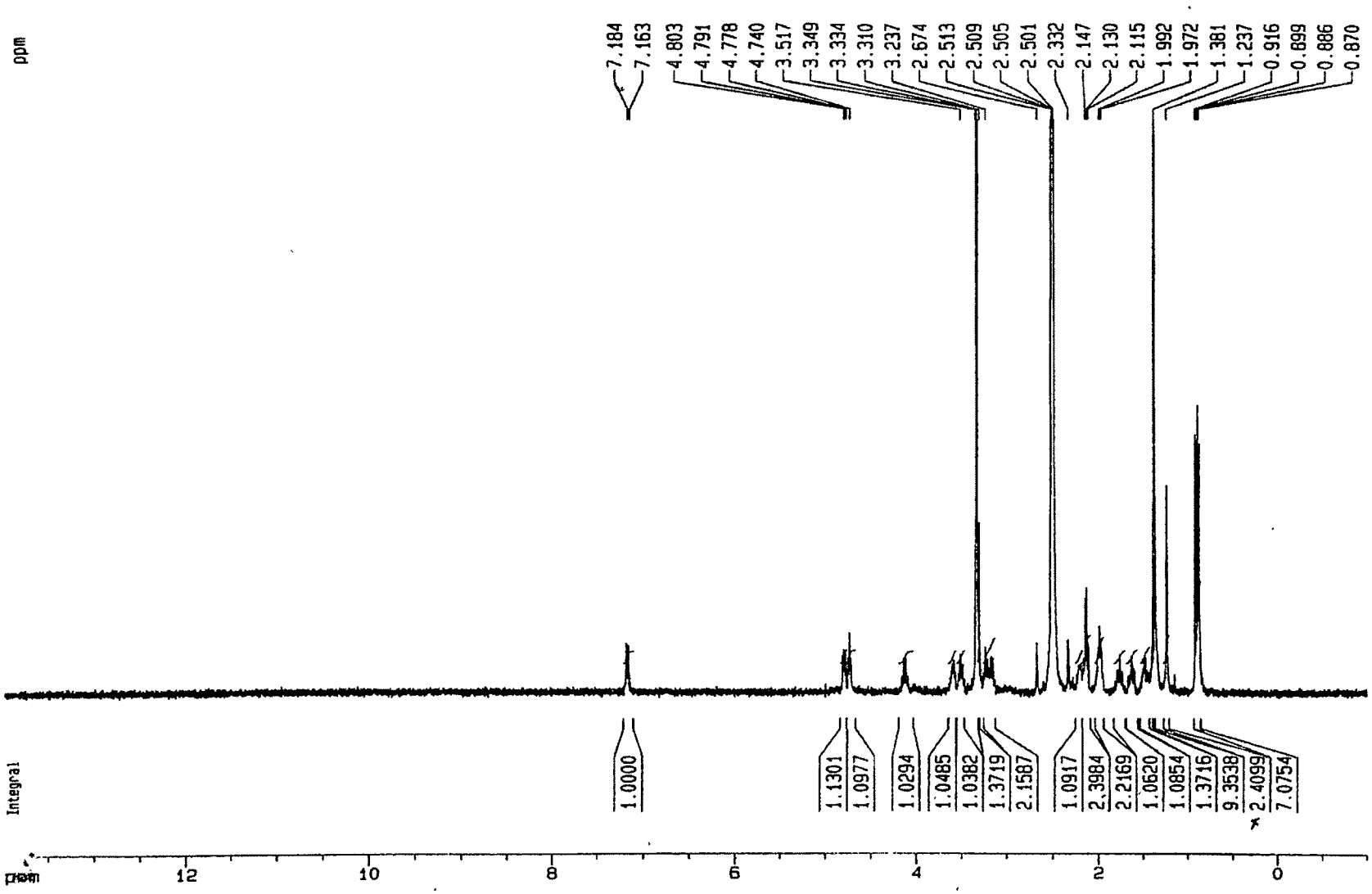
dipeptide **(87)** by sodium hydride in a mixture of DMF and DCM (1:9) at 0 °C. An improvement[117] in yield (from 40% to 60%) was observed when the proportion of DMF: DCM was changed from 1:1 to 1:9. Its IR spectrum showed peaks at 3395, 1704, 1675 and 1654 cm^{-1}. PMR spectrum showed peaks at δ 7.11-7.14 (d, 1H; N*H*), 4.46-4.51 (m, 1H; 5-C*H*), 4.17-4.20 (m, 1H; 1-C*H*), 3.14-3.20 (m, 2H; 4-CH_2), 2.17-2.25 (m, 1H; 2-C*H*), 1.49-1.82 (m, 4H; 3-C*H*, 6-CH_2 and 7-C*H*), 1.38 (s, 9H; $(CH_3)_3$) and two double doublets at δ 0.88-0.90 and 0.83-0.85 (6H; 8-CH_3 and 9-CH_3).

In order to convert the acid into amide function compound **(87)** was activated with BOP in dichloromethane at low temperatures followed by treatment with the amine salt **(47)** in presence of TEA to give crude amide **(88).** The amide **(88)** without further purification and characterization was used as such for the next step. The terminal amide group of compound **(88)** was dehydrated with cyanuric chloride in DMF at 0 °C to afford the desired nitrile **(89)**. IR spectrum of compound **(89)** showed characteristic peaks at 3355, 2232,

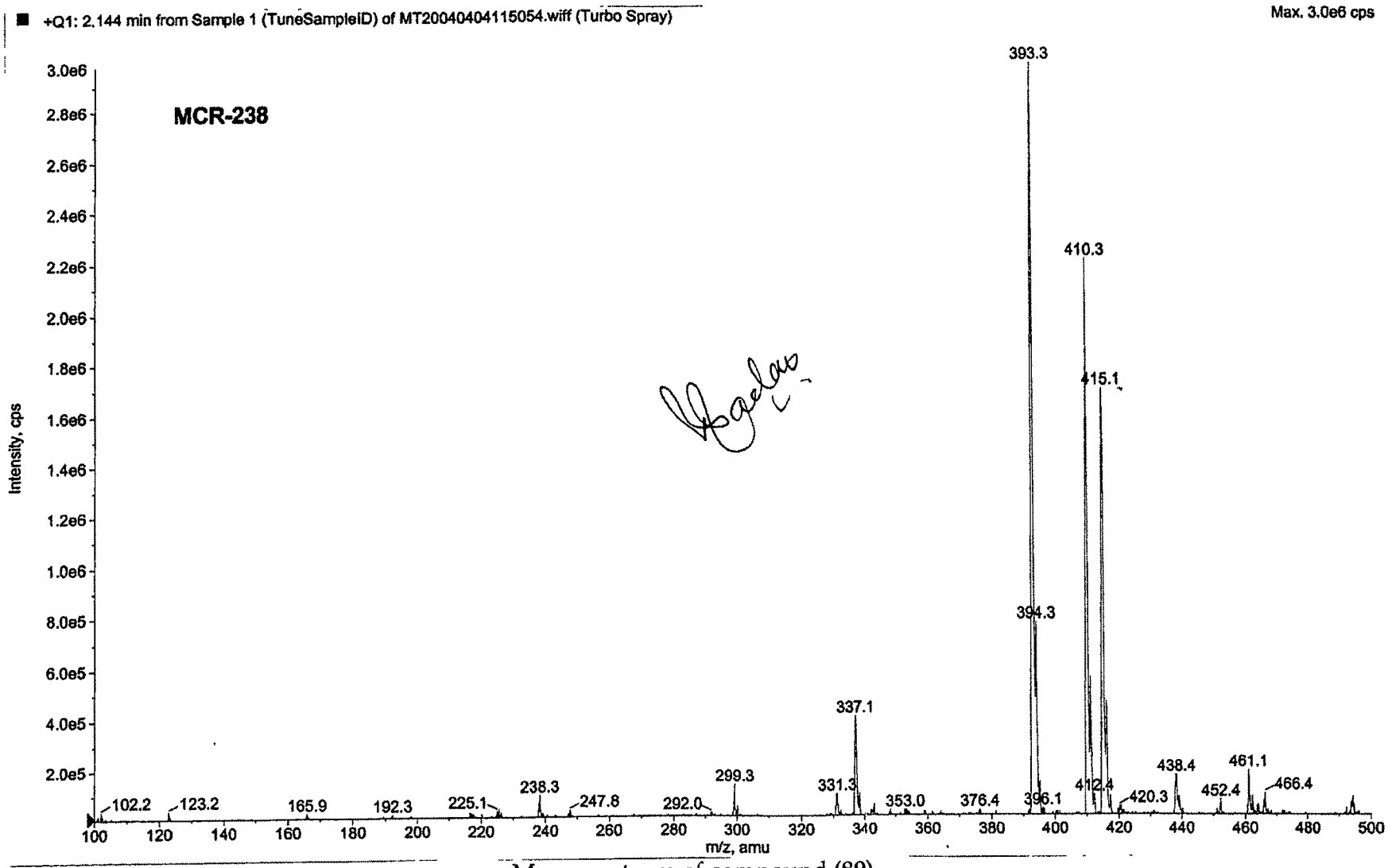


(89)

1712, 1691 and 1658 cm^{-1} for NH, CN, lactam C=O, carbamate C=O and C=O attached directly to pyrrolidine ring, respectively. Its PMR spectrum showed peaks at δ 7.16-7.18 (d, 1H; N*H*), 4.77-4.81 (m, 1H; 10-C*H*), 4.72-4.75 (t, 1H; 5-C*H*), 4.09-4.16 (m, 1H; 1-C*H*), 3.57-3.63 (m, 1H; 13-C*H*), 3.49-3.54 (m, 1H; 14-C*H*), 3.16-3.26 (m, 2H; 4-CH_2), 2.19-2.24 (m, 1H; 2-C*H*), 2.15-2.19 (m, 2H; 11-CH_2), 1.97-1.99 (m, 2H; 12-CH_2), 1.74-1.79 (m, 1H; 3-C*H*), 1.44-1.67 (m, 3H; 6-CH_2 and 7-C*H*), 1.38 (s, 9H; $(CH_3)_3$) and 0.84-0.92 (dd, 6H; 8-CH_3 and 9-CH_3). The quasi molecular ion peak was observed at 393.3.



