Synthesis of New Opioid Analgesic Peptide Analogues to Enkephalin (Leucine- and Methionine-Enkephalin)

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Abstract

A small number of researches were done in the design and synthesis of enkephalin analogues that are able to resist degradation effect of proteolytic enzymes with good bioavailability and half-lives.Through studying structure activity relationships we tried to incorporate phthalyl group, tryptophan and lysine amino acids in different positions in the basic backbone structure of the naturally occurring opioid Leu^5- and Met^5- enkephalin, in the hope that such insertion of these amino acids could induce interesting addition in the biological activity of these analogues with enhancement of their bioavailability, in addition to decrease side effects as addiction liability.

These synthesized peptides are:

1- Analogue I: phthalyl-tyrosyl-glycel-tryptophan methyl ester.

2- Analogue II: Boc-tyrosyl-glycyl-phenylalanyl-lysine ethyl ester. HBr.

According to the designed structures, the analogues were synthesized following the conventional solution method and they were identified using the following techniques: melting point, optical rotation, thin layer chromatography (TLC), infrared spectroscopy (IR), elemental analysis (CHN) and amino acid analysis.

Key words: peptide, phthalyl, opoioid analgesics

الخلاصة

إن عدداً قليلاً من البحوث تم إنجازها في مجال تصميم وتحضير مركبات جديدة مشابهة للببتيدات المسكنة الطبيعية)الانكفالين(، والتي لها القدرة على مقاومة تأثير الأنزيمات المحللة لها في داخل الجسم مع توافر حيوي وعمر نصفي جيد .وهذا البحث تم إنجازه على هذا الأساس. من خلال الدراسة المستفيضة للعلاقة بين الفعالية الحيوية والتركيب الكيمياوي لهذه المركبات تم اجراء تحويرات على البنية الجزيئية للببتيدات المورفينية بادخال مجموعة الفثاليل والاحماض الامينية التربتوفان واللايسين في مواقع مختلفة للبنية الجزيئية للببتيد المسكن الخماسي ليوسين^5 -وميثيونين^5-انكفالين أملاً بالحصول على مركبات شبيهة بالانكفالينات لم يسبق تحضيرها من قبل ولها فعالية حياتية وفسيولوجية مهمة مع زيادة توافرها الحيوي، على أمل ايضاً من تقليل الأعراض الجانبية مثل الإدمان .

والمركبات المحضرة هي :

1- المشتق الاول :فثاليل-تايروسيل-كلايسيل-تربتوفان مثيل استر .

2- المشتق الثاني :بوك-تايروسيل-كلايسيل-فنيل الانيل-لايسين اثيل استر هيدروبرومايد.

والتي خلقت بطريقة الطور السائل التقليدية، وتم استخدام التقنيات التالية بهدف التوصل الى الخواص المميزة لتلك الببتيدات ولإثبات بنيتها التركيبية :قياس درجة الانصهار، كروماتوغرافيا الطبقة الرقيقة، الاستدارة البصرية، التحليل الدقيق للعناصر، تحليل الأحماض الامينية، مطياف الأشعة تحت الحمراء.

Introduction

The opium group of narcotic drugs is among the most powerfully acting and clinically useful drugs producing depression in the central nervous system [(1)]. Opiates are drugs derived from opium (*Papver somniferum F. Papaveraceae*), and include morphine, codeine and a wide variety of semisynthetic congeners derived from them and thebain (another compound of opium) [(2)]. The term opioid is more inclusive, applied to all agonists and antagonists with morphine-like activity as well as to naturally occurring (endogenous) and synthetic opioid peptides [(3)]. Opioid peptides defined as peptides with opiate-like pharmacological effect [(4,5)]. The discovery of endogenous opioids has been followed shortly after the identification of opioid receptors [(6)]. The main clinical uses of opioid peptides are as analgesics (enkephalins) [(7,8)], as antioxidants (enkephalins) [(9)], as anticancers (dalargin) [(10)], as antibacterials and antifungals (phthalyl serine, phthalyl arginine) [(11)].

