

**SYNTHESIS OF DIPEPTIDE CONTAINING ASPIRIN AS A PRODRUG****Priti Anil Waje<sup>1</sup> and Kundan J. Tiwari<sup>2\*</sup>**<sup>1</sup>Student, SMBT Institute of Diploma Pharmacy, Dhamangaon, Nashik, India.<sup>2</sup>Asst. Prof., SMBT Institute of Diploma Pharmacy, Nashik, India.

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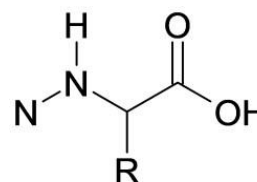
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**\*Corresponding Author****Kundan J. Tiwari**Asst. Prof., SMBT Institute of  
Diploma Pharmacy, Nashik,  
India.**ABSTRACT**

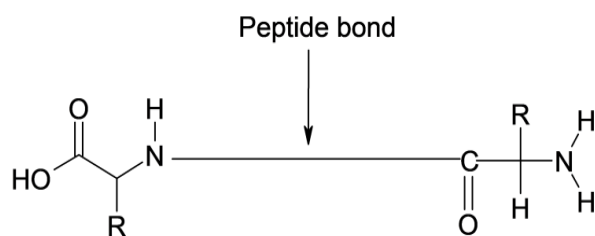
Proteins and Peptides which are continuously to grow in the medication for their possible use in the current drug therapy and protein drug market. Peptides are one of the best applicants of drug development due to their higher specificity and lower toxicity. Mostly peptides are obtained from chemical synthesis or other biological technique. The peptide based drugs are use to as a antimicrobial agent as well as cure of cancer. Most of peptides when attach to the Heterocyclic compounds shows most of activity such as antifungal, antimicrobial, antibacterial, anti-inflammatory activity etc. Most of synthetic molecules have been design to prevent the cell proliferation. The widely varieties of the biopeptides has been discovered by the last two decades. In chemical synthesis of peptides, mainly two procedure are used, one is a solid-phase synthesis technique which are carried out on a solid support such as a resin and other also solution phase synthesis technique. Condensation of Heterocyclic component or group like as P-amino salicylic acid (PAS), Coumarin, Nicotinic acid, Furan, Quinoline, Imidazole, Thiazole with a peptide containing amino acid shows potent biological action. In addition to the biological and pharmacological activities of the Dipeptide were examined by prediction of activity spectra for substance (PAAS) Program.

**KEYWORD:** Coumarin, Imidazole, Nicotinic acid, Quinoline, Thiazole.**1. INTRODUCTION**

The first peptide Synthesis was discovered by the term 'peptide', Find out the scientist one Fischer and fourneau in 1901. Designing of the new drugs has always interesting for the scientific research and in Field of medicinal Chemistry. Peptide synthesis become most practical part of in the now days For Scientific Research. Bringing modification in the parent Compound Frequently serves to enhance the activity of the Compound, along With this, In the most cases, it eliminate or remove adverse effect or toxic effect along with the parent drug. Scientific interpretation of the drug action is required to design a compound and that will be producing a specified therapeutic effect of drug. The term "Amino acid" are the structural unit of proteins having an amino group(-NH<sub>2</sub>) and carboxylic group (-COOH) attached to the  $\alpha$ -carbon. This term used to any compound containing an amino group and acidic function. The  $\alpha$ -carbon atom is the carbon atom to which amino and carboxylic group attached. Amino acid is the building blocks of which proteins are made up of amino acids while conjugated proteins have additional component. In this term frequently used with respective to  $\alpha$ -amino carboxylic acid which are isolated from the natural sources. The carbon atom which is the carboxylic group is attached is called as ' $\alpha$ -carbon' (Patrick G. L., 2003, Chatwal G. R., 2006).

**Structure 1: General structure of Amino acid.****1.1. The Peptide Bond**

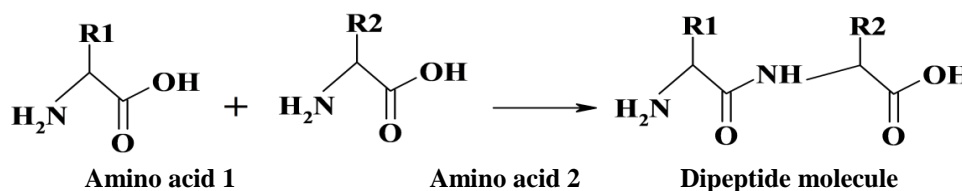
Protein and peptides are most similar in that which are made up of repeating units, or residues of  $\alpha$ -amino acids that are linked together by the peptide bonds also known as 'amide bonds' Where as peptide synthesis is the production of peptides molecule Where multiple amino acid are linkage together by the amide bonds, also known as peptide bonds (Lemke TL, Williams DA, Roche VF, 2008). Peptides are the molecule Where as two amino acids are linked together through a peptide bond, known as peptide linkage or amide linkage, With the Removal of water molecule. Peptide bond is also a covalent bond. This bond is a special linkage in which Nitrogen atom of one amino acid binds to the Carboxylic carbon atom of another amino acid (Shinde N.V., Dhake A.S., 2013, et al).



