

Journal of Comprehensive Pharmacy

Research Article

Available Online at: www.jcponline.in

ISSN NO: 2349-5669

Synthesis of 2-Substituted Enkephalin Derivatives for Analgesic Activity

Bharatha D Namadevan*, Jayakumar Annamalai.

Department of Pharmaceutical Chemistry, Adhiparasakthi College of Pharmacy, Melmaruvathur-603319, Tamil Nadu, India.

ARTICLE INFO

Article history:

Received 15 May 2014

Accepted 25 June 2014

Available online 21 August 2014

*Corresponding author:

Bharatha D Namadevan

E-mail:

gayatri.minnu@gmail.com

m.

Tel.: +91-9489390363

ABSTRACT

Aim: the present study was aimed to synthesis 2-substituted Enkephalin derivatives for analgesic activity.

Method: Two derivatives of each of Leucine (Leu) and Methionine (Met) enkephalin were synthesized by solution phase technique using EDC as a coupling agent. The 2nd position glycine is a suitable place to modify and enhance the analgesic activity of enkephalins. In our study Gly² was replaced with more hydrophobic para amino benzoic acid (PABA) and Leucine. The structures of the four synthesized compounds (EP1 - EP4) were consistent with IR, NMR and Mass spectroscopy.

Result: Out of these the Leucine substituted derivatives showed better analgesic activity when tested in Eddy's hot plate using pentazocine as a standard drug.

Conclusion: Enkephalin derivatives showed better analgesic activity when tested in Eddy's hot plate

Key words: Enkephalin, Pentapeptide and Analgesic activity.

INTRODUCTION

Endomorphin, enkephalin and dynorphin are the endogenous opioid pentapeptides possessing analgesic properties. They are described as endogenous ligands for the opioid receptors. Enkephalin is the simple pentapeptides and it is existing in two forms: Leu-Enkephalin (Tyr-Gly-Gly-Phe-Leu) and Met-Enkephalin-(Tyr-Gly-Gly-Phe-Met) [1]. It acts as a natural pain killer and also having fewer side effects compared to other opioid peptides [2]. Apart from analgesic activity, it also possesses antidepressant, antianxiety, anticonvulsant activities [3]. A basic amino terminal of Tyr is essential for the opioid activity of enkephalins and removal of the same results in practically inactive peptide [4]. It is well known from the reference that the most feasible position for

modifying the enkephalin is 2nd glycine unit and next priority to the 4th position Phe unit.

EXPERIMENTAL

Melting points were determined on Tosniwal electric melting point apparatus and are uncorrected. The completion of the reaction was checked by Thin layer chromatography using silica gel G and Chloroform: Methanol (7: 3) as stationary and mobile phase respectively and the spots were detected by Iodine vapours. The I.R spectra of the synthesized compounds were recorded in JASCO FT-IR spectrophotometer and the NMR spectra in BRUKER 500 MHz NMR spectrometer and the Mass spectra were recorded in JEOL GC mate by Electron impact method as ionization mode. The analgesic activity of

the synthesized compounds was screened by Eddy's Hot plate method.

Coupling of Amino acid [5]

Triethyl amine 4 mL was added at 0°C to amino acid methyl ester hydrochloride (10 mmol) in DCM (20 mL). It was stirred for 15 mins and BOC- amino acid (10 mmol) in chloroform (20 mL) followed by EDC (10 mmol) were added with stirring. After 8 hrs the reaction mixture was filtered and residue was washed with chloroform (30 mL) and added to the filtrate. The filtrate was washed with 5% sodium bicarbonate (20 mL) and saturated sodium chloride (20 mL) and water. Organic layer was dried over anhydrous sodium sulphate and the product was recrystallized by chloroform and petroleum ether.

Deprotection of Carboxylic Group

To a solution of the protected peptide in Tetrahydrofuran: water (1:1), LiOH was added at 0°C. The mixture was stirred for 1 hr at room temperature and then acidified to pH - 3.5 with 1N sulphuric acid. The aqueous layer was extracted with Diethyl ether (3 x 20 mL). The combined organic extract was dried over anhydrous sodium sulphate and concentrated under reduced pressure.

