Synthesis and Biological Evaluation of 2-Aminothiazole Analogues of N^{α}-Protected Amino Acids

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ABSTRACT: *Synthesis of 2-amino-thiazoles from N^{α}-protected bromomethyl ketones obtained from N^{α}-protected diazomethyl ketones has been reported in this study. N-protected amino acids were converted to diazomethylketones using (Benzotriazol-1-yloxy) tris (dimethylamino) phosphonium hexafluorophosphate (BOP) as a carboxylic acid activator and N-nitroso-N-methyl urea (NMU) as diazomethane source. Thus, prepared diazomethylketones were treated with aqueous HBr to get bromomethyl ketones in high yields. The 2-amino-thiazoles of protected-amino acids were prepared by sonicating the bromomethyl ketones with thiourea in acetone, using Hantzsch's procedure. The products were obtained in good yields and were fully characterized. The purity of the synthesized compounds was analyzed by collecting the RP-HPLC data for two sets of compounds. Kirby Bauer well diffusion technique was employed to test the antibacterial activity of the compounds, Boc-Arg-thiazole, Cbz-Asp-thiazole, Cbz-Trp-thiazole, Fmoc-Phe-thiazole, and Fmoc-Trp-thiazole. The test leads to the promising activity with Streptomycin sulfate as standard and the compound Fmoc-Phe-thiazole was susceptible to Staphylococcus aureus.*

KEYWORDS: *Diazomethylketone; Bromomethylketone; 2-Amino-thiazole; Antioxidant property; Antibacterial property.*

INTRODUCTION

The nuclei of aromatic heterocyclic compounds such as *1H*-1,2,4-triazole[1], *1H*-tetrazoles[2], 1,3,4 oxadiazole [3] and 1,3-thiazole[4] show biological importance of essential materials (pharmacophores) and gained attention due to their unique properties. The structural features embedded in the heteroatom containing *in-vivo* stable cyclic

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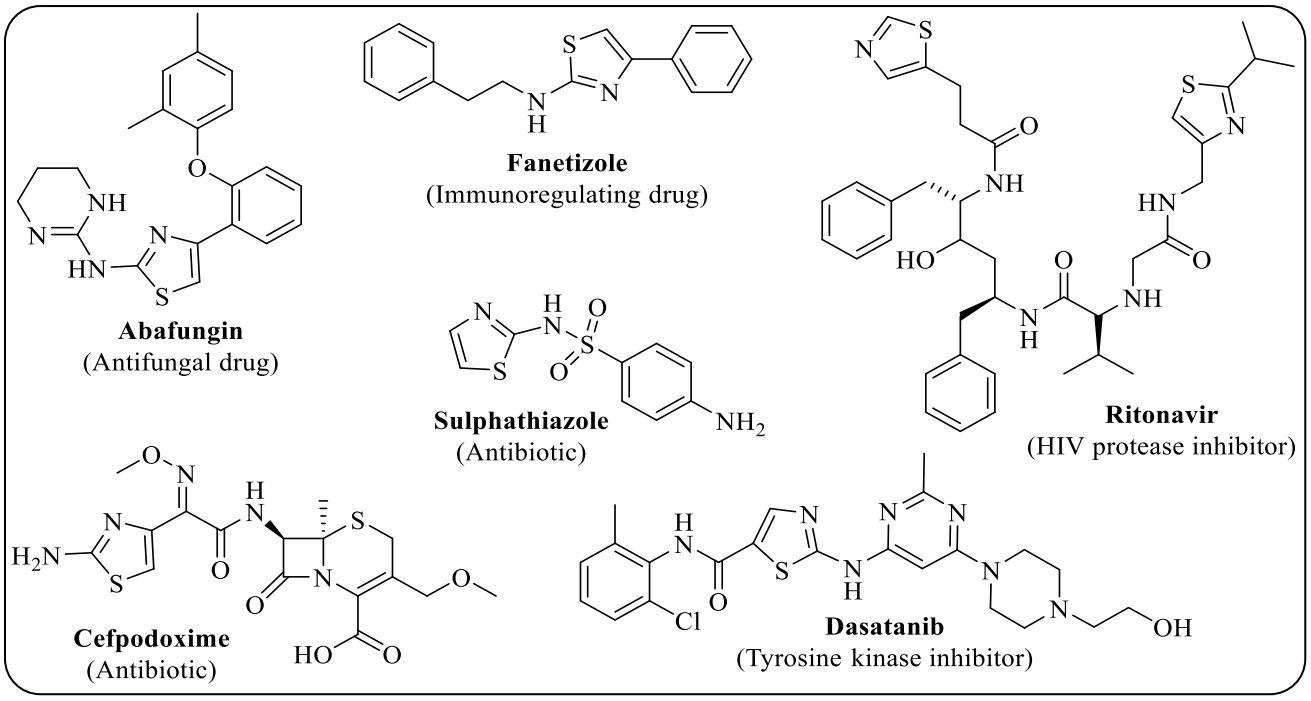


