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Supporting Information

Efficient synthesis of novel conjugated 1,3,4-oxadiazole-peptides

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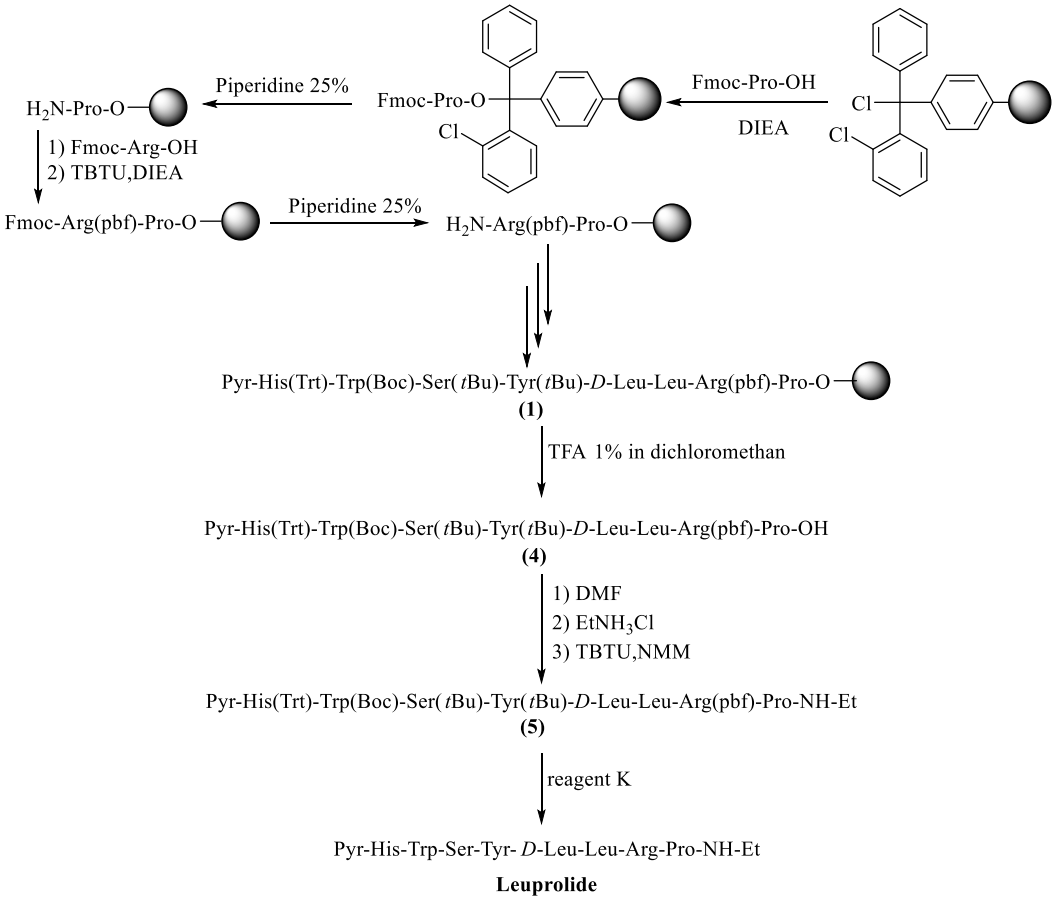
Peptide Synthesis:

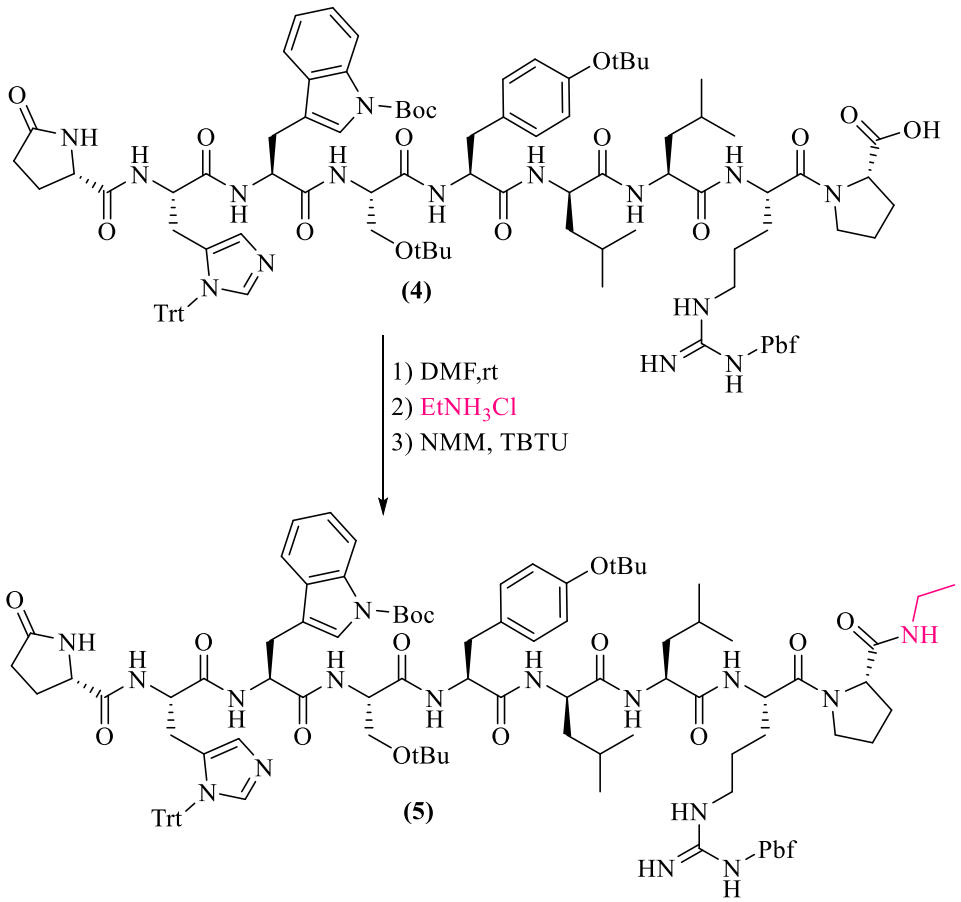


General Method 1: synthesis of nonapeptide Pyr-His(Trt)-Trp(Boc)- Ser(*t*Bu)-Tyr(*t*Bu)-*D*-Leu-Leu-Arg(Pbf)-Pro-OH (4):

The nonapeptides were synthesized using known Fmoc solid phase peptide synthesis strategy.[41]The synthesis of the peptides was carried out using 2-chlorotrityl chloride resin (1.0 mmol/g) following standard Fmoc strategy. At first resin was swelled with DCM (3×10 ml) for 30 min. Then Fmoc-Pro-OH (0.34 g, 0.10 mmol) was attached to the 2-CTC resin (1.0 g) with DIPEA (0.25 ml, 8 mmol) in anhydrous DCM: DMF (10 ml, 1:1) at room temperature for 2 h. After filtration, the remaining trityl chloride groups were capped by a solution of DCM/MeOH/DIPEA (17:2:1, 24 ml) for 30 min. The resin was filtered and washed thoroughly with DMF (3× 10 ml). The loading capacity was determined by weight after drying the resin under vacuum and was 1.0. The resin-bound Fmoc-amino acid was washed with DMF (3× 10 ml) and treated with 25% piperidine in DMF (14.5 ml) for 30 min and then

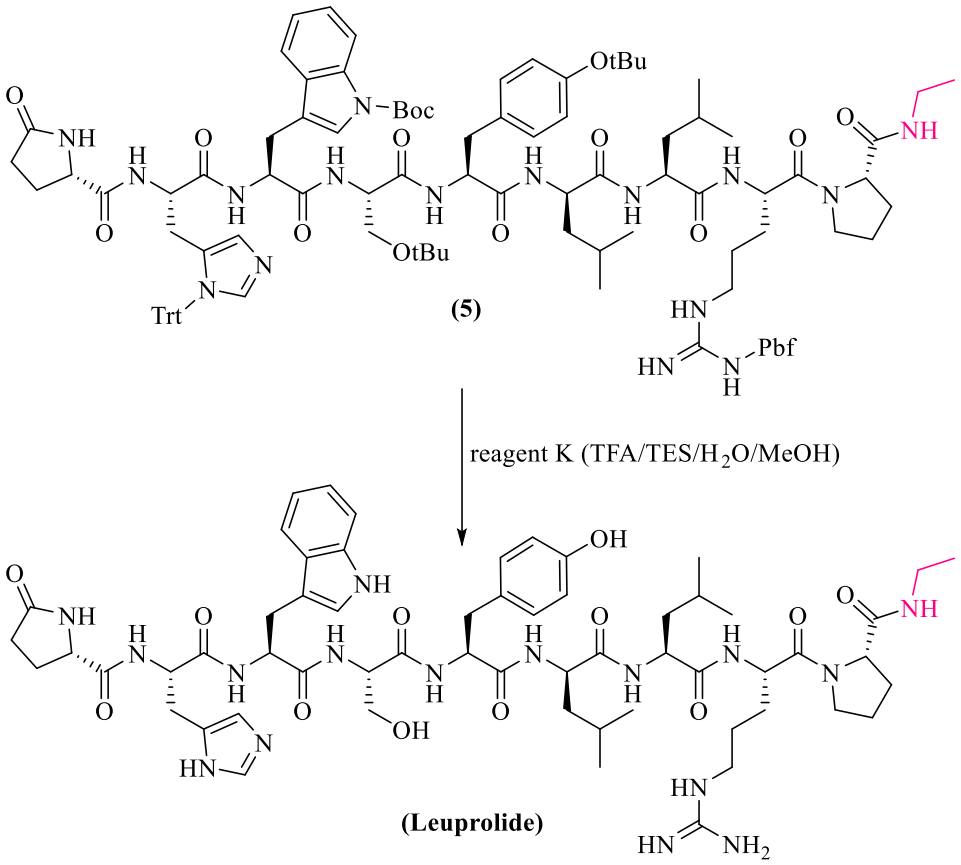
was washed with DMF (3× 10 ml). Then a solution of Fmoc-Arg(pbf)-OH (2.0 mmol), TBTU (0.64 g, 2.0 mmol), DIPEA (0.6 ml, 3.5 mmol) in 10 ml DMF was prepared and added to the resin-bound free amine and shaken for 1.5 h at room temperature. After completion of coupling, resin was washed with DMF (3× 10 ml). The coupling was repeated as the same methods for other amino acids of the sequence. In all cases, the coupling was confirmed using Kaiser to detect the presence or absence of free the primary amino groups. Fmoc determination was done using UV spectroscopy method. After completion of all couplings, resin was washed with DMF (3×10 ml). The produced nonapeptide (**4)** was cleaved from resin by treatment with TFA (1%) in DCM (100 ml) and neutralization with pyridine (4%) in MeOH (50 ml). The solvent was evaporated under reduced pressure and precipitated in water. The yield was 62% (1.17 g of nonapeptide **4**).

General Method 2: Ethylamidation of *C*-terminal of nonapeptide (4) to preparation of (5):



To a solution of nonapeptide **(4)** (1.2 g, 0.64 mmol) in DMF (1.8 ml), NMM (0.39 ml, 3.2 mmol) was added and stirred for 15 min. Then TBTU (0.306 g, 0.96 mmol) was added and allowed to stir for 30 min to preactivate the carboxylic acid group at *C*-terminal, after that ethylammonium chloride (0.106 g, 1.3 mmol) was added to the reaction mixture. The mixture was stirred for 24 h at room temperature and the progress of reaction was monitored using TLC (EtOAc: MeOH: H_2O 10:2:1). The desired *C*-terminal amidated unprotected nonapeptide **(5)** was precipitated in water and dried with 89 % yield. (1.09 g (**5**)**)**

General Method 3: Final deprotection of *C*-terminal amidated nonapeptide (5) to synthesis of leuprolide:

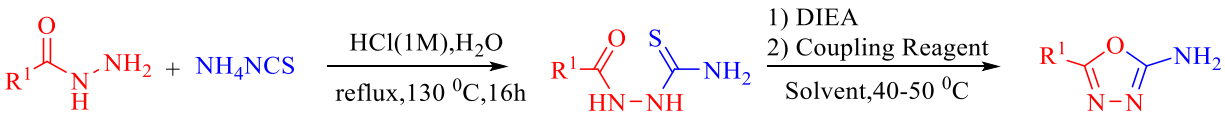


C-terminal amidated protected nonapeptide **(5)** (1.1 g, 0.58 mmol) was treated with TFA/TES/H_2O/MeOH (95:2.5:1.25:1.25) for 2 h at room temperature, and in this way all of protecting groups were removed. Then the mixture was evaporated, and precipitated with Et_2O. The final peptide was dried under vacuum at 40 ºC (Isolated yield 85%).

High Performance Liquid Chromatography:

The samples were dissolved in solvent A for liquid chromatography. The mobile phase for all HPLC analysis and purifications consisted of solvent A (100% water 0.1% Trifluoroacetic.acid, v/v) and solvent B (70% acetonitrile 30% water 0.1% Trifluoroacetic acid, v/v/v) and separations were performed on a HPLC system (Knauer, Germany) equipped with a pump 1000 (Knauer, Germany), UV detector 2500 (Knauer, Germany) and a 20 µL injection valve. Analytical RP-HPLC separation was carried out using a MZ C18 column (250 mm × 4.6 mm, 5 µm, 100 Å) at a flow rate of 1 mL min^{-1} , and The employed elution program started at 95 % A and remained at this point for 5 min before changing to 45 % of solvent A over 55 min. at 1% min^{-1}. peptide purification was performed at preparative scale using Macherey-nagel C18 column (250 mm × 21mm, 10 µm, 100 Å) The flow rates was set to 20 mL min^{-1} and the peptides were separated with linear gradients of solvent A between 95 and 45% at 0.5% min^{-1} (preparative) including pre- and postgradient equilibration steps. Elution profile was monitored via UV absorbance at 220 nm and peptides were collected manually according to their absorbance at 220 nm.

Synthesis of 2-amino-oxadiazoles (9a-e):

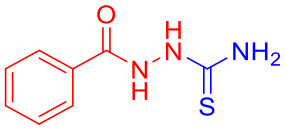


(6a-e) **7a** **(8a-e)** **(9a-e)**

General procedure for the synthesis of thiosemicarbazide intermediate (8a-e):

To a solution of ammonium thiocyanat **7a** (0.19 g, 2.5 mmol) in water (2ml), hydrochloric acid 1M (2ml) was added. The reaction mixture was magnetically stirred at 110-130 °C for 45 min to in situ produce Isothiocyanic acid intermediate. Then arylhydrazide (1 mmol) was added to the reaction mixture with continued stirring and heated for 5h. The progress of the reaction was monitored by TLC (*n*-hexane:EtoAc:MeOH 3:1:1). After the completion of the reaction, the ice was added to reaction mixture and the resulting precipitate of thiosemicarbazide was colected by filtration, washed with water and dried.

2-Benzoylhydrazine-1-carbothioamide (8a):

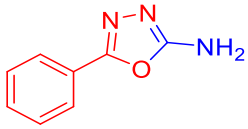


mp: 179-181°C; IR (KBr, cm^{-1}) *v* = 1545, 1701, 3388,3526 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ(ppm) 3.35 (brs, NH_2, exchange with water), 7.42-7.68 (m, 5H, 3H-Ar, NH_2), 7.89 (d, 2H, *J* = 7.7, H-Ar), 9.34 (s, 1H, NH), 10.38 (s, 1H, NH); ^{13}C-NMR (75 MHz, DMSO-d_6): δ(ppm) 127.9, 128.2, 131.8, 132.5, 165.9, 182.1.

General procedure for the synthesis of 2-amino-oxadiazoles (9a-e):

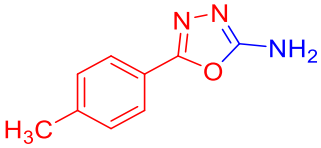
A one-necked flask was charged with DIC (Coupling reagent) (0.24 ml, 1.5 mmol), thiosemicarbazide (1mmol), diisopropylethylamine (0.27 ml, 1.5 mmol) and CH_3CN (5 ml). The reaction mixture was stirred under reflux for 24h. The progress of reaction was monitored by TLC (*n*-hexane: EtOAc 3:1). After completion of reaction, the product was isolated by filtration, washed with MeOH and dried. For further purification, the product was recrystallized in EtOH to afford of desired products (**9a-e**) in 52%-83% yields.

5-phenyl-1,3,4-oxadiazol-2-amine (9a):



White powder (0.113 g, 0.7 mmol, yield 70%). mp: 242-243 °C; IR (KBr, cm^{-1}) *v* = 1680, 3196 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ(ppm) 7.25 (s, 2H, NH_2), 7.42-7.58 (m, 3H, H-Ar), 7.75-7.80 (m, 2H, H-Ar); ^{13}C-NMR (75 MHz, DMSO-d_6): δ(ppm) 124.4, 125.1, 129.2, 130.3, 157.3, 163.9.

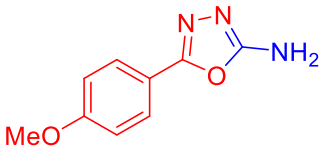
5-(*p*-tolyl)-1,3,4-oxadiazol-2-amine (9b):



White powder (0.137 g, 0.78 mmol, yield 78%). mp: 260-263 °C; IR (KBr, cm^{-1}) *v* = 1671, 3280 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ(ppm) 2.34 (s, 3H, -CH_3), 7.19, (s, 2H, NH_2), 7.31 (d, 2H, *J* = 8.1 Hz, H-Ar), 7.67 (d, 2H, *J* = 8.1 Hz, H-Ar); ^{13}C-NMR (75 MHz, DMSO-

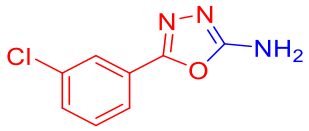
d_6): δ 21.1, 121.7, 125.1, 129.8, 140.2, 157.4, 163.7.

5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-amine (9c):



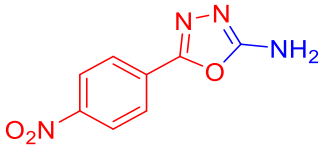
White powder (0.159 g, 0.83 mmol, yield 83%). mp: 251-253 °C; IR (KBr, cm^{-1}) *v* = 1666, 3137, 3325 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ(ppm) 3.79 (s, 3H, -OCH_3), 7.06 (d, 2H, *J* = 8.8 Hz, H-Ar), 7.13 (s, 2H, NH_2), 7.71 (d, 2H, *J* = 8.8 Hz, H-Ar); ^{13}C-NMR (75 MHz, DMSO-d_6): δ(ppm) 55.4, 114.7, 116.9, 126.8, 157.3, 160.8, 163.5.

5-(3-chlorophenyl)-1,3,4-oxadiazol-2-amine (9d):



White powder (0.102 g, 0.52 mmol, yield 52%). mp: 242-245 °C; IR (KBr, cm^{-1}) *v* = 1673, 3096, 3299 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ(ppm) 7.35 (brs, 2H, NH_2), 7.50-7.60 (m, 2H, H-Ar), 7.70-7.78 (m, 2H, H-Ar); ^{13}C-NMR (75 MHz, DMSO-d_6): δ(ppm) 123.6, 124.5, 126.3, 130.1, 131.3, 133.9, 156.1, 164.0, 164.1.

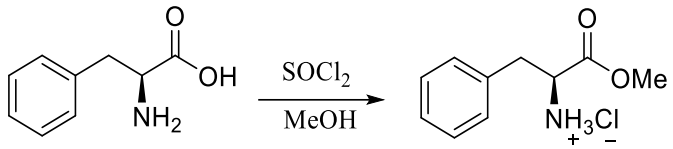
5-(4-nitrophenyl)-1,3,4-oxadiazol-2-amine (9e):



Yellowish powder (0.118 g, 0.57 mmol, yield 57%). mp: 250-252 °C; IR (KBr, cm^{-1}) *v* = 1345, 1523, 1673, 3098, 3283 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ(ppm) 7.54, (s, 2H, NH_2), 8.0 (d, 2H, *J* = 8.7 Hz, H-Ar), 8.35 (d, 2H, *J* = 8.7 Hz, H-Ar); ^{13}C-NMR (75 MHz, DMSO-d6): δ(ppm) = 124.6, 126.0, 129.8, 147.9, 156.1, 164.54, 164.59.

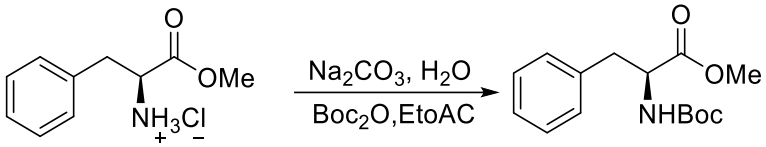
General Method for the Synthesis of Phenylalanine oxadiazole (9f):

a- Synthesis of phenylalanine methyl ester:



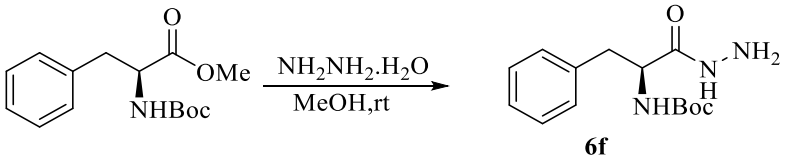
To a suspension of L-phenylalanine (1.6 g, 10 mmol) in methanol (10 ml), thionyl chloride (2.6 ml, 35 mmol) was added slowly via dropping funnel during 15 min at -10 °C. Then the reaction mixture was stirred at room temperature for 24 h. and the solvent was evaporated under vacuum. The resultant precipitate was filtered, and further purification was done using recrystallization in diethyl ether (Yield 94%).

b- Synthesis of Boc-Phenylalanine methyl ester:



0.215 g phenylalanine methyl ester hydrochloride (0.215 g, 1 mmol) was added to a solution of anhydrous sodium carbonate (0.13 g, 1.2 mmol) in water (5 ml) in a 100-ml round-bottom flask, and allowed to dissolve and stirred for 15 min. Then Boc anhydride (0.218 g, 1mmol) was dissolved in ethylacetate (2 ml) and added to the reaction mixture and was allowed to stirred for 24 h at room temperature. The progress of the reaction was monitored with TLC (*n*-hexane: EtOAc 2:1) and ninhyrdin test. After reaction completion, the pH was adjusted at 5.5 with citric acid and extracted with ethylacetate (EtOAc, 20 ml). The aqueous layer was back-extracted with EtOAc (3 × 10 ml). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated under reduced pressure to afford the Boc-phenylalanine methyl ester as colorless oil (94% yield).

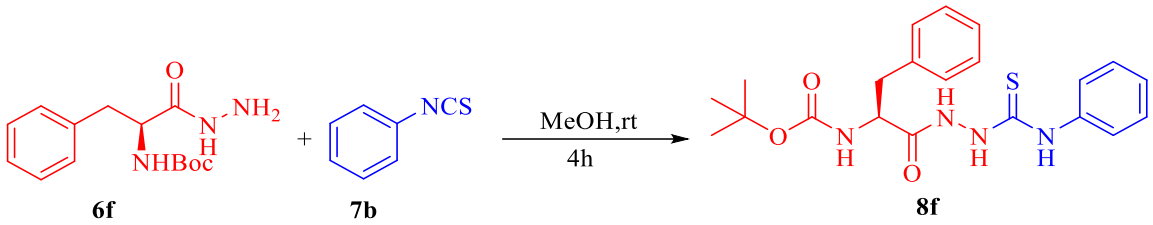
c- Synthesis of Boc- phenylalanine hydrazide **(6f):**



6f

Hydrazine monohydrate (0.16 ml, 3 mmol) was added to stirred solution of Boc-phenylalanine methyl ester (0.28 g, 1mmol) in 5ml of methanol. The reaction mixture was stirred at room temperature for 48 h. The progress of the reaction was monitored by TLC (*n*-hexane: EtOAc 3:1). After reaction completion the solvent was evaporated under vacuum, and the precipitate was washed with methanol (Yield 93%).

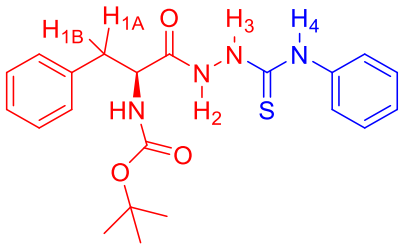
d- Synthesis of Boc- phenylalanine thiosemicarbazide **(8f):**



6f **7b** **8f**

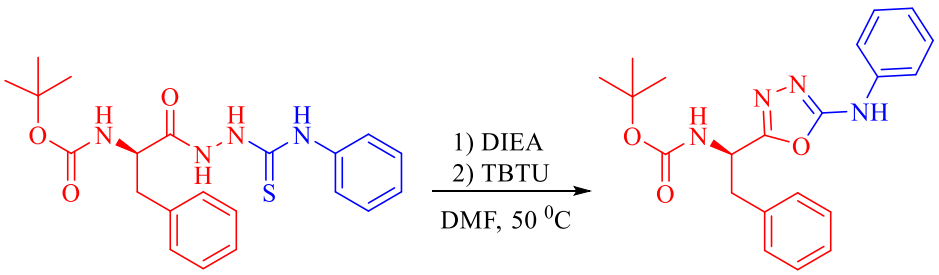
Phenylisothiocyanate (0.135 g, 1 mmol) was added to a stirred solution of Boc-phenylalanine hydrazide (0.28 g, 1 mmol) in MeOH (5ml) at room temperature for 4 h. The progress of the reaction was monitored with TLC (*n*-Hexane:EtOAc 1:1). After completion reaction, the precipitated thiosemicarbazide was filtered, washed with MeOH and dried (Yield 82%).

***t*ert-Butyl (S) – (1-Oxo-3-Phenyl-1- (2- (Phenylcarbamothioyl) hydrazinyl) Propan-2-yl) Carbamate (8f):**



mp: 168-171 °C; IR (KBr, cm^{-1}) ν = 1162, 1697, 3257, 3345 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ: 1.24 (s, 9H, *t*-Bu), 2.77-2.85 (dd, 1H, J = 13.4, 9.6 Hz, H_{1A}), 2.94-3.21 (m, 1H, H_{1B}), 4.00-4.10 (m, 1H ,-CHN chiral), 7.14-7.19 (m, 2H, H-Ar), 7.20-7.34 (m, 7H, H-Ar, NH), 7.5-7.53 (m, 2H, H-Ar), 9.16 (brs, 1H, H_2, NH), 9.77 (brs, 1H, H_3, NH), 10.34 (brs, 1H, H_4, NH); ^{13}C-NMR (75 MHz, DMSO-d_6): δ: 28.1, 36.5, 54.9, 78.6, 125.0, 126.3, 128.1, 129.3, 137.7, 138.9, 155.8, 171.3, 180.5.

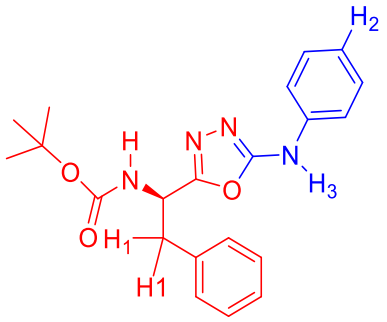
a- Synthesis of tert-butyl (R)-(2-phenyl-1-(5-(phenylamino)-1,3,4-oxadiazol-2 yl) ethyl) carbamate **(B):**



8f **B**

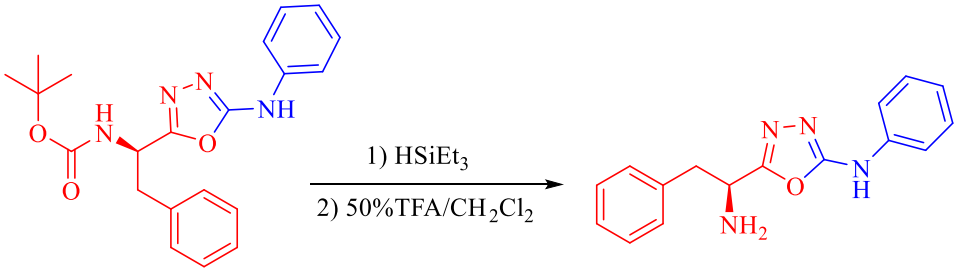
Coupling reagent TBTU (0.48 g, 1.5 mmol) was added to stirred solution of thiosemicarbazide **8f** (0.4 g, 1 mmol) and DIEA (0.27 ml, 1mmol) in DMF (3ml) in a 100-ml round-bottom flask. The reaction mixture was stirred at 50 °C for 24 h. The progress of the reaction was monitored with TLC (*n*-Hexane:EtOAc 3:1). After completion, the reaction mixture was cooled to room temperature and the solvent was evaporated under vacuum. Then water was added to the residue and the solid formed was filtered, washed with methanol and dried. For more purification, the product was recrystallized with ethanol to give 0.322 g (88%) of **B**.

tert-Butyl (R) – (2-Phenyl-1- (5-(Phenylamino) -1,3,4-Ozadiazole-2-yl)ethyl) carbamate (B):



mp: 168-172 °C; IR (KBr, cm^{-1}) ν = 1632, 1696, 3342, 3280 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ: 1.29 (s, 9H, *t*-Bu), 3.03-3.11 (dd, 1H, J = 13.4, 9.1 Hz, H_1), 3.19-3.25 (dd, 1H, J = 13.8, 5.9 Hz, H_1), 4.88-4.96 (m, 1H, -CHN chiral), 6.95-7 (t, 1H, J = 7.3 Hz, H-Ar, H_2), 7.19-7.35 (m, 7H, H-Ar, NH), 7.51-7.54 (m, 2H, H-Ar), 7.62-7.68 (m, 1H, H-Ar), 10.47 (s, 1H, NH, H_3); ^{13}C-NMR (75 MHz, DMSO-d_6): δ: 28.1, 37.4, 47.9, 78.4, 109.7, 116.8, 119.1, 121.7, 126.5, 127.1, 128.1, 129.1, 129.2, 137.2, 138.6, 138.7, 154.9, 159.8.

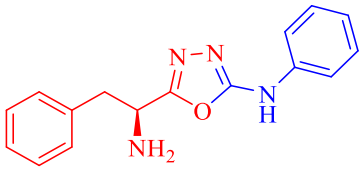
b- Synthesis of phenylalanine-1,3,4-oxadiazole **(9f):**



B **9f**

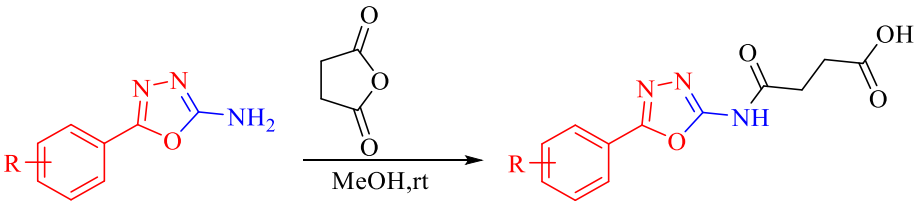
Boc-phenylalanine-1,3,4-oxadiazole **(B)** (0.38 g, 1 mmol) and triethylsilane (0.16 ml, 1 mmol) was added to 100 ml solution of 50% TFA in DCM. The reaction mixture was stirred at room temperature for 10 h. When the reaction was completed as monitored by TLC (*n*-Hexane:EtOAc 1:1) and also ninhydrin test, the solvent was evaporated under vacuum to reach 2-3 ml. The mixture obtained after evaporation was neutralized with saturated $NaHCO_3$ to pH 7 and the resulting precipitate filtered and washed with water. (81% yield)

(S) -5-(1-Amino-2-Phenyl ethyl) –N-Phenyl-1,3,4-Oxadiazole-2-amine (9f):



Pale gray powder (0.227 g, 0.81 mmol, yield 81%). mp: 188-191 °C; IR (KBr, cm^{-1}) ν = 1590, 3106, 3158, 3296; ^{1}H-NMR (300 MHz, DMSO-d_6): δ(ppm) = 3.05 (dd, 2H, J = 11.2, 6.7 Hz, H_1), 3.38 (s, 2H, NH_2), 4.26-4.31 (t, 1H, J = 7.2 Hz, -CHN chairal), 6.94-6.99 (t, 1H, J = 7.2 Hz, H-Ar), 7.07-7.41 (m, 7H, H-Ar), 7.52 (d, 2H, J = 8.2 Hz, H-Ar), 10.46 (s, 1H, NH); ^{13}C-NMR (75 MHz, DMSO-d_6): δ(ppm) = 41.4, 49.3, 116.8, 121.6, 126.4, 128.2, 129.0, 129.2, 137.8, 138.9, 159.6, 162.3.

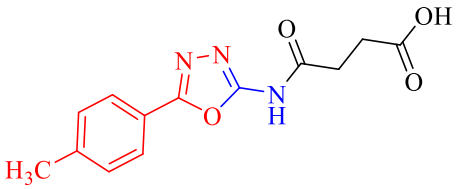
General procedure for the synthesis of 1,3,4 –oxadizaole (11a-e) using succinic anhydride:



(9b-f) **(11a-e)**

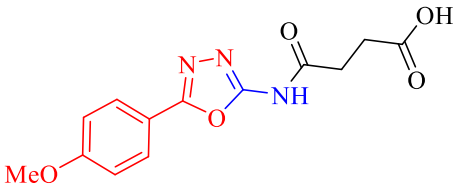
1 mmol of 2-amino oxadiazole **(9b-f)** and succinic anhydride (0.1 g, 1 mmol) were combined in MeOH (5 ml), in a 20-ml round-bottom flask at room temperature and stirred for 10 h. The progress of the reaction was monitored using TLC (*n*-Hexane: EtOAc 1:1). After completion the reaction, solvent was evaporated under vacuum and the resultant precipitate was washed with diisopropylether and filtered. The yields of products (**11a-e**) were 68%-96%.

4-Oxo-4-((5-(p-tolyl)-1,3,4-oxadiazol-2-yl) amino) butanoic acid (11a):



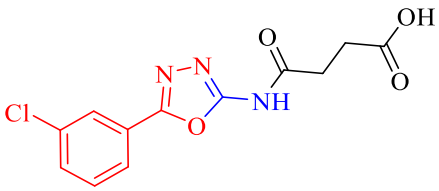
White powder (0.259 g, 0.94 mmol, yield 94%). mp: 209-213 °C; IR (KBr, cm^{-1}) *v* = 1655, 1734, 3448 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ(ppm) = 2.34 (s, 3H, -CH_3), 2.46-2.49 (m, 4H, -CH_2), 7.12 (s, 1H, NH), 7.31 (d, 2H, *J* = 8.1 Hz, H-Ar), 7.67 (d, 2H, *J* = 8.1 Hz, H-Ar), 12.0 (brs, 1H, -COOH); ^{13}C-NMR (75 MHz, DMSO-d_6): δ(ppm) = 21.1, 28.5, 28.7, 121.7, 125.1, 129.8, 140.3, 157.5, 163.7, 172.7, 173.5.

4-((5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl) amino)-4-oxobutanoic acid (11b):



White powder (0.28 g, 0.96 mmol, yield 96%). mp: 196-200 °C; IR (KBr, cm^{-1}) *v* = 1606, 1657, 1739, 3212 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ(ppm) = 2.40-2.49 (m, 4H, -CH_2), 3.80 (s, 3H, -OCH_3), 7.06 (d, 2H, *J* = 8.7 Hz, H-Ar), 7.13 (s, 1H, NH), 7.71 (d, 2H, *J* = 8.7 Hz, H-Ar), 12.23 (s, 1H, -COOH); ^{13}C-NMR (75 MHz, DMSO-d_6): δ(ppm) = 28.5, 28.7, 55.4, 114.7, 116.9, 126.8, 157.3, 160.9, 163.5, 172.7, 173.5.

