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Synthesis of novel compounds from Harmine (-carboline derivatives) isolated from Syrian *Peganum Harmala* L. Seeds

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ABSTRACT:

In the present work, we report the synthesis and structural characterizations of new -carboline derivatives of harmine isolated from Syrian Harmala Seeds (Peganum harmala L.)collectedfrom The Syrian Desert Area (Palmyra desert).Series of harmine derivatives were designed and synthesized via modification of position- 9 of β-carboline nucleusWe imployed three amino acids (L-Lysine, L-Ornithine, L-Alanine) to form amide bonds with Harmine skeleton;NH_2-groups in amino acids were protected by Boc-group.Therafter, TFA in 40% DCM was used to removeBoc-group. Thenew synthesized compounds and protected amino acids were purified bycolumn chromatographyand recrystallized. We characterized these new compounds and protected amino acids by utilizing spectroscopic technics (IR, LC-MS,GC-MS,^{1}H-NMR and ^{13}C-NMR). The yields ofnovel synthesized compounds and protected amino acids are in high percentage78.80%, 72.73%,85.32%, 84.33%, 81.63% and 91.85% of IIId (compound1),IIIe (compound2), IIIf(compound3),IIIa, IIIb and IIIc, respectively.

Keywords: *Harmine; new -carboline derivatives; synthesis;Boc; L-Lysine; L-Ornithine; L-Alanine; Syrian Harmala Seeds.*

1. INTRODUCTION

Natural and synthetic tetrahydro- -carbolines and -carbolines are class of alkaloids with a large spectrum of important pharmacological properties[1]. -carboline alkaloids are a large group of natural and synthetic indole alkaloids with different degrees of aromaticity, some of which are widely distributed in nature, including various plants, foodstuffs, marine creatures, insects, mammalians as well as human tissues and body fluids. These compounds are of great interest due to their diverse biological activities. Particularly, these compounds have been shown to intercalate into DNA, to inhibit CDK, Topisomerase, and monoamine oxidase, and to interact with benzodiazepine receptors and 5-hydroxy serotonin receptors. Furthermore, these chemicals also demonstrated a broad spectrum of pharmacological properties including sedative, anxiolytic, hypnotic, anticonvulsant, antitumor, antiviral, antiparasitic as well as antimicrobial activities [2]. -carbolines have been reported to possess significant antitumor activities [3-8], in addition these compounds are widely studied for their bioactivity in antibacterial, anti-radialization, anti-trypanosome, and neural activities as well as mutagenic and co-mutagenic properties. Also they are potent and specific inhibitors of cyclin-dependent kinases [8-14]. The extracts containing beta-carbolines from the plant *Peganumharmala* have been widely used in Northwest China as a very potent antitumor folk medicine, in which the main effective contents are beta-carboline alkaloids such as harmaline, harmine, harmalol, and Harman [3,5,9,11-18]. Aboriginal in South Africa use it to ease pain and hyperkinesias [19]. Also the plant *Peganum harmala* is used as medicinefor cancers of digestive system[19]. Evidence shows that beta-carbolines interact with DNA but it remains unclear about the way these compounds bind to the receptor and how they operate [12]. The structural simplicity of -carboline alkaloids

hides a multitude of in vitro and in vivo effects and makes these molecules interesting from both a biophysical and a medicinal perspective [20-22].

Harmal (*Peganumharmala* L. family *Zygophyllaceae*) is a perennial, glabrous plant which grows spontaneously in semi-arid conditions, steppe areas and sandy soils, native to eastern Mediterranean region. It is a shrub, 0.3-0.8 m tall with short creeping roots, white flowers and round seed capsules carrying more than 50 seeds. The plant is well-known in Iran and is widely distributed and used as a medicinal plant in Central Asia, North Africa and Middle East. It has also been introduced in America and Australia. This plant is known as “Espand” in Iran, “Harmel” in North Africa and Syria, “African rue,” “Mexican rue” or “Turkish rue” in the United States. Various parts of *P. harmala* including its seeds, fruits, root, and bark, have been used as folk medicine for a long time in Iran and other countries. Many pharmacological surveys have shown different effects of *P. harmala* and its active alkaloids (particularly harmine) [3,23-27].

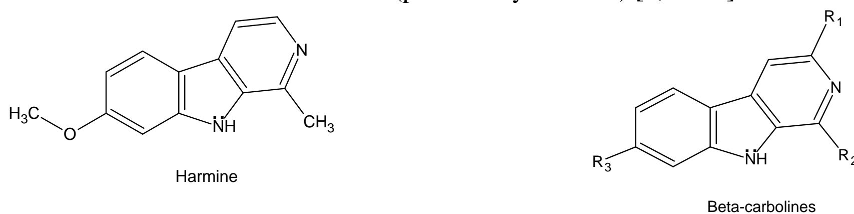


Figure (1): The structure of -carbolines and Harmine compound

Various -carboline alkaloids (figure 1,2) bind at BZRs and act as full, partial or mixed agonists, antagonists or inverse agonists, achieving either sedative, tremorgenic, anxiolytic, anxiogenic, proconvulsant or convulsant effect [28]. The structure-affinity analysis suggested that the presence of a 3-position substituent (e.g. amide, ester, carbinol) and a fully aromatic ring system are optimal for BZR binding; while the tetrahydro- -carbolines demonstrated considerably less affinity for BZR than their fully aromatic counterparts. In accordance with this rule, found that the DH Cs lacked the affinity for BZR, even when a 3-position ester group was incorporated into the ring ofharmalan (9), the compound did not bind to BZRs [28-36].

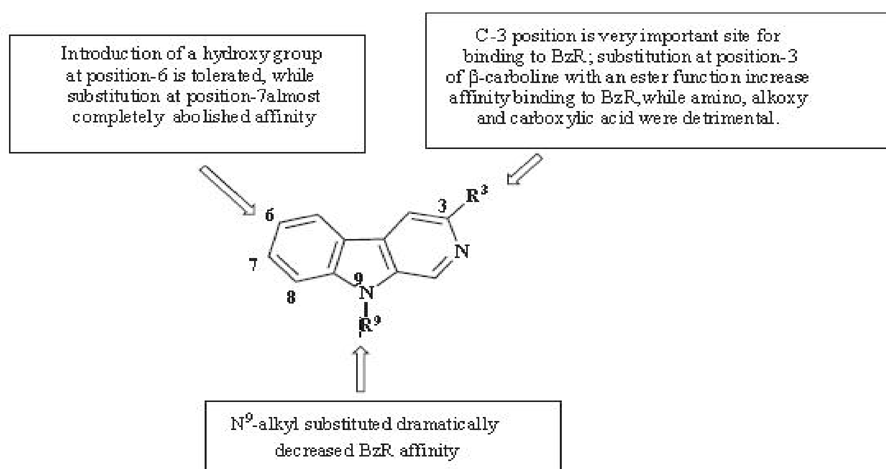


Figure (2):The structure –affinity relationships of Beta-carbolines binding to BzR

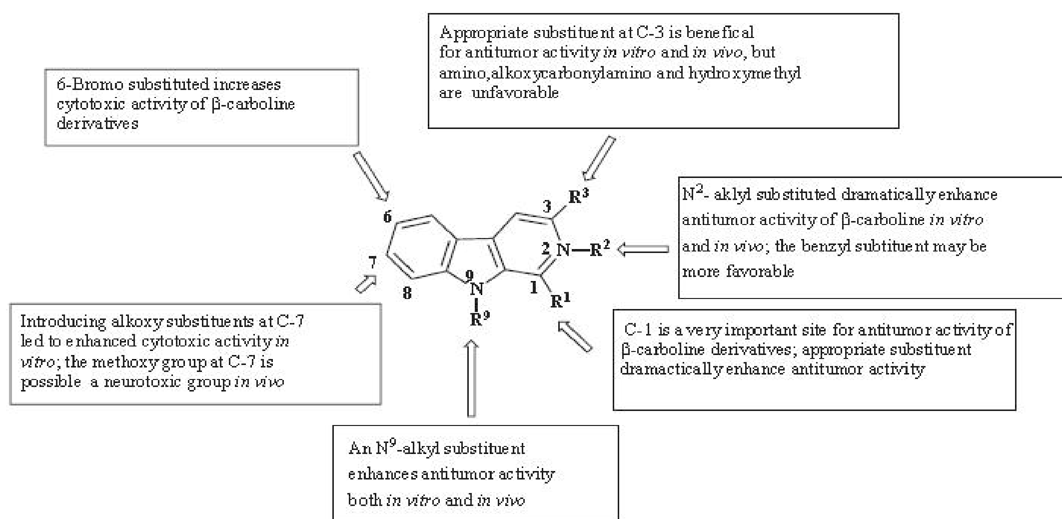
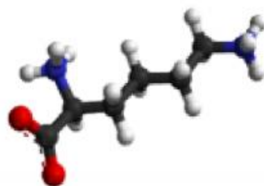


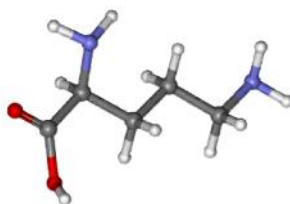
Figure (3): The structure-activity relationships of -carboline derivatives against tumor cells

Amino acid structure depends on both the side-chain functionality and the surrounding environment. Basic amino acids, such as arginine and lysine, often participate in intra- and intermolecular salt bridges with acidic residues. Whether these ionic interactions result in the stabilization of a conformer or complex depends on the solvent accessibility of these residues, the identity and position of nearby residues, and other factors [37].