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Materials and Methods:

All amino acids and their derivatives were optically active and of L-configuration and supplied from Fluka AG/Switzerland. All of the solvents and materials used were of Analar type and used without further purification. The method used for synthesis of these analogues was conventional solution method in which we used *N,N'*-dicyclohexyl carbodiimide (DCCI) and 1-hydroxy-benzotriazole (HBT) as a coupling agent and to prevent racemization, respectively. We used *tert*-butyoxy carbonyl (Boc) group and benzyloxy carbonyl (Z) group as terminal amino protecting group. Boc-tyrosine and lysine ethyl ester-N^{ε}-Z were obtained fully protected from Fluka AG/Switzerland; while we use methyl and ethyl ester to protect the carboxyl moiety in peptide synthesis. The final analogues were purified using gel filtration on sephadex LH-20 column eluted with 0.1N acetic acid. The synthesis of analogues (I and II) include the following general procedures:

a. N-terminal protection: the phtalylamide derivative of amino acid was used for the intermediate cpd (1.3) in analogue I by fusion method [12], and the common amino protecting group which is tert-butoxycarbonyl (Boc), (Boc-Tyrosine) and N-benzyloxycarbonyl (Lys-OEt-N-Z)were obtained fully protected for analogue II.

b. C-terminal protection: methyl and ethyl ester were used to protect the carboxyl group of amino acid in peptide synthesis.

c. Coupling method: conventional solution method was used as a coupling method between the protected amino acid for peptide synthesis. DCCI was used in the peptide bond formation as a coupling agent, while HBT was used to decrease racemization and to increase the yield.

d. De-protection of C-terminals: the removal of methyl or ethyl ester (de-esterification) was carried out with 1.5 equivalent of 1N sodium hydroxide solution (saponification).

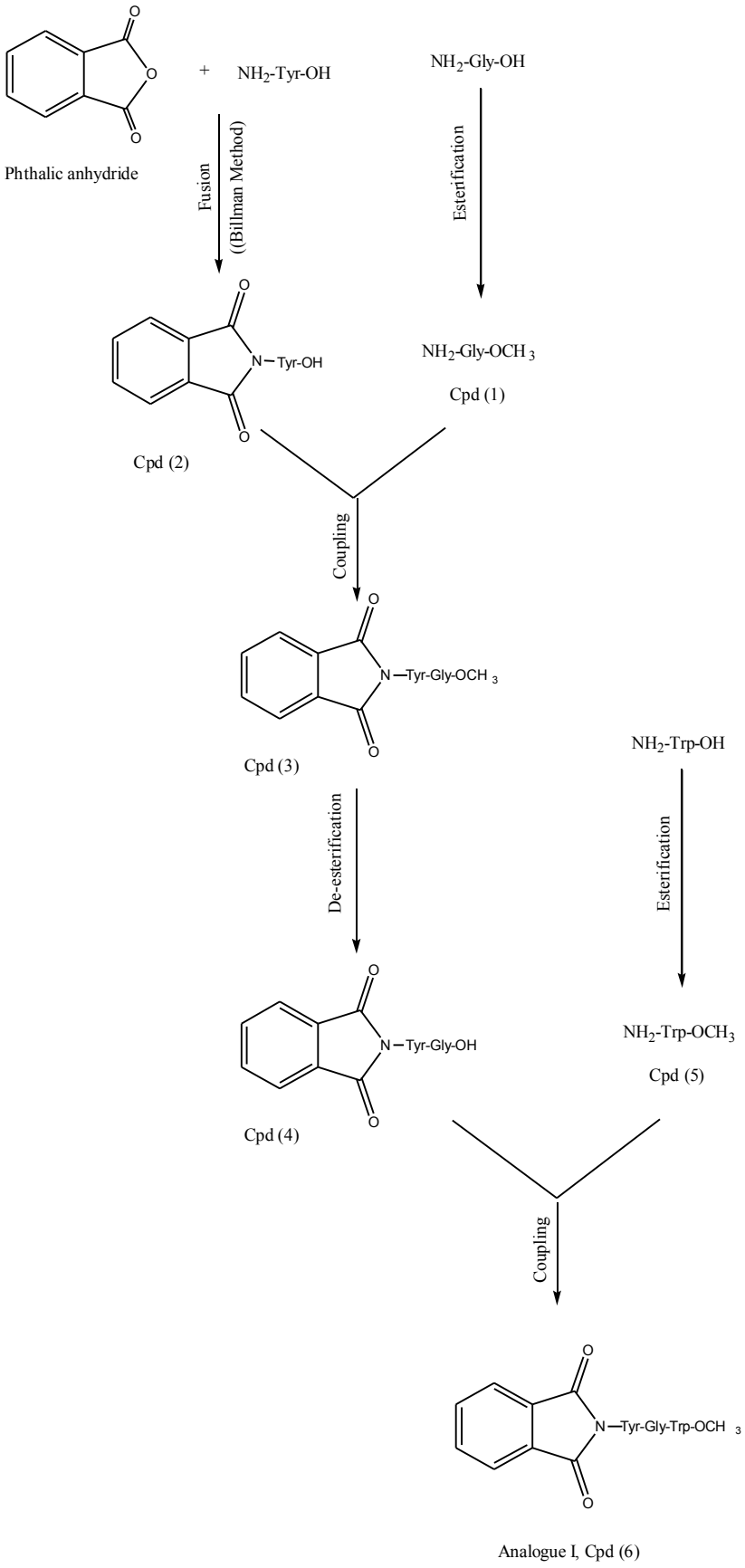
e. De-protection of N-terminal protecting groups: this step was performed with strong acidic conditions in which the benzyloxycarbonyl (Z) groups were removed with equimolar quantities of HBr in glacial acetic acid.

