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Research Article

DESIGN, SYNTHESIS AND ANTIMICROBIAL SCREENING OF AMINO ACIDS CONJUGATED 2-AMINO-4-ARYLTHIAZOLE DERIVATIVES

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ABSTRACT

An alarming clinical resurgence of multidrug resistance microbes has lent an imminent but unmet medical need for novel antimicrobials with clinically unexploited novel mode of action. In literature, thiazole derived amino acids/peptides have demonstrated potential antimicrobial effect with a unique mode of action. The present work describes *in vitro* antimicrobial evaluation of novel amino acid conjugated thiazole derivatives. Totally two series of amino acids conjugates were synthesized by coupling different *Boc*-amino acids with 2-amino-4-phenylthiazole and 2-amino-4-(4'-cholorophenyl)thiazole motifs using IBCF/NMM as coupling agents. The synthesized compounds were characterized by standard spectroscopic techniques, evaluated their antibacterial and antifungal activity by agar well diffusion method. Results show that, conjugation of amino acids to the thiazole moieties has resulted enhanced antibacterial and antifungal activities. Among the two series of amino acid conjugates, chloro substituted analogs has showed better antibacterial and antifungal activities. Tryptophan analogs showed highest antibacterial activity where as lysine and arginine analogs showed highest antifungal activity compare to other amino acid conjugates.

Keywords: 2-Amino-4-arylthiazole, Amino acids, Antimicrobial activity and Thiopeptides.

INTRODUCTION

Today, antibiotic-resistant microbes are making their inexorable march and medicinal chemists have now realized that the discovery of more powerful antibiotics is not the only an answer to this threat. But, a real need exists in searching a novel antimicrobial that express antimicrobial properties, possibly acting through mechanisms different from those of existing drugs. In this context, it is very essential to successfully develop a novel, efficient antimicrobial agents with clinically unexploited mode of action. [1]

Thiazole is a versatile bioactive heterocycle with biocidal S-C=N entity having their wide presence in many synthetic drugs of several infective diseases such as allergies,[2] malaria, [3] inflammation, [4] fungal and bacterial infection. [5] Interestingly, in recent decades numerous classes of *'thiopeptide antibiotics'*, a macrocyclic arrays of thiazole bearing amino acid/peptidic residues have been discovered from both microbial and marine origin. [6] They display a very promising antimicrobial activity and were recognized as one of the most privileged antibiotics that are not yet clinically exploited. They inhibit protein synthesis in bacteria by binding to the complex formed between 23S rRNA and ribosomal protein L11, thereby restricting the action of GTP dependent elongation factors. [7] It was revealed that, during penultimate stage of their interaction, certain amino acid moieties of thiopeptide antibiotics undergo heterocyclization in to thiazole ring bearing amino acid residues. [8] These thiazolyl amino acid residues have been identified as probable partners with DNA and thiazole moieties presumed to play a dominant role during their interaction. [9]

Despite the novel antimicrobial mode of thiopeptide antibiotics, total synthesis of 'thiopeptide antibiotics' have succumbed because of their complex molecular structure. However, owing to their potential biological applications, several new investigations have been reported towards the synthesis and structure activity relationship study of newly designed or modified shorter thiazolyl amino acids/peptides as novel anti-infective agents. [10] From these studies, it was evident that, the nature of both thiazole motifs and amino acid/peptidic residues, and the kind of thiazole-amino acid linkage is ought to be crucial in their interactions. This led to the continuous development involving different kind of conjugations of structurally diverse bioactive thiazoles with amino acid/peptide residues hoping to raise novel antimicrobial with high potential.

On the other hand, amino acids are the fundamental components of living organisms playing a crucial role in metabolism. Taking the advantage of low toxicity, biocompatibility and likeliness/structural diversity of amino acidic residues with the biological system, currently there is huge tendency of conjugating amino acid residues with small bioactive heterocyclic motifs in the field of biomedical research. In literature, there are several successful evidences where a simple known coupling reaction between amino acids with heterocyclic moiety has resulted enhanced potency, selectivity, *in vivo* stability, solubility, cell permeability and decreased toxicity of the bioactive heterocycle.[11]

By keeping all the above rational points in the mind and continuing our ongoing work on heterocyclic conjugated amino acids/peptides,[12] we found that 2-aminothiazole and related fused systems are one of the most reoccurring thiazole motifs in the field of modern drug discovery.[13] Especially, 2-amino-4-arylthiazoles have got a significant place in modern drug discovery due to their vast biological activities.[14] From ever since till to date there appears to be no reports available in the literature on amino acids conjugated 2-amino-4-arylthiazole. In this regard, the present investigations was taken to study the effect of conjugation of different amino acidic residues on the antimicrobial activity of 2-amino 4-phenylthiazole and 2-amino-4-(4'-cholorophenyl) thiazole.

