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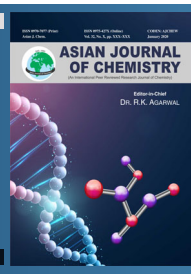

Synthesis, Characterization and Docking Studies of Anti-HCV Molecules

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The present study reports the synthesis and characterization of novel molecules inhibiting the spread of hepatitis C. The molecules were designed as to block the NS3/4A protease enzyme on HCV RNA. The molecules were synthesized using usual peptide synthesis techniques. Compounds with purity more than 95% were characterized and docking studies were also performed. All the compounds were characterized using physico-chemical techniques such as determination of melting point by DSC and NMR, mass, IR spectral studies. The docking studies were also conducted to assess the activity of molecules for inhibition of hepatitis C virus.

Keywords: Hepatitis C virus, Protease enzyme NS3/4A, Telaprevir, Amino acids.

INTRODUCTION

The hepatitis C virus has (HCV) [1-3] grown multifold in last few decades, but few options for the treatment of patients are available. The drugs available in the market are not effective against all the HCV genotype [4,5]. Researches to find effective anti HCV drugs is going on fast pace. In recent years drugs are found in market targeting HCV with different mechanism of action. Though many drugs failed to achieve desired activity but one such drug sofosbuvir [6,7] was found successful in achieving effective anti HCV treatment. These types of drugs which targets nonstructural protein 5B (NS5B) [8] of HCV are called NS5B nucleotide polymerase inhibitor. Sofosbuvir is also an option of treatment in combination [9,10] with other drugs such as daclatsavir. Other proteins are also present in HCV RNA which can be blocked to achieve anti HCV activities. One such protein is nonstructural protein 3/4A (NS3/4A) [11] serine protease and it is known to be responsible for cleavage of the HCV protein. If this protein site is blocked it will inhibit ribonucleic acid (RNA) replication. Many drugs came into market as HCV NS3/4A protease inhibitors *e.g.* telaprevir [12,13] but they all failed to give an effective alternative option for hepatitis C. This study is also based on anti HCV drugs targeting NS3/4A protein. We have synthesized various molecules having four different amino acids natural or synthetic in peptide chain having end capping C-terminal and N-terminal end with cyclic amines and pyrazine carboxylic or pyridine acids, respectively. The amino acids chosen are such that they fit into the cavity of NS3/4A protein thus blocking the site and interfere the process of HCV RNA replication.

To study the activity of molecules *in vitro* a lot of time and resources are needed, hence it was decided to screen molecules by docking studies. The tool to assess molecules nowadays various computer aided drug design programs are used. One such program is given by Schrödinger known as Glide. The molecules are docked into structure of protein and the energies are calculated by software on the basis of interaction between molecules and active site. In docking studies more the negative energy the more effective is the molecule against the disease.

EXPERIMENTAL

Solvents were of LR grade and further purified by distillation before use. Amino acids and other key raw materials were of LR grade and also purified before use. The completion of reaction was monitored by pre-coated TLC plates (TLC silica gel 60 F_{254} aluminium sheet by Merck). Synthesized compounds were purified by column chromatography (using

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silica gel of 60-120 mesh). NMR (Bruker Avance III 400 MHz), LC/MS (Agilent HP 1100 Series LC/MS) and FT-IR (Perkin Elmer Spectrum One FT-IR) were used to characterize the synthesized compounds. Differential scanning calorimetry (DSC) of Mettler Toledo, Model: DSC 821 e was used to determine the melting point of molecules. HPLC (Waters E2695, PDA Detector: 2998) was used to determine the purity of the molecules. Karl-Fisher Titrator (Analab Scientific Instrument, μAquaCal50) was used to determine moisture content in raw materials and in synthesized compounds whenever required.

General procedure for the synthesis of anti-HCV molecules

Protection of amino acids with *N*-benzyloxycarbonyl (CBz): Amino acid (50 g) was added to 100 mL toluene and the solution was then cooled to 0 ºC and 500 mL lithium hydroxide solution (11% in water) was added dropwise at 0-10 ºC. Benzyl chloroformate solution (240 mL, 50% in toluene) was then added to the reaction mixture. Reaction mass was stirred at 15-20 ºC for 15 h. Layers were separated and aqueous layer was washed with 100 mL toluene followed by the addition of 2N HCl, pH was adjusted to1.5. It was then extracted with 400 mL ethyl acetate. Organic extract was treated with 200 mL water and concentrated under vacuum to afford solid residue. Hexane was added to the solid residue, stirred and filtered. The solid was dried in oven under vacuum at 40 ºC to obtain CBz-L-amino acid.

Coupling reaction/amide formation using TBTU/HBTU: O-(Benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate (TBTU) or *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU) 1.2 mol. equiv was added to a solution of amino acid (1 mol eq) in acetonitrile. Mixture was stirred for 15 min and then 1.0 mol equiv. amine was added followed by the addition of triethylamine (1.0 mol equiv.) drop-wise at 20-25 ºC. Reaction mixture was stirred at room temperature for 16 h. DCM was added and reaction mass was then washed successively with 1N HCl, 5% $NaHCO_3$ and H_2O. The layers were separated and the organic extract was concentrated to yield solid residue. The solid residue was purified using hexane and ethyl acetate to yield desired compound which was dried in vacuum oven at 37 ± 2 ºC.

Deprotection of *N*-benzyloxycarbonyl (CBz): Palladium on carbon (50% wet with water) was added to the solution of starting material in methanol (10 vol. equiv.). Reaction mixture was stirred in high pressure autoclave under hydrogen pressure of 2 bar at 30 ± 2 ºC for 2-3 h. Reaction mass was then filtered through the bed of hyflo to remove carbon and the filtrate was concentrated under vacuum at 36 ± 2 ºC. Solid residue obtained was further purified using hexane and dried in vacuum oven at 37 ± 2 ºC to afford desired compound.

Coupling reaction/amide formation using EDC·HCl: Acid derivative (1 mol) was charged to a round bottom flask and then dichloromethane (10 to 20 vol eq), 1-hydroxybenzotriazole (1 mol) and EDC·HCl (1.2 mol) were then added to the reaction mixture. Reaction mixture was stirred at ambient temperature for 30 min to get clear solution. Amine (1.0 mol) was added to the reaction mixture. Reaction mixture was stirred at ambient temperature for 4 to 24 h. Progress of reaction was monitored by TLC/HPLC. Reaction was quenched by adding water (10 vol. equiv.). Layers were separated and the organic extract was washed with 10 times of 5% $NaHCO_3$ solution and 10 times of 1 N HCl and finally concentrated under vacuum to afford solid residue. The whole reactions chain is presented in **Scheme-I**.