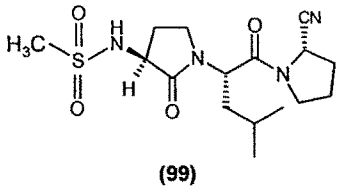
PMR spectrum of compound (89)



Mass spectrum of compound (89)

The Boc-protected compound **(89)** was treated with trifluroacetic acid to deprotect the amino group resulting in the formation of the TFA salt of amine, which was used as such in all further reactions. This amine salt was treated with various sulfonyl/carbonyl chlorides **(71-73, 90)** resulting into the formation of various sulfonamides **(99-101)** and amide **(102)**.

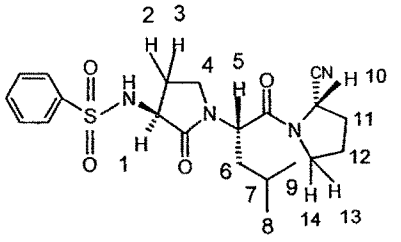
Compound **(99)** was synthesized by reacting TFA salt of compound **(89)** with mesyl chloride **(71; R = i)** in presence of TEA. Work up of the reaction



(99)

mixture followed by chromatographic purification yielded **(99; R = i)** as sticky mass. IR spectrum of this showed peaks at 3176 (NH), 2232 (CN), 1699 (lactam C=O), 1645 (amide C=O), and 1343 and 1168 cm^{-1} (SO_2 symmetric and asymmetric stretching vibrations).

The TFA salt of compound **(89)** on treatment with benzenesulphonyl chloride **(72; R = j)** as described above for compound **(99)** followed by crystallization using acetone-pet ether (60-80) yielded compound **(100; R = j)**. Characteristic absorption bands at 3258, 2232, 1694, 1650, 1334 and 1173 cm^{-1} were observed in IR spectrum. PMR spectrum of the compound **(100)** showed peaks at

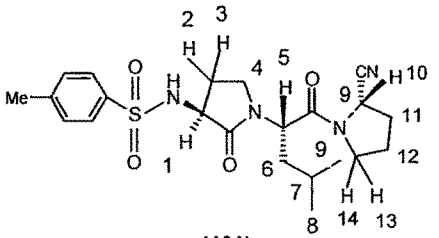


(100)

δ 7.89-7.92 (m, 2H), 7.52-7.61 (m, 3H), 5.34 (b, N*H*), 4.80-4.84 (m, 1H; 10-C*H*), 4.68-4.73 (m, 1H; 5-C*H*), 4.10-4.20 (m, 1H; 1-C*H*), 3.52-3.79 (m, 2H; 13-C*H* and 14-C*H*), 3.14-3.30 (m, 2H; 4-CH_2), 2.18-2.27 (m, 4H; 11-CH_2 and 12-CH_2), 1.95-2.10 (m, 1H;

3-C*H*), 1.55-1.78 (m, 3H; 6-CH_2 and 7-C*H*), and 0.87-0.94 (dd, 6H; 8-CH_3 and 9-CH_3). The quasi molecular ion peak was observed at 433.1.

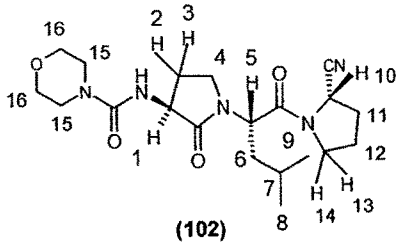
Compound **(101)** was prepared by reacting TFA salt of compound **(89)** with tosyl chloride **(73; R = k)** as described above for **99**. Work up of the reaction mixture followed by crystallization from ethyl acetate in *n*-hexane afforded **(101; R = k)**. The IR peaks at 3175, 2245, 1700, 1643, 1343 and 1167 cm^{-1} for NH, CN, lactam C=O, amide C=O and sulfonyl groups respectively, confirm the formation of compound **(101)**. PMR spectrum showed aromatic protons at δ 7.77-7.83 (d,



(101)

2H) and δ 7.26-7.36 (d, 2H). Other peaks in the spectrum were at δ 5.30 (s, 1H; N*H*), 4.81-4.84 (m, 1H; 10-C*H*), 4.68-4.73 (m, 1H; 5-C*H*), 4.10-4.15 (m, 1H; 1-C*H*), 3.71-3.80 (m, 2H; 13-C*H* and 14-C*H*), 3.14-3.28 (m, 2H; 4-CH_2), 2.48-2.56 (m, 1H; 2-C*H*), 2.44 (s, 3H; Ar-CH_3), 2.12-2.30 (m, 4H; 11-CH_2 and 12-CH_2), 1.97-2.08 (m, 1H; 3-C*H*), 1.56-1.77 (m, 3H; 6-CH_2 and 7-C*H*) and 0.88-0.95 (dd, 8-CH_3 and 9-CH_3). The quasi molecular ion peak was observed at 447.1.

Compound **(102)** was prepared by reacting TFA salt of compound **(89)** with morpholine-4-carbonyl chloride **(90; R = m)** as described above for comp-

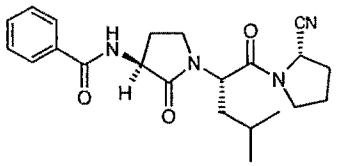


(102)

-ound **(99)**. Work up of the reaction mixture followed by crystallization from ethyl acetate gave **(102; R = m)**. Characteristic peaks at 3341 (NH), 2232 (CN), 1698 (lactam C=O stretching), 1656 (amide C=O attached to pyrrolidine ring) and

at 1638 cm^{-1} (amide C=O attached to morpholine ring) were observed in IR spectrum. PMR spectrum of compound **(102)** showed signals at δ 5.36-5.38 (d, 1H; N*H*), 5.00-5.05 (m, 2H; 16-CH_2), 4.92-4.97 (m, 2H; 16-CH_2), 4.84-4.90 (m, 1H; 10-C*H*), 4.73-4.75 (m, 1H; 5-C*H*), 4.30-4.40 (m, 1H; 1-C*H*), 3.81-3.85 (m, 2H; 15-CH_2), 3.59-3.63 (m, 1H; 13-C*H*), 3.33-3.39 (m, 1H; 14-C*H*), 3.20-3.31 (m, 2H; 4-CH_2), 2.60-2.75 (m, 2H; 15-CH_2), 2.26-2.33 (m, 1H; 2-CH_2), 2.15-2.20 (m, 2H; 11-CH_2), 1.90-2.10 (m, 2H; 12-CH_2), 1.82-1.85 (m, 1H; 3-C*H*), 1.32-1.63 (m, 3H; 6-CH_2 and 7-C*H*) and 0.93-1.01 (dd, 6H; 8-CH_3 and 9-CH_3).

TFA salt of the amine obtained on deprotection of Boc-derivative **(89)** was used as such for condensation with various acids **(51, 53, 69, 91-98)** to obtain the carboxamides **(103-113)**. For preparation of compound **(103)**, benzoic acid **(51; R = a)** was activated with BOP treatment followed by reaction with the TFA salt of



(103)

compound **(89)** in presence of TEA. Work up of the reaction mixture followed by chromatographic purification afforded compound **(103; R = a)**. Its IR spectrum showed bands at 3365 for NH, 2232 for CN, 1700 for lactam C=O, 1659 for amide attached to pyrrolidine ring and at 1647 cm^{-1} for keto of benzamide.

Compounds **(104-113)** were synthesized in a similar fashion as described above. Reaction between TFA salt of **89** and *trans*-cinnamic acid **(53; R = c)** followed by chromatographic purification yielded **(104; R = c)**. IR spectrum of compound **(104)** showed peaks at 3294, 2245, 1701, 1660 and 1634 cm^{-1} for NH, CN, lactam C=O, amide C=O attached to pyrrolidine ring and C=O of unsaturated carboxamide, respectively. Molecular ion peak was obtained at 422 (quasimolecular ion peak at 423.1). Other major fragments were obtained at 327.2, 299.2, 131.1 and 103.1 (Figure 12) in its mass spectrum.

