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SYNTHESIS AND BIOLOGICAL EVALUATION OF NICOTINIC ACID PEPTIDE DERIVATIVES

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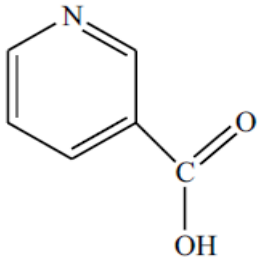
ABSTRACT:

The present study deals with the synthesis of nicotinic acid peptide derivatives and comparative evaluation of biological activities, such as antibacterial and antifungal. Among hetrocyclic aromatic compounds, pyridine nucleus is found in many bioactive products and incorporation of amino acids and peptides into the hetrocyclic aromatic congeners have resulted in compounds with potent activities. All the compounds were synthesized by coupling of nicotinic acids with amino acid methyl esters/dipeptides/tripeptides/tetrapeptides in presence of DCC as coupling agent and NMM as base under continuous stirring for 36 hrs. All synthesized peptide derivatives were identified on the basis of melting point range, Rf values, solubility studies, IR and 1H NMR spectral data. The antimicrobial activity of synthesized compounds was determined against bacterial strainsviz. E. Coli and S. Aureus and fungal strains viz. C. albicans and A. Niger using ciprofloxacin and fluconazole as standard respectively. All the synthesized compounds showed good to moderate antimicrobial activity at 40, 80 and 160 µg/ml. The comparative studies showed the following order of activity profile: nicotinic acid <nicotinic acid methylesters>dipeptide >tripeptide>tetrapeptide.

Keywords: Nicotinic acid, Peptides, Antimicrobial activity.

INTRODUCTION

Nicotinic acid (pyridine-3carboxylic acid) also called niacin which has a water-soluble vitamin B complex. It is found in variety of foods, including chicken, beef, fishes, peanuts and legumes. Nicotinic acid is a pyridine-3carboxylic acid with the chemical formula C5NH5O2. Nicotinic acid has a carboxyl group. In 1867 nicotinic acid was synthesized by oxidation of nicotine with nitric acid. Commercially, nicotinic acid obtained from beta-picoline, which is obtainable from coal tar. Nicotinic acid itself and its derivatives are of special importance because of their broad spectrum biological and pharmacological activities. They are widely used in various medicinal applications as an analgesic1, antibacterial2, anticonvulsant3, antifungal4, insecticidal5, antiinflammatory6, antioxidant7, antituberculosis8and antiviral9 activities. It also lowers the blood cholesterol and fat level.



PEPTIDES:

Peptides10 are made of amino acids linked into linear chain with overall length up to 100 amino acids. Peptides are short polymers of amino acid monomers linked by peptide bonds. Every peptide has N-terminus and C-terminus residue on the ends of peptide. Peptides are synthesized by coupling11 the carboxyl group or C-terminus of one amino acid to the amino group or N-terminus of another. Thus, keeping in mind the pharmacological potential of nicotinic acid and its derivatives as well as taking advantage of biodegradability and biocompatibility

of peptides, peptide12 derivatives of nicotinic acid were prepared to increase therapeutic efficacy and to decrease adverse effect. In present research, compound or moiety coupled with different peptide methyl ester using DCC and NMM in THF to afford peptide derivatives. Peptide derivatives of nicotinic acid shows good antibacterial activity and antifungal activity. The newly synthesized nicotinic-peptide derivatives were characterized by TLC, IR and NMR analysis.

MATERIALS AND METHODS:

The chemicals used were obtained from Spectrochem Pvt. Ltd, Mumbai and Sd fine-chem Limited, Mumbai. All the melting points were determined by open capillary method and uncorrected. The reactions were monitored by TLC on silica gel G plates using Chloroform: Methanol as developing solvent system in the ratio of 9: 1 and brown spots was detected on exposure to iodine vapours in tightly closed chamber. Final peptide derivatives were purified by recrystallisation from mixture of chloroform: methanol (1:1). IR spectrum of compounds in KBr pellet was recorded on a FTIR-RXI spectrophotometer13 (PERKIN ELMER). 1H-NMR Spectra of compounds was recorded on Bruker NMR spectrometer in deuterium-substituted chloroform using TMS as internal Standard (Chemical Shift in δ ppm). The biological activity of nicotinic acid derivatives and its methylester/ dipeptide/ tripeptide/ tetrapeptide on different bacterial and fungal strains were tested using Ciprofloxacin (MIC: 40 µg/ml) as antibacterial standards and Fluconazole (MIC: 40 µg/ml) as antifungal standard.

