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Synthesis, Antimicrobial and Anthelmintic Activity of 2-Amino-4-phenylthiazole Derivatives of Amino Acids and Peptides

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A novel series of 2-amino-4-phenylthiazole derivatives of amino acids and peptides were synthesized by solution phase technique. The synthesized compounds were characterized by FTIR, ^{1}H NMR and mass spectral analysis and evaluated for their antibacterial, antifungal and anthelmintic activities. The compounds exhibited significant antifungal and anthelmintic activities as compared to standard drugs fluconazole and mebendazole, respectively.

Key Words: Thiazole, Peptides, Antibacterial, Antifungal, Anthelmintic activities.

INTRODUCTION

Thiazoles and their derivatives are found to be associated with various biological activities such as antibacterial[1,2], antifungal[3], antiviral[4], antiinflammatory[5], antipyretic[6], antitubercular[7], analgesic[8], anticonvulsant and tranquilizer activities[9]. In view of the diverse biological activities associated with thiazoles, we wish to report the synthesis and antimicrobial activity of amino acids and peptides incorporated with 2-amino-4-phenylthiazole.

The molecule 2-amino-4-phenylthiazole derivatives of amino acids and peptides were synthesized by using DCC/Et_3N mediated solution phase technique of peptide synthesis. The amino group was protected with tertiary butyloxycarbonyl (Boc-) group and the carboxyl group was protected by esterification process. The Boc-amino acids were coupled with amino acid methyl ester hydrochlorides by dicyclohexylcarbodiimide (DCC) as a coupling agent and triethylamine (Et_3N) as a base to get protected dipeptides. The starting material 2-amino-4-phenylthiazole was prepared by condensation of acetophenone with thiourea in presence of iodine. The 2-amino-4-phenylthiazole was coupled with Boc-dipeptides using DCC to get 2-amino-4-phenylthiazole derivatives of amino acid and dipeptide followed by hydrolysis of Boc- group with trifluoroacetic acid.

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EXPERIMENTAL

All the reactions requiring anhydrous conditions were conducted in a flame dried apparatus. Melting points were determined by the open capillary method and were uncorrected. L-Amino acids, *tert*-butyloxycarbonyl (Boc), dicyclohexylcarbodiimide (DCC), trifluoroacetic acid (TFA) and triethyl amine (Et_3N) were obtained from Spectrochem Limited (India). IR spectra were recorded using KBr disc method on Thermo Nicolet 330 FTIR spectrometer. ^1H NMR spectra were recorded on Jeol-JMS D-300 (MHz) spectrometer. Chemical shifts were reported in ppm down field from tetramethyl silane as internal standard. The FAB-MASS spectra were recorded on a Jeol SX 102/DA-6000 mass spectrometer using xenon as the carrier gas. Purity of all compounds was checked by TLC on precoated silica gel G plates (Kiesel gel 0.25 mm, 60G F_{254}, Merck, Germany). Chloroform/methanol (9:1, v/v) was used as the developing solvent system and dark brown spots were detected on exposure to iodine vapours in a tightly closed chamber.

Preparation of 2-amino-4-phenylthiazole (1): A mixture of acetophenone (12 g, 0.1 mol), thiourea (15.22 g, 0.2 mol) and iodine (25.33 g, 0.1 mol) was taken in a round bottom flask and refluxed for 12 h. The reaction mixture was cooled and washed with diethyl ether to remove the excess of unreacted acetophenone and iodine. The reaction mixture was allowed to cool at room temperature and poured into the solution of ammonium hydroxide. The crude product obtained was recrystallized by methanol.

Preparation of dipeptides (2): Amino acid methyl ester hydrochloride (10 mmol) was dissolved in chloroform (20 mL). To this, triethylamine (4 mL, 28.7 mmol) was added at 0 ºC and the reaction mixture was stirred for 15 min. Boc-amino acid (10 mmol) in $CHCl_3$ (20 mL) and DCC (10 mmol) were added with stirring. After 12 h, the reaction mixture was filtered and the residue was washed with $CHCl_3$ (30 mL) and added to the filtrate. The filtrate was washed with 5 % $NaHCO_3$ (20 mL) and saturated NaCl (20 mL) solutions. The organic layer was filtered and evaporated in vacuum. To remove the traces of the dicyclohexyl urea, the product was dissolved in minimum amount of chloroform and cooled to 0 ºC. The crystallized dicyclohexyl urea was removed by filtration. Petroleum ether was added to the filtrate at 0 ºC to recrystallize the pure product.