Fig. 1: Structures of some important thiazole-containing drugs

molecules are known for their pharmacological and therapeutic properties[5] such as antiviral[6], analgesic[7], anticancer[8], antimicrobial[9], anti-inflammatory[10], antitumor[11], antifungal[12], antibacterial[13], anti convulsant[14] etc. Amongst the many heterocycles, thiazoles are considered natural products and drugs (Fig. 1) which are derived from a variety of peptides like steroidal receptor ligands with thiazole moiety as an essential pharmacophore[15]. Peptides having thiazole subunits are identified by reduction of multi-drug resistance of certain types of lymphoblasts[16], antifungal, antibacterial, antimicrobial, and anti-tubercular activities[17]. Various methods have been developed for the synthesis of peptides containing thiazole moieties. Few of them consist of (a) Hantzsch's protocol which utilizes thioamide as an intermediate[18] (b) oxidation of dihydrothiazoles to thiazoles Cu(I)/Cu(II) of an intermediate with ethyl cysteinate[19] (c) base catalyzed treatment of *N*-protected thioamide with ethyl bromopyruvate followed by dehydration along with $(Tf)_2O$[20] (d) condensation reactions of cysteine ester with esters of *N*-protected amino acids succeeded by oxidation of subsequent thiazoles[21] (e) by using Mitsunobu conditions or Burgess reagent cyclodehydration of *α*-hydoxylthioamide[22]. Some of the methods reported involve disadvantages such as; the use of strong oxidation and dehydration conditions, which may lead to racemization in the final products, lower yields, and use of expensive solvents. To expand our work and develop new synthetic methods for the synthesis of a variety of minor peptides having heterocyclic nuclei [23], synthesis of thiazole derivatives of α-amino acids under milder reaction conditions was taken up. The reaction was carried out without oxidation and dehydration steps. A method to prepare to thiazole derivatives of α-amino acids which uses N^{α}-Fmoc protected α-halomethylketones as key intermediate was devised. Fmoc group which is widely used in SPPS, is now being discovered for solution phase peptide and peptidomimetics synthesis. The *α*-halomethylketones are widely used in the preparation of pharmaceutically important compounds[24] and as precursors to the hydroxyethylamine isostere subunits present in various enzymes[25], rennin[26] and HIV protease inhibitors nelfinavir, nelfinavir, amprenavir[27]. They are transformed into chlorohydrins and epoxides, which are mainly utilized in the preparation of various enzyme inhibitors like caspase[24]. In the synthesis of N^{α}-protected halomethylketones, due to the complete acceptance of the protected Fmoc moiety towards acid treatment, the protected Fmoc- chemistry is more appropriate than the Boc and Z counter parts, which is vital for bubbling of acid gas to the diazomethylketones.

Several Fmoc protected α-amino, α-halomethylketones have been reported in the literature.

Just to mention a few, synthesis of halomethylketones include in the stereochemical change of *N*-Fmoc-(2S,3S)-trans-Pro-CHN_2 to the respective bromo/chloromethylketone by treating it with lithium bromide in the existence of CH_3COOH and H_2O for two hours at the room temperature[28] and the essential chloromethylketone is obtained after workup or the acidolysis of Fmo-Aaa-CHN_2 has been carried out with anhydrous HCl in AcOH[29] at 0 °C through the THF/ethyl acetate solution of diazomethylketone over a period of time to extract chloromethylketones which consume more time and basically tedious.

Keeping in mind the future scope, the present study has been aimed to design the thiazoles derived from N^{α}-protected amino acids using dynamics approach along with an in *vitro* synthesis of thiazoles and their importance followed by evaluation of antioxidant and anti-microbial attribute study.

EXPERIMENTAL SECTION

Instrumentation

TLC analysis was carried out by using silica gel coated on aluminium plate under UV chamber. ^{1}H NMR was recorded on a regular standard instrument. Melting points were recorded in a standard apparatus in open capillaries. Functional groups were detected by typical FT-IR instrument. Mass spectra were recorded on a Micromass Q-Tof Micro Mass Spectrometer. RP-HPLC was recorded over Agilent 1100 series having G1311A VWD at λ=240 nm; flow: 1.0 mL/min; with C18 Column; Acetonitrile: Water in gradient mode for 30 min.

Chemicals

All the chemicals used were procured from Merck and the solvents were distilled prior to use. Fluorenylmethyloxycarbony (Fmoc) /*t*-butyloxycarbonyl (Boc) /benzyloxycarbonyl (Cbz)-protected amino acids (99%), benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) (97%), diisopropylethylamine (DIEA) (98%), aq. HBr (47%), HCl (98%), Na_2SO_4 (99%), NaCl (99%), and thiourea (99%)were procured from Sigma-Aldrich. Microbial strains were purchased from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India.

