HYGEIA
JOURNAL FOR DRUGS AND MEDICINES
October 2011- March 2012
www.hygeiajournal.com

A half yearly scientific, international, open access journal for drugs and medicines. Research article section: Pharmaceutical chemistry / Design of drugs and medicines

Synthesis and Analgesic activity studies of Simple new Aspirin Enkephalin analogues

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Article history: Received: 02 October, 2010, revised: 27 November, 2010, accepted: 23 July, 2011, Available online: 01 October 2011

Abstract

*Aspirin amino acid amides **1(a-f)**, Aspirin dipeptide amides **2(a-f).** tri peptide amides **3(a-f)** and tetra peptide amides **4a** and **4c** were Synthesized. The synthesis of dipeptide amides were carried out in solution by stepwise elongation of the peptide chain from the C-terminal amino acid by coupling one amino acid at a time using DCC/HOBt method. The analgesic activity was studied by acetic acid induced writhing test. From the mean writhing values, it is evident that the entire sequence of the tetrapeptideamide namely, Gly-Gly-Phe-Met-NH_2 is essential for the observed analgesic activity of compound A. And, the entire sequence of the tetra peptide amide namely, Gly-Gly-Phe-Leu-NH_2 is essential for the observed analgesic activity of compound C. The decrease in analgesic activity of these compounds is in the order, aspirin tripepitide amides > aspirin dipeptide amides > aspirin amino acid amides.*

***Keywords**:* Aspirin, analgesic activity, amino acid amide, di, tri, and tetra peptides

1. Introduction

It has been found in our studies that the incorporation of inactive tetrapeptide amides of enkephalins into aspirin do not affect its peripherally medicated analgesic activity and interestingly has increased its potency by about 10 times.

It was now proposed to synthesize aspirin derivatives of aminoacid amides, dipeptide amides and tripeptide amides and study their analgesic activity. This is an attempt to know whether the entire tetra peptide amide sequence is responsible for its enhanced activity or any specific amino acid, dipeptide or tripeptide of the tetra peptide amide causes this enhancement. The following compounds were synthesized and their analgesic activity was studied.

Analgesics[1] are drugs which relieve pain without causing loss of consciousness. Analgesic drugs act on the central and peripheral nervous systems and relieve pain by direct effects on pain and related path ways but do not affect the underlying pathology. The term “analgesic” covers only those agents which when administered systematically provide non-specific relief from pain without loss of consciousness by increasing the threshold of pain.

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J. Balamani &M.Sekar, H.J.D.Med. Vol. 3 (2), 2011, pp.1-15.

Beluzzi et al [2, 3] reported the analgesic effect of Met- and Leu-enkephalins through injection into lateral ventricles of rats, using the tail flick procedure. This effect lasts only for a short duration and can be antagonized by naloxone.

I. Aspirin – Met – NH_2
II. Aspirin – Phe – NH_2
III. Aspirin – Phe – Met – NH_2
IV. Aspirin – Gly – Phe – NH_2
V. Aspirin – Gly – Phe – Met – NH_2
VI. Aspirin – Gly – Gly – Phe – NH_2
VII. Aspirin – Leu – NH_2
VIII. Aspirin – Gly – NH_2
IX. Aspirin – Gly – Gly – NH_2
X. Aspirin – Phe – Leu – NH_2
XI. Aspirin – Gly – Phe – Leu – NH_2

Both enkephalins are less potent than morphine. The lower potency of enkephalins and short duration of activity has been found to be due to the rapid cleavage of the Tyr1-Gly2 bond, by the action of enzymes present in brain. The biochemical role of the enkephalins is to inhibit the synthesis of the enzyme adenylcyclase. This enzyme catalyzes the conversion of 5'-adenosine triphosphate (ATP) to cyclic 3, 5'-adenosine monophosphate (cAMP).

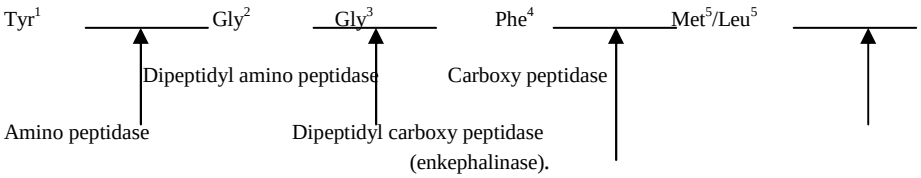
It is assumed that the lowering of the level of cAMP which is accompanied by a simultaneous increase in the level of cyclic 3, 5'-guanine monophosphate (cGMP) is related to analgesia. [1]

Enkephalins produced moderate to profound sedation and immobility, which can be prevented or reversed by naloxone. [4, 6] Leu-enkephalin elicits a contralateral rotational behavior, which cannot be prevented by naloxone. Enkephalins have received considerable attention as a result of the search for a drug with opiate-like activity and still be free of addiction liability[7]. Enkephalins induce dose-independent decrease in diastolic blood pressure and heart rate after intravenous administration of pentobarbitone anaesthetized dogs [8]. Enkephalins are also known to have antidiarrhoeal effects. [9, 10]

Enzymatic cleavage of Enkephalins

All the four peptide bonds in enkephalins are known to be susceptible to the action of peptidases which cause rapid degradation. However, amino peptidases as well as enkephalinase-A are the only two well defined peptidases which are responsible for the physiological inactivation of the pentapeptides [11, 12].