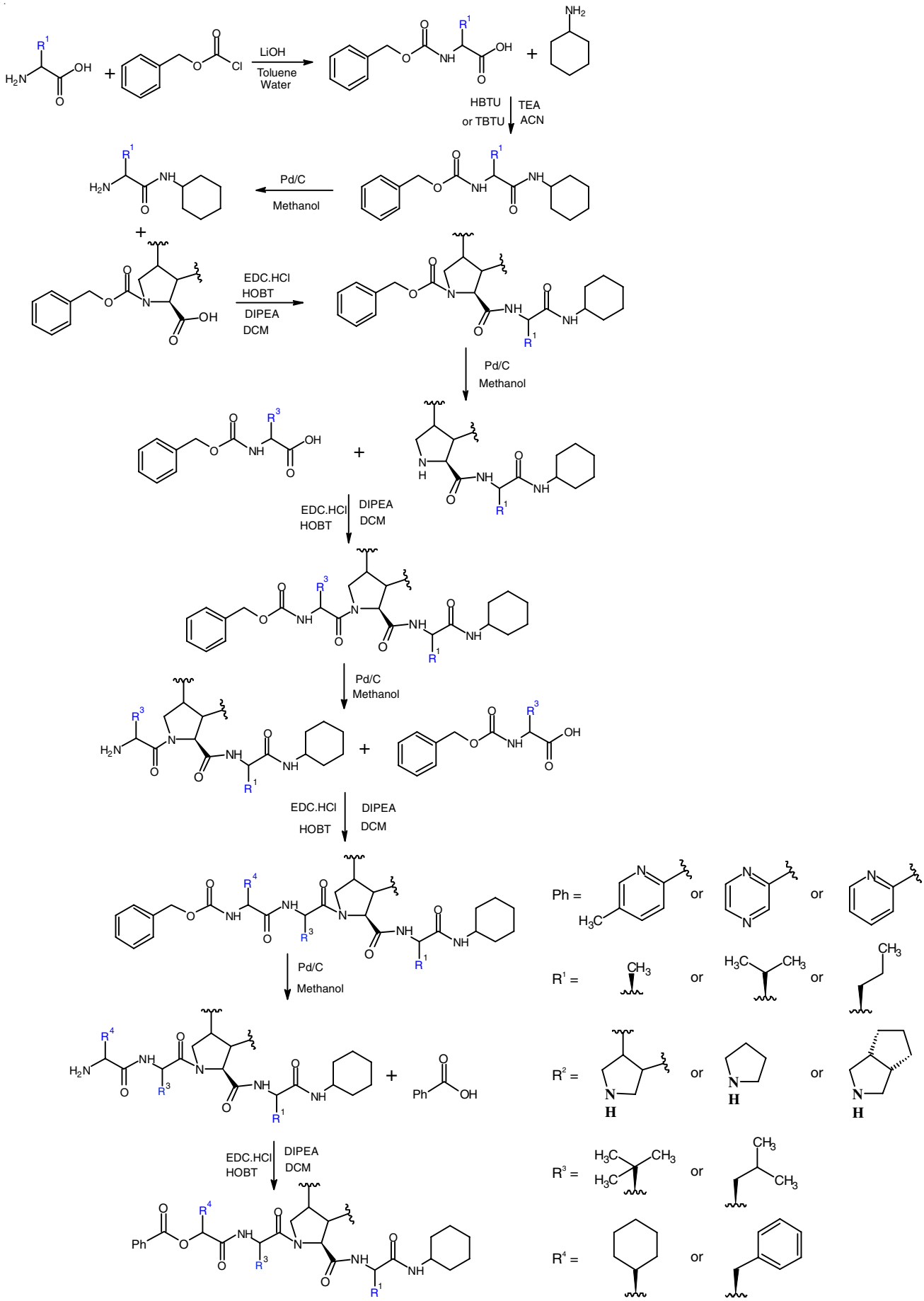
Spectral data

***N*-(Pyrazin-2-ylcarbonyl)-L-phenylalanyl-3-methyl-L-valyl-L-prolyl-*N*-cyclohexyl-L-norvalinamide (B-2):** White solid, Yield: 20%, m.f.: $C_{36}H_{51}N_7O_5$; m.w.: 661.83; purity by HPLC: 97.20%; IR (KBr, ν_{max}, cm^{-1}): 3386 (N-H), 3311 (N-H), 3063 (C-H), 2933 (C-H), 2856 (C-H), 1651 (C=O), 1632 (C=O), 1520 (C=O), 1446 (C-H), 1400 (C-N), 1370 (C-N), 1231 (C-N), 1020 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.14 (s, 1H), 8.88 (d, *J* = 2.40 Hz, 1H), 8.72 (m, 2H), 8.24 (d, *J* = 8.92 Hz, 1H), 7.85 (d, *J* = 8.16 Hz, 1H), 7.70 (d, *J* = Hz, 1H), 7.20 (m, 5H), 4.97 (q, *J* = 7.84 Hz, 1H), 4.53 (d, *J* = 8.92 Hz, 1H), 4.44 (m, 1H), 4.17 (q, *J* = 6.04 Hz, 1H), 3.63-3.48 (m, 3H), 3.31 (d, *J* = Hz, 2H), 2.02-1.13 (m, 18H), 0.98 (s, 9H), 0.85 (t, *J* = 7.16 Hz, 3H); MS (ESI-MS) *m/z*: 662.6 (MH^+).

***N*-(Pyrazin-2-ylcarbonyl)-L-phenylalanyl-L-leucyl-L-prolyl-*N*-cyclohexyl-L-norvalinamide (B-4):** White powder, Yield: 18%, m.f.: $C_{36}H_{51}N_7O_5$; m.w.: 661.83; purity by HPLC: 97.71%; IR (KBr, ν_{max}, cm^{-1}): 3382 (N-H), 3304 (N-H), 3064 (C-H), 2932 (C-H), 2856 (C-H), 1651 (C=O), 1632 (C=O), 1528 (C=O), 1450 (C-H), 1400 (C-N), 1368 (C-N), 1201 (C-N), 1020 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.12 (s, 1H), 8.88 (d, *J* = 2.40 Hz, 1H), 8.73 (m, 1H), 8.66 (d, *J* = 8.52 Hz, 1H), 8.51 (d, *J* = 8.04 Hz, 1H), 7.77 (d, *J* = 8.16 Hz, 1H), 7.56 (d, *J* = 7.88 Hz, 1H), 7.19 (m, 5H), 4.84 (m, 1H), 4.61 (q, *J* = 4.96 Hz, 1H), 4.37 (m, 1H), 4.16 (q, *J* = 8.12 Hz, 1H), 3.64 (m, 1H), 3.59 (m, 2H), 3.15 (dd, *J* = 4.56 Hz, 1H), 3.06 (q, *J* = 8.40 Hz, 1H), 2.01-1.13 (m, 21H), 0.91 (m, 9H); MS (ESI-MS) *m/z*: 662.6 (MH^+).

***N*-(Pyrazin-2-ylcarbonyl)-L-phenylalanyl-L-leucyl-L-prolyl-*N*-cyclohexyl-L-valinamide (B-8):** White powder, Yield: 21%, m.f.: $C_{36}H_{51}N_7O_5$; m.w.: 661.83; purity by HPLC: 94.98%; IR (KBr, ν_{max}, cm^{-1}): 3380 (N-H), 3309 (N-H), 3065 (C-H), 2958 (C-H), 2932 (C-H), 2856 (C-H), 1651 (C=O), 1630 (C=O), 1518 (C=O), 1450 (C-H), 1404 (C-N), 1369 (C-N), 1201 (C-N), 1020 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.12 (s, 1H), 8.88 (d, *J* = 2.44 Hz, 1H), 8.73 (m, 1H), 8.67 (d, *J* = 8.52 Hz, 1H), 8.54 (d, *J* = 7.96 Hz, 1H), 7.74 (d, *J* = 7.84 Hz, 1H), 7.68 (d, *J* = 8.92 Hz, 1H), 7.19 (m, 5H), 4.84 (m, 1H), 4.62 (q, *J* = 4.82 Hz, 1H), 4.48 (m, 1H), 4.04 (t, *J* = 8.72 Hz, 1H), 3.63 (m, 1H), 3.51 (m, 2H), 3.11 (dd, *J* = 4.28 Hz, 1H), 3.07 (q, *J* = 8.44 Hz, 1H), 2.04-1.03 (m, 18H), 1.00-0.87 (m, 12H); MS (ESI-MS) *m/z*: 662.5 (MH^+).

***N*-(Pyrazin-2-ylcarbonyl)-L-phenylalanyl-3-methyl-L-valyl-L-prolyl-*N*-cyclohexyl-L-alaninamide (B-14):** White powder, Yield: 21%, m.f.: $C_{34}H_{47}N_7O_5$; m.w.: 633.78; purity by HPLC: 97.42%; IR (KBr, ν_{max}, cm^{-1}): 3381 (N-H), 3311 (N-H), 3063 (C-H), 2957 (C-H), 2933 (C-H), 2855 (C-H), 1652 (C=O), 1632 (C=O), 1523 (C=O), 1447 (C-H), 1400 (C-N), 1370 (C-N), 1231 (C-N), 1020 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.15 (s, 1H) 8.88 (d, *J* = 2.44 Hz, 1H), 8.72 (m, 2H), 8.21 (d, *J* = 8.84 Hz, 1H), 7.91 (d, *J* = 7.44 Hz, 1H), 7.63 (d, *J* = 7.84 Hz, 1H), 7.19 (m, 5H), 4.98 (q, *J* = 7.92 Hz, 1H), 4.53 (d, *J* = 8.92 Hz, 1H), 4.43 (m, 1H), 4.20 (p, *J* = 7.20 Hz, 1H),



Scheme-I: General synthetic reaction of anti-HCV compounds

3.66-3.47 (m, 3H), 3.11 (d, *J* = 6.84 Hz, 2H), 2.03-1.03 (m, 17H), 1.09 (s, 9H); MS (ESI-MS) *m/z*: 634.7 (MH^+).