L-Lysine (Lys)is an -amino acidthat is used in the biosynthesis of proteins. It contains an -amino group (which is in the protonated $–NH_3^+$ form under biological conditions), an -carboxylic acid group (which is in the deprotonated $–COO^-$form under biological conditions), and a side chain lysyl ($(CH_2)_4NH_2$), classifying it as a charged (at physiological pH), aliphatic amino acid. It is essential in humans, meaning the body cannot synthesize it and thus it must be obtained from the diet. Lysine is a base, as are arginine and histidine and has the second highest proton affinity of the naturally occurring amino acids [38], and it is an essential amino acid needed for the synthesis of protein in growth, milk production, tissue maintenance and repair and gestation. Lysine is additionally a constituent of carnitine, a compound needed to transport fat into cellular structures. Animal by-products are rich sources of lysine, while vegetable sources are not [39]. L-Lysine is the only amino acid to have two different biosynthetic pathways. One is the aspartate (5) pathway present in bacteria, plants, and algae. The other starts from -ketoglutarate (6) and is present in fungi. Lysine is an essential amino acid for humans [40,41].



L-Ornithine (Orn) is not an amino acid coded for by DNA and is a non-proteinogenic amino acid, produced from L-glutamic acid in plants and from L-arginine in animals. L-Ornithine plays a central role in the urea cycle in terrestrial vertebrates [42,43].



L-Alanine (Ala) is an -amino acid that is used in the biosynthesis of proteins. It contains an -amino group (which is in the protonated $-NH_3^+$ form under biological conditions), an -carboxylic acid group (which is in the deprotonated $-COO^-$ form under biological conditions), and a side chain methyl group, classifying it as a nonpolar (at physiological pH), aliphatic amino acid. It is non-essential in humans, meaning the body can synthesize it [43].

The purpose of this study is to synthesis new bioactive compounds by the reaction of harmine structure with the basic amino acids such as L-Lysine, L-Ornitine and neutral amino acids such as L-Alanine. Harmine is a natural component that has beenisolatedfrom Syrian Peganum harmala seeds.Then the new compounds and protected amino acidshave been identified by using spectroscopic techniques(IR, GC-MS, LC-MS, ^{1}H-NMR and ^{13}C-NMR).

2. EXPERIMENTAL

2.1 Instruments and Apparatus

The HR GC-MS analyses were carried out on Agilent-5975C operating in electron ionization mode at 70eV. Helium was used as carrier gas and the injection split ratio was 1:100. Separation was achieved on DB-5 capillary column (30m×0.25mm×0.25µm) using the following temperature program: 1 min at 60°C, 20°C/min until 260°C, then 10 min at 260°C; Ion source and injection temperaturewere at 260°C; The FT-IR spectrum was recorded on Jasco 4100 spectrometer (KBr pellets); LC-MS spectrophotometer 2010 (SHIMADZU, Japan); pH-meter 744 (METROHM, Swiss); electro-thermal melting point (ELECTROTHERMAL, England); ^{1}H-NMR spectrometer AC-400MHz (BRUKER, USA) using TMS as internal standard and DMSO-d_6 as solvent;electro-magnetic stirrer L32 (LABINCO, Poland); analytical balance 3432 (SARTORIUS, Germany), with accuracy ±0.1mg; drying oven E28 (HERAEUS, Germany); Thin Layer backed sheets coated with (silica gel 60 G254),Merck, Germany; UV lamp (DESAGA, Germany) with 245nm and 336nm wave-lengths; bi-distill water apparatus (JANAT, Syria).

2.2 Chemicals

The compounds of the test set were prepared from the initial structure of harmine. Harmine (purity 96%) was extracted from Syrian Peganum Harmala Seeds [1]. Compounds (protected amino acids) were synthesized, purified, and characterized as previously described [مرجع الحماية]. All reagents were purchased from commercial suppliers, dried and purified when necessary.L-Lysine, L-Ornitine, L-Alanine, $(Boc)_2O$ (di-tert-butyl-oxy-carbonyl),HOBt (1-hydroxybenzotriazole) and DCC (N,N-dicyclohexylcarbodiimide) were obtained from Merck, Germanyand were used without further purification. Trifluroacetic acid TFA, N,N-dimethyl-formamide DMF, tetrahydoforane THF,dichloromethane DCM, acetonitrile ACN, ethylacetateEtOAc, ethanol EtOH, methanol MeOH,diethylether Et_2O and other chemicals were obtained from Sigma Aldrich and purified by re-distillation before use.All other solvents and chemicals used during the practical work were obtained from the Merck (Pvt.) ltd, and supplied (Analytical or HPLC grade), without prior purification. Di-distidlled water

was used for chemical reactions. 'Petrol' refers to the fraction of light petroleum ether boiling in the range 40-60 ºC. All reactions using anhydrous conditions were performed using flame-dried apparatus under an atmosphere of argon or nitrogen. 'Brine' refers to the saturated solution of sodium hydroxide.Anhydrous magnesium sulfate ($MgSO_4$) or sodium sulfate (Na_2SO_4) were obtained from Merck, Germanyand were used as drying agents after reaction workup, as indicated. Potassium Bromide (Uvasol, for IR spectroscopy, Merck, Germany).

2.3 Methods

2.3.1*Preparation ofprotected amino acids (L-Lysine, L-Ornitine and L-Alanine) with Boc-group:*

In this paper, the amine functional groups of the three amino acids L-Lys (ia), L-Orn(ib) and L-Ala(ic) were protected with 4-*tert*-butoxycarbonyl (Boc) to form Boc-L-lys(boc)-OH (IIIa), Boc-L-Orn(boc)-OH (IIIb) andBoc-L-ala-OH (IIIc), respectively. The crude products were purified by column chromatography over (silica gel, DCM: MeOH, 20:1) and by reprecipitation.

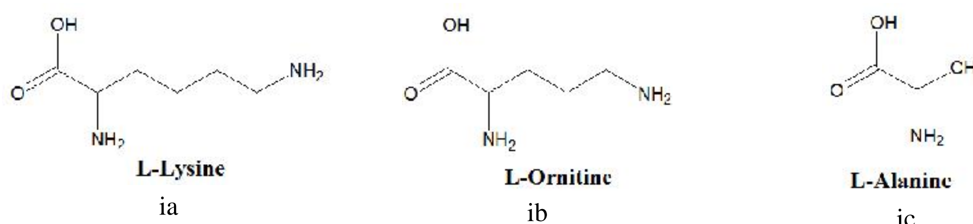
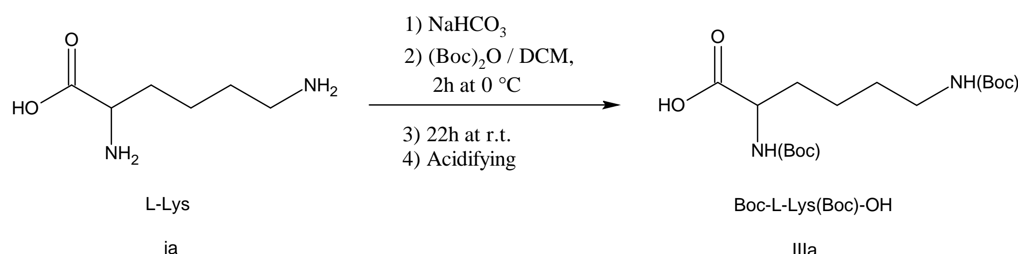


Figure (4): The structure of amino acids were used in this paper

Synthesis of Boc-L-Lys(Boc)-OH (IIIa):

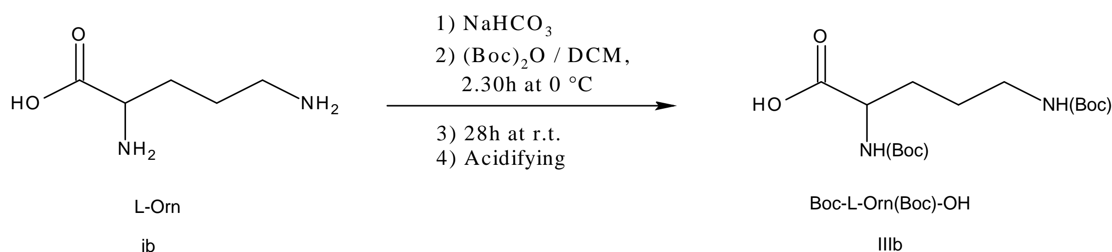
L-Lysine hydrochloride (15g, 102.74mmol) and $NaHCO_3$(10g)were dissolved in round buttoned flask containing 200ml di-distilled water and cooled in an ice-water bath. Then, a solution of $(Boc)_2O$(40.48g, 185.69mmol) in 100mL DCMwas dropwise with vigorousstirring for 2h in anice-water bath, then, the mixture was stirredfor22h at room temperature. The mixture was transferred to 250ml separation flask, water layer washed with Et_2O(30ml × 3) and the organic layers were discarded.The pH value of aqueous layer was adjusted to 2~3 with hydrochloric acid HCl 10M, then withbrine. After that, it was extracted with EtOAc (25ml × 3). The organic phase was combined and dried with anhydrousNa_2SO_4. The solution was concentrated and dried under vacuum by rotary evaporator [44]. Then, Theresidueobtained was purified on a column chromatography (silica gel, DCM: MeOH, 20:1) to afford the Boc-L-Lys(Boc)-OH (IIIa)which was purified by reprecipitation from Petrol as yellowish white solid. Yield: 29.12g (84.33%).



