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Research Article

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

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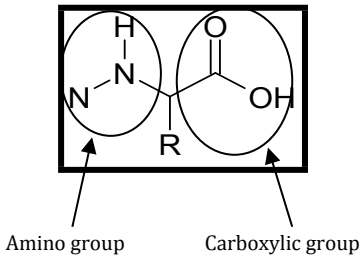
ABSTRACT

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.

Key Words: *Furan, Coumarin, Nicotinic acid, Quinoline, Thiazole, Imidazole.*

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].

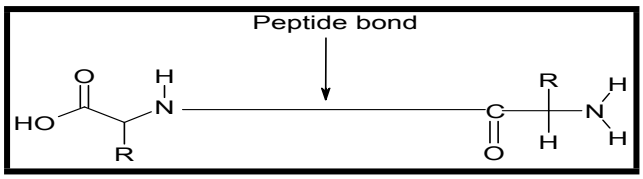


Structure 1: General structure of Amino acid

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.

1. The Peptide Bond:

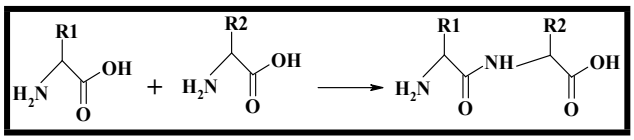
A peptide bond is a covalent bond that is formed between two molecules when the carboxyl group of one molecule reacts with the amino group of the molecule, releasing a molecule of water. This is a condensation reaction and usually occurs between amino acids. The resulting CO-NH bond is called a peptide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



Structure 2: Peptide bond

2. 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.



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.

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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].

Methods of synthesis of peptides:

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]

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

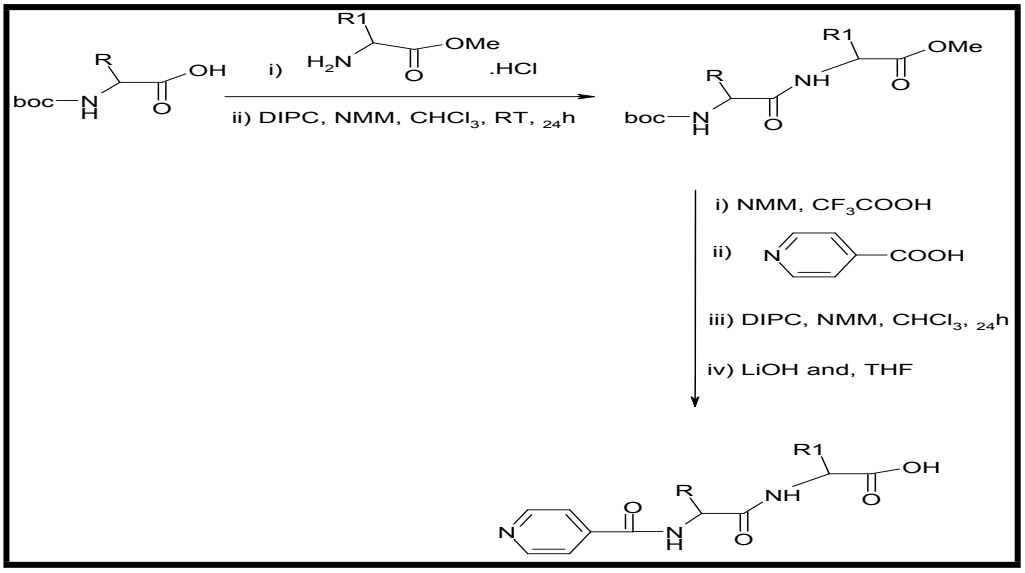
Disadvantages: [6]

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

Experimental work:

2. 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.



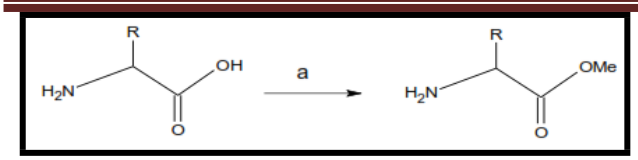
Scheme 1: Scheme for Synthesis of Dipeptide containing Iso-nicotinic acid

Table No. 1: Substitution groups for R & R_1

Molecule Id	R	R_1
INA 01	—H	—H
INA 02	—H	—CH_2—C$_6$H$_5$
INA 03	—H	—CH(CH$_3$)$_2$
INA 04	—CH_3	—CH_2—CH(CH$_3$)$_2$

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.