General procedure for the synthesis of N^{α}-protected amino acid derived diazoketones (2a-2j)

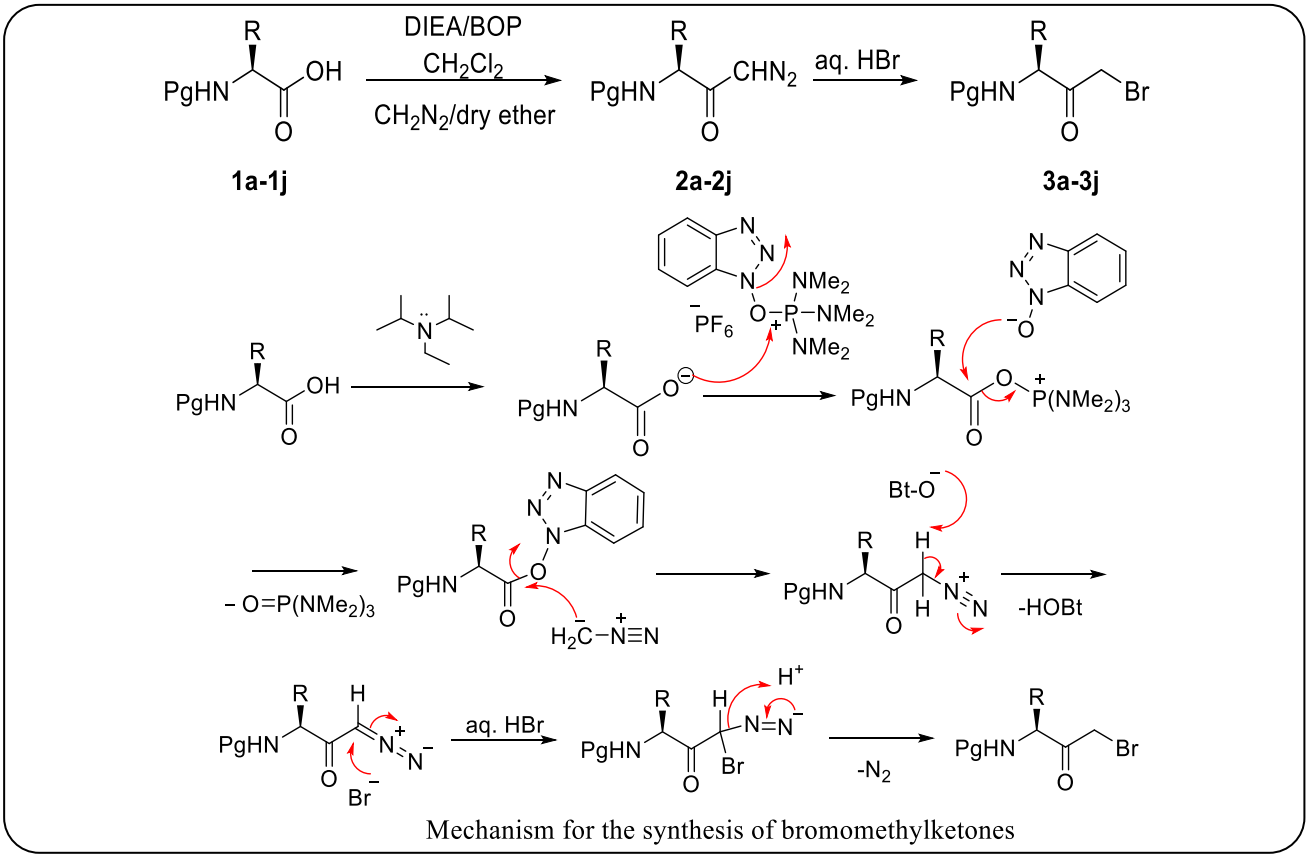
A solution of the N^{α}-protected amino acid (1 mmol) in dry THF (10 mL) was taken in a 100 mL round bottom flask and DIEA (1.2 mmol) was added at 0 °C and stirred for 5 min. To the reaction mixture, BOP (1.1 mmol) was added and stirring was continued. After the completion stirring for 30 min, a solution of diazomethane (2.5 mmol) in ether was added in drop-wise at 0 °C and the stirring was continued till the completion of the reaction which was monitored by thin layer chromatography (TLC) using EtOAc:*n*-Hexane (8:2).

After the completion of the reaction, the solvent was evaporated under reduced pressure and the crude sample was dissolved in EtOAc, the organic layer was washed with 5% HCl (in the case of Boc-protected amino acids, 5% citric acid was used), 5% Na_2CO_3, water, and brine respectively, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness to isolate the product. The crude was further purified by column chromatography with EtOAc: *n*-Hexane (8:2) and characterized by NMR and Mass spectroscopy (Scheme 1). The diazoketones 2g, 2i, and 2j were obtained as gums whereas rest all diazoketones were obtained as fine white solids.