The possible modes of degradation of enkephalin molecules are shown in figure below



In enkephalins presence of Tyr in position 1 seems to be an absolute requirement, since enkephalins are hydrolysed by an aminopeptidase to give L-Tyrosine and Gly-Gly-Phe-Leu/Met, which are biologically inactive. Also loss of activity is observed on changing the L-tyrosyl to a D-tyrosyl configuration. These evidences show the tyrosyl residue is a key-binding element in enkephalin sequence..

Other studies such as masking the hydroxyl group in tyrosyl residue or removing it shows the loss of activity. Above proofs stress the idea that the tyrosyl residue and phenolic hydroxyl group are responsible for analgesia [13]

2. Materials and Methods

Aspirin was prepared by refluxing a freshly recrystallised sample of salicylic acid with a mixture of Ac_2O and AcOH.

Aspirin was coupled with tripeptide amide, dipeptide amide and aminoacid amide using its p-nitrophenyl (Np) ester. Coupling of aspirin using DCC/HOBt was observed to be incomplete even after prolonged stirring. Thus, its aforementioned, ester was used for coupling. Aspirin p-nitro phenyl ester (Aspirin-ONp) was prepared by treating aspirin with 1.1 equivalent of p-nitrophenol in presence of DCC in EtOAc. Aspirin – ONp was precipitate using EtOH.

The synthesis of dipeptide and tripeptide amides were carried out in solution by stepwise elongation of the peptide chain from the c-terminal aminoacid by coupling one aminoacid at a time using DCC/HOBt method Boc group was used for N^{α} protection of all aminoacids. The Boc-group cleavage was carried out using 50% TFA / CH_2Cl_2. The amidation of C-terminal aminoacids was carried out by treating the corresponding Boc-aminoacids with dry NH_3 in presence of DCC and HOBt.

Then, Boc-aminoacid, Boc-aminoacid-NH_2, Boc-dipeptide-NH_2, Aspirin-dipeptide-NH_2, Boc-tripeptide-NH_2, Aspirin-tripeptide-NH_2 are prepared as follows.

2.1.**Boc-Amino acids**

A mixture of amino acid (10 mmoles), Boc-N_3 (2ml, 13 mmoles), Et_3N (3.4 ml, 25 mmoles) DMF (4ml) and water (5ml) was stirred at room temperature for 12-15 h. Water (50ml) was added to the reaction mixture and extracted with ether (3 x 30ml).

The aqueous phase was acidified with $KHSO_4$ to pH2 and the liberated Boc-aminoacid was extracted into EtOAc (3 x 20 ml). The combined extracts were washed with water (4 x 30 ml), dried over Na_2SO_4 and evaporated *in vacuo*. The residue was crystallized using petroleum ether.

The yield, melting point and the R_f values of the Boc-amino acid prepared are given below:

Boc-aminoacid	**Yield**	**M.p.**	**Rf values**	
	%	**°C**	**A**	**B**
Boc-Met	91	Oil	0.64	0.67
Boc-Phe	83	Oil	0.59	0.63
Boc-Gly	88	87-88	0.48	0.53
Boc-Leu	81	85-86	0.61	0.73

2.2. **Boc-aminoacid-NH_2**

To a mixture of Boc-aminoacid (10 mmoles), HOBt (1.5g, 11 mmoles), in CH_2Cl_2 (40ml), and DMF (10ml), a solution of DCC (2.1g, 10.5 mmoles) in CH_2Cl_2 (20ml) was added and stirred for 30 min. Then dry NH_3 was passed through the solution till saturation and stirred for 4-5h. The solvent was then evaporated *in vacuo* and the residue was diluted with EtOAc (100 ml). A few drops of AcOH were added to destroy any unreacted DCC. The mixture was cooled and filtered to remove DCU. The filtrate was washed successively with 10% aq. Na_2CO_3 (3 x 30 ml), water (3 x 30 ml), 1N HCl (1 x 30 ml), water (3 x 30 ml) and saturated NaCl (30 ml). It was then dried over Na_2So_4 and evaporated *in vacuo*. The residue was crystallized form petroleum ether.