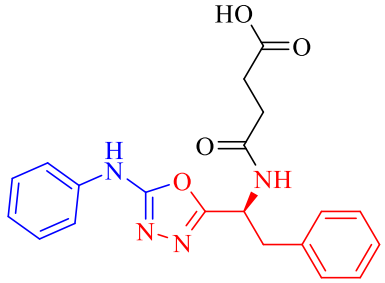
4-((5-(3-chlorophenyl)-1,3,4-oxadiazol-2-yl) amino)-4-oxobutanoic acid (11c):



White powder (0.201 g, 0.68 mmol, yield 68%). mp: 208-211 °C; IR (KBr, cm^{-1}) *v* = 1596, 1657,

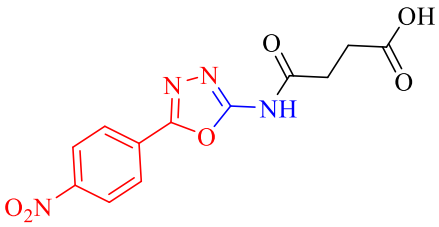
1740, 3106, 3299 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ(ppm) = 2.41-2.51 (m, 4H, -CH_2), 7.35 (s, 1H, NH), 7.50-7.58 (m, 2H, H-Ar), 7.70-7.74 (m, 2H, H-Ar), 11.8 (brs, 1H, -COOH); ^{13}C-NMR (75 MHz, DMSO-d_6): δ(ppm) = 28.6, 28.7, 123.7, 124.6, 126.3, 130.1, 131.3, 134.0, 156.3, 164.2, 172.7, 173.5.

4-Oxo-4-((2-phenyl-1-(5-(phenylamino)-1,3,4-oxadiazol-2-yl) ethyl) amino) butanoic acid (11d):



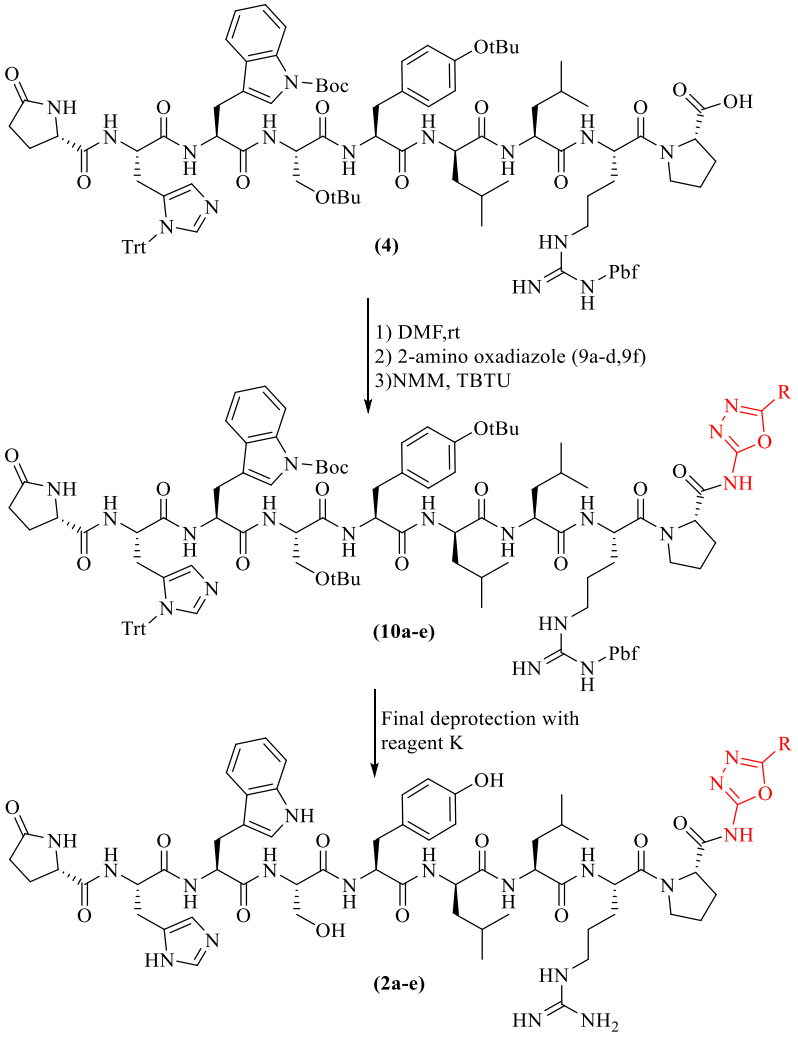
White powder (0.337 g, 0.886 mmol, yield 88.6%). mp: 211-213 °C; IR (KBr, cm^{-1}) *v* = 1654, 1730, 3070, 3199, 3306 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-*d_6*): δ(ppm) = 2.26 (t, 2H, *J* = 5.9 Hz, -CH_2), 2.34 (t, 2H, *J* = 5.9 Hz, -CH_2), 3.08 (dd, 1H, *J* = 13.6, 6.8 Hz, -CH_2Ph), 3.24 (dd, 1H, *J* = 13.6,6.8 Hz, -CH_2Ph), 5.2 (q, 1H, *J* = 7.9 Hz, -CHN), 6.97 (t, 1H, *J* = 7.2 Hz, H-Ar), 7.1-7.4 (m, 7H, H-Ar), 7.52 (d, 2H, *J* = 8.1 Hz, H-Ar) 8.61-8.66 (d, 1H, *J* = 8.1Hz, -NH), 10.48 (s, 1H, -NH), 11.9 (brs, 1H, -COOH); ^{13}C-NMR (75 MHz, DMSO-d_6): δ(ppm) = 28.9, 29.8, 37.5, 46.2, 116.9, 121.8, 126.6, 128.3, 129.1, 129.2, 137.1, 138.7, 159.4, 159.8, 170.8, 173.7.

4-((5-(4-Nitrophenyl)-1,3,4-oxadiazol-2-yl) amino)-4-oxobutanoic acid (11e):

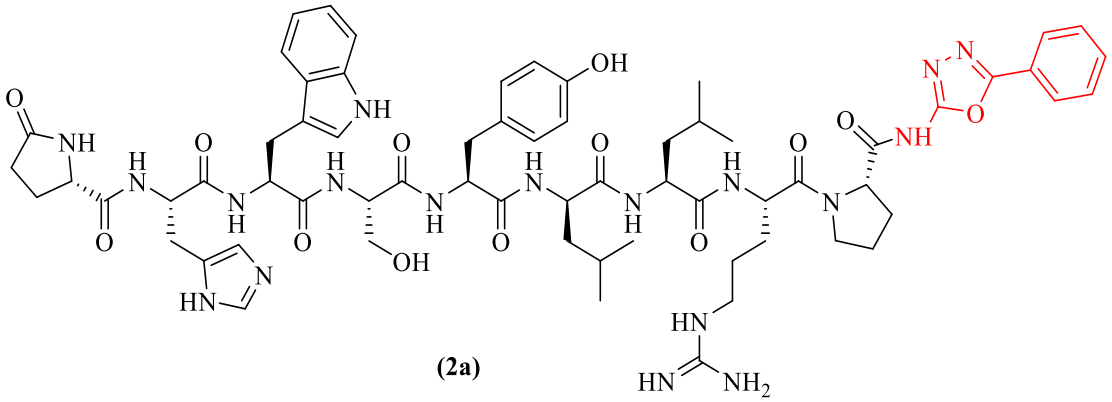


Yellowish powder (0.227 g, 0.74 mmol, yield 74%). mp: 238-241 °C; IR (KBr, cm^{-1}) *v* = 1350,1524, 1591, 1668, 1740, 3137, 3346 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ(ppm) = 2.38-2.53 (m, 4H, 2-CH_2), 7.53 (s, 1H, NH), 7.99 (d, 2H, *J* = 8.8 Hz, H-Ar), 8.33 (d, 2H, *J* = 8.8 Hz, H-Ar), 12.20 (brs, 1H, -COOH); ^{13}C-NMR (75 MHz, DMSO-d_6): δ(ppm) = 28.5, 28.7, 124.6, 126.0, 129.9, 147.9, 155.1, 164.6, 172.6, 173.4.

General Method for the synthesis of leuprolide analogues (2a-e) by coupling oxadiazole (9a-d,9f) to the peptide sequence (4) at *C*-terminal:

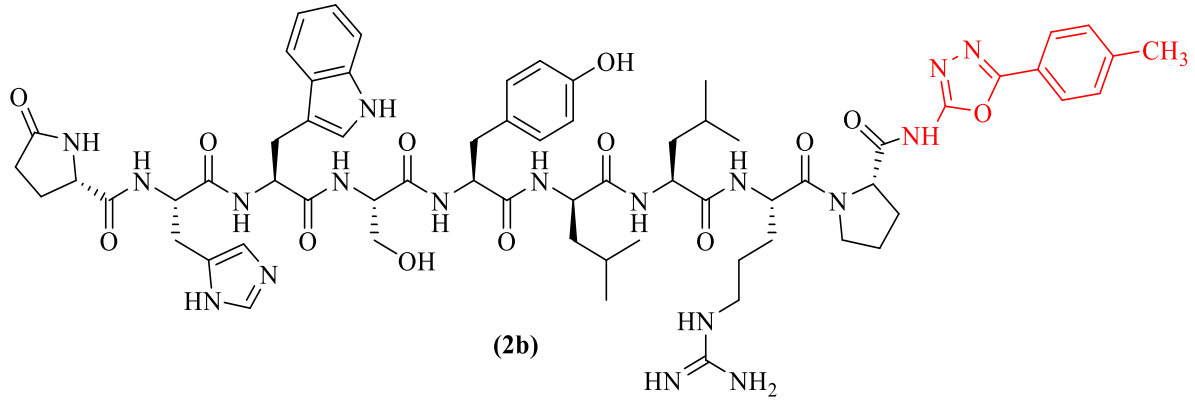


To a solution of nonapeptide **(4)** (1.2 g, 0.64 mmol) in DMF (1.8 ml), NMM (0.39 ml, 3.2 mmol) was added and stirred for 15 min. Then TBTU (0.306 g, 0.96 mmol) was added and The reaction mixture was magnetically stirred for 30 min to activate the carboxylic acid group at *C*-terminal, after that 1.3 mmol of oxadiazole **(9a-d,9f)** was added to the reaction mixture. The mixture was stirred for 24 h at room temperature and the progress of reaction was monitored using TLC (EtOAc: MeOH: H_2O 10:2:1). The desired C-terminal amidated unprotected nonapeptides **(10a-e)** was precipitated in water and dried. Final deprotection of the peptides **(10a-e)** was done by the same procedure mention previously to give leuprolide analogues **(2a-e)**, followed further purification was done using Prep-HPLC by the same procedure of purification.



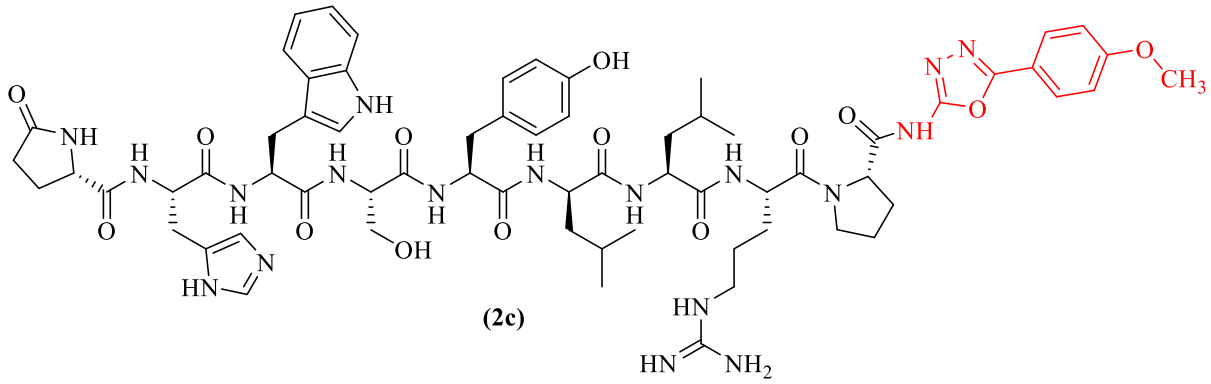
(2a)

HPLC analysis found that peptide **(2a)** was obtained in 94%< purity (R_t: 45.497 min). mp: 107-110 °C; HR-Mass (ESI): $C_{69}H_{92}N_{18}O_{13}$*m/z* = $[M+ (CH_3CN+H_2O) +H]^+$ Found 1434.6868, Calc. 1434.6867; $[M+ (CH_3CN+H_2O) +2H]^+/2$ Found 717.8472, Calc. 717.8472.



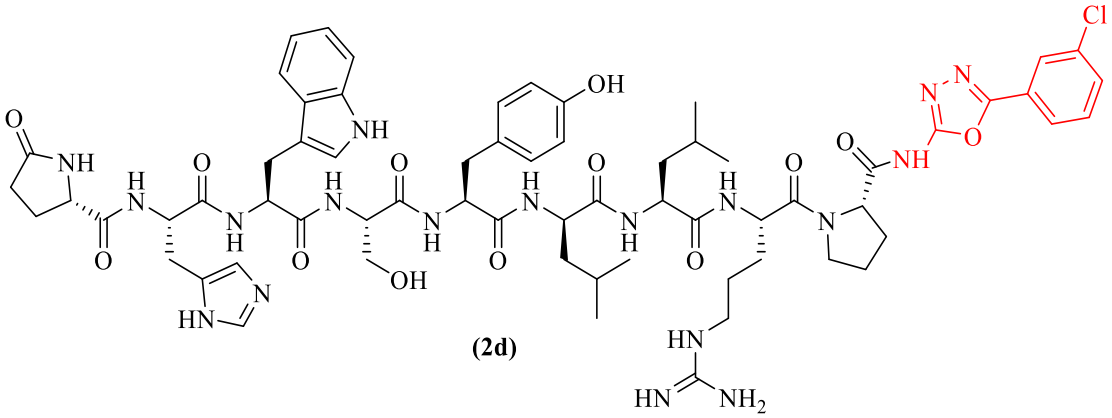
(2b)

HPLC analysis found that peptide **(2b)** was obtained in 96%< purity (R_t: 32.883 min). mp: 182-184 °C; HR-Mass (ESI): $C_{66}H_{86}N_{18}O_{13}$*m/z* =$[M-C_8H_7N_2O+H]^+$ For Found 1196.6205, Calc. 1196.6203; $[M-C_8H_7N_2O +2H]^+/2$ Found 598.8142, Calc. 598.8143.



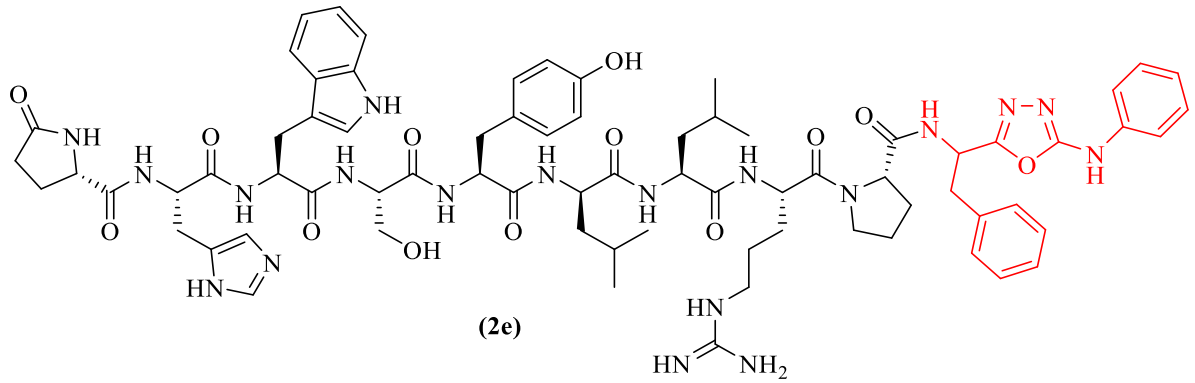
(2c)

HPLC analysis found that peptide **(2c)** was obtained in 95%< purity (R_t: 46.048 min). mp: 112-114 °C; HR-Mass (ESI): $C_{66}H_{86}N_{18}O_{14}$ *m/z* = $[M+HOAc+H_2O+H]^+$ Found. 1434.6869, Calc. 1434.6871; $[M+HOAc+H_2O+2H]^+/2$ Found 717.8474, Calc. 717.8472.



(2d)

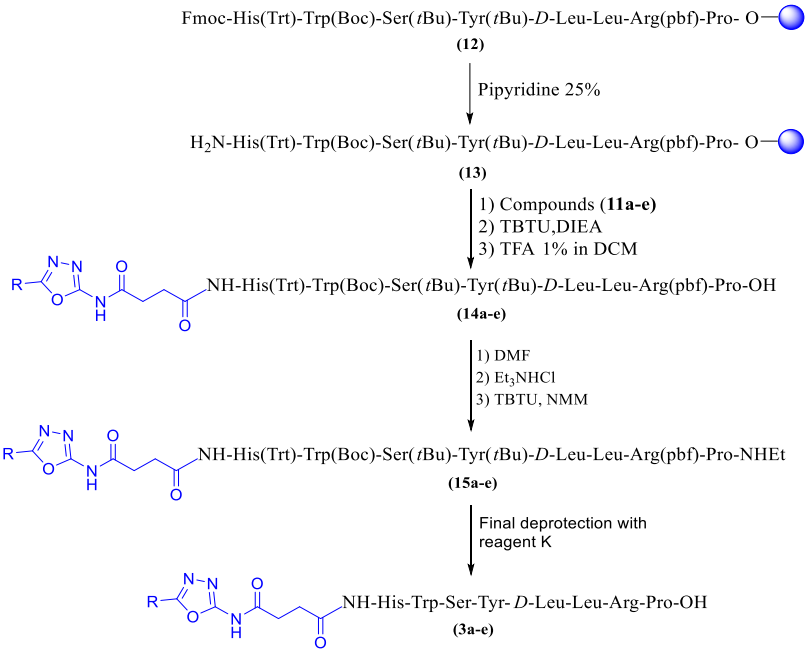
HPLC analysis found that peptide **(2d)** was obtained in 97%< purity (R_t: 32.258 min). mp: 214-218 °C; HR-Mass (ESI): $C_{65}H_{83}ClN_{18}O_{13}$ *m/z* =$[M-C_8H_4ClN_2O+H]^+$ Found 1182.6048, Calc. 1182.6050; $[M-C_8H_4ClN_2O+2H]^+/2$ Found 591.8063, Calc. 591.8065.



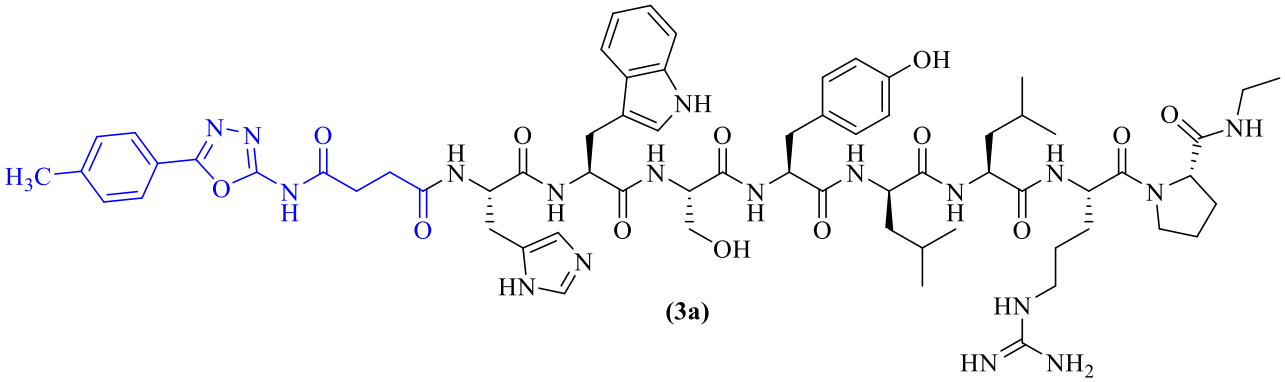
(2e)

HPLC analysis found that peptide **(2e)** was obtained in 96%< purity (R_t: 39.063 min). mp: 94-97 °C; HR-Mass (ESI): $C_{73}H_{93}N_{19}O_{13}$ *m/z* =$[M+H]^+$Found 1444.7265, Calc. 1444.72644; $[M+2H]/2^+$ Found 722.8669, Calc. 722.8668.

General procedure for the synthesis of leuprolide analogues (3a-e) by attaching functionalized oxadiazole 11a-e to the *N*-terminal of the peptidyl-resin sequence (12):

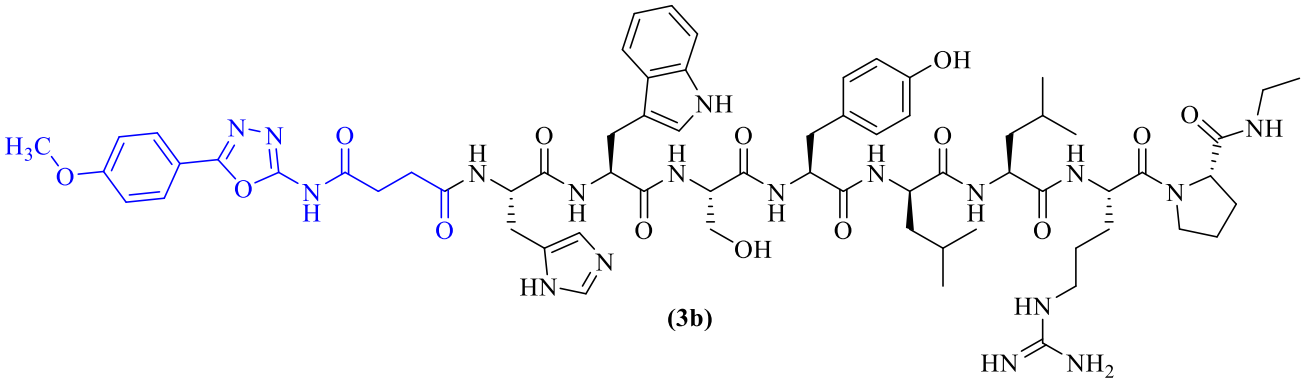


After synthesis of the peptidyl-resin sequence **(12)** as mentioned previously, p-Glu amino acid was replaced with the functionalized oxadiazole **(11a-e)**. In this way at first deprotection of the Fmoc-His was made and then a solution of oxadiazole **(11a-e)** (2 mmol), TBTU (0.64 g, 2.0 mmol), DIPEA (0.6 ml, 3.5 mmol) in 10 ml DMF was added to the resin-bound free amine **(13)** and shaken for 2 h at room temperature. Completion of the coupling was monitored by the Kaiser test. The resin was washed with DMF (3×10ml). Cleavage, ethyl amidation and final deprotection of the peptide were done by the same procedure mention previously to give leuprolide analogues **(3a-e)**, followed further purification was done using Prep-HPLC by the same procedure of purification.



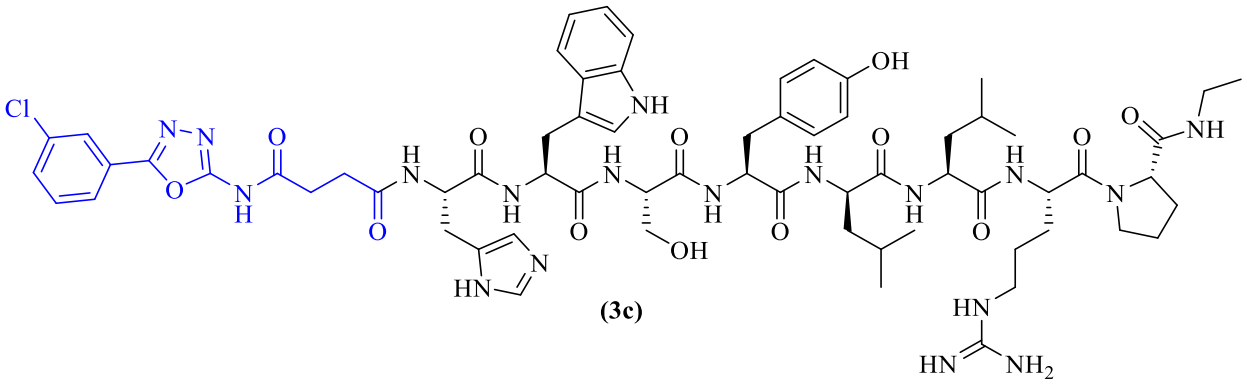
(3a)

HPLC analysis found that peptide **(3a)** was obtained in 95%< purity (R_t: 34.77 min). mp: 142-145 °C; HR-Mass (ESI): $C_{67}H_{90}N_{18}O_{13}$ *m/z* = $[M\text{-}C_9H_7N_3O+H]^+$ Found 1185.6048, Calc. 1185.6049; $[M\text{-}C_9H_7N_3O+2H]^+/2$ Found 593.3061, Calc. 593.3062.



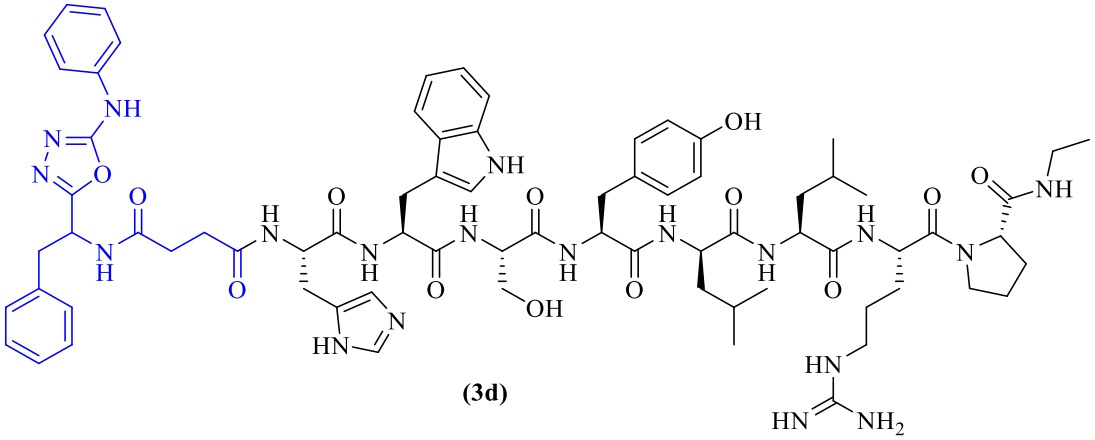
(3b)

HPLC analysis found that peptide **(3b)** was obtained in 96%< purity (R_t: 34.428 min). mp: 119-121 °C; HR-Mass (ESI): $C_{67}H_{90}N_{18}O_{14}$ *m/z* = $[M\text{-}C_8H_7N_2O+H]^+$Found 1212.6524, Calc. 1212.6525; $[M\text{-}C_8H_7N_2O+2H]^+/2$ Found 606.8296, Calc. 606.8295.



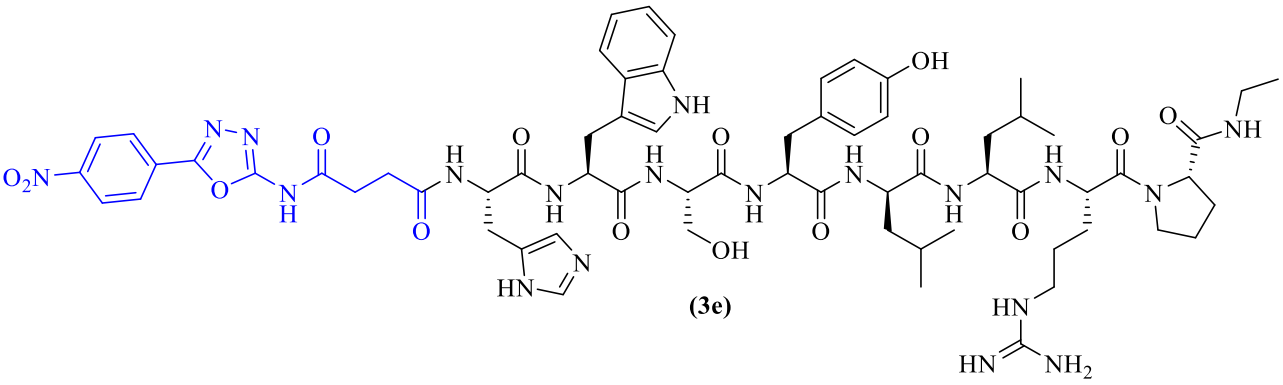
(3c)

HPLC analysis found that peptide **(3c)** was obtained in 94%< purity (R_t: 35.398 min). mp: 121-123 °C; HR-Mass (ESI): $C_{66}H_{87}N_{18}ClO_{14}$*m/z* = $[M\text{-}C_8H_5ClN_3O+H]^+$ Found 1185.6050, Calc. 1185.6051; $[M\text{-}C_8H_5ClN_3O+2H]^+/2$ Found 593.3062, Calc. 593.3061.



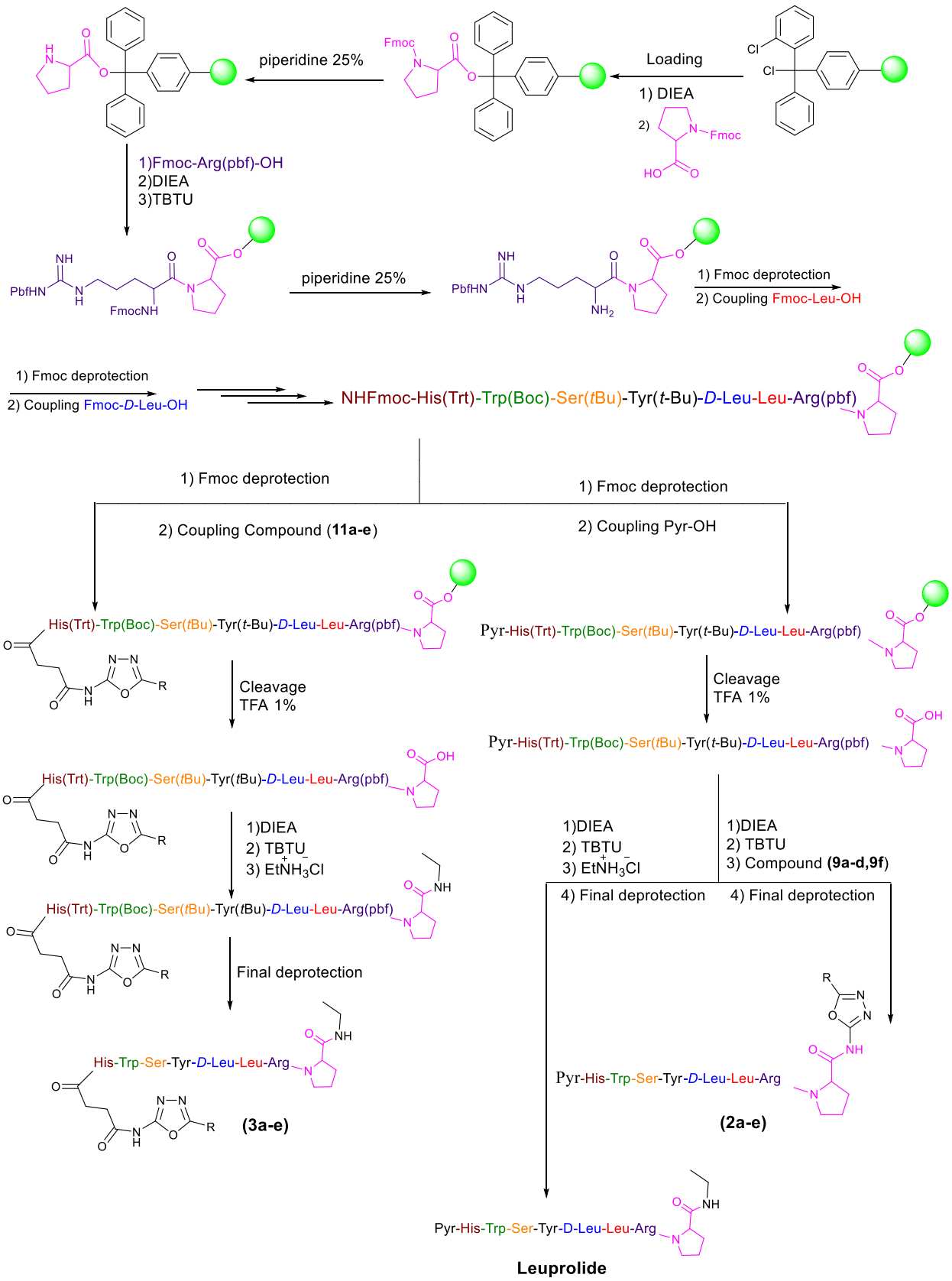
(3d)

HPLC analysis found that peptide **(3d)** was obtained in 97%< purity (R_t: 43.682 min). mp: 104-106 °C; HR-Mass (ESI): $C_{74}H_{97}N_{19}O_{13}$*m/z* =$[M+H]^+$Found 1460.7585, Calc. 1460.7585; $[M+2H]^+/2$ Found 730.8824, Calc. 730.8824.

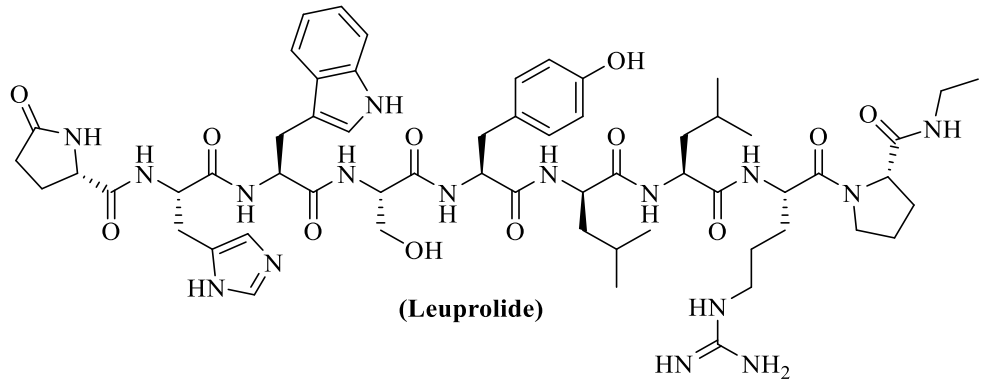


(3e)

HPLC analysis found that peptide **(3e)** was obtained in 96%< purity (R_t: 35.988 min). mp: 108-110 °C; HR-Mass (ESI): $C_{66}H_{87}N_{19}O_{15}$*m/z* = $[M\text{-}C_8H_5N_3O_3+H]^+$ Found 1185.6047, Calc. 1185.6046; $[M\text{-}C_8H_5N_3O_3+2H]^+/2$ Found 593.3061, Calc. 593.3060.