Structure 2: Structure of Peptide bond.

### 1.2 Structure of Dipeptide

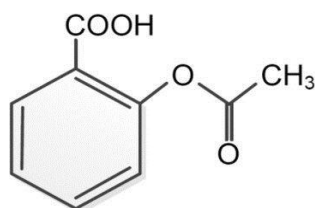
In this dehydration synthesis, a water molecule is removed and the peptide bond connect to the nitrogen of one amino(-NH<sub>2</sub>) group to the carbon of the other carboxylic(-COOH) group. The resulting molecule called as a 'Dipeptide' (Dr. Yogesh Ushir, Dr.



Structure 3: Structure of Dipeptide.

### 1.3 Prodrug

The drug which undergoes biotransformation before showing its desired or greater pharmacological activity are known as prodrug. It is also known as proagents. The Prodrugs are generally the ester or amide of the parent drugs. Prodrugs are more useful in the improving stability, solubility and bioavailability of drug, which are masking unpleasant taste and odour of parent compound and also it reduces the drug toxicity. Prodrug can be used to mask the side-effects and toxicity of drugs such as Aspirin (Block J.H, Beale J.M, 2008, R. M. Mehta, 2019).



Structure 4: Structure of Aspirin.

Sudharshan singh, 2016). This is a condensation type of reaction which is occurs between two amino acid. Dipeptide should have different chemical and biological properties. The dipeptide analogue with heterocyclic ring shows good antimicrobial activity also they may shows antifungal, antiemetic, and anticancer activities. The drug from any class if combine with dipeptide moiety it shows increase in the action. In order to develop molecule with better effect an attempt was made to couple various dipeptides with Aspirin as modifications in the parent compound serves to enhance the activity of the compounds and also in most cases it eliminates adverse effects or toxicity associated with the parent drug ((Block J.H, Beale J.M, 2008).

**Chemical Formula:** C<sub>9</sub>H<sub>8</sub>O<sub>4</sub>

**Molecular weight:** 180.158 g/mol

Most of the peptides act as the therapeutic agents which are mostly obtained from the natural sources in the minor or less quantity. The number of heterocyclic Compounds which are Found to shows the various biological activity Such as anti-inflammatory, antifungal, analgesic, antipyretic, anti-rheumatic, antineoplastic, Insecticidal, melanin production inhibitory activities. Synthesis of new peptide derivatives as atherapeutic agents was quickly expanded with the discovery of Peptide as a pituitary hormones (Reddy VS., Haripriya K., 2010).

### 1.4 PEPTIDE FUNCTION AND ITS EXAMPLE: (Block J.H, Beale J.M, 2008).

Table no -1

SR. NO.	PEPTIDE FUNCTION	EXAMPLE
1	Hormones	Insulin
2	Growth inhibitors	Ascorbic acid or Vitamin-C
3	Neurotransmitter	Mealonocyte-Stimulating hormone
4	Target enzyme	Cyclo-oxygenase
5	Immunomodulators	lenalidomide, Thalidomide, pomalidomide

Synthesis of new and more potent analogs of molecule with already established activities forma key part of the research in the Medicinal chemistry and pharmaceutical field. A great number of drugs are heterocyclic

compounds Most Commonly are of Synthetic origin Few obtained from natural Sources which includes alkaloids, vitamins, Xanthenes, cardiac Glycosides etc. Heterocyclic compounds are widely distributed in the

nature which is essential for the life Genetic material of DNA is also made up of the heterocyclic based pyrimidine and purines. A large number of heterocyclic Compounds both like as natural and Synthetic are pharmacologically active and in the clinical use. In many heterocyclic compounds have application in the agriculture as insecticides, pesticides, herbicide, and Fungicides etc. They also find out in the applications as sensitizers, antioxidants, developers and Copolymers etc. (Gupta R.R., Kumar M., 2005, Shinde N. V, Dhake AS, 2013).

## 2. METHODS OF SYNTHESIS OF PEPTIDES

### 2.1 Solution Phase Synthesis

This method is completely depends on the efficiency of Solid Support. In this method is synthesized by the chemical methods which are Valuable for large-scale production or Manufacture and For the Specialized laboratory application. The most of the ordinary Synthetic Chemistry takes place in the solution. When reaction must be modified to accommodate the Solid Support, it takes place in time and resources to develop and optimize the reaction condition of State. A Combinatorial Chemistry may be occupy in months designing a solid-phase reaction and gathering essential materials but then conduct entire Synthesis in a few hours or days. Many reactions cannot be support on the Solid Supports because of failed reactions or to produce poor material. In these reasons, there has been most interest for using Solution phase chemistry For preparation of the combinatorial libraries Solution phase Combinatorial Chemistry Mostly leads to the mixture of a products. Consider reacting Set of amines with acid chloride all take in one Flask, and with a reactant and Condition choose so that is no reaction of amine with amine or Chlorides with chlorides occurs. An only reaction takes place in between amine and chlorides.<sup>[2]</sup> So the result would be a mixture amides one for the each possible combination of amines and acid chlorides. Then the resultant mixture could be tested for activity, under the assumption that is the inactive amides didn't interfere with the binding of active molecule. If the activity are found Smaller Subset of amines and chlorides Can be tested to the eventually Find out the structure which is responsible for activity (Block J.H, Beale J.M, 2008).