The yield, melting points and R_f values of the Boc-aminoacid-amides prepared are given below:

Boc-aminoacid -NH_2	Yield %	M.p. °C	Rf values A	B
Boc-Met-NH_2	86	109-111	0.67	0.77
Boc-Phe-NH_2	92	120-122	0.63	0.69
Boc-Leu-NH_2	87	139-141	0.55	0.62
Boc-Gly-NH_2	82	115-117	0.49	0.53

2.3. **Aspirin-aminoacid-NH_2**

Boc-aminoacid-NH_2 (5 mmoles) was deprotected by treating with a 1:1 mixture of TFA / CH_2Cl_2 (10ml) for 30 min at room temperature. The solvent was removed under reduced pressure and the residue was treated with 5M HCl/THF (10ml) for 30 min to get HCl. Aminoacid-NH_2. The solvent was removed under reduced pressure and the residue was dried over KOH pellets *in vacuo*.The above HCl salt was dissolved in dry DMF (10ml) and neutralized with Et_3N. To this a solution of aspirin-ONp (1.5, 5 mmoles) and HOBt (0.7 g, 5 mmoles) in CH_2Cl_2 (10ml) was added. The reaction mixture was stirred for 24h at room temperature. TLC monitored the completion of the reaction.

The solvent was then evaporated under reduced pressure and the residue was diluted with EtOAc. Further workup process is same as given for Boc-aminoacid-NH_2. Crystallization was done using petroleum ether.

The yield, melting points and R_f values of the aspirin-amino acid-NH_2 prepared are give below:

Asp-aminoacid – NH_2	Yield %	M.p. °C	Rf values A	B
Aspirin-Met-NH_2 (I)	88	175-177	0.57	0.63
Aspirin-Phe-NH_2 (II)	87	176-178	0.61	0.66
Aspirin-Leu-NH_2 (VII)	81	171-173	0.58	0.64
Aspirin-Gly-NH_2 (VIII)	70	173-175	0.63	0.73

2.4. **Boc-dipeptide-amide**

Boc-aminoacid-NH2 (5 mmoles) was deprotected by treating with a 1:1 mixture of TFA / CH2Cl2 (10ml) for 30 min at room temperature.

The solvent was removed under reduced pressure and the residue was treated with 5M HCl / THF (10ml) for 30 min to get HCl. Dipeptide-NH_2. The solvent was removed under reduced pressure and the residue was dried over KOH pellets *in vacuo*.

The above HCl salt was dissolved in dry DMF (10ml) and neutralized with Et_3N. To this a solution of Boc-aminoacid (5 mmoles) and HOBt (0.7g, 5 mmoles) in CH_2Cl_2 (20ml) was added.

This mixture was cooled to 0°C and a cold solution of DCC (1g, 5 mmoles) in CH_2Cl_2 (10ml) was added dropwise under stirring. The reaction mixture was the stirred for about 1h at 0°C and for about 15 h at room temperature. TLC monitored the completion of the reaction. The solvent was then evaporated under reduced pressure and the residue was diluted with EtOAc. Further workup process is same as given for Boc-aminoacid-NH_2. Crystallization was done using petroleum ether.

The yield, melting point and R_f values of the Boc-dipeptide-NH2 prepared are given below:

Boc-aminoacid – NH_2	Yield	M.p.	Rf values	
	%	°C	A	B
Boc-Phe-Met-NH_2	80	118-120	0.76	0.83
Boc-Gly-Phe-NH_2	81	144-146	0.71	0.75
Boc-Phe-Leu-NH_2	81	165-167	0.53	0.61
Boc-Gly-Gly-NH_2	71	136-138	0.46	0.54
Boc-Gly-Met-NH_2	78	145-147	0.67	0.71

2.5. **Aspirin-dipeptide-NH_2**

Boc-dipeptide-NH_2 (5 mmoles) was deprotected by treating with a 1:1 mixture of TFA/CH_2Cl_2 (10 ml) for 30 min at room temperature. The solvent was removed under reduced pressure and the residue was treated with 5M HCl/THF (10 ml) for 30 min to get HCl. dipeptide-NH_2. The solvent was removed under reduced pressure and the residue was dried over KOH pellets *in vacuo.*

The above HCl salt was dissolved in dry DMF (10ml) and neutralized with Et_3N. To this a solution of aspirin-ONp (1.5g, 5 mmoles) and HOBt (0.7g, 5 mmoles) in CH_2Cl_2 (10ml) was added. The reaction mixture was stirred for 24 h at room temperature. TLC monitored the completion of the reaction. The solvent was then evaporated under reduced pressure and the residue was diluted with EtOAc. Further workup process is same as given for Boc-aminoacid-NH_2. Crystallization was done using petroleum ether.

The yield, melting point and R_f values of the aspirin-dipeptide-NH_2 prepared are given below:

Asp-dipeptide NH_2	Yield %	M.p. °C	Rf values A	B
Aspirin-Phe-Met-NH_2 (III)	82	172-174	0.54	0.59
Aspirin-Gly-Phe-NH_2 (IV)	83	173-175	0.56	0.61
Aspirin-Phe-Leu-NH_2 (x)	81	171-173	0.59	0.65
Aspirin-Gly-Gly-NH_2 (IX)	77	176-178	0.54	0.61

2.6. **Boc-tripeptide-NH_2**

Boc-dipeptide-NH_2 (5 mmoles) was deprotected by treating with a 1:1 mixture of TFA / CH_2Cl_2 (10ml) for 30 min at room temperature. The solvent was removed under reduced pressure and the residue was treated with 5M HCl/THF (10ml) for 30 min to get HCl.dipeptide-NH_2. The solvent was removed under reduced pressure and the residue was dried over KOH pellets in vacuum.

