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ULTRASOUND-MEDIATED SYNTHESIS PYRAZINE-2-CARBOXYLAMINO ACIDS AND DIPEPTIDES AS POTENT INSECTICIDAL AND ANTHELMINTIC AGENTS

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ABSTRACT

A series of pyrazine-2-carboxylamino acids and dipeptides were synthesized by solution-phase peptide synthesis by conventional as well as by using an ultrasonic bath-type sonicator. The reaction time was drastically reduced by sonication with good yields. The synthesized compounds were characterized by IR, ^{1}H NMR and Mass spectral analysis and evaluated for anthelmintic and insecticidal activities.

KEYWORDS: Pyrazine, Pyrazine-2-carboxylic acid, Anthelmintic, Insecticidal activity.

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INTRODUCTION

Pyrazines and its derivatives play an important role in the drug discovery realm especially as structural analogues of purines derivatives of pyrazines exhibit various pharmacological activities such as antibacterial[1,2], anti-inflammatory[3] uterine relaxing activity[4], antibronchospastic[5], antiulcer[6], cardiac stimulating[7], antidepressant[8], hypoglycemic activity[9] and antiproliferative activity[10]. Here in, we report the synthesis and evaluation of a series of pyrazine-2-carboxylamino acid and dipeptides as potent antimicrobial agents. A new series of pyrazine-2-carboxylamino acids and dipeptides were synthesized by solution-phase peptide synthesis by conventional (EDC/Et_3N) as well as by using an ultrasonic bath-type sonicator. The reaction time was drastically reduced by sonication with good yields.

MATERIALS AND METHODS

All the reactions requiring anhydrous conditions were conducted in flame dried apparatus. The amino acids used are L-amino acid, except D-alanine, purchased from Spectrochem Private Limited, Mumbai, India. Solvents and reagents were purified by standard methods. Boc-amino acids, amino acid methyl ester hydrochlorides and nitro-arginine were prepared by standard procedures. N-methylvaline was prepared using NaH/CH_3I by Benoiton method[11]. Organic extracts were dried over anhydrous sodium sulphate. Melting points were determined by an open capillary method and are uncorrected. Completion of the reaction and purity of the compounds were checked by thin layer chromatography. IR spectra were recorded on Nicolet impact 400 FT/IR spectrometer using KBr pressed pellet technique. ^{1}H NMR spectra were recorded on GEOL-JMS D–300 (MHz) NMR spectrometer. MASS spectra were recorded on Shimandzu GC-MS (at 70 eV) Mass Spectrometer using xenon as the carrier gas.

Preparation of the Dipeptides (Conventional method)

Amino acid methyl ester hydrochloride (10mmol) was dissolved in chloroform (20ml)**.** To this, triethylamine (0.7ml) was added at 0^{o}C and the reaction mixture was stirred for 15 minutes**.** Boc-amino acid (10mmol) in $CHCl_3$ (20ml) and EDC (Ethyl-3,3-dimethylaminopropylcarbodiimide) (10mmol) were added with stirring**.** After 12 hours, the reaction mixture was filtered to remove the byproduct EDU(ethyl-3,3-diaminopropylurea). The filtrate was washed with 5% $NaHCO_3$ (20ml), 5% HCl (20ml) and distilled H_2O (20ml)**.** The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated in vacuum**.** The residue was purified by recrystallization from $CHCl_3$ and petroleum ether (**Scheme 1**).

Preparation of pyrazine-2-carboxyl-amino acids and peptides(sonication method)

Pyrazine 2-carboxylic acid (10mmol), amino acid methyl ester/dipeptides (10mmol), EDC (10mmol) and $CHCl_3$ (15ml) were added in a Pyrex flask. The mixture was stirred in the water bath of an ultrasonic cleaner and the product formation was monitored by TLC. The reaction was completed after stirring for 11-12 minutes. The reaction mixture was filtered to remove the byproduct EDU and was washed with 5% $NaHCO_3$ (20ml), 5% HCl (20ml) and distilled H_2O (20ml)**.** The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated in vacuum**.** The residue was purified by recrystallization from $CHCl_3$ and petroleum ether (Scheme-1). The ester group was hydrolysed by LiOH by standard method. All the compounds were obtained as semisolid mass (**Table 1**).

Compound-1: Pyrazine-2-carboxyl-glycine: Molecular formula : $C_8H_9N_3O_3$,Molecular weight :195.06 , FT**IR (KBr) in cm^{-1}:** 3243.60 (NH Stretch), 2926.59, (CH Strech, asym.),2865.06 (CH Stretch, sym.),1790.71 (C=O Stretch) **^{1}H NMR (500 MHz, $CDCl_3$):** δ 9.0 (1H, d, Ar-H); 8.8 (1H, d, Ar-H); 8.7 (1H, d, Ar-H); 6.0 (1H, s, N-H); 4.1(1H, s, C-H); 3.7 (3H, s, Me-H) **MASS (in m/z):**195.06(M^+).