Synthesis of BOC-amino acids:

L-amino acid (20mmol) was dissolved in 1 mmol/litre sodium hydroxide (20ml) and isopropanol (20ml). Ditert-butylpyrocarbonate (Boc) (26mmol) in isopropanol (10ml) was added followed by 1 mmol/litre sodium hydroxide (20ml) to the resulting solution. The solution was stirred at room temperature for 2 hrs, washed with light petroleum ether (b.p 40-60°C) (20ml), acidified to pH 3.0 with 1 mmol/litre H2SO4 and finally extracted with chloroform (3x20ml).The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure to give the crude product, which was crystallized from chloroform and petroleum ether (b.p 40-60 ºC) to get semi-solid mass of compound.

Synthesis of L-amino acid methyl ester hydrochlorides:

Thionyl chloride (1.4ml, 20mmol) was slowly added to methanol (100ml) at 0°C and L-amino acid (2.3g, 20mmol) was added to above solution. The resulting mixture was refluxed for 12 hrs at 70°C. Methanol was evaporated and the residue was triturated with ether at 0°C, until excess dimethyl sulphate was removed. The crude solid was crystallized from methanol and ether at 0°C to give pure amino acid methyl ester.

Synthesis of linear peptide fragments:

Amino acid methyl ester hydrochloride/peptide methyl ester (10mmol) was dissolved in chloroform (20mL). To this, N-methylmorpholine (21mmol) was added at 0 °C and the reaction mixture was stirred for 15 minutes. Boc-amino acid/peptide (10mmol) in chloroform (20mL) and N, N-dicyclohexylcarbodiimide (10 mmol) were added with stirring. After 36hrs, the reaction mixture was filtered and the residue was washed with chloroform (30mL) and added to the filtrate. The filtrate was washed with 5% sodium bicarbonate and saturated sodium chloride solutions. The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated in vacuum. The crude product was crystallized from a mixture of chloroform and petroleum ether followed by cooling at 0 °C. Deprotection at carboxyl terminal was done by adding lithium hydroxide (0.36 g, 15mmol) to a solution of Boc-di/tripeptide methyl ester (10mmol) in tetrahydrofuran: Water (1: 1, 36ml) at 0 °C. Resulting mixture was stirred at RT for 1 hr and then acidified to pH 3.5 with 1N H2SO4. The aqueous layer was extracted with diethyl ether (3 × 25mL). The combined organic extracts were dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product was crystallized from methanol and ether to get pure Boc-di/tripeptides. Deprotection at amino terminal was accomplished by treatment of Boc di/ tripeptides (10 mmol) dissolved in chloroform (15mL) with trifluoroacetic acid (2.28g, 20mmol). The resulting solution was stirred at RT for 1 hr, washed with saturated sodium bicarbonate solution (25mL). The organic layer was dried over anhydrous sodium sulphate

and concentrated under reduced pressure. The crude product was purified by crystallization from chloroform and petroleum ether (B.p. 40-60 °C) to get pure di/tri/tetrapeptide methyl esters.