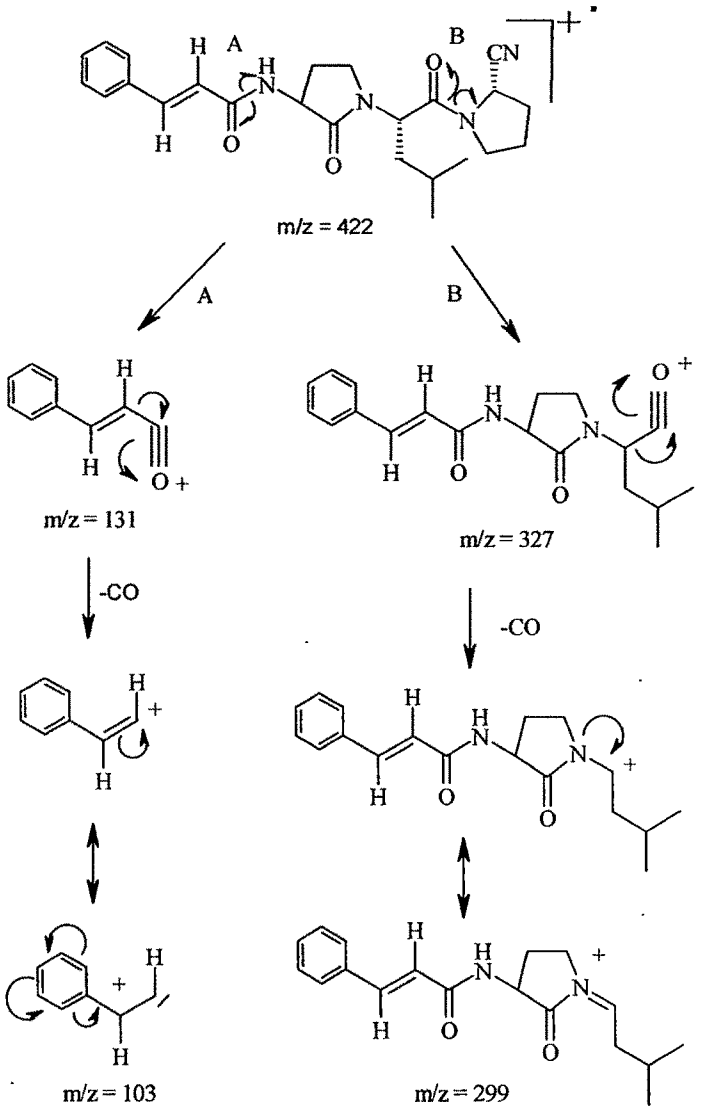
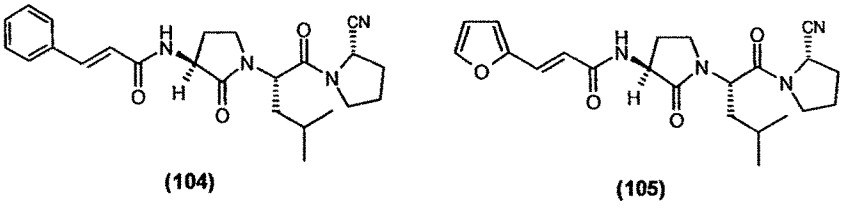
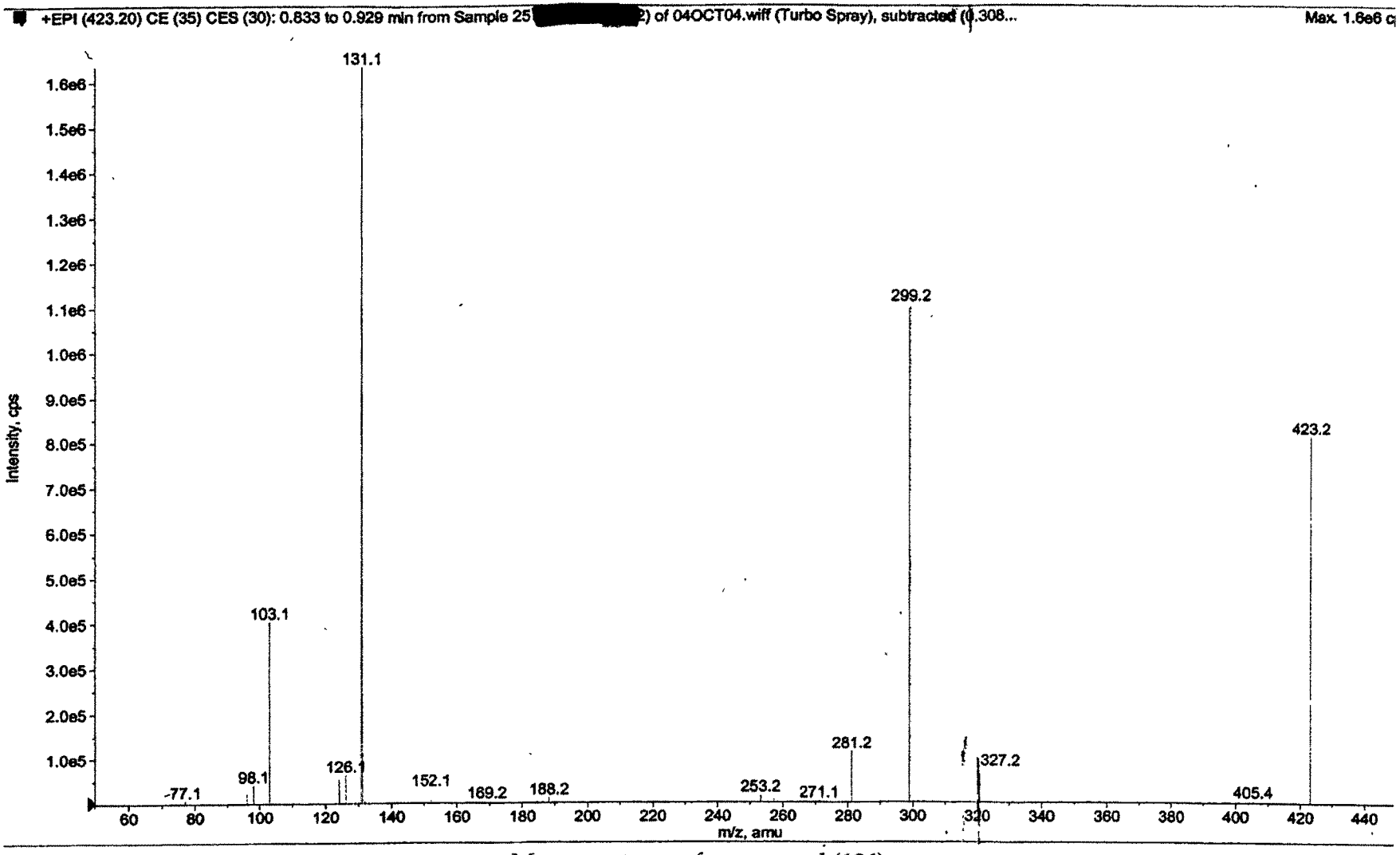


Figure 12: Mass spectral fragmentation pattern of compound **(104)**

Compound **(105)** was prepared by reacting TFA salt of compound **(89)** with 2-furylacrylic acid **(69; R = g)**. Work up of the reaction mixture followed by



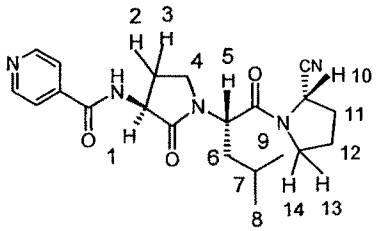
(104) **(105)**



Mass spectrum of compound (104)

chromatographic purification yielded compound **(105; R = g)**. The IR spectrum showed peaks at 3308 (NH stretching), 2232 (CN), 1693 (lactam C=O), 1660 (C=O attached to pyrrolidine ring) and at 1630 cm^{-1} (amide C=O).

Compound **(106)** was obtained by reacting TFA salt of compound **(89)** with isonicotinic acid **(91; R = n)** as per scheme-IV. Work up of the reaction mixture followed by crystallization from ethyl acetate in *n*-hexane yielded **(106; R = n)**. The IR spectrum of the compound showed peaks at 3232 (NH stretching), 2246 (CN), 1699 (lactam C=O stretching), 1666 (C=O attached to pyrrolidine ring) and at 1655 cm^{-1} (amide C=O). PMR spectrum of the compound **(106)** showed

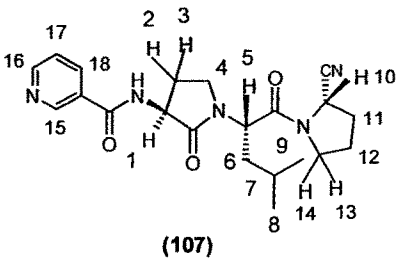


(106)

aromatic protons at δ 8.74-8.77 (m, 2H) and 7.62-7.66 (m, 2H), and the N*H* proton appeared as a doublet at δ 7.18-7.20. Other peaks were at δ 4.92-5.03 (m, 2H; 5-C*H* and 10-C*H*), 4.10-4.18 (m, 1H; 1-C*H*), 3.63-3.72 (m, 2H; 13-C*H* and 14-C*H*), 3.29-3.38 (m, 2H; 4-CH_2), 2.65-2.80 (m, 1H; 2-C*H*), 2.23-2.37 (m, 4H; 11-CH_2 and 12-CH_2), 1.85-1.88 (m, 1H; 3-C*H*), 1.25-1.55 (m, 3H; 6-CH_2 and 7-C*H*) and 0.95-1.03 (dd, 6H; 8-CH_3 and 9-CH_3).

Reaction between TFA salt of compound **(89)** with nicotinic acid **(92; R = o)** afforded compound **(107)**. IR spectrum of the compound showed peaks at 3354, 2232, 1700, 1653 and 1631 cm^{-1} for NH, CN, lactam C=O, amide C=O attached to pyrrolidine ring and carboxamide, respectively. PMR spectrum of the compound **(107)** showed four aromatic protons at δ 9.03-9.04 (d, 1H; 16-ArC*H*), 8.72 (s, 1H; 15-ArC*H*), 8.09-8.12 (m, 1H; 18-ArC*H*) and 7.35-7.39 (m, 1H; 17-ArC*H*). Other signals were at δ 7.09-7.11 (d, 1H; N*H*), 5.08-5.18 (m, 1H; 10-C*H*), 4.90-5.00 (m, 1H; 5-C*H*), 3.81-3.90 (m, 1H; 1-C*H*), 3.65-3.71 (m, 1H; 13-C*H*), 3.59-

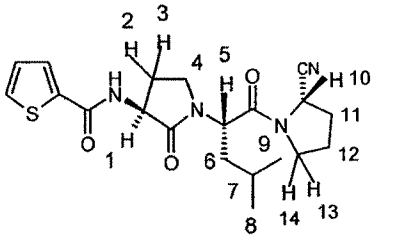
3.64 (m, 1H; 14-C*H*), 3.33-3.45 (m, 2H; 4-CH_2), 2.68-2.81 (m, 1H; 2-C*H*), 2.11-2.37 (m, 4H; 11-CH_2 and 12-CH_2), 1.85-1.90 (m, 1H; 3-C*H*), 1.45-1.66 (m, 3H; 6-CH_2 and



(107)

7-C*H*) and 0.93-1.01 (dd, 6H; 8-CH_3 and 9-CH_3). It gave M+H peak at 398.2 in its mass spectrum. Other prominent peaks were at 302, 274 and 256.