The solution-phase synthesis is beneficial as compare to solid-phase synthesis are as follow: The Large number of protecting group reagents is commercially available whereas the Number of solid-phase synthesis resins,

which are often to use the solid-phase protecting reagents, is limited. In the solution-phase technique, the range of organic reactions is very large, whereas on solid phases there are certain limitations (Chaudhary S, Singh R. K, 2012).

In solution phase synthesis only three steps are involved as shown follows,

**Step 1: Protection-** An amino acid is an acid with a basic group at one end and an acid group at the other. To prevent an amino acid from reacting with itself, one of these groups is reacted with something else to make it unreactive (Kundan J. Tiwari, N.V. Shinde, 2016).

**Step 2: Coupling-** The protected amino acid is then reacted with the amino acid attached to the polymer to begin building the peptide chain (Kundan J. Tiwari, N.V. Shinde, 2016).

**Step 3: Deprotection-** The protection group is now removed from the acid at the end of the chain so it can react with the next acid to be added on. The new acidic then protected (Step 2) and the cycle continue until a chain of the required length has been synthesized(Kundan J. Tiwari, N.V. Shinde, 2016).

### Advantages

1. Easy method for synthesis of dipeptides.
2. Less solvent is required as compare to Solid phase synthesis.

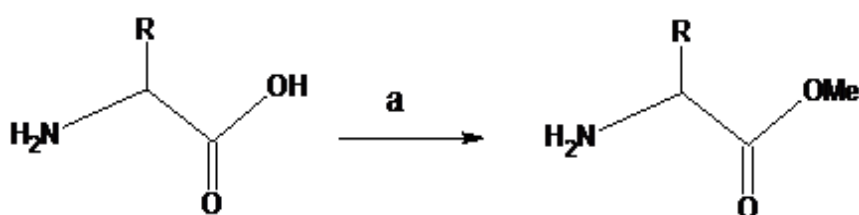
### Disadvantages

1. Solution phase synthesis is more time consuming process.
2. It required more time for condensation as well as for stirring.

## 3. EXPERIMENTAL WORK

### 3.1 Preparation of Amino acid methyl ester hydrochlorides -

Thionyl chloride (0.7ml, 10.0 mmol) was added to methanol (100ml) slowly at 0°C and the amino acid (10.0mmol) was added to this solution and the solution was refluxed for 8-10 hours. The solvent was evaporated to give the amino acid methyl esterhydrochloride which was triturated with ether at 0°C until excess dimethyl sulphite was removed. The resulting solid was recrystallized from methanol and diethyl ether at 0°C.

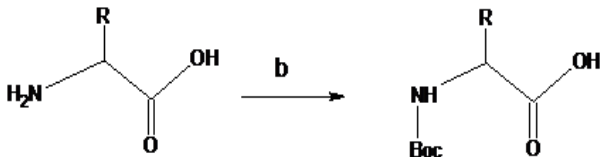


Where, a- SOCl<sub>2</sub>, MeOH, Reflux, 8-10hr,

**Scheme i: General reaction of Ester formation.**

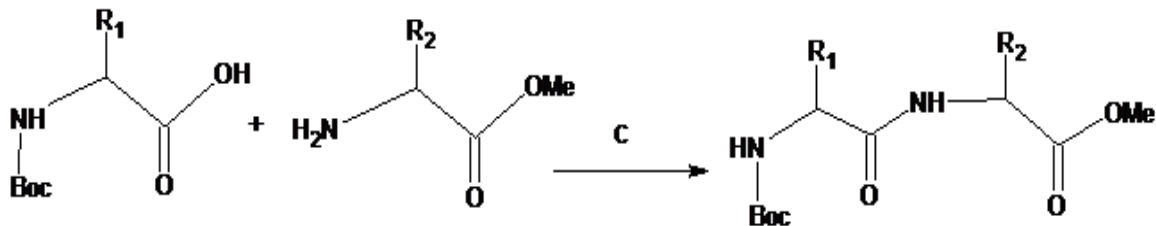
### 3.2 Preparation of BOC-amino acid

Amino acid 10 mmol dissolve in 1N NaOH (20 ml) and isopropanol (20 ml) and BOC (3 ml) stir for 2 hr. wash with light petroleum ether then acidified with to PH 3 with H<sub>2</sub>SO<sub>4</sub>. Extract with CHCl<sub>3</sub> (20x3ml) dry the layer over anhydrous NaSO<sub>4</sub>.



Where, b- (Boc)<sub>2</sub>O, 1N NaOH, isopropanol, RT, 2hr

**Scheme- ii: General reaction of Boc-amino acid formation.**



Where, C- DIPC, CHCl<sub>3</sub>, NMM, RT, 24h.