f. Peptide purification: the intermediates and the peptides have been purified by repeating re-crystallization several times (2-4 times) using different solvents as diethyl ether, petroleum ether, ethyl acetate, absolute ethanol, distilled water and chloroform. The final analogues were purified using gel filtration on sephadex LH-20 column eluted with 0.1N acetic acid.

Synthesis of analogue I:

Scheme (1) shows the steps of synthesis of analogue I which include:

a- Synthesis of glycine-methyl ester (cpd. 1): a suspension containing (1.5gm, 0.02 mol) of glycine in methanol (15ml) was cooled down to (-15 ^{o}C), then thionyl chloride (1.46ml, 0.02 mol) equimolar was added drop wise to the suspension, keeping the temp below (-10 ^{o}C). Then the reaction mixture was kept at (40 ^{o}C) for (3 hrs), followed by refluxing for (3 hrs), and left at room temperature overnight. After solvent evaporation to dryness in vacuum, the product was purified by re-crystallization from methanol-diethylether (1:10) mixture. Physical appearance, melting point, and R_f value are listed in table (1) [13].



Scheme (1): Synthesis of analogue I.

Table (1): Physical appearance, M.Ps, and R_f values of intermediates and final analogues.

Compound No.	Physical appearance	Melting points (°C) Found	Melting points (°C) Reported	R_f values *
5	White crystals	209-210	213-214	0.88 (D) 0.71 (A)
1	White crystals	171-173	175	0.78 (D) 0.86 (E)
2	Off-white powder	188-190	-	0.84 (B) 0.85 (C) 0.76 (D)
3	Off-white Powder	140-142	-	0.39 (B) 0.68 (D)
4	White powder	180-182	-	0.8 (C) 0.92 (D)
6 (Analogue I)	White powder	170-173	-	0.75 (D) 0.9 (E)
9	White crystals	154-155	157-158	0.76 (A) 0.94 (D)
7	White powder	115-118	-	0.69 (D) 0.88 (E)
8	White powder	155-158	-	0.68 (C) 0.95 (D) 0.83 (E)
10	White powder	148-150	-	0.8 (A) 0.36 (B) 0.6 (D)
11	Faint yellow powder	218-220	-	0.7 (C) 0.8 (D)
12	White powder	158-160	-	0.55 (B) 0.67 (C)
13 (Analogue II)	Needle shaped crystals	196-198	-	0.45 (B) 0.37 (C)

* Solvent system used in TLC were:
A: Butanol: Acetic acid : D.W. (4:1:5).
B: Chloroform: Methanol: Acetic acid (4:5:1).
C: Chloroform: Methanol: Ether (5:3:3).
D: Chloroform: Methanol (7:3).
E: C: Chloroform: Methanol: Benzene (4:3:2).