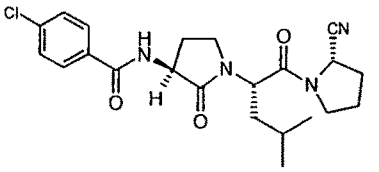
The compound **(108)** was prepared as described for **(103)** by reacting the TFA salt of **91** and 2-thiophenecarboxylic acid **(93; R = p)**. Work up of reaction mixture followed by crystallization from ethyl acetate yielded **(108; R = p)**. Its IR spectrum showed peaks at 3354 (NH stretching), 2245 (CN), 1700 (lactam C=O stretching), 1652 (C=O attached to pyrrolidine ring) and 1630 cm^{-1} (amide C=O



(108)

stretching). PMR showed signals at δ 7.47-7.52 (m, 2H), 7.04-7.07 (m, 1H), 6.88-6.93 (d, 1H; N*H*), 4.90-5.00 (m, 1H; 10-C*H*), 4.51-4.58 (m, 1H; 5-C*H*), 3.83-3.90 (m, 1H; 1-C*H*), 3.65-3.71 (m, 1H; 13-C*H*), 3.59-3.64 (m, 1H; 14-C*H*), 3.30-3.42 (m, 2H; 4-CH_2), 2.25-2.30 (m, 1H; 2-C*H*), 2.13-2.20 (m, 2H; 11-CH_2), 1.95-2.08 (m, 2H; 12-CH_2), 1.79-1.88 (m, 1H; 3-C*H*), 1.35-1.60 (m, 3H; 6-CH_2 and 7-C*H*) and 0.92-1.00 (dd, 6H; 8-CH_3 and 9-CH_3). It showed quasi molecular ion peak at 403.5. Other prominent peaks in its mass spectrum were at 307 and 279.

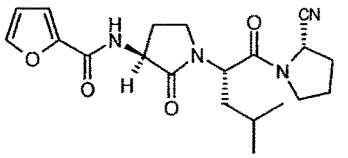
Compound **(109)** was prepared by reacting TFA salt of compound **(89)** with p-chlorobenzoic acid **(94; R = q)**. Characteristic absorption bands were observed at 3323, 2246, 1693, 1653 and 1631 cm^{-1} for NH, CN, lactam C=O, amide



(109)

attached to pyrrolidine ring and carboxamide, respectively in its IR spectrum.

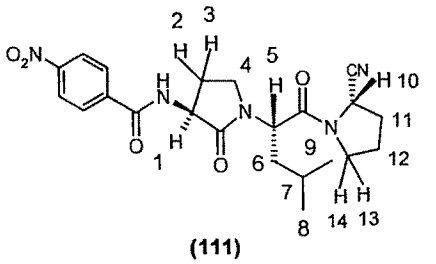
Compound **(110)** was prepared by reacting TFA salt of **89** and 2-furoic acid **(95; R = r)**. Work up of the reaction mixture followed by crystallization from ethyl acetate yielded **(110; R = r)**. Peaks in IR spectrum at 3341, 2232, 1698, 1655



(110)

and 1638 cm^{-1} for NH, CN, lactam keto, amide attached to pyrrolidine ring and keto amide respectively, confirm the formation of compound **(110)**. The quasi molecular ion peak was observed at 387.3 and other ion peaks were at 291 and 263 in the mass spectrum of the compound **(110)**.

The synthesis and work up of the reaction between TFA salt of **89** and p-nitro benzoic acid **(96; R = s)** was carried out in same fashion as described above

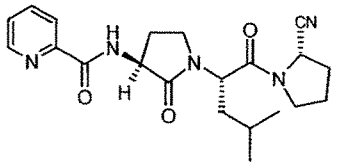


(111)

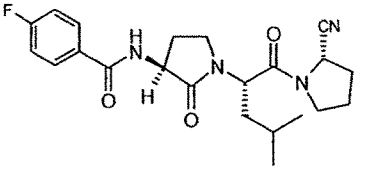
for compound **(103)** to yield compound **(111; R = s)**. IR spectrum of this compound showed peaks at 3323 (NH stretching), 2246 (CN), 1701 (lactam C=O), 1666 (C=O attached to pyrrolidine ring), 1647 (amide C=O) and 1525 and 1346

cm^{-1} (NO_2). PMR spectrum showed proton signals at δ 8.26-8.30 (d, 2H), 7.94-7.98 (d, 2H), 7.14-7.16 (d, 1H; N*H*), 4.89-5.02 (m, 2H; 5-C*H* and 10-C*H*), 4.58-4.64 (m, 1H; 1-C*H*), 3.44-3.65 (m, 1H; 13-C*H*), 3.28-3.60 (m, 3H; 4-CH_2 and 14-C*H*), 2.68-2.73 (m, 1H; 2-C*H*), 2.10-2.35 (m, 4H; 11-CH_2 and 12-CH_2), 1.83-1.87 (m, 1H; 3-C*H*), 1.25-1.58 (m, 3H; 6-CH_2 and 7-C*H*) and 0.93-1.02 (dd, 6H; 8-CH_3 and 9-CH_3). Mass spectrum of the compound **(111)** showed prominent peaks at 442.1 (M+H), 346.1 and 318.1.

Compound **(112)** was prepared by reacting TFA salt of compound **(89)** with 2-picolinic acid **(97; R = t)**. Work up of the reaction mixture followed by chromatographic purification yielded compound **(112; R = t)**. Its IR spectrum showed peaks at 3203 (NH stretching), 2232 (CN), 1690 (lactam C=O), 1660 (C=O attached to pyrrolidine ring) and at 1647 cm^{-1} (amide C=O). Compound **(113)**



(112)

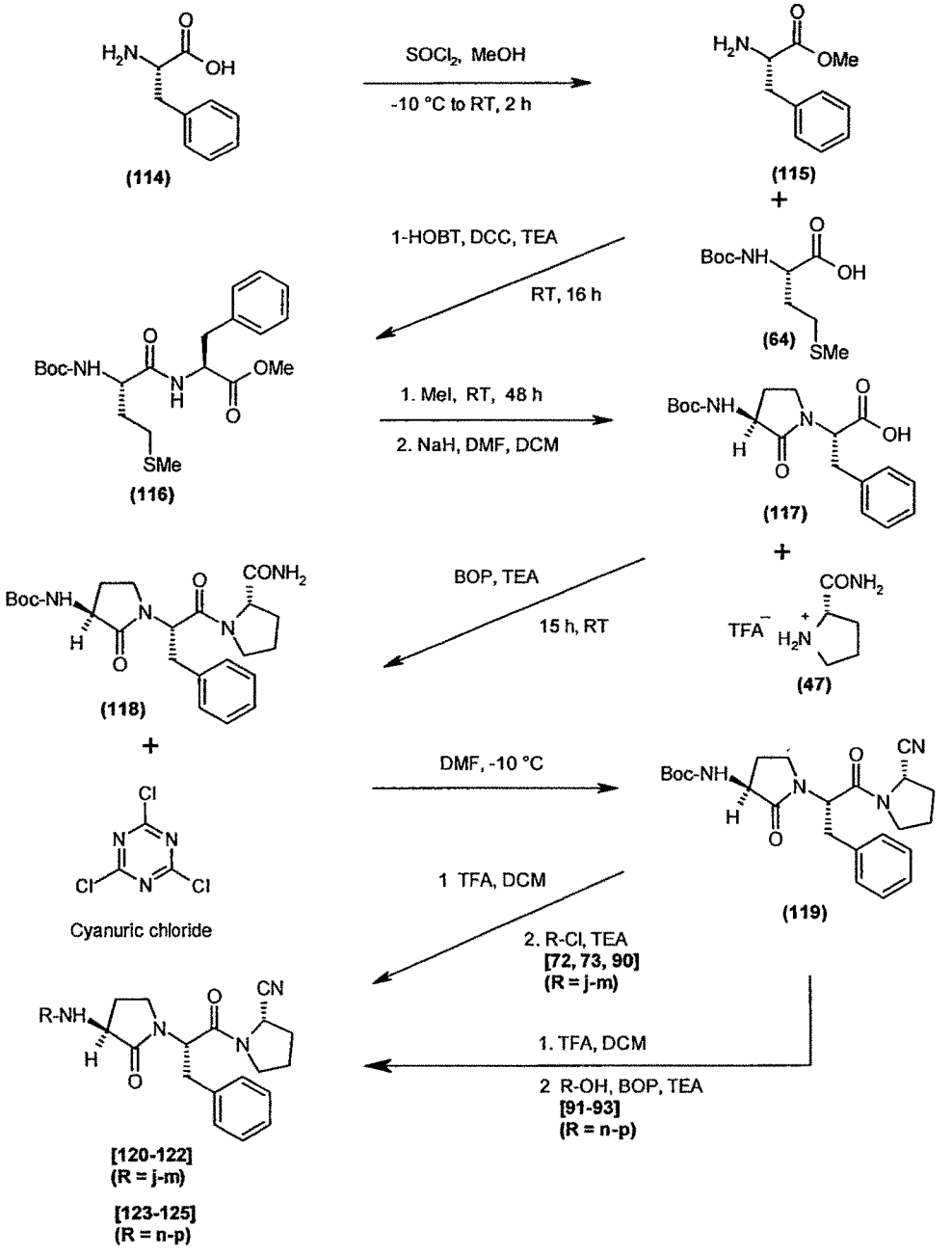


(113)

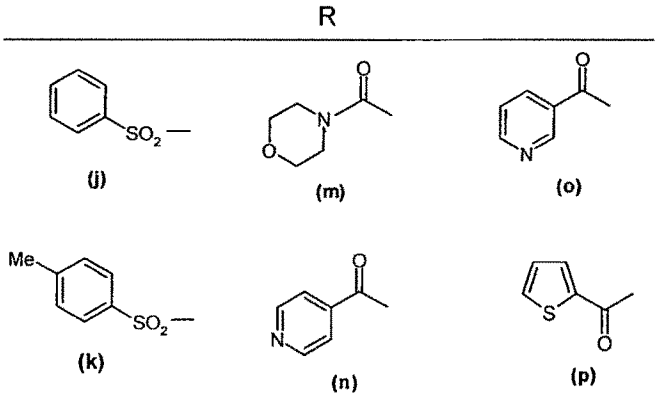
was prepared by reacting TFA salt of compound **(89)** with p-flurobenzoic acid **(98; R = u)**. Characteristic absorption bands were observed at 3354, 2245, 1700, 1659 and 1648 cm^{-1} for NH, CN, lactam C=O, amide attached to pyrrolidine ring and amide C=O, respectively.