The above HCl salt was dissolved in dry DMF (10ml) and neutralized with Et3N. To this a solution of Boc-aminoacid (5 mmoles) and HOBt (0.7g, 5 mmoles) in CH_2Cl_2 (20ml) was added. This mixture was cooled to 0°C and a cold solution of DCC (1g, 5 mmoles) in CH_2Cl_2 (10ml) was added dropwise under stirring. The reaction mixture was then stirred for about 1h at 0°C and for about 15 h at room temperature. TLC monitored the completion of the reaction. The solvent was then evaporated under reduced pressure and the residue was diluted with EtOAc. Further workup process is same as given for Boc-aminoacid-NH_2. Crystallization was done using petroleum ether.

The yield, melting point and R_f values of the Boc-tripeptide-NH_2 prepared are given below:

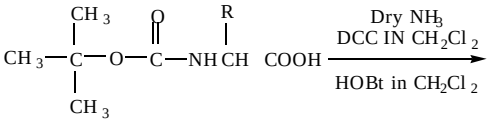
Boc-tripeptide –NH_2	Yield %	M.p. °C	Rf values A	B
Boc-Gly-Phe-Met-NH_2	91	145-147	0.59	0.64
Boc-Gly-Gly-Phe-NH_2	86	166-168	0.55	0.62
Boc-Gly-Phe-Leu-NH_2	82	165-167	0.63	0.68
Boc-Gly-Gly-Met-NH_2	83	165-167	0.74	0.70

2.7. **Aspirin-tripeptide-NH_2**

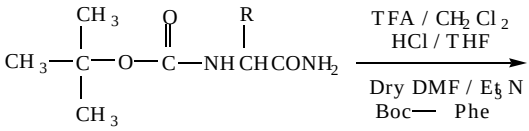
Boc-tripeptide-NH_2 (5 mmoles) was deprotected by treating with a 1:1 mixture of TFA/CH_2Cl_2 (10 ml) for 30 min at room temperature. The solvent was removed under reduced pressure and the residue was treated with 5M HCl/THF (10 ml) for 30 min to get HCl. tripeptide-NH_2. The solvent was removed under reduced pressure and the residue was dried over KOH pellets *in vacuo.*

The above HCl salt was dissolved in dry DMF (10ml) and neutralized with Et_3N. To this a solution of aspirin-ONp (1.5g, 5 mmoles) and HOBt (0.7g, 5 mmoles) in CH_2Cl_2 (10ml) was added. The reaction mixture was stirred for 24 h at room temperature.

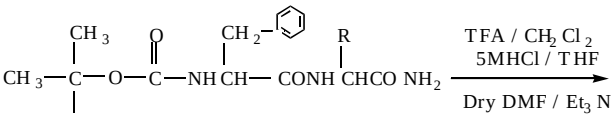
3. Scheme of synthesis



Boc – amino acid 1(a – b)

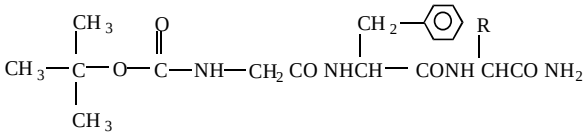


Boc – aminoacid amide 2(a – b)

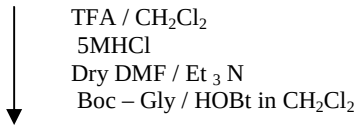


Boc – Phe – aminoacid amide 3(a – b)

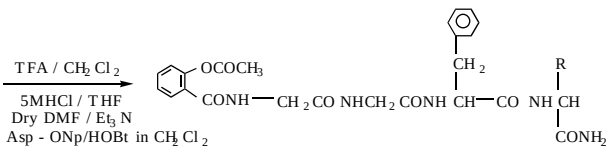
Boc – Gly/HOBt in CH_2Cl_2
DCC in CH_2Cl_2



Boc – Gly – Phe – aminoacid amide 4(a – b)



Boc – Gly – Gly – Phe – aminoacid amide 5 (a – b)

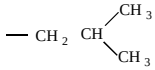


Asp – Gly – Gly – Phe – aminoacid – NH_2

1 and 3

1a, 2a, 3a, 4a, 5a & 1,

R = $CH_2\ CH_2\ SCH_3$

1b, 2b, 3b, 4b, 5b & 3 R = 

TLC monitored the completion of the reaction. The solvent was then evaporated under reduced pressure and the residue was diluted with EtOAc. Further workup process is same as given for Boc-aminoacid-NH_2.