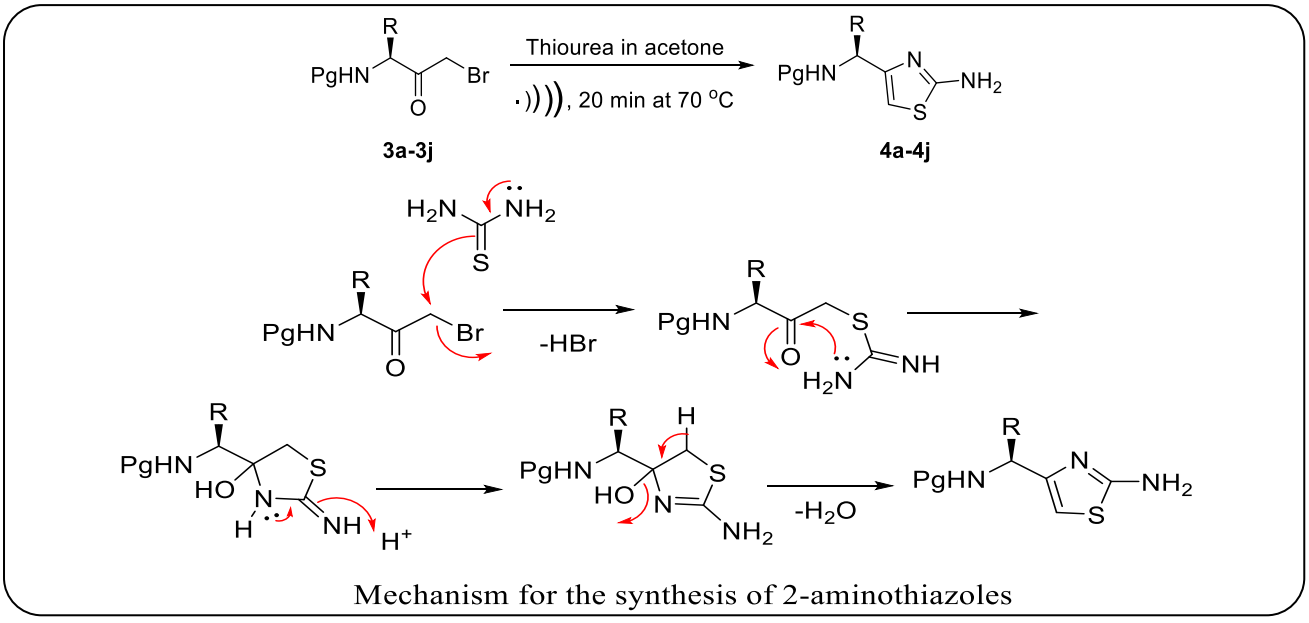
General procedure for the synthesis of N^{α}-protected amino acid-derived aminothiazoles (4a-4j)

To a solution of N^{α}-protected amino acid-derived diazoketone (1 mmol) in THF (5 mL) 45% aqueous HBr was added dropwise at 0 °C and stirred for 1 h at rt. The progress of the reaction was monitored by TLC with EtOAc:*n*-Hexane (8:2) and the bromoketone derivatives were isolated after the evaporation of the solvent followed by filtration of the product. All the bromoketones (3a-3j) were obtained in good yields as fine solids and were used as such without further purification (Scheme 1).

The above bromoketones (3a-3j) (1 mmol) in a dry 100 mL conical flask were charged with acetone (20 mL) and thiourea (1.5 mmol) and subjected to ultrasonication at 70 °C for about 20 min to give the aminothiazole derivatives and the progress of the reaction was monitored by TLC (EtOAc:*n*-Hexane 7:3). After the completion of the reaction, the solvent was evaporated and the crude product was diluted in EtOAc and the organic layer was washed with water (10 mL X 2), brine (10 mL), dried over anhydrous



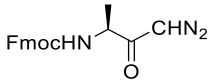
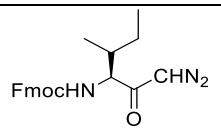
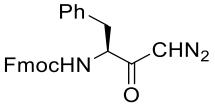
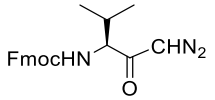
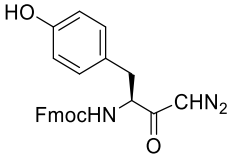
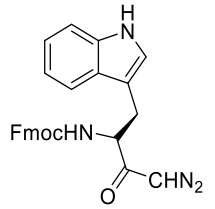
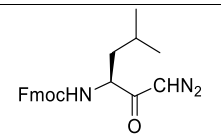
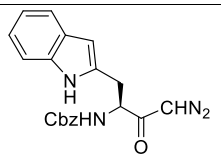
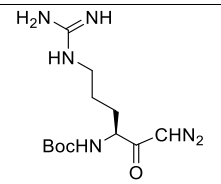
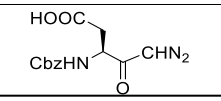
Scheme 1: Synthesis of N^{α}-protected bromomethylketone



Scheme 2: Synthesis of 2-amino-thiazole derivatives of N^{α}-protected amino acids

Na_2SO_4 and evaporated to dryness. All the products (4a-4j) obtained were characterized by NMR and Mass spectroscopic analysis (Scheme 2). The synthesized amino thiazoles 4a-4f and 4h were obtained as white solids and compounds 4g, 4i, and 4j were obtained as gummy syrups.