3.1.5 *N*-[(*S*)1-[(*S*)1-Benzyl-2-((*S*)2-cyano-1-pyrrolidino)-2-oxoethyl]-2-oxo-3-pyrrolidino]sulfonamides/carboxamides (120-125)

It was planned to attach a more bulky group like phenyl in place of isopropyl in compounds **(120-125)**. For this purpose a synthetic strategy (Scheme-V) similar to that adopted in Scheme-IV was followed replacing *L*-leucine with *L*-phenylalanine as the starting material. *L*-Phenylalanine **(114)** on treatment with

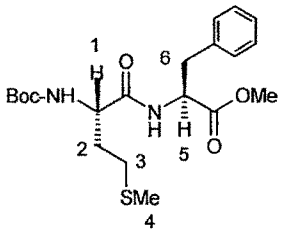

(114)
SOCl2, MeOH
-10 °C to RT, 2 h
(115)
+
1-HOBT, DCC, TEA
RT, 16 h
(64)
(116)
1. MeI, RT, 48 h
2. NaH, DMF, DCM
(117)
+
BOP, TEA
15 h, RT
(47)
(118)
+
DMF, -10 °C
Cyanuric chloride
(119)
1 TFA, DCM
2. R-Cl, TEA
[72, 73, 90]
(R = j-m)
1. TFA, DCM
2 R-OH, BOP, TEA
[91-93]
(R = n-p)
[120-122]
(R = j-m)
[123-125]
(R = n-p)

Contin ..



Scheme-V

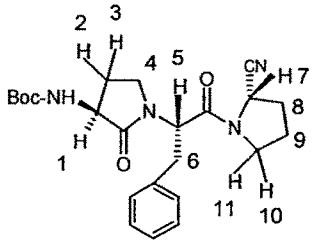
thionyl chloride in methanol afforded *L*-phenylalanine methyl ester hydrochloride **(115)**. The ester **(115)** so obtained was treated with Boc-*L*-methionine **(64)** in presence of DCC, 1-HOBT and TEA to afford the dipeptide **(116)**. Characteristic peaks at 3346 (NH), 3300 (NH), 1740 (ester C=O stretching),



(116)

1687 (carbamate C=O stretching) and at 1666 cm^{-1} (amide C=O stretching) were observed in its IR spectrum. PMR spectrum of compound **(116)** showed signals for aromatic protons at δ 7.20-7.30 (m, 3H) and 7.07-7.11 (m, 2H). Other signals were at δ 6.58-6.60 (d, 1H; N*H*), 5.12-5.15 (d, 1H; N*H*), 4.80-4.87 (m, 1H; 5-C*H*), 4.23-4.25 (m, 1H; 1-C*H*), 3.71 (s, 3H; OCH_3), 3.09-3.13 (m, 2H; 6-CH_2), 2.50-2.54 (t, 2H; 3-CH_2), 2.04 (s, 3H; SCH_3), 1.80-2.03 (m, 2H; 2-CH_2) and 1.43 (s, 9H; $(CH_3)_3$). Synthesis of compound **(117)** was effected by an intramolecular alkylation route[117]. Boc protected dipeptide **(116)** was dissolved in methyl iodide and stirred at room temperature to give methyl sulfenium iodide salt. This salt was then cyclized *in situ* to γ-lactam acid dipeptide **(117)** by sodium hydride in DMF:DCM (1:9) at 0 °C[117].

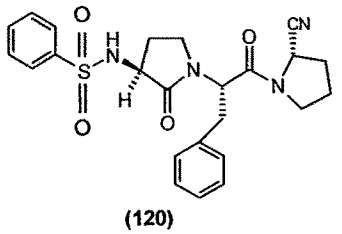
Compound **(117)** was activated with BOP in dichloromethane and then reacted with compound **(47)** in presence of TEA to give amide **(118).** IR spectrum of compound **(118)** showed peaks at 3382 and 3300 (NH_2), 3200 (NH), 1705 (lactam C=O), 1680 (carbamate C=O), 1652 (C=O attached to pyrrolidine ring) and 1624 cm^{-1} ($CONH_2$). The terminal amide group of **(118)** was dehydrated



(119)

using cyanuric chloride in DMF at 0 °C giving the nitrile **(119).** The IR spectrum of compound **(119)** showed peaks at 3436, 2232, 1722, 1692 and 1646 cm^{-1} for NH, CN, lactam C=O, carbamate C=O and C=O attached to pyrrolidine ring, respectively. PMR spectrum showed signals at 7.25-7.37 (m, 5H; Ar-*H*), 5.03-5.07 (m, 1H; 5-C*H*), 4.84-4.85 (d, 1H; N*H*), 4.64-4.67 (m, 1H; 7-C*H*), 4.09-4.11 (m, 1H; 1-C*H*), 3.60-3.65 (m, 1H; 10-C*H*), 3.41-3.47 (m, 1H; 11-C*H*), 3.29-3.37 (m, 2H; 4-CH_2), 2.97-3.05 (m, 2H; 6-CH_2), 2.58-2.64 (m, 1H; 2-C*H*), 2.11-2.18 (m, 2H; 8-C*H*), 1.86-1.99 (m, 3H; 3-C*H* and 9-CH_2) and 1.45 (s, 9H; $(CH_3)_3$). Quasi molecular ion peak for the compound appeared at 427 in its mass spectrum.

Compound **(119)** was treated with trifluoroacetic acid in dichloromethane to give its TFA salt. This salt was reacted with benzenesulfonyl chloride **(72; R**

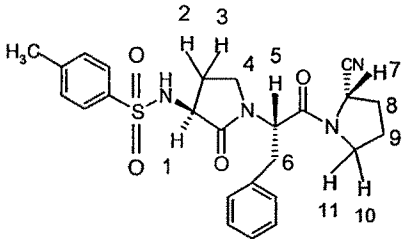


(120)

= j) in presence of TEA. Work up of the reaction mixture followed by chromatographic purification afforded **(120; R = j).** IR spectrum of compound **(120)** showed peaks at 3258, 2232, 1700, 1651, 1330 and 1130 cm^{-1} for NH, CN, lactam C=O, amide attached to pyrrolidine ring and sulfonyl groups,

respectively. Quasi molecular ion peak for the compound appeared at 467 in its mass spectrum.

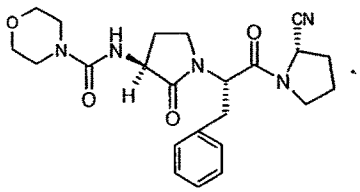
The synthesis and work up of the reaction between TFA salt of compound **(119)** and tosyl chloride **(73; R = k)** was carried out in same fashion as described above for compound **(120)** to yield compound **(121; R = k)**. Peaks at 3218 (NH), 2232 (CN), 1700 (lactam C=O), 1657 (amide C=O attached to pyrrolidine ring) and 1335 and 1163 cm^{-1} (sulfonyl group) were observed in its IR spectrum. PMR spectrum of the compound **(121)** provided peaks at δ 7.70-7.91



(121)

(d, 2H; Ar-*H*), 7.19-7.35 (m, 7H; Ar-*H*), 5.15-5.16 (d, 1H; N*H*), 4.93-4.97 (m, 1H; 5-C*H*), 4.63-4.70 (m, 1H; 7-C*H*), 3.98-4.03 (m, 1H; 1-C*H*), 3.63-3.68 (m, 1H; 10-C*H*), 3.49-3.55 (m, 1H; 11-C*H*), 3.28-3.42 (m, 2H; 4-CH_2), 2.87-2.95 (m, 2H; 6-CH_2), 2.50-2.57 (m, 1H; 2-C*H*), 2.45 (s, 3H; Ar-CH_3) and 1.90-2.16 (m, 5H; 8-CH_2 and 9-CH_2).

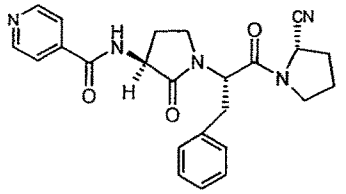
The reaction between TFA salt of compound **(119)** and morpholine-4-carbonyl chloride **(90; R = m)** was carried out in same fashion as described above



(122)

for compound **(120)** to yield compound **(122; R = m)**. Peaks in its IR spectrum at 3218, 2245, 1700, 1666 and 1653 cm^{-1} for NH, CN, lactam C=O, amide attached to pyrrolidine ring and amide C=O respectively, confirm the formation of compound **(122)**. Mass spectrum of the compound showed peaks at 440.1 (M+H), 353, 344, 316, 229 and 211.

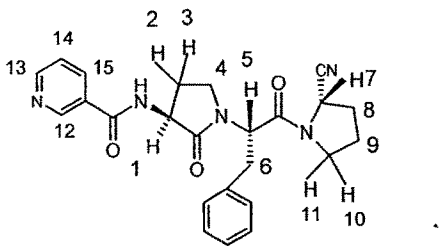
TFA salt of compound **(119)** was reacted with isonicotinic acid **(91; R = n)** in presence of BOP and TEA. Work up of the reaction mixture followed by crystallization from ethyl acetate-*n*-hexane afforded compound **(123; R = n)**.



(123)

Characteristic absorption peaks at 3364 (NH), 2232 (CN), 1700 (lactam C=O), 1659 (C=O attached to pyrrolidine ring) and 1647 cm^{-1} (amide C=O) were observed in its IR spectrum. The quasi molecular ion peak was observed at 432.1 in its mass spectrum.