Preparation of 2-amino-4-phenylthiazole of peptides (3): 2-amino-4-phenylthiazole (1.76 g, 10 mmol) was dissolved in chloroform (20 mL). To this, triethylamine (4 mL, 28.7 mmol) was added at 0 ºC and the reaction mixture was stirred for 15 min. Boc-amino acid (10 mmol) in $CHCl_3$ (20 mL) and DCC (10 mmol) were added with stirring. After 12 h, the reaction mixture was filtered and the residue was washed with $CHCl_3$ (30 mL) and added to the filtrate. The filtrate was washed with 5 % $NaHCO_3$ (20 mL) and saturated NaCl (20 mL) solutions. The organic layer was filtered and evaporated in vacuum. To remove the traces of the dicyclohexyl urea, the product was dissolved in minimum amount of chloroform and cooled to 0 ºC. The crystallized dicyclohexyl urea was removed by filtration. Petroleum ether was added to the filtrate at 0 ºC to recrystallize the pure product.

2-(Alanyl)-amino-4-phenylthiazole (a): Brown sticky mass, % yield: 60, m.f. $C_{12}H_{13}N_3OS$, m.w. 247, FTIR (KBr, ν_{max}, cm^{-1}): 3293.5 (-NH), 3015 (-CH aromatic), 2931 (-CH aliphatic), 2885.4 (-CH) of thiazole, 1693.4 (-CO). ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: δ 8.0 (1H, s, -NH) δ 7.9-7.4 (5H, m, aromatic-H), δ 6.2 (1H, s, -CH of thiazole) δ 4.9 (1H, m, α-H) δ 1.7 (3H, m, CH_3). Mass m/z: $[M + 1]^+$ 248.