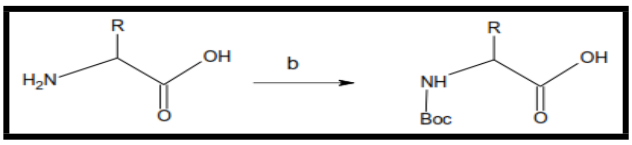
Where, a →$SOCl_2$, MeOH, Reflux, 8-10h.

Structure 4: General reaction of Ester formation

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]

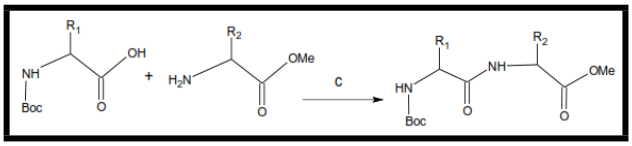


Where, b → $(Boc)_2O$, 1N NaOH, isopropanol, RT, 2h

Structure 5: General reaction of Boc-amino acid formation

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. 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.

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.

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.

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].

RESULT AND DISCUSSION

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-Glycine:

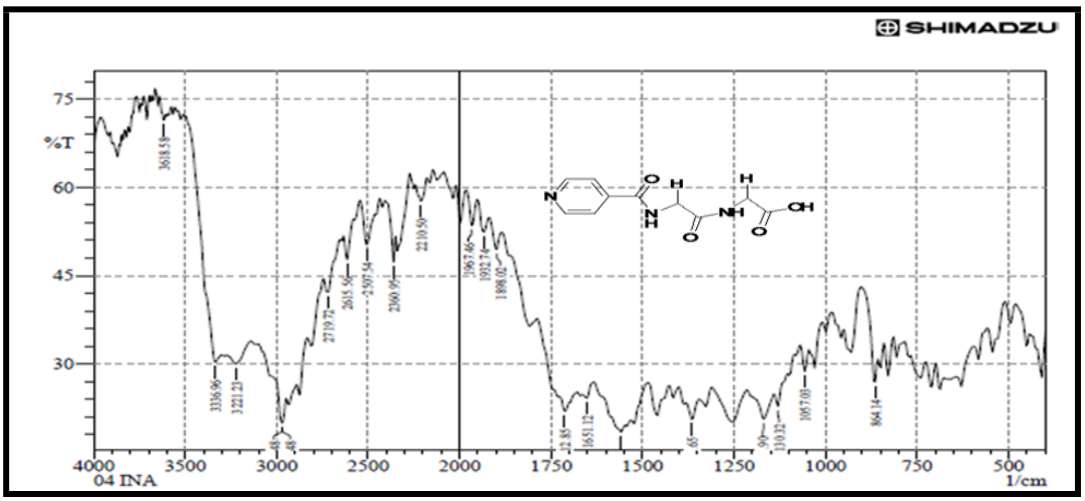


Fig. 1: Infrared spectra of INA-Glycine-Glycine

Interpretation of IR: [14] Above IR spectra show C-H Str (2970.48), -CO- Str (1712.85), -CO-NH Str (1651.12), -COOH Str (2615.56).

INA-Glycine-Phenylalanine:

