ejpmr, 2020,7(1), 469-474

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

SYNTHESIS OF SOME DIPEPTIDES CONTAINING HETEROCYCLE ISO-NICOTINIC ACID AS AN ANTIMICROBIAL AGENT

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Article Received on 21/11/2019 **Article Revised on 12/12/2019** **Article Accepted on 01/01/2020**

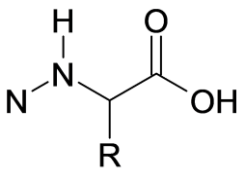
ABSTRACT

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 are use to cure cancer as well as antimicrobial agent. Most of the synthetic molecules have been design to prevent cell proliferation, Multiplication of microbial cells. Most of the peptide when attach to heterocyclic compounds shows most of the activity like antimicrobial activity, antifungal, antiemetics etc. The wide varieties of biopeptides have been discovered from last two decades. Condensation of heterocyclic moiety viz nicotinic acid, thiazole, coumarin, quinoline, furan, imidazole etc with peptides containing amino acids shows potent biological activities.

KEYWORDS: Coumarin, furan, imidazole, nicotinic acid, quinoline, thiazole.

• INTRODUCTION

Designing of the new drugs has always been interesting for scientific research and in the field of medicinal chemistry. Bringing modifications in the parent compound often serves to enhance the activity of the compound, along with this, in most cases, it eliminates adverse effects or toxicity associated with the parent drug. Scientific understanding of the drug action is required to design a compound that will produce a specified therapeutic effect. Peptides and proteins are very similar in that they are made up of repeating units, or residues, of α-amino acids that linked together by peptide bonds, also known as amide bonds.[1] Amino acids are building blocks of which proteins are made up of amino acids while conjugated proteins have additional component. In principle, the term, "Amino Acid" could be used to refer to any compound containing an amino group and acidic function, in actual practice, this term is often used with reference to α- amino carboxylic acids which are isolated from natural sources. α- amino acids have following general structure by convention. The carbon atom to which the carboxylic group is attached is call α- carbon.[5]



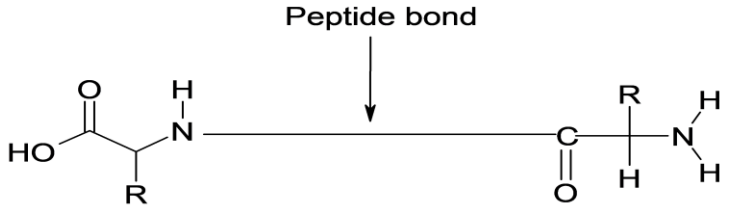
Amino group **Carboxylic group**

Structure 1: General structure of Amino acid.

Peptides are the molecules where two or more amino acids are linked together through a peptide bond, known as amide linkage or peptide 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.

2.1 The Peptide Bond

A peptide bond is a covalent bond that is formed between two molecules when the carboxyl group of one molecule reacts with the amino group of the molecule, releasing a molecule of water. This is a condensation reaction and usually occurs between amino acids. The resulting CO-NH bond is called a peptide bond, and the resulting molecule is an Amide.



Structure 2: Peptide bond.

2.2 Structure of the Peptide Bond

X-ray diffraction studies of crystals of small peptides by Linus Pauling and R. B. Corey indicated that the peptide bond is rigid, and planer. Pauling pointed out that this is largely a consequence of the resonance interaction of the amide, or the ability of the amide nitrogen to delocalize its lone pair of electrons onto the carbonyl oxygen.

Amino acid 1 **Amino acid 2** **Dipeptide molecule**

Structure 3: Structure of dipeptide.

Synthesis of new peptide derivatives as therapeutic agent was suddenly expanded with the discovery of peptides as pituitary hormones. Many peptides function as hormones, enzymes, enzyme inhibitor substrates, Growth promoters or inhibitors, neurotransmitters and immunomodulators etc.[15]

Most of the peptides which act as therapeutic agents are obtained from natural sources in less quantity. The numbers of heterocyclic compounds are found to show various biological activities like antifungal, antibacterial, antineoplastic, insecticidal, anti-inflammatory, melanin production inhibitory activities.

