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Synthesis of peptide derivatives of aspirin and their antibiogram

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ABSTRACT

Peptide derivatives of Aspirin (1 to 8) were synthesized by using Ac_2O/AcOH reaction with Salicyclic acid. Aspirin was coupled with amino acid amide and dipeptide amide and tripeptide amide using its p-nitro phenyl (N_P) ester. The ester (Aspirin–ON_P) was prepared using p-nitro phenol and DCC in EtOAc and was precipitated using EtOH. The synthesis of dipeptide amides were carried out in solution by stepwise elongation of the peptide chain from the C-terminal amino acid by coupling one amino acid at a time using DCC/HOBt method. Boc-group was used for N^{α} protection of all amino acids. The Boc-group cleavage was carried out using 50% TFA/CH_2Cl_2. The amidation of C-terminal amino acid was carried out by treating the corresponding Boc–amino acid–ONp esters with dry NH_3 in presence of DCC and HOBt. These compounds were subjected to antibiotic susceptibility studies against the *Pseudomonas fluorescens, Escherichia coli and Staphylococcus aureus*. Methionine amide of aspirin was active on *S. aureus*. Glycyl-glycyl-phenylalanyl-leucine amide of aspirin was moderately active on all the three bacterial strains used. Glycyl-Phenylalanyl-leucine amide of aspirin and glycyl-glycyl-methionine amide of aspirin were active on *P. fluorescens.* Leucine amides of aspirin was moderately active on *S. aureus* Methionine amides of aspirin was highly active on *E. coli*. Phenylalanyl-leucine amides of aspirin and glycyl-glycyl-phenylalanyl-methionine amide of aspirin were active on none of the organisms used in this study. In general the peptide derivatives of aspirin (1,3,4,5,6,7) were not active on all the three microbes used. Compounds 2 and 8 were sensitive to none of the organisms used in this study. By the major study, we observed that the derivative of aspirin showed a greater influence in inhibiting the test organisms used.

Keywords: Aspirin–ONp, Boc–N_3 (t-butyl azido formate), HOBt (1-Hydroxy benzotriazole), DCC (N, N^1–Dicyclo hexyl carbodiimide) and DCU (N, N^1–Dicyclohexyl urea).

INTRODUCTION

Salicylic acid was found to possess analgesic activity (Patric, 2000) and it was used for the treatment of integumental pain, headache and reduces fever. Nevertheless, it was not used medicinally due to its side-effect of producing gastric disturbances. But their derivatives are found to be useful. Aspirin is used for musculo-skeletal disorders. In a sufficiently larger dose, aspirin acts as an anti-rheumatic and anti-platelet agent. In a single dose, aspirin produces only analgesic action. Use of analgesics requires an understanding of the biochemical and physiological mechanism of analgesics (Williams et al., 1981). To improve the solubility and to reduce the side effects of aspirin (Wilson et al., 1968) various salts like calcium salicylate, aluminium salicylate were introduced by blocking the free carboxyl group. Sodium and potassium salts of aspirin are unstable in contact with moisture. Fujii et al. (1982) were successful in

the synthesis of peptides by coupling a large number of small segments (total of 30) ranging from di to octa peptides one by one starting from the carboxyl terminus. The incomplete cleavage of protecting groups leads to loss of biological activity (Kenner et al., 1979).

The most commonly used protecting group combination of Z and Boc are cleaved by catalytic hydrogenolysis. Beluzzi et al. (1975) and Birscher et al. (1976) were first to report the analgesic effect of Met-and Leu-enkephalins through injection into lateral ventricles of rats, using the tail flick procedure. The peptides are found not only in the pituitary but also in neurons in the CNS where they probably serve as neuro-transmitters with a wide spectrum of biological activity (Snyder, 1980). Infectious diseases are world's leading cause of premature death, killing almost 50,000 people every day (Anonymous, 2000). Resistance to antimicrobial agents is emerging in a wide variety of pathogens and multiple drug resistance is becoming common in diverse organisms such as *S. aureus, S. sepidemidis, S. typhi,* and *S. paratyphi* A (Threlfall et al., 1996; Gowan, 1999; Ahamed et al., 2001), this has necessitated a search for new antimicrobial substances from other sources including plants. Over the past 20 years, there has been a lot of investigation on plants as sources of new antimicrobial agents. But still there is an immediate need to identify novel substances active towards pathogens with high resistance (Reciomc, 1978; 1988; Cragg et al., 1997; Samy et al., 2000). Recent studies have shown that several alcoholic extracts of various traditional medicinal plants exhibit antibacterial activity (Ahamed et al., 2001; Akinyemiko et al., 2005). Anti Staphylococcal and anti – Salmonella activities of plants have also been reported (Perez et al., 1990; Polombo et al., 2002; Rani et al., 2004). *P. aeruginosa* is a leading cause of hospital acquired infections giving to a wide range of opportunistic infections. Its high intrinsic resistance to antibiotics and ability to develop multidrug resistance pose serious therapeutic problems (Livermore, 2002).