***N*-(Pyrazin-2-ylcarbonyl)-L-phenylalanyl-L-leucyl-L-prolyl-*N*-cyclohexyl-L-alaninamide (B-18):** White powder, Yield: 19%, m.f.: $C_{34}H_{47}N_7O_5$; m.w.: 633.78; purity by HPLC: 94.68%; IR (KBr, ν_{max}, cm^{-1}): 3382 (N-H), 3302 (N-H), 3064 (C-H), 2950 (C-H), 2932 (C-H), 2856 (C-H), 1651 (C=O), 1631 (C=O), 1527 (C=O), 1450 (C-H), 1402 (C-N), 1369 (C-N), 1201 (C-N), 1020 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.12 (s, 1H), 8.87 (s, 1H), 8.72 (m, 1H), 8.66 (d, *J* = 8.40 Hz, 1H), 8.49 (d, *J* = 7.88 Hz, 1H), 7.87 (d, *J* = 7.40 Hz, 1H), 7.49 (d, *J* = 7.68 Hz, 1H), 7.18 (m, 5H), 4.84 (q, *J* = 4.44 Hz, 1H), 4.60 (q, *J* = 7.24 Hz, 1H), 4.33 (m, 1H), 4.14 (p, *J* = 7.16 Hz, 1H), 3.64-3.49 (m, 3H), 3.13 (m, 2H), 2.03-1.08 (m, 20H), 1.09 (d, *J* = 4.68 Hz, 6H); MS (ESI-MS) *m/z*: 634.7 (MH^+).

***N*-(Pyridin-2-ylcarbonyl)-L-phenylalanyl-3-methyl-L-valyl-L-prolyl-*N*-cyclohexyl-L-valinamide (C-1):** White powder, Yield: 20%, m.f.: $C_{37}H_{52}N_6O_5$; m.w.: 660.84; purity by HPLC: 93.49%; IR (KBr, ν_{max}, cm^{-1}): 3313 (N-H), 3062 (C-H), 2959 (C-H), 2932 (C-H), 2872 (C-H), 2855 (C-H), 1730 (C=O), 1650 (C=O), 1625 (C=O), 1591 (C=O), 1494 (C-H), 1434 (C-H), 1229 (C-N), 997 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.75 (d, *J* = 4.96 Hz, 1H), 8.63 (d, *J* = 8.28 Hz, 1H), 8.22 (d, *J* = 7.96 Hz, 1H), 7.99 (d, *J* = 6.52 Hz, 2H), 7.77 (dd, *J* = 4.00 Hz, 2H), 7.60 (d, *J* = 4.36 Hz, 1H), 7.20-7.10 (m, 5H), 4.95 (q, *J* = 6.48 Hz, 1H), 4.54 (m, 2H), 4.09 (t, *J* = 8.2 Hz, 1H), 3.62 (m, 2H), 3.51 (m, 1H), 3.09 (d, *J* = 5.88 Hz, 2H), 2.08-1.10 (m, 17 H), 0.97 (s, 9H), 0.86 (d, *J* = 6.4, 6H); MS (ESI-MS) *m/z*: 661.4 (MH^+).

***N*-(Pyrazin-2-ylcarbonyl)-L-phenylalanyl-3-methyl-L-valyl-L-prolyl-*N*-cyclohexyl-L-valinamide (C-2):** White powder, Yield: 20%, m.f.: $C_{36}H_{51}N_7O_5$; m.w.: 661.83; m.p.: 81.9 ºC; purity by HPLC: 93.74%; IR (KBr, ν_{max}, cm^{-1}): 3382 (N-H), 3313 (N-H), 3063 (C-H), 2959 (C-H), 2933 (C-H), 2872 (C-H), 1668 (C=O), 1653 (C=O), 1626 (C=O), 1466 (C-H), 1444 (C-H), 1400 (C-H), 1370 (C-H), 1247 (C-N), 1199 (C-N), 1019 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.14 (s, 1H), 8.88 (m, 1H), 8.74 (m, 2H), 8.26 (d, *J* = 9.00 Hz, 1H), 7.77 (m, 2H), 7.17 (m, 5H), 4.96 (q, *J* = 7.08 Hz, 1H), 4.54 (m, 2H), 4.08 (t, *J* = 8.00 Hz, 1H), 3.64-3.59 (m, 3H), 3.10 (d, *J* = 5.88 Hz, 2H), 2.08-1.10 (m, 15H), 0.97 (s, 9H), 0.86 (d, *J* = 6.44 Hz, 6H); MS (ESI-MS) *m/z*: 662.4 (MH^+).

***N*-[(5-Methylpyrazin-2-yl)carbonyl]-L-phenylalanyl-3-methyl-L-valyl-L-prolyl-*N*-cyclohexyl-L-valinamide (C-3):** Light brown powder, Yield: 20%, m.f.: $C_{37}H_{53}N_7O_5$; m.w.: 675.86; m.p.: 79.2 ºC; purity by HPLC: 93.9%; IR (KBr, ν_{max}, cm^{-1}): 3314 (N-H), 3063 (C-H), 2959 (C-H).2932 (C-H), 2872 (C-H), 2855 (C-H), 1652 (C=O), 1626 (C=O), 1475 (C-H), 1443 (C-H), 1228 (C-N), 1031 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.99 (s, 1H), 8.68 (d, *J* = 8.32 Hz, 1H), 8.60 (s, 1H), 8.21 (d, *J* = 8.84 Hz, 1H), 7.77 (m, 2H), 7.20-7.10 (m, 5H), 4.96 (q, *J* = 6.48 Hz, 1H), 4.53 (m, 2H), 4.48 (m, 1H), 4.08 (t, *J* = 8.04 Hz, 1H), 3.62 (m, 2H), 3.51 (m, 1H), 3.09 (d, *J* = 5.92 Hz, 2H), 2.58 (s, 3H), 2.01-1.10 (m, 15 H), 0.97 (s, 9H), 0.85 (d, *J* = 6.36, 6H); MS (ESI-MS) *m/z*: 676.4 (MH^+).

***N*-(Pyridin-2-ylcarbonyl)-L-phenylalanyl-L-leucyl-L-prolyl-*N*-cyclohexyl-L-valinamide (C-4):** Off white powder, Yield: 20%, m.f.: $C_{37}H_{52}N_6O_5$; m.w.: 660.84; m.p.: 80.5 ºC; purity by HPLC: 94.3%; IR (KBr, ν_{max}, cm^{-1}): 3305 (N-H), 3063 (C-H), 2958 (C-H), 2932 (C-H), 2871 (C-H), 2855 (C-H), 1650 (C=O), 1625 (C=O), 1590 (C=O), 1450 (C-H), 1434 (C-H), 1233 (C-N), 997 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.63 (m, 2H), 8.54 (d, *J* = 7.88 Hz, 1H), 7.98 (d, *J* = 3.76 Hz, 2H), 7.74 (d, *J* = 7.76 Hz, 1H), 7.69 (d, 8.84 Hz, 1H), 7.61 (dd, *J* = 4.20 Hz, 1H), 7.20-7.15 (m, 5H), 4.83 (q, *J* = 4.52 Hz, 1H), 4.60 (m, 1H), 4.48 (m, 1H), 4.05 (t, *J* = 7.44 Hz, 1H), 3.66 (m, 1H), 3.51 (m, 2H), 3.13 (dd, *J* = 9.64 Hz, 1H), 3.02 (d, *J* = 8.32 Hz, 1H), 1.98-1.14 (m, 18 H), 0.89 (d, *J* = 6.48, 6H), 0.83 (d, *J* = 6.48, 6H); MS (ESI-MS) *m/z*: 661.4 (MH^+).