Deprotection of Amino Group

The protected peptide was dissolved in chloroform (15 mL) and treated with Trifluoroacetic acid (2 mmol). The solution was stirred at room temp for 1hr and washed with saturated sodium bicarbonate (5 mL) and the organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure.

Analgesic activity

Mice (20-30 g) of either sex were selected and kept for one week to acclimatize to laboratory conditions before starting the experiment. They were subjected to standard diet and water *ad libitum*, but 12 h prior to an experiment, the rats were deprived of food but not water. The study was approved by the Institutional Animal Ethics Committee (IAEC) (Ref No. IAEC/XII/12/ SVP/2010). A 50 mg/kg was taken as effective dose for enkephalins to evaluate the analgesic activity [6].

The mice were divided into 8 groups of six each. The test was carried out using Eddy's hot plate apparatus maintaining the temperature $55 \pm 1^\circ\text{C}$. Mice were placed on hot plate and the reaction time that is licking of hind paw or jumping whichever appears first was recorded in seconds. The cut off time kept was 15 seconds and the animal not showing any response after 15 min are discarded from the study. The reaction time

was measured at 0, 30, 90 and 180 min after the administration of test drugs and standard drug pentazocine [7].

RESULTS AND DISCUSSION

IR Spectra:

The IR spectra of synthesized compounds clearly shows the presence of important functional groups N-H, C=O, C-S, OH, NH₂ and COOH in the molecule. The IR spectral results of the compound **EP-1**: 3434 (OH Str in COOH), 2925 (Aliphatic CH Str), 1700 (C=O Str in Amide), 1605, 1517, 1437 (Ar, C=C str), 1517 (C=O Str in COOH), **EP-2**: 3333 (NH Str in Amide), 2960 (Aliphatic CH Str), 1663, 1517, 1454 (Ar, C=C Str), 1517 (C=O Str in COOH), 1415 (Ar, CH Str), **EP-3**: 3369 (OH Str in COOH), 2928 (Aliphatic CH Str), 1637 (C=O in Amide), 1603, 1517, 1437 (Ar C=C Str), 1437(C-S), **EP-4**: 3368 (NH Str in Amine), 2926 (Aliphatic CH Str), 1675 (C=Ostr in Amide), 1616, 1518,1455 (Ar C=C Str), 1455 (C-S Str in Met).

¹H NMR Spectra:

The proton NMR spectra of synthesized compounds showed characteristic peaks. **EP-1** (CDCl₃): 7.8 (Protons in Amide group), 7.2 (Protons in phenyl group of Phe), 6.6 (Protons in phenyl group of tyr), 3.5-3.8 (Methylene protons in adjacent to amide group), 1.2-1.6 (protons in Methylene group of leu); **EP-2** (CDCl₃): 7.1-7.3 (Amide protons), 6.7-6.8 (protons in Tyr), 5.0 (Protons in OH group of Tyr), 4.3-4.6 (Ali protons in Amide linkage), 3.92 (Ali protons in Tyr), 1.2-1.6 (Protons in Alkyl group of leu); **EP-3** (CDCl₃): 7.8 (2H, d, NH), 7.2 (Protons in phenyl group of Phe), 6.6 (Protons in Phenyl group of Tyr), 3.5-3.8 (Ali protons in Amide linkage), 1.2-1.6 (C-S of Met); **EP-4** (CDCl₃): 7.2-7.4 (2H, d, NH), 7.0-7.1 (Protons in phenyl group of Phe), 6.7 (Protons in Phenyl group of Tyr), 3-3.2 (Methylene proton in Tyr and Phe), 1.2-1.5 (Protons in Alkyl group of leu), 2.1 (Protons in amide group of Tyr).

Mass Spectra:

The mass spectra of the compounds were showed molecular ion peaks corresponding to their molecular weight. The molecular ion peaks of the compound are **EP-1**: 617.44, **EP-2**: 611.68, **EP-3**: 635.36, **EP-4**: 625.61.

