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SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME N-METHYLATED DERIVATIVES OF THIAZOLYLAMINO ACIDS AND PEPTIDES

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ABSTRACT
A series of substituted thiazole containing N-methylated amino acids and peptides were synthesized by solution phase technique and were subjected to evaluation of biological activities. In this project 2-amino-4-phenylthiazole is synthesized and then coupled with protected amino acid. In case of dipeptide, protected amino acids are condensed together to get dipeptide using DIPC (di-isopropylcarbodiimide) and then dipeptide is condensed to 2-amino-4-phenyl thiazole using coupling agent to get the final compound. Synthesized compounds are characterized and anthelmintic and insecticidal activity studies were performed.
Keywords: Solution Phase Technique, Thiazole, Linear Peptides, Anthelmintic, Insecticidal.

INTRODUCTION

Thiazole has drawn attention of many researchers because thiazole and its derivatives are constituents of many biomolecules including antibiotics, vitamins, penicillin and sulfathiazole. Thiazole is used for manufacturing biocides, fungicides, pharmaceuticals, and dyes.

A study of literature showed that thiazole & its derivatives possess variety of biological activities such as antifungal[1], anti-inflammatory & antibacterials[2], anthelmintic, antitubercular, anticancer, algicidal, and analgesic activity. Benzo[d]-isothiazole and benzothiazole derivatives has shown antiviral, antimicrobial & antiproliferatives[3] activities.

Combination of a relative small number of amino acids is the strategy used by nature to construct peptides, which are most variable and adaptable molecules of life. Peptides functions as hormones, enzymes, enzyme inhibitors or substrate, growth factors, neurotransmitters & immunomodulators[4]. Their extreme variability makes them suitable mediators of specific molecular interactions. An interaction between peptides or peptides and other molecules controls all the physiological or biological & pathological phenomena of life. This characteristic feature allows bioactive peptides to act as therapeutic agents and is used to design peptide drugs[5]. Bacteriocins[6] are small peptide molecules which have drawn attention due to its antimicrobial activity and other medicinal applications.

Many naturally occurring thiazole peptides such as GE2270A[7], Nocathiacins[8], micrococcin P[9], thiostrepton[10], nosiheptide[11], and GE37468 A[12] have been found to be associated with diverse biological activities. Most of the thiopeptide antibiotics inhibit protein synthesis in bacteria, and share a common mode of action. They act by inhibiting the action of GTP-dependant elongation factors. The thiopeptides have been demonstrated to be active against Gram-positive bacteria and anaerobes, including pathogens resistant to antibiotics currently in use, and micrococcin is a potent growth inhibitor of the human malarial parasite *Plasmodium falciparum*[13].

N-methylated amino acids are commonly found in natural occurring peptide antibiotics. The N-methylated peptide antibiotics are found to possess enhanced activity[14] compared to the unmethylated forms because, the hydrogen bonding pattern in N-methylated peptides is different from that of the unmethylated peptides.

Therefore, an attempt is made to synthesize new series thiazole derivatives of N-methylated amino acids and peptides by condensation of peptides to the thiazole ring system and study its biological activity.

MATERIALS AND METHOD

All the reactions carried out in hot air oven dried apparatus. All the reactions were magnetically stirred manufactured by REMI. Organic extracts dried over anhydrous sodium sulphate. Melting points were determined by capillary method. Amino acids, di-tert-butylpyrocarbonate, Trifluoroacetic acid, Di-isopropylcarbodiimide, N-methyl morpholine, methyl iodide and solvents were obtained from Spectrochem Ltd. Mumbai, Nice chemicals Kochi, Himedia, Qualigens. IR spectra recorded on Avatar Thermo Nicolett-400 FT-IR-spectrometer using a thin film support on KBr pellets for solids and chloroform as a solvent for semisolids. Boc-amino acids, amino acid methyl ester hydrochlorides were prepared by standard procedures[15]. N-methylated amino acids were prepared using NaH/CH_3I by Bentoin method[16].The values are reported as V_{max} (cm^{-1}). ^1H NMR spectra were recorded on GEOL-JMS D-300 (MHz) NMR spectrometer. The spectra were obtained in $CDCl_3$ and the chemical shift values are reported as values in ppm relative to TMS (δ=0) as internal standard. The ES spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer. The spectra were recorded at room temperature.