The yield, melting point and R_f values of the aspirin-tripeptide-NH_2 prepared are given below:

Aspirin-tripeptide-NH_2	**Yield**	**M.p.**	**Rf values**	
	%	**°C**	**A**	**B**
Aspirin-Gly-Phe-Met-NH_2 (V)	78	175-177	0.56	0.60
Aspirin-Gly-Gly-Phe-NH_2 (Vi)	82	170-172	0.56	0.62
Aspirin-Gly-Phe-Leu-NH_2 (XI)	78	170-173	0.61	0.66

The Structures of compounds A, C, I to XI were confirmed by IR, NMR Data and elemental analysis.

4. Acetic acid induced writhing test

Analgesic effects of the compounds were studied using ACOH-inducing writhing in mice. The mouse weighing 25-30g was separated into 25 groups of 5 each. The animal studies were conducted according to the institutional animal ethical committee (IAEC) and CPCSEA guidelines, (No.659/02/a/CPCSEA).After 18h fasting, the test was conducted as follows: 0.5% carboxymethyl cellulose was administered orally to one group of mouse considered as control. Another gp was administered orally with aspirin (100mg/ kg body, weight) and the seven gps were administered orally with compounds I to VI (100mg/kg body weight).

Compound 'Aspirin – Gly – Gly – Phe – Met – NH_2 (A) is the standard. Another six groups were administered orally with compounds VII to XI (100mg/kg body weight). Compound Asp – Gly – Gly – Phe – Leu – NH_2 (C) is the standard and the remaining ten groups were administered orally with compounds (1) to (10). An hour after administration of standard and analogues, AcOH (1%) v/v (1ml/100g body weight) was injected intraperitonially. Then the number of writhing responses of each mouse in a group was noted during a period of 1h and mean writhing scores of each group was calculated.

5. Results and discussion

The present work was carried out with the aim of studying structure activity relationship in Aspirin-Gly-Gly-Phe-Met-NH_2 (A). Six compounds incorporating aminoacid amide, dipeptide amide and tripeptide amide related to A were synthesized. Their analgesic activity was determined and compared with that of A.

Aspirin prepared using the reported procedure could be obtained in highly pure form after recrystallaization twice or thrice in hot water. The dipeptide sequences Boc-Phe-Met-NH_2 , Boc-Gly-Phe-NH_2 and the tripepitide sequences Boc-Gly-Phe-Met-NH_2, Boc-Gly-Gly-Phe-NH_2 were synthesized by DCC/HOBt method and were obtained in good yield and purity.

The glycine aminoacid sample that usually contains small quantities of glycylglycine was removed after N^{α} protection using Boc group by using column packed with silica gel and solvent system(a) chloroform : methanol : acetic acid (40:2:1). Aspirin-ONp and Boc-aminoacids were prepared using the reported procedure. Boc-Met-NH_2 and Boc-Phe-NH_2 were prepared using Boc-Met or Boc-Phe / dry NH_3 / DCC / HOBt and was obtained in about 87% yield.

The structure of Boc-Met, Boc-Met-NH_2, Boc-Phe, Boc-Phe-NH_2, Boc-Gly, Boc-Gly-NH_2, Boc-Leu, Boc-Leu-NH_2, were confirmed by IR data. The structure of A and C were confirmed by ^1H NMR Spectral date.

Boc-Met: The IR Spectrum of Boc-Met shows a broad band around 3345 cm^{-1}, which may be due to O-H stretching of carboxyl group. The N-H stretching of the secondary amide expected in the range of 3300 – 3100 cm^{-1}, might have superimposed with O-H stretching. The bands at 2578 and at 2372 cm^{-1} may be due to C-H stretching. The sharp band at 1720 cm^{-1} may corresponds to the C=O stretching of carboxyl group and the band at 1652 cm^{-1} may corresponds to the C=O stretching in secondary amide. The N-H bending bands might have superimposed on the C=O stretching. The IR Spectrum of this compound is given in Table 1.

Boc-Met-NH_2: In this spectrum no broad band occurs at 3345 cm^{-1}. This shows the absence of O-H group.

Two sharp bands at 3390 and 3188 cm^{-1} observed may correspond to the asymmetric and symmetric N-H stretching frequencies of the primary amide. The sharp band at 3342 cm^{-1} may be due to the N-H stretching of the secondary amide.

The bands at 2927 and 2850 cm^{-1} may corresponding to C-H stretching frequencies. Some of the C-H stretching might have superimposed. The bands at 1678 and 1658 cm^{-1} may be due to C=O stretching.

Boc-Leu-NH_2: In this spectrum no broad band occurs at 3345 cm^{-1}. This shows the absence of O-H group. Two sharp bands at 3405 and 3180 cm^{-1} observed may correspond to the asymmetric and symmetric N-H stretching frequencies of the primary amide.