SYNTHESIS OF NICOTINIC ACID DERIVATIVE

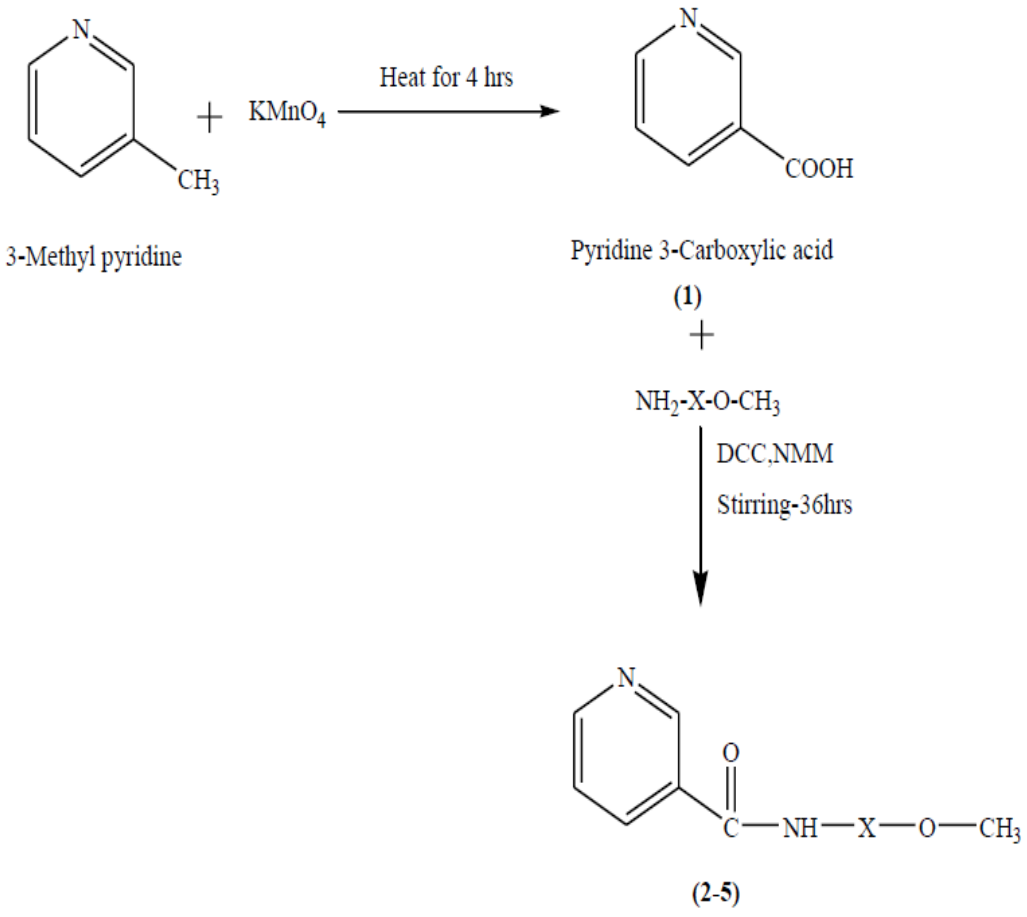
Synthesis of nicotinic acid (1)

To the conical flask, 100 mg of 3-methyl pyridine in 1 litre of water was added and oxidized with 450 gm of potassium permanganate, over a period of 4-5 hrs, maintain the temperature at 700C Then solid mass was formed. Then wash the solid cake with (4× 500 ml) portion of water, evaporated the combined filtrate. Allow to cool overnight, collect the precipitates of compound 32 by suction filtration. Recrystalized from hot water, yield of pure compound 32 is 67g.

Synthesis of Nicotinic acid peptide derivatives (2-5):

To a mixture of compound Di/tri/tetra-peptide methyl ester (10 mmol) in THF (75 ml), 2.3 ml of NMM was added at 0 °C with stirring of nicotinic acid (10 mmol) in THF (75 ml) and DCC (2.1 g, 10 mmol) were added to the above mixture and stirring was done for 36 h. After 36 h, the reaction mixture was filtered and residue was washed with THF (50 ml). Then, filtrate was washed with 5% sodium hydrogen carbonate and saturated sodium chloride solution (30 ml) and dried over anhydrous Na2SO4, filtered and evaporated in vacuum. The crude product obtained was crystallized from a mixture of chloroform and n-hexane followed by cooling at 0 0C to give pure compound (2-5).