Heterocyclic compounds are widely distributed in nature which is essential to life. Genetic material DNA is also composed of heterocyclic based-pyrimidines and purines. A large number of heterocyclic compounds, both synthetic and natural are pharmacologically active and are in clinical use. Several heterocyclic compounds have applications in agriculture as insecticides, fungicides, herbicides, pesticides etc. They also find applications as sensitizers, developers, antioxidants, copolymers etc.[3,15]

Cancer has been an ever-growing public problem since its appearance and the estimated worldwide new incidence of it is about 6 million cases per year.[7,8] It is the second major cause of death after cardiovascular disease[9] this disease is now well characterized by unregulated proliferation of cells.[10,11] Synthesis of newer and more potent analogs of molecules with already established activities form a key part of research in the pharmaceutical field. Bringing about modifications in the parent compound swerves to enhance the activity of the compounds and also in most cases eliminates adverse effects or toxicity associated with parent drug. A great number of drugs are heterocyclic compounds, mostly are of synthetic origin few have obtained from natural resources which include alkaloids, cardiac glycosides, xanthenes, vitamins etc.[4,15]

3. Methods of synthesis of peptides

3.1. Solution phase synthesis

The most ordinary synthetic chemistry takes place in solution. When a reaction must be modified to accommodate a solid support, it takes time and resources to develop and optimize the reaction conditions. Indeed, a combinatorial chemistry may spend months designing a solid-phase reaction and gathering the necessary materials but then conduct the entire synthesis in a matter of hours or days. Many reactions cannot ever be run on solid supports because of poor yields or failed reactions. For these reasons, there has been much interest in using solution-phase chemistry for the preparations of combinatorial libraries. Solution-phase combinatorial chemistry often leads to a mixture of products. Imagine reacting a set of 10 amines with 10 acid chlorides, all in one flask, and with the reactants and conditions chosen so that no reaction of amines with amines or chlorides with chloride occure, only reactions between amines and chlorides. The result would be mixture of 100 amides one for each possible combination of amine and acid chloride. The resultant mixture could then be tested for activity, under the assumption that the inactive amides did not interfere with binding of active molecules. If activity is found, smaller subsets of amines and chlorides can be tested to eventually find the structure responsible for activity.[2,6,15]

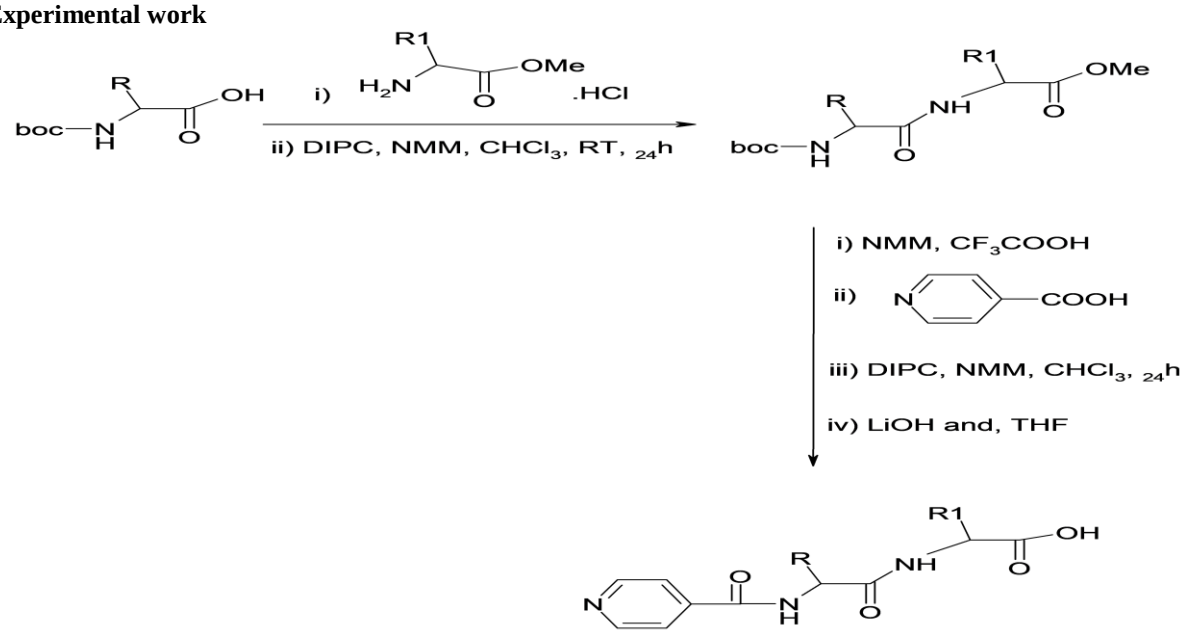
Advantages[6]

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

Disadvantages[6]

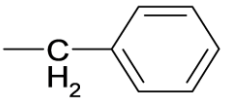
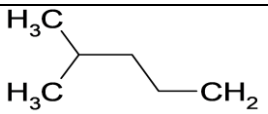
- Solution phase synthesis is more time consuming process.
- It required more time for condensation as well as for stirring.