***N*-(Pyrazin-2-ylcarbonyl)-L-phenylalanyl-L-leucyl-L-prolyl-*N*-cyclohexyl-L-valinamide (C-5):** Off white powder, Yield: 20%, m.f.: $C_{36}H_{51}N_7O_5$; m.w.: 661.84; m.p.: 102.3 ºC; purity by HPLC: 94.6%; IR (KBr, ν_{max}, cm^{-1}): 3379 (N-H), 3307 (N-H), 3064 (C-H), 2958 (C-H), 2932 (C-H), 2871 (C-H), 1668 (C=O), 1650 (C=O), 1465 (C-H), 1450 (C-H), 1401 (C-H), 1369 (C-H), 1248 (C-N), 1200 (C-N), 1019 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.12 (s, 1H), 8.88 (d, *J* = 2.08 Hz, 1H), 8.74 (m, 1H), 8.67 (d, *J* = 8.52 Hz, 1H), 8.54 (d, *J* = 7.92 Hz, 1H), 7.74 (d, *J* = 7.72 Hz, 1H), 7.69 (d, *J* = 8.88 Hz, 1H), 7.19 (m, 5H), 4.84 (m, 1H), 4.60 (m, 1H), 4.48 (m, 1H), 4.05 (t, *J* = 7.52 Hz, 1H), 3.67 (m, 1H), 3.51 (m, 2H), 3.15 (dd, *J* = 4.24 Hz, 1H), 3.06 (q, *J* = 8.52 Hz, 1H), 1.99-1.11 (m, 18H), 0.91-0.82 (m, 12H); MS (ESI-MS) *m/z*: 662.4 (MH^+).

***N*-[(5-Methylpyrazin-2-yl)carbonyl]-L-phenylalanyl-L-leucyl-L-prolyl-*N*-cyclohexyl-L-valinamide (C-6):** Light brown powder, Yield: 20%, m.f.: $C_{37}H_{53}N_7O_5$; m.w.: 675.89; m.p.: 109.3 ºC; purity by HPLC: 94.4%; IR (KBr, ν_{max}, cm^{-1}): 3306 (N-H), 3030 (C-H), 2958 (C-H), 2932 (C-H), 2870 (C-H), 2855 (C-H), 1649 (C=O), 1620 (C=O), 1475 (C-H), 1449 (C-H), 1232 (C-N), 1031 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.98 (s, 1H), 8.60 (m, 2H), 8.53 (d, *J* = 7.96 Hz, 1H), 7.74 (d, *J* = 7.80 Hz, 1H), 7.69 (d, *J* = 8.84 Hz, 1H), 7.20-7.14 (m, 5H), 4.83 (q, *J* = 7.96 Hz, 1H), 4.60 (q, *J* = 8.36 Hz, 1H), 4.48 (m, 1H), 4.05 (t, *J* = 7.60 Hz, 1H), 3.67 (m, 1H), 3.51 (m, 2H), 3.15 (dd, *J* = 4.16 Hz, 1H), 3.05 (dd, *J* = 8.40 Hz, 1H), 2.58 (s, 3H), 2.99-1.11 (m, 18 H), 0.91-0.82 (m, 12H); MS (ESI-MS) *m/z*: 676.3 (MH^+).

***N*-{(2*S*)-2-Cyclohexyl-2-[(pyridin-2-ylcarbonyl)amino]-acetyl}-3-methyl-L-valyl-L-prolyl-*N*-cyclohexyl-L-valinamide (C-7):** Off white powder, Yield: 20%, m.f.: $C_{36}H_{56}N_6O_5$; m.w.: 652.86; m.p.: 84.6 ºC; purity by HPLC: 95.6%; IR (KBr, ν_{max}, cm^{-1}): 3314 (N-H), 3060 (C-H), 2958 (C-H), 2931 (C-H), 2854 (C-H), 1653 (C=O), 1628 (C=O), 1591 (C=O), 1448 (C-H), 1435 (C-H), 1232 (C-N), 997 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.70 (m, 2H), 8.17 (d, *J* = 8.56 Hz, 1H), 8.05-7.99 (m, 2H), 7.76 (d, *J* = 7.72 Hz, 1H), 7.71 (d, *J* = 8.92 Hz, 1H), 7.63 (dd, *J* = 5.32 Hz, 1H), 4.61 (t, *J* = 7.56 Hz, 1H), 4.51 (d, *J* = 8.60 Hz, 1H), 3.40 (m, 1H), 4.06 (t, *J* = 7.48 Hz, 1H), 3.70 (m, 1H), 3.61 (m, 1H), 3.50 (m, 1H), 1.93-0.99 (m, 26 H), 0.96 (s, 9H), 0.84 (d, *J* = 6.64, 6H); MS (ESI-MS) *m/z*: 653.4 (MH^+).

***N*-{(2*S*)-2-Cyclohexyl-2-[(pyrazin-2-ylcarbonyl)amino]-acetyl}-3-methyl-L-valyl-L-prolyl-*N*-cyclohexyl-L-valinamide (C-8):** Off white powder, Yield: 22%, m.f.: $C_{35}H_{55}N_7O_5$; m.w.:

653.85; m.p.: 85.6 ºC; purity by HPLC: 95.1%; IR (KBr, ν_{max}, cm^{-1}): 3380 (N-H), 3316 (N-H), 3061 (C-H), 2956 (C-H), 2931 (C-H), 2854 (C-H), 1668 (C=O), 1650 (C=O), 1627 (C=O), 1466 (C-H), 1448 (C-H), 1399 (C-H), 1369 (C-H), 1234 (C-N), 1202 (C-N), 1019 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.19 (s, 1H), 8.91 (m, 1H), 8.76 (m, 1H), 8.66 (d, *J* = 9.28 Hz, 1H), 8.17 (d, *J* = 8.60 Hz, 1H), 7.76 (d, *J* = 7.84 Hz, 1H), 7.72 (d, *J* = 8.88 Hz, 1H), 4.62 (t, *J* = 7.92 Hz, 1H), 4.51 (d, *J* = 8.64 Hz, 1H), 4.40 (m, 1H), 4.06 (t, *J* = 7.72 Hz, 1H), 3.70 (m, 1H), 3.61 (m, 1H), 3.50 (m, 1H), 1.93-1.10 (m, 26H), 0.96 (s, 9H), 0.84 (d, *J* = 6.64 Hz, 6H); MS (ESI-MS) *m/z*: 654.4 (MH^+).

***N*-[(2*S*)-2-Cyclohexyl-2-{[(5-methylpyrazin-2-yl)carbonyl]amino}acetyl]-3-methyl-L-valyl-L-prolyl-*N*-cyclohexyl-L-valinamide (C-9):** Light brown powder; Yield: 23%, m.f.: $C_{36}H_{57}N_7O_5$; m.w.: 667.88; m.p.: 84.6 ºC; purity by HPLC: 95.3%; IR (KBr, ν_{max}, cm^{-1}): 3316 (N-H), 3075 (C-H), 2959 (C-H), 2931 (C-H), 2854 (C-H), 1649 (C=O), 1627 (C=O), 1475 (C-H), 1448 (C-H), 1247 (C-N), 1202 (C-N), 1031 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.05 (s, 1H), 8.64 (s, 1H), 8.60 (d, *J* = 9.40 Hz, 1H), 8.15 (d, *J* = 8.52 Hz, 1H), 7.76 (d, *J* = 7.72 Hz, 1H), 7.71 (d, *J* = 8.80 Hz, 1H), 4.62 (t, *J* = 7.72 Hz, 1H), 4.50 (d, *J* = 8.72 Hz, 2H), 4.39 (m, 1H), 4.06 (t, *J* = 7.64 Hz, 1H), 3.60 (m, 1H), 3.50 (m, 1H), 2.60 (s, 3H), 1.92-0.99 (m, 26 H), 0.96 (s, 9H), 0.84 (d, *J* = 6.68 Hz, 6H); MS (ESI-MS) *m/z*: 668.4 (MH^+).

***N*-{(2*S*)-2-Cyclohexyl-2-[(pyridin-2-ylcarbonyl)amino]acetyl}-L-leucyl-L-prolyl-*N*-cyclohexyl-L-valinamide (C-10):** Off white powder; Yield: 20%, m.f.: $C_{36}H_{56}N_6O_5$; m.w.: 652.86; m.p.: 81.9 ºC; purity by HPLC: 95.6%; IR (KBr, ν_{max}, cm^{-1}): 3305 (N-H), 3066 (C-H), 2958 (C-H), 2930 (C-H), 2854 (C-H), 1650 (C=O), 1625 (C=O), 1591 (C=O), 1449 (C-H), 1435 (C-H), 1235 (C-N), 997 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.68 (d, *J* = 4.56 Hz, 1H), 8.59 (d, *J* = 9.32 Hz, 1H), 8.45 (d, *J* = 7.60 Hz, 1H), 8.06-8.00 (m, 2H), 7.72 (d, *J* = 7.84 Hz, 1H), 7.63 (m, 2H), 4.55-4.40 (m, 3H), 4.03 (t, 7.40 *J* = Hz, 1H), 3.71 (m, 1H), 3.52 (m, 2H), 1.86-1.34 (m, 29H), 0.89-0.81 (m, 12H); MS (ESI-MS) *m/z*: 653.4 (MH^+).