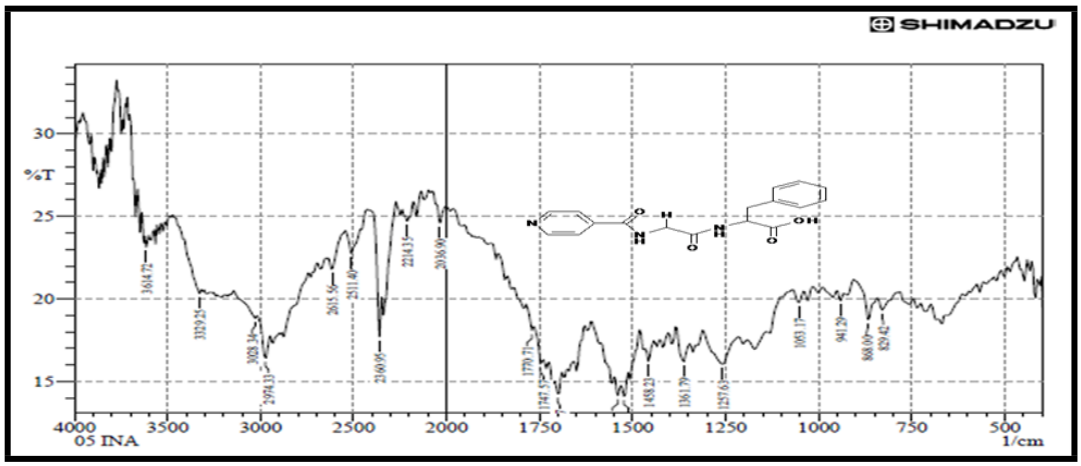


Fig. 2: Infrared spectra of INA-Glycine-Phenylalanine

Interpretation of IR: [14] Above IR spectra show C-H Str (2974.33), -CO- Str (1701.27), -CO-NH Str (1539.25), -COOH Str (2615.56).

INA-Glycine-Valine:

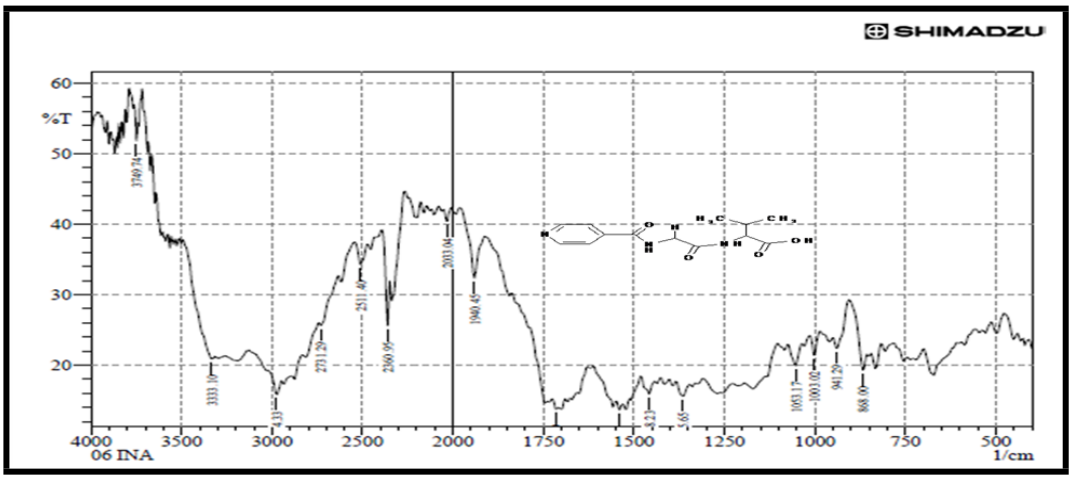


Fig. 3: Infrared spectra of INA-Glycine-Valine

Interpretation of IR: [14] Above IR spectra show C-H Str (2974.33), -CO- Str (1716.70), -CO-NH Str (1539.25), -COOH Str (2511.40).

INA-Alanine-Leucine:

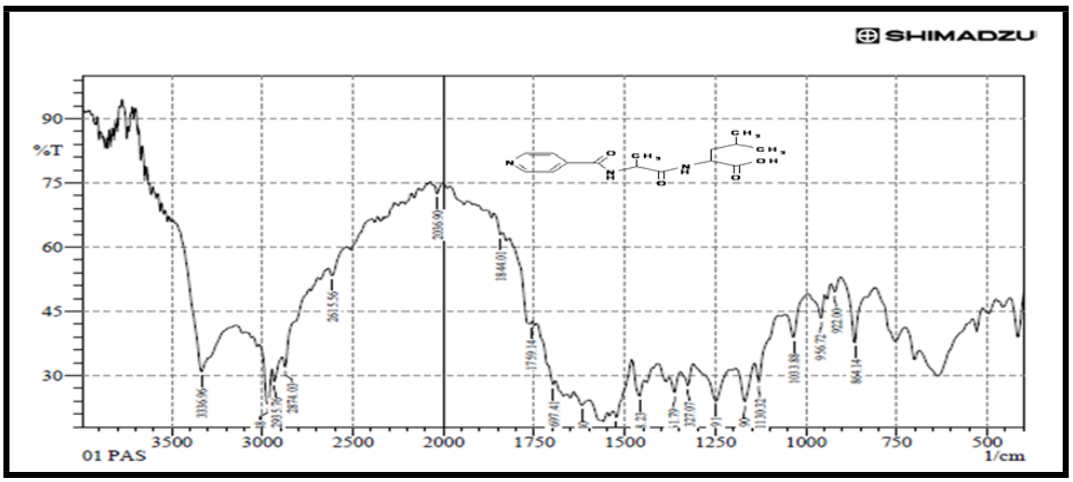


Fig. 4: Infrared spectra of INA-Alanine-Leucine

Interpretation of IR: [14] Above IR spectra show C-H Str (2970.48), -CO- Str (1697.41), -CO-NH Str (1543.10), -COOH Str (2615.56).