**Scheme-iii: General reaction of Dipeptide formation.**

### 3.4 Deprotection of the Carboxyl Group

To a solution of the protected peptide (1.0 mmol) in THF: H<sub>2</sub>O (1:1) (36ml), LiOH (1.5 mmol) was added at 0°C. The mixture was refluxed at 55-60°C for 15 min and then acidified to pH 3.5 with 1N H<sub>2</sub>SO<sub>4</sub>. The mixture was extracted with solvent ether (3x15ml). The combined ether extracts were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure (Chaudhary S, Singh R. K, 2012).

### 3.5 Deprotection of the Amino group

The protected peptide (1 mmol) was dissolved in CHCl<sub>3</sub> (15ml) and treated with CF<sub>3</sub>COOH (2mmol, 0.228 g). The solution was stirred at room temperature for 1 hour, washed with saturated NaHCO<sub>3</sub> (5ml). The organic layer

### 3.3 Preparation of Dipeptide

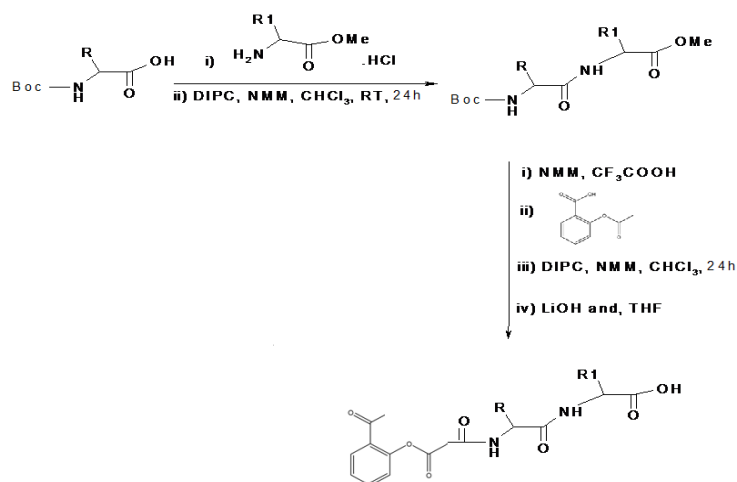
The dipeptides were prepared by using Boc-amino acids and amino acid methyl hydrochloride. The 10mmol of BOC amino acid in 20ml of Chloroform and 10mmol of amino acid methyl hydrochloride in 20ml of chloroform were prepared. 10mmol of DIPC was added to the above reaction mixture with stirring. After 24hr stirring, washed the residue and filtrate with 5% NaHCO<sub>3</sub> and saturated NaCl solution. Dried the organic layer over Na<sub>2</sub>CO<sub>3</sub> evaporated the mixture in vacuum (Chaudhary S, Singh R. K, 2012).

was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The product was purified by recrystallization from CHCl<sub>3</sub> and petroleum ether (Chaudhary S, Singh R. K, 2012).

### 3.6 Synthesis of Dipeptide molecule by Coupling with Aspirin

The dipeptides were dissolved in 20ml of chloroform and 10mmol of Aspirin dissolved in 20ml of chloroform in that 10mmol of DIPC was added to the above reaction mixture with stirring. After 24hr stirring, washed the residue and filtrate with 5% NaHCO<sub>3</sub> and saturated NaCl solution. Dried the organic layer over Na<sub>2</sub>CO<sub>3</sub> evaporated the mixture in vacuum.

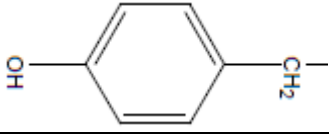
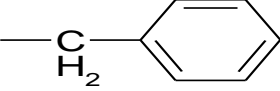
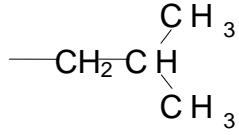
## 4. SCHEME-iv



**Scheme for Synthesis of Dipeptide containing Aspirin.**

4.1 Substitution groups of R & R<sub>1</sub>

Table no- 2.

Molecule Id	R	R <sub>1</sub>
ASP-01	—H	—CH <sub>3</sub>
ASP-02		
ASP-03		—CH <sub>3</sub>

## 5. RESULTS AND DISCUSSION

## 5.1 Step-I,

Table no- 3: Physical data of amino acid methyl ester hydrochloride.

Sr. no	Name of the Amino acid Methyl esters HCL	Molecular Formula	Molecular weight Kg/mol	M.P (°C)	Theoretical yield in Kg	Practical yield in Kg	% Practical yield
1	Gly-OMe.HCl	C <sub>3</sub> H <sub>8</sub> NO <sub>2</sub>	0.07507	145-147	0.00083	0.00062	74.69%
2	Tyr-OMe.HCl	C <sub>10</sub> H <sub>13</sub> NO <sub>3</sub>	0.18119	195	0.00022	0.00019	86.36%
3	Leu-OMe.HCl	C <sub>7</sub> H <sub>16</sub> NO <sub>2</sub>	0.113171	145	0.00051	0.00040	78.43%

## 5.2 Step II,

Table no-4: Physical data of Boc-Amino acids.