4. Experimental work



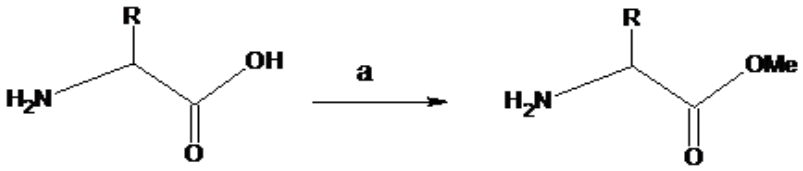
Scheme for Synthesis of Dipeptide containing Iso-nicotinic acid.

Table no 1: Substitution groups for R & R_1.

Molecule Id	R	R_1
INA 01	—H	—CH_2 
INA 02	—H	—CH(CH_3)(CH_3)
INA 03	—CH_3	H_3C  CH_2

4.1 Preparation of Amino acid methyl ester hydrochlorides

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

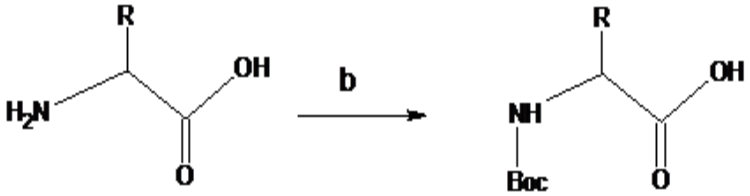


Where, a $SOCl_2$, MeOH, Reflux, 8-10h.

Structure 4: General reaction of Ester formation.

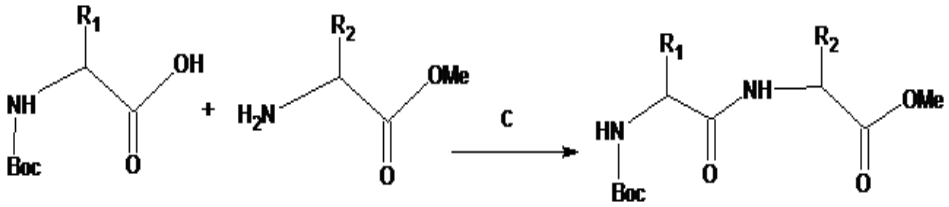
4.2 Preparation of BOC-amino acid

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_2SO_4. Extract with $CHCl_3$ (20x3ml) dry the layer over anhydrous $NaSO_4$.



The Boc-amino acids were prepared by the following route.[12]
Where, b $(Boc)_2O$, 1N NaOH, isopropanol, RT, 2h
Structure 5: General reaction of Boc-amino acid formation.

4.3 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% $NaHCO_3$ and saturated NaCl solution. Dried the organic layer over Na_2CO_3 evaporated the mixture in vacuum.



c DIPC, $CHCl_3$, NMM, RT, 24h.
Structure 6: General reaction of Dipeptide formation.

4.4 Deprotection of the Carboxyl Group
To a solution of the protected peptide (1.0 mmol) in THF**:** H2O (1**:**1) (36ml), LiOH (1.5 mmol) was added at 0^0C**.** The mixture was refluxed at 55-60°C for 15 mins and then acidified to pH 3.5 with 1N H_2SO_4. The mixture was extracted with solvent ether (3x15ml)**.** The combined ether extracts were dried over Na_2SO_4 and concentrated under reduced pressure.

4.5 Deprotection of the Amino group
The protected peptide (1 mmol) was dissolved in $CHCl_3$ (15ml) and treated with CF_3COOH (2mmol, 0.228 g)**.** The solution was stirred at room temperature for 1 hour, washed with saturated $NaHCO_3$ (5ml)**.** The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure**.** The product was purified by recrystallization from $CHCl_3$ and petroleum ether.

4.6 Synthesis of Titled compounds by Coupling with Iso-nicotinic Acid
The dipeptides were dissolved in 20ml of chloroform and 10mmol of Iso-nicotinic acid 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% $NaHCO_3$ and saturated NaCl solution. Dried the organic layer over Na_2CO_3 evaporated the mixture in vacuum.