Table 1: List of N^{α}-protected diazomethyl ketones

Entry	Diazomethyl ketone	Yield (%)	M.P./ °C
2a		89	118
2b		91	203
2c		91	198
2d		90	188
2e		88	169
2f		86	198
2g		88	Gum
2h		85	181
2i		85	Gum
2j		88	Gum

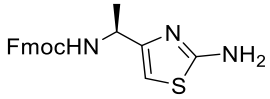
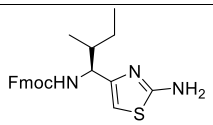
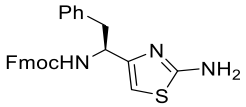
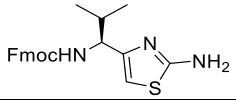
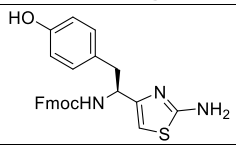
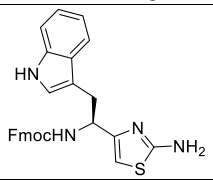
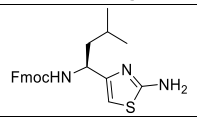
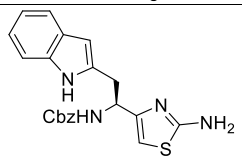
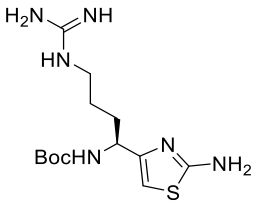
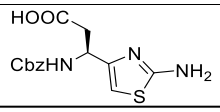
RESULTS AND DISCUSSION

An effective synthesis of N^{α}-protected diazomethyl ketone using the (benzotriazol-1-yloxy) tris (dimethylamino) phosphonium hexafluorophosphate (BOP) and *N*-nitroso-*N*-methyl urea (NMU) has been developed (Scheme 1). For this, the N^{α}-protected amino acid was treated with BOP, and the intermediate was treated with *an insitu-generated* diazomethane solution in ether. The method with BOP as coupling gives high yields of diazo ketone while the conventional dicyclohexyl carbodiimide (DCC), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and mixed anhydrides gives significant quantities of corresponding methyl esters which may further require column chromatographic purifications. The diazoketones (Table 1) obtained through the procedure developed by us were generally pure enough for further steps and the loss due to amino acid getting converted into the methyl esters is avoided. The diazoketones were then converted into corresponding bromomethyl ketones by treating diazomethylketone in THF with 45% aqueous HBr at 0 °C and stirred for 1 h at rt. All the bromomethyl ketones were obtained as fine solids and were isolated by filtration followed by the water wash. The solid products were recrystallized and taken into further steps without further purification (Scheme 1).

At the end of our studies, the bromomethyl ketones were utilized for the synthesis of 2-amino-thiazole analogs of N^{α}-protected amino acids under ultrasonication with thiourea using acetone as solvent at 70°C for 20 min (Scheme 2).After the completion of the reaction, TLC (EtOAc:*n*-Hexane (7:3) examination was carried out to determine the formation of the product. TLC indicates the consumption of the bromomethylketone and formation of a new spot for the thiazole. Then the solvent was removed using a rotatory evaporator. The crude compound was diluted with EtOAc washed with dil. Na_2CO_3, water and brine prior to column chromatography to obtain the pure aminothiazoles (Table 2).

An effective, convenient approach for the preparation of 2-amino-thiazole correspondents of N^{α}-protected amino acids from bromomethylketone using thiourea has been presented in the current study. Bromomethylketones act as a good precursor for the synthesis of thiazole derivatives of protected amino acids. Overall, the proposed protocol is a simple, efficient, and straightforward method to afford the thiazole derivatives of amino acids. All the reactions were monitored by TLC. Products obtained were characterized by ^{1}H NMR, ^{13}C NMR, and mass spectrophotometric analysis. RP-HPLC for the synthesized samples (2a-c and 3a-c) was collected and it demonstrates the purity as well as the difference in the R_f values between the synthesized compounds. For example, bromoketone compounds

Table 2: List of N-protected thiazole analogues

Entry	Thiazoles	Yield (%)	M.P./ ºC
4a		89	118
4b		91	203
4c		91	198
4d		90	188
4e		88	169
4f		86	198
4g		88	Gum
4h		85	181
4i		85	Gum
4j		88	Gum

(3a/3c) exhibits signals at 11.035 min/ 14.221 min, diazoketone compounds (2a/2c) shows signals at 17.845 min/ 19.886 min, and the amino thiazole compounds (4a/4c) at 20.229 min/ 24.573 min respectively (Fig. 2). This clearly shows the difference between the R_t values and also the purity that can be obtained through the report generated.