General Procedure for the Synthesis of of 2-amino-4-Phenylthiazole:

Although various methods are available for the synthesis of amino thiazoles we followed the method[15] in which acetophenone is treated with thiourea in presence of iodine. A mixture consisting of acetophenone (0.2mol, 24g), thiourea (0.4mol, 30.44g) and Iodine (0.2mol, 50.76g) was heated overnight on electric mantle. The crude reaction mixture was cooled to RT; the semisolid mass was then washed with diethyl ether to remove unreacted acetophenone and iodine. The residue was then dissolved in boiling water and filtered to remove sulphur. Then the solution was cooled to RT and made basic with ammonium hydroxide. The

aminophenylthiazole which separated was recrystallized from alcohol.

Preparation of amino acid methyl ester:

Thionyl chloride (0.7ml, 10mmol) was added drop wise to methanol (50ml) with constant stirring slowly at 0°C and the amino acid (10mmol) was added to this solution and the solution was refluxed for 8-10 hrs. The solvent was distilled off to give the residue of amino acid methyl ester hydrochloride which was washed with ether at 0°C to remove excess dimethyl sulphite. The resulting solid was recrystallized from methanol and diethyl ether at 0°C.

Preparation of Boc-amino acid:

The amino acid (10mmol) was dissolved in 1N NaOH (10ml) and isopropanol (10ml). Di-tert-butylpyrocarbonate $(Boc)_2O$ (13 mmol, 3ml) in isopropanol (5ml) was added followed by 1N NaOH (10ml) to the resulting solution. The solution was stirred at room temperature for 2 hr.

After stirring it was washed with light (40-60 °C) petroleum ether (20ml), and acidified to pH 3.0 with 2N H_2SO_4 and finally extracted with chloroform (3x20ml).

The organic layer was dried over anhydrous sodium sulphate and the organic layer was evaporated over water bath to give Boc-amino acid. The crude product was recrystallized from chloroform.

Preparation of Boc-amino acid methyl ester from amino acid methyl ester:

Amino acid methyl ester (10 mmol) dissolved in chloroform (30ml) was taken, to this NMM (21mmol 2.3ml) and $(Boc)_2O$ (13mmol 3ml) was added. The solution was stirred for 2hr at room temperature. The stirred solution was diluted with diethyl ether (50ml). The solution was washed with 10% $NaHCO_3$ followed by 5% HCl and water. The organic layer was separated and was dried over anhydrous sodium sulphate, and concentrated to give the product.

Preparation of Boc-(N-Me) amino acid methyl ester from Boc-amino acid methyl ester:

Boc-amino acid ester (5mmol) was dissolved in DMF (30ml), to this NaH (15mmol) was added at room temperature. Sodium hydride was first activated by washing with sodium-dried ether. (The ether was then decanted into a dry container containing methanol and then discarded). The activated NaH was then transferred into the reaction mixture, followed by MeI (8.1ml 40mmol).

The solution was stirred for 4hr and then diluted with diethyl ether. The solution was washed with saturated NH_4Cl (20ml), 20% $Na_2S_2O_3$ (20ml) and water. The organic layer was dried with anhydrous Na_2SO_4 and concentrated to give viscous oil[16].

Preparation of (N-Me) dipeptides:

(N-Me) amino acid ester (10mmol) dissolved in chloroform (20ml), to this NMM (21mmol 2.3ml) was added and reaction mixture was stirred for 15min at RT. To the resulting solution Boc-amino acid (10mmol) dissolved in chloroform (20ml) and di-isopropylcarbodiimide (10mmol 1.5ml) were added.

The solution was stirred for 24 hr and the reaction mixture was washed with 5% $NaHCO_3$ (20ml), 5% HCl and water. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated on water bath. The residue was purified by recrystallization from $CHCl_3$ at 0 °C.