Table (2): Infrared values for analogue I and II.

Analogue number	IR value
Analogue I	3600-3460, 3340, 3074, 2983, 2858, 2806, 1738, 1677, 1569, 1504, 1440, 1373, 1244, 846 and 786
Analogue II	3690-3384, 3328, 3031, 2927, 2850, 1736, 1670, 1628, 1610, 1569, 1508, 1448, 1340, 1311, 1277 and 640

b- Synthesis of phthalyl-tyrosine (cpd. 2): Compound 2 is prepared by Billman *et al.* method [14], in which L-tyrosine (18.119gm, 0.1mol) and phthalic anhydride (114.8gm, 0.1mol) fused together to give cpd. 2. Physical appearance, melting point, and R_f value are listed in table (1). Elemental analysis, amino acid analysis and optical rotation are listed in table (3), (4) and (5), respectively.

c- Synthesis of phthalyl-tyrosyl-glycin methyl ester (cpd. 3): a stirred solution of cpd. 2 (1 mmol) in DMF (5ml) and NMM (1 mmol) were added with stirring for (10 min). Then eqimolar amount of cpd. 1 previously dissolved in DMF (5 ml) was also added, the mixture was cooled down to (-10 °C). HBT (2 mmol) and DCCI (1 mmol) were added with stirring. Stirring was continued for (3 days) at (0 °C) and then at room temperature for (7 days). Then DCU was filtered, the filtrate was concentrated under vacuum, and then the residue was re-dissolved in ethyl acetate washed several times. The product was collected after solvent evaporization. Physical properties, elemental analysis, amino acid analysis and optical rotation

are listed in table (1), (3), (4) and (5), respectively.

d- Synthesis of Phthalyl-tyrosyl-glycine (cpd 4): to a stirred solution of cpd 3 (0.5mmol) dissolved in dioxan (5 ml): water mixture (5:1) at (18 ^{o}C), sodium hydroxide solution (1N, 0.75ml) was added drop wise over (30 min). The reaction was allowed to proceed for additional (3 hrs). Then the reaction mixture was acidified with equimolar quantity of hydrochloric acid. After the addition of ice-water, a precipitate was obtained. The physical properties are listed in table (1).

e- Synthesis of Trptophane-methyl ester (cpd 5): a suspension of tryptophan (9.8 mmol) in methanol (20ml) was cooled down to (-15^{o}C), and continue the procedure as in the synthesis of cpd 1.

f- Synthesis of analogue I (Phthalyl-glycyl-tryptophane methyl ester (cpd 6)): the same procedure was performed as in the synthesis of cpd 3. Physical properties, elemental analysis, amino acid analysis and optical rotation are listed in table (1), (3), (4) and (5), respectively. IR values for analogue I are shown in table (2).

Table (3): Elemental analysis of some intermediates and final analogues.