***N*-{(2*S*)-2-Cyclohexyl-2-[(pyrazin-2-ylcarbonyl)amino]acetyl}-L-leucyl-L-prolyl-*N*-cyclohexyl-L-valinamide (C-11):** Off white powder; Yield: 20%, m.f.: $C_{35}H_{55}N_7O_5$; m.w.: 653.85; m.p.: 82.9 ºC; purity by HPLC: 95.8%; IR (KBr, ν_{max}, cm^{-1}): 3389 (N-H), 3309 (N-H), 3072 (C-H), 2956 (C-H), 2931 (C-H), 2854 (C-H), 1648 (C=O), 1465 (C-H), 1449 (C-H), 1401 (C-H), 1368 (C-H), 1248 (C-N), 1157 (C-N), 1019 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.19 (s, 1H), 8.91 (d, *J* = 1.92 Hz, 1H), 8.76 (m, 1H), 8.52 (d, *J* = 9.24 Hz, 1H), 8.44 (d, *J* = 7.48 Hz, 1H), 7.72 (d, *J* = 7.76 Hz, 1H), 7.61 (d, *J* = 8.80 Hz, 1H), 4.55-4.40 (m, 3H), 4.03 (t, *J* = 7.24 Hz, 1H), 3.72 (m, 1H), 3.52 (m, 2H), 1.98-0.96 (m, 29H), 0.93-0.80 (m, 12H); MS (ESI-MS) *m/z*: 654.4 (MH^+).

***N*-[(2*S*)-2-Cyclohexyl-2-{[(5-methylpyrazin-2-yl)carbonyl]amino}acetyl]-L-leucyl-L-prolyl-*N*-cyclohexyl-L-valinamide (C-12):** Light brown powder; Yield: 20%, m.f.: $C_{36}H_{57}N_7O_5$; m.w.: 667.88; m.p.: 82.9 ºC; purity by HPLC: 95.63%; IR (KBr, ν_{max}, cm^{-1}): 3308 (N-H), 3076 (C-H), 2959 (C-H), 2931 (C-H), 2854 (C-H), 1650 (C=O), 1627 (C=O), 1474 (C-H), 1449 (C-H), 1248 (C-N), 1204 (C-N), 1031 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.05 (s, 1H), 8.64 (s, 1H), 8.45 (m, 2H), 7.72 (d, *J* = 7.72 Hz, 1H), 7.61 (d, *J* = 8.80 Hz, 1H), 4.54 (m, 1H), 4.47 (t, *J* = 7.76 Hz, 1H), 4.42 (m, 1H), 4.03 (t, *J* = 7.44 Hz, 1H), 3.70 (m, 1H), 3.52 (m, 2H), 2.60 (s, 3H), 1.97-0.95 (m, 29 H), 0.95 (m, 12H); MS (ESI-MS) *m/z*: 668.5 (MH^+).

(1*S*,3a*R*,6a*S*)-*N*-[(2*S*)-1-(Cyclohexylamino)-3-methyl-1-oxobutan-2-yl]-2-[(2*S*)-4-methyl-2-({(2*S*)-3-phenyl-2-[(pyridin-2-ylcarbonyl)amino]propanoyl}amino)pentanoyl]-octahydrocyclopenta[*c*]pyrrole-1-carboxamide (D-1): White powder, Yield: 21%, m.p. by DSC: 79.3 ºC; m.f.: $C_{40}H_{56}N_6O_5$; m.w.: 700.9; purity by HPLC: 92.53%; IR (KBr, ν_{max}, cm^{-1}): 3303 (N-H), 3063 (C-H), 2957 (C-H), 2933 (C-H), 2868 (C-H), 2855 (C-H), 1649 (C=O), 1629 (C=O), 1590 (C=O), 1451 (C-H), 1433 (C-H), 1225 (C-N), 997 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.65 (m, 2H), 8.57 (d, *J* = 7.88 Hz, 1H), 7.98 (d, *J* = 3.68 Hz, 2H), 7.74 (dd, *J* = 3.64 Hz, 2H), 7.60 (dd, *J* = 4.68 Hz, 1H), 7.19 (m, 5H), 4.83 (m, 1H), 4.59 (q, *J* = 7.32 Hz, 1H), 4.36 (s, 1H), 4.07 (t, *J* = 7.60 Hz, 1H), 3.72 (dd, *J* = 9.00 Hz, 1H), 3.54 (m, 2H), 3.10 (dd, 9.36 *J* = Hz, 1H), 3.02 (dd, *J* = 5.68 Hz, 1H), 2.68 (m, 1H), 1.92-1.10 (m, 21H), 0.86 (m, 12H); MS (ESI-MS) *m/z*: 701.4 (MH^+).

(1*S*,3a*R*,6a*S*)-*N*-[(2*S*)-1-(Cyclohexylamino)-3-methyl-1-oxobutan-2-yl]-2-[(2*S*)-4-methyl-2-({(2*S*)-3-phenyl-2-[(pyrazin-2-ylcarbonyl)amino]propanoyl}amino)-pentanoyl]octahydrocyclopenta[*c*]pyrrole-1-carboxamide (D-2): Off white powder, Yield: 21%, m.p. by DSC: 79.9 ºC; m.f.: $C_{39}H_{55}N_7O_5$; m.w.: 701.8; purity by HPLC: 91.02%; IR (KBr, ν_{max}, cm^{-1}): 3381 (N-H), 3302 (N-H), 3064 (C-H), 2957 (C-H), 2933 (C-H), 2869 (C-H), 1662 (C=O), 1650 (C=O), 1629 (C=O), 1466 (C-H), 1451 (C-H), 1400 (C-H), 1368 (C-H), 1249 (C-N), 1152 (C-N), 1019 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.19 (s, 1H), 8.87 (d, *J* = 1.92 Hz, 1H), 8.72 (m, 1H), 8.67 (d, *J* = 8.48 Hz, 1H), 8.57 (d, *J* = 7.92 Hz, 1H), 7.74 (m, 2H), 7.16 (m, 5H), 4.84 (m, 1H), 4.58 (q, *J* = 7.28 Hz, 1H), 4.35 (m, 1H), 4.05 (t, *J* = 7.64 Hz, 1H), 3.72 (t, *J* = 9.28 Hz, 1H), 3.54 (m, 2H), 3.14-3.02 (m, 2H), 2.68 (m, 1H), 1.92-1.10 (m, 21H), 0.90-0.82 (m, 12H); MS (ESI-MS) *m/z*: 702.4 (MH^+).

(1*S*,3a*R*,6a*S*)-*N*-[(2*S*)-1-(Cyclohexylamino)-3-methyl-1-oxobutan-2-yl]-2-[(2*S*)-4-methyl-2-{[(2S)-2-{[(5-methylpyrazin-2-yl)carbonyl]amino}-3-phenylpro-panoyl]amino}-pentanoyl]octahydrocyclopenta[*c*]pyrrole-1-carboxamide (D-3): Light brown powder, Yield: 21%, m.p. by DSC: 91.8 ºC; m.f.: $C_{40}H_{57}N_7O_5$; m.w.: 715.9; purity by HPLC: 91.8%; IR (KBr, ν_{max}, cm^{-1}): 3304 (N-H), 3064 (C-H), 2957 (C-H), 2933 (C-H), 2869 (C-H), 1649 (C=O), 1630 (C=O), 1474 (C-H), 1451 (C-H), 1249 (C-N), 1226 (C-N), 1031 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.97 (s, 1H), 8.60-8.53 (m, 3H), 7.75 (d, *J* = 9.24 Hz, 2H), 7.17 (m, 5H), 4.82 (q, *J* = 4.24 Hz, 1H), 4.58 (q, *J* = 7.20 Hz, 1H), 4.35 (m, 1H), 4.05 (t, *J* = 7.56 Hz, 1H), 3.72 (t, *J* = 8.96 Hz, 1H), 3.53 (m, 2H), 3.13-3.01 (m, 2H), 2.68 (m, 1H), 2.58 (s, 3H), 1.92-1.10 (m, 21 H), 0.90-0.82 (m, 12H); MS (ESI-MS) *m/z*: 716.4 (MH^+).