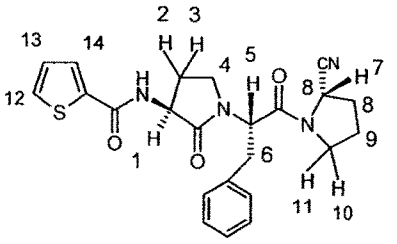
Compound **(124; R = o)** was prepared by reacting TFA salt of compound **(119)** with nicotinic acid **(92; R = o)** as described above for **(123)**. Its IR spectrum showed peaks at 3463 for NH, 2232 for CN, 1690 for lactam C=O, 1660 for amide attached to pyrrolidine ring and 1639 cm^{-1} for keto amide. PMR showed aromatic protons at δ 9.00 (s, 1H; 12-ArC*H*), 8.74-8.77 (d, 1H; 13-ArC*H*), 8.09-8.14 (m, 1H; 15-ArC*H*) and 7.24-7.42 (m, 6H; 14-ArC*H* and Ar*H*). Other signals were present



(124)

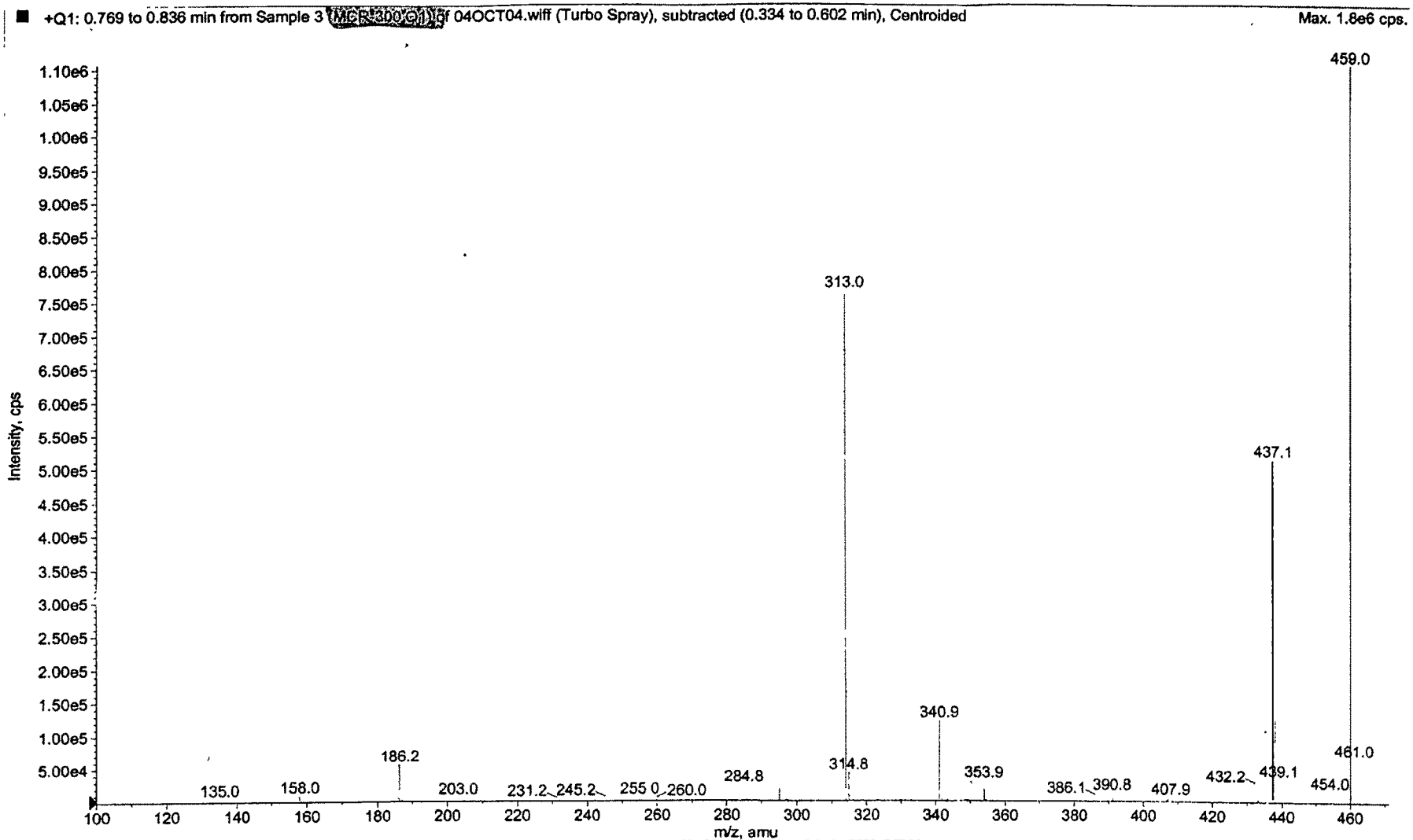
at 6.67-6.68 (d, 1H; N*H*), 5.04-5.09 (m, 1H; 5-C*H*), 4.67-4.73 (m, 1H; 7-C*H*), 4.07-4.17 (m, 1H; 1-C*H*), 3.53-3.67 (m, 2H; 10-C*H* and 11-C*H*), 3.29-3.41 (m, 2H; 4-CH_2), 3.06-3.11(m, 2H; 6-CH_2), 2.82-2.89 (m, 1H; 2-C*H*), 2.15-2.21 (m, 2H; 8-CH_2) and 1.88-2.09 (m, 3H; 3-C*H* and 9-CH_2). The quasi molecular ion peak was observed at 432.1 in its mass spectrum.

Compound **(125)** was prepared by reacting TFA salt of compound **(119)** with 2-thiophenecarboxylic acid **(93; R = p)** as described above for **(123)**. Work up of the reaction mixture followed by crystallization with ethyl acetate yielded



(125)

(125; R = p). IR spectrum of compound **(125)** showed peaks at 3354 (NH stretching), 2232 (CN), 1700 (lactam C=O stretching), 1653 (amide C=O attached to pyrrolidine ring) and 1631 (keto amide). In the PMR spectrum aromatic protons appeared at δ 7.49-7.53 (m, 1H; 14-ArC*H*), 7.23-7.39 (m, 6H) and 7.06-7.08 (m, 1H; 12-ArC*H*). Other signals were at δ 6.56-6.57 (d, 1H; N*H*), 5.04-5.08 (m, 1H; 5-C*H*), 4.68-4.75 (m, 1H; 7-C*H*), 4.01-4.06 (m, 1H; 1-C*H*), 3.30-3.67 (m, 4H; 4-CH_2, 10-C*H* and 11-C*H*), 3.03-3.09 (m, 2H; 6-CH_2), 2.78-2.83 (m, 1H; 2-C*H*), 2.15-2.21 (m, 2H; 8-CH_2) and 1.88-2.08 (m, 3H; 3-C*H* and 9-CH_2). Prominent ion peaks in the mass spectrum were at 437.1 (M+H), 341, 313 and 111 (Figure 13).



Mass spectrum of compound (**125**)

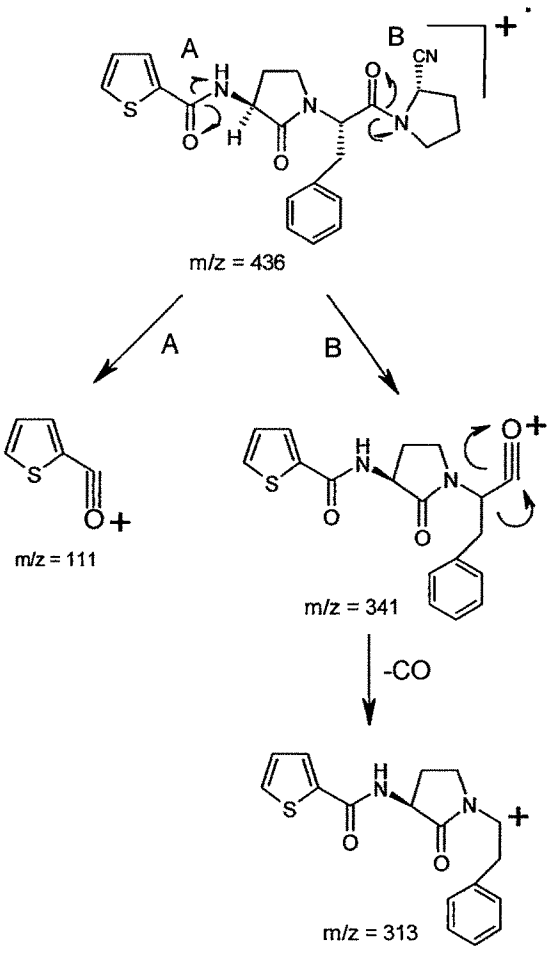
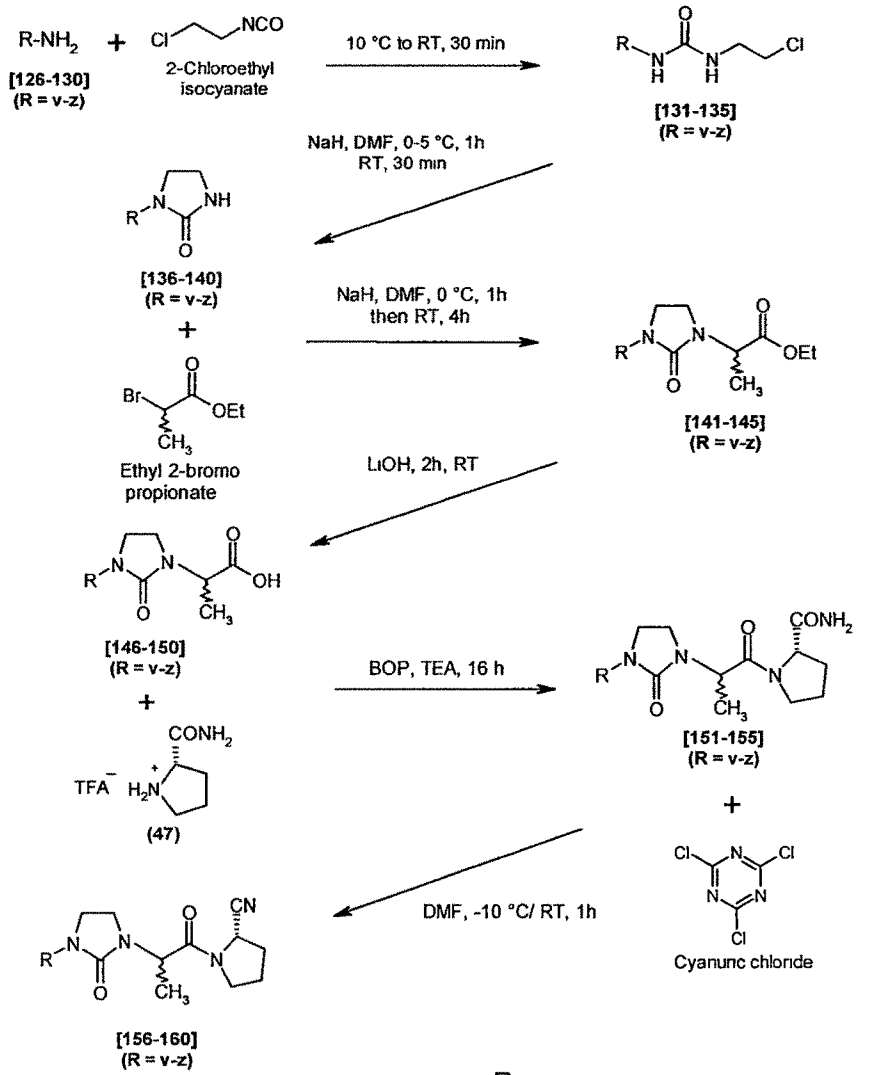