MATERIALS AND METHODS

Reagents

The course of reaction and purity were ascertained by performing TLC. Melting points were determined in open capillaries and are uncorrected. IR spectra were recorded in JASCO FT-IR 4100 spectrophotometer with KBr and only significant absorption levels (reciprocal centimeter) are listed. 1H-NMR spectra were recorded at 300 MHz Bruker FT-NMR Spectrometer in $CDCl_3$ using tetra methyl silane (TMS) as internal standard. Elemental analysis (C, H, N and S) were carried out on an Elementar Vario EL III CHNS analyzer. The results are within 0.4% of the theoretical values.

Unless, otherwise stated all the starting materials and reagents were obtained from Aldrich (USA), Spectrochem Pvt. Ltd (India) and Rankem Pvt. Ltd. (India) and were used without further purification. The amino acids used were *L*- series unless mentioned.

Synthesis:

Synthesis of 2-Amino-4-phenylthiazole and 2-amino-4-(4'-cholorophenyl) thiazole: [14f]

A finely powdered thiourea (15.2 g, 0.2 mol) and iodine (25.4 g, 0.1 mol) mixed with acetophenone/4-chloroacetophenone (0.1mmol) in an 250 mL round bottom flask was refluxed on a water bath for 6

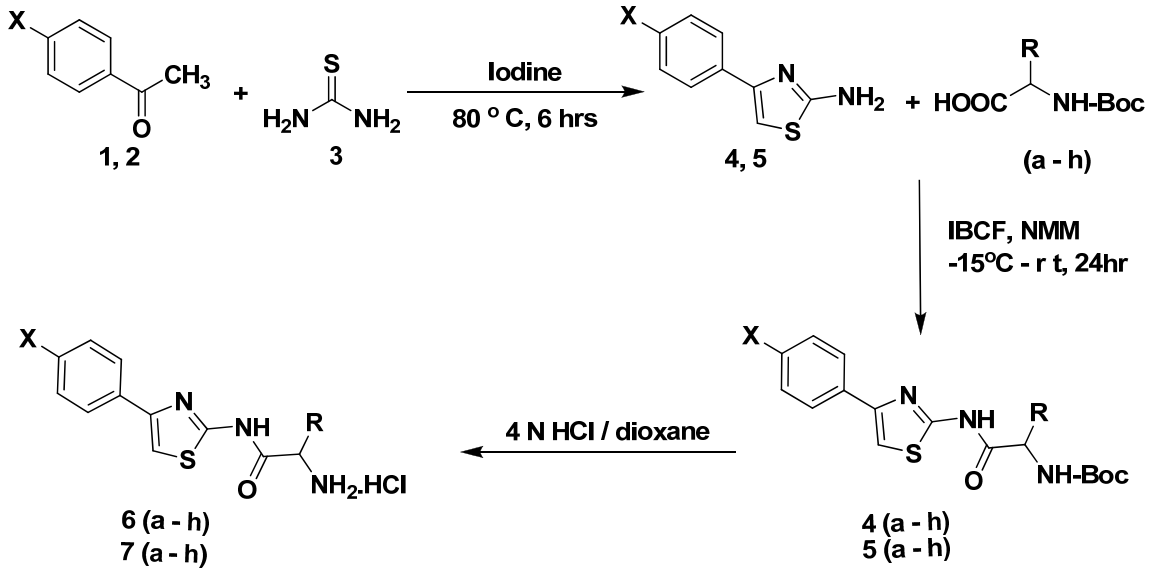
hrs. The obtained solid was triturated with diethyl ether to remove unreacted acetophenone, washed with aqueous sodium thiosulphate to remove excess of iodine and then with water. The crude product was dissolved in hot water, filtered to remove the sulphones; 2-amino-4-phenylthiazole/2-amino-4-(4'-cholorophenyl) thiazole was precipitated by addition of ammonia. Solid separated was filtered, washed with water and recrystalized from benzene.