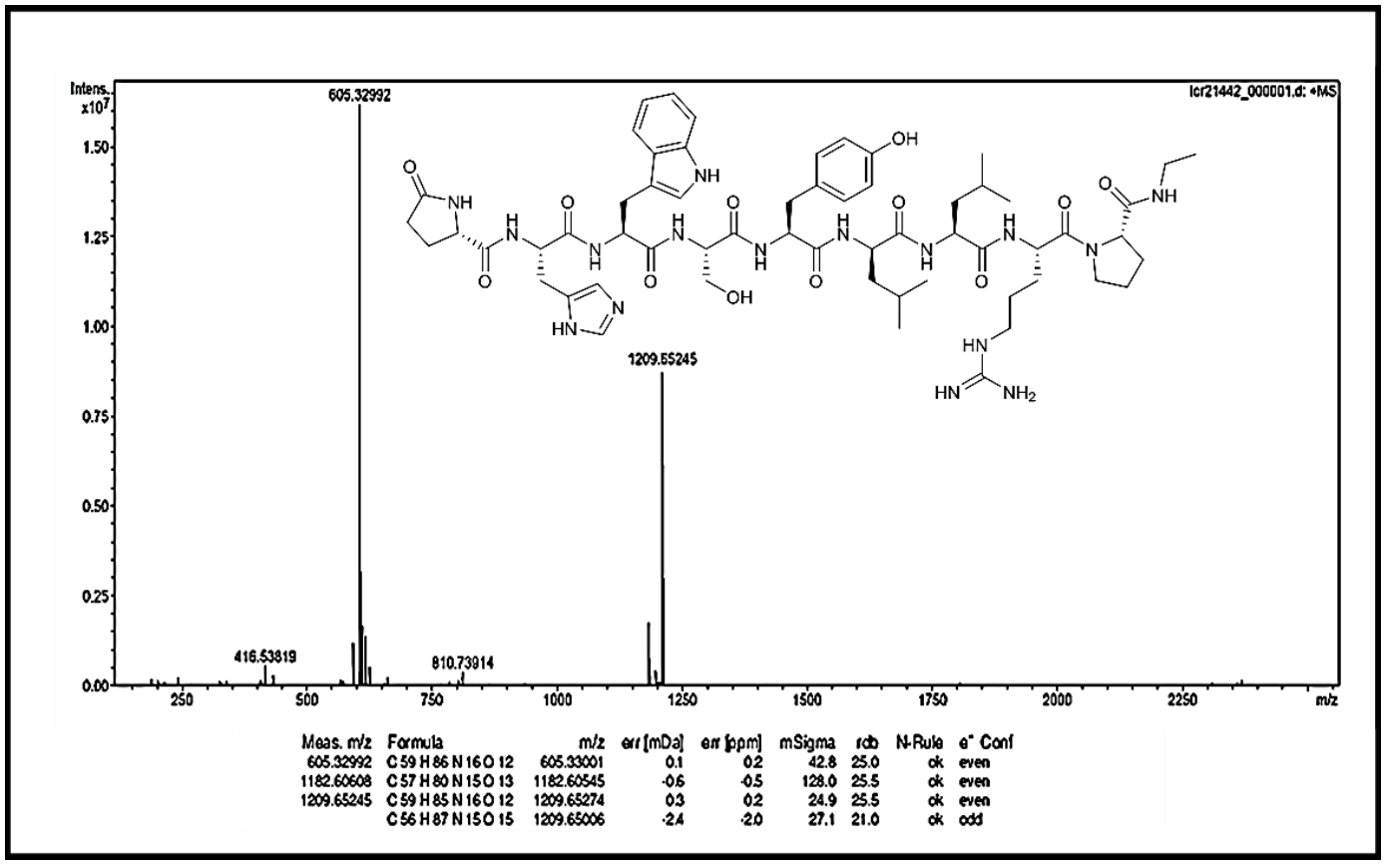

piperidine 25%
Loading
1) DIEA
2)
1)Fmoc-Arg(pbf)-OH
2)DIEA
3)TBTU
piperidine 25%
1) Fmoc deprotection
2) Coupling Fmoc-Leu-OH
1) Fmoc deprotection
2) Coupling Fmoc-D-Leu-OH
NHFmoc-His(Trt)-Trp(Boc)-Ser(tBu)-Tyr(t-Bu)-D-Leu-Leu-Arg(pbf)
1) Fmoc deprotection
2) Coupling Compound (11a-e)
1) Fmoc deprotection
2) Coupling Pyr-OH
His(Trt)-Trp(Boc)-Ser(tBu)-Tyr(t-Bu)-D-Leu-Leu-Arg(pbf)
Pyr-His(Trt)-Trp(Boc)-Ser(tBu)-Tyr(t-Bu)-D-Leu-Leu-Arg(pbf)
Cleavage
TFA 1%
Cleavage
TFA 1%
Pyr-His(Trt)-Trp(Boc)-Ser(tBu)-Tyr(t-Bu)-D-Leu-Leu-Arg(pbf)
His(Trt)-Trp(Boc)-Ser(tBu)-Tyr(tBu)-D-Leu-Leu-Arg(pbf)
1)DIEA
2) TBTU
3) EtNH3Cl
1)DIEA
2) TBTU
3) EtNH3Cl
1)DIEA
2) TBTU
3) Compound (9a-d,9f)
His(Trt)-Trp(Boc)-Ser(tBu)-Tyr(tBu)-D-Leu-Leu-Arg(pbf)
4) Final deprotection
4) Final deprotection
Final deprotection
His-Trp-Ser-Tyr-D-Leu-Leu-Arg
(3a-e)
Pyr-His-Trp-Ser-Tyr-D-Leu-Leu-Arg
(2a-e)
Pyr-His-Trp-Ser-Tyr-D-Leu-Leu-Arg
Leuprolide

Pyr-His-Trp-Ser-Tyr-*D*-Leu-Leu-Arg-Pro-NH-ET (leuprolide)



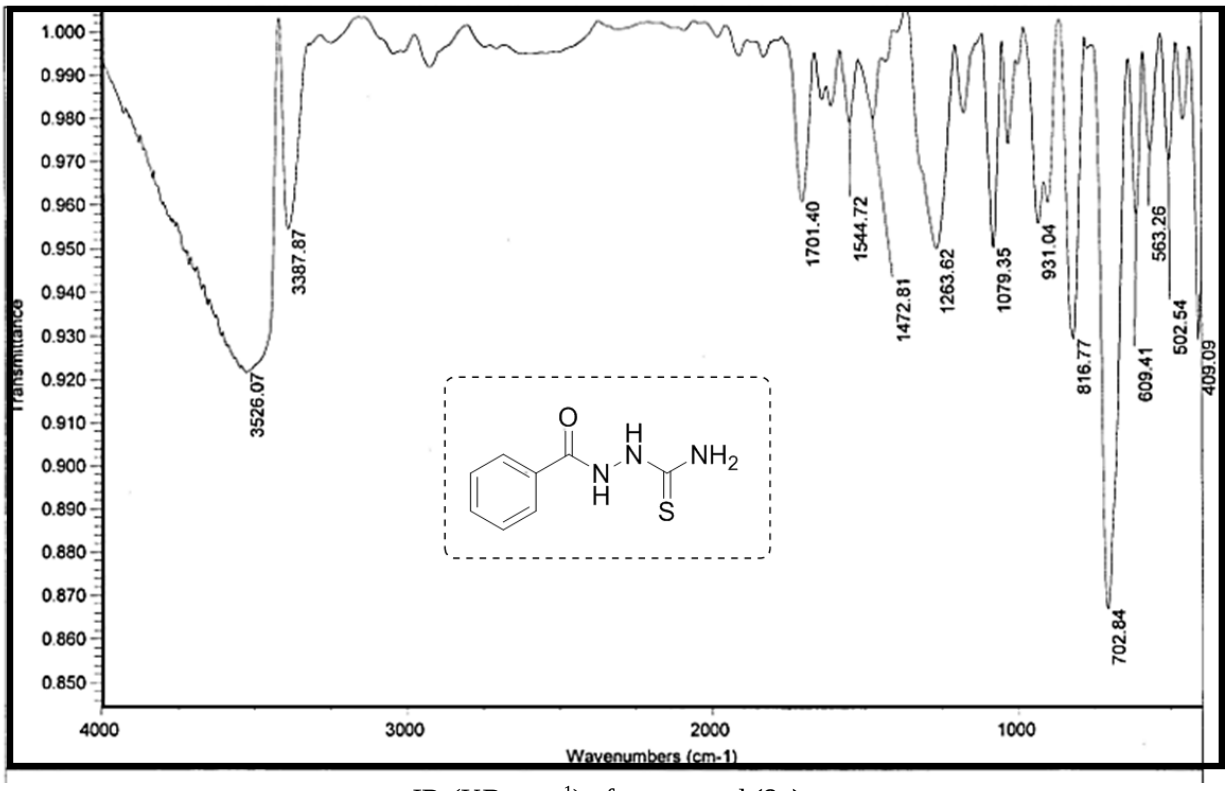
(Leuprolide)

HPLC analysis found that peptide **(Leuprolide)** was obtained in 96%< purity (R_t: 32.995 min). HR-Mass (ESI): $C_{54}H_{84}N_{16}O_{12}$ *m/z* =[M+H] $^{+}$Found 1209.6525, Calc. 1209.6524; [M+2H] $^{+}$/2Found 605.3299, Calc. 605.3299.

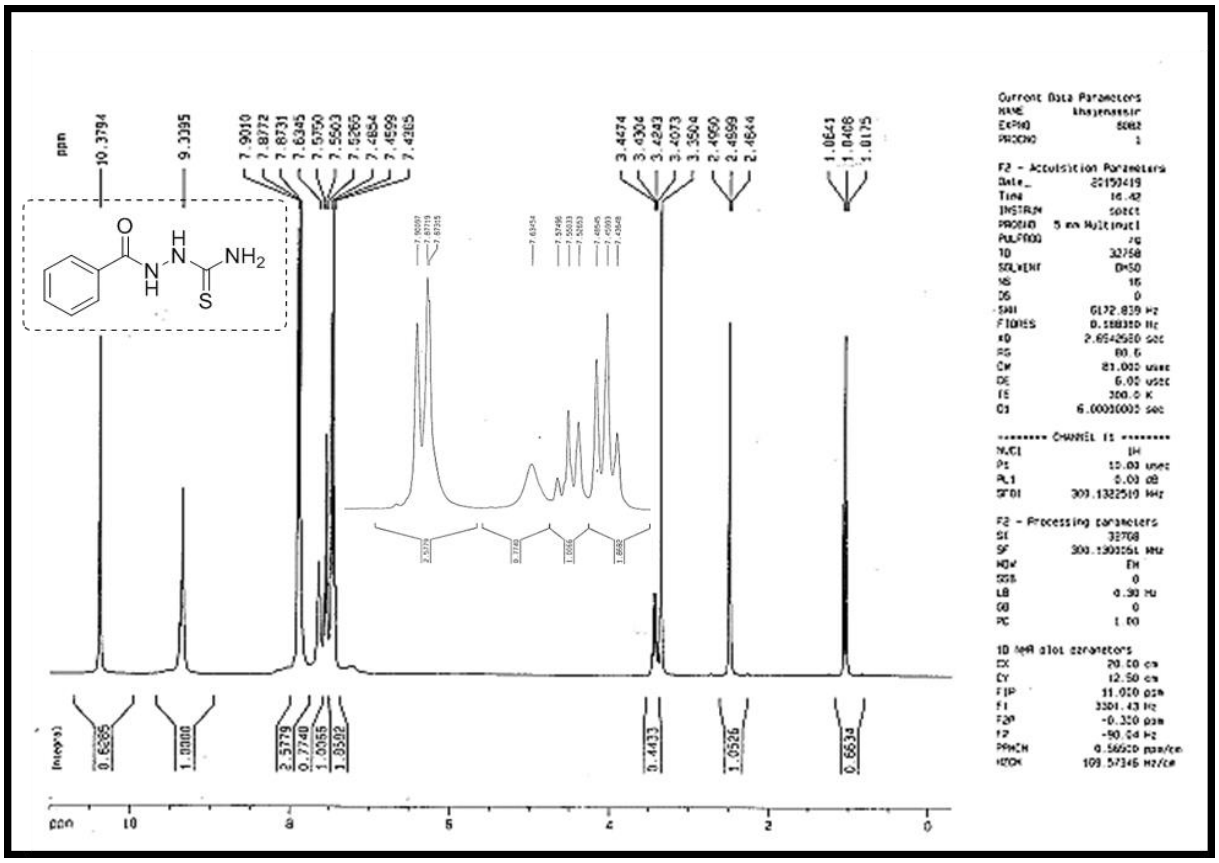


Meas. m/z	Formula	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
605.32992	C 59 H 86 N 16 O 12	605.33001	0.1	0.2	42.8	25.0	ok	even
1182.60608	C 57 H 80 N 15 O 13	1182.60545	-0.6	-0.5	128.0	25.5	ok	even
1209.65245	C 59 H 85 N 16 O 12	1209.65274	0.3	0.2	24.9	25.5	ok	even
	C 56 H 87 N 15 O 15	1209.65006	-2.4	-2.0	27.1	21.0	ok	odd

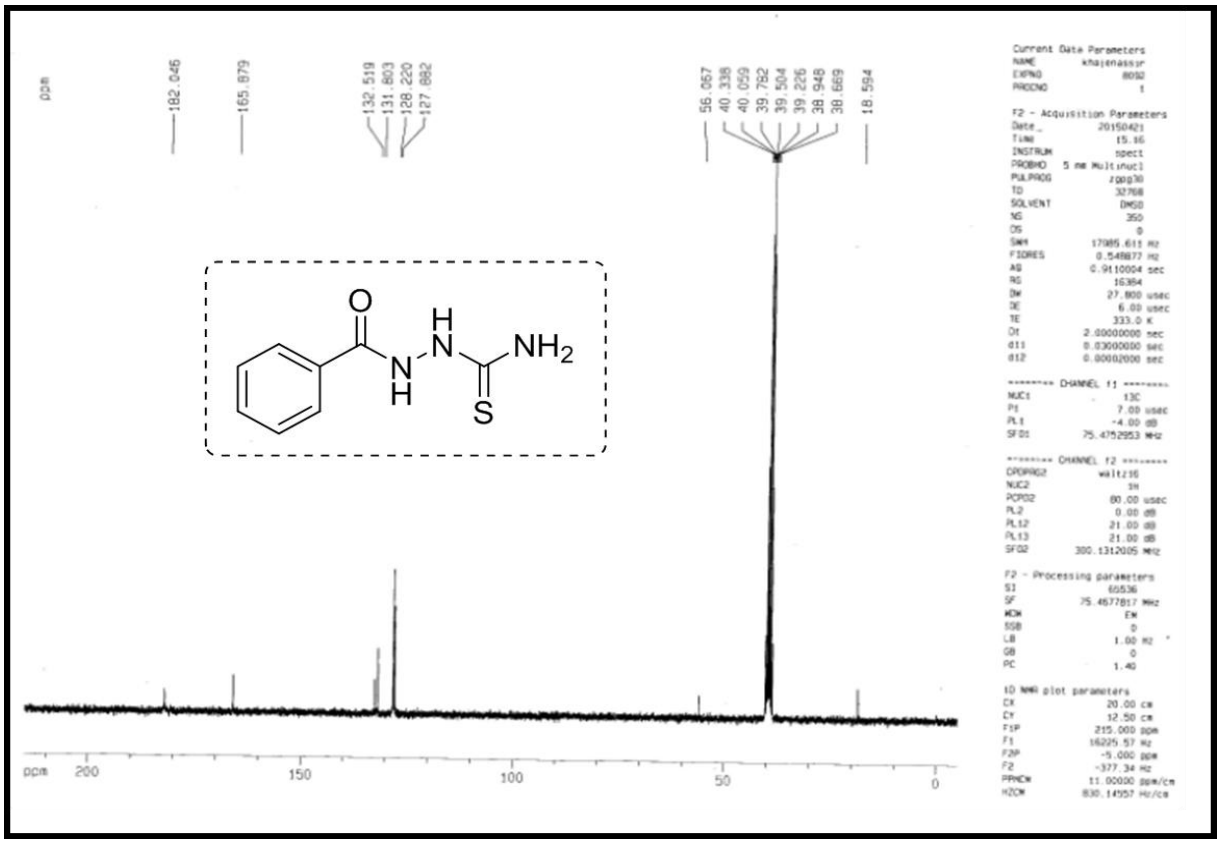
HR-MS (ESI) of Leuprolide



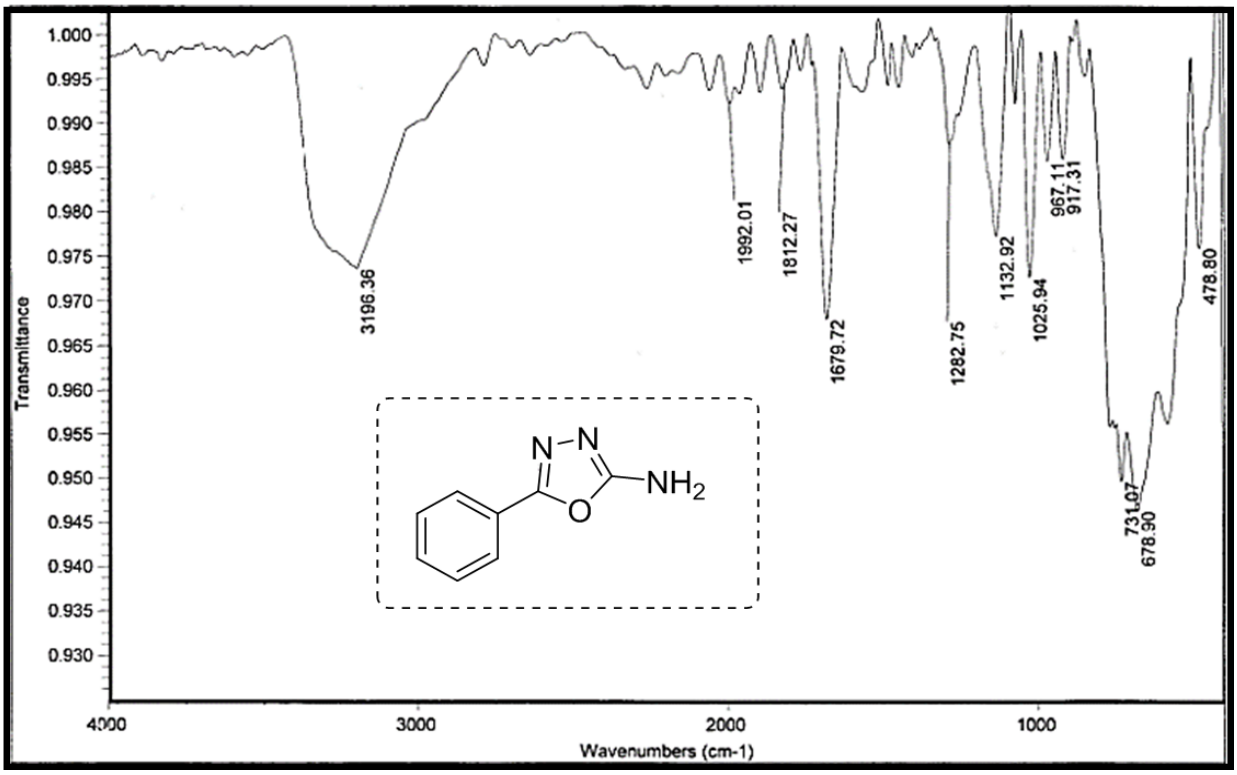
IR-(KBr-cm^{-1}) of compound **(8a)**



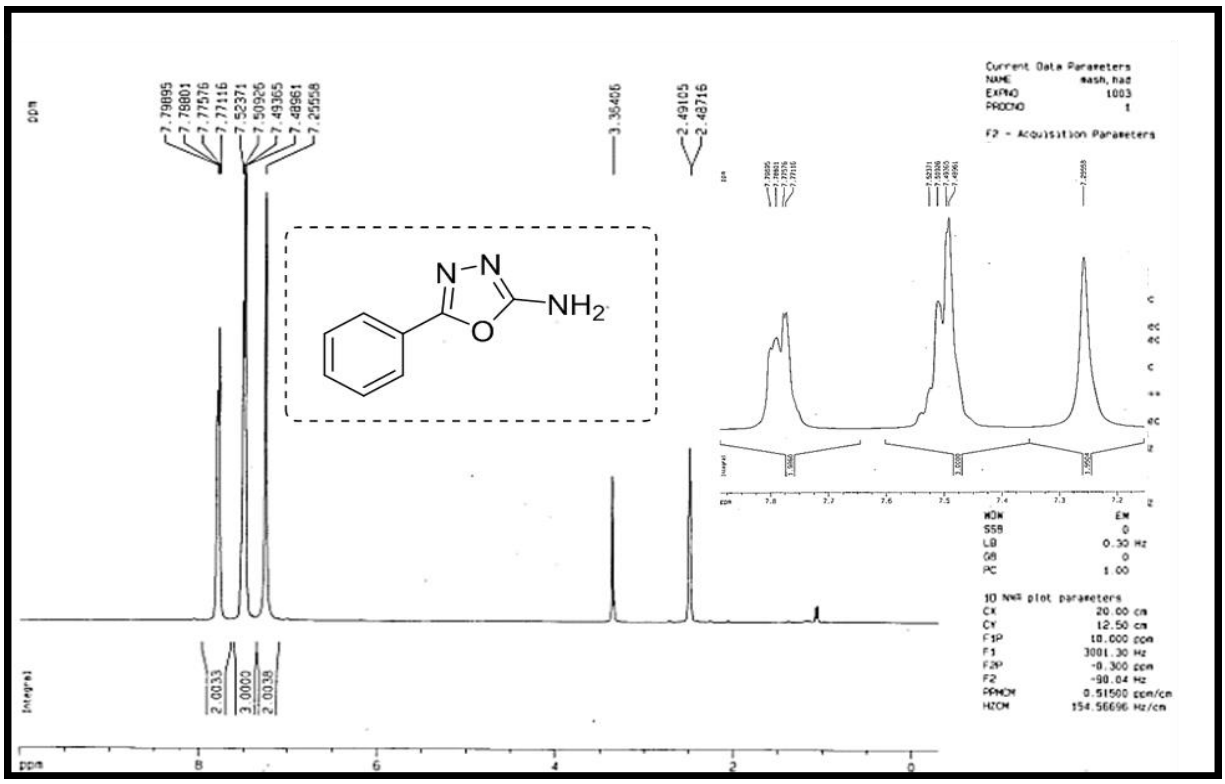
^{1}H-NMR of compound **(8a)** (300 MHz, DMSO-*d*$_6$)



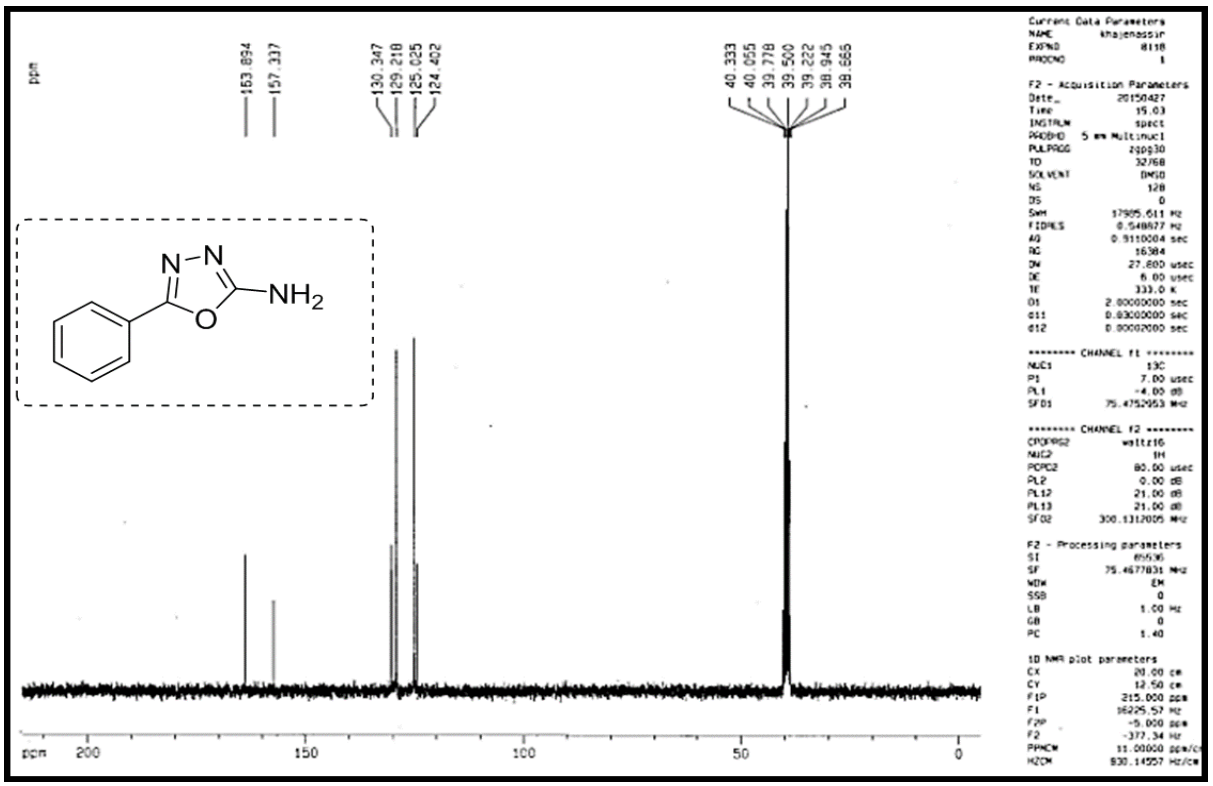
^{13}C-NMR of compound **(8a)** (75 MHz, DMSO-d_6)



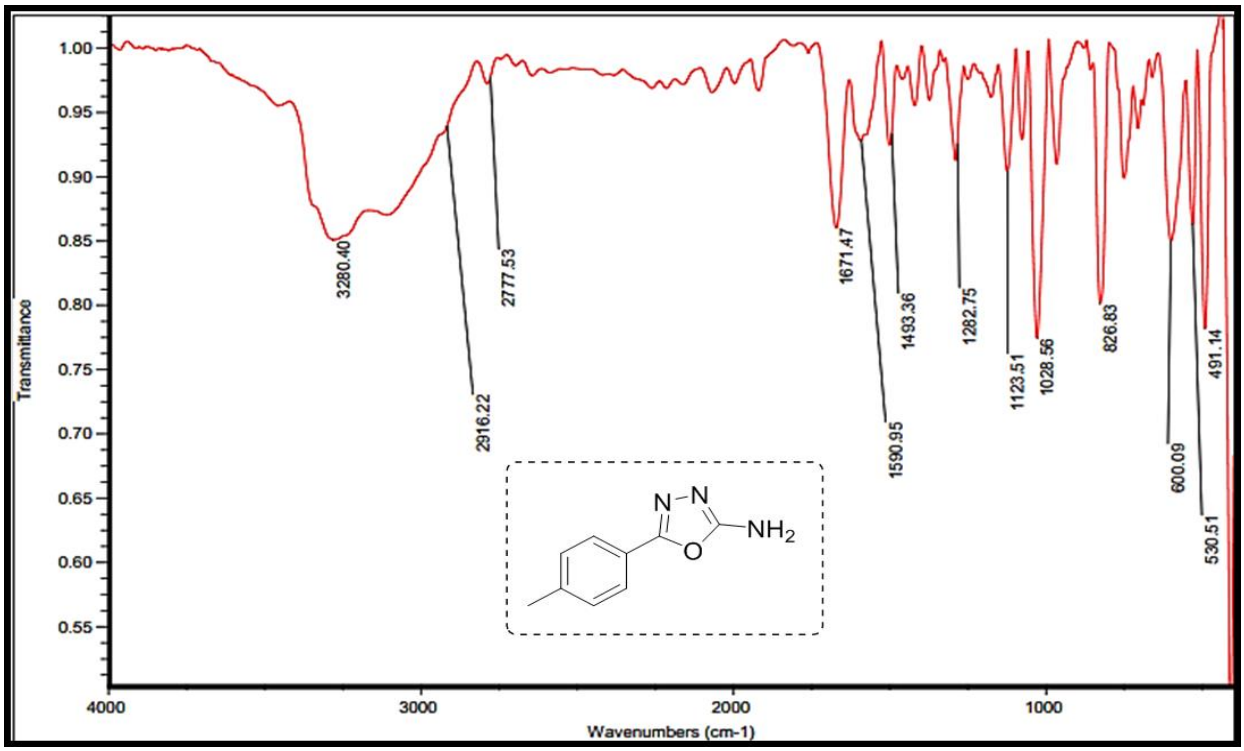
IR(KBr-cm^{-1}) of compound **(9a)**



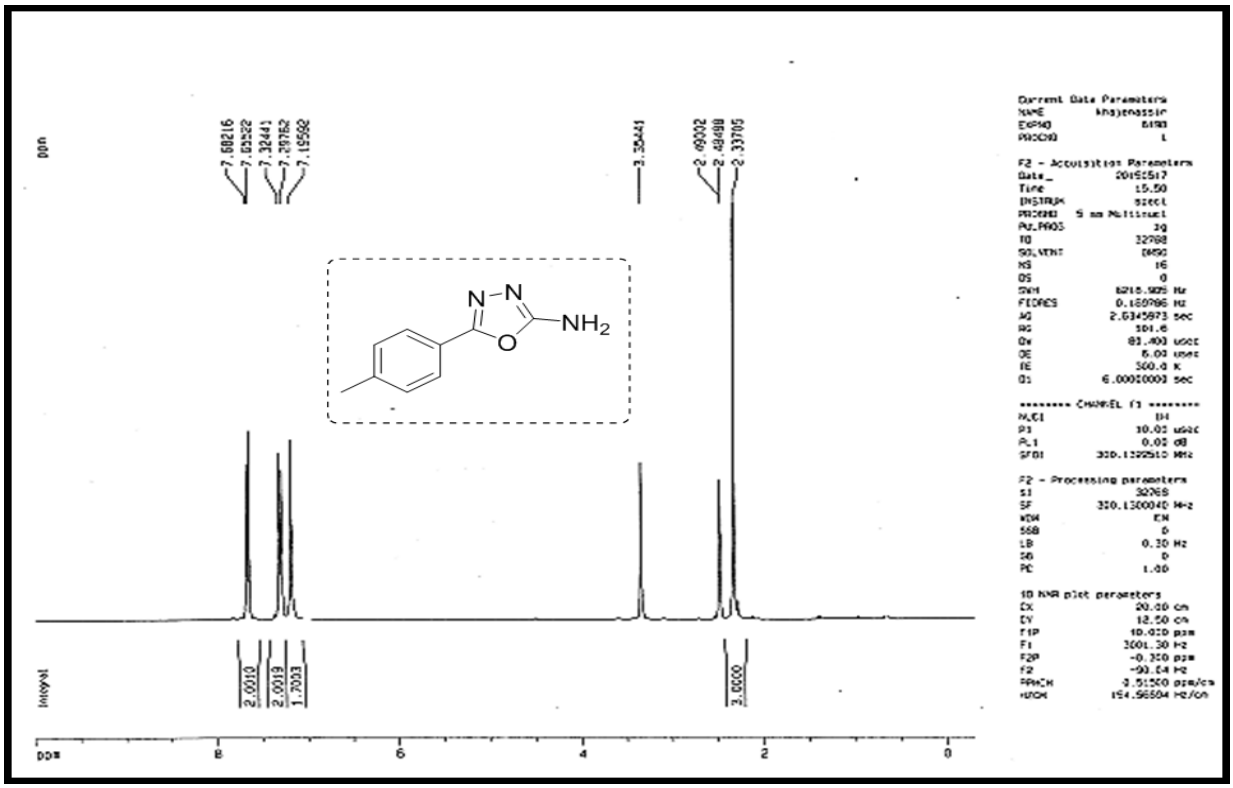
^{1}H-NMR of compound **(9a)** (300 MHz, DMSO-d_6)



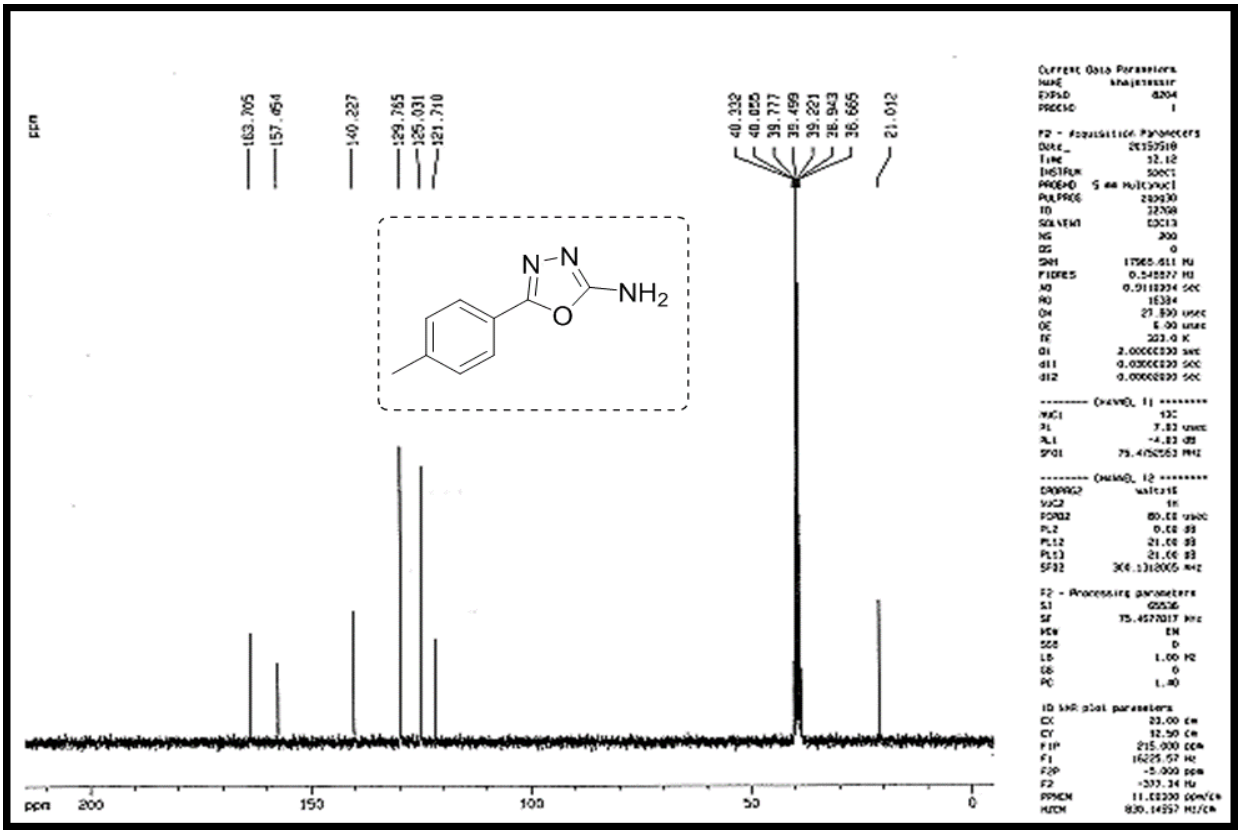
^{13}C-NMR of compound **(9a)** (75 MHz, DMSO-d_6)