Synthesis of Boc-L-Orn(Boc)-OH (IIIb):

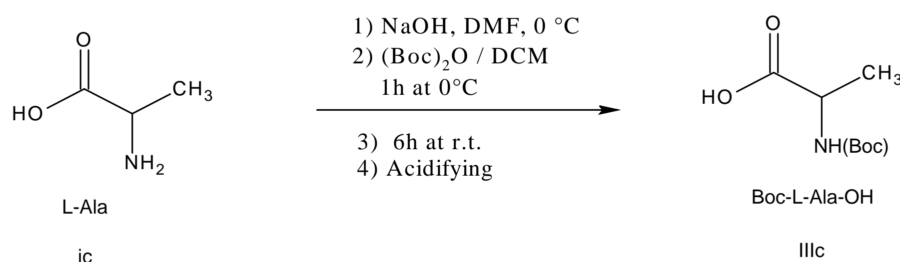
L-Orn hydrochloride(17g, 128.8mmol) and $NaHCO_3$(6.4g) of were dissolved in round buttoned flask containing 200mldi-distilled water and cooled in an ice-water bath. Then, a solution of $(Boc)_2O$ (64.57g, 296.13mmol) in 100mL DCM was added dropwisewith vigorous stirring for 2.30h in an ice-water bath, then, the mixture was stirred for22h at room temperature. The mixture was transferred to 250ml separation flask,

water layer washed with Et_2O(30ml × 3) and the organic layers were discarded. The pH value was adjusted to 2~3 with dilute hydrochloric acid HCl, then with brine.After that, it was extracted with EtOAc (25ml × 3). The organic phase was combined and dried with anhydrousNa_2SO_4. The solution was concentrated and dried under vacuum by rotary evaporator [44]. The residue obtained was purified on a column chromatography(silica gel, DCM: MeOH, 20:1) to affordBoc-L-Orn(Boc)-OH (IIIb) which was purified by reprecipitation from 25%EtOAc/Et_2O as white solid. Yield: 34.94g (81.63%).



Synthesis of Boc-L-Ala-OH (IIIc):

L-Ala hydrochloride (1.35g, 15mmol) was dissolved in round buttoned flask containinga mixtureof 30ml DMF and 15ml di-distilled water and 15ml of 1M NaOH, and it was stirred and cooled in an ice-water bath. Then, a solution of $(Boc)_2O$ (3.5g, 16mmol) in 25mL DCM was added dropwise with stirring in an ice-water bathfor 1h then at room temperature for6h. After that, the solution was concentrated in vacuum to about 40-60 ml, cooled in an ice-water bath again, covered with a layer of EtOAc (about 50 ml) and acidified with a solution of hydrochloric acid HCl 10M to pH 2~3. The aqueous phase was extracted with EtOAcand this operation was done repeatedly. The EtOAcextracts were cooled, washed with water and dried over anhydrous Na_2SO_4 and evaporated in vacuum [45]. Then, the residue obtained was purified on a chromatographic column (silica gel, DCM: MeOH, 20:1) to give the product that was purified byreprecipitation from 10% EtOAc/Et_2O. Pure material was obtained as white solid. Yield: 25.36 g (91.85%).



2.3.2 Synthesis of new bioactive harmine derivatives (-carboline derivatives):

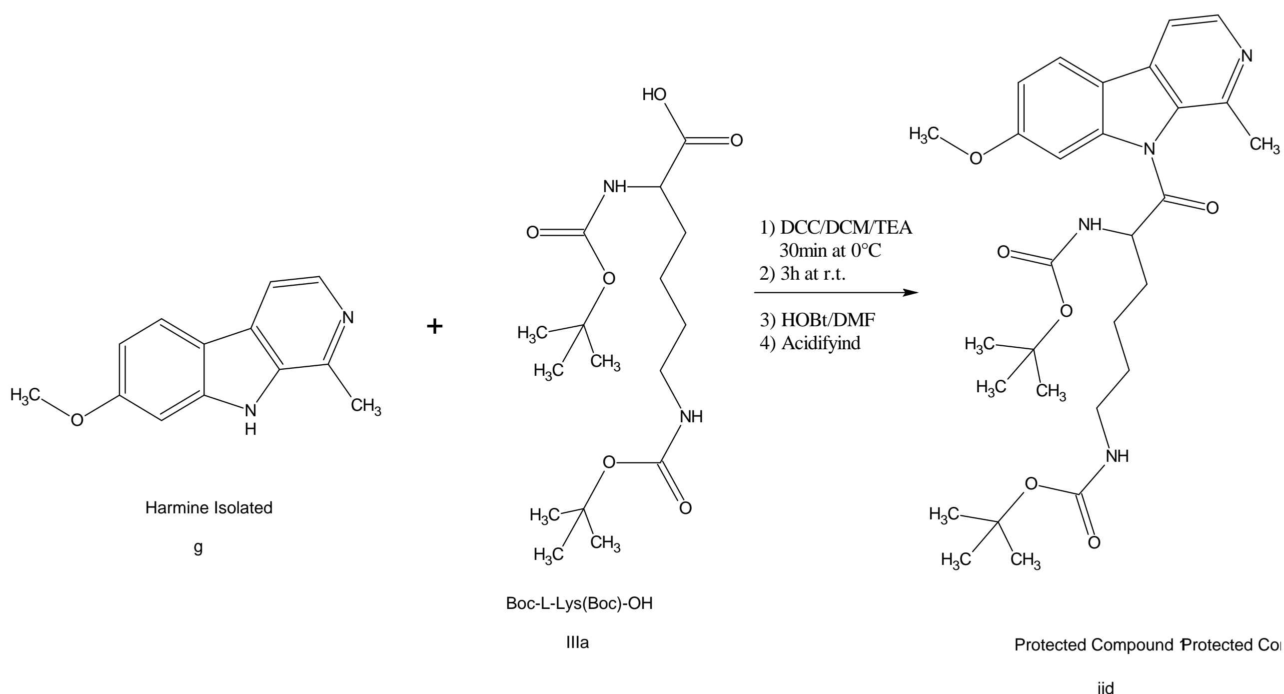
All our synthesized compounds were characterized by their spectroscopic data (IR, GC-MS,^{1}H-NMR and ^{13}C-NMR), which are described in the experimental section. Carbodiimide activation DCC of amino acid derivatives often causes a partial racemization of the amino acid. In peptide synthesis, adding an equivalent of HOBt (1-hydroxybenzotriazole) minimizes this problem [46].