General procedure for the conjugation of *Boc*-amino acids with 2-amino-4-phenylthiazole/2-amino-4-(4'-cholorophenyl)thiazole:

To Boc-amino acid (2 mmol) dissolved in acetonitrile (15 mL) and cooled to 0 °C was added NMM (0.25 mL, 2 mmol). The solution was cooled to -15 ± 1 ºC and IBCF (0.275mL, 2 mol) was added under stirring while maintaining the temperature at -15 °C. After stirring the reaction mixture for 10 min at this temperature, HOBt (0.27 g, 2 mmol) was added. The reaction mixture was stirred for an additional 10 min and a pre-cooled solution of 2-amino-4-phenylthiazole/2-amino-4-(4'-cholorophenyl)thiazole (**4/5**, 2 mol) and NMM (0.25 mL, 2 mol) in DMF (10 mL) was added slowly. After 20 min, the pH of the solution was adjusted to 8 by the addition of NMM and the reaction mixture stirred over night at room temperature. The solvent was removed under reduced pressure and the residual DMF solution was poured into about 200 mL ice-cold 90% saturated $KHCO_3$ solution and stirred for 30 mins. The product precipitated was extracted into $CHCl_3$. The organic layer was washed with water, 0.1 N HCl in cold, 5% $NaHCO_3$ solution, water and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure; the obtained amino acid conjugated 2-amino-4-phenylthiazole/2-amino-4-(4'-cholorophenyl)thiazole [**4(a-h) & 5(a-h)**] was triturated with dry ether/petroleum ether mixture and dried under vacuum.

Deprotection of Boc group:

The Boc group of the synthesized compounds (1 mmol) was deblocked by treating with 4*N* HCl in dioxane (10 mL / g of the compound) for 1.5 hrs. Excess HCl and dioxane were removed under reduced pressure, triturated with ether, filtered, washed with ether and dried to afford hydrochloride salts of amino acid conjugates [**6(a-h) & 7(a-h),** Yield (100%)].



Where,

Sl. No.	X	R = Side chains of amino acids a	b	c	d	e	f	g	h
4, 6	H	*							
5, 7	Cl			O(2,6 Cl_2-Bzl)	NH	NBom	OBzl	NH-Z	HN, NH, HN, NO_2

* R is the closed pyrrolidine structure of the amino acid 'proline', which extends from α-carbon to amine group.

Scheme: Synthesis of the amino acid conjugated 2-amino-4-phenylthiazole/2-amino-4(4'-choloro)-phenylthiazole analogs

Antimicrobial assay

All the synthesized compounds *viz* 2-amino-4-phenylthiazole (**4**), 2-amino-4-(4'-cholorophenyl)thiazole (**5**) and their respective amino acid conjugates [**6(a-h) & 7(a-h)**] were evaluated for their *in vitro* antibacterial activity against gram +ve and gram -ve bacterial strains *viz, Escherichia coli, Staphylococcus aereus, Klebeliessa pneumoniae, Pseudomonas areginosa* and *Bacillus substilis* by using the agar well diffusion method. [15] The bacterial strains were maintained on LB agar medium at 28 °C. The bacteria were grown in LB broth, centrifuged at 10,000 rpm for 5 minutes; a pellet was dissolved in double distilled water and used to inoculate the plates. The autoclaved molten media (20 mL) was poured in each 90 mm sterilized petriplate and allowed to solidify. A circular well of diameter 6 mm was made exactly at the center of the plates by using cork borer and each well was filled with 0.1 mL of the test solution (10mg/mL). Streptomycin and DMSO were used as positive control and negative control respectively. All the compounds were tested in

triplicate and inhibition zones were measured in mm after 24 hrs of incubation. The results were presented in **table-1.**

Antifungal activity:

In vitro antifungal assays of all the synthesized compounds *viz* 2-amino-4-phenylthiazole (**4**), 2-amino-4-(4'-cholorophenyl)thiazole (**5**) and their respective amino acid conjugates [**6(a-h) & 7(a-h)**] were performed against fungal strains *Aspergillus niger, Aspergillus flavus* and *Fusarium monoliforme* using agar well diffusion method.[16] The fungal cultures were raised by growing on potato dextrose agar media at pH 7.4 for six days at 25 °C. The spores were harvested in sterilized normal saline (0.9% NaCl in distilled water) and its concentration was adjusted to 1 x 10^6 / mL with a Haemometer. The autoclaved molten media (20 mL) was poured in each 90 mm sterilized petriplate and allowed to solidify. To study the growth response of fungi species, 0.4 mL of the synthesized compound solution (5mg/mL) was poured into each plate and spread over the agar media. 10 µL spore suspension was poured in to small depression made at the center of the plate and kept for 6 days at 25 °C. After six days of incubation, the fungal growth were measured and compared with the control. The control plates contained only DMSO for which fungal growth is taken as 100% (without inhibition). The fungal activity of all the synthesized compounds was assessed by comparing the zone of fungal growth in treated plates with that of control plates in mm. The results were presented in **table-2.**

RESULTS AND DISCUSSION

Synthesis and Characterization:

2-Amino-4-phenylthiazole (**4**) and 2-amino-4-(4'-cholorophenyl)thiazole (**5**) were prepared by the literature method [13f] and their respective amino acid conjugates [**4(a-h)** & **5(a-h)**] were synthesized by coupling different Boc-amino acids with using IBCF/HOBt and NMM as coupling reagents. The Boc group of the resulting amino acid conjugates was removed with 4*N* HCl in dioxane and they [**6(a-h) & 7(a-h)**] were used as such for antimicrobial screening. In all the steps, yield was quantitative (>80%) and purity was satisfactory. The structures of Boc protected amino acids conjugate were confirmed by the IR, ^{1}H-NMR and elemental analysis.

Antimicrobial Activity:

All the synthesized compounds *viz* 2-amino-4-phenylthiazole (**4**), 2-amino-4(4'-choloro) phenylthiazole (**5**) and their respective amino acid conjugates [**6(a-h) & 7(a-h)**] were evaluated for their *in vitro* antibacterial activity and antifungal activity. The results are depicted in **table 1** and **2**. It is clearly evident from the results that conjugation of amino acids has enhanced antibacterial and antifungal activity of both 2-amino-4-phenylthiazole (**4**) and 2-amino-4-(4'-cholorophenyl)thiazole (**5**) motifs. In comparison, amino acid conjugates of 2-amino-4-(4'-cholorophenyl)thiazole are moderately more potent than their respective counterparts of 2-amino-4-phenylthiazole. The enhanced activity of 2-amino-4-(4'-cholorophenyl)thiazole analogs might be due to the electron withdrawing property of chloro group on *p*-position of phenyl ring which is absent in the case of 2-amino-4-phenylthiazole. Furthermore, amino acid conjugates of both the series follows a parallel trend towards all the bacterial/fungal strains tested.

In vitro antibacterial activities were performed against *Escherichia coli, Staphylococcus aereus, Klebsiella pneumoniae, Pseudomonas areginosa* and *Bacillus substilis*. Among the two series of amino acid conjugates tested for antibacterial activity, tryptophan derivatives (**6d** & **7d**) showed highest degree of antibacterial activity followed by tyrosine (**6c** & **7c**) and phenylalanine (**6b** & **7b**) analogs of respective series. The highest activity shown by the tryptophan conjugated derivatives might be due to presence of indole, which can readily be protonated and can bind firmly to the negatively charged surface of bacterial cell wall, which possesses a negatively charged surface and thus making easier passage of the conjugates inside the bacterial cell. Also, it is very interesting to note that, the potency of amino acid conjugates towards gram-negative bacteria is fairly higher than that of gram-positive bacteria, may be due to the easy passage of conjugates through thin peptidoglycan layer of gram-negative bacterial cell wall when compared to the gram-positive bacteria, which is very much thicker.[17]

In vitro antifungal activity was performed against *Aspergillus niger*, *Aspergillus flavus* and *Fusarium monoliform*. All the amino acid conjugates except serine conjugate have shown better fungicidal effect against all the fungal strains tested when compare to 2-amino-4-phenyl thiazole and 2-amino-4(4'-choloro)-phenylthiazole. Among the conjugates, lysine (**6g** & **7g**) and arginine (**6h** & **7h**) derivatives exhibited notable antifungal activity when compared to the standard drug bavistin. The highest fungicidal activity of lysine (**6g** & **7g**) and arginine (**6h** & **7h**) derivatives might be due to their high lipophilic nature which serves to penetrate through the fungi surface.