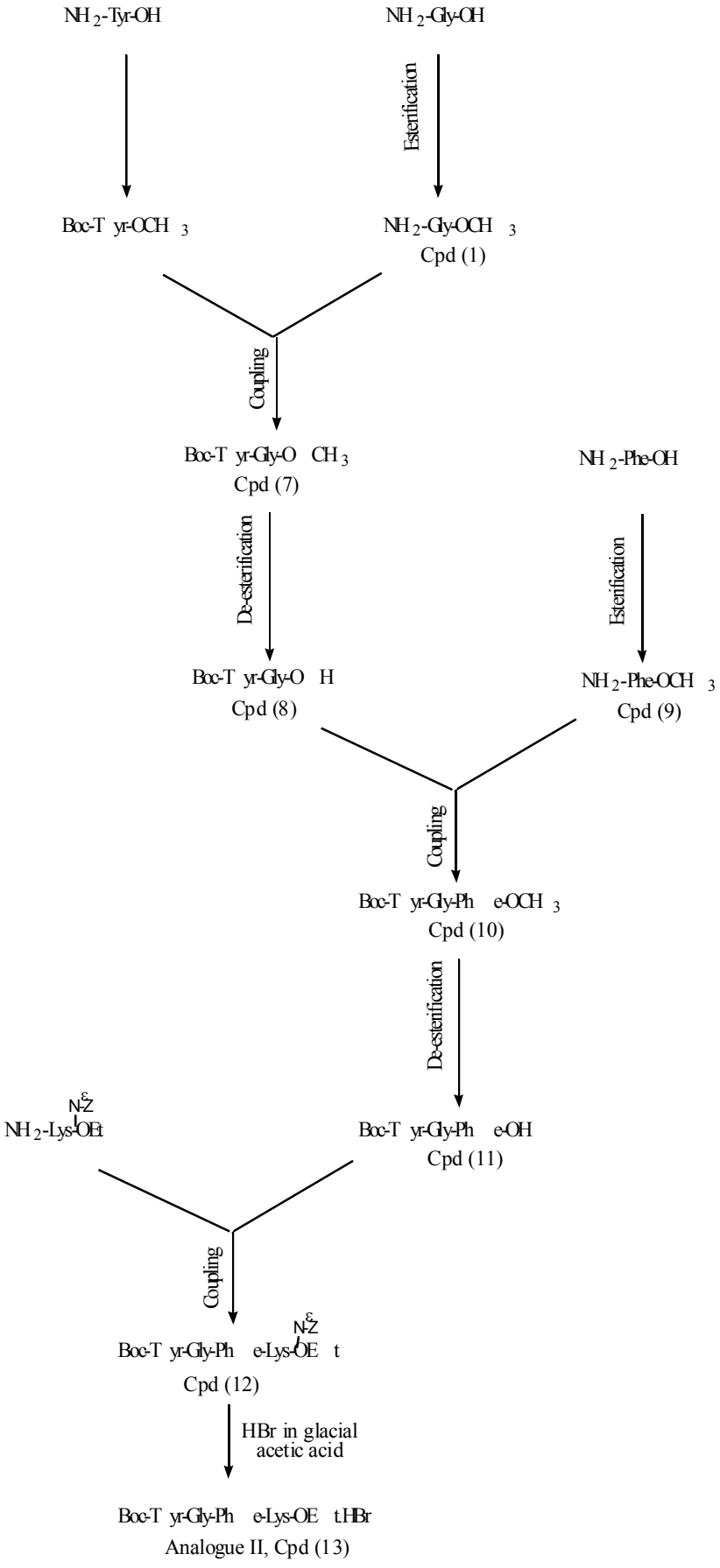
Cpd. no.	Compound	Chemical formula	Calculated/Found		
			C%	H%	N%
2	Phthalyl-N-Tyr	$C_{17}H_{13}N_1O_5$	65.59 65.89	4.21 4.45	4.49 4.31
3	Phthalyl-N-Tyr-Gly.OMe	$C_{20}H_{18}N_2O_6$	62.82 63.2	4.74 4.55	7.32 7.59
6	Phthalyl-N-Tyr-Gly-Trp.OMe (Analogue I)	$C_{31}H_{22}N_2O_6$	65.48 66.01	4.96 5.50	9.85 9.66
8	Boc-Tyr-Gly	$C_{16}H_{22}N_2O_6$	56.79 57.45	6.55 6.11	8.27 8.75
10	Boc-Tyr-Gly-Phe.OMe	$C_{26}H_{33}N_3O_7$	62.51 62.81	6.65 6.60	8.41 8.91
12	Boc-Tyr-Gly-Phe-Lys.OEt (-N$^{\varepsilon}$-Z)	$C_{41}H_{53}N_5O_{10}$	63.46 66.1	6.88 6.79	9.02 9.35
13	Boc-Tyr-Gly-Phe-Lys.OEt.HBr (Analogue II)	$C_{33}H_{48}N_5O_8Br$	54.84 54.81	6.55 5.99	9.69 9.21

Table (4): Amino acid analysis of some intermediates and final analogues.