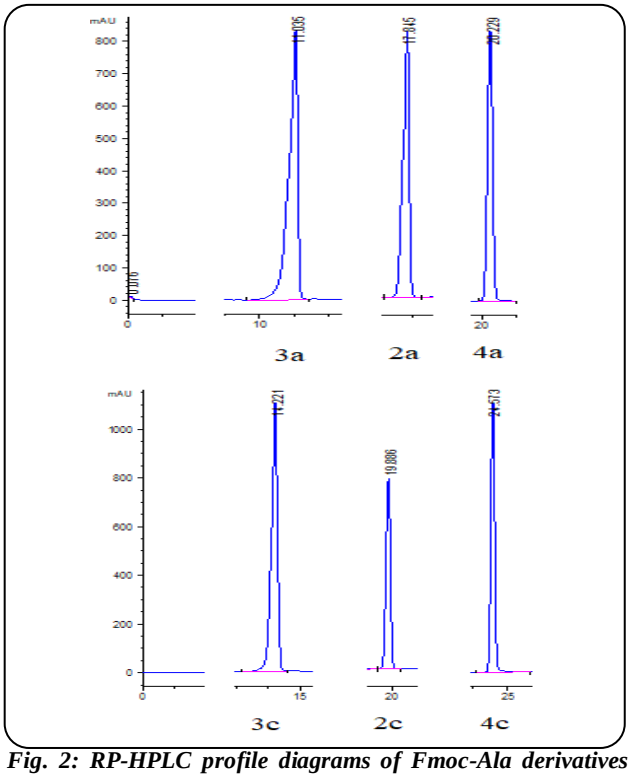


Fig. 2: RP-HPLC profile diagrams of Fmoc-Ala derivatives (2a, 3a, 4a) and Fmoc-Phe derivatives (2c, 3c 4c)

BIOLOGICAL STUDIES

Antioxidant property

An antioxidant is the one that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that includes the loss of electrons or increases in the oxidation state. These reactions can be pointers to the formation of chain reactions as they produce free radicals. Some of the chain reactions which occur in the cell lead to damage or death of the cell. In order to avoid these types of reactions by eliminating the radical intermediates, antioxidants are used. Antioxidants can capture free radical intermediates. Some of the free radicals which are highly reactive and species like oxygen are present in a wide variety of sources in biological systems. Oxidation of proteins, lipids, or DNA which is initiated by the free radicals may be a pointer to progressive diseases. Being a stable free radical 1,1-diphenyl-2-picryl hydrazyl (DPPH) is considered a pointer of the radical scavenger nature. It has a strong absorption band centered at about 520 nm, initially the DPPH radical has a deep violet colour, after decolorisation it turns pale green (Fig 3). This antioxidant stuff permits detection and the change in the strong optical absorption band at 520 nm indicates the number of initial radicals. We tested the antioxidant

Table 3: Tabular column showing the measured zones of antioxidant activity

Sl. No	Sample	% Radical scavenging activity
1	Fmoc-Ala-ψ[$COCHN_2$] (2a)	46.34
2	Fmoc-Phe-ψ[$COCHN_2$] (2c)	58.53
3	Fmoc-Phe-ψ[$COCH_2Br$] (3c)	46.43