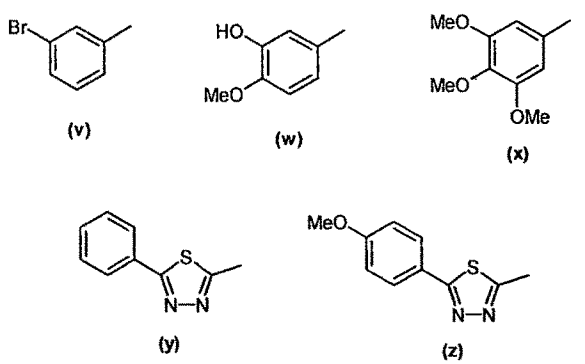
Figure 13: Mass spectral fragmentation pattern of compound **(125)**

3.1.6 (*S*)1-[2-[3-(Substituted)-2-oxo-1-imidazolidinyl]propionyl]pyrrolidine-2-carbonitrile (156-160)

After synthesizing some conformationally constrained peptidomimetic compounds having a γ-lactam ring in the chain providing a fixed conformation, it was planned to isosterically replace the γ-lactam with imidazolidinone ring system. The free NH group was planned to be blocked by substituting various aromatic/heteroaromatic groups to provide compounds **(156-160)**. In order to achieve this target, a synthetic strategy was designed as shown in scheme-VI. 2-Chloroethyl isocyanate was reacted with 3-bromoaniline **(126; R = v)** to offer


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
Cyanunc chloride
[156-160]
(R = v-z)

R


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

Scheme-VI

the desired disubstituted urea **(131; R = v)**. It showed strong absorption bands at 3315 (NH) and 1639 cm^{-1} (C=O of urea) in its IR spectrum. In a similar fashion other urea derivatives **(132-135)** were also prepared.

The urea derivative **(131; R = v)** so obtained was cyclized into the imidazolidinone **(136; R = v)** by giving it sodium hydride treatment in DMF. The compound gave strong peaks at 3259 (NH) and 1684 cm^{-1} for (C=O stretching) in its IR spectrum. Other urea derivatives **(132-135)** were similarly cyclized into their imidazolidinone analogs **(137-140)**.

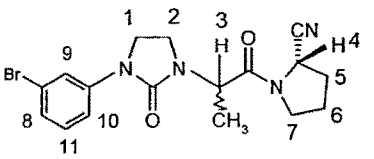
The imidazolidinones **(136-140)** were alkylated by treating them with ethyl 2-bromopropionate in presence of a strong base like sodium hydride to afford the esters **(141-145)**. Compound **(142; R = w)** showed strong peaks at 3176 (OH), 1736 (C=O, ester) and 1665 cm^{-1} (C=O, amide) in its IR spectrum. All the above reactions were monitored by TLC and IR spectroscopy. Since the product **(141-145)** obtained were all distereomeric mixtures, it was tried to resolve them by fractional crystallization but, failure was faced in all these attempts.

The ethyl esters **(141-145)** were hydrolyzed in basic conditions to offer the free acids **(146-150)**. Compound **(147; R = w)** gave absorption bands at 3408 (OH), 1741 (C=O, acid) and 1646 cm^{-1} (C=O, urea) in its IR spectrum. Other compounds offerd similar pattern of peaks in their IR spectra.

The imidazolidinone acids **(146-150)** so obtained were coupled with TFA salt of prolinamide **(47)** in presence of BOP and TEA to give amides **(151-155)** as diastereomeric mixtures. Compound **(151; R = v)** offered peaks at 3395 and 3204 for NH_2, 1693 for imidazolidinone C=O stretching, 1666 for C=O attached to pyrrolidine ring and 1646 cm^{-1} for $CONH_2$ in its IR spectrum. Following the procedure as described above for **(151)** compounds **(152-155)** were obtained from **(147-150)** as a distereomeric mixture. IR spectrum of compound **(152; R = w)** offered peaks at 3367 (OH), 3280 and 3203 (NH_2), 1706 (C=O, urea), 1674 (C=O attached to pyrrolidine ring), 1642 ($CONH_2$). Peaks at 3368 and 3190 (NH_2), 1713

(imidazolidinone C=O), 1666 (C=O attached to pyrrolidine ring), 1646 cm^{-1} ($CONH_2$) were observed in the IR spectrum of compound **(153; R = x)**.

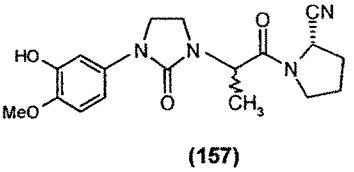
Dehydration of terminal amide function in compound **(151; R = v)** was effected by cyanuric chloride in DMF to give the desired nitrile **(156; R = v)** as diastereomeric mixture. Its IR spectrum showed peaks at 2232, 1694 and 1653 cm^{-1} for CN, imidazolidinone C=O, and C=O attached to pyrrolidinone ring.



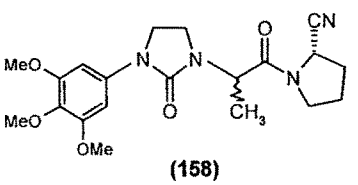
(156)

Signals were obtained at δ 7.76-7.80 (d, 1H; 8-ArC*H*), 7.43-7.50 (m, 1H; 11-ArC*H*), 7.17-7.25 (m, 2H; 9- and 10-ArC*H*), 4.87-4.96 (m, 1H; 3-C*H*), 4.73-4.78 (m, 1H; 4-C*H*), 3.59-3.93 (m, 6H; 1-CH_2, 2-CH_2 and 7-CH_2), 2.11-2.36 (m, 4H; 5-CH_2 and 6-CH_2) and two doublets at δ 1.47-1.49 and 1.39-1.41 for CH_3 in its PMR spectrum. The compound offered prominent peaks at 391.5, 296 and 268 in its mass spectrum (Figure 14).

Compound **(157; R = w)** was synthesized from compound **(152; R = w)** as described above for **(156)**. Its IR spectrum showed peaks at 3450 (OH), 2232