Boc-Leu: The IR spectrum of Boc-Leu shows a broad band around 3345 cm^{-1}, which may be due to O-H stretching of carboxyl group. The N-H stretching of the secondary amide expected in the range of 3300 – 3100cm^{-1}, might have superimposed with OH-stretching. The bands at 2950, 2850 and 2560 cm^{-1} may be due to C-H stretching in $–CH_2$ and –CH groups.

The sharp band at 1725 cm^{-1} may corresponds to the C=O stretching of carboxyl group and the band at 1655 cm^{-1} may corresponds to the C=O stretching in secondary amide. The N-H bending bands might have superimposed on the C=O stretching. The IR spectrum of this compound is given in Table 2.

Table. 1 IR Spectral data (ν in cm^{-1})

Compound	$\nu_{O-H\ str}$	$\nu_{N-H\ str}$	$\nu_{C-H\ str}$	$\nu_{C=O\ str}$
Boc-Met	3345	-CONH-superimposed on O-H stretching	2578 2372	-COOH-1720 -CONH-1652
Boc-Met-NH_2	---	$-CONH_2$ 3390, asym 3188, symm -CONH- 3342	2927 2850	-CONH-1658 $-CONH_2$ 1678

Table. 2 IR Spectral data (ν in cm^{-1})

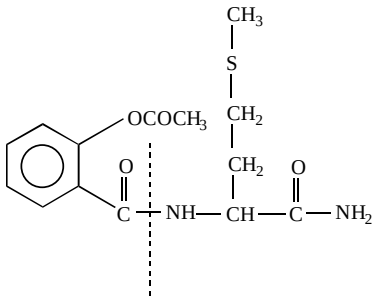
Compound	$\nu_{O-H\ Str}$	$\nu_{N-H\ Str}$	$\nu_{C-H\ Str}$	$\nu_{C=O\ Str}$
Boc-Leu	3345	-CONH-superimposed on O-H stretching	2950 2850 aromatic ring; 2560	-COOH-1725 -CONH-1655
Boc-Leu-NH_2	---	$-CONH_2$ 3405, asym 3180, symm -CONH-3325	2945 2875	-CONH-1642 $-CONH_2$ 1675

The sharp band at 3325 cm^{-1} may be due to the N-H stretching of the secondary amide. The bands at 2945 and 2875 cm^{-1} may correspond to C-H stretching frequencies. Some of the C-H stretching might have superimposed. The bands at 1675 and 1642 cm^{-1} may be due to C=O stretching frequencies corresponding to the primary and secondary amide. The $\nu_{C=O}$ stretch which appears at 1720 cm^{-1} in the carboxyl group of Boc-Leu may now be appearing at a lower frequency of 1675 cm^{-1} due to the +M effect of primary amide group.

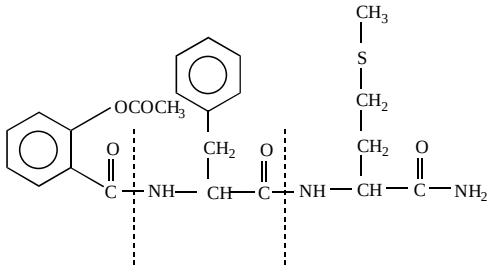
The above pattern of bands thus confirms the conversion of Boc-Leu to Boc-Leu-NH_2. The IR spectrum of this compound is given in Table 2.

The structures of compounds I to XI were given as below:

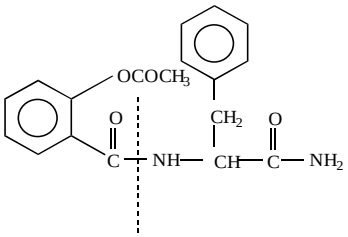
Aspirin-Met-NH_2 (I)



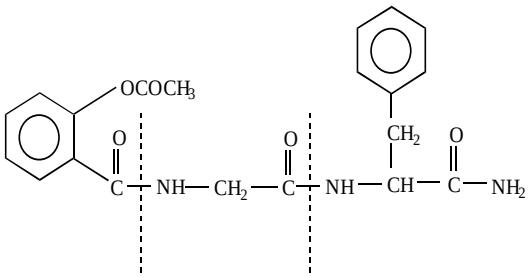
Aspirin-Phe-Met-NH_2 (III)

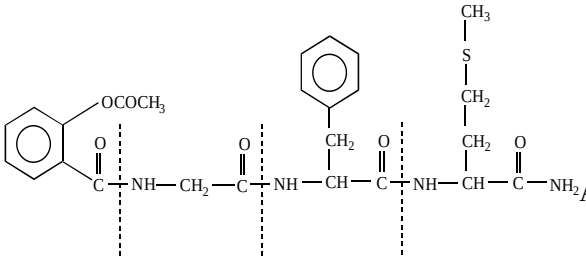


Aspirin-Phe-NH_2 (II)