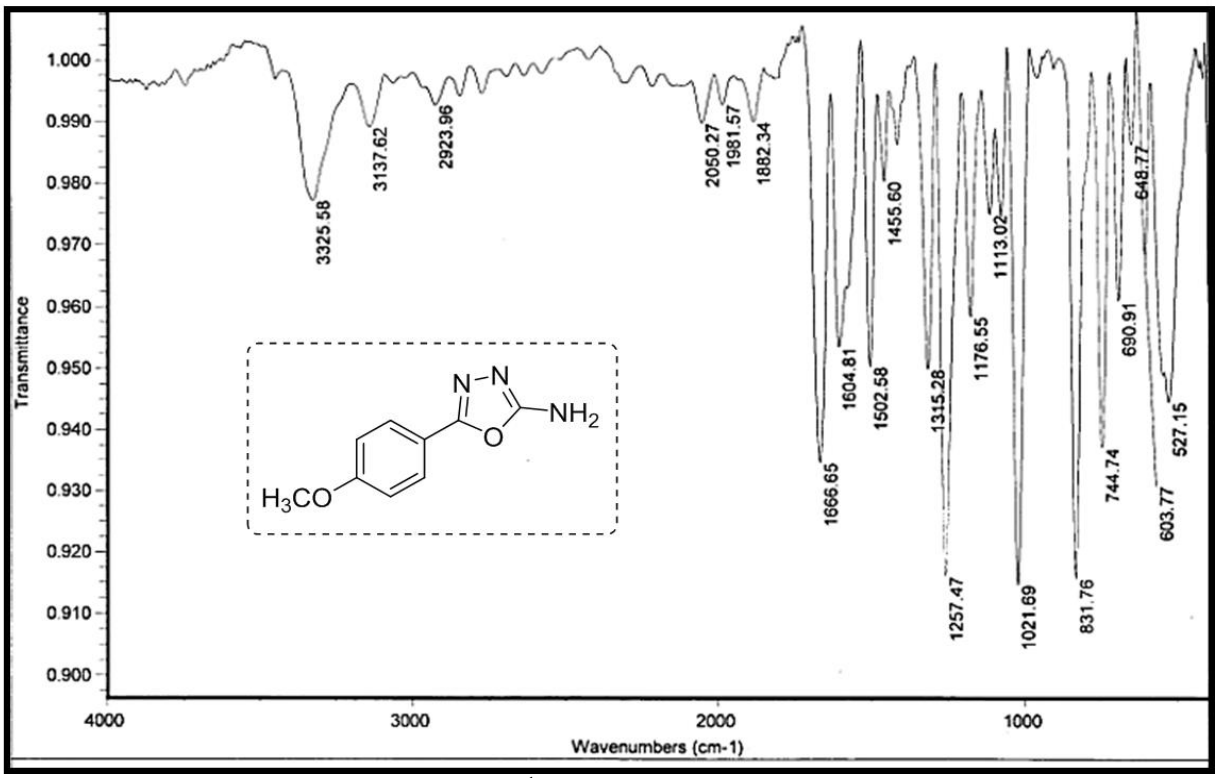
IR(KBr-cm^{-1}) of compound **(9b)**



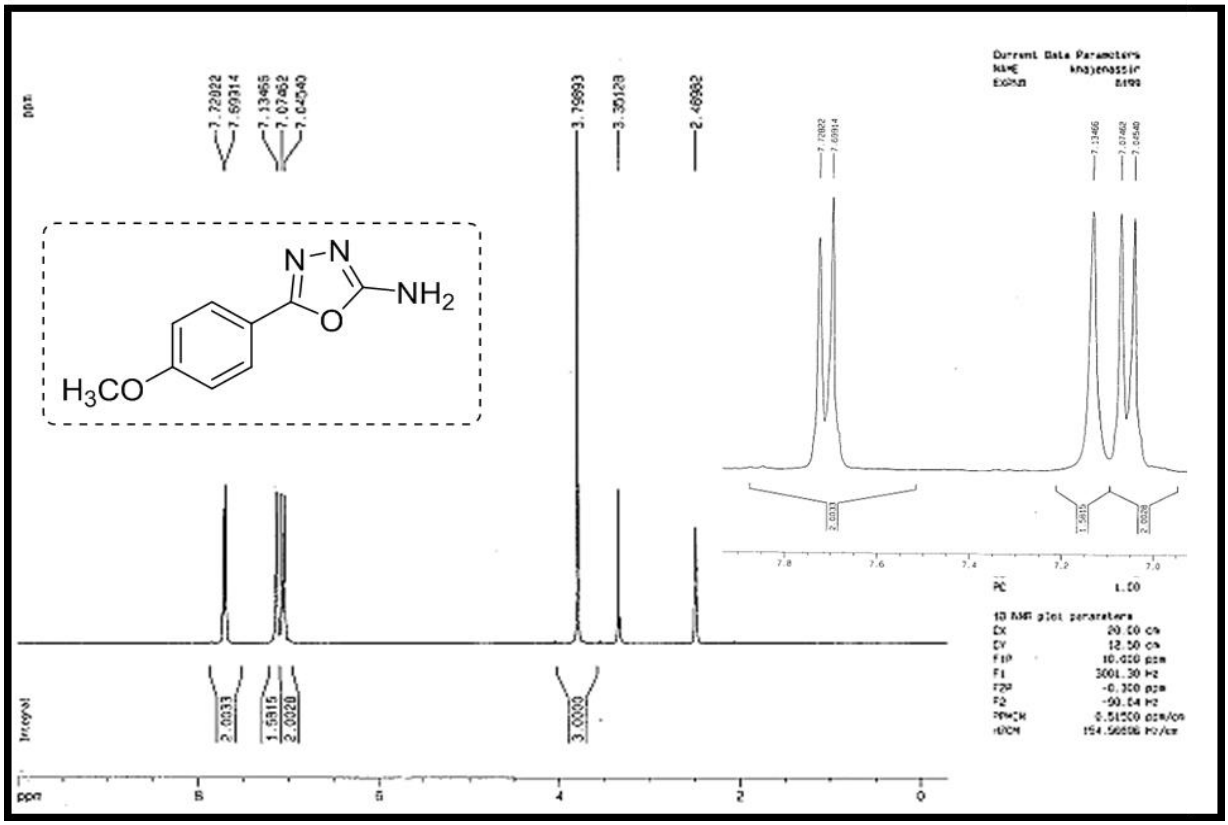
^{1}H-NMR of compound **(9b)** (300 MHz, DMSO-*d*$_6$)



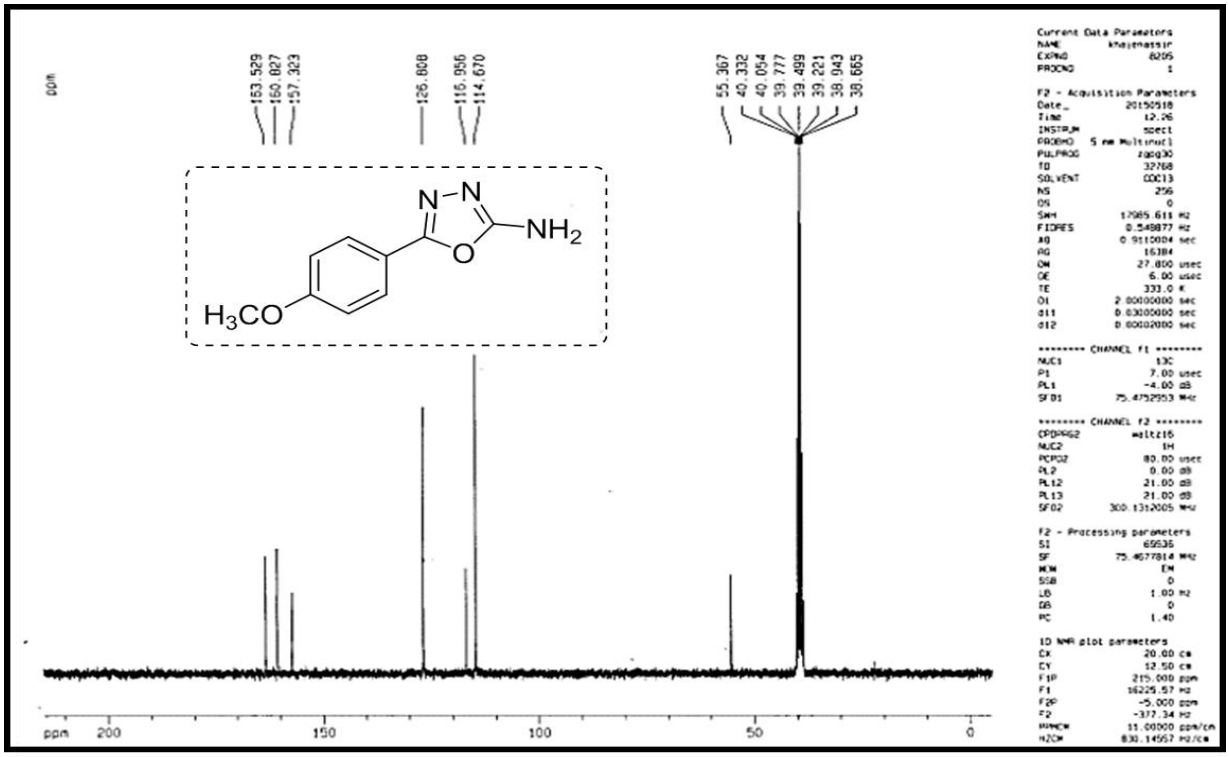
^{13}C-NMR of compound **(9b)** (75 MHz, DMSO-*d*$_6$)



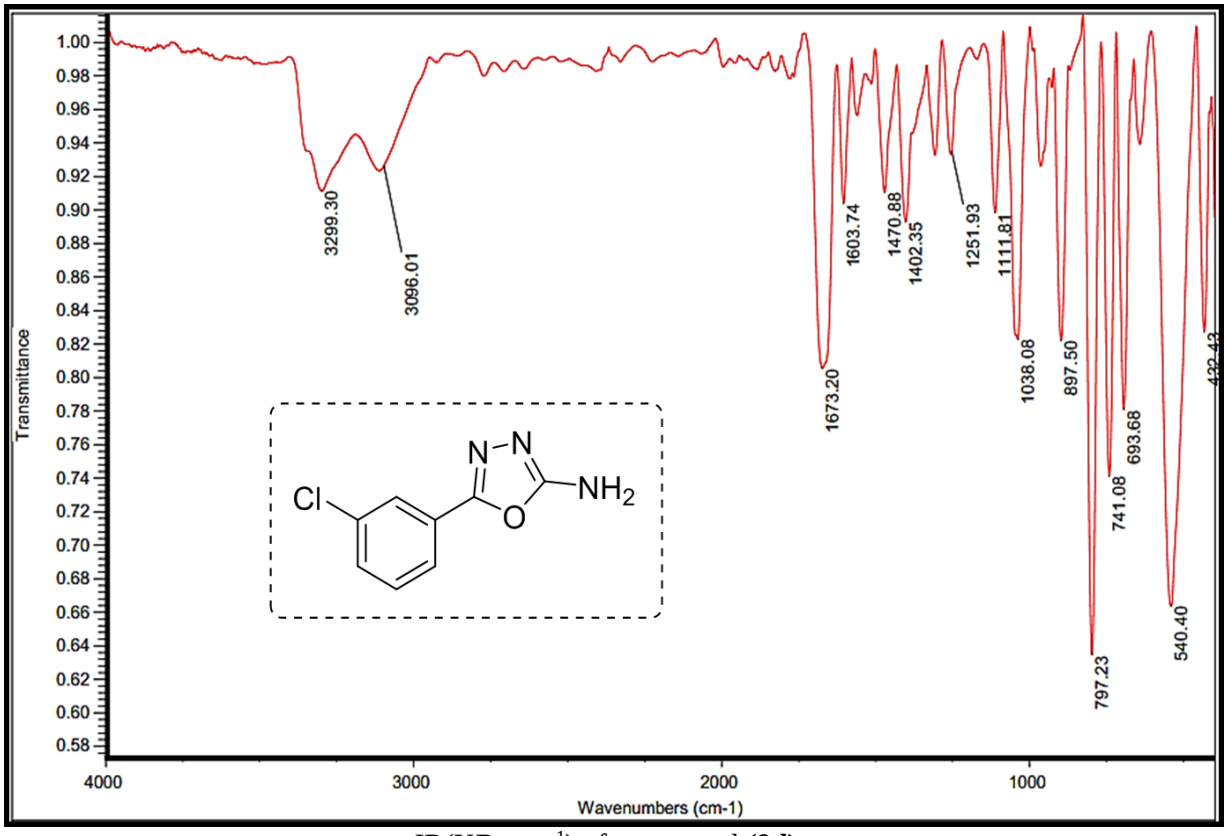
IR(KBr-cm^{-1}) of compound **(9c)**



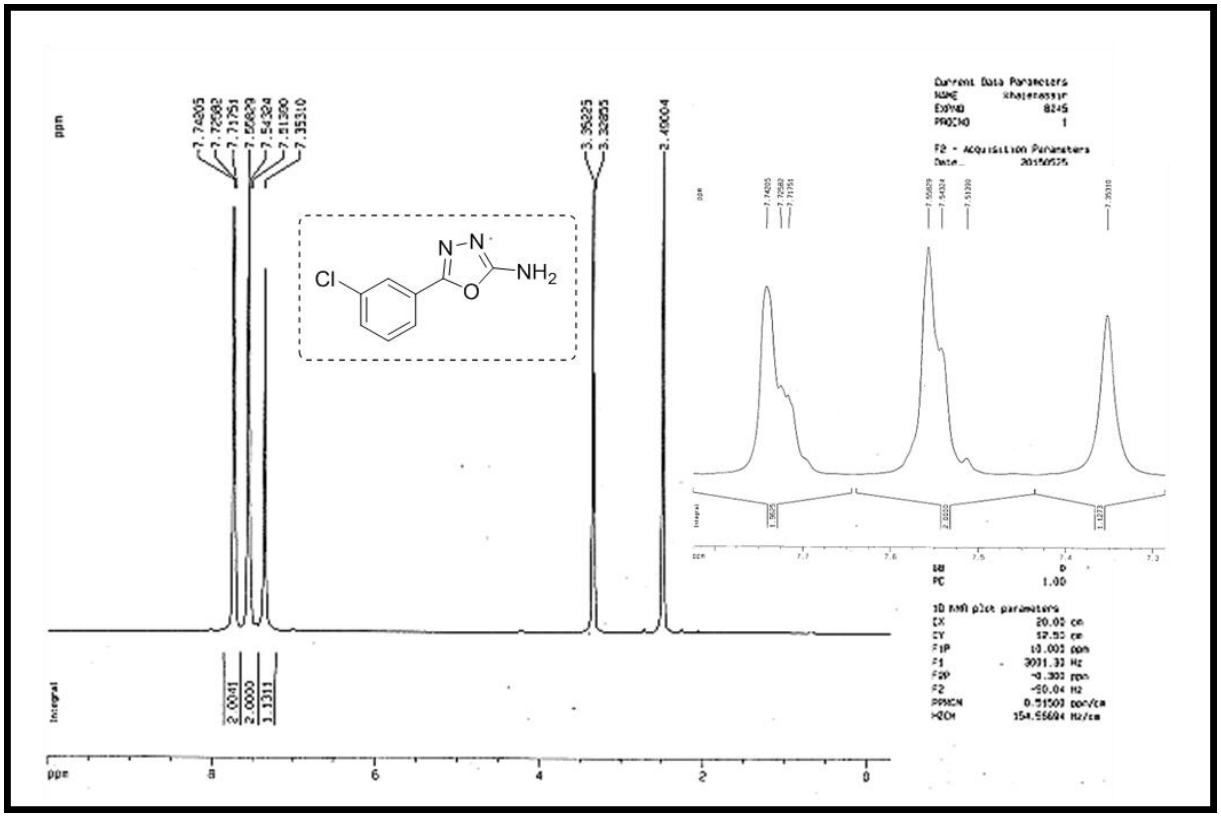
^{1}H-NMR of compound **(9c)** (300 MHz, DMSO-*d*$_6$)



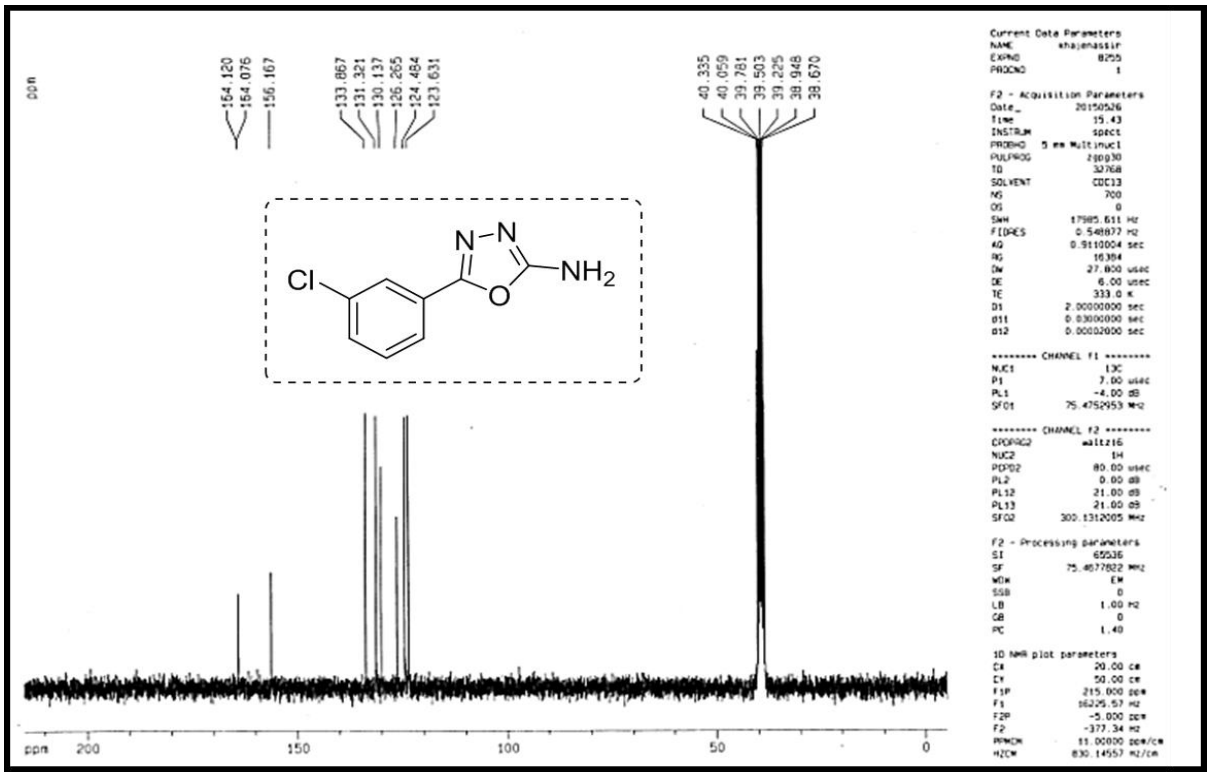
^{13}C-NMR of compound **(9c)** (75 MHz, DMSO-*d*$_6$)



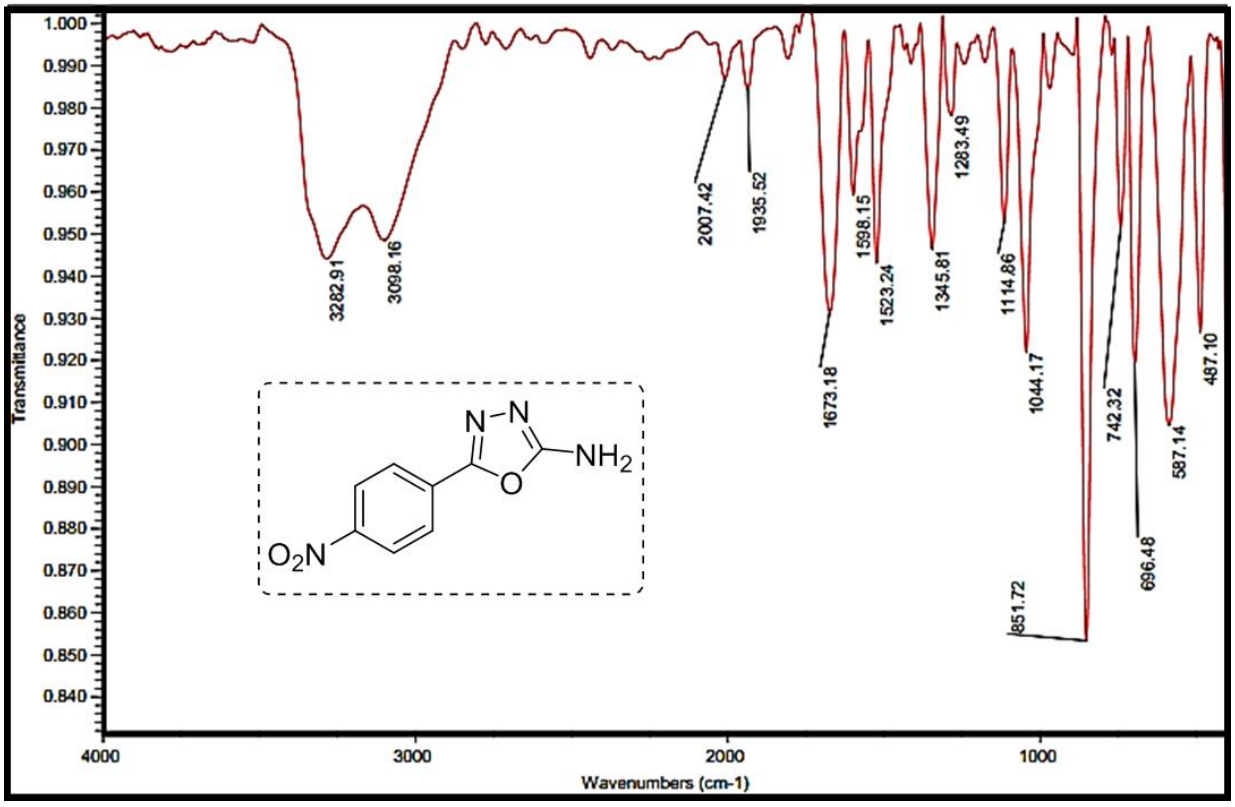
IR(KBr-cm^{-1}) of compound **(9d)**



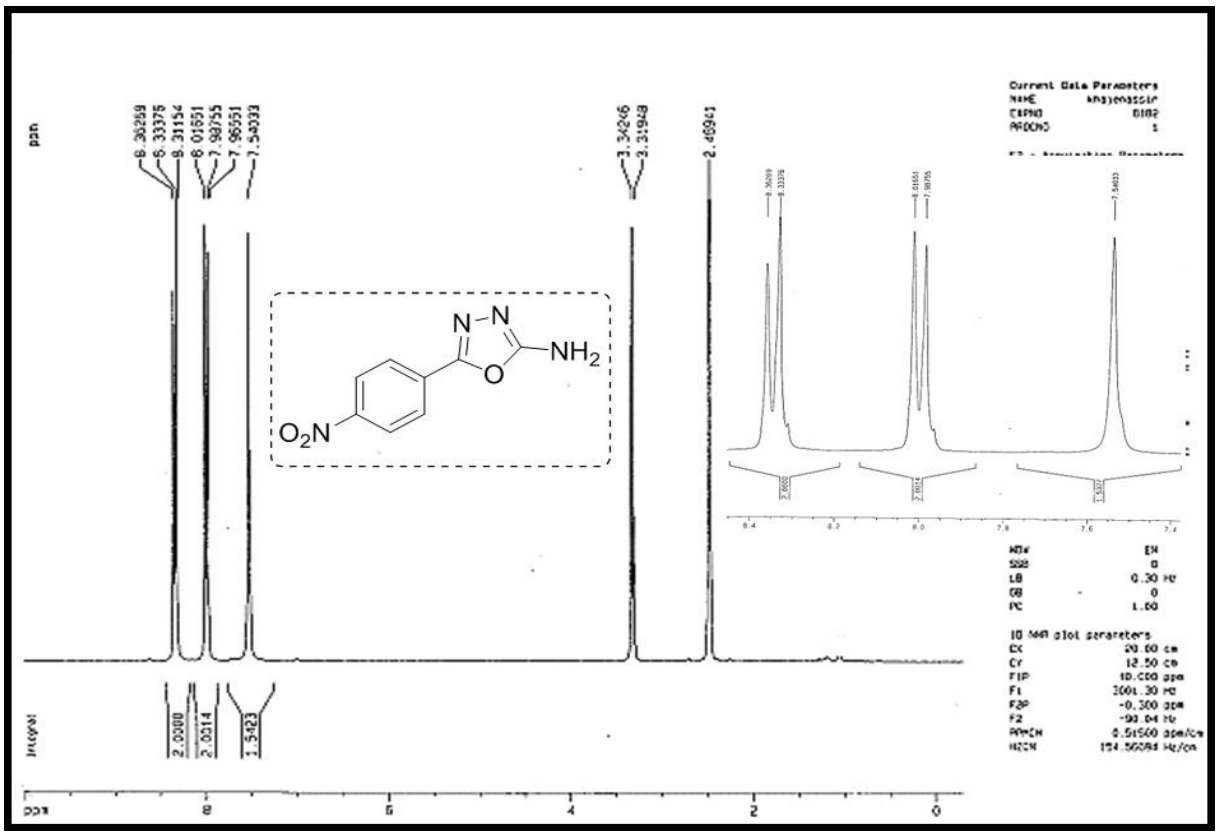
^{1}H-NMR of compound **(9d)** (300 MHz, DMSO-d_6)



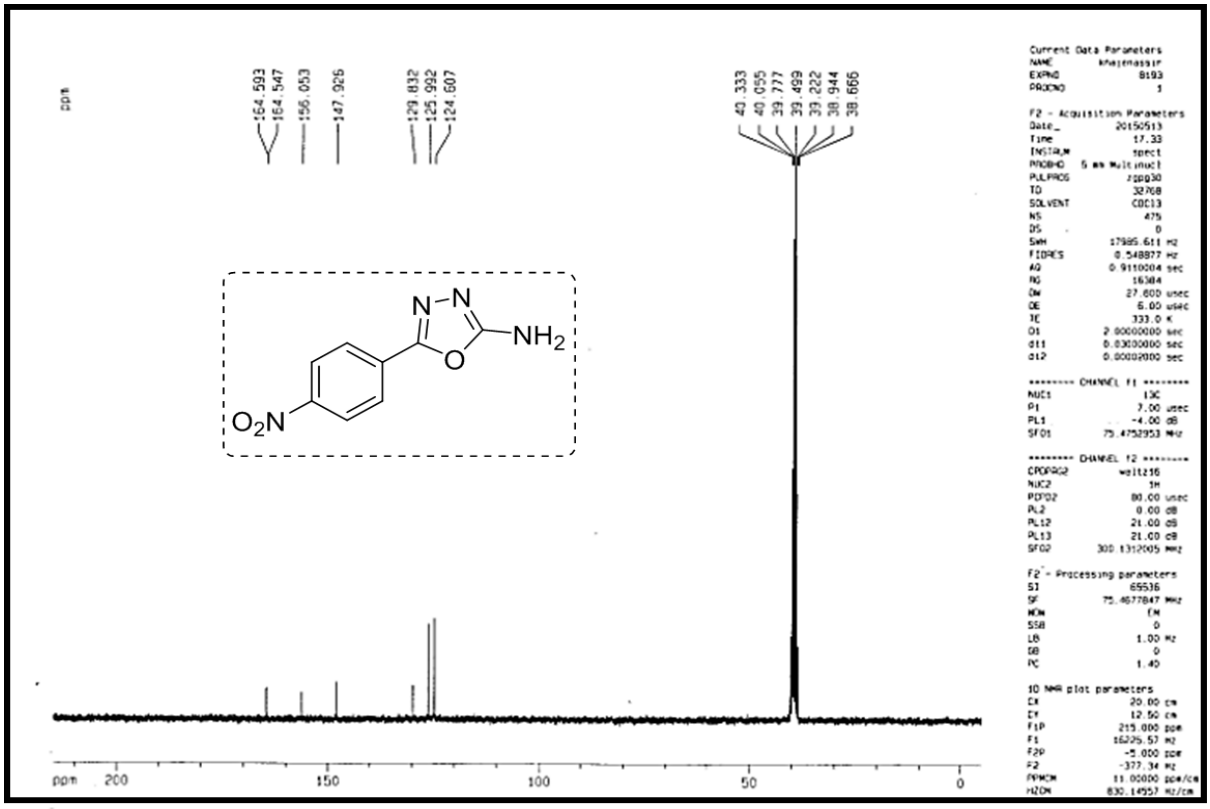
^{13}C-NMR of compound **(9d)** (75 MHz, DMSO-*d_6)*



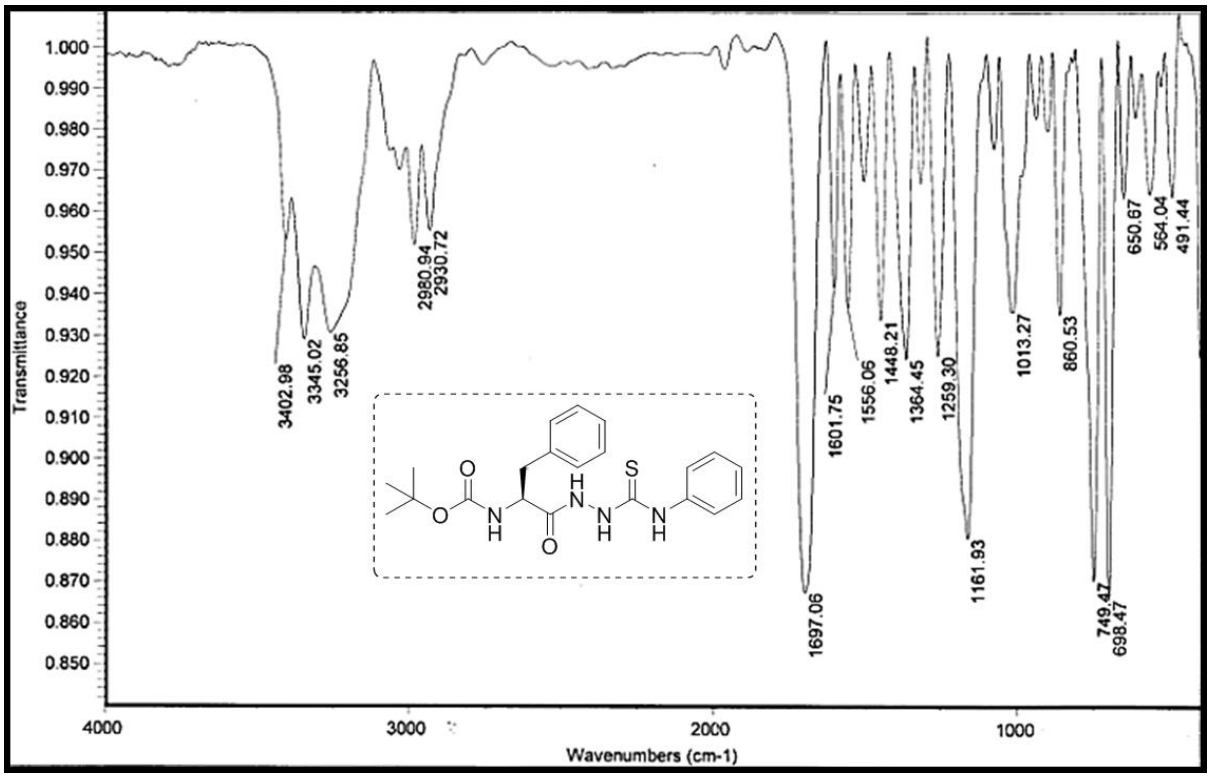
IR-(KBr-cm^{-1}) of compound **(9e)**



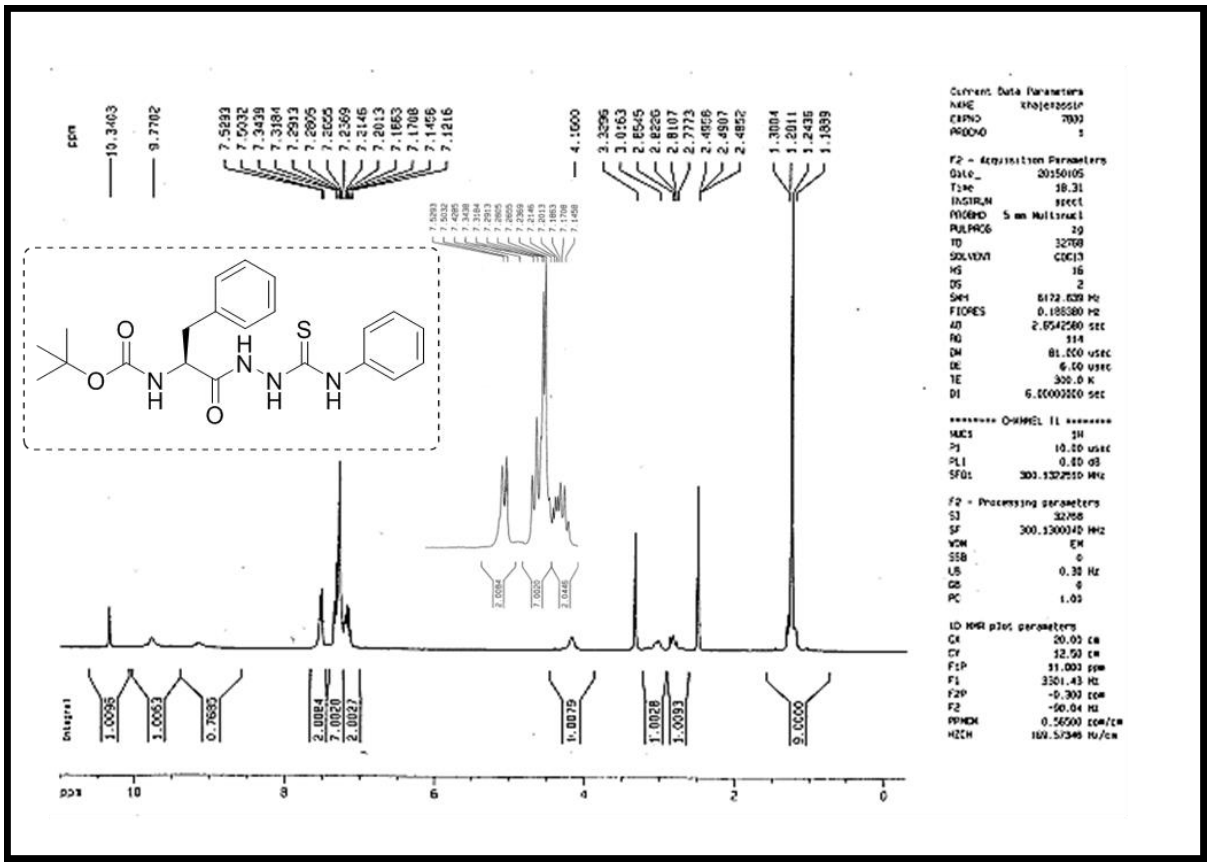
^{1}H-NMR of compound **(9e)** (300 MHz, DMSO-d_6)



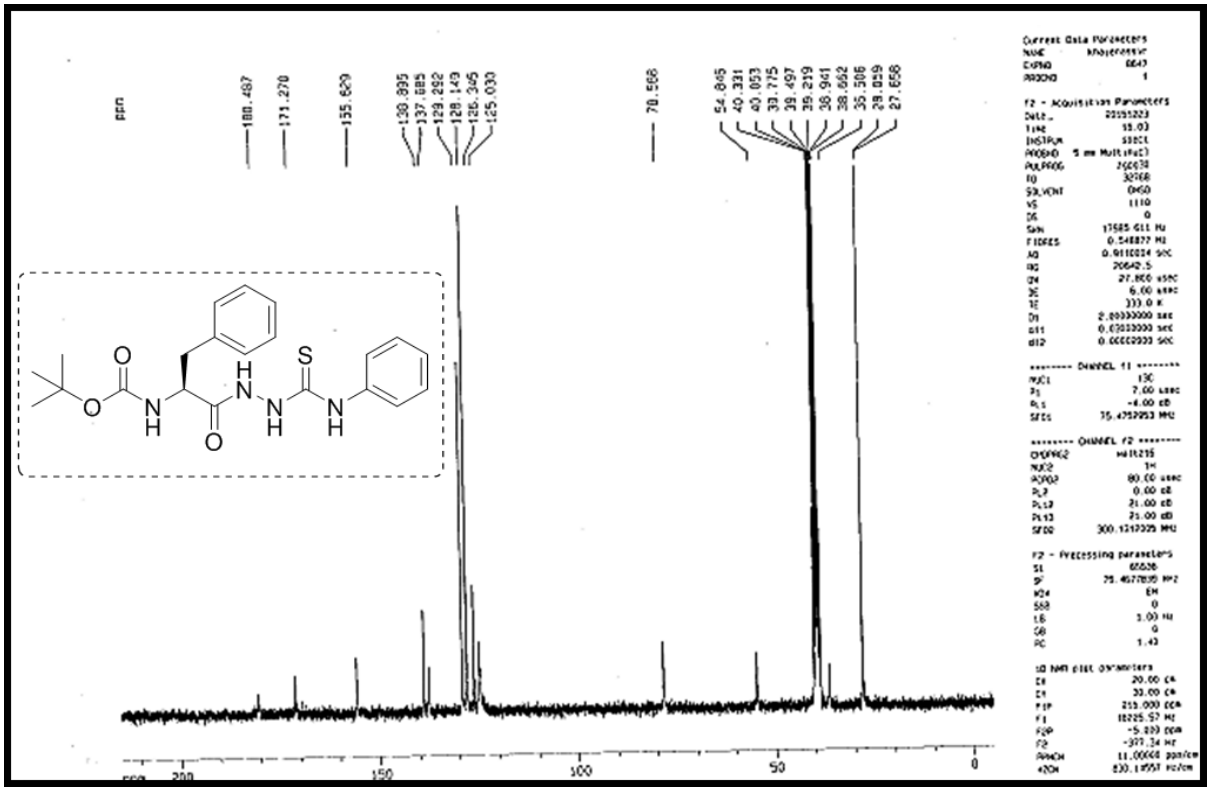
^{13}C-NMR of compound **(9e)** (75 MHz, DMSO-d_6)