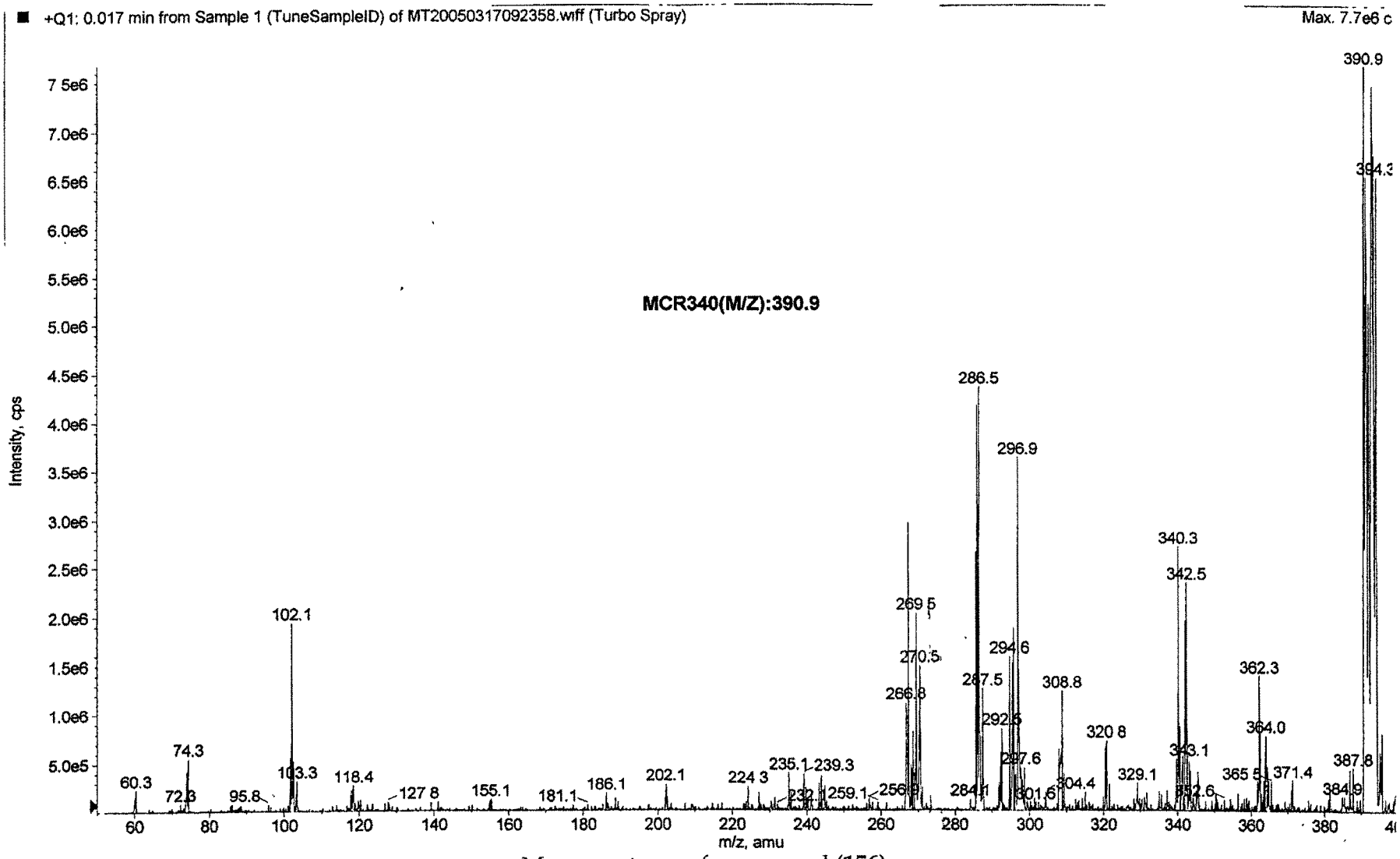


(157)



(158)

(CN), 1700 (imidazolidinone C=O stretching) and 1652 cm^{-1} (C=O attached to pyrrolidine ring). Compound **(158; R = x)** was obtained from compound **(153; R = x)** as described above for compound **(156)**. Its IR spectrum showed peaks at 2232, 1700 and 1651 cm^{-1} for CN, imidazolinone C=O stretching and C=O attached directly to pyrrolidine ring, respectively.



Mass spectrum of compound (156)

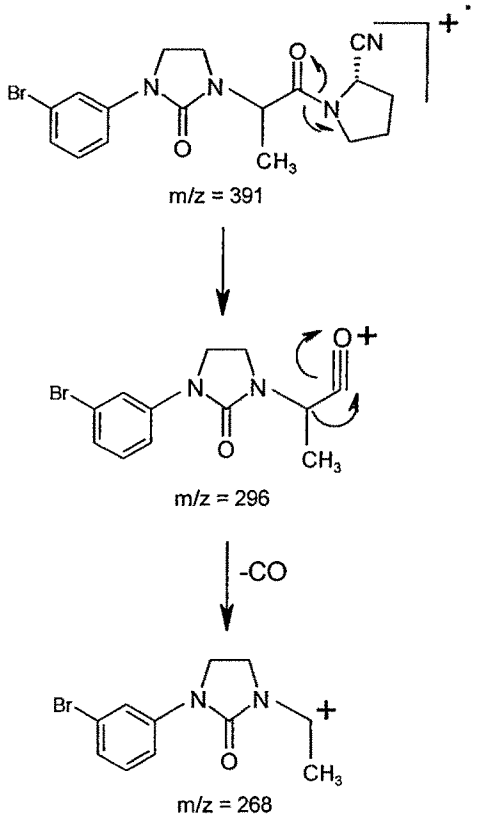
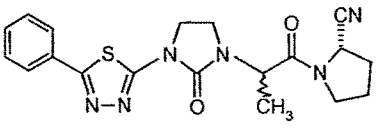
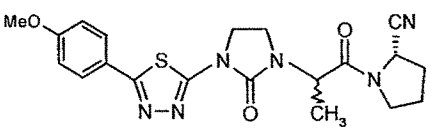


Figure 14: Mass spectral fragmentation pattern of compound **(156)**

Compounds **(159, 160)** were also synthesized from compounds **(154, 155)** as described above for compound **(156)**. Characteristic absorption bands at 2245, 1707 and 1647 cm^{-1} for CN, imidazolidinone C=O and C=O directly attached to pyrrolidine ring, respectively confirm the formation of **(159; R = y)**. For comp-



(159)



(160)

-ound **(160; R = z)** peaks were observed at 2245 (CN), 1705 (imidazolinone C=O) and 1655 cm^{-1} (C=O directly attached to pyrrolidine ring in the IR spectrum. It offered prominent peaks at 427.5 (M+H) and 303 in its mass spectrum.

3.2 BIOLOGICAL EVALUATION

The aim of our study was to develop new antimalarial agents, ideally directed against the targets, which were not previously exploited in the antimalarial chemotherapy. Malarial cysteine protease falcipain is such a target. The synthesized derivatives were tested for falcipain inhibition (*in vitro* inhibition assay) following the reported procedure[130]. To determine the selectivity for falcipain inhibition over other related cysteine proteases the test compounds were also screened against cathepsin-B and L (*in vitro* inhibition assay) [131].

3.2.1 Assay of falcipain inhibition

IC_{50} value against recombinant falcipain-2 was determined as described previously[132]. Equal amount of recombinant enzyme was incubated for 30 min at room temperature in 100 mM sodium acetate (pH 5.5) 10 mM dithiothreitol (DTT) with different concentrations of test inhibitors. Inhibitor solutions were prepared from a stock solution in DMSO (maximum concentration of DMSO in the assay was 1 %). After 30 min incubation the substrate Z-Leu-Arg-AMC (Bezyloxycarbonyl-Leu-Arg-7-amino-4-methyl coumarin) in the same buffer was added to final concentration (25 mM). Fluorescence was monitored for 15 min at room temperature in a Labsystems Fluoroskan Ascent spectrofluorometer. IC_{50} values were determined from plots of percents of activity over the compound concentration using Graphpad Prism Software. The results of the study are summarized in Table 2.

3.2.2 Assay of cathepsin inhibition

The substrate Cbz-Arg-Arg-MCA and the reversible inhibitor E-64 were purchased from Bachem (King of Prussia, PA) and Peptides International (Louisville, KY), respectively. Human cathepsin B and L were expressed and purified as described previously[131] Fluorescence was monitored on a Varian Gemini spectrofluorometer with the excitation and emission wavelenghts at 380 and 440 nm, respectively. The enzyme, stored at 4 °C in inhibited form by MMTS, was preactivated by incubation with 2mM DTT in the same buffer as the reaction mixture. The concentration of active enzyme was determined by

titration with E-64. All kinetic measurements were carried out at 23 °C at pH 6.0 in the presence of 50 mM sodium phosphate, 5mM EDTA, 2mM DTT, 0.2 M NaCl, and 5% DMSO. Enzyme concentration in the assay was 150 pM and 100 μM was used for Z-R-R-MCA. The Ki values were obtained from a graph of 1/vs vs [I] by measuring the initial rate of substrate hydrolysis (vs) in the presence of different concentrations of inhibitor [I] and at substrate concentration kept well below Km. The results of the study are summarized in Table 3.

Table 2: Inhibition of falcipain by test compounds

Sr. no.	Comp no.	IC_{50} (μM)	Sr. no.	Comp no.	IC_{50} (μM)
1	59	> 50	**23**	105	> 50
2	60	> 50	**24**	106	> 50
3	61	> 50	**25**	107	> 50
4	62	> 50	**26**	108	> 50
5	75	> 50	**27**	109	> 50
6	76	> 50	**28**	110	> 50
7	77	> 50	**29**	111	> 50
8	78	> 50	**30**	112	> 50
9	79	> 50	**31**	113	> 50
10	80	> 50	**32**	119	> 50
11	81	> 50	**33**	120	> 50
12	82	> 50	**34**	121	> 50
13	83	> 50	**35**	122	> 50
14	84	> 50	**36**	123	> 50
15	87	> 50	**37**	124	> 50
16	89	> 50	**38**	125	> 50
17	99	> 50	**39**	156	> 50
18	100	> 50	**40**	157	> 50
19	101	> 50	**41**	158	> 50
20	102	> 50	**42**	159	> 50
21	103	> 50	**43**	160	> 50
22	104	> 50			

Table 3: Inhibition of cathepsin-B and L by test compounds

Sr. no.	Comp no.	IC_{50} (μM)		Sr. no.	Comp no.	IC_{50} (μM)	
		Cat B	Cat L			Cat B	Cat L
1	59	NI	8.6	**21**	103	NI	NI
2	60	NI	5.3	**22**	104	NI	NP
3	62	92	67	**23**	105	NI	NP
4	66	NI	NI	**24**	106	NI	NP
5	75	NI	NI	**25**	107	NI	NP
6	76	NI	NI	**26**	108	NI	NP
7	77	NI	NI	**27**	109	NI	NI
8	78	NI	NI	**28**	110	NI	NP
9	79	NI	NI	**29**	111	77.3	NI
10	80	NI	NI	**30**	112	NI	NP
11	81	NI	NI	**31**	113	NI	NI
12	82	NI	NI	**32**	119	NI	NP
13	83	79	NI	**33**	120	NI	NP
14	84	NI	NI	**34**	121	NI	NP
15	87	NI	NI	**35**	122	NI	NP
16	89	NI	NI	**36**	123	NI	NP
17	99	NI	NP	**37**	124	NI	NP
18	100	NI	NP	**38**	125	NI	NP
19	101	NI	NP	**39**	158	NI	NP
20	102	NI	NP				

NI = No Inhibition

NP = Not Performed

Any enzyme inhibitors should posses as low an IC_{50} as possible (may be upto picogram levels) in order to be accepted as a potential drug for further developmental studies. All the compounds tested for falcipain inhibitory activity (Table-2) showed IC_{50} more than 50 μM. This clearly indicated that the tested compounds have shown a poor binding affinity for the enzyme falcipain. Similarly, all of these compounds have shown no inhibition towards Cathepsin-B and L except for compounds **(59, 60** and **62),** which have shown some inhibition of Cathepsin-L, and compounds **(62, 83** and **111)** of Cathepsin-B.

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.