The standard drug pentazocine showed increase in reaction time to the stimulus which was found to be significant at 90 min, compared to control. The peak analgesic activity was observed at 90th min for both standard and test drug and the activity started to abolish thereafter.

Table 1: Physical Data of Synthesized Compounds

Compound	Molecular formula	Molecular weight	Physical state	Colour	Yield (%)	R _f Values*
EP-1	C ₃₃ H ₃₉ N ₂ O ₇	617.44	Semisolid	Light Brown	77.62	0.634
EP-2	C ₃₂ H ₄₅ N ₂ O ₇	611.68	Semisolid	Dark brown	83.23	0.576
EP-3	C ₃₂ H ₃₇ N ₂ O ₇ S	635.36	Semisolid	Brown	78.94	0.553
EP-4	C ₃₁ H ₄₃ N ₂ O ₇ S	625.61	Semisolid	Reddish brown	75.45	0.627

Table 2: Results of Analgesic Activity

Group	Sample	Paw licking or jumping in seconds			
		0 min	30 mins	90 mins	180 min
I	Control (Normal saline, 10 ml/kg, p.o)	2.603±0.22	2.753±0.28	2.827±0.21	2.758±0.20
II	Pentazocine (5mg/kg, p.o)	2.717±0.18	11.58±0.62**	14.40±0.37**	11.85±0.34**
III	EP 1 (50mg/kg, p.o)	2.718±0.24	6.713±0.15*	7.522±0.21**	5.788±0.41*
IV	EP 2 (50mg/kg, p.o)	2.752±0.13	7.022±0.35**	10.108±0.23**	8.480±0.33**
V	EP 3 (50mg/kg, p.o)	2.717±0.21	6.468±0.24**	8.890±6.29**	6.977±0.28**
VI	EP 4 50(mg/kg, p.o)	2.703±0.14	9.965±0.37**	13.81±0.39**	10.12±0.44**

Pentazocine reaction time at 90th min is 14.40 ± 0.37. The EP-4 showed comparable activity to that of standard drug whose reaction time is 13.8 ± 0.39.

The EP-2 showed moderate activity whereas EP-1 and EP-3 could show only less activity. The Leu substituted compounds showed more activity than PABA substituted compounds in both the cases i.e., Leu and Met Enkephalins. The incorporation of more hydrophobic moiety in the place of Gly² enhances the analgesic action. But however this statement is true only upto the carbon number of four to five beyond that there is a marked reduction in the activity. This can be justified from the activity data of synthesized compounds.

Acknowledgement

The authors are thankful to the management and principal of Adiparasakthi College of Pharmacy, melmaruvathur for their support on the study and also IIT, madras for the spectral data.

REFERENCES

- Bodnar RJ. Endogenous opiates and behavior: 2006. *Peptides* 2007;**28(12)**:2435-2513.
- Hughes J, Smith TW, Kosterlitz HW, Fothergill LA, Morgan BA, Morris HR. Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature* 1975;**258(5536)**:577-580.
- Plotnikoff NP, Kastin AJ, Coy DH, Christensen CW, Schally AV, Spirtes MA. Neuropharmacological actions of enkephalin after systemic administration. *Life Sci*. 1976;**19(8)**:1283-1288.
- Frederickson RC. Enkephalin pentapeptides-- a review of current evidence for a physiological role in vertebrate neurotransmission. *Life Sci* 1977;**21(1)**:23-42.
- Bodanszky M, Bodanszky A. *Practice of Peptide Synthesis*, Springer verlog, Newyork: 1984. P.78.
- OECD (Organization for Economic Cooperation and Development) Guideline 425 Acute Oral Toxicity. *Environmental Health and Safety Monograph series on Testing and Assessment*, 2000, 24.
- EDDY NB, LEIMBACH D. Synthetic analgesics. II. Dithienylbutenyl and dithienylbutylamines. *J Pharmacol Exp Ther* 1953;**107(3)**:385-93.

Cite this Article as: Namadevan BD, Annamalai J. Synthesis of 2-Substituted Enkephalin Derivatives for Analgesic Activity. *J Compr Phar* 2014;1(3):91-94.