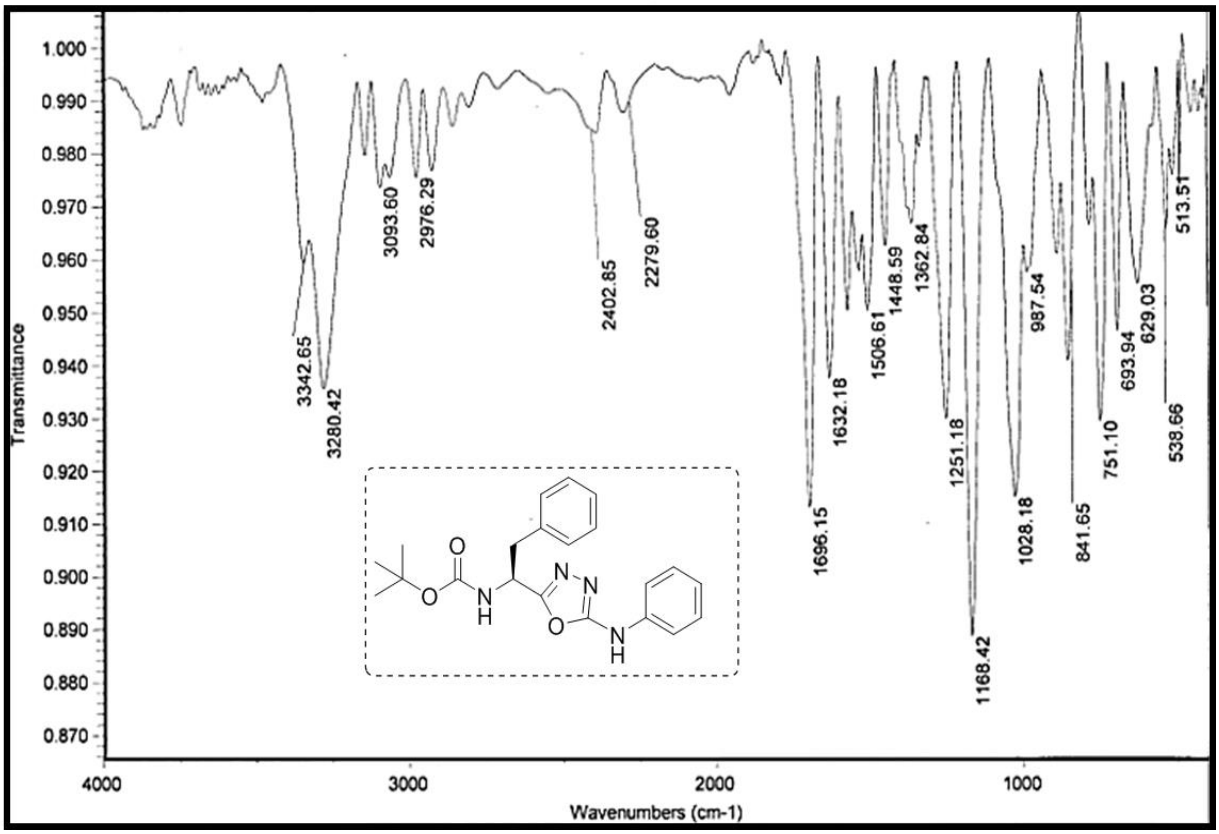
IR(KBr-cm^{-1}) of compound **(8f)**



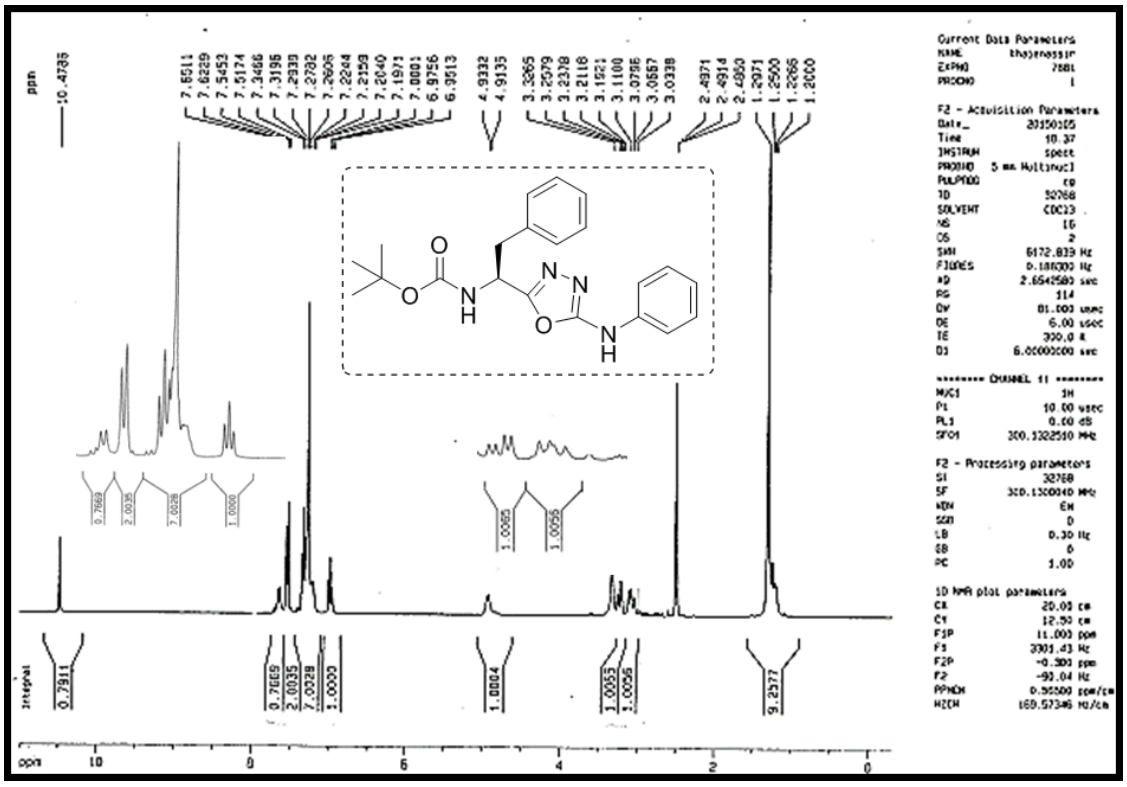
^{1}H -NMR of compound **(8f) (**300 MHz, DMSO-d_6*)*



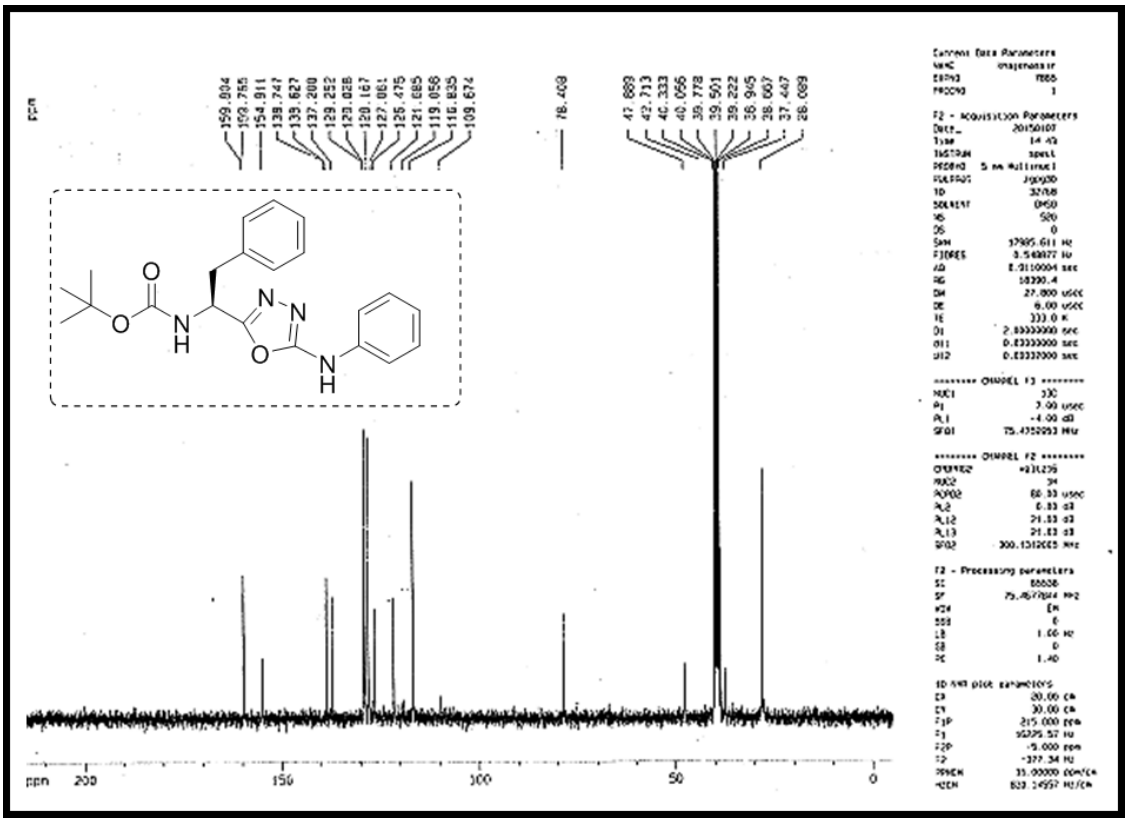
^{13}C-NMR of compound **(8f) (75**MHz, DMSO-d_6**)**



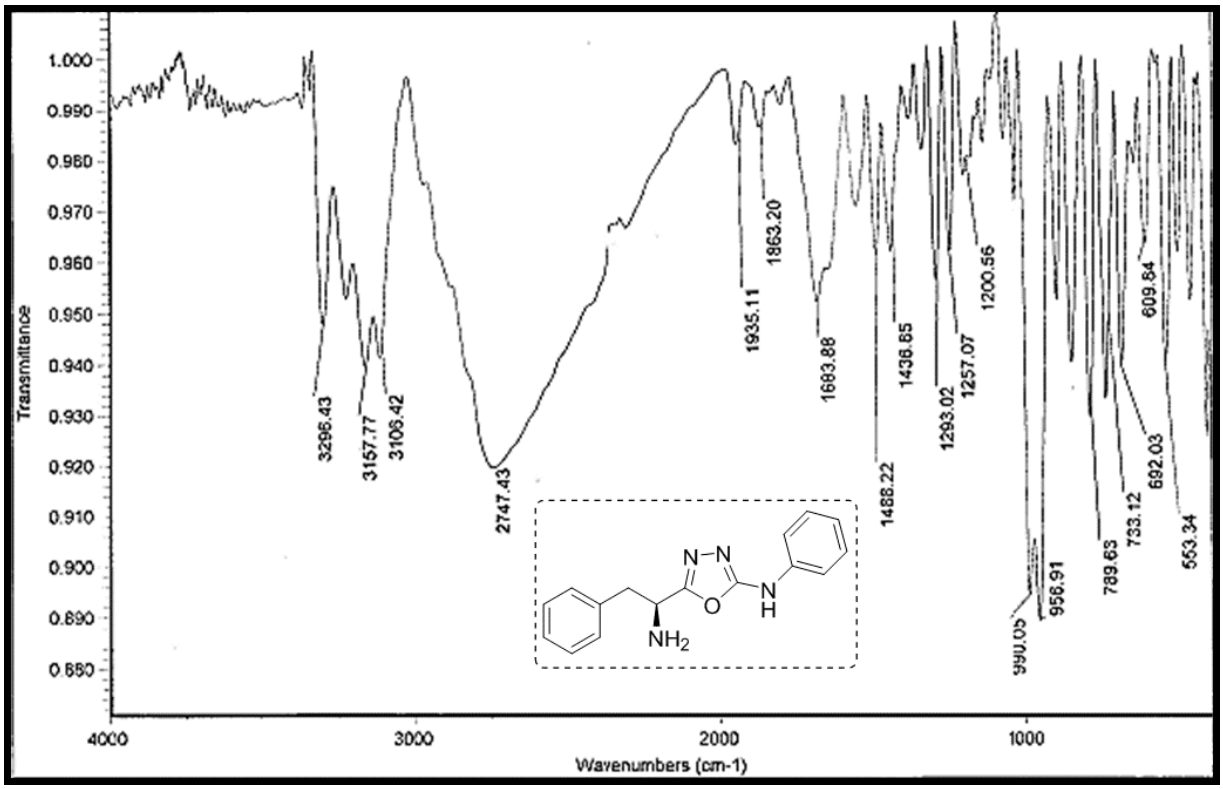
IR-(KBr-cm^{-1}) of compound **(B)**



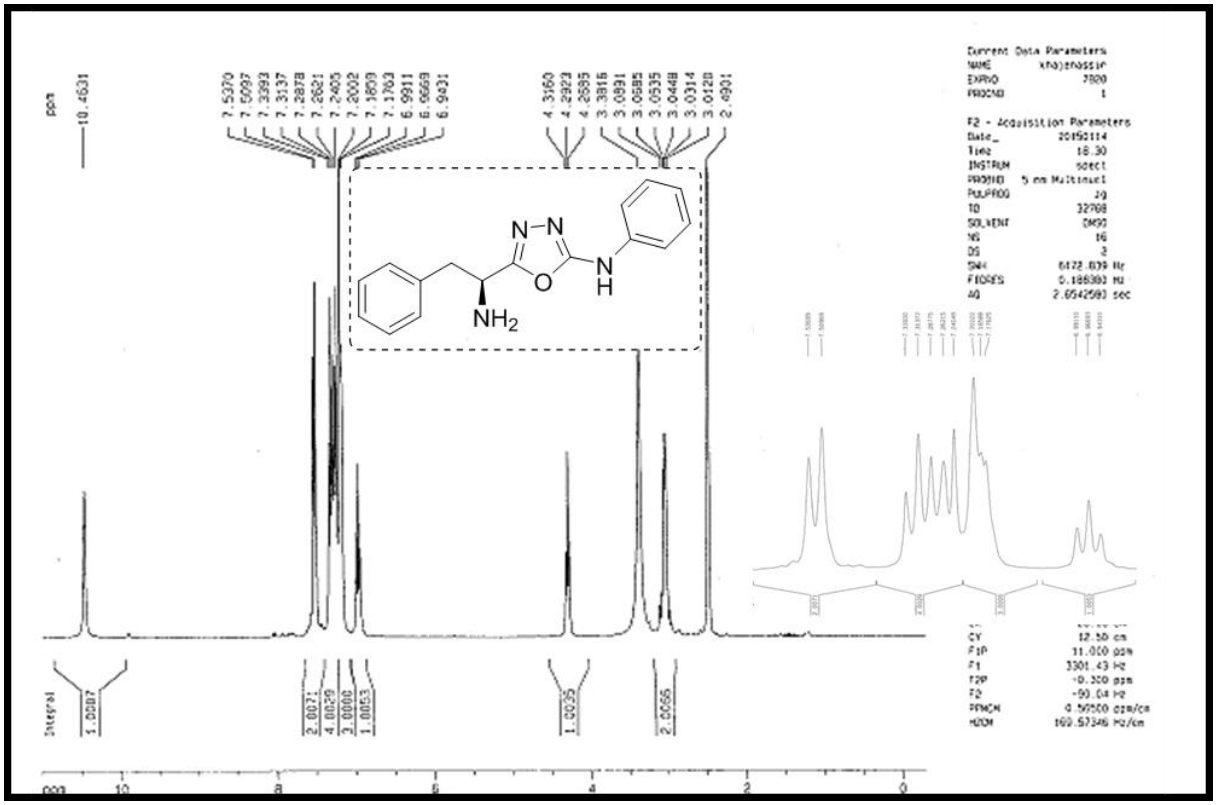
^{1}H-NMR of compound **(B)** (300 MHz, DMSO-d_6)



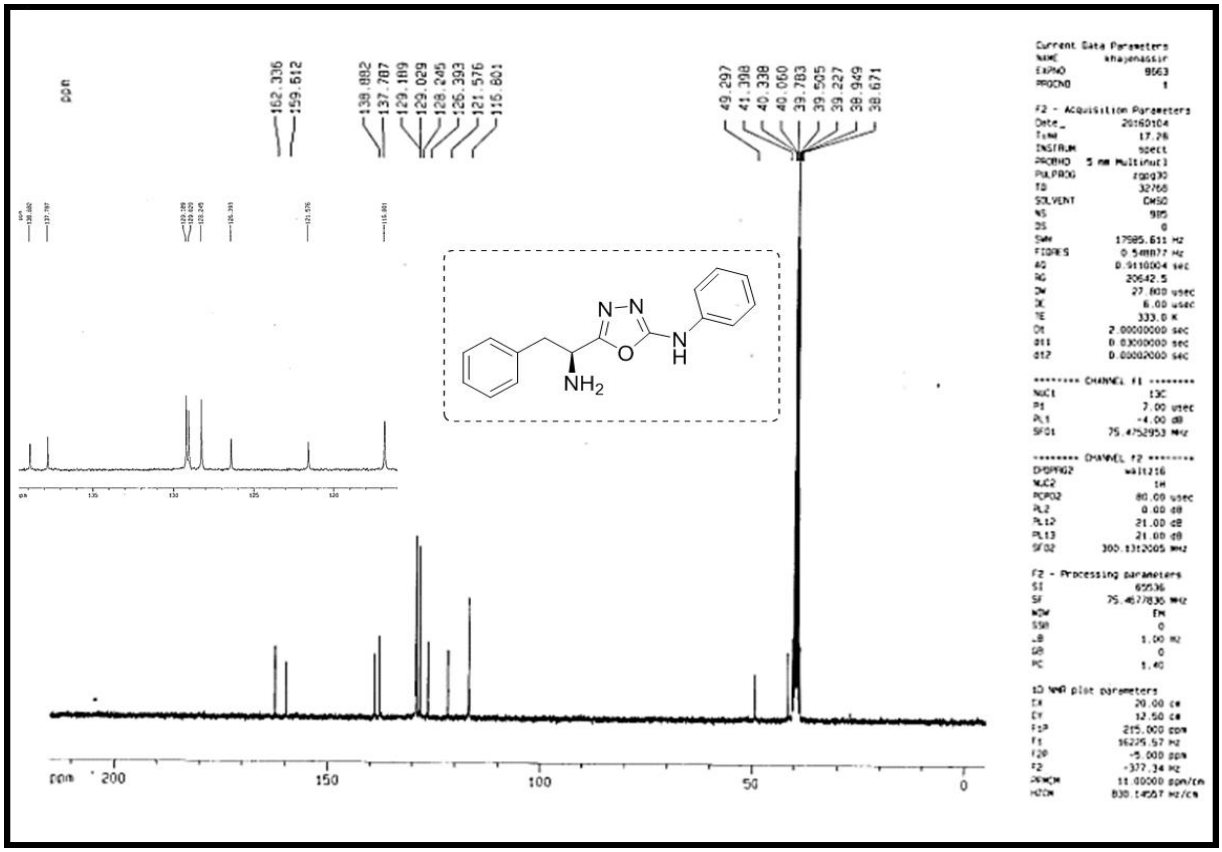
^{13}C-NMR of compound (**B)** (75 MHz, DMSO-d_6)



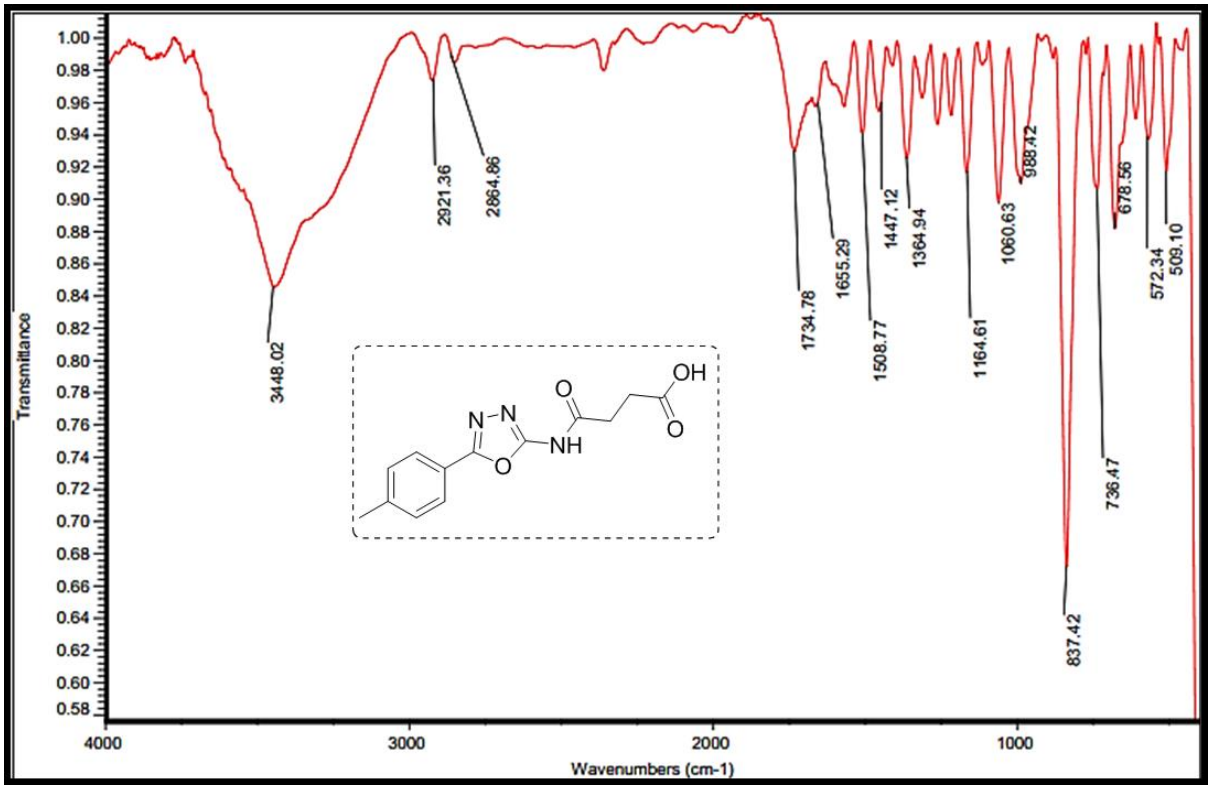
IR(KBr-cm^{-1}) of compound **(9f)**



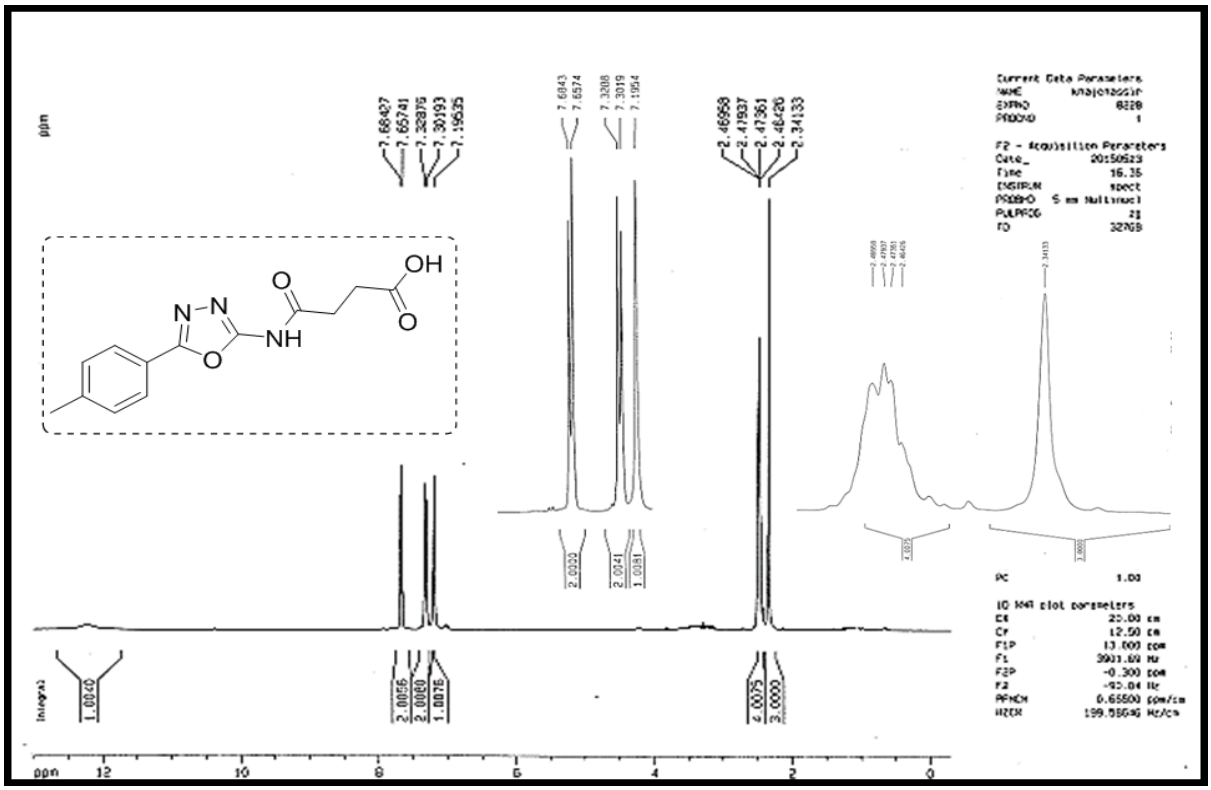
^{1}H-NMR of compound **(9f)** (300 MHz, DMSO-d_6)



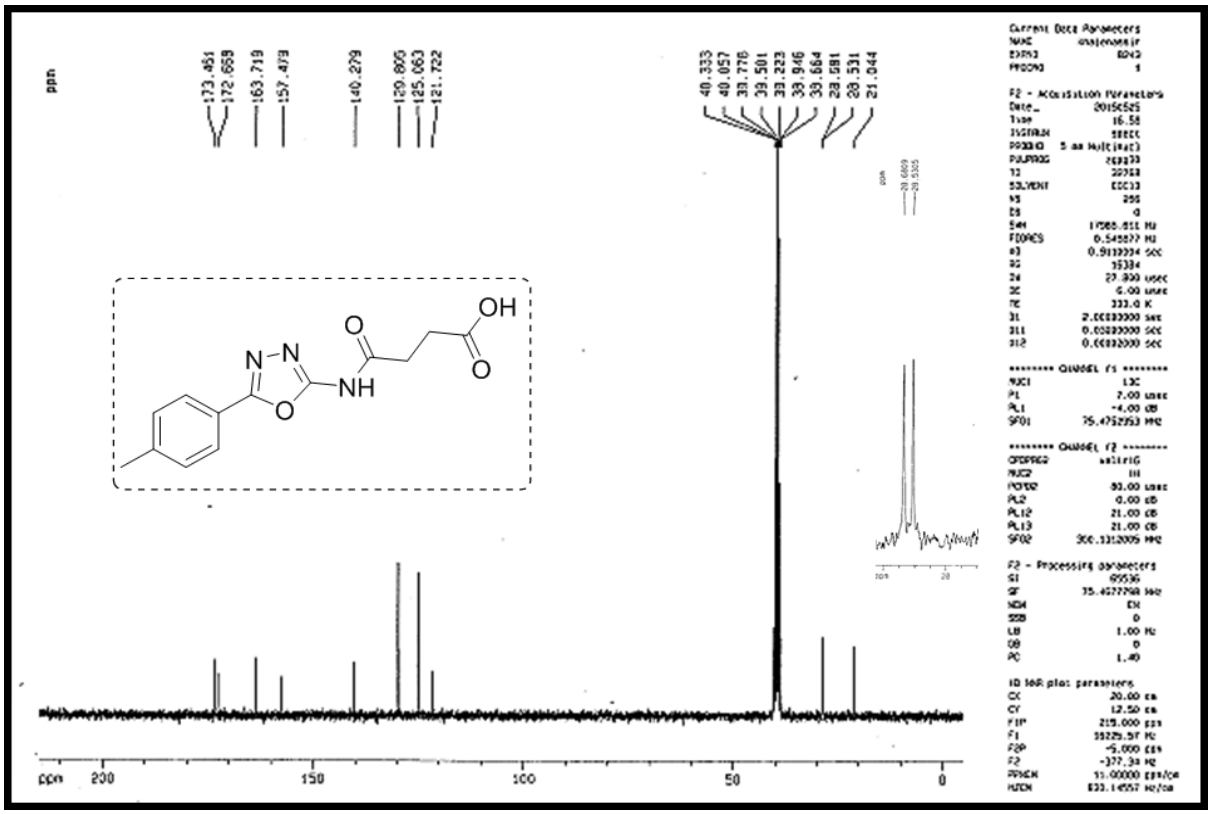
^{13}C-NMR of compound **(9f)** (75 MHz, DMSO-d_6)



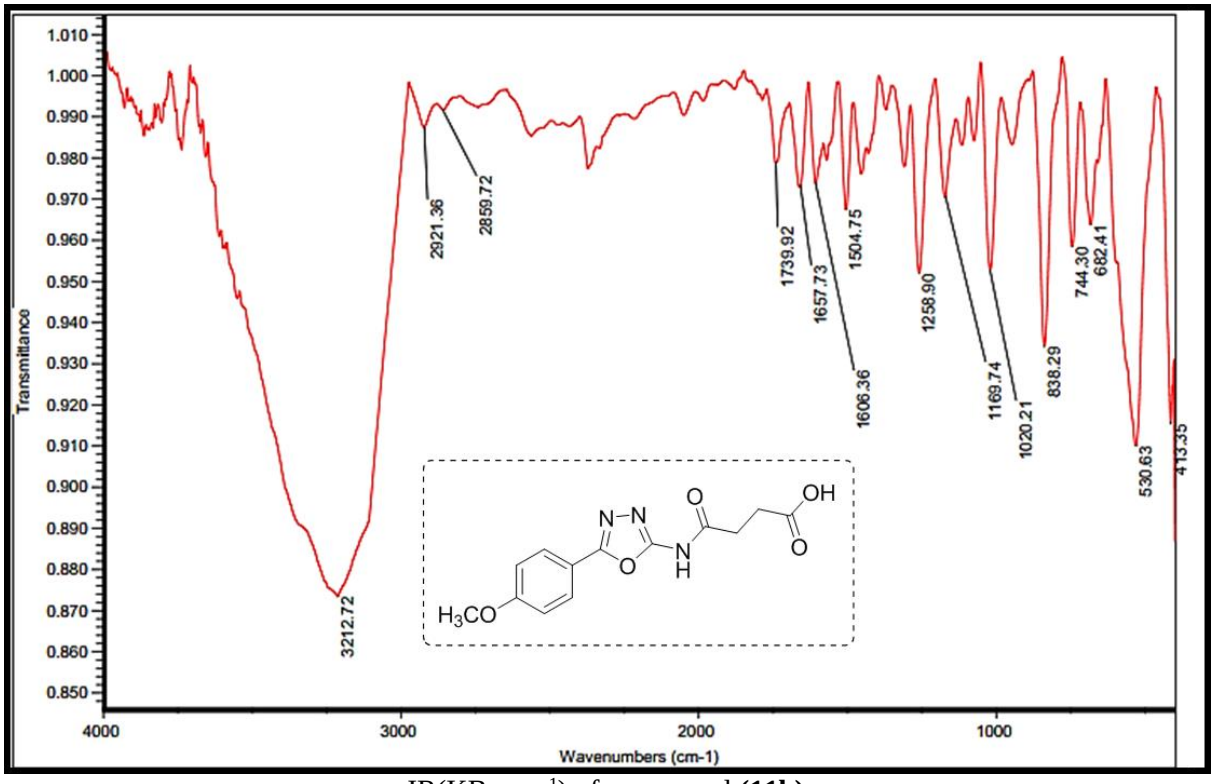
IR(KBr-cm^{-1}) of compound **(11a)**



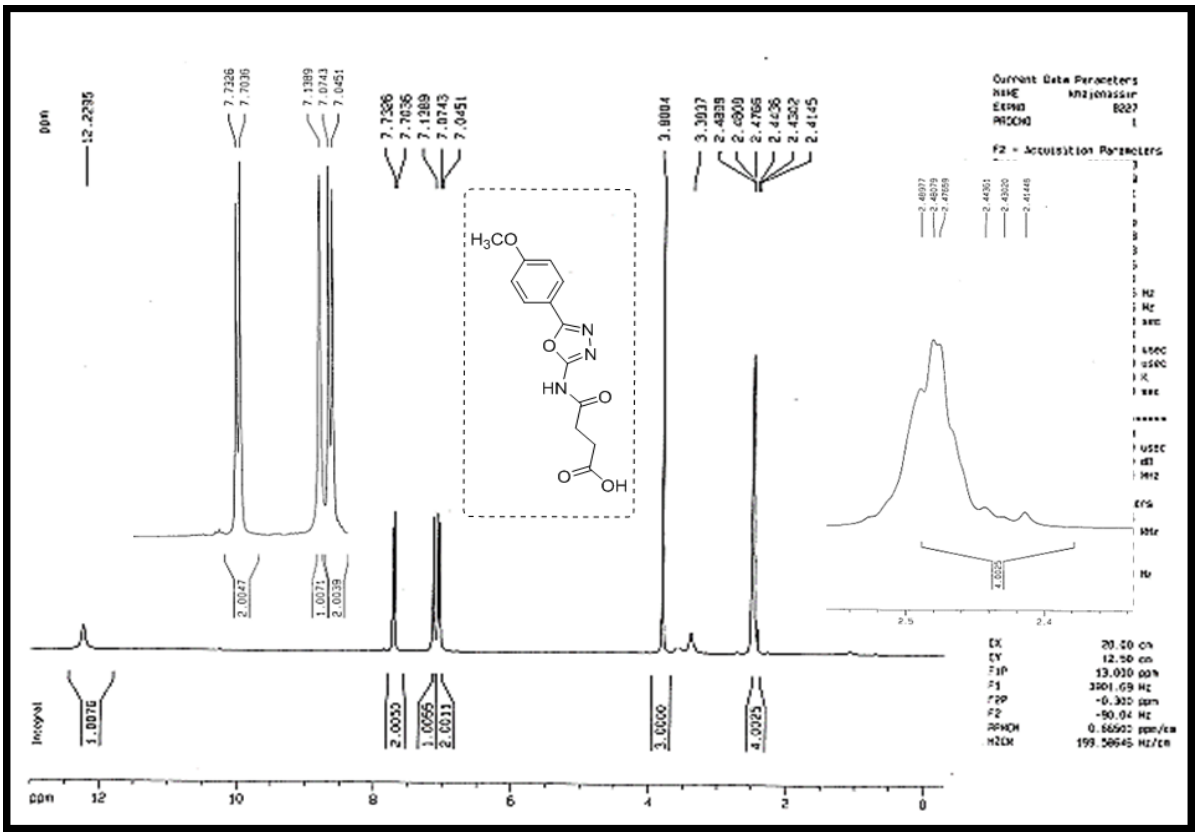
^{1}H-NMR of compound **(11a)** (300 MHz, DMSO-d_6)