Sr. no	Boc-Amino acid	Molecular Formula	Molecular weight Kg/mol	M.P (°C)	Theoretical yield in Kg	Practical yield in Kg	% Practical yield
1	Boc-Ala	C <sub>8</sub> H <sub>15</sub> NO <sub>4</sub>	0.189	80-82	0.0012	0.001	83.00%
2	Boc-Phe-ala	C <sub>14</sub> H <sub>19</sub> NO <sub>4</sub>	0.179	38-40	0.0003	0.00035	85.36%

## 5.3 Step III,

Table no- 5: Physical data of the Dipeptides.

Sr. no	Compound	Molecular Formula	Molecular weight Kg/mol	M.P (°C)	Theoretical yield in Kg	Practical yield in Kg	% Practical yield
1	Boc-Gly-Ala-OMe	C <sub>11</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub>	0.26Kg/mol	52-54	0.00040	0.00032	80.00%
2	Boc-Tyr-Phe-Ala-OMe	C <sub>18</sub> H <sub>22</sub> N <sub>2</sub> O <sub>5</sub>	0.32Kg/mol	47-49	0.00055	0.00035	63.63%

## 6. Analytical Spectroscopy

## 6.1 Interpretation of spectral data

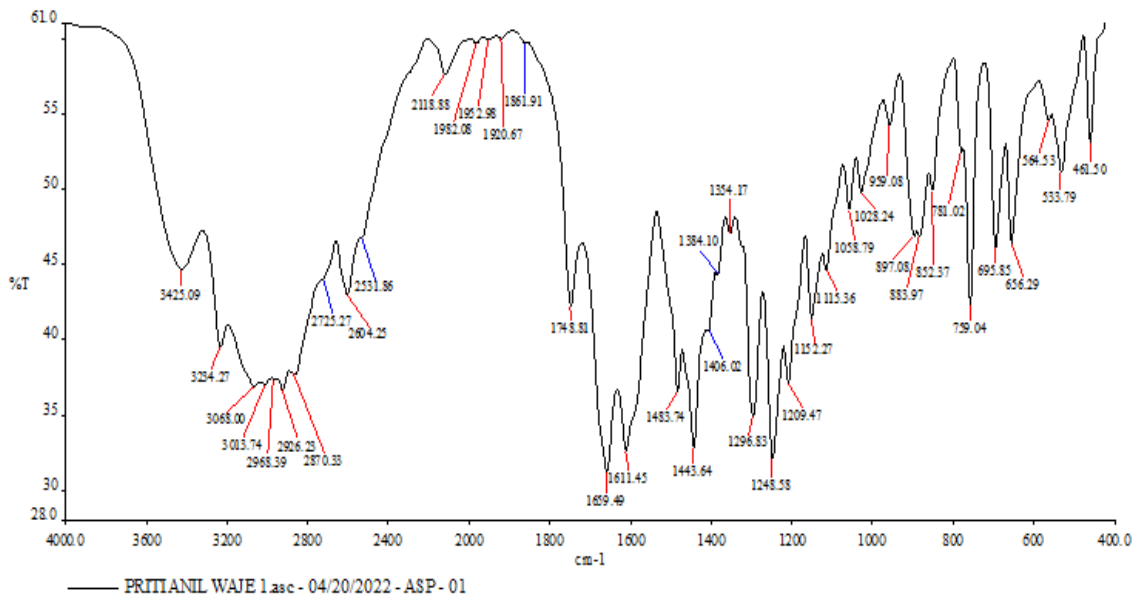
Table no- 6: Kundan Tiwari, Amol A Deshmukh, Nirmal Shinde, 2015, et al.

Type of spectroscopy	Interpretation Code	Interpretation
IR	ASP-01	C-H str (2968.39), -CO-str (1748.81), -CO-NH str(1659.49), -COOH str (2604.25), -C=C str (3068.00), C=O str (1748.81).
	ASP-02	C-H str (2945.05), -CO- str (1745.99), -CO-NH str (1587.94), -COOH str (2.625.01), -OH str (3340.65), C=O str (1745.99).
NMR	ASP-01	<sup>1</sup> HNMR: 4H of Ar-H (m, 8.5318), 1H of R-OH(s, 1.4094), 2H of H-C-COOH (s, 3.7244-3.8003), 2H of CH-NH <sub>2</sub> (7.3712 -7.3913), H-C-OH (3.7244-3.8003), R-OH(1.4094), 1H of CH-COOH (s,2.5076), 2H of CH-NH <sub>2</sub> (d, 6.8446-6.8274)
	ASP-01	<sup>13</sup> CNMR: 167.93(C-O), 161.29(C-O), 134.38(C-N), 118.30(C-N), 47.84(CH <sub>2</sub> ), 39.58(CH <sub>2</sub> ), 47.84(CH <sub>2</sub> )
Mass	ASP-01	Ion Peak: 397.03, Base peak: 137.02