4.7 Broth Dilution method
Prepare nutrient broth (double strength) test tubes and label first tube (UT), inoculums is not added which is used for checking sterility of medium and as a negative control. Other all test tubes, inoculums (three to four drops) is added to reach the final concentration of microorganism is 10^6 cells/ml. in all test tubes, test microbial compound is added ranging from 0.54 to 5 ml except uninoculated (negative control) and control (positive) tube. The positive control tube is used to check the suitability of the medium for growth of the test microorganism and the viability of the inoculums. Adjust the final volume (10 ml) in all test tubes by using sterile water. All test tubes are properly shaken and then incubated at 37º C for two days.[13]

4.8 Antimicrobial study
Minimum Inhibitory concentration is the minimum concentration of antimicrobial compound found to inhibit the growth of a particular test microorganism. It is applied to disinfectant, antiseptic, preservative, antibiotics. Minimum inhibitory concentration (MIC) values are usually expressed in terms of µm/ml. or units/ml. MIC of different antimicrobial is determine by broth dilution method.

5. RESULT AND DISCUSSION
5.1 Infra-Red Spectrum
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. Fig 1 gives the IR spectra of the pure drug.

- **INA-Glycine-Phenylalanine**

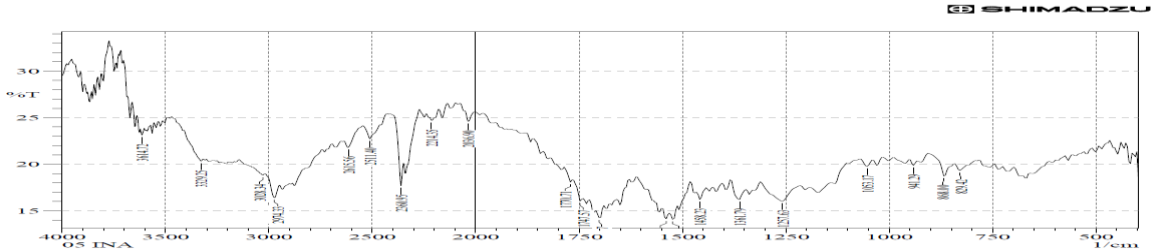


Fig. 1: Infrared spectra of INA 05.

Interpretation of IR[14]: Above IR spectra show C-H Stretching (2974.33cm^{-1}), C=C Stretching (3028.34), -CO- Stretching (1701.27), -CO-NH Stretching (1539.25), -COOH Stretching (2615.56).

- **INA-Glycine-Valine**

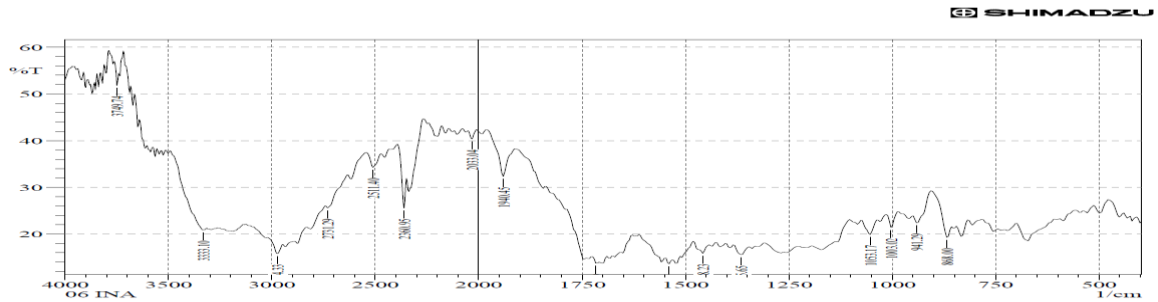


Fig. 02: Infrared spectra of INA 06.

Interpretation of IR[14]: Above IR spectra show C-H Stretching (2974.33cm^{-1}), -CO- Stretching (1716.70), -CO-NH Stretching (1539.25), -COOH Stretching (2511.40).