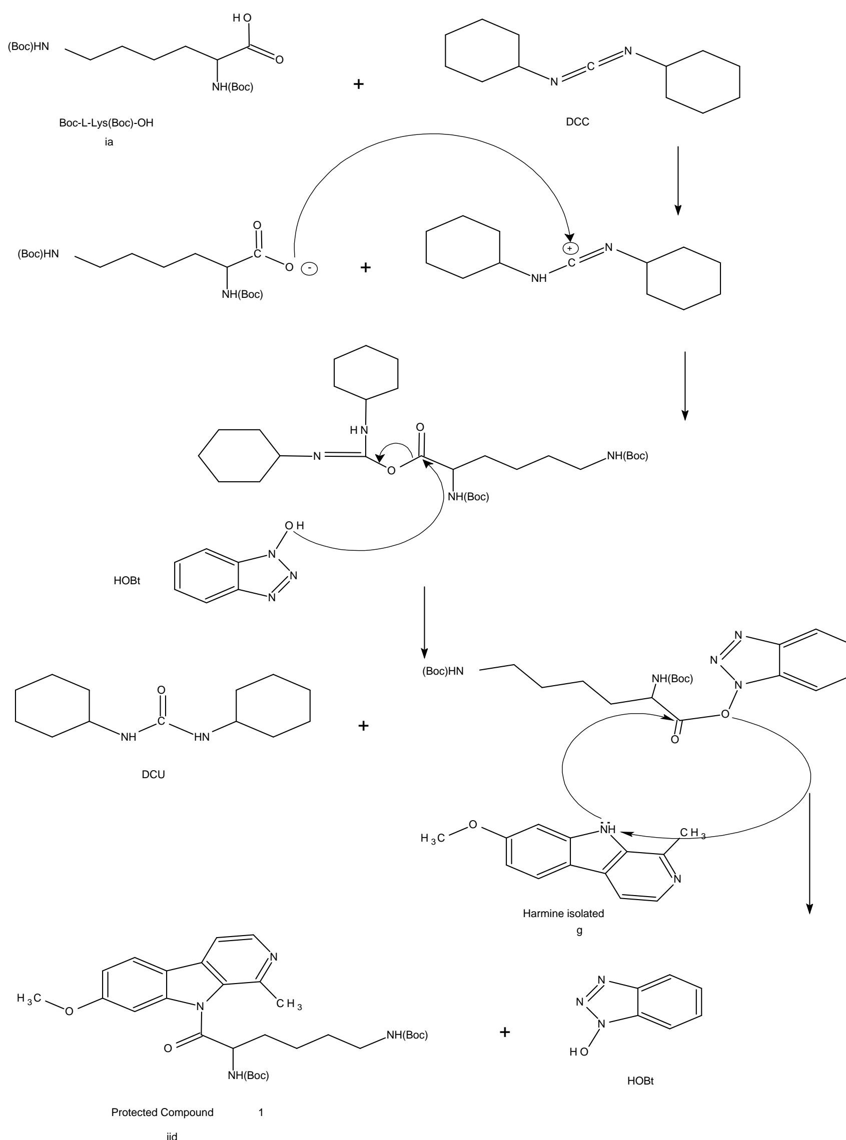
2.3.2.1 Synthesis of Compound 1 (IIId)[5-(7-methoxy-1-methyl-9*H*- -carboline-9-yl)-5-oxohexane-1,4-diamine:

Step-1: Reacting Harmine with synthesized Boc-L-Lys-Boc-OH:

(2.42g, 7mmol) of synthesized Boc-L-Lys-Boc-OH was dissolved in a minimum amount of DMF and mixed with (1.44g, 7mmol) of DCC in 15mlof DCM that wasadded dropwiseand basified with 12.7ml of TEA,

the solution was stirring at 0°C for 30min, followed by stirring for 3h at room temperature. This step was repeated with (1.44g, 7mmol)of DCC in DCM. After that,in a dropping funnel (3.22g, 21mmol) of HOBt was dissolved in a minimum amount of DMF and addeddropwise to the previous mixture with stirring for 2h at room temperature and placed under Argon. The precipitate formed of DCU (di-cyclo-hexyl urea) was filtrated out, followed by evaporation of the solvent from the filtrate to about 10mL. Then, (1.35g, 6.37mmol) of harmine (isolated from Syrian harmala seeds) [47]in 15ml of DMFwas added to thefiltrate. The completion of the reaction was indicated by TLC (silica gel, $CHCl_3$: MeOH,8.5:1.5).Then, the solution was concentrated in vacuum to about 10-20ml. The reaction mixture was acidified with 2ml of HCl4Nunder cold condition, and the solution was extracted with EtOAc (3×100ml) after adding solid NaCl to the acidified solution. The organic extract was washed withHCl4N and 10ml of brineand dried over anhydrous $MgSO_4$. The solventswere evaporated under vacuumto get the **protected Compound 1**which was carried on to the next step without further purification. The Synthesis mechanism ofprotected Compound 1aredescribed in **scheme 1**.





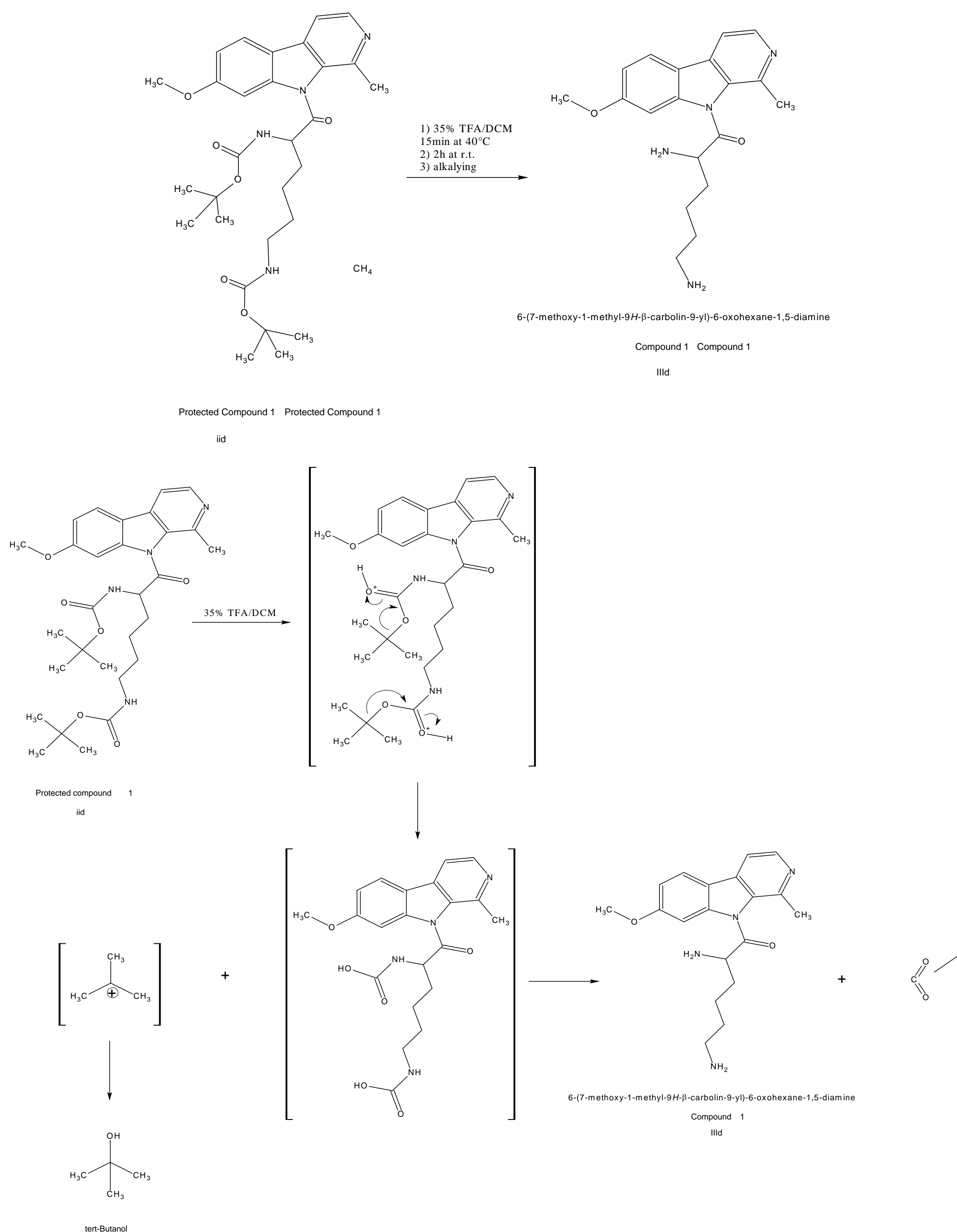
Scheme 1:Synthesis mechanism of protected Compound 1

Step-2: Deprotecting the protection group (N-Boc)and purifying of synthetic protected compound 1 (iid):
Adding TFA: The N-protecting groups of N-Boc are usually removed with (25-50)% trifluoroacetic acid TFA in dichloromethane DCM in the final step. This process is necessaryto keep the Synthesized Compound 1 stable in these conditions.

Trifluoroacetic acid TFA is toxic, so the method of removing the N-Boc group depending on how the peptide will be used, it may be necessary to exchange TFA for a more biologically benign acid. Recently several common methods of exchanging or removing trifluoroacetate were evaluated [48,49].

The method: In a round bottom flask, thereaction mixtureof **SynthesizedCompound 1 (iid)** was dissolved in 20ml DMF, then 10ml of 35% TFA in DCM was dropped to the previose reaction mixture,which was stirred at 40ºC for 15min, thenat room temperature for 2h. After that, the reaction mixture was cooled in an ice-water bath. The resulting crystals were filteredanddissolved in hot water and shaken.Then, it was adjusted to

pH~7.5-8 with aqueous sodium hydroxide solution. The solvent was removed and the residue obtained was purified on a chromatographic column (silica gel, 100% petrol to 15% EtOAc in petrol) to give the titled **Compound 1** (IIId) thatwas purified by reprecipitation from 20% MeOH/Et_2O and collected as white needle-like crystals with(1.71g, 78.80%) yield.The synthesis mechanism of de-protection of Compound 1isdescribed in **scheme 2**.