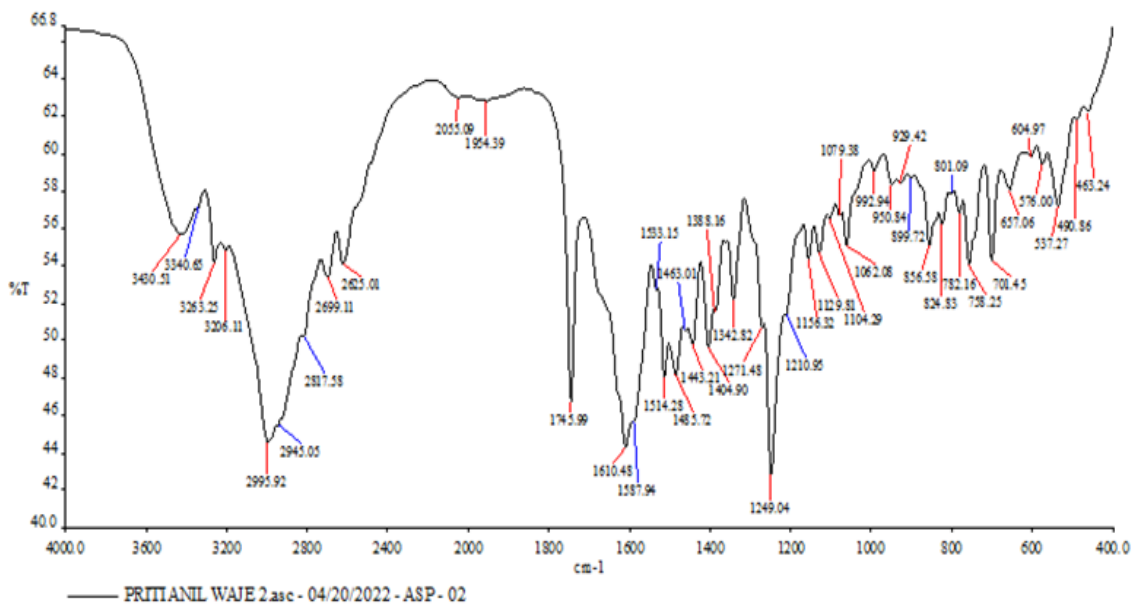
**6.2 Infra-Red Spectrum**

Infra-red spectrum is a important record which gives sufficient information about the structure of a compound. The IR spectrum of the sample was recorded and the

functional groups were interpreted as per the structure and where found to be appropriate or matching the structure of the drug (Y.R. Sharma, 1980).



**Fig. 1: IR Spectroscopy of Sample ASP 01.**



**Fig. 2: IR Spectroscopy of Sample ASP 02.**

**6.3 Nuclear magnetic resonance spectroscopy**

NMR means Nuclear magnetic resonance spectroscopy is the study of compounds by recording the interaction between radiofrequency (Rf) electromagnetic radiations and the nuclei of molecules placed in a strong magnetic

field. There are two common type of NMR: First is  $H^1$  NMR and second is  $C^{13}$  NMR. There is considerable difference in between  $H^1$  NMR and  $C^{13}$  NMR spectra in the mode of recording as well as in their appearance (Y.R. Sharma, 1980).

ASP-01  
<sup>1</sup>H\_8scan DMSO {D:\Spectra} nmr 27

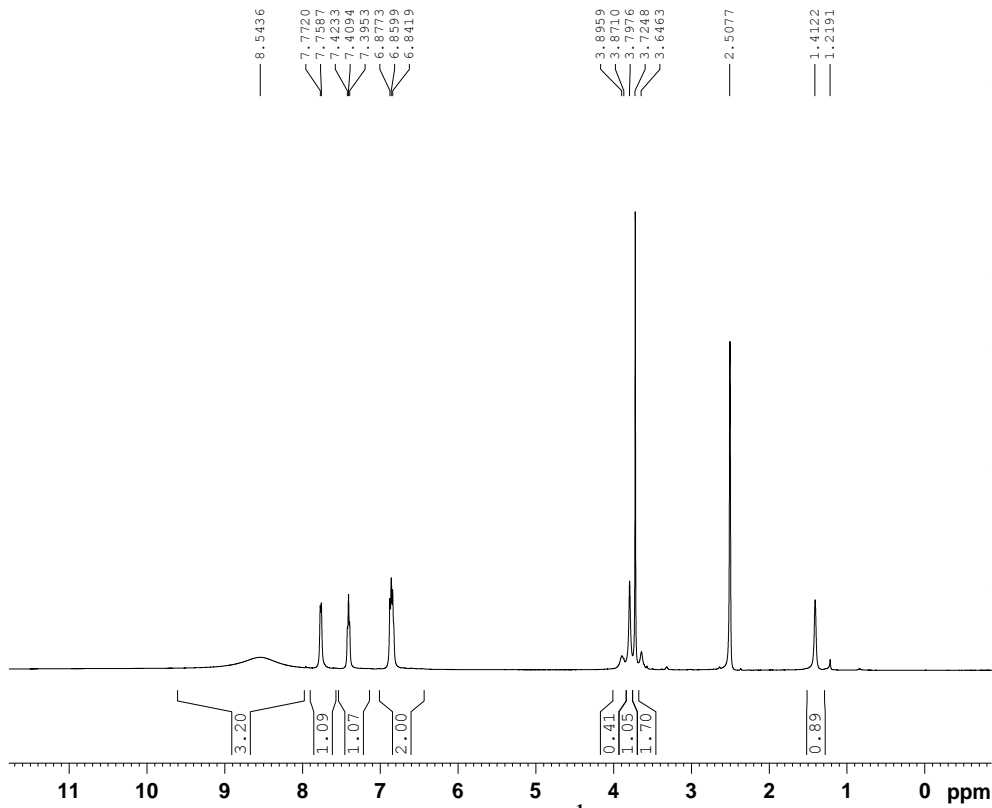


Fig 3: <sup>1</sup>H NMR of ASP 01.