Deprotection of the carboxylic group:

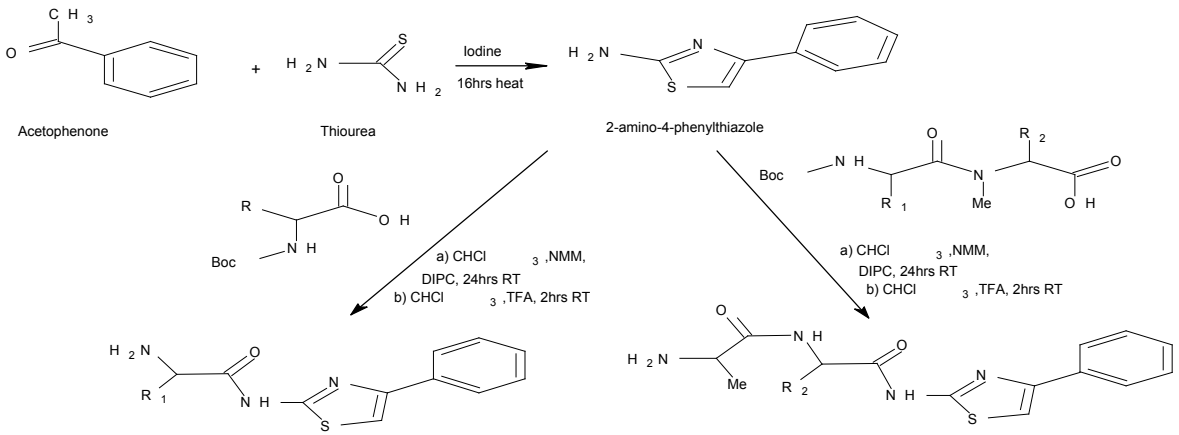
To the protected peptide / amino acid (5mmol), (1:1) THF: H_2O (20ml) and LiOH (7.5mmol .31g) was added. The mixture was refluxed for 15-20min and then acidified to pH 3.5 with 1N H_2SO_4. The aqueous layer was extracted with solvent $CHCl_3$ (3x15ml). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated over water bath.

Deprotection of the amino group:

Boc protected peptide (5mmol) was dissolved in $CHCl_3$ (15ml) and treated with TFA (10mmol, 0.8ml). The solution was stirred at room temperature for 2 hr, then washed with saturated $NaHCO_3$ (20ml) solution and water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The product was recrystallized from $CHCl_3$ at 0 °C.

Synthesis of 2-amino-4-Phenylthiazole derivatives of peptides:

2-Amino-4-phenylthiazole (5mmol) was dissolved in chloroform (20ml). To this, NMM (10.5mmol, 1.15ml) was added and the reaction mixture was stirred for 15 min. Boc-amino acids / peptides (5mmol) in chloroform (20ml) and DIPC (5mmol 0.8ml) were added and the solution was stirred for 24hr at RT. After stirring, the resulting solution was washed with 5% $NaHCO_3$ (20ml) and distilled water. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated over water bath. The residue was purified by recrystallization from $CHCl_3$ at 0°C. The above residue was dissolved in $CHCl_3$ (15ml) and TFA was added. The reaction mixture was stirred for 2hr at room temperature and washed with saturated $NaHCO_3$ (20ml) solution and water. The organic layer was dried over anhydrous Na_2SO_4 and was concentrated to give the product. The overall synthesis is mentioned in scheme 1.



R_1 and R_2 are the side chain of amino acids

Scheme 1

Anthelmintic Activity

Anthelmintic activity studies were carried out against earthworms (Eudrilus *eugeniea)* by Garg's method[17]. Suspensions of the samples were prepared by mixing the samples with 15% tween 80 and distilled water and the resultant mixtures were stirred using a stirrer for 30 minutes. The resulting suspensions were used for the activity studies. Standard drug (Mebendazole) was also prepared with the same concentration in a similar way. An earthworm was placed in a beaker containing 20ml of suspension of the standard drug (Mebendazole) at RT. An earthworm was kept as control in 20ml suspension of distilled water and 15% tween 80. 20ml each of the suspensions of the test compounds were added into separate beaker containing an earthworm in each. The time required for the paralysis and death of the worms was noted. The death time was ascertained by placing the earthworm in warm water at 50°C, which stimulated the movement if the worm was alive.