Cpd. no.	Compound	Amino acids				
		Tyr	Gly	Phe	Lys	Trp
2	Phthalyl-N-Tyr	1.1				
3	Phthalyl-N-Tyr-Gly.OMe	0.99	1.02			
6	Phthalyl-N-Tyr-Gly-Trp.OMe (Analogue I)	0.96	0.99			1.01
8	Boc-Tyr-Gly	1.09	1.11			
10	Boc-Tyr-Gly-Phe.OMe	1.06	0.98	1.08		
12	Boc-Tyr-Gly-Phe-Lys.OEt (-N$^{\varepsilon}$-Z)	1.01	0.95	1.1	0.89	
13	Boc-Tyr-Gly-Phe-Lys.OEt.HBr (Analogue II)	0.89	1.13	1.1	1.18	

Table (5): Optical rotation of some intermediates and final analogues.

Cpd. no.	Compound	Optical rotation $[\alpha]_D^{25}$, c=1 in DMF
2	Phthalyl-N-Tyr	-32 ^{o}
3	Phthalyl-N-Tyr-Gly.OMe	-16 ^{o}
6	Phthalyl-N-Tyr-Gly-Trp.OMe (Analogue I)	-36 ^{o}
8	Boc-Tyr-Gly	+48 ^{o}
10	Boc-Tyr-Gly-Phe.OMe	+80 ^{o}
12	Boc-Tyr-Gly-Phe-Lys.OEt (-N$^{\varepsilon}$-Z)	-29 ^{o}
13	Boc-Tyr-Gly-Phe-Lys.OEt.HBr (Analogue II)	-27 ^{o}



Scheme (2): Synthesis of analogue II.

Synthesis of Analogue II:

Scheme (2) shows the steps of synthesis of analogue II which include:

a- Synthesis of phthalyl alanine methyl ester (cpd. 9): the same procedure was carried out as in synthesis of cpd. 1 and cpd. 5.

b- Synthesis of Boc-tyrosyl-glycine methyl ester (cpd. 7), Boc-tyrosyl-glycyl-phenylalanyl-lysine etheyl ester-N^{ε}-Z (cpd. 12): again the same procedure was applied as in the synthesis of cpd. 3 and cpd. 6.

c- Synthesis of Boc-tyrosyl-glycine (cpd. 8) and Boc-tyrosyl-glycyl-phenylalanine (cpd. 11): The de-esterification was performed as in the synthesis of cpd. 4 and the physical results are listed in table(1).

d- Synthesis of analogue II (Boc-tyrosyl-glycyl-phenylananyl-lysin ethyl ester. HBr (cpd. 13)): cpd. 12 (0.337 mmol) was dissolved in HBr (2.5N, 3ml) in glacial acetic acid, the mixture was left at room temperature for (30 min), then it was poured into (200ml) of diethyl ether with stirring. The separated oily material was evaporated under reduced pressure. The precipitated HBr salt was re-crystallized from ethyl acetate-petroleum ether mixture (1:7). The physical properties, elemental analysis, amino acid analysis and optical rotation are shown in tables (1), (3), (4) and (5), respectively. IR values are shown in table (2) .

Results and Discussion:

The results of our work were been shown in schemes (1 and 2), figures (1 and 2) and tables (1, 2, 3, 4 and 5). The methodology that has been adapted in this work seems to be successful according to the results indicated previously, in addition a biological activity study has been done on cpd. 4 (phthalyl-tyrosyl-glycine) and give significant positive results for analgesic activity relative to morphine [15].The structural modification that has been made on the backbone of Leu-enkephalin in this work to synthesize these new analogues and the positive results mentioned indicate that an enhanced analgesic activity has been achieved from this modification and this would open the door for further modifications or studies of these two analogues to discover a new biological activities as antimicrobials and anticancers.

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