Physical and analytical data of 2-amino-4-phenylthiazole, 2-amino-4-(4'-cholorophenyl) thiazole and their conjugates linked to the Boc-amino acids:

2-Amino-4-phenylthiazole (4):

Yield: 96%; mp: 148°C (Lit: 146-148 °C)[13f]; IR(KBr, cm^{-1}): 1640, 1670, 3320; ^{1}HNMR($CDCl_3$) δ: 7.35-7.80 (6H, m, Ar-H), 7.02 (2H, s, NH_2). Elemental Analysis (C, H, N, S in %) found (calculated) for $C_9H_8N_2S$: 61.35 (61.34), 4.63 (4.58), 151.52 (15.90), 18.15 (18.19).

2-Amino-4-(4'-chlorophenyl)thiazole (5):

Yield: 96%; mp: 145°C (Lit: 145°C)[13b]; IR(KBr, cm^{-1}): 1643, 1674, 3322; ^{1}HNMR($CDCl_3$) δ: 7.42-7.82 (5H, m, Ar-H), 7.06 (2H, s, NH_2); Elemental Analysis (C, H, N, S in %) found (calculated) for $C_9H_7ClN_2S$: 61.35(61.34), 4.63(4.58), 151.52(15.90), 18.15(18.19).

***tert*-Butyl 2-(4-phenylthiazol-2-ylcarbamoyl)pyrrolidine-1-carboxylate (4a):**

Yield: 87%; mp: 175°C; IR(KBr, cm^{-1}): 1511, 1463, 1043; ^{1}HNMR($CDCl_3$) δ: 12.48 (1H, s, CONH-thiazole), 7.34-7.82 (6H, m, Ar-H), 4.42-4.44 (1H, m, $^{\alpha}$CH, Pro), 3.39-3.48 (2H, m, $^{\delta}CH_2$, Pro), 2.25-2.55 (2H, m, $^{\beta}CH_2$, Pro), 1.99-2.05 (2H, m, $^{\gamma}CH_2$, Pro), 1.39 (9H, s, $3CH_3$-Boc); Elemental Analysis (C, H, N, S in %) found (calculated) for $C_{19}H_{23}N_3O_3S$: 61.15(61.10), 6.16(6.21), 11.29(11.25), 8.55(8.59).

***tert*-Butyl 2-(4-(4'-chlorophenyl)thiazol-2-ylcarbamoyl)pyrrolidine-1-carboxylate (5a):**

Yield: 86%; mp: 172°C; IR(KBr, cm^{-1}): 1512, 1464, 1047; ^{1}HNMR($CDCl_3$) δ: 12.50 (1H, s, CONH-thiazole), 7.45-7.87 (5H, m, Ar-H), 4.43-4.46 (1H, m, $^{\alpha}$CH, Pro), 3.39-3.46 (2H, m, $^{\delta}CH_2$, Pro), 2.27-2.56 (2H, m, $^{\beta}CH_2$, Pro), 2.00-2.07 (2H, m, $^{\gamma}CH_2$, Pro), 1.40 (9H, s, $3CH_3$-Boc). Elemental Analysis (C, H, N, S in %) found (calculated) for $C_{19}H_{22}ClN_3O_3S$: 55.95(55.94), 5.46(5.44), 10.29(10.30), 7.85(7.86).

***tert*-Butyl 1-oxo-3-phenyl-1-(4-phenylthiazol-2-ylamino) propan -2-ylcarbamate (4b):**

Yield: 89%; mp: 175°C; IR(KBr, cm^{-1}): 1510, 1460, 1045, 1640; ^{1}HNMR($CDCl_3$) δ: 12.48 (1H, s, CONH- thiazole), 7.34-7.84 (11H, m, Ar-H), 7.04 (1H, d, Boc-NH), 4.51 (1H, t, $^{\alpha}$CH-Phe), 3.38 (2H, d, $^{\beta}$ CH_2-Phe), 1.36 (9H, s, $3CH_3$-Boc); Elemental Analysis (C, H, N, S in %) found (calculated) for $C_{23}H_{25}N_3O_3S$: 65.19(65.23), 5.95(5.95), 9.90(9.92), 7.56(7.57).