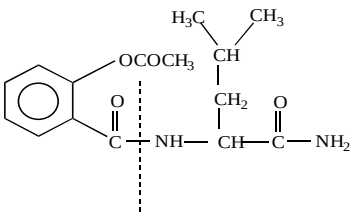


Aspirin-Gly-Phe-NH_2 (IV)



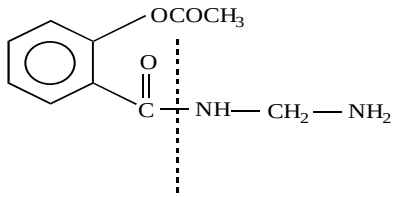


Aspirin-Gly-Phe-Met-NH_2 (V)

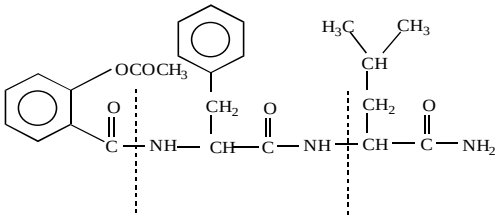


Aspirin-Leu-NH_2 (VII)

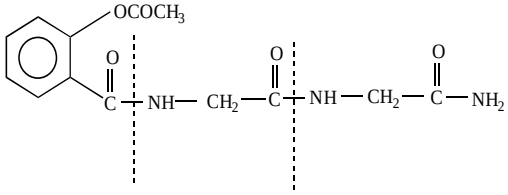
Aspirin-Gly-NH_2 (VIII)



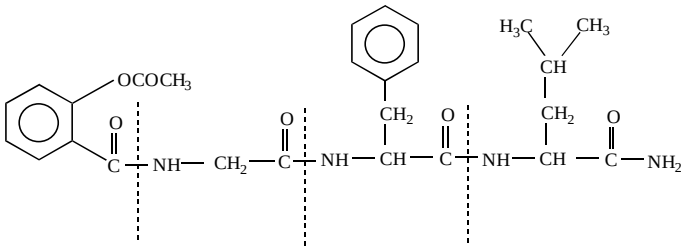
Aspirin-Phe-Leu-NH_2 (IX)



Aspirin-Gly-Gly-NH_2 (X)



Aspirin-Gly-Phe-Leu-NH_2



5.1. **Data pertaining to a few selected compounds are as follows:**

A. **Asp-Gly-Gly-Phe-Met-NH_2**

M.Pt.(175-178° C);

IR(KBr)3390 cm^{-1} $_{(A}$symm.NH),3188cm^{-1}(symmNH), 3342cm^{-1} $_{(GONH),1678cm}{}^{-1}$(>c=o),

^{1}HNMR;(400MHZ)(DMSO-d_6)δ2.27(-$COCH_3$),δ7.8(-NH-) and δ8.4(-NH_2);

Elemental analysis: Glycyl – Glycyl – phenylalanyl – Methionine amide of Aspirin. $C_{27}H_{33}O_7N_5S$ (571) is found to have C 56.74%, H = 5.78% and N 12.26%.;calculated C 56.71% H5.32% and N 11.75%.

C. **Asp-Gly-Gly-Phe-Leu-NH_2**:-

M.Pt(165 to 168° C); IR(KBr);3394cm^{-1} $_{(A}$symm.NH),3207cm^{-1} $_{(}$symm.NH),

3351cm^{-1} $_{(GONH),1717cm}{}^{-1}$(>c=o),

^{1}HNMR;(400MHZ)(DMSO-d_6) δ2.5(-$COCH_3$),δ8(-NH-) and δ7.95(-NH_2);

Elemental analysis: Glycyl – Glycyl – phenylalanyl – Leucine amide of Aspirin.

$C_{28}H_{35}O_7N_5$ (553) is found to have C 60.76%, H = 6.33% and N 12.66%.;

calculated C 60.2% H5.98% and N 12.32%.

Boc- Methionine (Boc-Met)

M.Pt. 109-111°C; IR (KBr); 3394cm^{-1} (asymmetric NH), 3207cm^{-1} (sym. NH), 3351 cm^{-1} (–CONH)., 1717 cm^{-1}, (>C=O),

^{1}HNMR; (400 MHz) (DMSO-d_6); δ2.5 (–$COCH_3$), δ8 (N-H) and δ7.95 (-NH_2).

Elemental analysis: $C_{10}H_{19}O_4S$ (235) is found to have C 51.06%, H = 8.09% and S 13.62%.;calculated C 50.9% H 9.12% and S 12.9%.

Boc-Leucine (Boc-Leu)

M.P. 85-88°C; IR (KBr); 3390cm^{-1} (Asymm.NH); 3188cm^{-1} (symm.NH)., 3342cm^{-1} (CONH), 1678cm^{-1} (>C=O),

^{1}H NMR: δ2.27 (-$COCH_3$), δ8.2 (-NH-) δ8.4 (-NH_2).

Elemental analysis: $C_{11}H_{21}O_4$ (195) is found to have C 67.69%, H = 10.77% and O 32.82%.;calculated C 66.9% H10.12% and O 31.93%.