Radical scavenging activity (%) = $[(A_o - A_1)/A_oX100]$

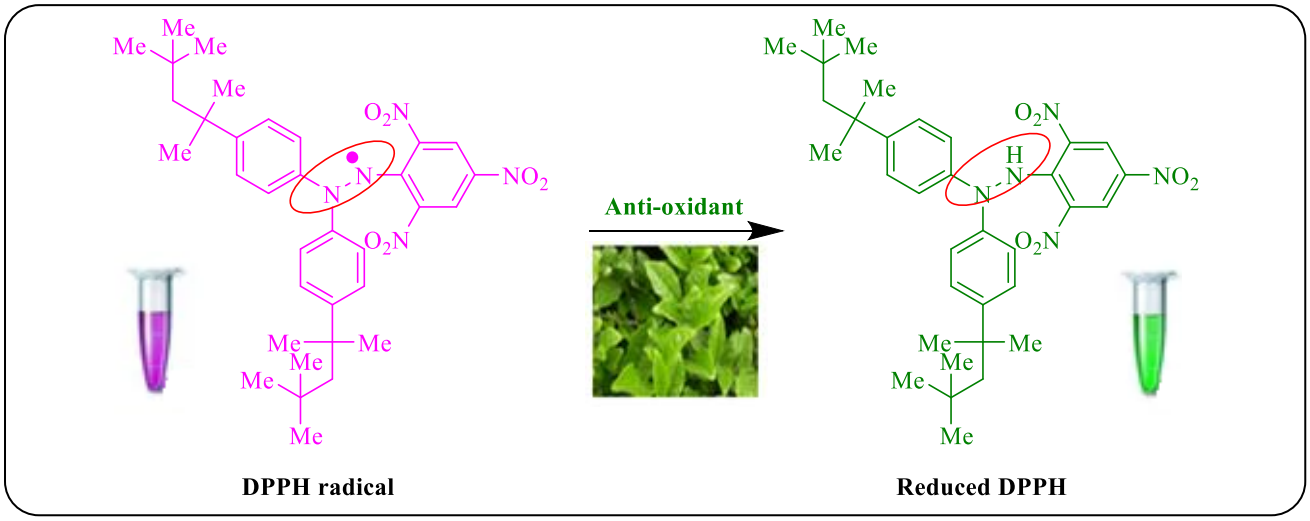


Fig. 3: Scheme showing the DPPH scavenging activity

property for the three synthesized derivatives such as Fmoc-Ala-ψ[$COCHN_2$] (2a), Fmoc-Phe-ψ[$COCHN_2$] (2c) Fmoc-Phe-ψ[$COCH_2Br$] (3c). Among the three compounds tested, the compound 2c shows maximum (58.53) and compound 2a with minimum (46.34) antioxidant activity. These results can be comparable with the reported thiazole metal complex analogs [30].

Where, A_o is the calculated absorbance of the control (blank, without sample) and A_1 is the calculated absorbance of the sample.

Antibacterial property

We further demonstrated the efficacy of the synthesized compounds towards their antibacterial activity[31]. The effect of antibacterial action of the synthesized molecules depends on various mechanisms. Firstly, peptidoglycan membrane disruption is by remodeling of phospholipid bilayers of the bacteria, by the action of synthetic peptidomimetics. Other mechanism is pore formation which is due to electrostatic potential created between the synthesized compounds and membrane. Apart from these mechanisms, the synthetic compounds can also target on receptors, active enzymes, proteins, DNA and RNA leads to change in the metabolism of the bacteria. In the present study, we employed a method called Kirby Bauer well diffusion technique [32]. The strains used for this method were *Escherichia coli* MTCC 443 and *Staphylococcus aureus* MTCC 5823. *E. coli* was sub-cultured in Luria Bertoni broth and allowed to grow for 10 h. *S. aureus* was sub-cultured in nutrient broth and allowed to grow for 24 h. After the growth of the culture, Mac Farland's turbidity test of the culture at 600 nm was found to be 0.8-1.0 OD range. Both cultures were further swabbed on sterilized Mueller Hinton Agar plates and samples were loaded by Kirby Bauer well diffusion method at different volumes of samples and the plates were kept in incubation for 24-36 h. After incubation the zones formed (Fig. 4 and 5) were measured and tabulated.

From the above Table 4, the synthesized samples i.e. Boc-Arg-thiazole (4i), Cbz-Asp-thiazole (4j), Cbz-Trp-thiazole (4h), Fmoc-Phe-thiazole (4c), Fmoc-Trp-thiazole (4f) are showing activity which was checked with respect to Streptomycin sulphate. Based on the formation of zones the compounds Boc-Arg-thiazole (4i), Cbz-Asp-thiazole (4j), Cbz-Trp-thiazole (4h), Fmoc-Phe-thiazole (4c) are more susceptible than Fmoc-Trp-thiazole (4f) which is less susceptible to *E. coli* when compared with the standard Streptomycin sulphate. The reason for susceptible nature

Table 4: The inhibition zones of the synthesized samples against Escherichia coli

Sl. No.	Volume and concentration	Inhibitory zone (mm)				
		Boc-Arg-thiazole (4i)	Cbz-Asp-thiazole (4j)	Cbz-Trp-thiazole (4h)	Fmoc-Trp-thiazole (4f)	Fmoc-Phe-thiazole (4c)
1*	100 µL (2 mg/mL)	8.0	8.0	8.0	8.0	8.0
2	50 µL (50 mg/mL)	0.75	2.0	4.5	0.0	1.0
3	100 µL (50 mg/mL)	2.25	6.25	3.0	1.0	4.0
4	150 µL (50 mg/mL)	4.75	8.5	2.25	0.0	5.0
5	200 µL (50 mg/mL)	6.5	12.25	9.75	2.0	9.0
6	250 µL (50 mg/mL)	10.5	11.0	5.5	3.0	8.0
7	300 µL (50 mg/mL)	12.25	12.25	12.0	5.0	11.0

* indicates the inhibitory zone formed with the standard Streptomycin sulphate.