The term resistance implies that the organism is expected not to respond to a given drug irrespective of the dosage and of the location of the infection (Gales et al., 2001). The in vitro susceptibility patterns of the isolated bacteria to different antimicrobial agents were determined by disc diffusion technique (Cruckshank et al., 1975). Enterotoxigenic *Escherichia coli* (ETEC) strains are the major cause of diarrhoea in man and domestic animals. It has been estimated that only ETEC gives rise to 650 million cases of diarrhoea and 800,000 deaths annually in children under five years of age in developing countries (Black, 1986). Similarly, the impact of ETEC diarrhoea on young animals health is also of very high magnitude (Gyles, 1986). ETEC strains harbour several virulence factors, the important ones are plasmid encoded (heat labile, LT and or heat stable ST) enterotoxin(s) and plasmid or chromosome encoded adhesions which enable them to attach to and proliferate in small bowl (Blanco et al., 1991; Bertin, 1992; Gyles, 1992).

MATERIALS AND METHODS

Synthesis of peptide amides of aspirin

Boc-aminoacid NH_2 was prepared using Boc-aminoacid /dry NH_3 / DCC / HOBt and was obtained in about 85% yield. The synthesis of dipeptide amides, tripeptide amides, and tetrapeptide amides were carried out in solution by stepwise elongation of the peptide chain from the C-terminal amino acid by coupling one amino acid at a time using DCC/HOBt method (Kundu et al., 1986). Boc group (Carpino, 1957; Seigmund et al., 1957) was used for $N^{\propto}$ protection of all amino acids. The Boc-group cleavage was carried out using 50% TFA/CH_2Cl_2. The amidation of C-terminal amino acid was carried out by treating the corresponding Boc-aminoacid–ONp esters with dry NH_3 in presence of DCC and HOBt.

Antibiogram study

Swabs were prepared with absorbent cotton wool application stick. These swabs were then kept in suitable test tubes plugged with cotton wool and sterilized.

Collection of samples

The strains collected from the hospital environment were kept in transporting media and transported to the laboratory within 24

hours. Until further processing, it was preserved in refrigerator.

Identification of the organisms

The organism authenticity was found by performing biochemical reactions and sugar fermentation tests. The results of biochemical reactions and sugar fermentation tests are tabulated in table I.

The following peptide derivatives were subjected to antibiotic susceptibility studies against the bacteria *E. coli, P. fluorescens, S. aureus*. using aspirin as the control.

1. Asp – Leu – NH_2
2. Asp –Phe - Leu – NH_2
3. Asp – Gly –Phe - Leu – NH_2
4. Asp –Gly- Gly- Phe- Leu – NH_2
5. Asp – Met – NH_2
6. Asp – Gly- Met – NH_2
7. Asp – Gly- Gly- Met – NH_2
8. Asp –Gly- Gly- Phe- Met – NH_2

In Paper disc method, organisms were cultivated in nutrient broth for testing the antibacterial activity against the synthesized peptide derivatives. Muller–Hinton agar plates were prepared and the organisms were inoculated onto it following streak plate method (Pal et al., 1999).