SCHEME OF WORK: 1a



X=L-Tyr (**2**), X=L-Val-Ile (**3**), X=Arg-Tyr-Phe (**4**), L-Ala-Trp-Val-Ile (**5**)

Biological activity

An Anti-microbial agent14 is a substance that kills or inhibits the growth of microorganism such as bacteria, fungi, or protozoan. Disinfectants are antimicrobial substances used on non-living objects or outside the body. On the basis of their primary activity, they are more specifically called antibacterial, antifungal, antiprotozoal, anti parasitic or antiviral agents etc.

Antimicrobial activity:

The antibacterial and antifungal activity can be determined by two methods ie. turbidimetric method and disc diffusion method. The synthesized peptide derivatives were screened for antibacterial activity against Escherichia coli (Gram-negative bacteria) and Staphylococcus aureus (Gram-positive bacteria) and antifungal activity against fungal strain Aspergillus niger and Candida albicans. using modified Kirby-Bauer disc diffusion method. All synthesized compounds were dissolved separately to prepare stock solution of 1mg ml-1 using Dimethyl sulphoxide (DMSO) and chloroform as solvent. From this stock solution further dilutions of concentration 40, 80 and 160 µg/ml were made respectively. Firstly, nutrient agar medium was prepared by using the different ingredients mentioned above and then this medium was poured into the petri plates. Petri plates were inoculated with bacterial suspension and incubated at 370C for 24 hrs. After 24 hours, petri plates were taken out from incubator. Each petri plate was divided into equal portions along the diameter to place one disc. Three discs of test samples 40,80,160µg ml-1 were placed on three portions together and one disc with standard drug ciprofloxacin (40µg ml-1) and a disc impregnated with the solvent (DMSO and Chloroform) as negative control. The test samples tested at the concentration 40, 80, 160µg ml1 were checked, incubated and then the diameters obtained for the test samples were compared with the diameter obtained with standard drug ciprofloxacin (40µg ml-1) and Fluconazole (40µg ml-1).

RESULTS:

Table 1: TLC, IR, NMR ranges of compounds

Cpds	IR ranges (KBr) υ cm-1	NMR ranges(DMSO) δ ppm	TLC Rf value
1.	IR(KBR)υ : 3067 (C-H stretching, aromatic ring), 1360 (C-N stretching, aromatic ring), 1477 (C=C stretching, aromatic ring), 3184 (OH stretching, COOH), 1313 (C-C stretching, aromatic ring), 1700 (C=O stretching, COOH) 1302 (C-O stretching, COOH).	^{1}HNMR ($CDCl_3$) δ: 9.11 (s, 1H, pyridine ring), 8.28 (d,1H, pyridine ring), 8.68 (d,1H, pyridine ring),7.52 (m, 1H, pyridine ring) 11.1 (s, 1H, COOH) ppm.	R_f-0.82.
2.	IR(KBR)υ : 3169 (N-H stretching, amide), 3076 (C-H stretching, aromatic ring), 1738 (C=O stretching, ester), 1625(C=O stretching, amide), 1414(C=C stretching, aromatic ring), 1360 (C-N stretching, aromatic ring), 1461(N-H bending, amide), 3372 (O-H stretching, tyr) 1213 (C-O stretching, ester) cm^{-1}.	^{1}HNMR ($CDCl_3$) δ: 7.3 (s, 1H, NH, amide), 9.09 (s, 1H, pyridine ring), 8.21 (d, 1H, pyridine ring) 8.28 (d,1H, pyridine ring), 7.52 (m, 1H, pyridine ring) 6.69 (m, 4H, aromatic ring of tyr), 5.09 (s, 1H, OH, tyr), 4.42 (m, 1H, CH, tyr), 3.71 (d, 2H, CH_2, tyr), 3.77 (s, 3H, OCH_3, ester) ppm.	R_f-0.80.
3.	IR(KBR)υ :2927 (C-H stretching, aromatic), 2850.40(C-H stretching, alkanes), 1737 (C=O stretching, esters), 1574.29 (C-C stretch aromatics), 1317 (C-N stretching, aromatic), 2889 (C-H stretching, CH) 2857 (C-H stretching, CH_2),1453 (C=C stretching, aromatic), 1248 (C-N stretch amide),1213 (C-O stretch esters), 1668(C=O stretching, amide), 3300 (N-H stretching, amide) cm^{-1}.	^{1}HNMR ($CDCl_3$) δ: 9.06 (s, 1H, pyridine ring), 8.74 (s, 2H, NH, amide), 4.53 (m, 1H, CH val), 4.38(m, 1H, CH, Ileu), 3.71(s, 3H, OCH_3, ester), 2.5 (m, 1H, CH, Ileu), 2.3(m, 1H, CH, Val), 1.06 (m, 2H, CH_2, Ileu), 1.02 (d, 6H, CH_3,Val), 1.0(m, 6H, CH_3, Ileu) ppm.	R_f-0.79.