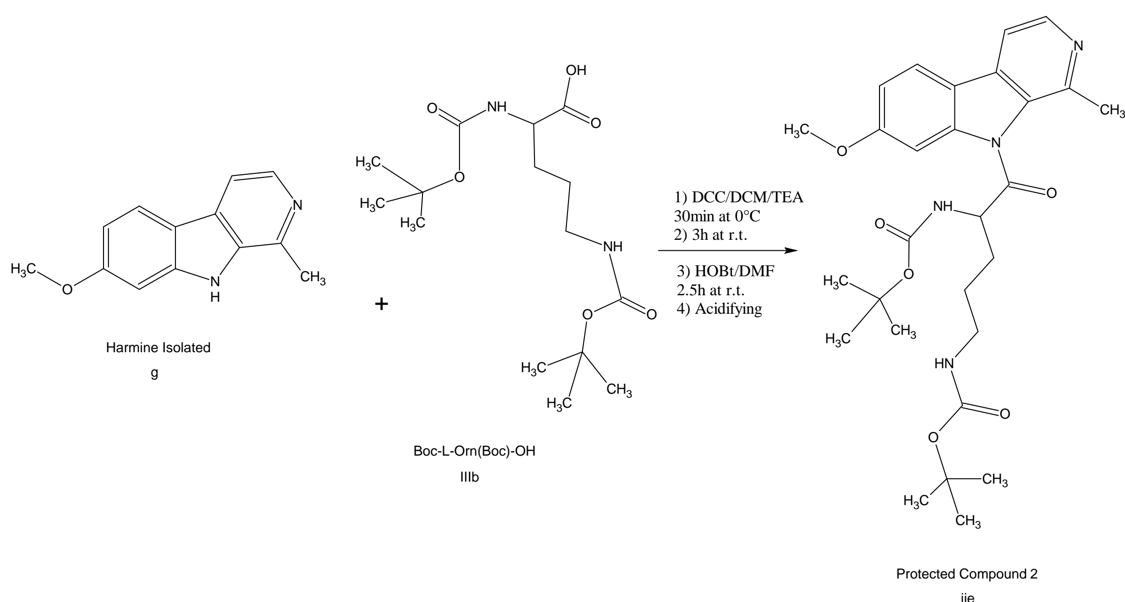


Scheme 2:Synthesis mechanism of de-protection of Compound 1 (IIId)

2.3.2.2Synthesis of Compound 2 (IIIe)([5-(7-methoxy-1-methyl-9*H*- -carboline-9-yl)-5-oxopentane-1,4-diamine:

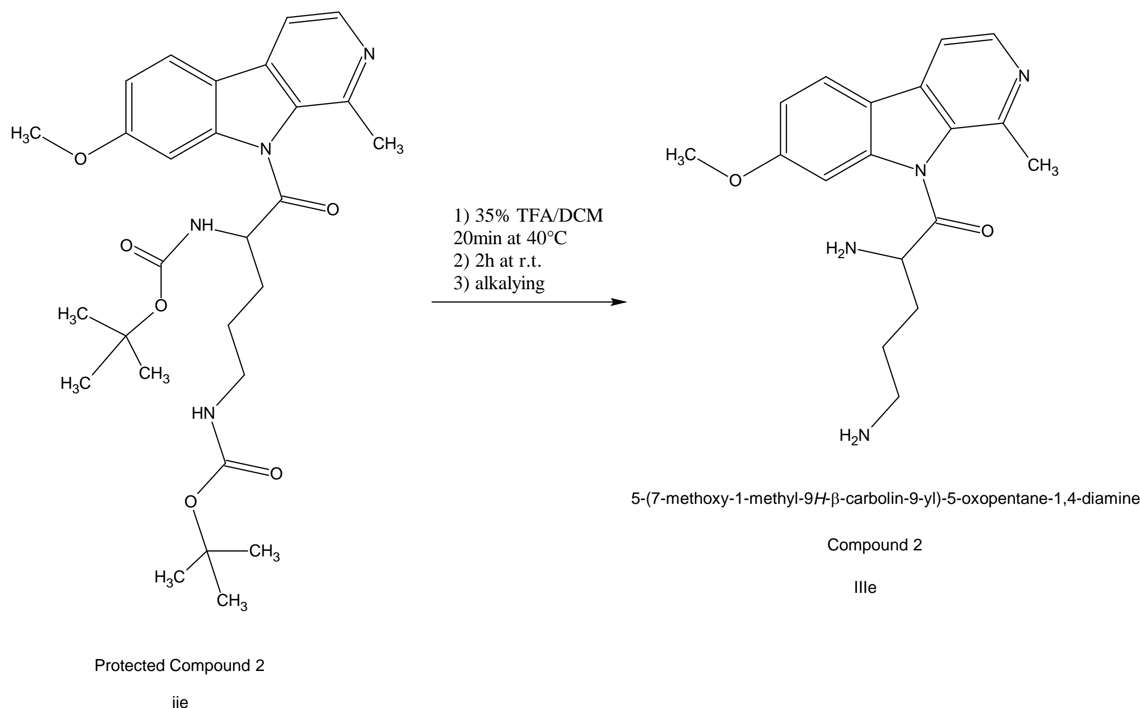
Step-1: Reacting Harmine with synthesized Boc-L-Orn-Boc-OH:

(1.52g, 4.58mmol) of synthesizedBoc-L-Orn-Boc-OH was dissolved in a minimum amount of DMF and mixed with (1.03g, 5mmol) of DCC in 15ml of DCM that was added dropwise with stirring at 0°C for 30min in the presence of 12.7ml of TEA, followed by stirring for 3h at room temperature. This step was repeated with a new amount of DCC (1.03g, 5mmol) in DCM. In a separate flask dissolve (2.30g, 15mmol) of HOBt in a minimum amount of DMF and stir the mixture until the HOBt dissolves. Then the HOBt was dropped to the reaction mixture and stirred at room temperature and placed under argon for 2.5h in the presence of TEA, then, filtrated of the DCU precipitate formed and removed it, followed by evaporation of the solvent from the filtrate. After that, (0.86 g, 4.06 mmol) of harmine (isolated from Syrian harmala seeds) [47] in 10ml of DMF was added to the reaction mixture.After completion of the reaction as indicated by TLC (silica gel, CHCl3: MeOH, 85:15).Then, the solution was concentrated in vacuum to about 10-20ml. The reaction mixture was acidified with 2ml ofHCl4N under cold condition, and the solution was extracted with EtOAc (3×100ml) after adding solid NaCl to the acidified solution. The organic extract was washed with 4N HCl and 10ml of Brine, then, dried over anhydrous $MgSO_4$. Evaporation of solvent under under vacuum to give the product **protecting Compound 2**(iie) which was carried on to the next step without further purification.



Step-2:Deprotecting the protection group (N-Boc) of synthetic protected compound 2 (iie) and purifying it:

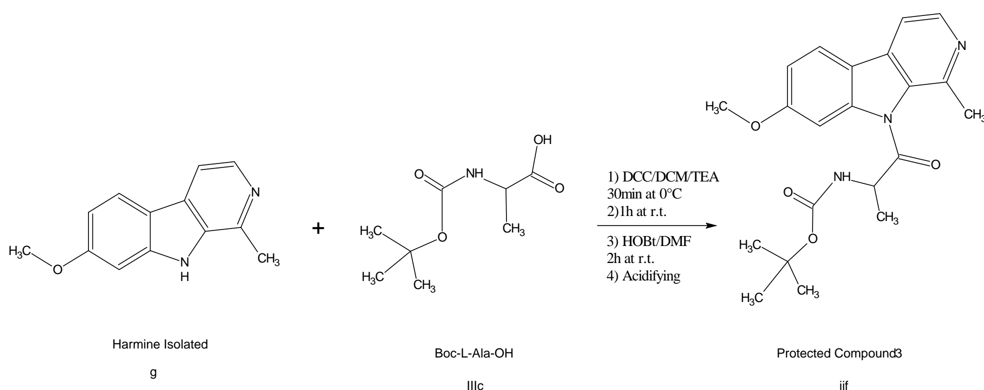
In a round bottom flask the reaction mixtureof **syntheticprotected Compound 2** was dissolved in DMF, then 10 ml of 35% TFA in DCM was dropped to the reaction. This reaction mixture was stirred at 40ºC for 20min, then at room temperature for 2h. After that, reaction mixture was cooled in an ice-water bath. The resulting crystals were filtered and removed, dissolved in hot water and the hot solution was adjusted to pH 8 with aqueous sodium hydroxide solution. The solvent was removed and the residue obtained purified on a chromatographic column (silica gel, 100% petrol to 15% EtOAc in petrol) to give the titled **Compound 2**(IIIe) whichwas purified by reprecipitation from 15% MeOH/Et_2O and collected as white to yellowish needle-like crystals in (0.96 g, 72.73%) yield.