^{1}H NMR parameters of the aminoacid residues (A) and (C) are Asp – Gly (1) – Gly (2) – Phe (3) – X (4) – NH_2

Groups	**Parameters**	**X(4) Leu**	**X(4) Met**
Gly (1)	δ NH	8.21	8.2
	δ CH_2	3.2	3.4
Gly (2)	δ NH	8	7.8
	δ CH_2	3.06	3.1
Phe (3)	δ NH	8	8
	δ $C^{\alpha}H$	4.5	5.3
	δ CH_2	3.6	3.4
	δ C_6H_5	7.3	7.25
– NH_2	δ	7.95	8.4
– $COCH_3$	δ	2.5	2.27

The methyl resonance of methionine at 2.04 ppm, the isopropyl resonance of leucine at 4.5 ppm[14].

The result of the analgesic activity studies carried out for the compounds using AcOH induced writhing test are given below:

S.No	**Compound**	**Writhing response for 1h**					**Mean**
		1	**2**	**3**	**4**	**5**	
1.	Control	69	65	63	67	68	66.4
2.	Aspirin	15	12	11	13	14	13.0
3.	Aspirin-Gly-Gly-Phe-Met-NH_2(A)	1	0	1	1	0	0.6
4.	Aspirin-Gly-Phe-Met-NH_2 (V)	2	1	2	2	1	1.6
5.	Aspirin-Gly-Gly-Phe- NH_2(VI)	2	3	2	2	3	2.4
6.	Aspirin-Phe-Met-NH_2(III)	4	4	5	4	4	4.2
7.	Aspirin-Gly-Phe- NH_2(IV)	5	3	4	5	3	4.0
8.	Aspirin-Met- NH_2(I)	8	7	9	8	7	7.8
9.	Aspirin-Phe- NH_2(II)	9	8	8	9	8	8.4
10.	Aspirin-Gly-Gly-Phe-Leu- NH_2(C)	1	2	3	1	2	1.8
11.	Aspirin-Gly-Phe-Leu- NH_2(XI)	3	2	1	3	2	2.2
12.	Aspirin-Phe-Leu- NH_2(IX)	5	4	5	5	4	1.6
13.	Aspirin-Gly-Gly- NH_2(X)	5	6	5	5	6	5.4
14.	Aspirin-Leu- NH_2(VII)	10	8	7	10	8	8.6
15.	Aspirin-Gly- NH_2(VIII)	7	8	9	7	8	7.8

5.2. *The table reveals that:*

1. The first group of mouse administered with the control showed an average writhing of 66.4.
2. The second group of mouse administered with the dispersion containing aspirin showed an average writhing of 13.
3. The third group of mouse administered with the dispersion containing A showed an average writhing of 0.6.
4. The fourth and fifth group of mouse administered with the dispersion containing V and VI showed an average writhing of 1.6 and 2.4 respectively.

5. The sixth and seventh group of mouse administered with the dispersion containing III and IV showed an average writhing of 4.2 and 4.0 respectively.
6. The eighth and ninth group of mouse administered with the dispersion containing I and II showed an average writhing of 7.8 and 8.4 respectively.
7. The tenth group of mouse administered with the dispersion containing compound C showed an average writhing of 1.8.
8. The eleventh group of mouse administered with the dispersion containing XI showed an average writhing of 2.2 respectively.
9. The twelth and thirteenth groups of mouse administered with the dispersion containing IX and X showed an average writhing of 4.6 and 5.4 respectively.
10. The fourteenth and fifteenth groups of mouse administered with the dispersion containing VII and VIII showed an average writhing of 8.6 and 7.8 respectively.

6. Conclusion

The present work was carried out with the aim of studying structure activity relationship in Aspririn- Gly-Gly-Phe- Met- NH_2 (A). and Aspririn- Gly-Gly-Phe-Leu- NH_2 (C). Six compounds incorporating aminoacid amide, dipeptide amide and tripeptide amide related to A and C were synthesized. Their analgestic activity were determined and compared with that of A and C.

From the mean writhing values, it is evident that the entire sequence of the tetrapeptideamide namely, Gly-Gly-Phe-Met-NH_2 is essential for the observed analgesic activity of compound A. And, the entire sequence of the tetrapeptide amide namely, Gly-Gly-Phe-Leu-NH_2 is essential for the observed analgesic activity of compound C. The decrease in analgesic activity of these compounds is in the order as follows,

Aspirin tripeptide amides > Aspirin dipeptide amides > aspirin aminoacid amides.

This shows that all the four amino acids are required for the observed activity of the parent compound (A) and(C) .Further research is warranted to investigate the pharmacokinetic parameters regarding the synthesized compounds.

Acknowledgement:

We thank the SIF, IISC, Bangalore for recording the NMR and IR Spectra. The author express thanks to the Secretary, the Principal and the management of Kongunadu Arts and Science College, Coimbatore, India for providing all the facilities to carry out this research work.

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