4.	IR(KBR)υ : 3327 (N-H stretching, amide), 3067 (C-H stretching, aromatic ring), 2933 (C-H stretching, CH_2), 2852 (C-H stretching, CH_2), 1743 (C=O stretching, ester), 1627 (C=O, amide), 1577 (C=C stretching, aromatic ring), 3401 (O-H stretching, tyr), 1456 (N-H bending, amide), 1208(C-O stretching, ester)cm^1.	^{1}HNMR ($CDCl_3$) δ: 8.76 (s, 3H, NH, amide), 9.03(s, 1H, pyridine ring), 7.23-7.29 (m, 5H, pheala), 5.01 (m, 1H, CH, Tyr), 5.09 (s, 1H, OH, tyr), 6.69 (m, 4H, aromatic ring of tyr), 4.58 (m, 1H, CH, Pheala), 4.59 (m, 1H, CH, arg), 3.91 (s, 2H, CH_2, arg), 3.7 (d, 2H, CH_2, Pheala), 3.77 (s, 3H, OCH_3, ester), 3.72 (d, 2H, CH_2, Tyr), 2.56 (m, 2H, CH_2, arg), 1.90 (s, 4H, NH Arg), 1.55 (m, 2H, CH_2, Arg) ppm.	R_f-0.75.
5.	**IR(KBR)υ :** 3298(N-H stretching, amide), 3071(C-H stretching, aromatic ring), 2935 (C-H stretching, CH_3), 2852 (C-H stretching, CH_2), 1739 (C=O stretching, ester), 1668 (C=O stretching, amide), 1464(C=C stretching, aromatic ring), 1516 (N-H bending, amide), 1246 (C-N stretching, amide) , 1217 (C-O stretching, ester) cm^{-1}.	**^{1}HNMR ($CDCl_3$) δ:** 8.99 (s, 4H, NH, amide), 9.11 (s, 1H, pyridine ring), 4.05 (m, 1H, CH, ala), 1.43 (d, 3H, CH_3, ala), 3.98 (s, 1H, CH, try), 3.63 (s, 2H, CH_2, trp), 7.71 (s, 1H, NH, Indole ring) ,6.39 (s, 1H, Indole ring), 6.69 (m, 4H, aromatic ring of tyr), 4.53 (m, 1H, CH, val), 4.38 (m, 1H, CH, Ileu), 3.86 (s, 3H, OCH_3, ester), 2.5 (m,1H, CH,Ileu), 2.3 (m,1H,CH, Val), 1.06 (m, 2H, CH_2, Ileu), 1.02 (d, 6H, CH_3 ,Val), 1.0 (m, 6H,CH_3, Ileu) ppm.	Rf-0.78

Table 2: The results of antibacterial activity of different compounds against *S.aureus* and *E.coli*

Compounds	*Staphylococcus aureus*		*Escherichia coli*	
	MIC µg/ml	**Zone of diameter(mm)**	**MIC µg/ml**	**Zone of diameter(mm)**
1.	40	3.5	160	3
2.	160	3	40	1.5
3.	40	4.1	80	2.5
4.	40	1.5	160	2
5.	160	1.1	80	**0.5**
Ciprofloxacin	40	7	40	6.5

Table 2: shows that all compounds give different MIC for different bacterial strains i.e. with *S. aureus* and *E.coli*, this reveals that compounds **1, 3, 4** are most effective in case of *S. aureus* and compounds **2** most effective in case of *E.coli*. at **40 µg/ml** (Shown in fig.)