Peptide derivatives (1 to 8) and aspirin (control) were dissolved in DMSO in equal proportions. Whatmann's No 1 filter paper was cut into disc shape using a punching machine and was dipped into this mixture. Then the discs were taken out from the mixture using sterile forceps. These discs were placed on the Muller-Hinton agar plates that were streaked with the test organisms. The plates were then incubated at 37 °C for 24 hours. After the incubation, the plates were observed for the presence of any zone of inhibition, and the diameters of the zones were measured using a scale keeping aspirin as the control.

RESULTS

The compounds synthesized were confirmed by the IR, NMR spectrum and elemental analysis studies and the data are given below:

Data pertaining to a few selected compounds are as follows:

1. M.P. 109-111 °C; IR (KBr); 3394cm^{-1} (asymmetric NH), 3207cm^{-1} (sym. NH), 3351 cm^{-1} (–CONH)., 1717 cm^{-1}, (>C=O), ^{1}HNMR; (400 MHz) (DMSO); δ2.5 (–$COCH_3$), δ8 (N-H) and δ7.95 (-NH_2).

2. M.P. 138-140 °C; IR (KBr); 3390cm^{-1} (Asymm.NH); 3188cm^{-1} (symm.NH)., 3342cm^{-1} (CONH), 1678cm^{-1} (>C=O), ^{1}H NMR: δ2.27 (-$COCH_3$), δ8.2 (-NH-) δ8.4 (-NH_2).

Elemental analysis

Compound 1 Glycyl – Glycyl – phenylalanyl – Methionine amide of Aspirin. $C_{27}H_{33}O_7N_5S$ (571) is found to have C 56.74%, H = 5.78% and N 12.26%.

Compound 2, Glycyl – Glycyl – Phenyl alanyl – leucine amide of Aspirin, $C_{28}H_{35}O_7$ N5(553) is found to have C 60.76%, H 6.33% and N 12.66%.

^{1}H NMR study

^{1}H NMR parameters of the amino acid residues (1) and (2) are Asp – Gly (1) – Gly (2) – Phe (3) – X (4) – NH_2 (Table 2).

Antibiogram

Upon studying the sensitivity pattern of *E. coli, P. fluorescens, S. aureus*. against the various amides of aminoacids, dipeptides, tripeptides, and tetrapeptides, the following results were obtained and tabulated in the table 3.

Aspirin was used as a positive control that showed zone size of 15 mm.

P. fluorescens showed a high sensitive activity against the aspirin methionine amide

Table 1: Results of Biochemical reactions and Sugar fermentation tests.

S.No	Organism	Indole	M.R.	V.P.	Citrate	Oxidase	Coagulase	Glucose	Lactose
1	*E.Coli*	+	+		-	-	-	A/G	A/G
2	*P.fluorecens*	-	-	-	+	+	-	A	-
3	*S.aureus*	-	+	+	-	-	+	A	A

Table 2: Proton NMR study.

	Parameters	X(4) Leu	X(4) Met
Gly (1)	δ NH δ CH_2	8.21 3.2	8.2 3.4
Gly (2)	δ NH δ CH_2	8 3.06	7.8 3.1
Phe (3)	δ NH δ $C^{\alpha}H$ δ CH_2 δ C_6H_5	8 4.5 3.6 7.3	8 5.3 3.4 7.25
– NH_2 – $COCH_3$	δ δ	7.95 2.5	8.4 2.27

The methyl resonance of methionine at 2.04 ppm, the isopropyl resonance of leucine at 4.5 ppm (Bundi et al., 1975).

Table 3: Results of sensitivity pattern of Microorganisms.

Compounds	*E-Coli*	*P. fluorescens*	*S.aureus*
Aspirin	*15*	*15*	*15*
Asp – Leu – NH_2	-	-	15
Asp –Phe - Leu – NH_2	-	-	-
Asp – Gly –Phe - Leu – NH_2	15	-	18
Asp –Gly- Gly- Phe- Leu – NH_2	15	14	18
Asp – Met – NH_2.	22	17	-
Asp – Gly- Met – NH_2	-	-	17
Asp – Gly- Gly- Met – NH_2	17	-	18
Asp –Gly- Gly- Phe- Met – NH_2	-	-	-

with 17 mm and the glycyl-glycyl-phenylalanyl-leucine amides of aspirin showed 14 mm and found to be moderately active whereas the remaining amides were not active. This is shown in plate 1 and figure 1.