2.3.2.3 Synthesis of Compound 3(IIIf) [1-(7-methoxy-1-methyl-9*H*- -carboline-9-yl)-1-oxopropane-2-amine)]:

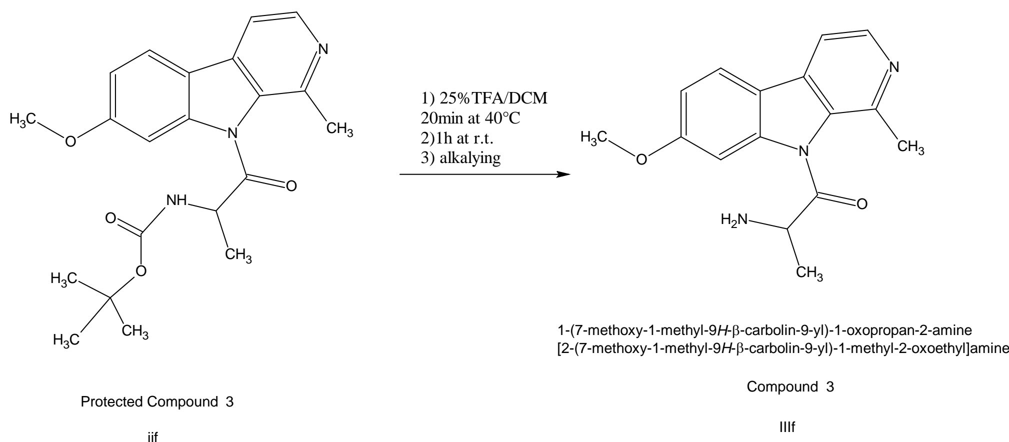
Step-1: Reacting Harmine with synthesized Boc-L-Ala-OH:

(1.85g, 9.79mmol) of synthesized Boc-L-Ala-OH was dissolved in a minimum amount of DMF and mixed with (1.24g, 6mmol) of DCC in 15ml of DCM that was added dropwise with stirring at 0°C for 30min and basified the mixture with 8ml of TEA, followed by stirring for 1h at room temperature. This step was repeated with a new amount of DCC (1.03g, 5mmol) in DCM. In a separate flask dissolve (2.76g, 18mmol) of HOBt in a minimum amount of DMF and stir the mixture until the HOBt dissolves. If the HOBt doesn't dissolve completely, add just enough DMF to bring it into solution. Then the HOBt was dropped to the reaction mixture and stirred at room temperatureand placed under argon for 2h in the presence of TEA, then, filtrated of the DCU precipitate formed and removed, followed by evaporation of the solvent from the filtrate. Then, (1.89g, 8.92mmol) of harmine (isolated from Syrian harmala seeds) [47]in 25ml of DMF was added to the reaction mixture. After completion of the reaction as indicated by TLC (silica gel, CHCl3: MeOH, 7:2).Then, the solution was concentrated in vacuum to about 20-30 ml. The reaction mixture was acitified with 4N HCl (2ml) under cold condition, and the solution was extracted with EtOAc (3×100ml) after adding solid NaCl to the acitified solution. The organic extract was washed with 10ml of HCl4N and 10ml of Brine, then, dried over anhydrous $MgSO_4$. Evaporation of solvent under under vacuum to give the product**protecting Compound 3 (iif)**which was carried on to the next step without further purification.



Step-2: Deprotecting the protection group (N-Boc) of synthetic protected compound 2 (iif) and purifying it:

In a round bottom flask the reaction mixtureof **syntheticprotected Compound 3** was dissolved in DMF, then 10 ml of 25% TFA in DCM was dropped to the reaction. This reaction mixture was stirred at 40ºC for 20min, then at room temperature for 1h. After that, reaction mixture was cooled in an ice-water bath. The resulting crystals were filtered, dissolved in hot water and the hot solution was adjusted to pH 8 with aqueous sodium hydroxide solution. The solvent was removed and the residue obtained purified on a chromatographic column (silica gel, ACN: Hex, 20:1) to give the titled **Compound 3**(IIIf) which was purified by reprecipitation from DCM/petrol and collected as white solid like a bright crystals in (2.15 g, 85.32%) yield.



3. Results and Discussion

3.1 Physical properties:

Some physical properties for synthesized compounds are shown in table (1).

Table (1): Thephysical properties for synthesized compounds (IIIa,IIIb,IIIc,IIId,IIIe and IIIf)

Sr. No.	Com.	Mol. Formula	Mol. Wt. g/mol	Yield %	M.P. ºC	R_f
1	IIIa	$C_{16}H_{30}N_2O_6$	346	84.33	179-181	0.22
2	IIIb	$C_{15}H_{28}N_2O_6$	332	81.63	155-157	0.29
3	IIIc	$C_8H_{15}NO_4$	189	91.85	81-84	0.36
4	iid	$C_{29}H_{40}N_4O_6$	540	-	-	0.15
5	iie	$C_{28}H_{38}N_4O_6$	526	-	-	0.17

6	iif	$C_{21}H_{25}N_3O_4$	383	-	-	0.31
7	IIId	$C_{19}H_{24}N_4O_2$	340	78.80	260-261	0.65
8	IIIe	$C_{18}H_{22}N_4O_2$	326	72.73	253-255	0.68
9	IIIf	$C_{16}H_{17}N_3O_2$	283	85.32	194-195	0.73

3.2 Identification of (IIIa-IIIf) Structures

The structures of the synthesized compounds (IIIa,IIIb,IIIc,IIId,IIIe and IIIf) were identified by the available spectroscopic methods: IR, LC-MS, GC-MS, ^{1}H-NMR and ^{13}C-NMR spectroscopies.

3.2.1 IR Spectrum

The compounds (IIIa,IIIb,IIIc,IIId,IIIe and IIIf) were scanned through wave numbers ranged between 400-4000cm^{-1} using KBr disk. The main absorption bands of the (IIIa,IIIb,IIIc,IIId,IIIe and IIIf)are shown in tables (2) and (3).

Table (2): The main absorption bands and the possible functional groups inprotected amino acids (IIIa,IIIb, and IIIc)

absorption bonds	wave number cm^{-1}		
	IIIa	IIIb	IIIc
-N-H stretching I band	3370	3453	3326
O-H stretching (COOH)	3012-3250	3074-3262	3092-3310
Aliphatic -C-H stretching (*sp*^{3})	2845,2981	2832,2925	2876,2963
$-CH_2-$ stretching	1466	1463	-
$-CH_3$bending	1369	1374	1379
Amide(-NH-C=O) II band	1580,1643	1566,1650	1557,1644
C=O, COOH stretching	1730	1737	1728
C-O, Urethane	1178,1280	1182,1245	11241292
C=O, Urethane	1701	1698	1715

Table (3): The main absorption bands and the possible functional groups in new bioactive compounds (IIId,IIIe, and IIIf)

absorption bonds	wave number cm^{-1}		
	IIId	IIIe	IIIf
$-NH_2$ stretching II band	3345	3362	3408
Aromatic -C-H stretching (*sp*^{2})	3022	3062	3018
Aliphatic -C-H stretching (*sp*^{3})	2882	2933	2954
$-CH_2-$ stretching	1469	1458	-
Aromatic C=C stretching	1488	1490	1493
Aromatic -C-N stretching	1532	1539	1545
$-CH_3$bending	1370	1363	1374
Amide (-N-C=O) II band	1545.1590	1532,1579	153,1582
C-O, Ether	1076,1130	1055,1166	1089,1145

IR spectrum gave information regarding the presence of twisting, bending and vibrating of the various functional groups in the synthesized compounds.

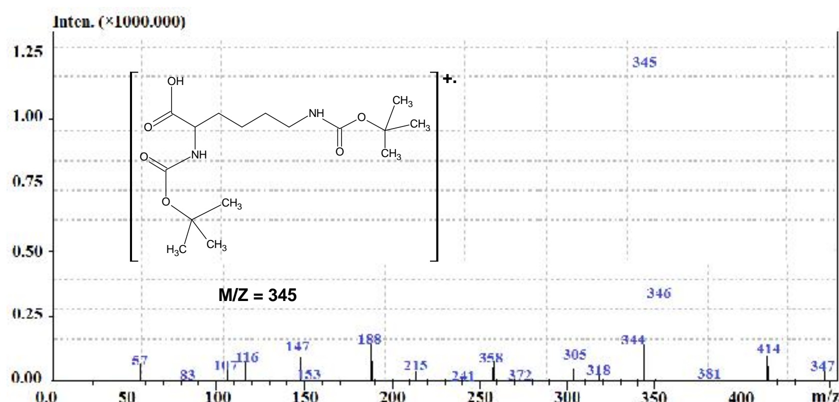
FTIR was used to characterize 'fingerprint' spectra of organic molecules. A nexus FTIR instrument was used under inert conditions to improve the signal: noise ratios, and obtain better spectra. The IR spectrum of

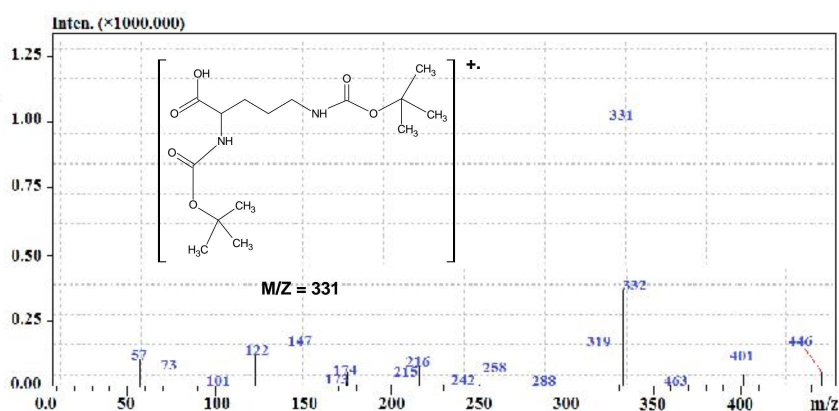
the new compounds shows the fingerprints of the molecular structure and functional groups with slight shifts up-field, for the carbonyl of wave-number ranges of 1730, 1737, 1728 cm^{-1} and there was an absence, with strong CO-NH signals in 1590, 1579, 1582 cm^{-1} range. There was strong signals for the amino groups in the wave-number regions of 3345, 3362, 3408 cm^{-1}, an absence of the ‘Boc’ protecting group, in the wave-number region 1280,1245,1292cm^{-1}.