(1*S*,3a*R*,6a*S*)-*N*-[(2*S*)-1-(Cyclohexylamino)-3-methyl-1-oxobutan-2-yl]-2-[(2*S*)-2-({(2*S*)-2-cyclohexyl-2-[(pyridin-2-ylcarbonyl)amino]acetyl}amino)-3,3-dimethylbutanoyl]-octahydrocyclopenta[*c*]pyrrole-1-carboxamide (D-7): Light

brown powder, Yield: 21%, m.p. by DSC: 121.9 ºC; m.f.: $C_{39}H_{60}N_6O_5$; m.w.: 792.93; purity by HPLC: 88.95%; IR (KBr, ν_{max}, cm^{-1}): 3317 (N-H), 3062 (C-H), 2959 (C-H), 2932 (C-H), 2855 (C-H), 1655 (C=O), 1623 (C=O), 1591 (C=O), 1449 (C-H), 1434 (C-H), 1228 (C-N), 1201 (C-N), 997 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.67 (d, *J* = 4.56Hz, 1H), 8.59 (d, *J* = 9.24 Hz, 1H), 8.25 (d, *J* = 9.20 Hz, 1H), 8.03 (m, 2H), 7.78-7.72 (m, 2H), 7.63 (d, *J* = 5.24 Hz, 1H), 4.67 (t, *J* = 2.72 Hz, 1H), 4.57 (d, *J* = 9.16 Hz, 1H), 4.32 (d, *J* = 3.00 Hz, 1H), 4.06 (q, *J* = 8.36 Hz, 1H), 3.76 (m, 1H), 3.65 (m, 1H), 3.32 (m, 1H), 2.74 (m, 1H), 1.90-1.00 (m, 29H), 0.93 (s, 9H), 0.84 (d, *J* = 6.72 Hz, 6H); MS (ESI-MS) *m/z*: 693.7 (MH^+).

(1*S*,3a*R*,6a*S*)-*N*-[(2*S*)-1-(Cyclohexylamino)-3-methyl-1-oxobutan-2-yl]-2-[(2*S*)-2-({(2*S*)-2-cyclohexyl-2-[(pyridin-2-ylcarbonyl)amino]acetyl}amino)-4-methylpentanoyl]-octahydrocyclopenta[*c*]pyrrole-1-carboxamide (D-10): Light brown powder, Yield: 21%, m.p. by DSC: 124.5 ºC; m.f.: $C_{39}H_{60}N_6O_5$; m.w.: 692.93; purity by HPLC: 80.82%; IR (KBr, ν_{max}, cm^{-1}): 3384 (N-H), 3311 (N-H), 3061 (C-H), 2961 (C-H), 2874 (C-H), 1727 (C=O), 1681 (C=O), 1660 (C=O), 1620 (C=O), 1460 (C-H), 1434 (C-H), 1240 (C-N), 1201 (C-N), 997 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.68 (d, *J* = 4.64 Hz, 1H), 8.57 (d, *J* = 9.44 Hz, 1H), 8.50 (d, *J* = 7.92 Hz, 1H), 8.03 (m, 2H), 7.65 (m, 3H), 4.67 (t, *J* = 2.70 Hz, 1H), 4.57 (dd, *J* = 9.13 Hz, 1H), 4.29 (d, *J* = 2.84 Hz, 1H), 4.02 (m, 1H), 3.72 (m, 1H), 3.63 (m, 1H), 3.54 (m, 1H), 2.72 (m, 1H), 1.90-1.00 (m, 32H), 0.85 (m, 12H); MS (ESI-MS) *m/z*: 693.5 (MH^+).

(1*S*,3a*R*,6a*S*)-*N*-[(2*S*)-1-(Cyclohexylamino)-1-oxopentan-2-yl]-2-[(2*S*)-3,3-dimethyl-2-({(2*S*)-3-phenyl-2-[(pyrazin-2-ylcarbonyl)amino]propanoyl}amino)-butanoyl]octahydrocyclopenta[*c*]pyrrole-1-carboxamide (V-2): White powder; Yield: 20%, m.f.: $C_{39}H_{55}N_7O_5$; m.w.: 701.89; m.p.: 133.6 ºC; purity by HPLC: 93.33%; IR (KBr, ν_{max}, cm^{-1}): 3380 (N-H), 3309 (N-H), 3063 (C-H), 2963 (C-H), 2934 (C-H), 2870 (C-H), 1654 (C=O), 1620 (C=O), 1527 (C=O), 1449 (C-H), 1399 (C-H), 1370 (C-H), 1228 (C-N), 1153 (C-N), 1020 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.13 (s, 1H), 8.88 (d, *J* = 2.24 Hz, 1H), 8.71 (m, 1H), 8.66 (d, *J* = 8.32 Hz, 1H), 8.27 (d, *J* = 8.88 Hz, 1H), 7.90 (d, *J* = 8.04 Hz, 1H), 7.66 (d, *J* = 8.00 Hz, 1H), 7.20 (m, 5H), 4.99 (m, 1H), 4.48 (d, *J* = 8.84 Hz, 1H), 4.29 (d, *J* = 3.56 Hz, 1H), 4.18 (m, 1H), 3.79 (q, *J* = 9.80 Hz, 1H), 3.64-3.48 (m, 2H), 3.08 (m, 2H), 2.66 (m, 1H), 1.84-1.10 (m, 21 H), 0.97 (s, 9 H), 0.83 (t, *J* = 6.92 Hz, 3H); MS (ESI-MS) *m/z*: 702.5 (MH^+).

(1*S*,3a*R*,6a*S*)-*N*-[(2*S*)-1-(Cyclohexylamino)-1-oxopentan-2-yl]-2-[(2*S*)-4-methyl-2-({(2*S*)-3-phenyl-2-[(pyrazin-2-ylcarbonyl)amino]propanoyl}amino)-pentanoyl]octahydrocyclopenta[*c*]pyrrole-1-carboxamide (V-4): White powder; Yield: 20%, m.f.: $C_{39}H_{55}N_7O_5$; m.w.: 701.89; m.p.: 67.9 ºC; purity by HPLC: 92.7%; IR (KBr, ν_{max}, cm^{-1}): 3378 (N-H), 3299 (N-H), 3064 (C-H), 2962 (C-H), 2933 (C-H), 2869 (C-H), 1651 (C=O), 1621 (C=O), 1530 (C=O), 1453 (C-H), 1385 (C-H), 1362 (C-H), 1228 (C-N), 1153 (C-N), 1020 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.11 (s, 1H), 8.88 (d, *J* = 2.36 Hz, 1H), 8.73 (d, *J* = 1.36 Hz, 1H), 8.65 (d, *J* = 8.60 Hz, 1H), 8.54 (d, *J* = 7.88 Hz, 1H), 7.87 (d, *J* = 8.04 Hz, 1H), 7.58 (d, *J* = 7.80 Hz, 1H), 7.20 (m, 5H), 4.86 (m, 1H), 4.58 (q, *J* = 7.44 Hz, 1H), 4.24 (d, *J* = 3.16 Hz, 1H), 4.10 (m, 1H), 3.72 (t, *J* = 9.68 Hz, 1H), 3.66-3.48 (m, 2H), 3.13 (dd, *J* = 4.32 Hz, 1H), 3.07 (q, *J* = 8.36 Hz, 1H), 2.68 (m, 1H), 1.92-1.01 (m, 24 H), 0.95-0.78 (m, 9 H); MS (ESI-MS) *m/z*: 702.5 (MH^+).