S. aureus was highly sensitive to glycyl-phenylalanyl-leucine amides of aspirin, glycyl-glycyl-phenylalanyl-leucine amides of aspirin and glycyl-glycyl-methionine amides of aspirin giving 18 mm of inhibition zone size and leucine amide of aspirin showed 15 mm, glycyl-methionine amides of aspirin showed 17 mm of inhibition of zone whereas the rest of the amides used phenylalanyl-leucine amides of aspirin, glycyl-glycyl-phenylalanyl-methionine amides of aspirin were not active. This is shown in plate 2 and figure 2

E. coli gave a very high sensitivity against methionine amides of aspirin with 22 mm, glycyl-glycyl-methionine amides of aspirin giving 17 mm, but glycyl-glycyl-phenylalanyl-leucine amides of aspirin and glycyl-phenylalanyl-leucine amides of aspirin inferred to be moderately active with 15 mm. This is shown in plate 3 and figure 3.

DISCUSSION

Aspirin has been shown by many authors to possess antimicrobial properties (Wang et al., 2003; Al-Bakri et al., 2009). It was used as the positive control in this study, and its activity was compared to those of its

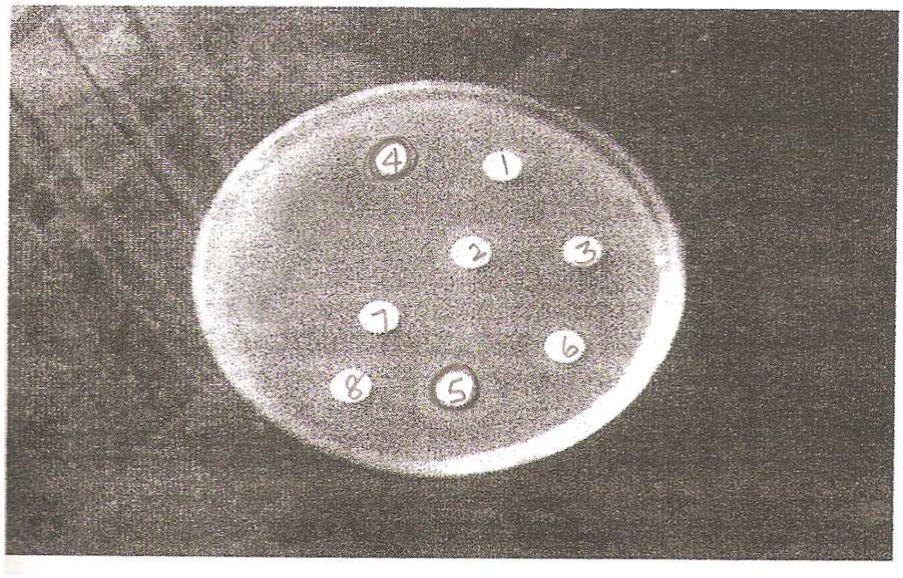

Plate 1: *Pseudomonas fluorescence*

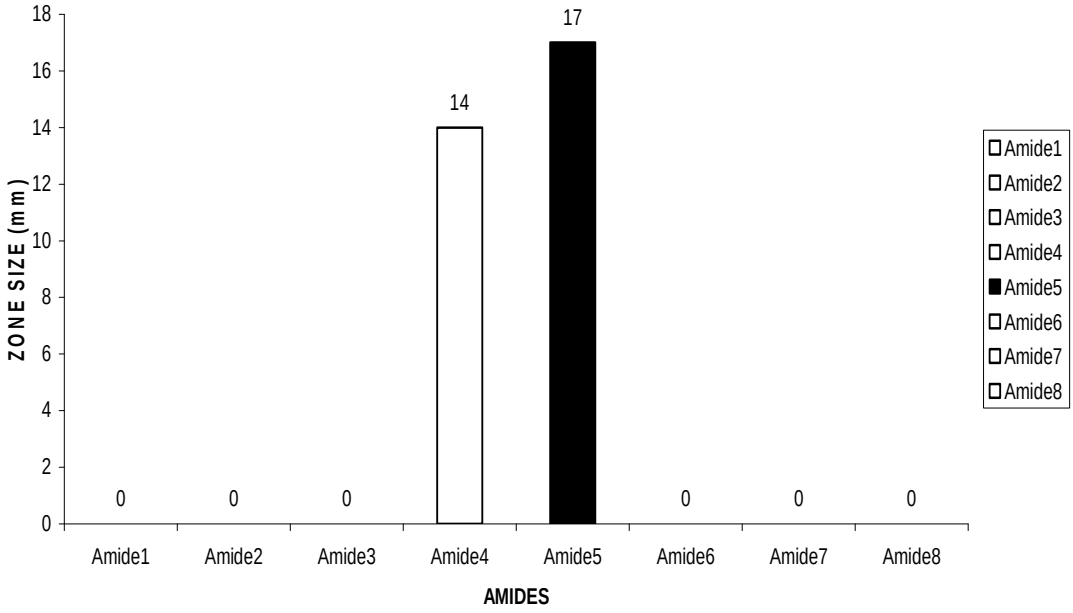

Figure 1: Antibiogram of *Pseudomonas fluorescence*.

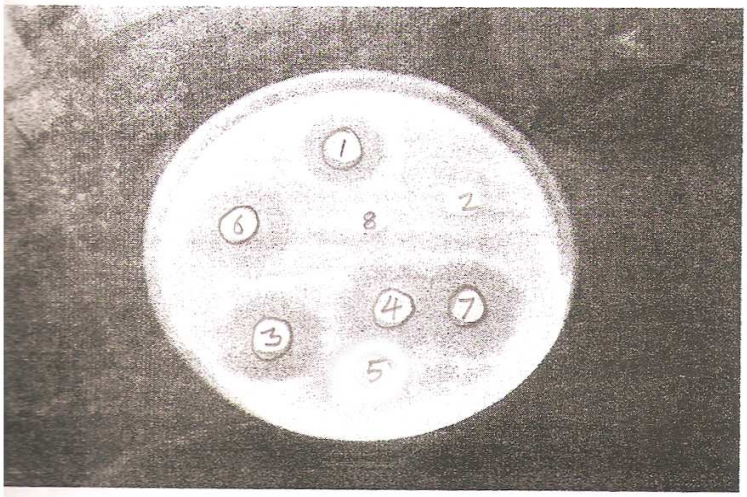

Plate 2 : *Staphylococcus aureus*

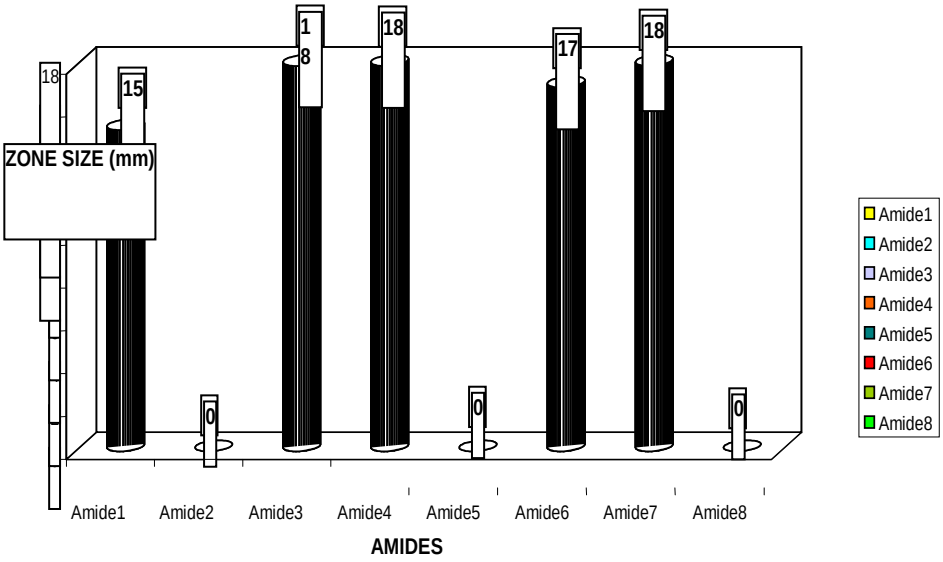

Figure 2: Antibiogram of *Staphylococcus aureus.*

derivatives. The peptide derivatives of aspirin having amide group acted well on the microorganisms used and showed a greater zone of inhibition ranging in size from 14 to 22 mm. On comparing the effects of all the amides used, methionine amides of aspirin were active on *S. aureus.* Glycyl–glycyl-phenylalanyl-leucine amides of aspirin was moderately active on all the three bacterial strains used. Glycyl-Phenylalanyl-leucine amides of aspirin and glycyl-glycyl-methionine amides of aspirin were not active on *P. fluorescens*. Leucine amides of aspirin was moderately active on *S. aureus*.