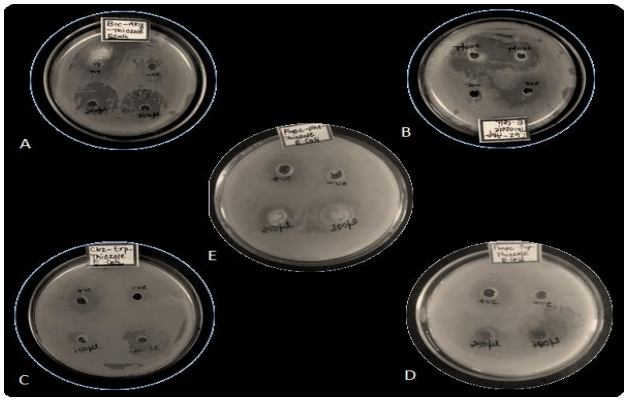


Fig.4: Antibacterial Activity of [A]Boc-Arg-thiazole (4i), [B] Cbz-Asp-thiazole (4j), [C] Cbz-Trp-thiazole (4h), [D] Fmoc-Trp-thiazole (4f) and [E] Fmoc-Phe-thiazole (4c) against E. coli

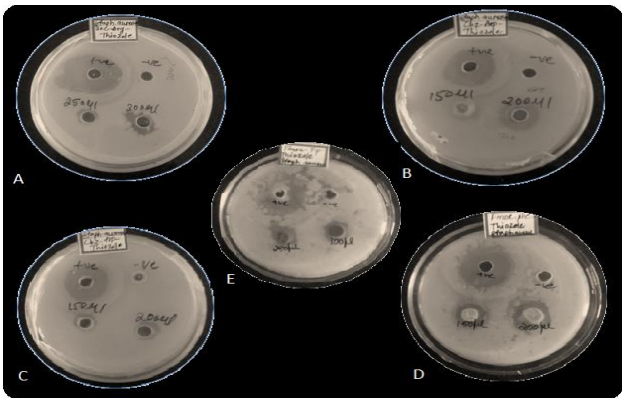


Fig. 5: Antibacterial activity of [A] Boc-Arg-thiazole (4i), [B] Cbz-Asp-thiazole (4j), [C] Cbz-Trp-thiazole (4h), [D] Fmoc-Phe-thiazole (4c) and [E] Fmoc-Trp-thiazole (4f) against Staphylococcus aureus.

for the synthesized compounds may be due to the interactions with the protein selected and also due the nature of penetration of the compounds into the bacterial cell.

From above Table 5, the synthesized samples *i.e.* Boc-Arg-thiazole (4i), Cbz-Asp-thiazole (4j), Cbz-Trp-thiazole (4h), Fmoc-Phe-thiazole (4c), Fmoc-Trp-thiazole (4f) are showing activity which was checked with respect to Streptomycin sulphate. Based on the formation of zones the compounds Boc-Arg-thiazole (4i), Cbz-Asp-thiazole (4j), Cbz-Trp-thiazole (4h), Fmoc-Phe-thiazole (4c) are more susceptible to *Escherichia coli* than Fmoc-Trp-thiazole (4f) which is less susceptible when compared with the standard Streptomycin sulphate. The reason for susceptibility may be envisaged as the penetration of the compound to the bacteria and reaction with the target protein, which provides scope for further investigation of these molecules.

CONCLUSIONS

In summary, we have established an efficient method for the synthesis of 2-amino-thiazolesusing *N*-protected α-amino acids employing BOP as a coupling agent for the preparation of diazoketones. This step eliminates the formation of the methyl ester byproduct thereby column purification was not inevitable. The synthesized thiazoles were characterized by spectrophotometric analysis and tested for antioxidant and antibacterial properties. The purity of the samples was determined by RP-HPLC spectrum of two series of the compounds and it was evident from the data that the compounds were of at most purity. The synthesized compounds Fmoc-Ala-ψ[$COCHN_2$] (2a), Fmoc-Phe-ψ[$COCHN_2$] (2c) Fmoc-Phe-ψ[$COCH_2Br$] (3c) exhibit promising antioxidant activity and compounds, Boc-Arg-thiazole (4i), Cbz-Asp-thiazole (4j), Cbz-Trp-thiazole (4h), Fmoc-Phe-thiazole (4c), Fmoc-Trp-thiazole (4f) were effective against E. Coli and Fmoc-Phe-thiazole (4c) showed clear zone inhibition against *Staphylococcus aureus* with the standard drug Streptomycin sulphate.

Table 5: The inhibition zones of the synthesized sample against Staphylococcus aureus

Sl. No.	Volume and concentration	Inhibitory zone (mm)				
		Cbz-Trp-thiazole (4h)	Cbz-Asp-thiazole (4j)	Boc- Arg-thiazole (4i)	Fmoc-Trp-thiazole (4f)	Fmoc- Phe-thiazole (4c)
1*	100 µL (2 mg/mL)	10.0	10.0	10.0	10.0	10.0
2	50 µL (50 mg/mL)	0.5	1.0	0.25	1.0	0.0
3	100 µL (50 mg/mL)	1.5	3.25	0.25	1.0	1.0
4	150 µL (50 mg/mL)	1.5	3.75	1.5	1.0	4.0
5	200 µL (50 mg/mL)	1.5	5.0	0.75	1.0	5.0
6	250 µL (50 mg/mL)	1	4.5	1.75	2.0	5.0
7	300 µL (50 mg/mL)	0.75	5.75	3.5	2.0	8.0

* indicates the inhibitory zone formed with the standard Streptomycin sulphate.

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