Table 3: Antifungal activity of different compounds against *A. niger* and *candida albicans*

Compounds	*Aspergillus niger*		*Candida albicans*	
	MIC µg/ml	**Zone of diameter(mm)**	**MIC µg/ml**	**Zone of diameter(mm)**
1.	40	2.9	40	4
2.	160	2.7	40	1.5
3.	40	2.6	80	3
4.	80	2.8	160	3.2
5.	80	4.0	80	2.1
Fluconazole	40	4.6	40	4.5

From table it is concluded that compounds **1 and 3** are most effective and Compounds **2, 4 and 5** are least effective in case of ***A.niger*** and compounds **1** and **2** are most effective and **3, 4**are least effective in case *C. albicans* fungal strain.

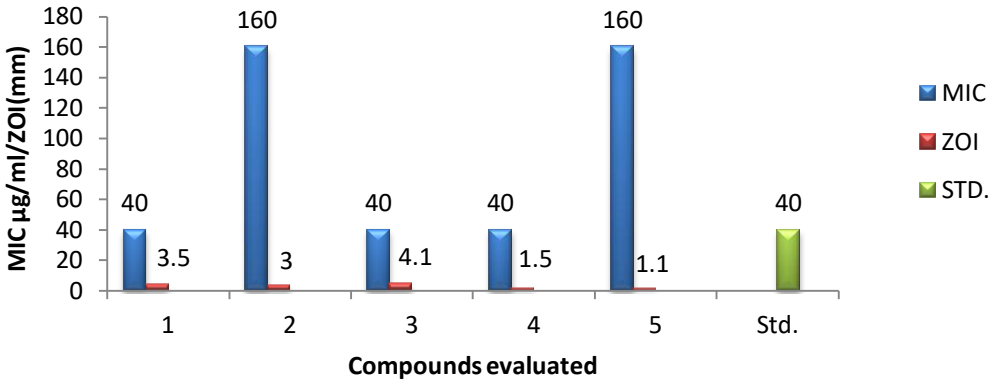


Figure 1: Comparison of minimum inhibitory concentration (µg/ml)/ZOI of standard Ciprofloxacin and nicotinic acid- peptide derivatives in case of *S.aureus.*

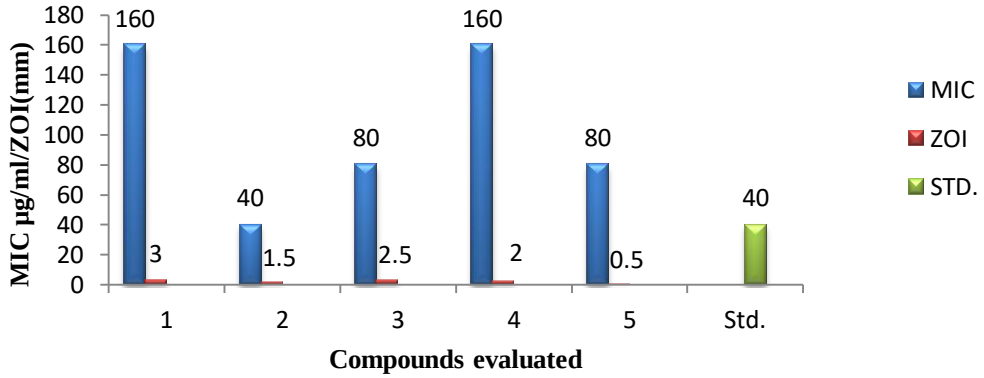


Figure 2: Comparison of minimum inhibitory concentration (µg/ml) /ZOI of standard Ciprofloxacin and nicotinic acid- peptide derivatives in case of *E coli.*