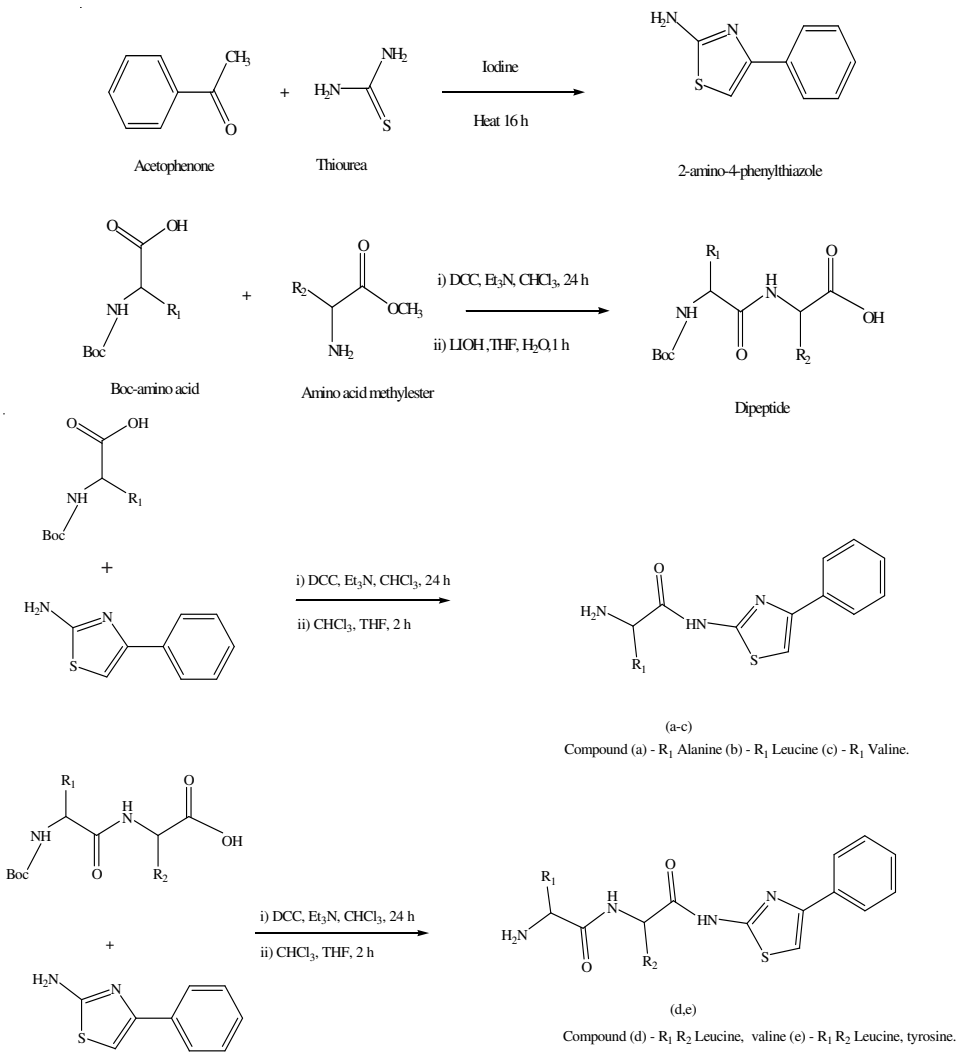
2-(Valyl)-amino-4-phenylthiazole (b): Brown sticky mass, % yield: 70, m.f. $C_{14}H_{17}N_3OS$, m.w. 275, FTIR (KBr, ν_{max}, cm^{-1}): 3292.1 (-NH), 3014 (-CH aromatic), 2931.1 (-CH aliphatic), 2887 (-CH) of thiazole, 1628.9 (-CO). ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: δ 8.0 (1H, s, -NH), δ 7.7-7.3 (5H, δ, aromatic-H), δ 6.2 (1H, s, -CH of thiazole), δ 4.6 (1H, m, α-H), δ 1.2 (1H, m, α-H) δ 1.01(6H, d, CH_3). Mass m/z: $[M + 1]^+$ 278.

2-(Leucyl)-amino-4-phenylthiazole (c): Brown sticky mass, % yield: 66, m.f. $C_{15}H_{19}N_3OS$, m.w. 289, FTIR (KBr, ν_{max}, cm^{-1}): 3305.5 (-NH), 3014 (-CH aromatic), 2930.9 (-CH aliphatic), 2870.1 (-CH aliphatic) 2886 (-CH) of thiazole, 1694 (-CO). ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: δ 7.9 (1H, s,-NH), δ 7.7 (1H, m, aromatic-H), δ 7.4 (2H, m, aromatic-H), δ 6.9 (2H, m, aromatic-H), δ 6.2 (1H, s, -CH of thiazole), δ 4.8 (1H, m, α-H), δ 1.4 (2H, d, $-CH_2$ of leucine), δ 1.2 (1H, m, α-H) δ 1.02 (6H, d, CH_3 of leucine). Mass m/z: M^+ 289.

2-(Leucyl-valyl)-amino-4-phenylthiazole (d): Brown sticky mass, % yield: 67.3, m.f. $C_{20}H_{28}N_4O_2S$, m.w. 388, FTIR (KBr, ν_{max}, cm^{-1}): 3338.9 (-NH), 3110 (-CH), 2967.1 (-CH), 2887.6 (-CH) of thiazole, 1666.5 (-CO). ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: δ 7.8 (1H, m, -NH), δ 7.5-7.3 (5H, m, aromatic-H), δ 6.7 (1H, s, aromatic-H), δ 6.2 (1H, s, -CH of thiazole), δ 4.4-4.3 (1H, m, α-H) δ 3.9-3.7 (1H, m, α-H) δ 2.9 (1H, m, β-H of valine) δ 1.7 (3H, β-H of leu and valine) δ 1.2-0.95 (13H, m, $-CH_3$ groups and γ-H of leu). Mass m/z: M^+ 388.