- **INA-Alanine-Leucine**

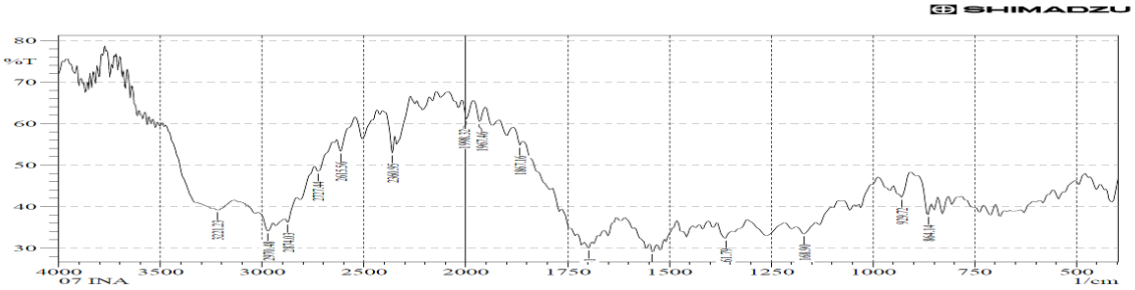


Fig. 3: Infrared spectra of INA 07.

Interpretation of IR[14]: Above IR spectra show C-H Stretching (2970.48cm^{-1}), -CO- Stretching (1697.41), -CO-NH Stretching (1543.10), -COOH Stretching (2615.56).

5.2 Nuclear Magnetic Resonance (NMR) study

NMR spectroscopy was done by using 200 mhZ in $CDCl_3$ shown as follows;

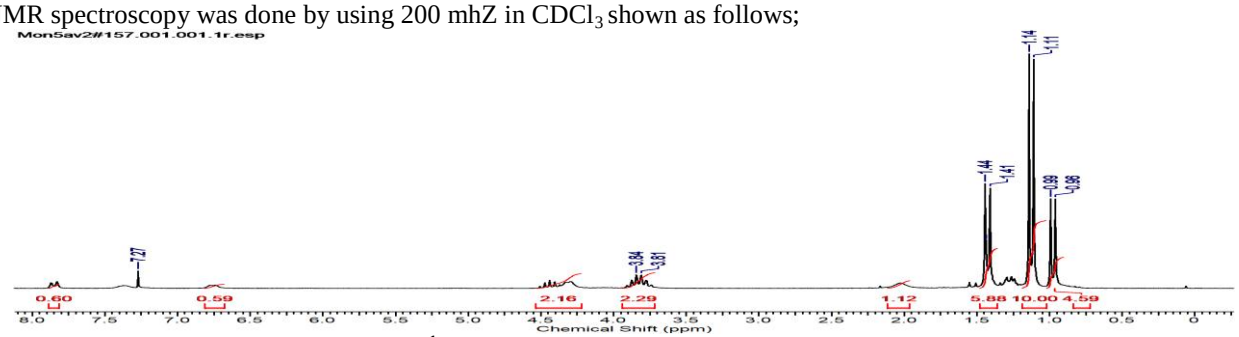


Fig. 4:-^{1}H NMR of INA-07 at 200 mhZ in $CDCl_3$.

Interpretation of ^{1}H NMR[14]: ^{1}H NMR, [200 mhZ in $CDCl_3$ δ ppm]: 7.27 (5H, Phenyl), 3.84 (H-C-OH), 3.81 (H-C-OR), 1.44 (R-OH), 0.99 (R-CH_3).

5.3 Antimicrobial study[13]

The antimicrobial study was done by using Nutrient Broth media by using *Staphylococcus aureus. Staphylococcus aureus* is the Gram Positive bacteria. Two series of dilutions were prepared likewise one containing Iso-nicotinic acid drug while another one containing Iso-nicotinic acid with dipeptide conjugate. The bacterial were inoculated in both series of test tube and incubated at 37º C for 48 hrs.

Compound shows good antimicrobial activity against gram –ve and gram +ve bacteria as compared with standard gentamycin. Compound **INA 01** shows good activity against gram –ve bacteria *Escherichia coli* and *Pseudomonas aeruginosa.* While compound **INA 02** shows good antimicrobial activity against gram +ve bacteria *Staphylococcus aureus* and *Bacillus substilis*.

Compounds **Ch01** and **Ch02** shows better antimicrobial activity as compare to standard.

Table no. 17: Antimicrobial activity.

Compound code	Gram negative Bacteria		Gram positive Bacteria	
	E. coli	***P. aureginosa***	***S. aureus***	***B. subtilis***
INA01	**6.25**	**12.5**	**12.5**	**6.25**
INA 02	**6.25**	**6.25**	**12.5**	25
INA 03	25	25	25	50
gentamycin.	**3.12**	**3.12**	**3.12**	**3.12**

6. CONCLUSION

As many peptides based molecules are shown to posses 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.

7. 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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