Compound-2: Pyrazine-2-carboxyl-valine: Molecular formula :$C_{11}H_{15}N_3O_3$,Molecular weight :237.26 , **FTIR (KBr) in cm^{-1}:** 3301.93 (NH Strech), 3067.41 (aromatic CH stretch), 2926.47 (CH Stretch, asym.), 2865.41 (CH Stretch, sym.), 1661.53 (C=0 Stretch). **^{1}H NMR (500 MHz, $CDCl_3$):** δ 9.0 (1H, s, Ar-H); 8.8

(1H, d, Ar-H); 8.7 (1H, d, Ar-H); 6.0 (1H, s, N-H); 4.2 (1H, m, C-H); 3.8 (3H, m, Me-H). **MASS (in m/z):**237.26 (M^+).

Compound-3: Pyrazine-2-carboxyl-proline: Molecular formula :$C_{11}H_{13}N_3O_3$,Molecular weight :235 , **FTIR (KBr) in cm^{-1}:** 3250.11 (aromatic CH stretch), 2928.21 (CH Strech, asym.), 2866.12 (CH Stretch, sym.), 1700.64 (C=0 Stretch) **^{1}H NMR (500 MHz, $CDCl_3$):** δ 9.0 (1H, , Ar-H); 8.7 (1H, d, Ar-H); 8.6 (1H, d, Ar-H); 6.0 (1H, s, N-H); 4.1(1H, s, C-H); 3.7 (3H, s, Me-H)**.MASS (in m/z):**235 (M^+).
Compound-4:Pyrazine-2-carboxyl-(N-Me-valine): Molecular formula :$C_{12}H_{17}N_3O_3$,Molecular weight :251.29 , **FTIR (KBr) in cm^{-1}:** 3301.93 (NH Strech), 3067.41 (aromatic CH stretch), 2926.47 (CH Stretch, asym.), 2865.41 (CH Stretch, sym.), 1661.53 (C=0 Stretch). **^{1}H NMR (500 MHz, $CDCl_3$):** δ 9.0 (1H, s, Ar-H); 8.8 (1H, d, Ar-H); 8.7 (1H, d, Ar-H); 6.0 (1H, s, N-H); 4.2 (1H, m, C-H); 3.8 (3H, m, Me-H). **MASS (in m/z):**251.29 (M^+).
Compound-5: Pyrazine-2-carboxyl-phenylalanyl-glycine : Molecular formula : $C_{11}H_{13}N_3O_3$, Molecular weight : 235 , **FTIR (KBr) in cm^{-1}:** 3281.99 (NH Strech), 2924.10 (CH Stretch, asym.), 2854.42 (CH Stretch, sym.), 1371.69 (aliphatic CH in plane bending), 1671.24 (C=0 Stretch).**^{1}H NMR (500 MHz, $CDCl_3$):** δ 9.0 (1H, s, Ar-H); 7.3(5H, m, Ar-H), 8.8 (1H, d, Ar-H); 8.7 (1H, d, Ar-H); 6.0 (1H, s, N-H); 4.1(1H, m, C-H); 3.7 (3H, s, Me-H). **MASS (in m/z):**235 (M^+).

Evaluation of Insecticidal activity

Insecticidal activity test of the title compounds was carried out as per the procedure of Hu et al[12] with modifications. 100mg of the test sample was dissolved in 4ml of acetone and was spread over a whatmann filter paper of 55mm diameter. It was allowed to dry in open air to give a uniform layer of the test compound on the filter paper. It was then placed in the petri dish base with the same inner diameter. The lid of the petri dish was stuffed with a thin layer of wet cotton. 6 nos of termites (*Coptotermes formosanus*) were placed in each petri plate and were covered with the lid containing wet cotton. A Standard drug (chloropyrifos) and a control were kept in a similar manner. Time was noted soon after introduction of the termites in the petri plates (**Table-2**).

Evaluation of Anthelmintic activity

Anthelmintic activity was carried out aganist earthworms (*Eudrilus sp.)* by Garg's method[13]. Suspensions of the samples were prepared by triturating the samples with 0.5% tween 80 and distilled water and the resultant mixtures were stirred using a mechanical stirrer for 30 mins. The resulting suspensions were used for the activity studies. The suspensions were diluted to contain 100 mg in 5ml of the test samples. Standard drugs, Mebendazole was also prepared with the same concentration in a similar way (**Table 3).**

RESULTS AND DISCUSSION

The dipeptides were conveniently synthesized by EDC/Et_3N method with less reaction time and good yields. The title compounds could be synthesized by sonication within 11-12 mins reaction time and with significant yields. The compounds did not show antimicrobial activity but exhibited potent anthelmintic and insecticidal activities. Pyrazine-2-carboxylproline (P3) has shown potent anthelmintic and insecticidal activities probably due to the presence of pyrrolidine moiety in addition to pyrazine in the molecule.