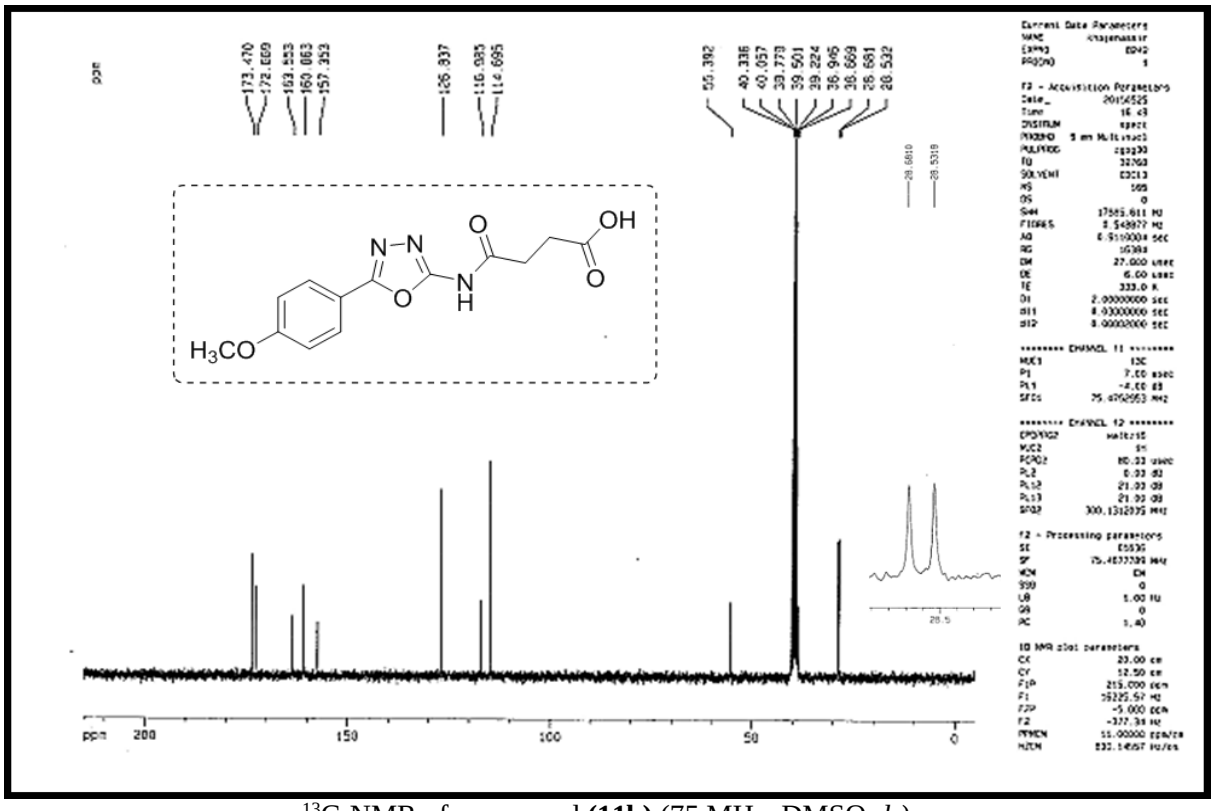
^{13}C-NMR of compound **(11a)** (75 MHz, DMSO-d_6)



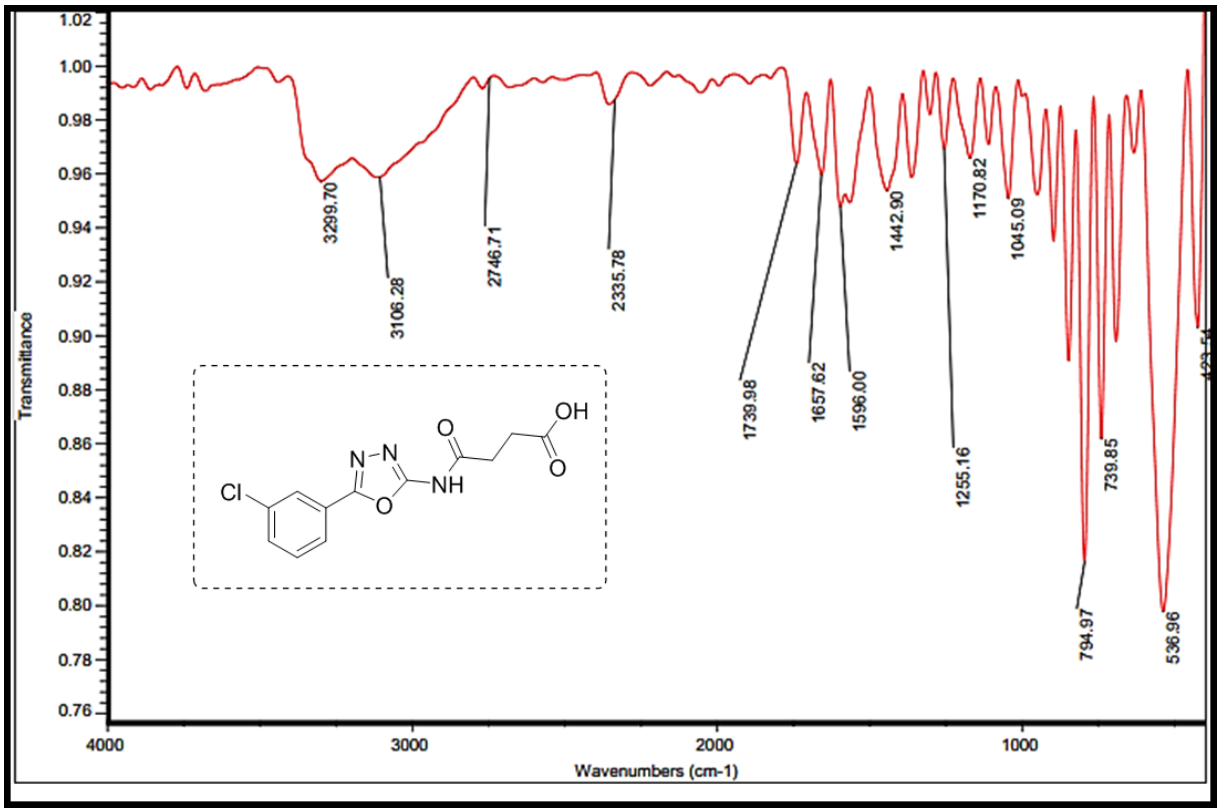
IR(KBr-cm^{-1}) of compound **(11b)**



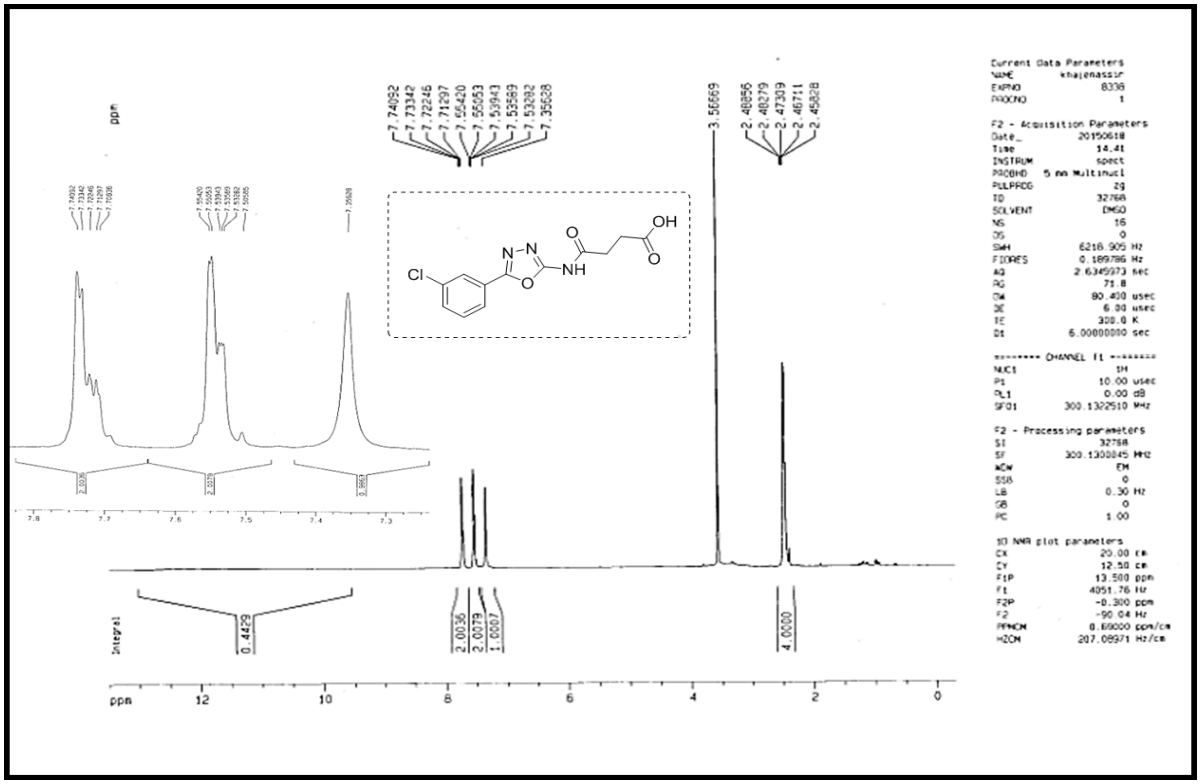
^{1}H-NMR of compound **(11b)** (300 MHz, DMSO-d_6)



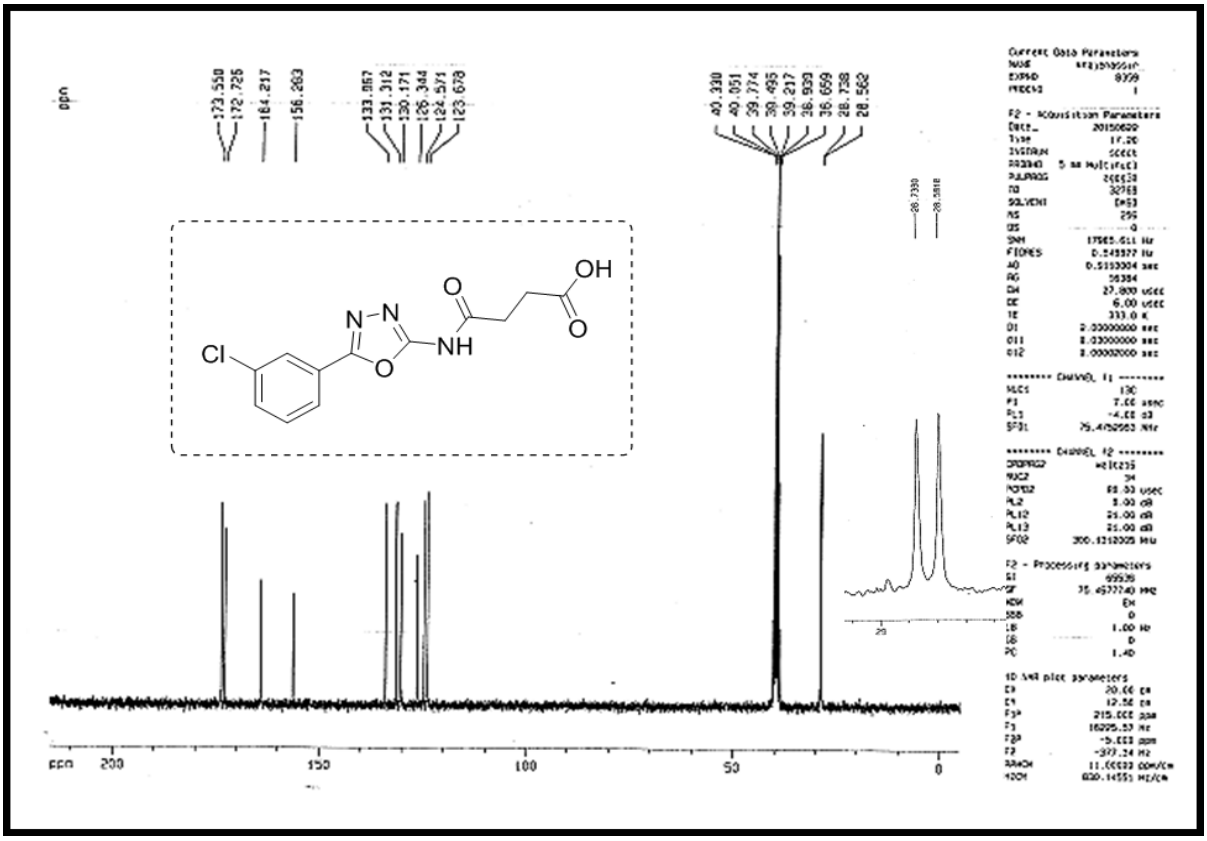
^{13}C-NMR of compound **(11b)** (75 MHz, DMSO-d_6)



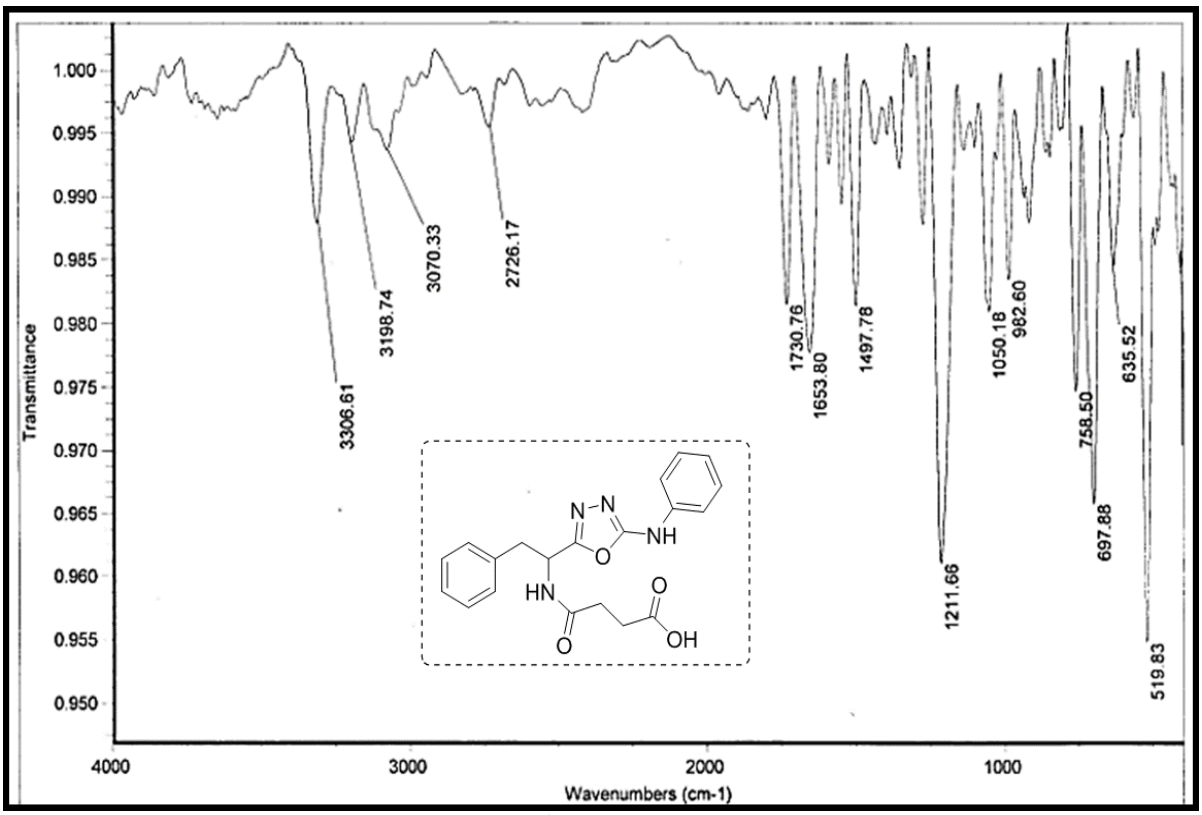
IR(KBr-cm^{-1}) of compound **(11c)**



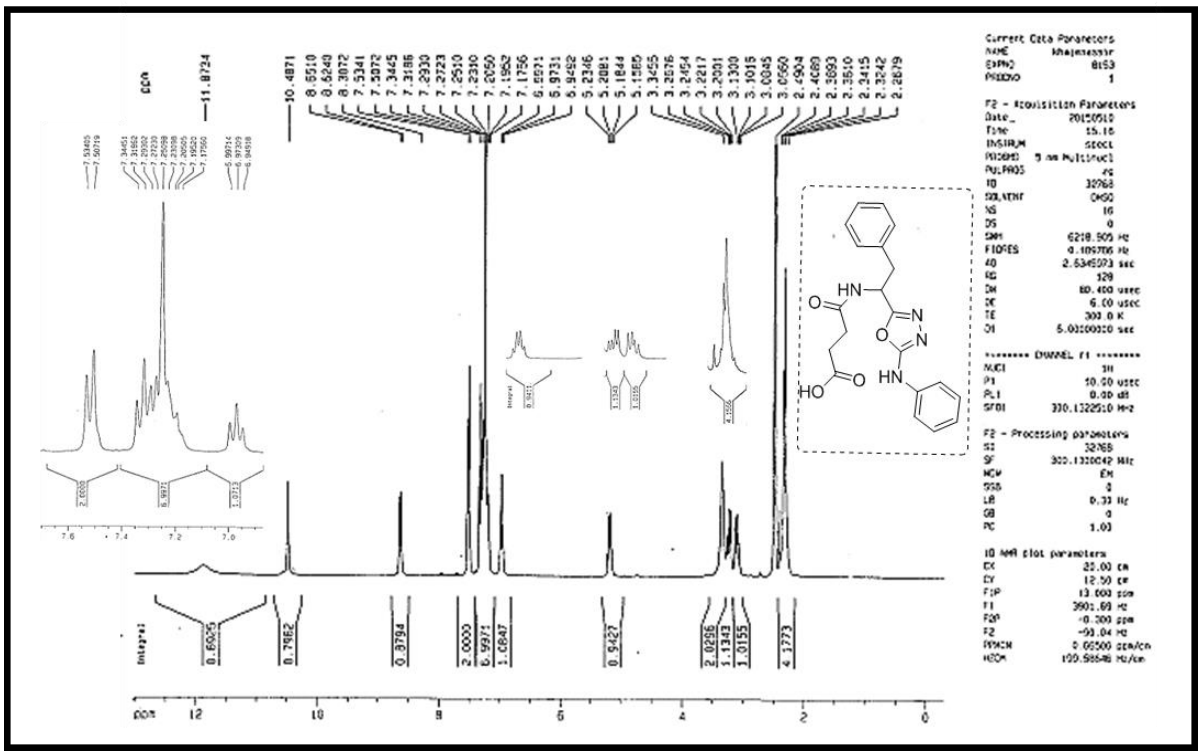
^{1}H-NMR of compound **(11c)** (300 MHz, DMSO-*d*$_6$)



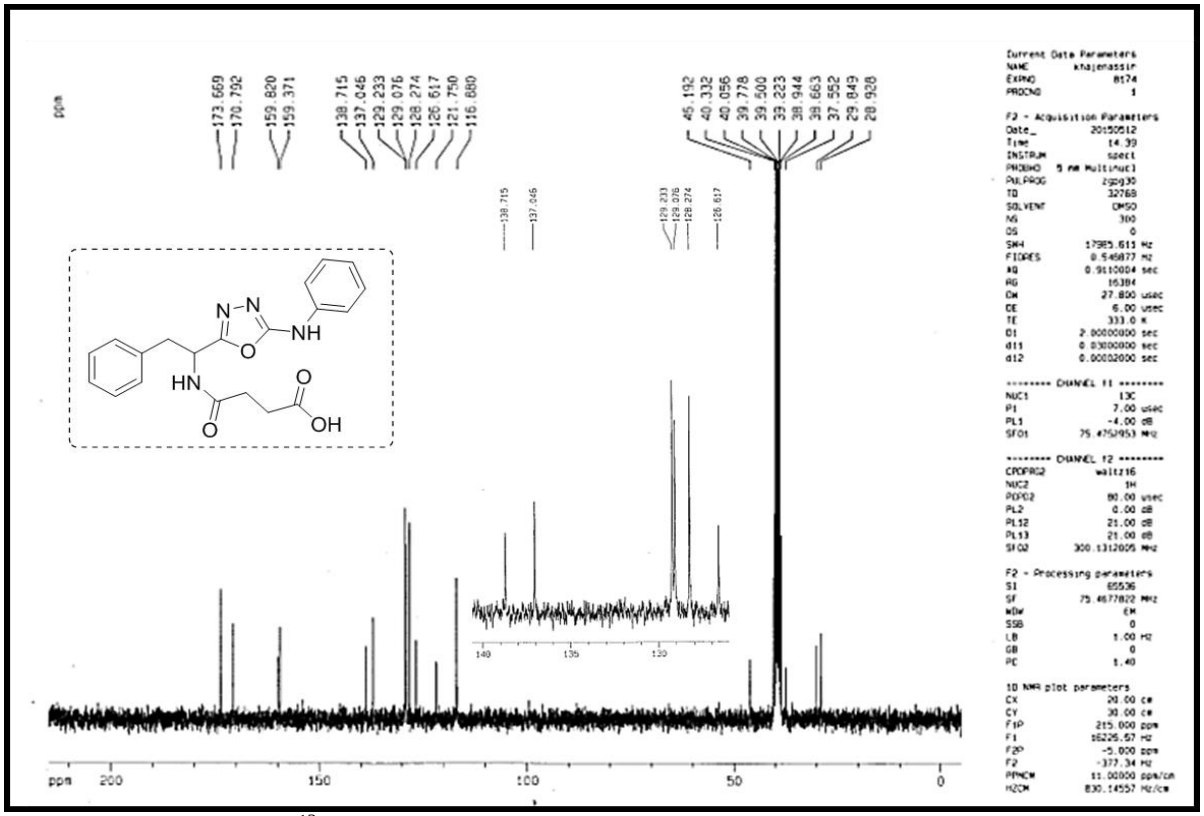
^{13}C-NMR of compound **(11c)** (75 MHz, DMSO-d_6)



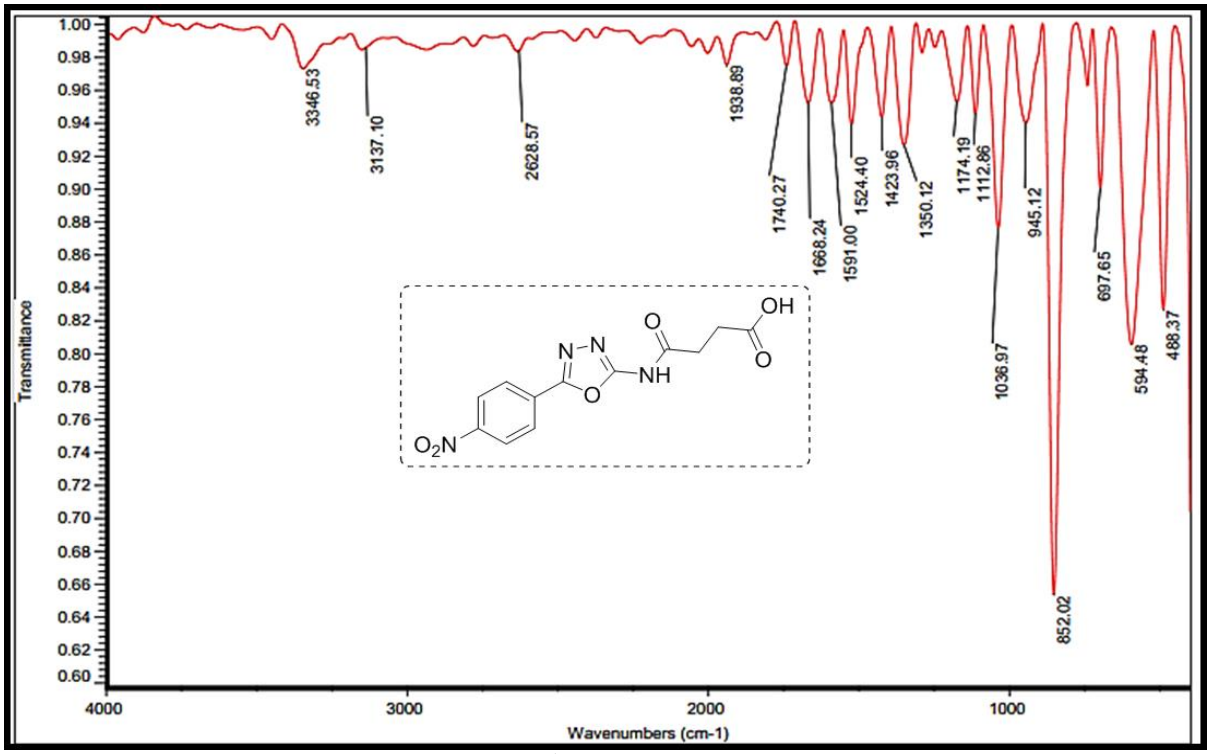
IR(KBr-cm^{-1}) of compound **(11d)**



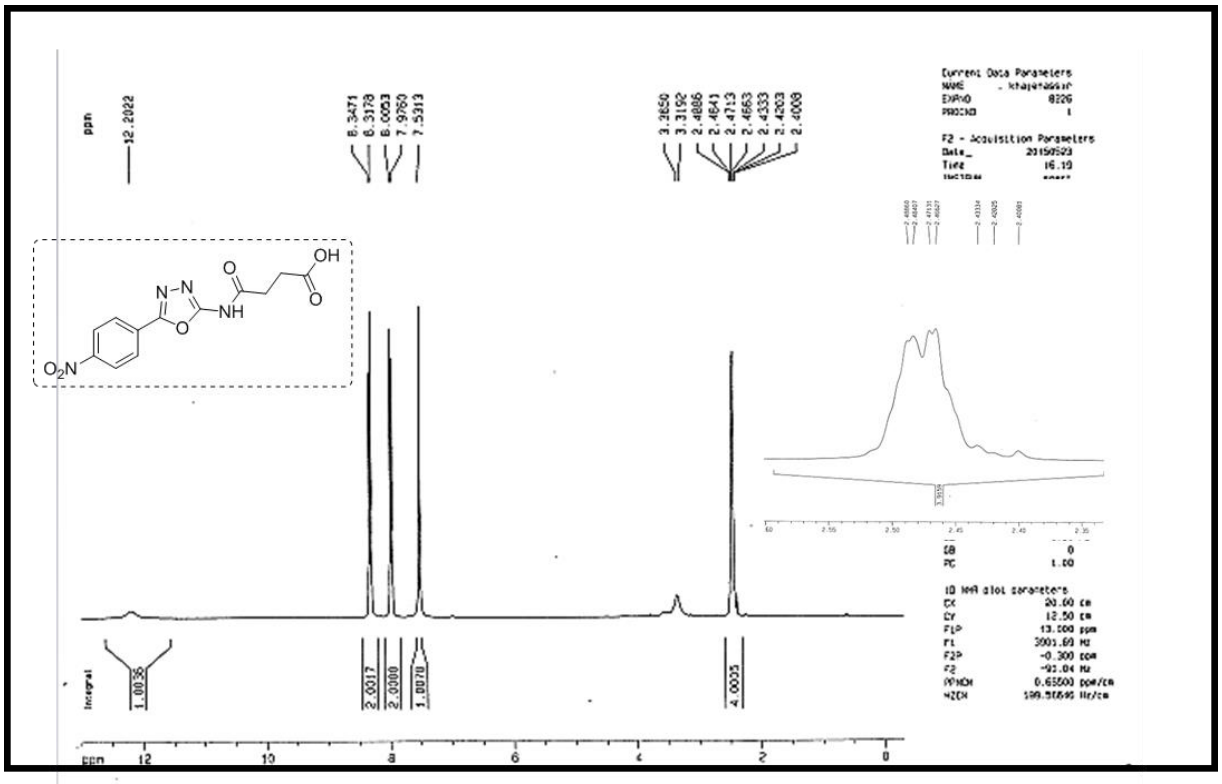
^{1}H-NMR of compound **(11d)** (300 MHz, DMSO-d_6)



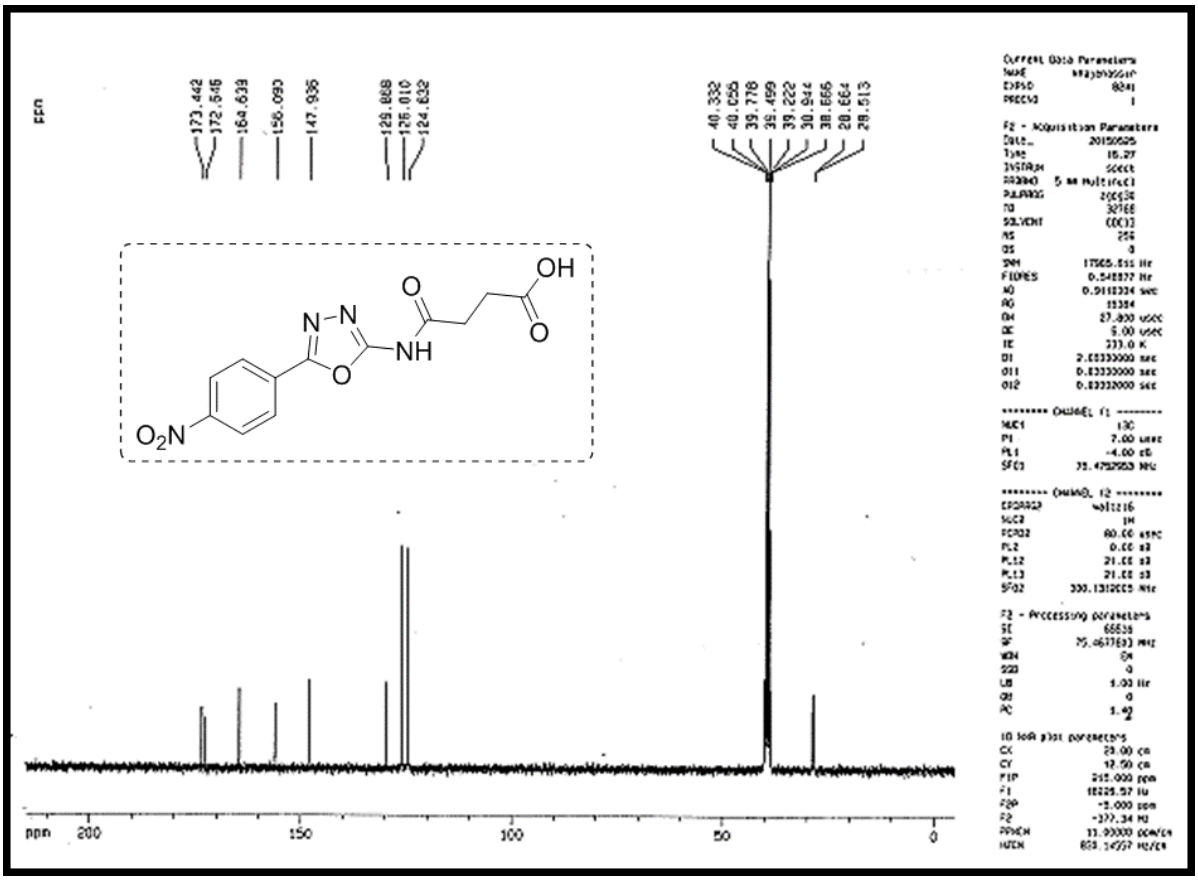
^{13}C-NMR of compound **(11d)** (75 MHz, DMSO-d_6)



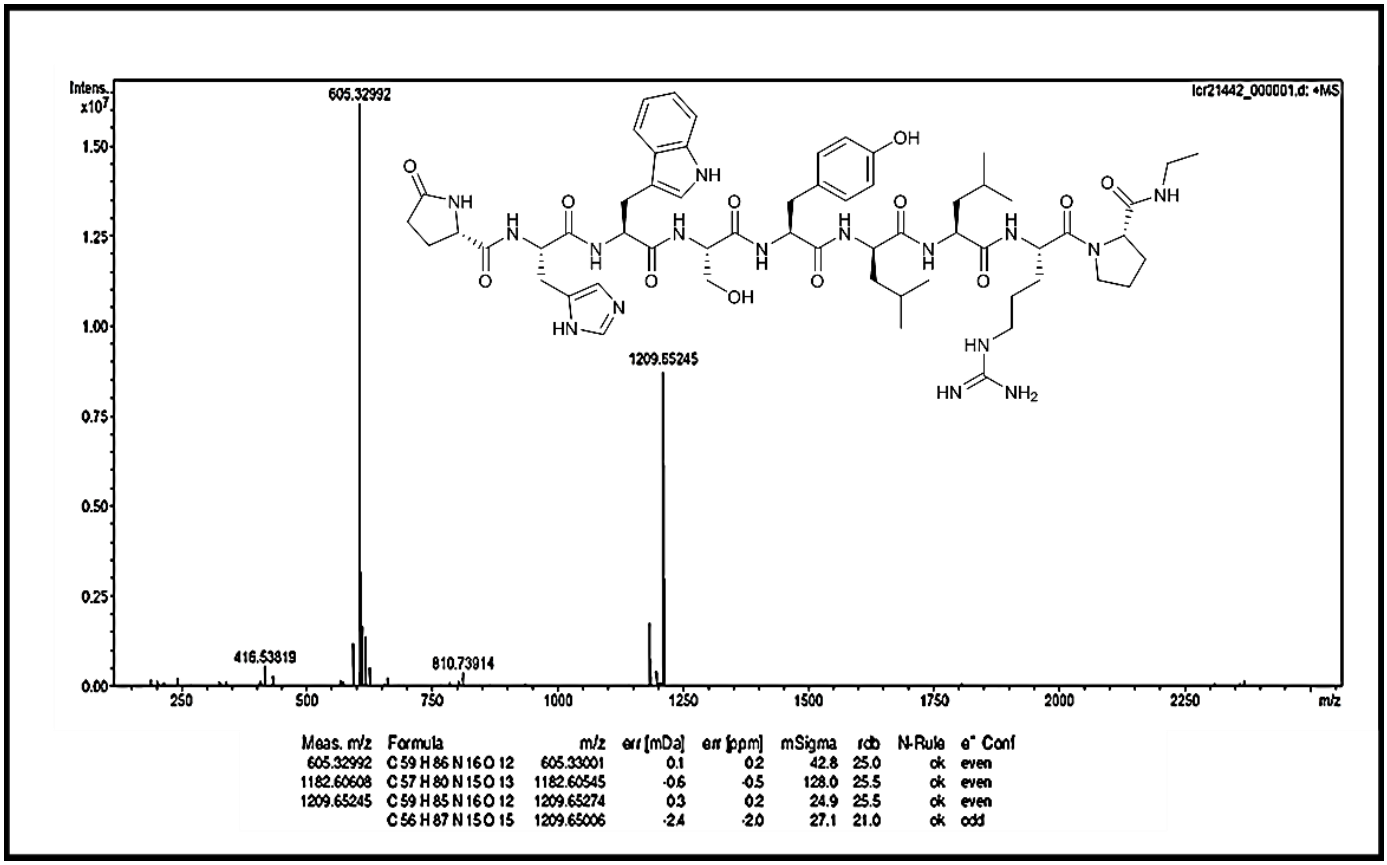
IR(KBr-cm^{-1}) of compound **(11e)**



^{1}H-NMR of compound **(11e)** (300 MHz, DMSO-d_6)