BRUKER  
 AVANCE NEO  
 500 MHz NMR  
 SPECTROMETER  
 SAIF, P.U.

Current Data Parameters  
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 EXPNO 270  
 PROCNO 1

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 PULPROG zg30  
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 SOLVENT DMSO  
 NS 16  
 DS 0  
 SWH 14705.883 Hz  
 FIDRES 0.448788 Hz  
 AQ 2.2282240 sec  
 RG 95.7854  
 DW 34.000 usec  
 DE 6.79 usec  
 TE 300.2 K  
 D1 1.00000000 sec  
 TD0 1  
 SFO1 500.1730885 MHz  
 NUC1 <sup>1</sup>H  
 P0 3.33 usec  
 P1 10.00 usec  
 PLW1 20.93000031 W

F2 - Processing parameters  
 SI 65536  
 SF 500.1700000 MHz  
 WDW EM  
 SSB 0  
 LB 0.30 Hz  
 GB 0  
 PC 1.00

ASP-01  
 C13CPD DMSO {D:\Spectra} nmr 27

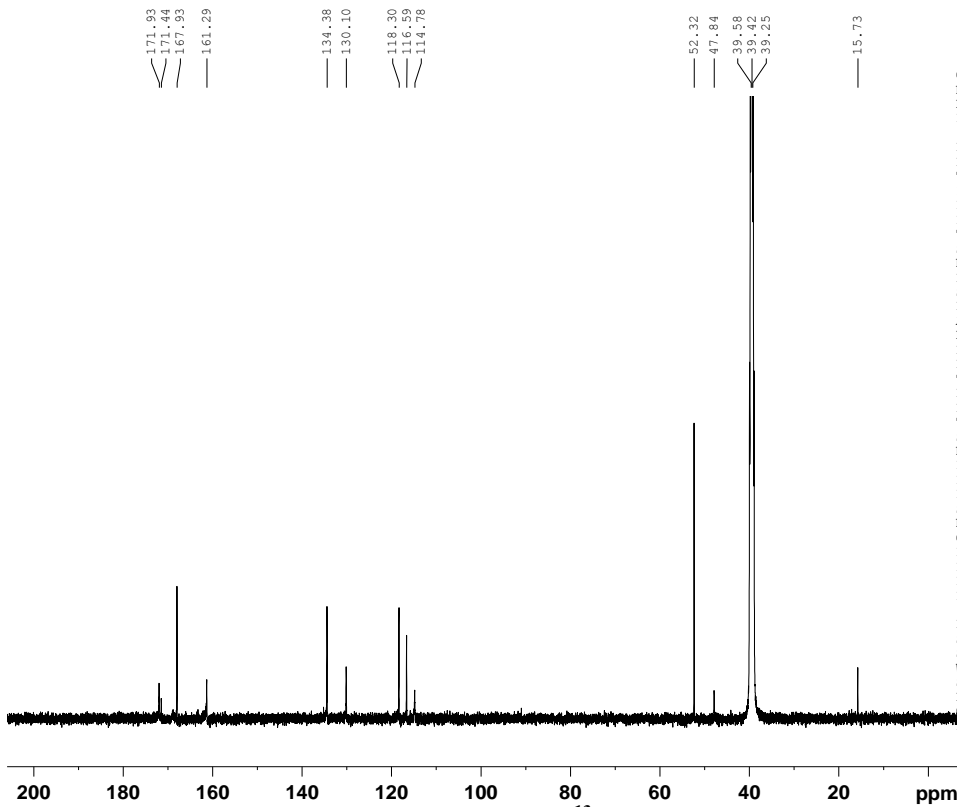


Fig 4: <sup>13</sup>C NMR of ASP 01.

BRUKER  
 AVANCE NEO  
 500 MHz NMR SPECTROMETER  
 SAIF, PANJAB UNIVERSITY,  
 CHANDIGARH

Current Data Parameters  
 NAME Mar03-2022  
 EXPNO 271  
 PROCNO 1

F2 - Acquisition Parameters  
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 Time\_ 23.58 h  
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 PROBHD Z119470\_0333 (  
 PULPROG zgpg30  
 TD 65536  
 SOLVENT DMSO  
 NS 512  
 DS 4  
 SWH 37037.035 Hz  
 FIDRES 1.130281 Hz  
 AQ 0.8847360 sec  
 RG 101  
 DW 13.500 usec  
 DE 6.50 usec  
 TE 301.1 K  
 D1 2.00000000 sec  
 D11 0.03000000 sec  
 TD0 1  
 SFO1 125.7804233 MHz  
 NUC1 <sup>13</sup>C  
 P0 3.33 usec  
 P1 10.00 usec  
 PLW1 83.14099884 W  
 SFO2 500.1720007 MHz  
 NUC2 <sup>1</sup>H  
 CPDPRG[2] waltz65  
 PCPD2 80.00 usec  
 PLW2 20.93000031 W  
 PLW12 0.32703000 W  
 PLW13 0.16449000 W