Insecticidal Activity

Insecticidal activity studies of the synthesized compounds were carried out against termites (Coptotermis *formasanus*) by Morita et al., method[18]. Whatmann filter paper was cut in accordance to the inner diameter of the petri plate, 100mg of compounds was dissolved in chloroform and were poured on the filter paper fitted in different petri plates. Standard drug (chloropyrifos) was also prepared in similar way. For control only the solvent was poured on filter paper in plate. Termites (about 5 nos) were placed on each of the petri plate and covered with the lid, with wet absorbent cotton attached to the upper lid of the plate. Set up was kept undisturbed and the death time was noted.

RESULTS AND DISCUSSION

The results of all the compounds along with physical properties have been shown in the Table 1. The spectral data of 2-amino-4-phenylthiazole derivatives of amino acid and peptides synthesized by the above described method are as follows.

Spectral Data:

Compound-I: IR (KBr Pallets): 3293.5 (NH stretch), 3015 (Ar CH stretch), 2931 (aliphatic CH), 1693.4 (C=O stretch) cm^{-1}. **^{1}H NMR (300 MHz, $CDCl_3$):** δ 7.9-7.4 (7H, m, Ar -H, -NH), δ 4.9 (1H, m, α-H), δ 1.7 (3H, m, $-CH_3$), δ 1.4(9H, s, t-Bu). **FAB Mass: m/z:** 348 (M+1).

Compound-III: IR (KBr Pallets): 3277 (NH stretch), 3064 (Ar CH stretch), 2930 (aliphatic CH), 1692 (C=O stretch) cm^{-1}. **^{1}H NMR (300 MHz, $CDCl_3$):** δ 8.2-7.1 (12H, m, Ar -H, -NH), δ 4.8 (1H, m, α-H), δ 3.2-2.9 (6H, m, β-H, N-CH_3), δ 2.7 (2H, m, $-NH_2$), δ 1.3 (3H, m, $-CH_3$). **FAB Mass: m/z:** 409 (M+1).

The results of Anthelmintic activity against *Eudrilus eugeniea* are shown in Table 2 and Insecticidal activity against Coptotermis *formasanus* are shown in Table 3.

CONCLUSION

All the synthesized compounds (1-4) were characterized by FT-IR, ^{1}H NMR, FAB-MASS spectral studies. All the 4 molecules were showing good docking scores and antitubercular activity when compare with the standard molecules.

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Table 1: Physical data of 2-amino-4-phenylthiazole derivatives of peptides

Sl No.	Product Name	Physical State	% Yield
1.	2-(D-alanyl)amino-4-phenyl thiazole	Light yellow sticky mass	90
2.	2-[leucyl-(N-Me)phenylalanyl] amino-4-phenylthiazole	Yellow sticky mass	80
3.	2-[D-alanyl-(N-Me)phenylalanyl] amino-4-phenylthiazole	Light brown sticky mass	91
4.	2-[(N-Me)Isoleucyl]amino-4-phenylthiazole	Yellow Pasty mass	77
5.	2-[ß-alanyl-(N-Me)Isoleucyl] amino-4-phenylthiazole	Brown pasty mass	62

Table 2: Anthelmintic activity of synthesized compounds

Sl No.	Compound	Conc. of Compound (100mg/20ml)	Paralyzing Time(mins)	Death Time (mins)
1	Compound I	100	60	70
2	Compound II	100	50	55
3	Compound III	100	45	50
4	Compound IV	100	55	60
5	Compound V	100	70	75
6	Control	-	No Effect	No Effect
7	Mebendazole	100	55	60

Table 3: Insecticidal activity of synthesized compounds

Sl No.	Compound	Conc. of Compound (100mg/2ml)	Death Time (Hr)
1	Compound I	100	2:50
2	Compound II	100	2:10
3	Compound III	100	1:45
4	Compound IV	100	2:30
5	Compound V	100	3:00
6	Chloropyrifos	100	2:40

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