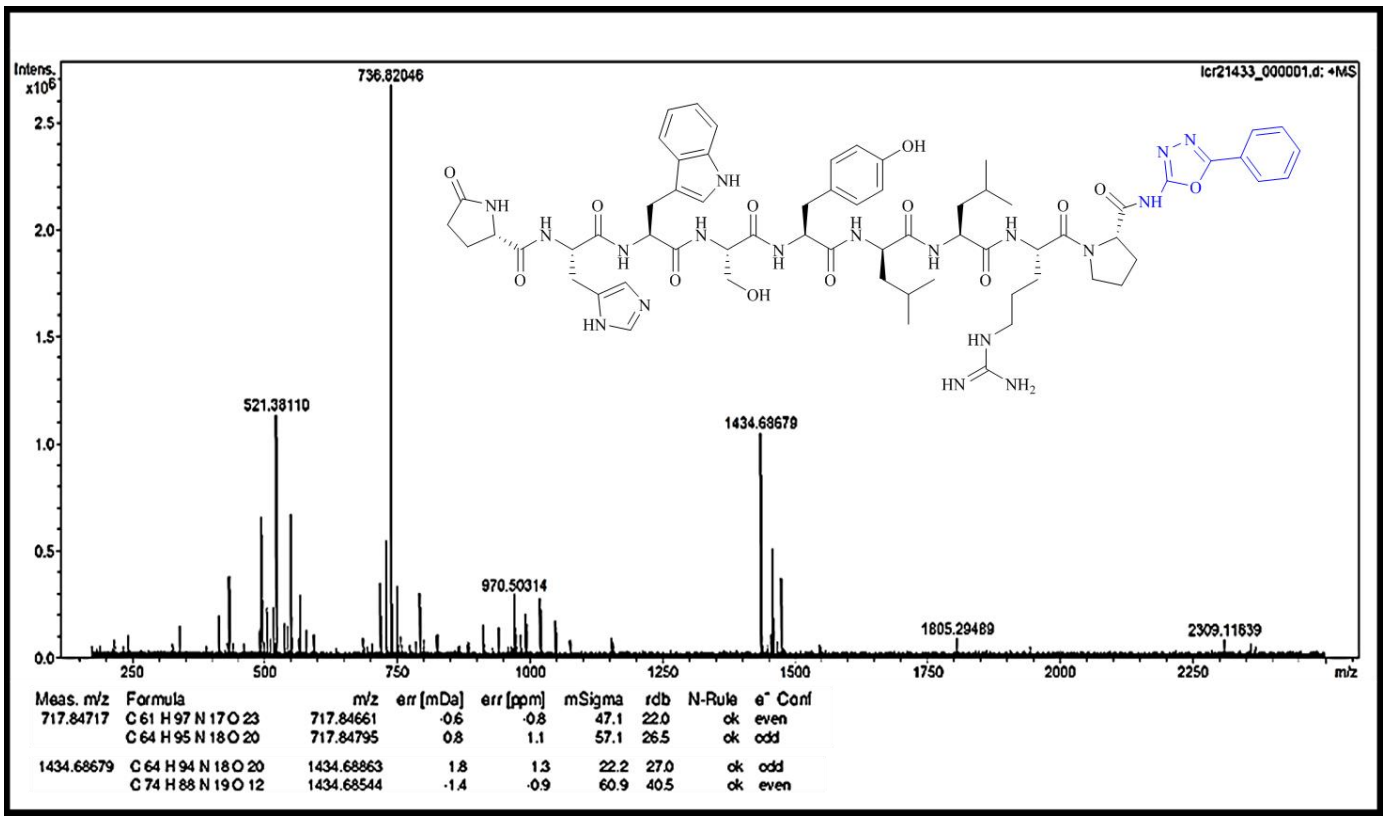


^{13}C-NMR of compound **(11e)** (75 MHz, DMSO-d_6)



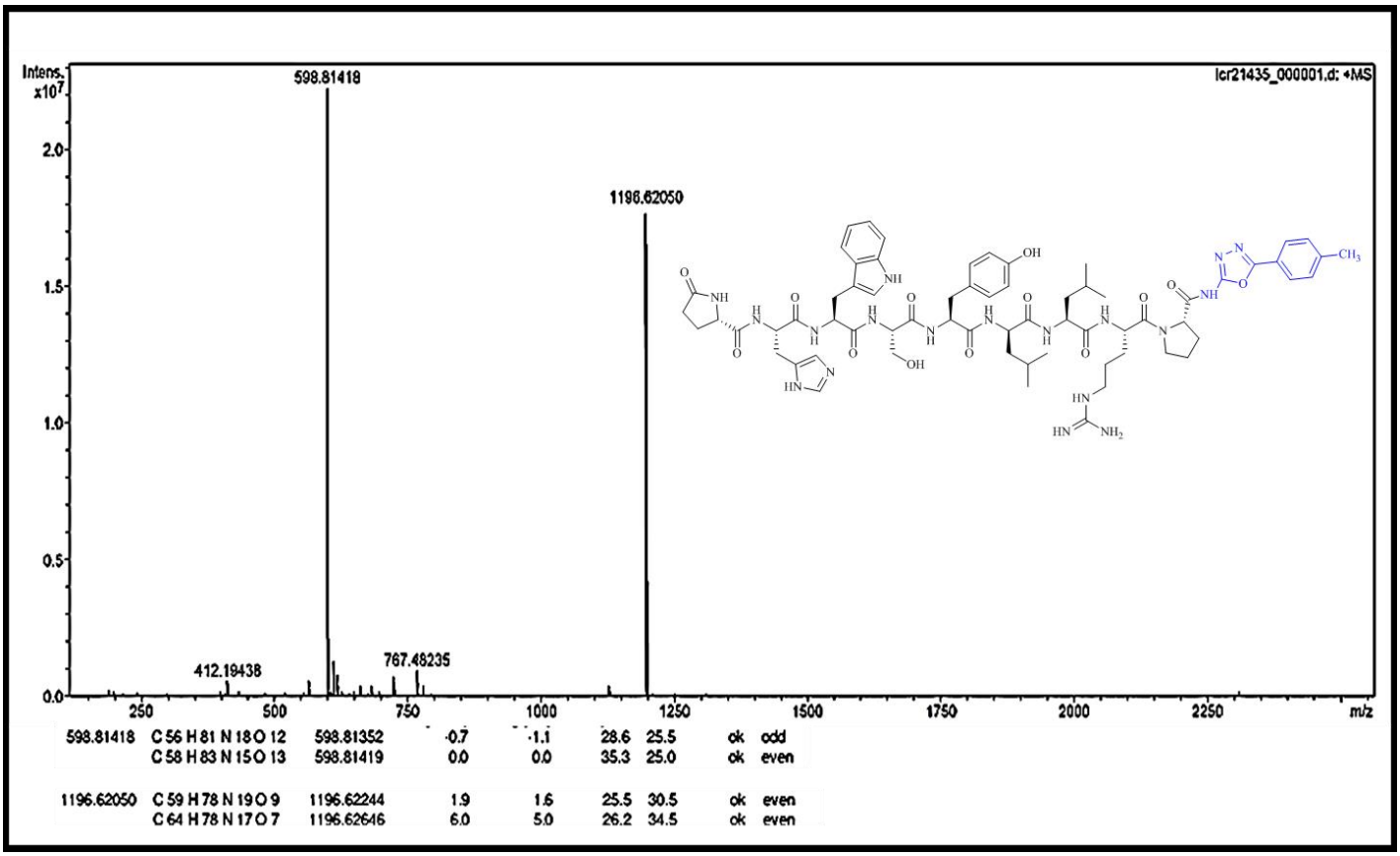
Meas. m/z	Formula	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
605.32992	C 59 H 86 N 16 O 12	605.33001	0.1	0.2	42.8	25.0	ok	even
1182.60608	C 57 H 80 N 15 O 13	1182.60545	-0.6	-0.5	128.0	25.5	ok	even
1209.65245	C 59 H 85 N 16 O 12	1209.65274	0.3	0.2	24.9	25.5	ok	even
	C 56 H 87 N 15 O 15	1209.65006	-2.4	-2.0	27.1	21.0	ok	odd

HR-MS (ESI) Leuprolide acetate

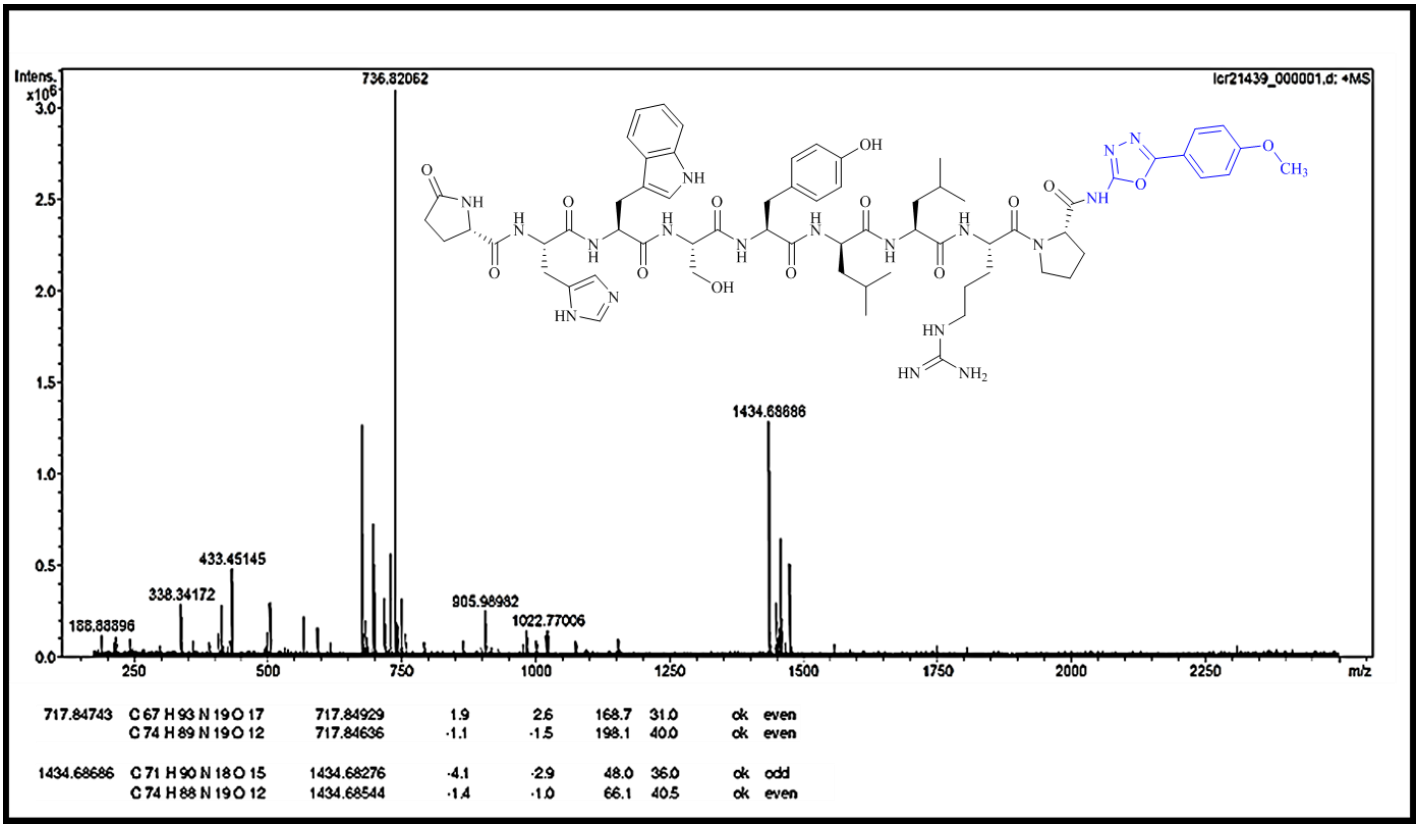


Meas. m/z	Formula	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
717.84717	C 61 H 97 N 17 O 23	717.84661	-0.6	-0.8	47.1	22.0	ok	even
	C 64 H 95 N 18 O 20	717.84795	0.8	1.1	57.1	26.5	ok	odd
1434.68679	C 64 H 94 N 18 O 20	1434.68863	1.8	1.3	22.2	27.0	ok	odd
	C 74 H 88 N 19 O 12	1434.68544	-1.4	-0.9	60.9	40.5	ok	even

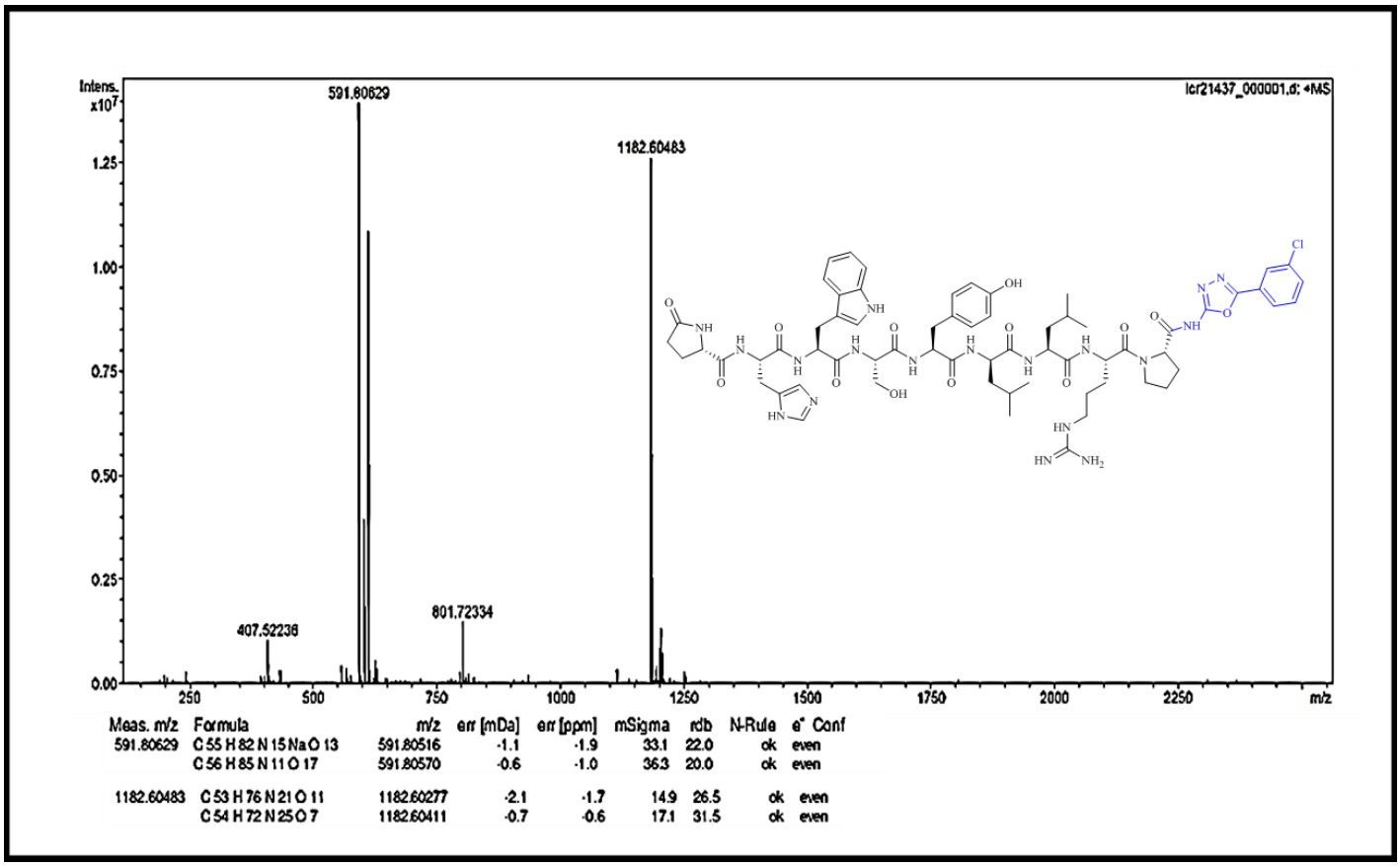
HR-MS (ESI) Leuprolide analogues **(2a)**



HR-MS (ESI) Leuprolide analogues **(2b)**

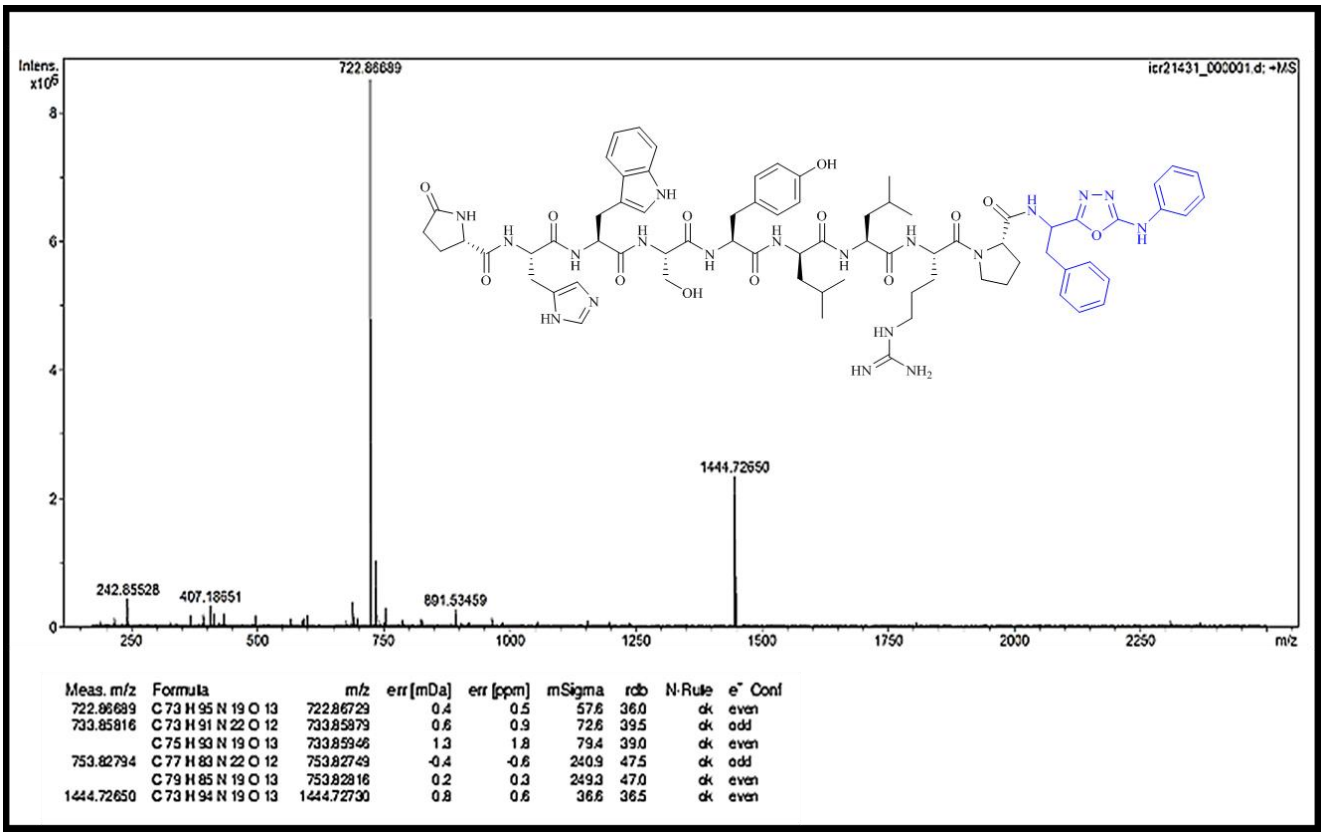


HR-MS (ESI) Leuprolide analogues **(2c)**



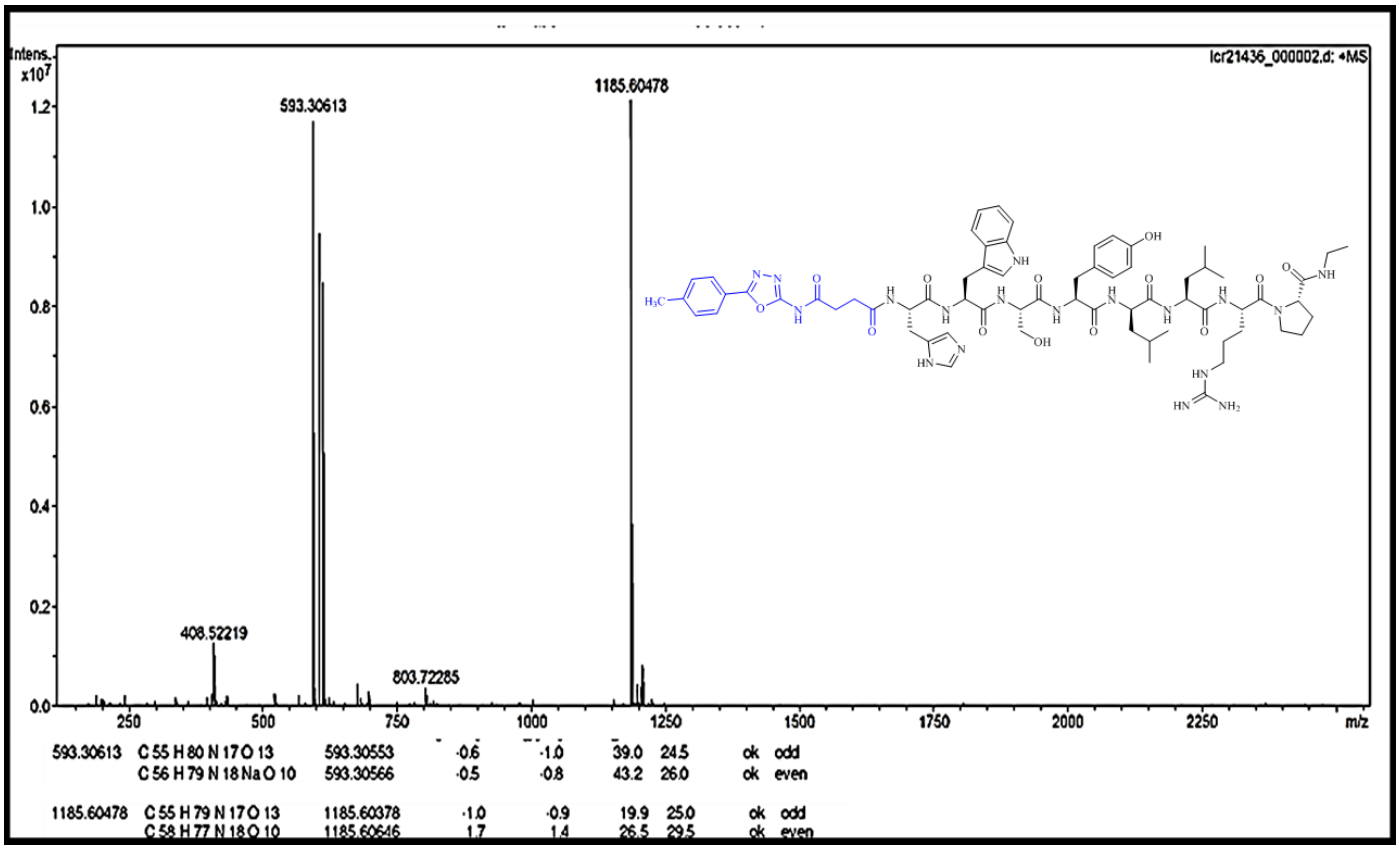
Meas. m/z	Formula	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
591.80629	C 55 H 82 N 15 Na O 13	591.80516	-1.1	-1.9	33.1	22.0	ok	even
	C 56 H 85 N 11 O 17	591.80570	-0.6	-1.0	36.3	20.0	ok	even
1182.60483	C 53 H 76 N 21 O 11	1182.60277	-2.1	-1.7	14.9	26.5	ok	even
	C 54 H 72 N 25 O 7	1182.60411	-0.7	-0.6	17.1	31.5	ok	even

HR-MS (ESI) Leuprolide analogues **(2d)**

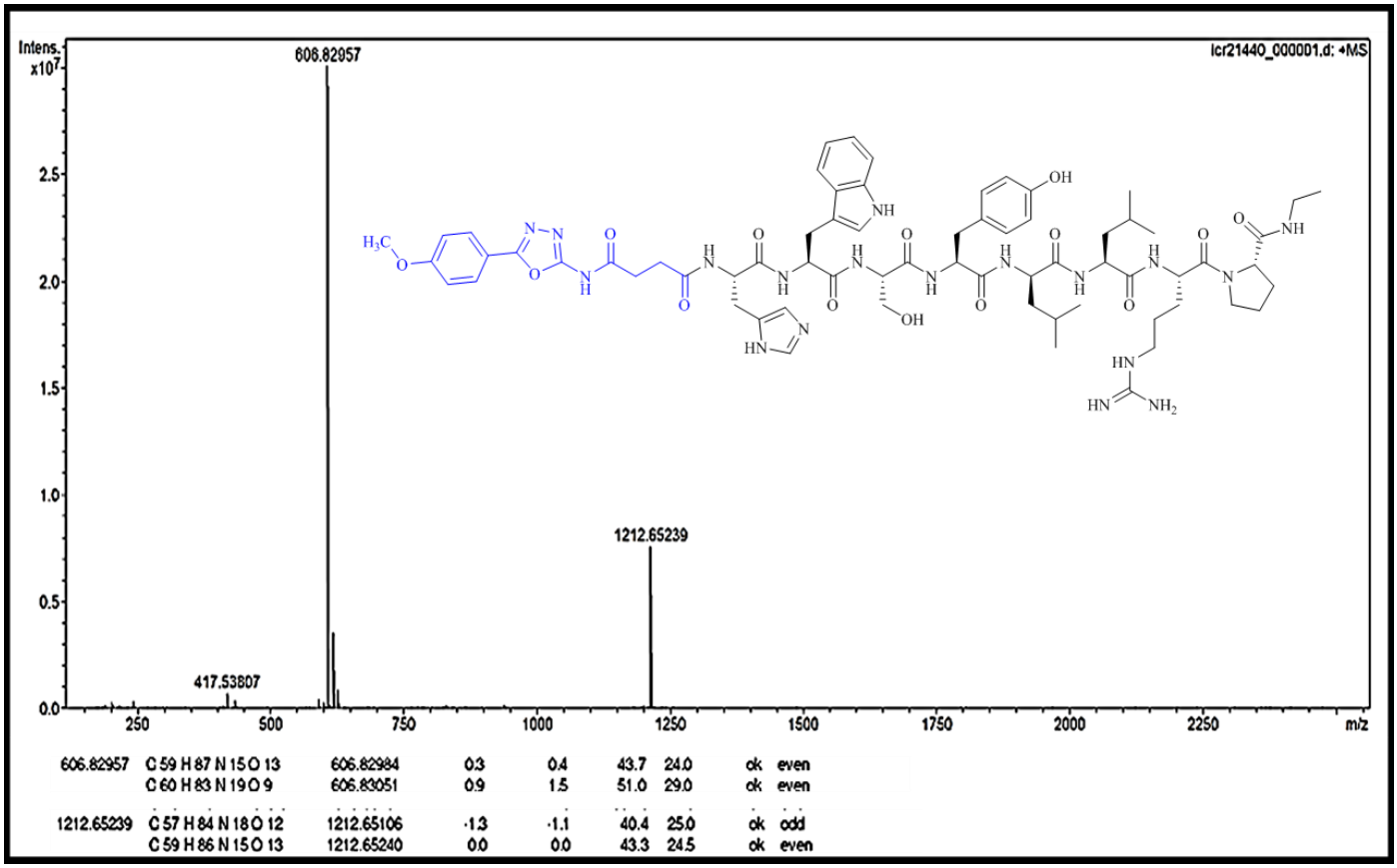


Meas. m/z	Formula	m/z	err [mDa]	err [ppm]	mSigma	rdb	N-Rule	e⁻ Conf
722.86689	C 73 H 95 N 19 O 13	722.86729	0.4	0.5	57.6	36.0	ok	even
733.85816	C 73 H 91 N 22 O 12	733.85879	0.6	0.9	72.6	39.5	ok	odd
	C 75 H 93 N 19 O 13	733.85946	1.3	1.8	79.4	39.0	ok	even
753.82794	C 77 H 83 N 22 O 12	753.82749	-0.4	-0.6	240.9	47.5	ok	odd
	C 79 H 85 N 19 O 13	753.82816	0.2	0.3	249.3	47.0	ok	even
1444.72650	C 73 H 94 N 19 O 13	1444.72730	0.8	0.6	36.6	36.5	ok	even

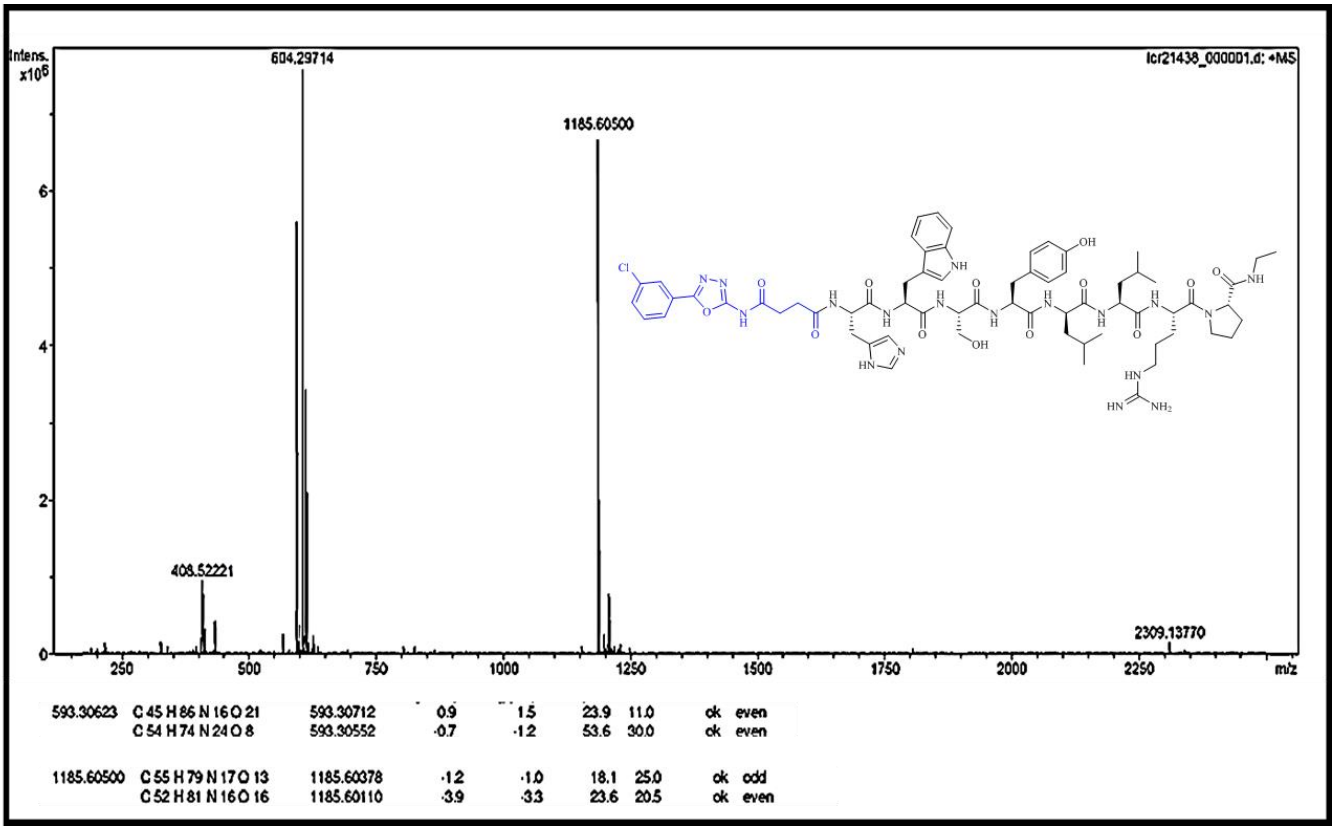
HR-MS (ESI) Leuprolide analogues **(2e)**



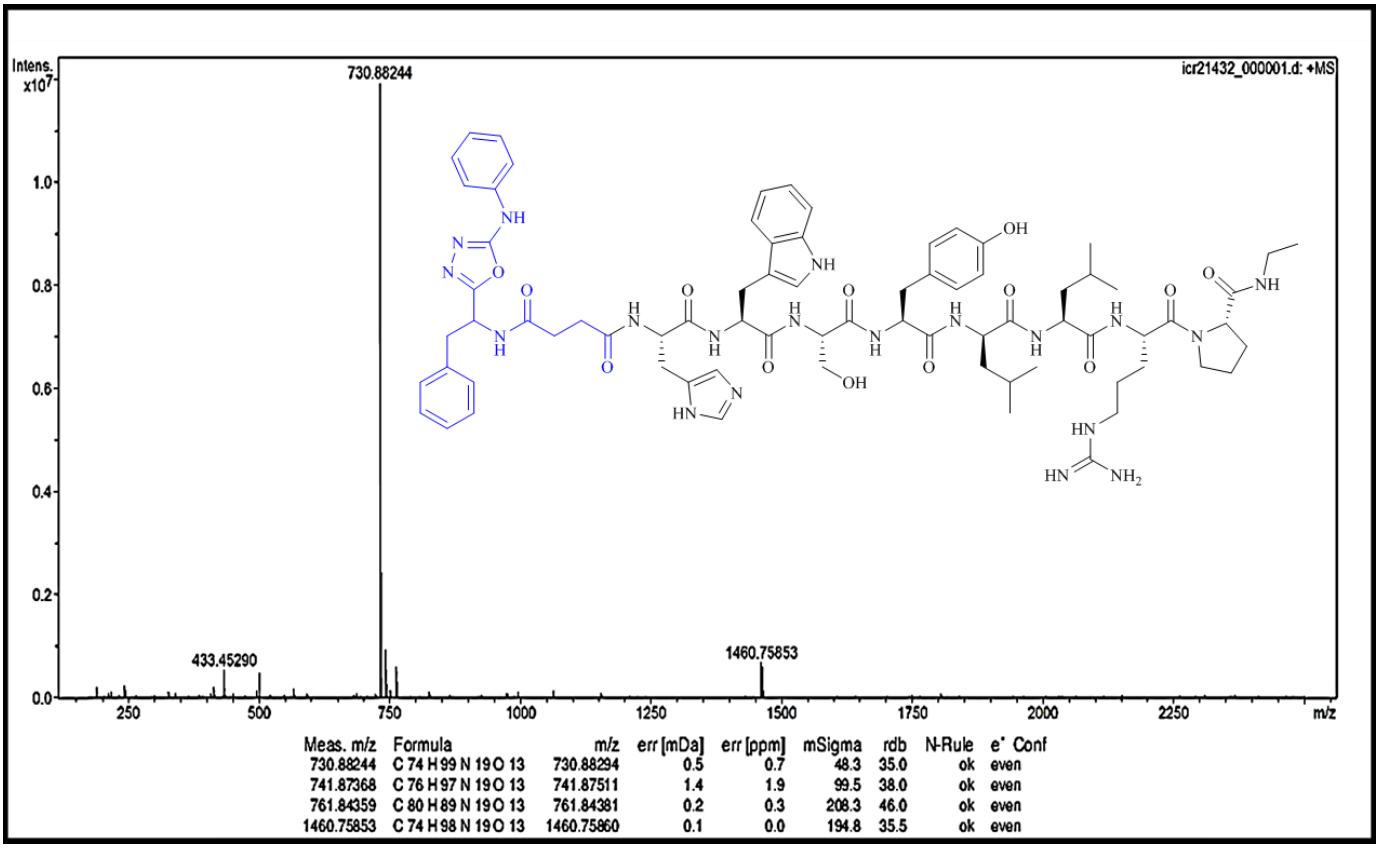
HR-MS (ESI) Leuprolide analogues **(3a)**