F2 - Processing parameters  
 SI 32768  
 SF 125.7679230 MHz  
 WDW EM  
 SSB 0  
 LB 1.00 Hz  
 GB 0  
 PC 1.40

## 6.4 Mass spectroscopy

Mass spectrometry is the most accurate method for determination of the molecular mass of the molecule and its elemental composition. The peak produced by an ion which formed by the removal of one electron from

analyzed molecule is called as 'ion peak'. The largest peak in the structure is called as the 'base peak'. It is the highest peak or most intense peak in the spectrum. (Y.R. Sharma, 1980).

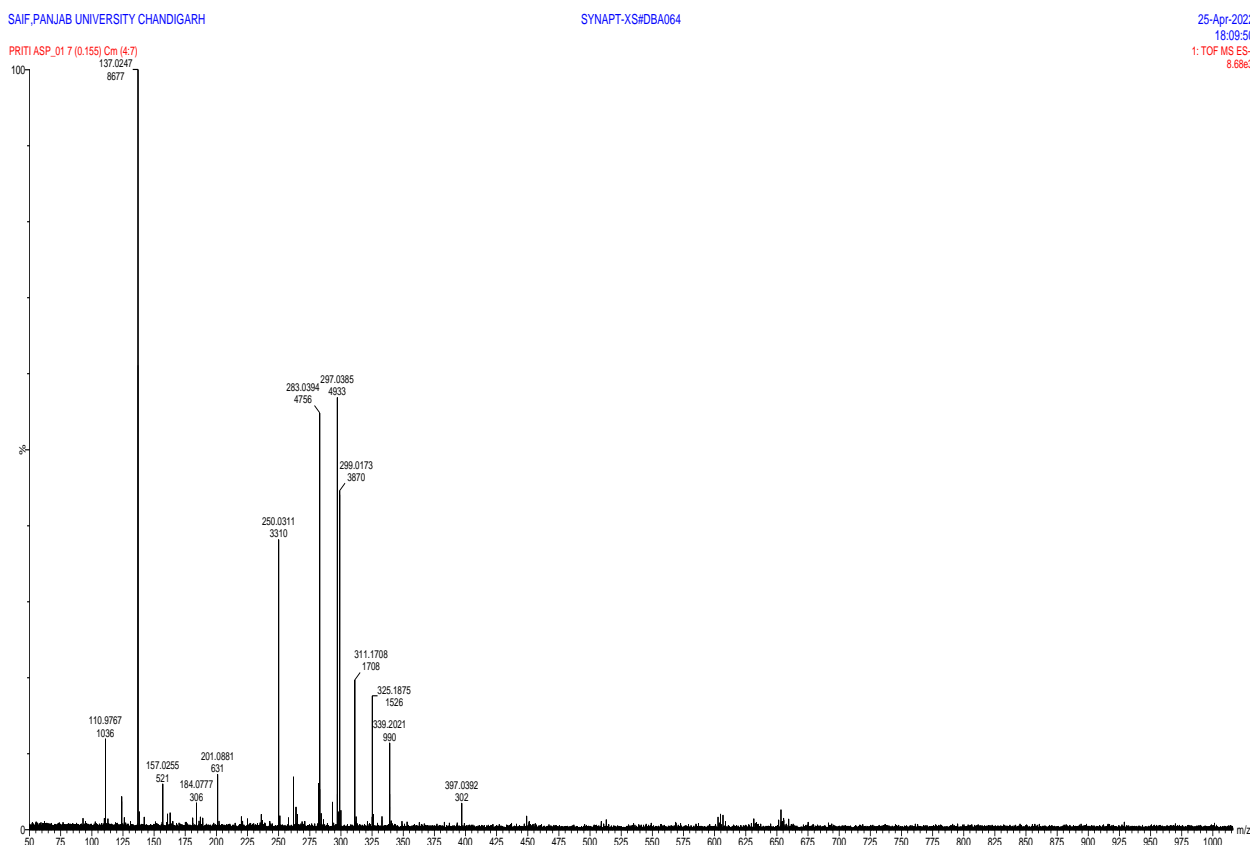


Fig 5: Mass Spectroscopy of ASP 01.

## 7. CONCLUSION

Proteins and peptides are continued to grow in medications for their potential use in current drug therapy and in protein drug market. The Peptide based drugs act as antimicrobial agent. As many peptides based molecules are shown to possess good biological activity like cytotoxic, antimicrobial, anticancer etc., the synthesized molecules even tested for biological activities. By taking into consideration, the activities possessed by the peptide based molecules there is a scope for the designing of new series of peptide molecules as Antimicrobial agent.

## 8. ACKNOWLEDGEMENT

It is a moment of gratification and pride to look back with a sense of contentment at the long traveled path, to be able to recapture some of the fine moments, to be able to thank Dr. Y.V.Ushir Principal SMBT IOP for your valuable guidance and Support also I thankful to SMBT Sevabhavi trust who supported us for completion of this research.

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