2-(Leucyl-tyrosyl)-amino-4-phenylthiazole (e): Brown sticky mass, % yield: 64, m.f. $C_{24}H_{28}N_4O_3S$, m.w. 452, FTIR (KBr, ν_{max}, cm^{-1}): 3256.3 ($-NH_2$), 3110 (-CH), 2962.1 (-CH), 2933.9 (-NH), 2874 (-CH) of thiazole, 1666.7 (-CO). ^{1}H NMR (300 MHz, $CDCl_3$) δ in ppm: δ 8.0 (1H, s, -NH), δ 7.8-6.5 (10H, m, aromatic-H), δ 6.1 (1H, s, -CH of thiazole) δ 4.2 (1H, m, α-H), δ 4.0 (1H, m, α-H), δ 3.8 (2H, m, β-CH_2 of tyrosine), δ 1.85 (2H, m, β-CH_2 of leu). Mass m/z: $[M + 1]^+$ 453.

Antimicrobial activity: The antimicrobial activity was determined using disc diffusion method by measuring the inhibition zone in mm. All the synthesized compounds were evaluated *in vitro* for their antibacterial and antifungal activities. The compounds were tested at a concentration of 50 μg/mL against bacterial strains (*Staphylococcus aureus*, *Bacillus substilis*, *Pseudomonas aeruginosa* and *Escherichia coli*) and the fungal strains (*Asperigillus flavus*, *Asperigillus fumigates* and *Candida albicans*) by disc diffusion method, respectively[10]. Ampicillin and fluconazole were served as standard drugs for comparison of the results. The culture media used were nutrient agar and sabourd's medium for bacteria and fungus strains, respectively. The results are presented in Table-1.



Scheme-I

TABLE-1
RESULTS OF ANTIMICROBIAL ACTIVITY

Comp. code	Diameter of zone of inhibition (mm)						
	B. subtilis	*S. aureus*	*E. coli*	*P. aeuruginosa*	*C. albicans*	A. flavus	A. fumigatus
a	–	10	05	–	15	13	14
b	10	08	08	09	14	14	15
c	07	12	10	07	13	12	13
d	08	09	08	07	13	15	12
e	11	13	11	10	10	14	11
Ampicillin	16	22	14	15	–	–	–
Fluconazole	–	–	–	–	16	17	17

(– indicates no activity).

Anthelmintic activity: The synthesized compounds were evaluated for their anthelmintic activity against *Eudrilus eugenia* by Garg's and Atal method[11] using mebendazole as a standard drug. All the synthesized compounds were found to be potent anthelmintics. The results are given in Table-2.

TABLE-2
RESULTS OF ANTHELMINTIC ACTIVITY

Compound code	Conc. of the compound (mg)	Mean paralyzing time (min) + SE	Mean death time (min) + SE
a	100	5.50 ± 0.34	7.15 ± 0.30
b	100	5.40 ± 0.25	6.30 ± 0.22
c	100	5.55 ± 0.28	7.20 ± 0.26
d	100	6.20 ± 0.41	7.40 ± 0.30
e	100	5.20 ± 0.28	6.45 ± 0.30
Control	–	–	–
Mebendazole	100	6.20 ± 0.23	7.45 ± 0.32

RESULTS AND DISCUSSION

A new series of 2-amino-4-phenylthiazole derivatives of amino acids and peptides was synthesized with good yields and the structures were confirmed by IR, ^{1}H NMR and mass spectral data. The synthesized compounds have shown good antimicrobial activities as compared to their standard drugs but the antifungal activity is more prominent. The leucine containing amino acids/dipeptide has shown potent antimicrobial activity compared to the alanine and valine containing compounds.

The increased activity of the compounds may be assumed due to the change in the hydrogen bond formation and increased liphophilic character of the molecule which enhances the permeability of the molecule into the bacteria and fungi. All the synthesized compounds have shown good anthelmintic activity against earthworm, *Eudrilus eugenia* strain as compared to the standard drug mebendazole. On passing toxicity tests, these compounds may prove to be good candidates for clinical studies and potential antimicrobial and anthelmintic agents of future.

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