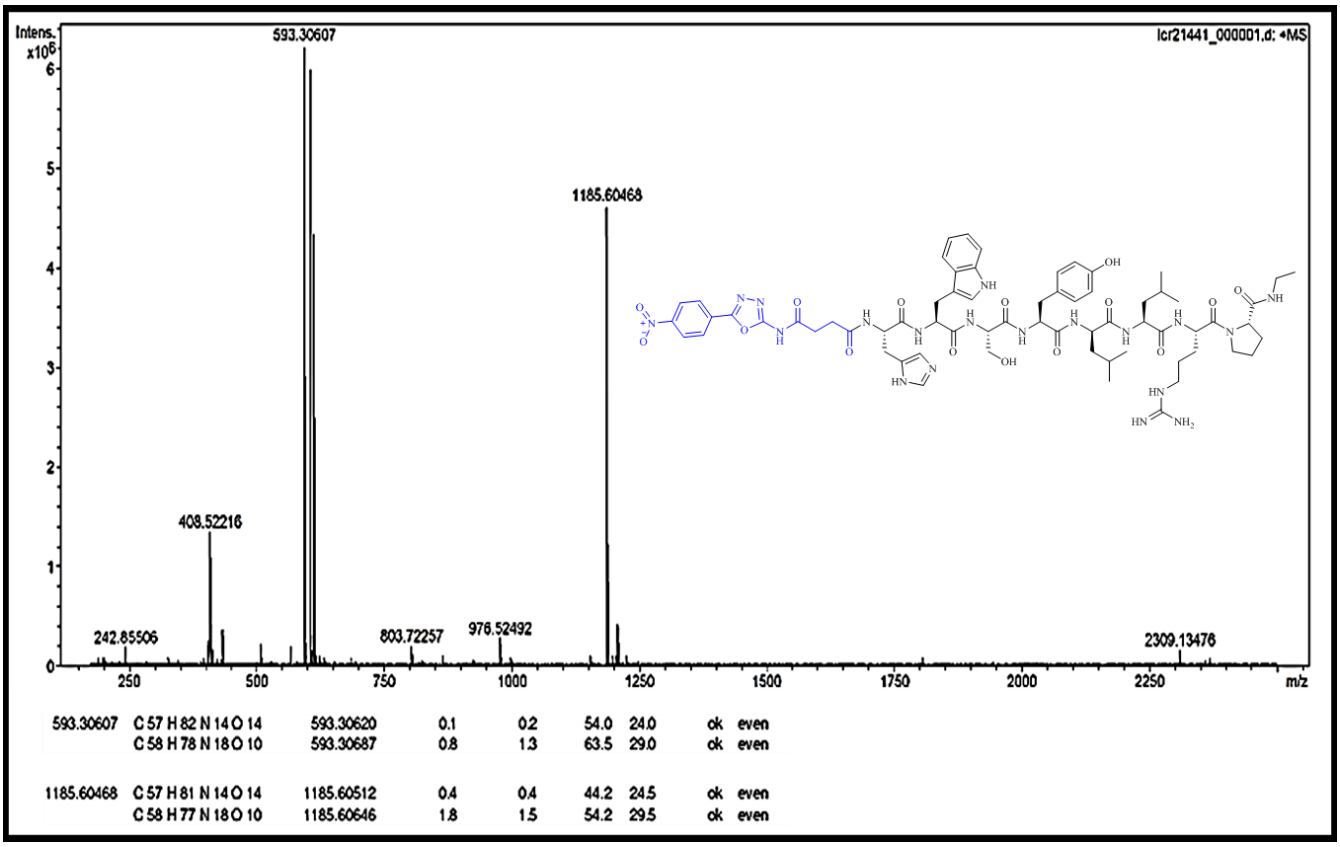
HR-MS (ESI) Leuprolide analogues **(3b)**



HR-MS (ESI) Leuprolide analogues **(3c)**

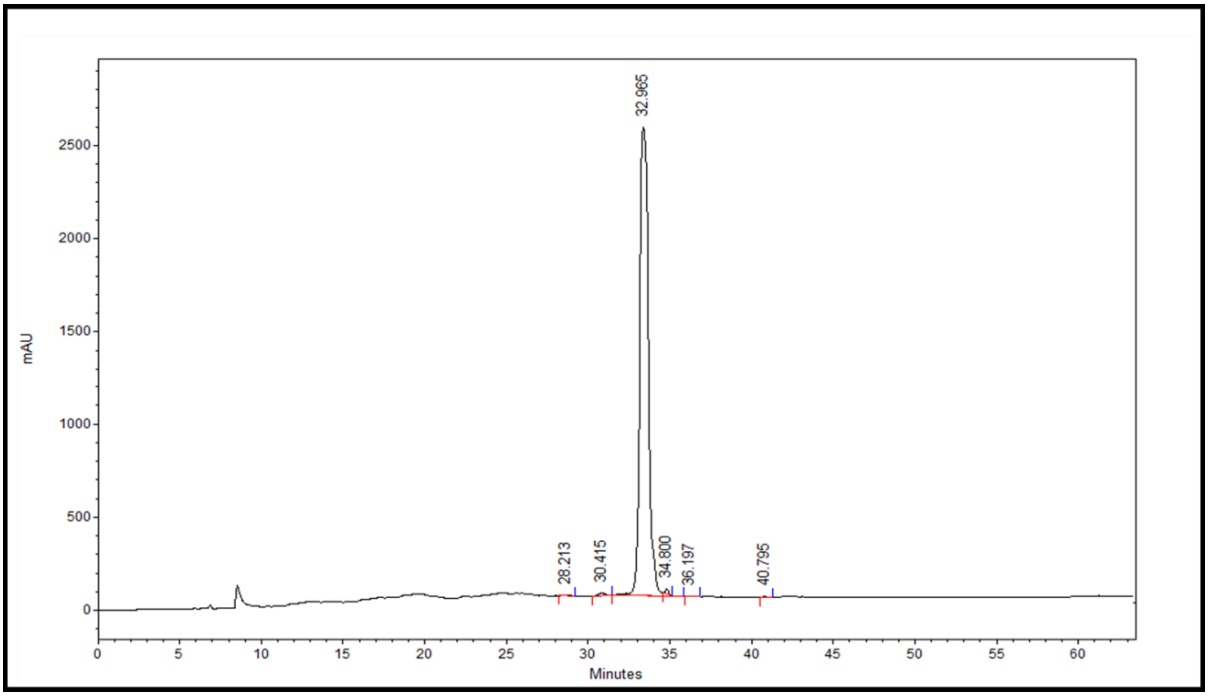


HR-MS (ESI) Leuprolide analogues **(3d)**

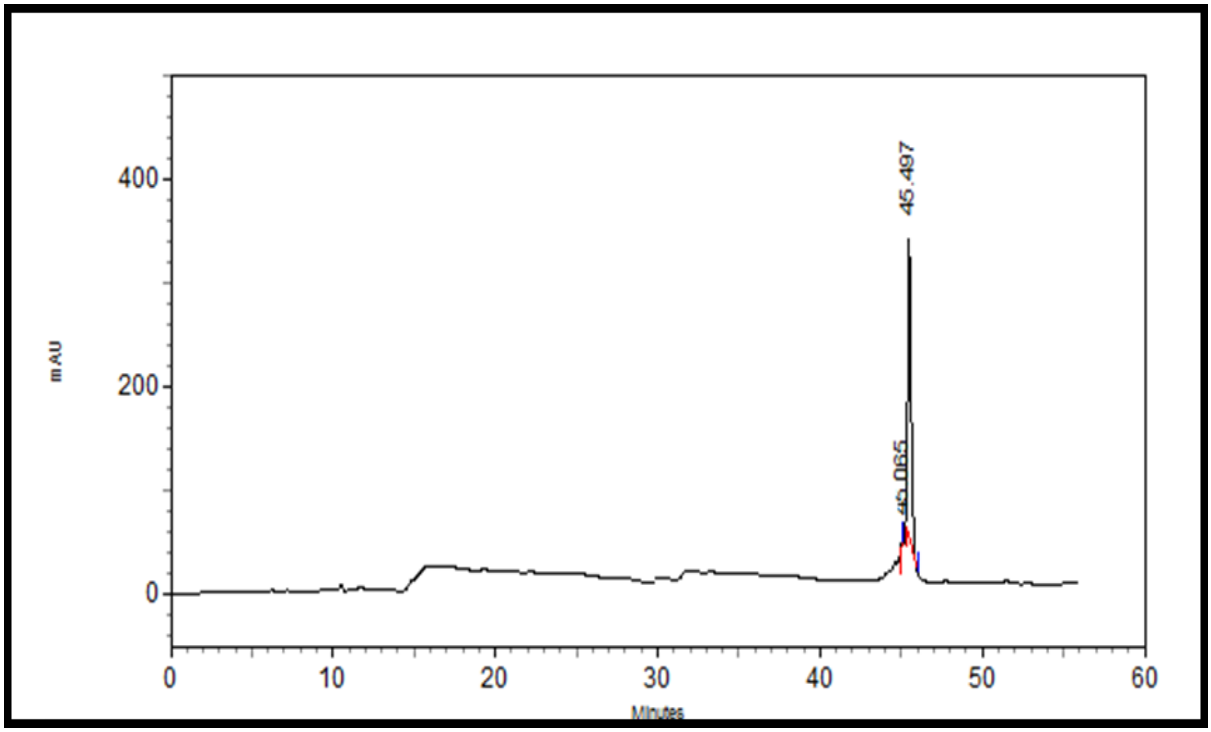


593.30607	C 57 H 82 N 14 O 14	593.30620	0.1	0.2	54.0	24.0	ok even
	C 58 H 78 N 18 O 10	593.30687	0.8	1.3	63.5	29.0	ok even
1185.60468	C 57 H 81 N 14 O 14	1185.60512	0.4	0.4	44.2	24.5	ok even
	C 58 H 77 N 18 O 10	1185.60646	1.8	1.5	54.2	29.5	ok even

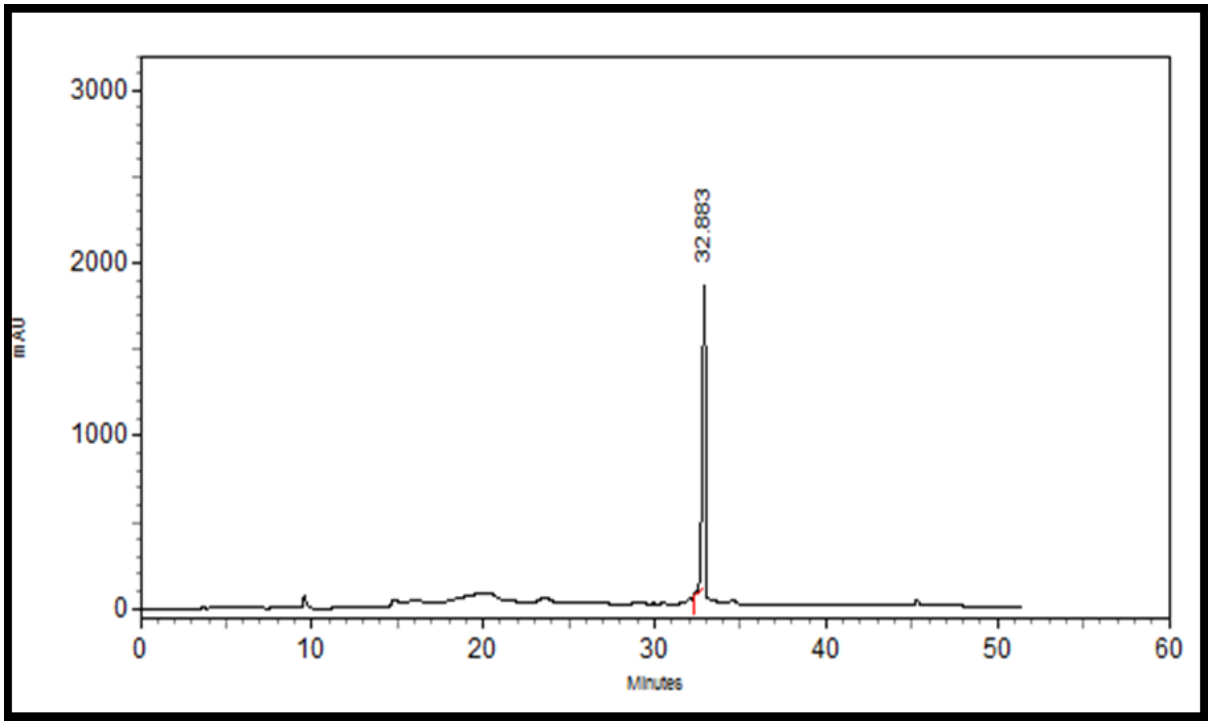
HR-MS (ESI) Leuprolide analogues **(3e)**



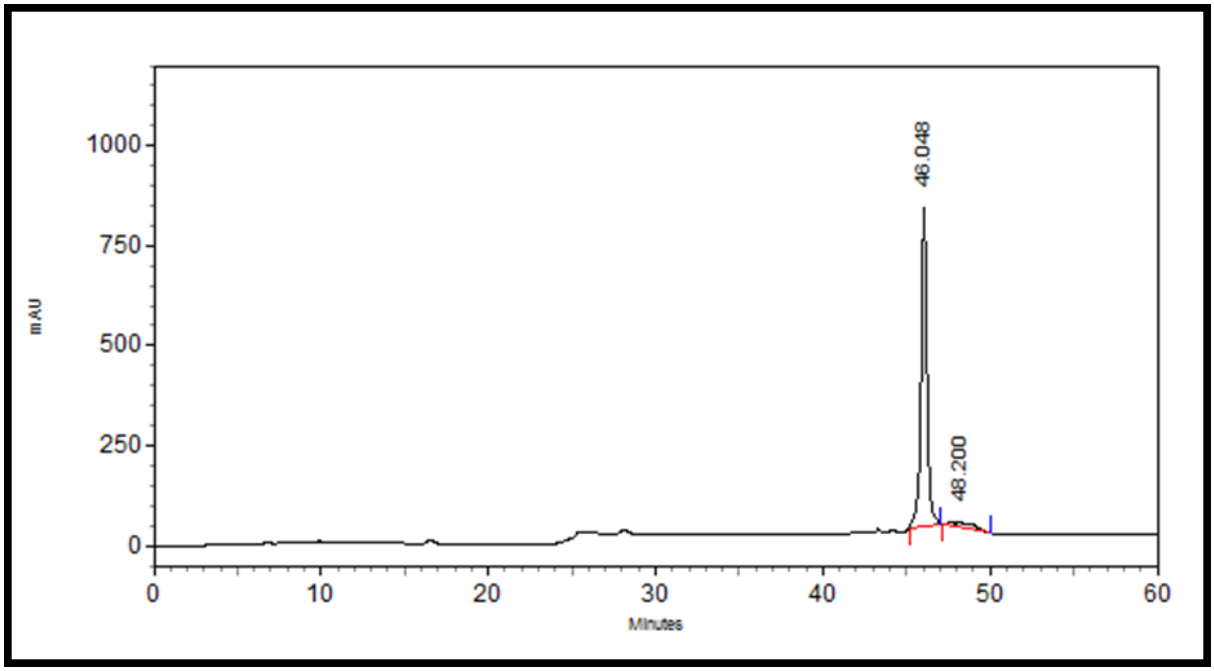
Analytical HPLC chromatogram of leuprolide acetate



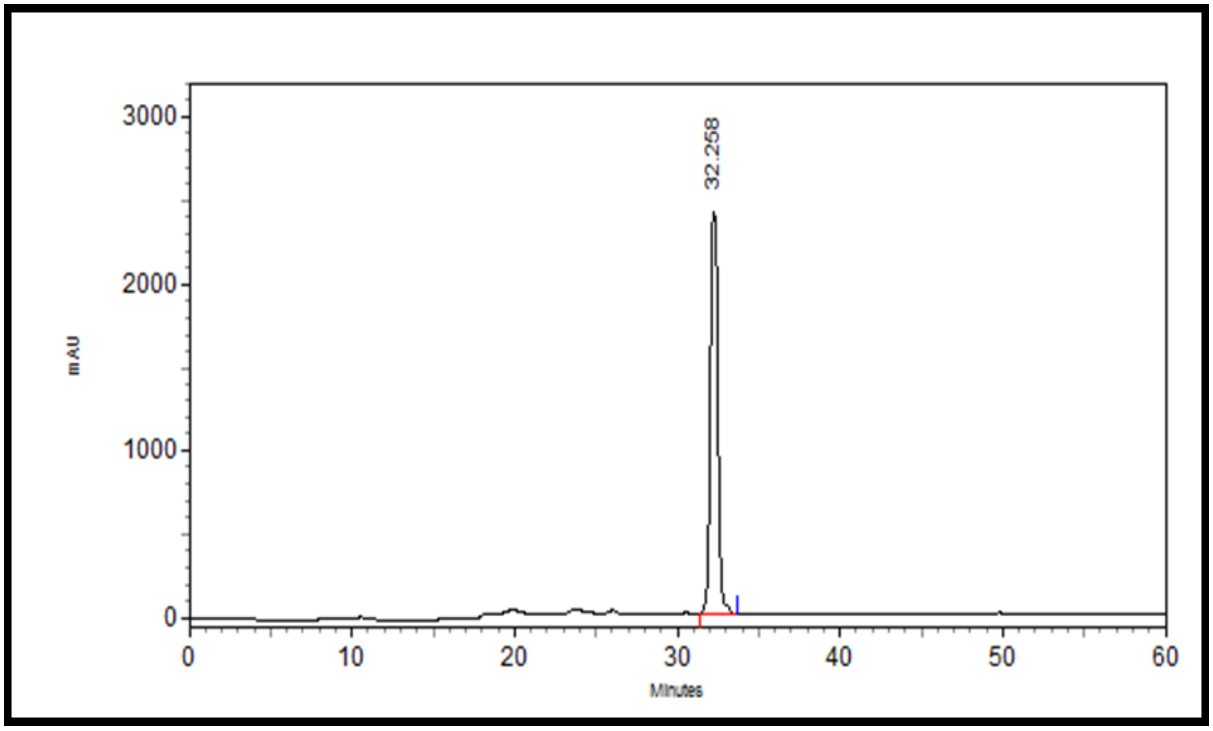
Analytical HPLC chromatogram of leuprolide analogues **(2a)**



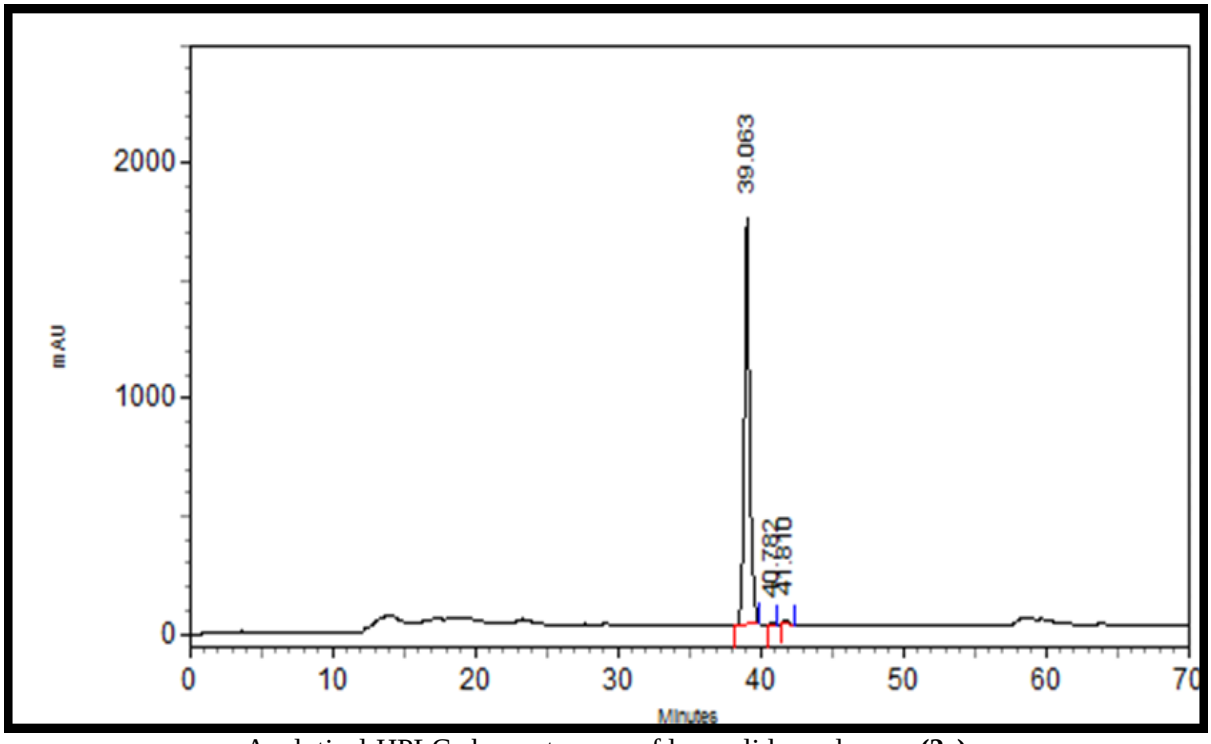
Analytical HPLC chromatogram of leuprolide analogues **(2b)**



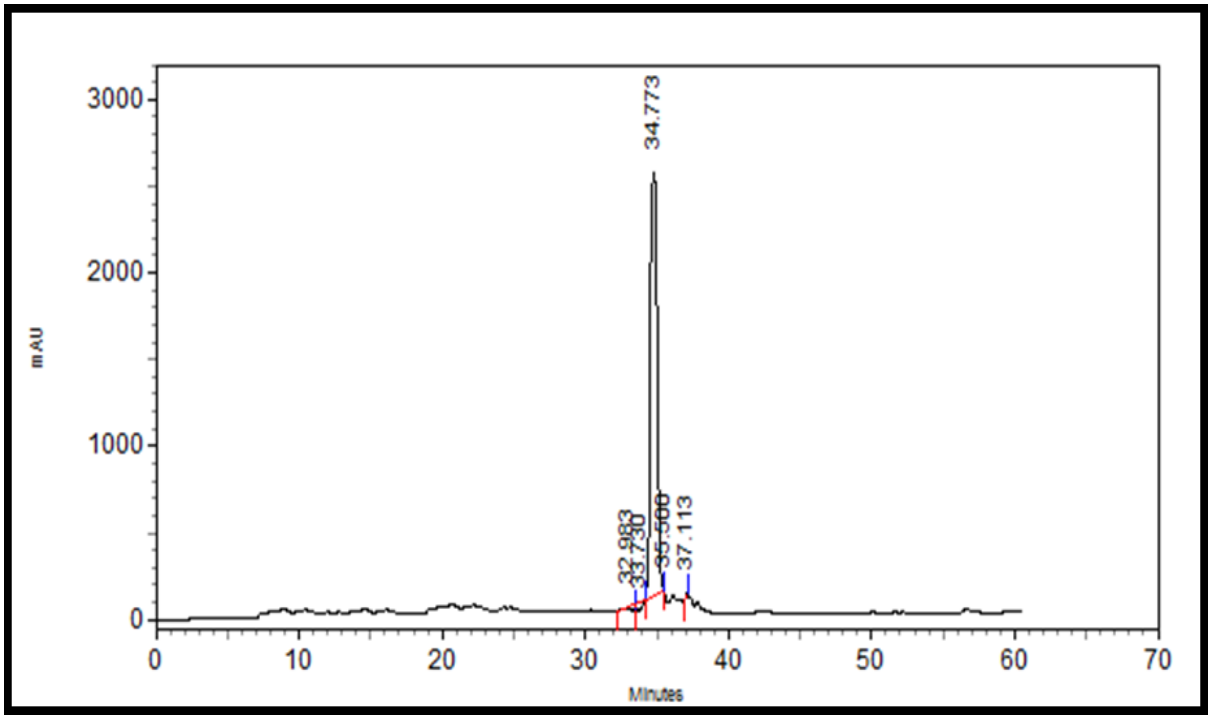
Analytical HPLC chromatogram of leuprolide analogues **(2c)**



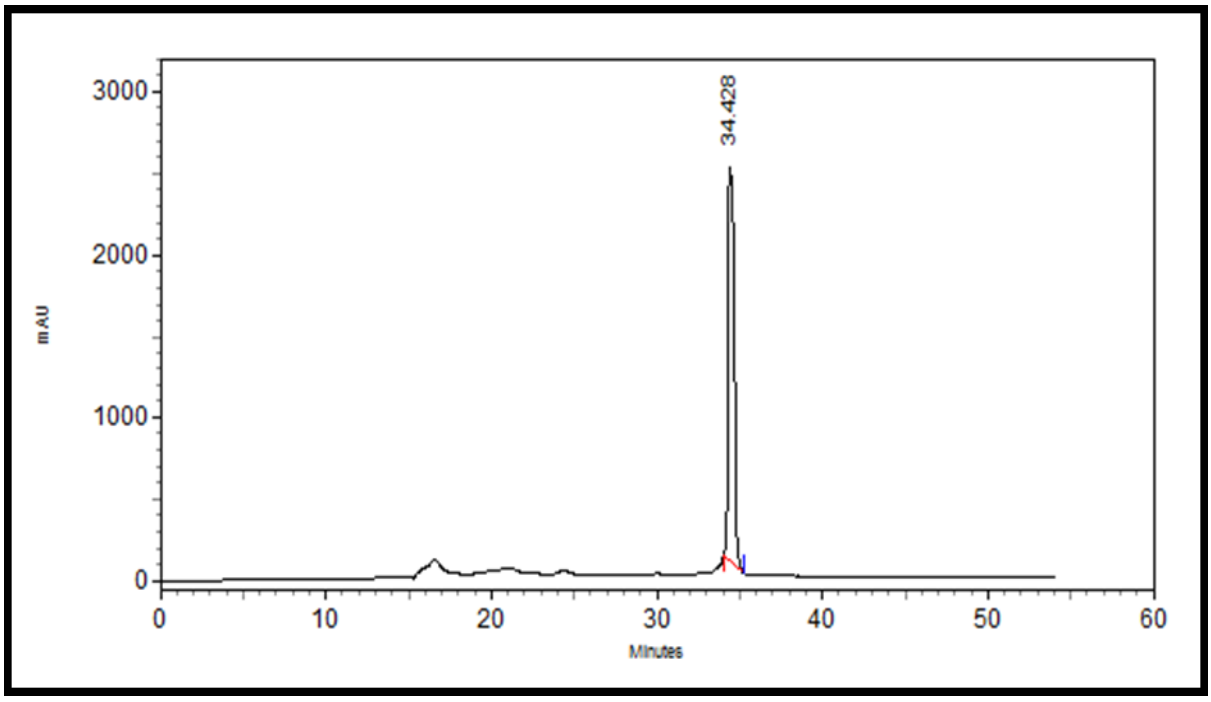
Analytical HPLC chromatogram of leuprolide analogues **(2d)**



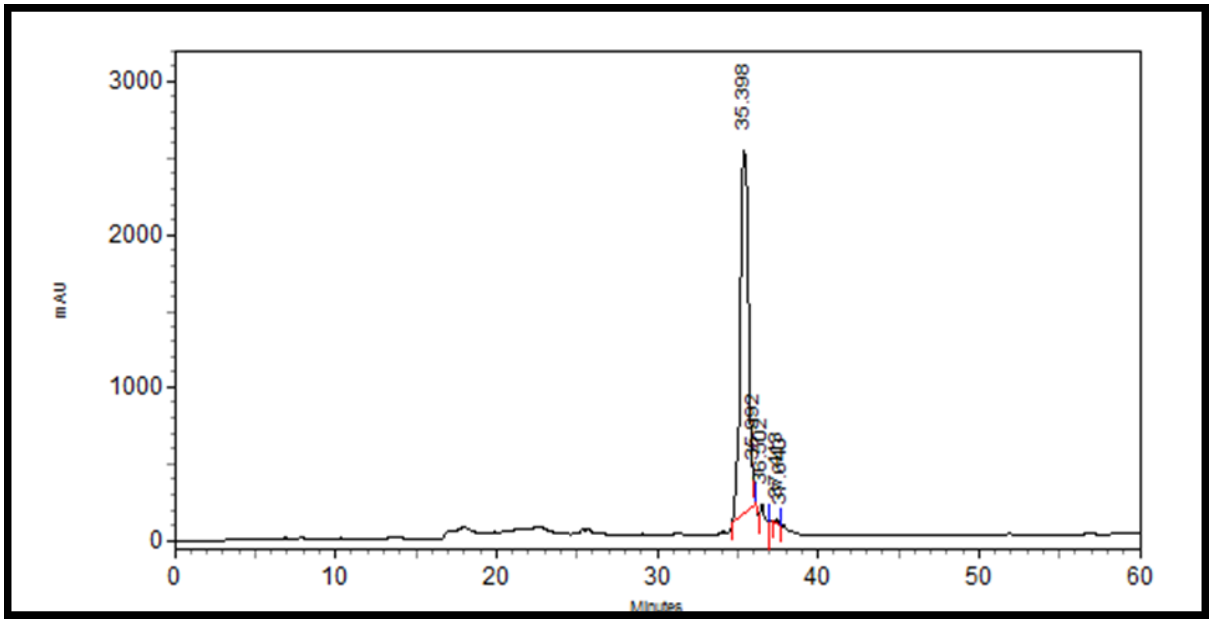
Analytical HPLC chromatogram of leuprolide analogues **(2e)**



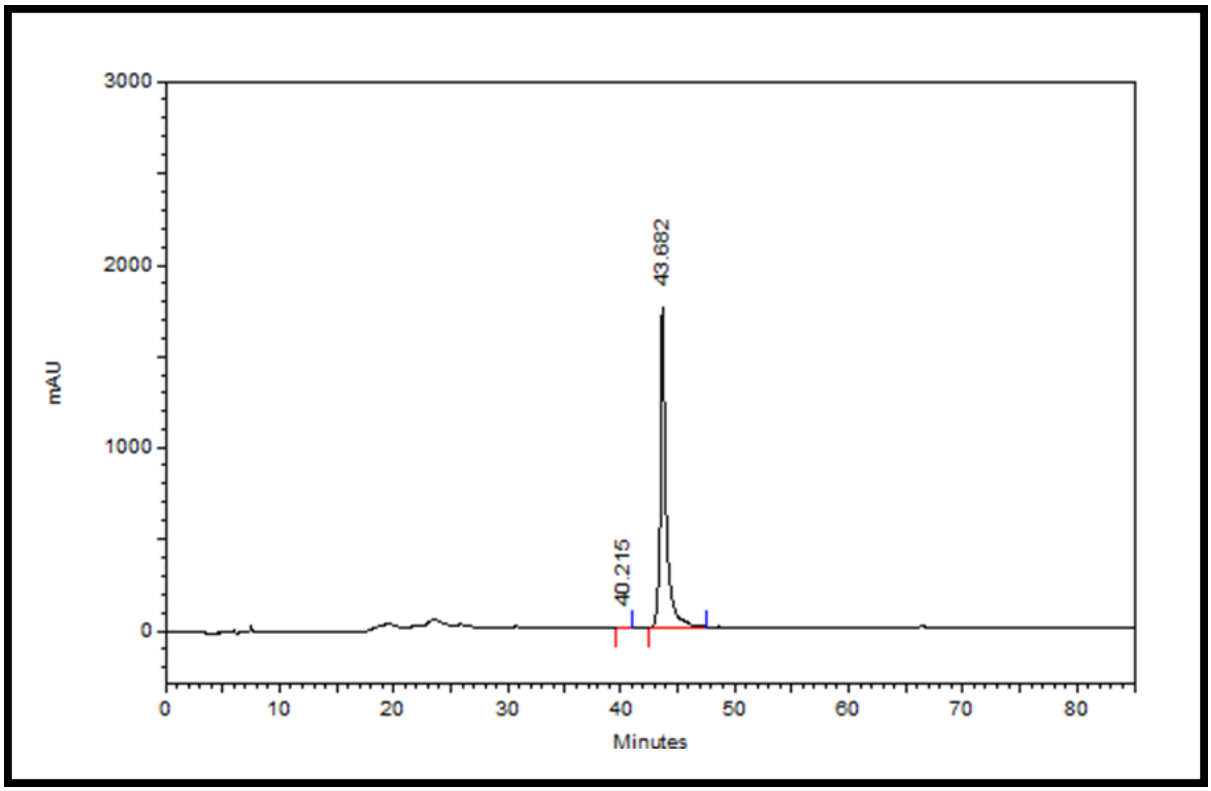
Analytical HPLC chromatogram of leuprolide analogues **(3a)**



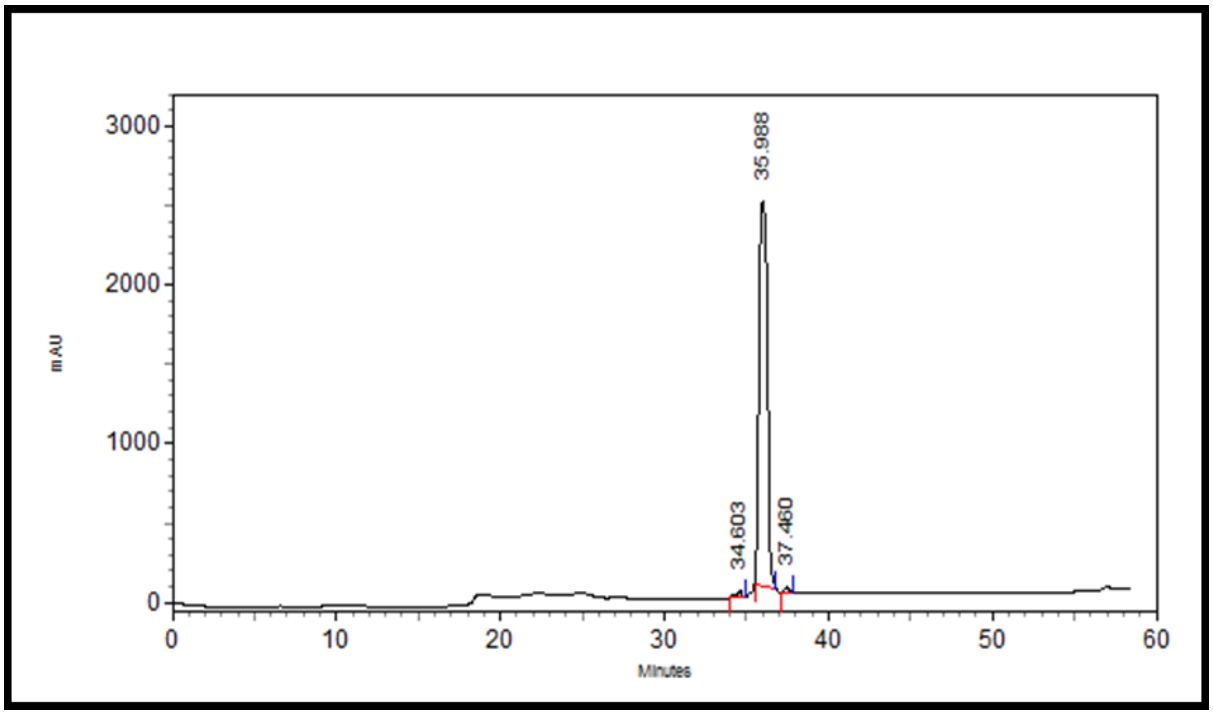
Analytical HPLC chromatogram of leuprolide analogues **(3b)**



Analytical HPLC chromatogram of leuprolide analogues **(3c)**



Analytical HPLC chromatogram of leuprolide analogues **(3d)**



Analytical HPLC chromatogram of leuprolide analogues **(3e)**