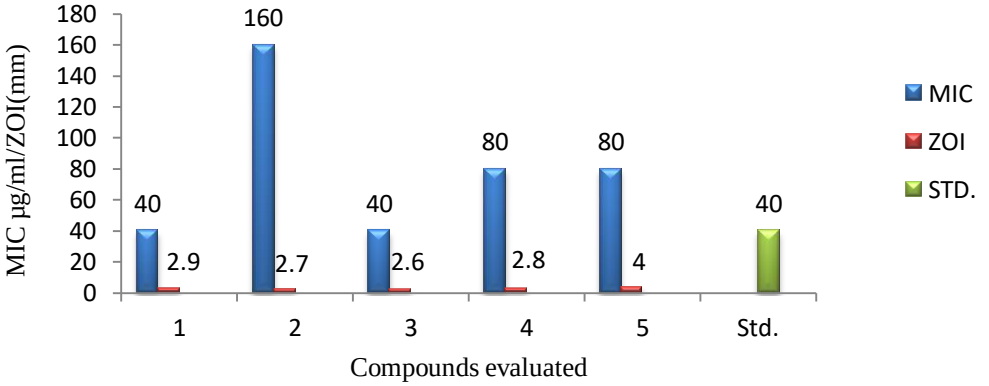


Figure 3: Comparison of minimum inhibitory concentration (µg/ml) /ZOI of standard fluconazole and Nicotinic acid- peptide derivatives in case of A.niger

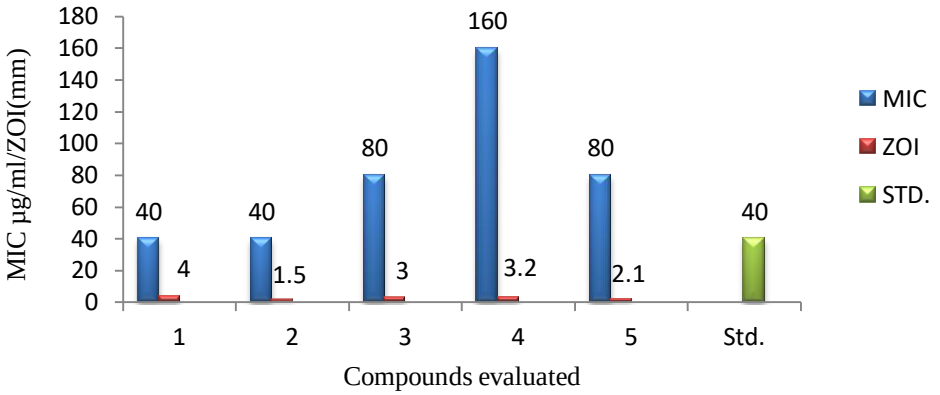


Figure 4: Comparison of minimum inhibitory concentration (µg/ml)/ZOI of standard fluconazole and Nicotinic acid- peptide derivatives in case of *C. albicans*

CONCLUSION

Synthesis of all peptide derivatives were carried out successfully via coupling reaction with good yields. N, N-dicyclohexylcarbodiimide proved to be a good coupling agent both economically and yield wise. The title compounds were synthesized by coupling of nicotinic acid derivatives with amino acid methyl esters/ dipeptides /tripeptides/tetrapeptides in the presence of N, N-dicyclohexylcarbodiimide as a coupling agent and N-methylmorpholine as a base under continuous stirring for 36 hrs. All synthesized peptide derivatives were identified on the basis of melting point range, Rf values, and IR and 1H NMR spectral data confirmed the identity of the synthesized compounds. Nicotinic acid and its peptide derivatives showed the presence of characteristic absorption bands in the region 3409-3363, 1768-1768, 1582-1517 cm-1 respectively. In 1H NMR spectra, the range 8.10-7.31 ppm corresponding to the (CO-NH) proton. Nicotinic acid derivatives and its methylester /dipeptide /tripeptide/tetrapeptide all showed mild to moderate antimicrobial activity. From the antibacterial studies, we concluded that newly synthesized peptide derivative 3 exhibits highest activity against S. aureus; compound 4 exhibits good activity against E. coli. at 40 µg/ml concentration. In case of fungal strains, compound 1 exhibits highest activity against C. albicans; compound 1 exhibits potent activity against A. niger.

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