The Boc group (*t*-BuOCO-) is used extensively to protect amino groups in peptide synthesis (new harmine derivatives), and is introduced with (di-butyl dicarbonate) which gave high yields with protected amino acid derivatives [50] .

3.2.2 LC-MS and GC-MS Spectrum

The mass spectra (positive polarity) of (IIIa,IIIb, and IIIc)are shown in the figure (5) by using HPLC-MS Spectroscopy. It shows the presence of the main peaks at m/z 346, 332 and 189, respectively corresponding to the radical cations ($M^{\bullet+}$) which are in agreement with the proposed structure.





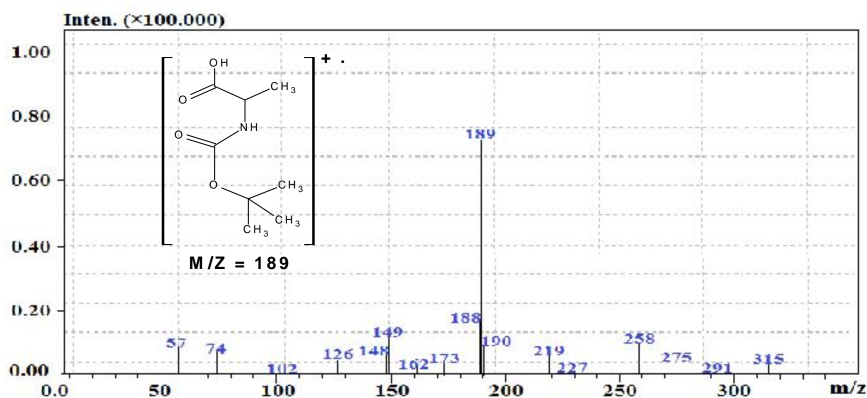
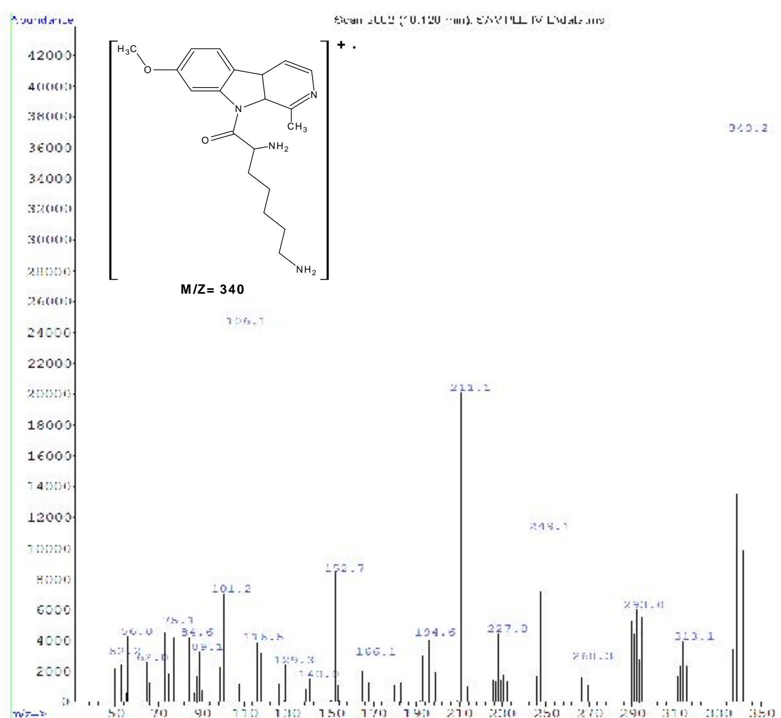
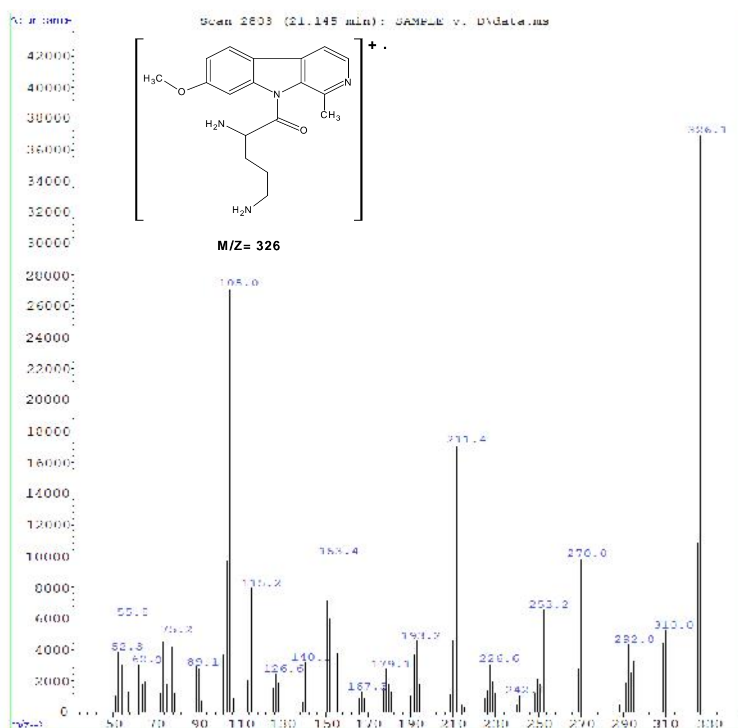


Figure (5): mass Spectrum of (IIIa,IIIb, and IIIc), respectively

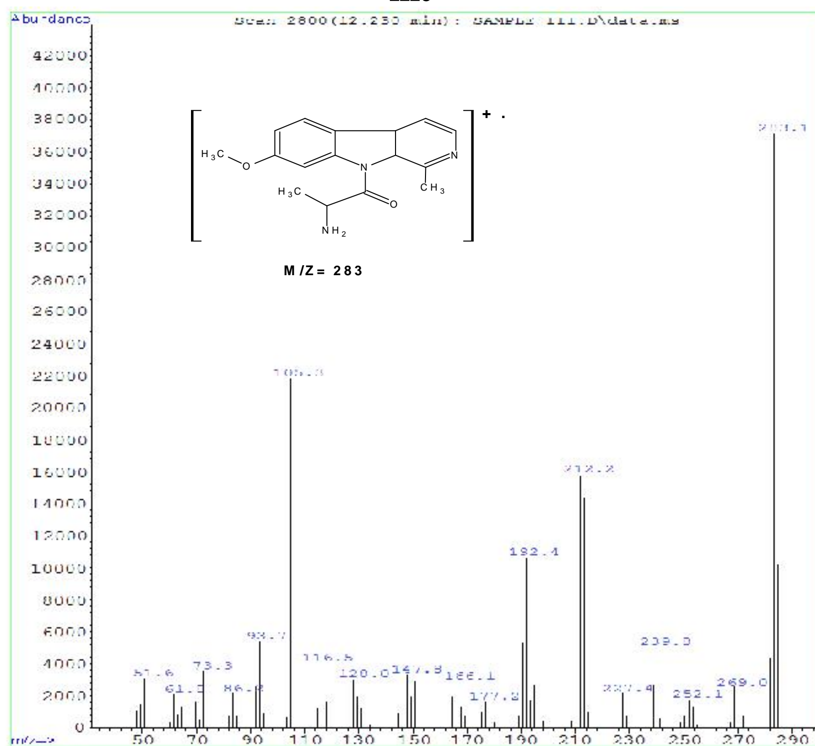
The mass spectra (positive polarity) of (IIId,IIIe, andIIIf)are shown in the figure (6) by using GC-MS Spectroscopy. It shows the presence of the main peaks at m/z 340, 326 and 283, respectively corresponding to the radical cations ($M^{•+}$) which are in agreement with the proposed structure.