(1*S*,3a*R*,6a*S*)-*N*-[(2*S*)-1-(Cyclohexylamino)-1-oxopropan-2-yl]-2-[(2*S*)-3,3-dimethyl-2-({(2*S*)-3-phenyl-2-[(pyrazin-2-ylcarbonyl)amino]propanoyl}amino)butanoyl]-octahydrocyclopenta[*c*]pyrrole-1-carboxamide (V-14): Off white powder; Yield: 21%, m.f.: $C_{37}H_{51}N_7O_5$; m.w.: 673.84; m.p.: 67.8 ºC; purity by HPLC: 98.5%; IR (KBr, ν_{max}, cm^{-1}): 3392 (N-H), 3319 (N-H), 3030 (C-H), 2961 (C-H), 2934 (C-H), 2856 (C-H), 1654 (C=O), 1624 (C=O), 1522 (C=O), 1449 (C-H), 1399 (C-H), 1370 (C-H), 1228 (C-N), 1154 (C-N), 1019 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.14 (s, 1H), 8.87 (d, *J* = 1.24 Hz, 1H), 8.72 (d, *J* = 1.32 Hz, 1H), 8.67 (d, *J* = 8.40 Hz, 1H), 8.24 (d, *J* = 8.72 Hz, 1H), 7.93 (d, *J* = 7.32 Hz, 1H), 7.60 (d, *J* = 7.76 Hz, 1H), 7.19 (m, 5H), 4.99 (q, *J* = 7.60 Hz, 1H), 4.49 (d, *J* = 8.68 Hz, 1H), 4.27 (d, *J* = 3.28 Hz, 1H), 4.17 (p, *J* = 7.12 Hz, 1H), 3.76 (t, *J* = 9.64 Hz, 1H), 3.58 (m, 1H), 3.49 (m, 1H), 3.08 (m, 2H), 2.63 (m, 1H), 2.54 (m, 1H), 1.52-1.23 (m, 18 H), 1.19 (d, *J* = 6.96 Hz, 1H), 0.98 (s, 9 H); MS (ESI-MS) *m/z*: 674.6 (MH^+).

(1*S*,3a*R*,6a*S*)-*N*-[(2*S*)-1-(Cyclohexylamino)-1-oxopropan-2-yl]-2-[(2*S*)-4-methyl-2-({(2*S*)-3-phenyl-2-[(pyrazin-2-ylcarbonyl)amino]propanoyl}amino)pentanoyl]-octahydrocyclopenta[*c*]pyrrole-1-carboxamide (V-18): Off white powder; Yield: 18%, m.f.: $C_{37}H_{51}N_7O_5$; m.w.: 673.84; m.p.: 99.31 ºC; purity by HPLC: 95.3%; IR (KBr, ν_{max}, cm^{-1}): 3392 (N-H), 3319 (N-H), 3030 (C-H), 2961 (C-H), 2934 (C-H), 2856 (C-H), 1654 (C=O), 1624 (C=O), 1522 (C=O), 1449 (C-H), 1399 (C-H), 1370 (C-H), 1228 (C-N), 1154 (C-N), 1019 (C-H); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.14 (s, 1H), 8.87 (d, *J* = 1.24 Hz, 1H), 8.72 (d, *J* = 1.32 Hz, 1H), 8.67 (d, *J* = 8.40 Hz, 1H), 8.24 (d, *J* = 8.72 Hz, 1H), 7.93 (d, *J* = 7.32 Hz, 1H), 7.60 (d, *J* = 7.76 Hz, 1H), 7.19 (m, 5H), 4.99 (q, *J* = 7.60 Hz, 1H), 4.49 (d, *J* = 8.68 Hz, 1H), 4.27 (d, *J* = 3.28 Hz, 1H), 4.17 (p, *J* = 7.12 Hz, 1H), 3.76 (t, *J* = 9.64 Hz, 1H), 3.58 (m, 1H), 3.49 (m, 1H), 3.08 (m, 2H), 2.63 (m, 1H), 2.54 (m, 1H), 1.52-1.23 (m, 18 H), 1.19 (d, *J* = 6.96 Hz, 1H), 0.98 (s, 9 H); MS (ESI-MS) *m/z*: 674.6 (MH^+).

RESULTS AND DISCUSSION

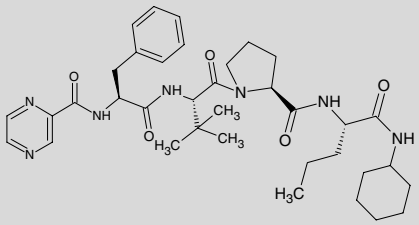
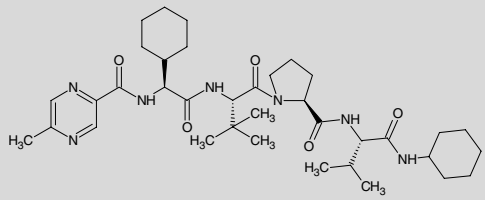
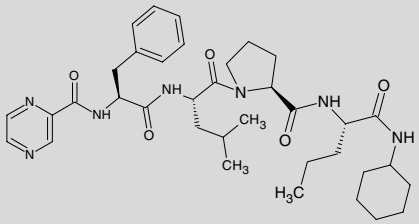
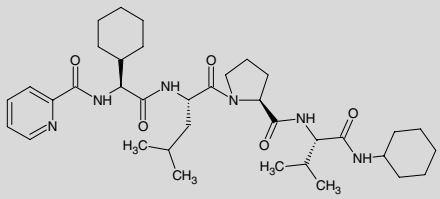
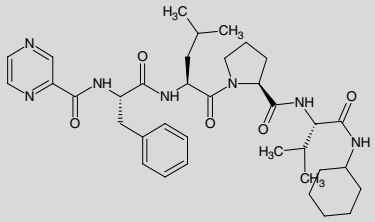
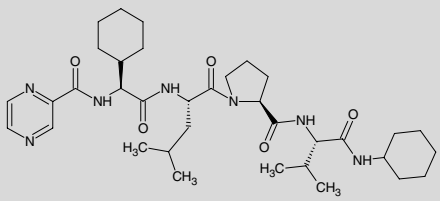
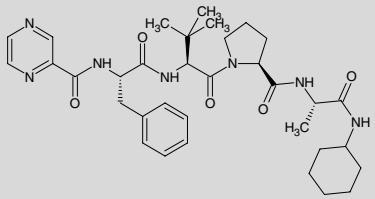
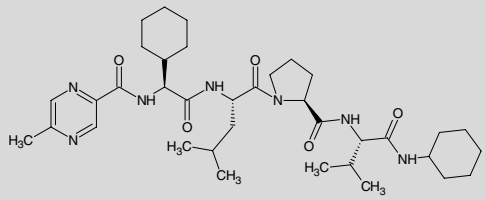
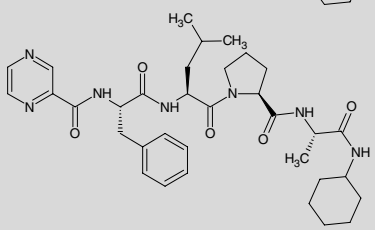
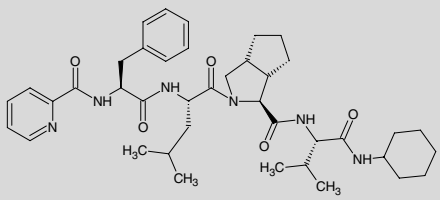
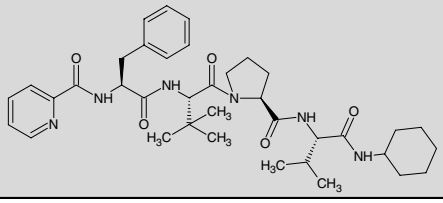
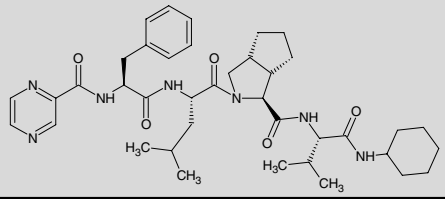
Using the above mentioned procedure, compounds were synthesized in 8-10 steps using natural and synthetic amino acids. The end capping for *C*-terminal of peptide chain was used is cyclohexyl amine. Pyrazine-2-carboxlic acid, pyridine-2-carboxylic acid and 4-methyl-2-pyrazine carboxylic acids were used to end cap *N*-terminal of peptide chain. The peptide bond formation was carried out in solution phase synthesis using EDC·HCl/HOBT or TBTU as coupling reagent and triethylamine or diisopropylamine as base. Palladium on carbon was used for deprotection of carbobenzyloxy group to get free amine group. The yield of peptide bond formation step ranged from 60-90% for different intermediate steps and the yield for deprotection of carbobenzyloxy group was between 80-95%. The overall yield of all the steps for different molecule ranged from 15-25%.