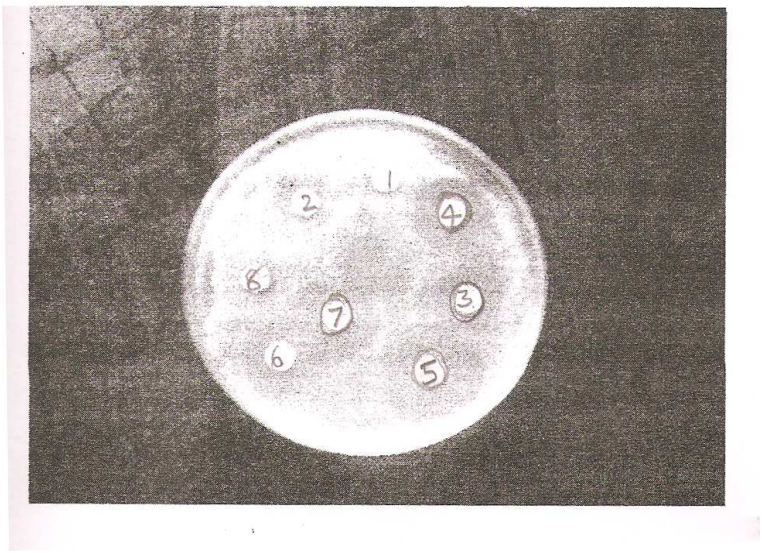

Plate 3: *E. Coli*

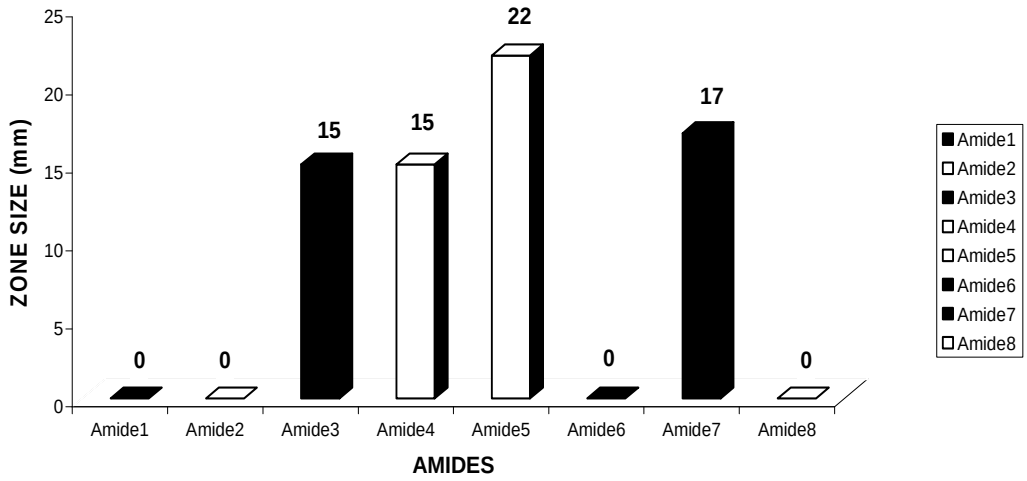

Figure 3: Antibiogram of *E. Coli*

Methionine amides of aspirin were highly active on *E. coli.* These results suggest that the addition of some amide groups to aspirin increases the antimicrobial activity. Phenylalanyl-leucine amides of aspirin and glycyl-glycyl-Phenylalanyl-methionine amide of aspirin were not active on any of the organisms used in this study.

Conclusion

From the results obtained, we can conclude that the derivative of aspirin showed a greater influence in inhibiting the test organisms used.

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