IIId



IIIe



IIIf

Figure (6): Mass spectrum of (IIId,IIIe, and IIIf), respectively

3.2.3 3 ^{1}H-NMR and ^{13}C-NMR Spectrum

The ^{1}H-NMR and ^{13}C-NMR spectral data of compounds (IIIa,IIIb,IIIc,IIId,IIIe and IIIf)consist the following results, where the chemical shift are given in ppm:

- ^{1}H-NMR (DMSO): = 11.23 (s, 1H, O–H), 7.13–7.01 (m, 2H, N–H), 4.19 (s, 1H, C–H), 3.30 (s, 2H, CH_2–NH), 2.32–1.55 (m, 6H, CH_2), 1.39 (s, 18H, CH_3);^{13}C-NMR (DMSO): = 175.43 (1C, C=O), 156.37–155.66 (2C, C=O(Boc)), 79.69–79.51 (2C, C–O(CH_3)$_3$), 54.02 (1C, C–H), 41.63–28.30 (4C, CH_2 groups), 23.30 (6C, CH_3 groups) for compound (IIIa).
- ^{1}H-NMR (DMSO): = 11.41 (s, 1H, O–H), 7.18–7.03 (m, 2H, N–H), 4.36 (s, 1H, C–H), 3.73 (s, 2H, CH_2–NH), 1.93–1.59 (m, 4H, CH_2), 1.41 (s, 18H, CH_3); ^{13}C-NMR (DMSO): = 173.62 (1C, C=O), 156.43–155.61(2C, C=O(Boc)), 80.01–79.59(2C, C–O(CH_3)$_3$), 53.35 (1C, C–H), 40.39–25.45 (3C, CH_2 groups), 23.30 (6C, CH_3 groups) for compound (IIIb).
- ^{1}H-NMR (DMSO): = 9.66 (s, 1H, O–H), 6.91 (s, 1H, N–H), 4.49 (s, 1H, C–H), 1.48 (s, 3H, CH_3–CH), 1.38 (s, 9H, CH_3); ^{13}C-NMR (DMSO): = 175.10 (1C, C=O), 155.90 (1C, C=O(Boc)), 79.64 (1C, C–O(CH_3)$_3$), 48.95 (1C, C–H), 28.33 (3C, CH_3 groups), 17.30 (1C, CH_3–CH)for compound (IIIc).
- ^{1}H-NMR (DMSO): = 8.24 (s, 2H, NH_2–CH), 8.12–6.66 (m, 5H, Ar–H), 5.44 (m, 2H, NH_2–CH_2),, 3.88 (s, 3H, CH_3-O), 3.77 (s, 1H, CH–NH_2), 2.79 (s, 3H, CH_3–Ar), 2.62 (m, 2H, CH_2–NH_2), 1.64–1.05 (m, 6H, CH_2); ^{13}C-NMR (DMSO): = 168.46 (1C, C=O), 161.22 (1C, C–O), 146.32–114.11 (10C, C–Ar), 55.56 (1C, CH_3–O), 52.47 (1C, CH–NH_2), 40.45 (1C, CH_2–NH_2), 32.77–22.73 (3C, CH_2 groups), 19.80 (1C, CH_3–C)for compound (IIId).
- ^{1}H-NMR (DMSO): = 8.31 (s, 2H, NH_2–CH), 8.20–6.63 (m, 5H, Ar–H), 5.31 (m, 2H, NH_2–CH_2), 3.91 (s, 3H, CH_3–O), 3.79 (s, 1H, CH–NH_2), 2.80 (s, 3H, CH_3–Ar), 2.65 (m, 2H, CH_2–NH_2), 1.62–0.91 (m, 4H, CH_2); ^{13}C-NMR (DMSO): = 168.91 (1C, C=O), 160.12 (1C, C–O), 147.12–112.78 (10C, C–Ar), 54.86 (1C, CH_3–O), 51.84 (1C, CH–NH_2), 40.10 (1C, CH_2–NH_2), 30.25–25.64 (2C, CH_2 groups), 19.16 (1C, CH_3–C)for compound (IIIe).
- ^{1}H-NMR (DMSO): =8.35(s, 2H, NH_2–CH), 8.18–6.65 (m, 5H, Ar–H), 3.88 (s, 3H, CH_3–O), 3.73 (s, 1H, CH–NH_2), 2.68 (s, 3H, CH_3–Ar), 1.38 (s, 3H, CH_3–CH); ^{13}C-NMR (DMSO): = 168.20 (1C, C=O), 159.65 (1C, C–O), 145.16–113.31 (10C, C–Ar), 55.03 (1C, CH_3–O), 49.58 (1C, C–NH_2), 19.80–18.44 (2C, CH_3 groups), for compound (IIIf).

The protection of amino acids were carried out at low temperatures 0-5°Cfor the first hour of the reactionand gradually brought to room temperature thereafter. The novel compounds at deprotection step of boc-group needed some heat to push the reaction forward; however they gave high yields with longer bench standing times[49].

The yields of protected amino acids were more than 80%, whereas yields of new -carboline derivatives were more than 70%, with melting points that were generally higher than protected amino acids. Factors such as the solvent/solvent mixture, type of base medium, as well as the TEA used, were amended in attempts to obtain higher yields of novel compounds.

Melting points of all synthesized compounds in this paper are generally upper than melting points of their precursors, possibly due to the closer packing of the rings (sp3 hybrid orbitals) and hence an augmented delocalization, which makes these novel compounds more labile.

However, it was also worthy to note that certain -carbolines were very dangerous. On the other hand, human are continuously exposed to endogenous and exogenous -carboline alkaloids. Thus, a rising need, the study on how to deal with them and how to utilize them, especially, their biological and pharmacological activities, should be brought into our mind instantly to reduce their potential risk and to develop new drugs. Moreover, further studies in vivo with respect to possible actions on human health are urgently required. [51].

Increasing evidences have substantially accumulated to support that -carboline and related derivatives widely occurred in nature, especially in various tissues and body fluids of human. And human beings are sufficiently exposed to various -carboline alkaloids, which are both present in plants used for the preparation of hallucinogenic materials and medicinal drugs, and in tobacco smoke and well-cooked food [52]. In addition, previous investigations indicated that human beings can endogenously form various -carboline alkaloids, such as harmine and harmalan. The proposed biosynthesis pathways of these "endogenous alkaloids" in human body fluids and tissues have attracted much concern because of their possible influence on the central nervous function in the last two decades. However, so far, it has been debated whether substantial amounts of them are derived from diet or physiologically [29]. Undoubtedly, the -carbolines had extensive biochemical activities and multiple pharmacological effects. Individual compounds might selectively interact with specific targets so as to lead to a variety of pharmacological actions in vitro and in vivo. Therefore, the -carboline alkaloids might be a particularly promising lead compounds for discovering and developing novel clinical drugs. Taking all those reports together, we might conclude that: (1) the -carboline structure was an important basis for the design and synthesis of novel clinical drugs; (2) various substituents at different positions of -carboline ring system might play a crucial role in determining their multiple pharmacological function; (3) the substituents at position-1, 2, 3 and 9 of -carboline might be important pharmacophore for their antitumor activities; while the substituents at position-3 might be vital for their exhibiting various neuropharmacological effect; the nature of substituents at position-1 and 3 might contritute to their antiparasitic activities or antithrombotic activities. However, it was also worthy to note that certain -carbolines were very dangerous. Harman and norharman were comutagens or precursors of mutagens; TaClo, TaBro and N-methylated -carboline derivatives were potent endogenous neurotoxins; and N-nitroso derivatives of -carboline and APNH derivatives were endogenous mutagens and carcinogens[29]. On the other hand, human are continuously exposed to endogenous and exogenous -carboline alkaloids. Thus, a rising need, the study on how to deal with them and how to utilize them, especially, their biological and pharmacological activities, should be brought into our mind instantly to reduce their potential risk and to develop new drugs. Moreover, further studies in vivo with respect to possible actions on human health are urgently required [51,53,54].

4. CONCLUSION

We have synthesized new -carboline derivatives (from Harmine) bearing a substituted amino acid group of position C-9, they have been designed and characterized by chromatographic and spectroscopies techniques. The Boc-protecting group (tert-butoxycarbonylating agents) of amino protection groups has some advantages compared to the other protecting groups due to its stability under basic conditions, which we need for synthesis in this paper.The carboxylic functional groups of the L-Lysine, L-Ornithine and L-Alanine were not protected with any carboxylic protecting groups. The spectral methods(IR, GC-MS, LC-MS, ^{1}H-NMR and ^{13}C-NMR) proved the proposed structures of the synthesized protected amino acids and new -carboline derivatives (IIIa,IIIb,IIIc,IIId,IIIe and IIIf).

Harmine is a natural product that is existed in Syrian Peganum Harmala Seeds (Palmyra Region). Harmine in our reactions was isolated and then we synthesized newderivatives ofHarmine in high quantitative yields. The reaction is rapid, secure, environmentally friendly and highly efficient.

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