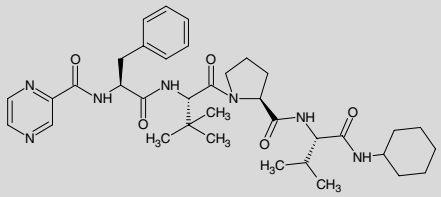
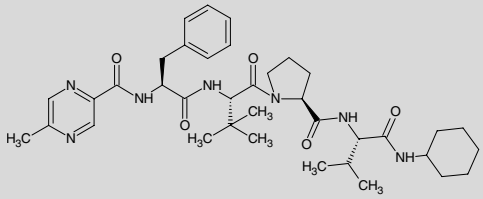
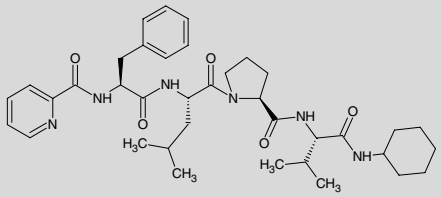
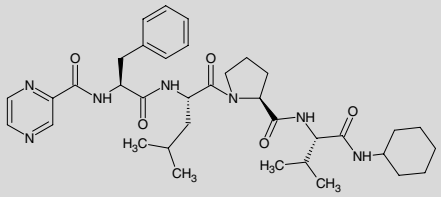
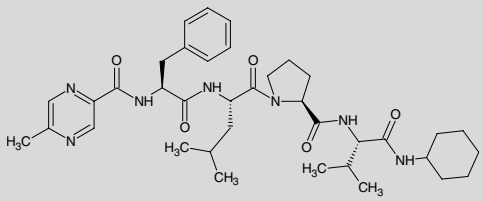
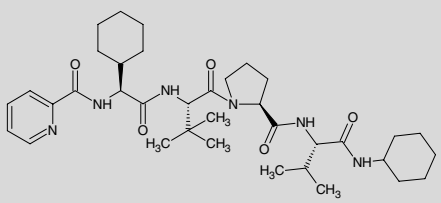
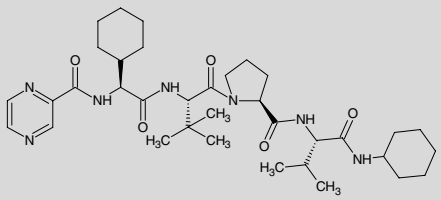
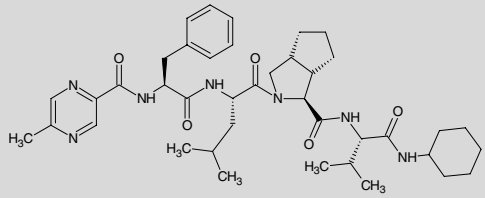
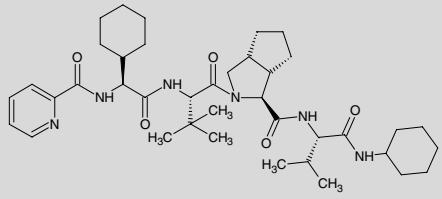
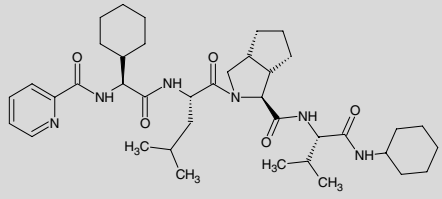
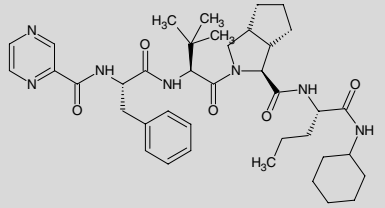
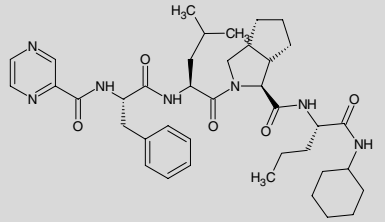
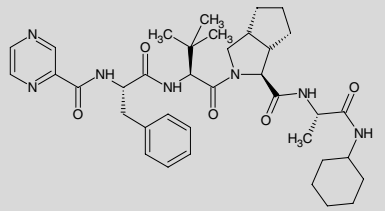
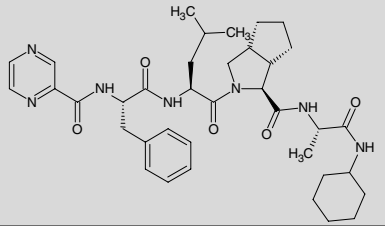
Sharp melting point and HPLC purity of synthesized molecules ensured the high purity of synthesized compounds. Synthesized molecules with purity more than 90% were reported in the present communication. Molecular mass observed in the mass spectrum of molecules supported the molecular formula and molecular formula of the synthesized compounds given in Table-1. Similarly, a characteristic group frequencies observed in the IR spectra and the position of chemical shifts for different protons, their intensities and their fine structure in the ^{1}H NMR spectra of synthesized compounds were substantially supported the suggested structure of synthesized compounds.

In the IR spectra of synthesized compounds, the peaks for the stretching vibration of NH of amide group were observed in the ranged 3393-3306 cm^{-1} and the peaks for the stretching vibration for CO present in amide group were observed in the ranged 1700-1650 cm^{-1}, which substantially supported the presence of peptide bond in the synthesized compounds. Stretching frequencies for ketonic C=O groups present in the molecules were observed at around 1625 cm^{-1}. Characteristic peaks for other functional group present in the molecules were also observed as these were reported in the elsewhere.

In ^{1}H NMR spectra of the synthesized compounds, a typical chemical shifts for -CH protons of pyrazine ring were observed

TABLE-1
STRUCTURE OF MOLECULES WITH DOCKING SCORE

Code	Structure	Docking score	Code	Structure	Docking score
B-2		-4.478265	**C-9**		-4.1746
B-4		-4.748429	**C-10**		-5.289434
B-8		-4.956415	**C-11**		-3.529662
B-14		-4.286729	**C-12**		-3.442027
B-18		-4.981793	**D-1**		-3.0721
C-1		-3.864045	**D-2**		-3.88466

C-2		-4.070298
C-3		-4.775948
C-4		-4.765459
C-5		-4.348648
C-6		-3.001531
C-7		-5.0455
C-8		-4.252889
D-3		-4.241075
D-7		-3.603116
D-10		-4.226773
V-2		-5.068024
V-4		-4.620229
V-14		-4.424154
V-18		-4.065631

in the range δ 9.5 to 8.5 ppm, peaks for phenyl protons were observed in the range of δ 6.0 to 8.5 ppm. Peaks of CH proton of amino acid beside amide bond were observed in the range δ 5.0 to 3.5 ppm. Peaks in the range δ 1.8 to 1.1 ppm confirmed the presence of cyclohexyl group. All these supported the structure of synthesized compounds given in Table-1.

The molecular docking studies of all the molecules were conducted to determine the affinity between molecules and active site of NS3/4A protein of hepatitis C virus. The docking studies were carried out by using Glide program of Schrödinger. The molecules were docked into the 3D structure of NS3/4A domain of HCV protein. The docking score of all the molecules are also given in Table-1. The docking of telaprevir drug was also conducted along with new molecules and the docking score of telaprevir as predicted by software was found to be -5.110482. More the negative the docking score, more is the interaction between molecule and binding site. There are many molecules which have comparable energies to that of telaprevir such as B-8, B-18, C-3, C-4, C-7 and V-2 and other molecules such as C-10 and V-8 have slightly more negative energies than telaprevir. This implies that these compounds may be potent as anti-HCV molecules.

Conclusion

All the molecules were characterized using physico-chemical techniques and supported the suggested structures of the synthesized compounds. Docking studies indicated that these molecules might have significant anti HCV properties on comparison with NS3/4A inhibitor HCV drug telaprevir. Few molecules were found to have either equal or more docking score, which means that they may be used as potent HCV inhibitor in future. There is need for further investigation of these molecules.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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