2. Mass Spectroscopy Study:

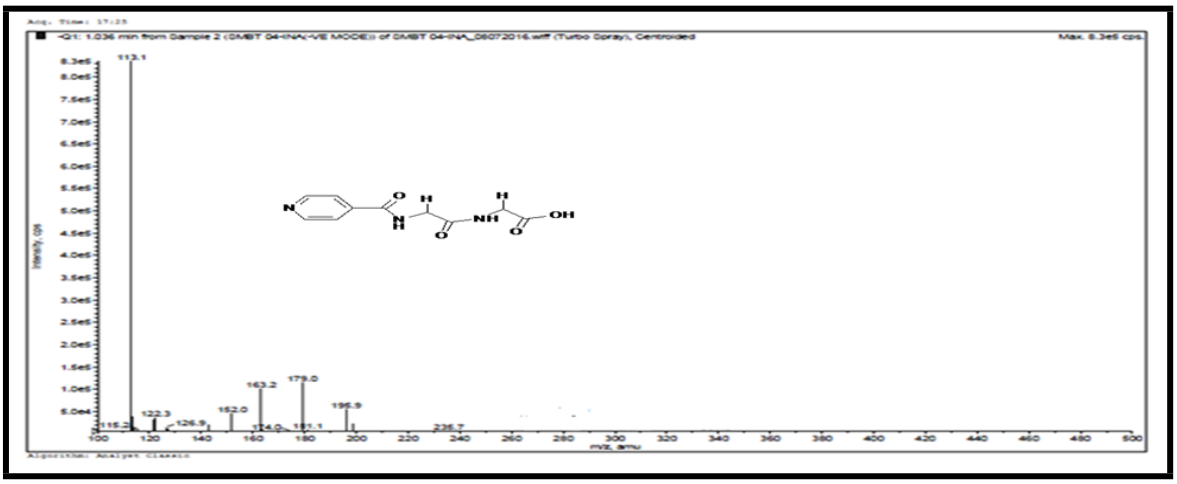


Fig. 5: Mass spectra of INA-Glycine-Glycine

Interpretation of Mass Spectra: [14] Above Mass spectra show Molecular Ion peak At 236.7 m/z, Base peak at 179.0 m/z.

3. Nuclear Magnetic Resonance (NMR) study: NMR spectroscopy was done by using 200 mhZ in $CDCl_3$ shown as follows;

^{13}C NMR Spectra of INA-Glycine-Glycine:

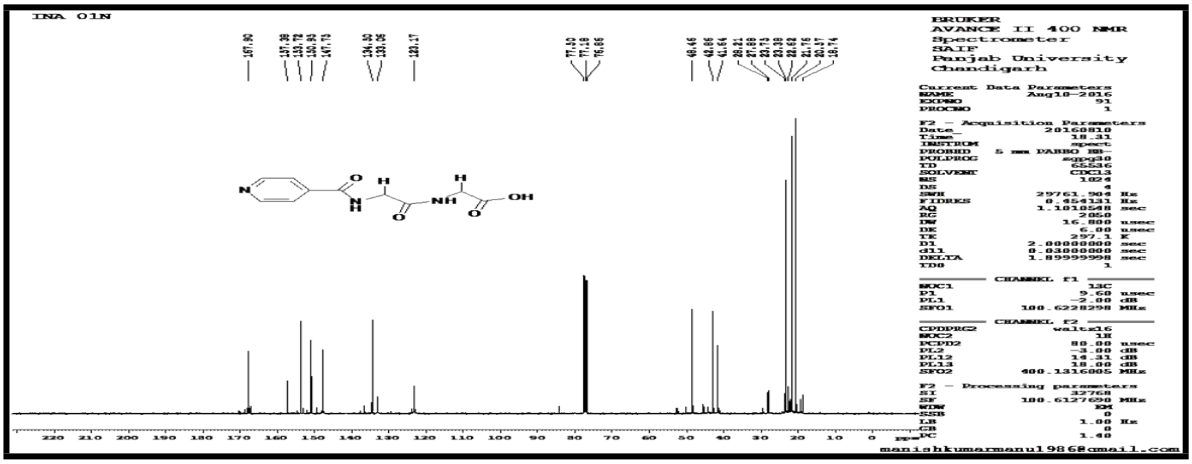


Fig. 6: ^{13}C NMR Spectra of INA-Glycine-Glycine

Interpretation of ^{13}C NMR Spectra: [13C] NMR: 167.90 (C-O), 157.38 (C-O), 153.72 (C-O), 134.50 (C-N), 133.06 (C-N), 123.17 (C-N), 76.86 (C-H), 41.64 (CH2), 27.88 (CH3).

^{1}H NMR Spectra of INA-Glycine-Glycine:

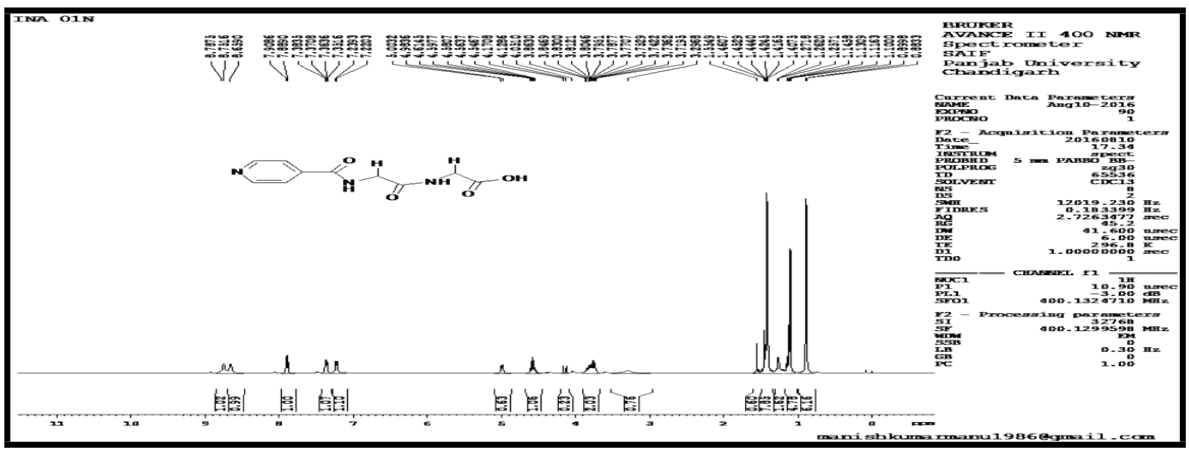


Fig. 7: ^{1}HNMR Spectra of INA-Glycine-Glycine

Interpretation of ^{1}H NMR: [14] ^{1}HNMR: 4H of Ar-H (m, 8.6590-8.7875), 1H of R-OH (s, 1.5549), 2H of H-C-COOH (s, 3.2968), 2H of CH-NH_2 (7.2203, 7.2393).

4. Antimicrobial study: [13]

Antimicrobial activity was performed on all synthesized compounds mensioned in table no. 2 *against Staphylococcus aureus, Pseudomonas aerinosa* and *Bacillus subtilis*. At the end of incubation period the tubes were examined for turbidity. Cloudiness indicates that bacterial growth has not been inhibited by the concentration of compound present in the medium. MIC was determined as the lowest concentration of the tested agent in which bacteria did not grow. The observed minimum inhibitory concentration for the respective microorganisms is listed in the table no. 2.

Table No. 2: Table showing MIC of Dipeptide containing Iso-nicotinic acid

Compound code	Microorganisms			
	E. coli	*P. aureginosa*	*S. aureus*	*B. subtilis*
INA 01	6.25	6.25	12.5	25
INA 02	12.5	25	`50	25
INA 03	6.25	12.5	12.5	6.25
INA 04	25	25	12.5	50
Streptomycin.	3.12	3.12	3.12	3.12

Compounds 1 and 3 showed highest activity compared to other compounds. Compounds 4 showed very poor activity for *B. subtilis.*

No compound achieves the activity as that of standard drug but till the dipeptide shows good antimicrobial activity.

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.

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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 infinite number of people, some who were with me from the beginning some who joined me at the some stage during the journey, whose kindness, love and blessing has brought this day, I wish to thanks each one of them from my heart.

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