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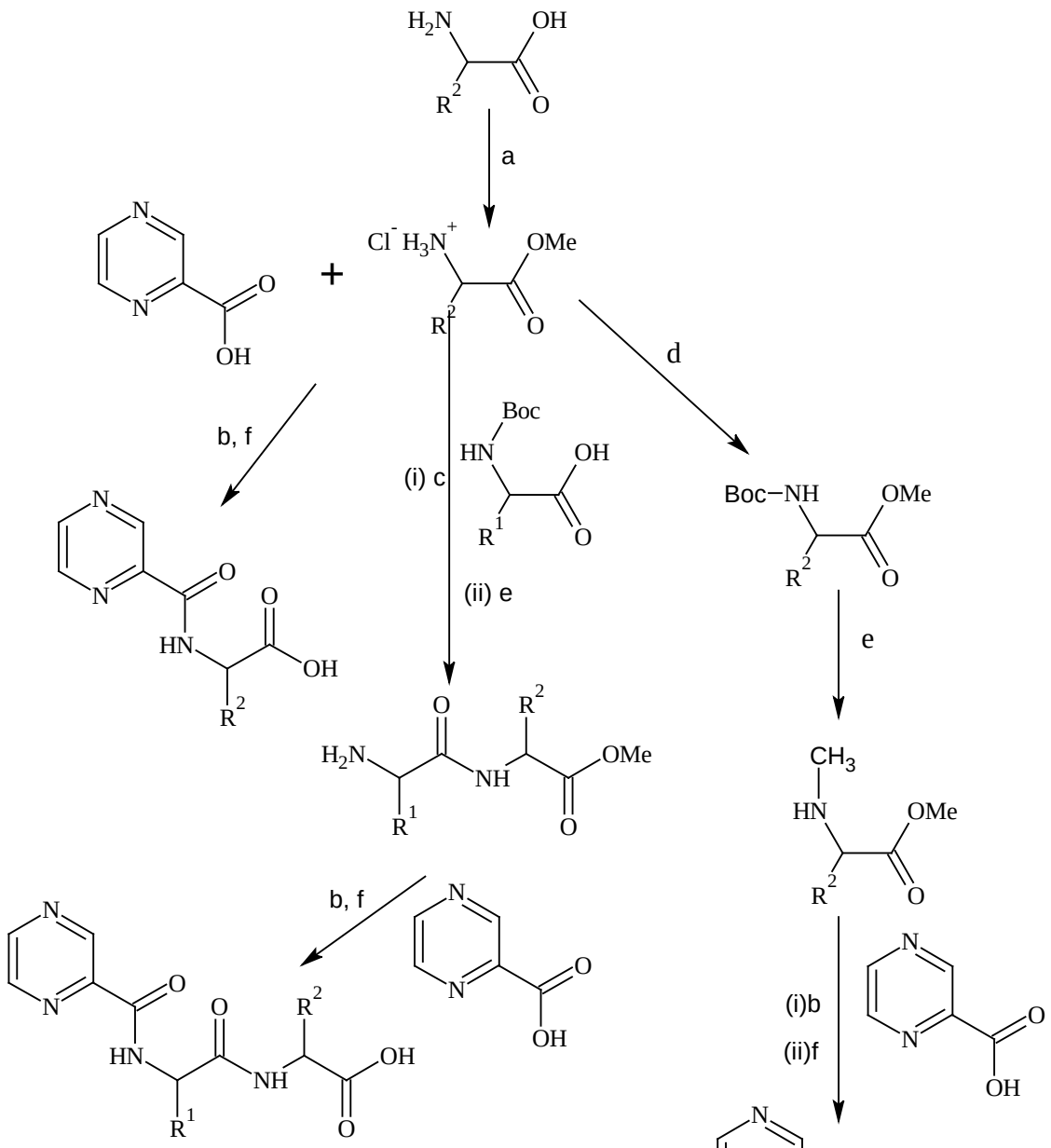

Side chains of amino acids

R^2 = side chain of Gly (P1), Val(P2), Pro(P3), N-Me-Val(P4)

R^1, R^2 = side chains of Phe-Val (P5)

SCHEME-1

S.No	Compound code	Amino acid/peptide	Reaction Time	% Yield
1.	P1	Gly	11	95
2.	P2	Val	11	93
3.	P3	Pro	12	92
4.	P4	(N-Me)Val	12	95
5.	P5	Phe-Val	11	93

Table 1: Physical Data of Pyrazole-2-carboxylamino acids/dipeptides

S.No	Compound code	Amino acid/peptide	Conc. (mg)	Mean death time(min)
1.	P1	Gly	100	92
2.	P2	Val	100	87
3.	P3	Pro	100	76
4.	P4	(N-Me)Val	100	89
5.	P5	Phe-Val	100	79
6.	(Chloropyrifos)	-	100	80
	CONTROL	-	Nil	No effect

Table 2: Insecticidal Activity of Pyrazole-2-carboxylamino acids/dipeptides

Sl.No	Compound code	Amino acid/peptide	Conc. (mg)	Mean paralyzing time (min)	Mean death time(min)
1.	P1	Gly	100	10	13
2.	P2	Val	100	9	12
3.	P3	Pro	100	7	10
4.	P4	(N-Me)Val	100	9	11
5.	P5	Phe-Val	100	8	12
6.	(Mebendazole)	-	100	5	8
7.	CONTROL	-	Nil	No effect	No effect

Table 3: Anthelmintic Activity of Pyrazole-2-carboxylamino acids/dipeptides

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