***tert*-Butyl-1-(4-(4-chlorophenyl)thiazol-2-ylamino)-1-oxo-3-phenylpropan-2-ylcarbamate (5b):**

Yield: 85%; mp: 112°C; IR(KBr, cm^{-1}): 1517, 1464, 1048, 1645; ^{1}HNMR($CDCl_3$) δ: 12.50 (1H, s, CONH- thiazole, 7.45-7.86 (10H, m, Ar-H), 7.04 (1H, d, Boc-NH), 4.54 (1H, t, $^{\alpha}$CH-Phe), 3.39 (2H, d, $^{\beta}$ CH_2-Phe), 1.37 (9H, s, $3CH_3$-Boc); Elemental Analysis (C, H, N, S in %) found (calculated) for $C_{23}H_{24}ClN_3O_3S$: 65.34(65.32), 5.33(5.28), 9.20(9.18), 7.00(7.00).

***tert*-Butyl-3-(4-(2,6-dichlorobenzyloxy)phenyl)-1-oxo-1-(4-phenylthiazol-2-ylamino) propan-2-ylcarbamate (4c):**

Yield 89%; mp:82°C; IR(KBr, cm^{-1}): 1512, 1461, 1043, 1646; ^{1}HNMR($CDCl_3$) δ: 12.49 (1H, s, CONH-thiazole), 7.33-7.79 (13H, m, Ar-H), 7.09 (1H, d, Boc-NH), 5.23 (2H, s, CH_2-OBzlCl_2), 4.49 (1H, t, $^{\alpha}$CH-Tyr), 3.68 (2H, d, $^{\beta}CH_2$-Tyr), 1.38 (9H, s, $3CH_3$-Boc); Elemental

Analysis (C, H, N, S in %) found (calculated) for $C_{30}H_{29}Cl_2N_3O_4S$: 60.20(60.20), 4.90(4.88), 7.00(7.02), 5.35 (5.36).

***tert*-butyl-1-(4-(4-chlorophenyl)thiazol-2-ylamino)-3-(4-(2,6-dichlorobenzyloxy)phenyl)-1-oxopropan-2-ylcarbamate (5c):**

Yield 91%; mp:139°C; IR(KBr, cm^{-1}): 1510, 1460, 1045, 1640 3360, 1240; ^{1}HNMR($CDCl_3$) δ: 12.50 (1H, s, CONH- thiazole), 10.85 (1H, d, NH, Trp), 7.35-7.86 (11H, m, Ar-H), 7.09 (1H, d, Boc-NH), 4.59 (1H, t, αCH-Trp), 3.38 (2H, d, βCH$_2$-Trp), 1.36 (9H, s, 3CH$_3$-Boc); Elemental Analysis (C, H, N, S in %) found (calculated) for $C_{30}H_{28}Cl_3N_3O_4S$: 56.93(56.92), 4.49(4.46), 6.65 (6.64), 5.06(5.07).

***tert*-Butyl-3-(1H-indol-3-yl)-1-oxo-1-(4-phenylthiazol-2-ylamino)propan-2-ylcarbamate (4d):**

Yield: 84%, mp: 126°C; IR (KBr, cm^{-1}): 1517, 1468, 1041, 1649; ^{1}HNMR($CDCl_3$) δ: 12.51 (1H, s, CONH-thiazole), 7.46-7.82 (12H, m, Ar-H), 7.10 (1H, d, Boc-NH), 5.23 (2H, s, CH$_2$-OBzlCl_2), 4.50 (1H, t, αCH-Tyr), 3.69 (2H, d, βCH$_2$-Tyr), 1.38 (9H, s, 3CH$_3$-Boc); Elemental Analysis (C, H, N, S in %) found (calculated) for $C_{25}H_{26}N_4O_3S$: 64.88(64.91), 5.69 (5.67), 12.18(12.11), 6.95(6.93).

***tert*-Butyl-1-(4-(4-chlorophenyl)thiazol-2-ylamino)-3-(1H-indol-3-yl)-1-oxopropan-2-ylcarbamate (5d):**

Yield: 80%; mp: 123°C; IR(KBr, cm^{-1}): 1514, 1462, 1049, 1646, 3364, 1245; ^{1}HNMR($CDCl_3$) δ: 12.50 (1H, s, CONH- thiazole), 10.85 (1H, d, NH, Trp), 7.45-7.87 (10H, m, Ar-H), 7.10 (1H, d, Boc-NH), 4.60 (1H, t, αCH-Trp), 3.39 (2H, d, βCH$_2$-Trp), 1.39 (9H, s, 3CH$_3$-Boc); Elemental Analysis (C, H, N, S in %) found (calculated) for $C_{25}H_{25}ClN_4O_3S$: 60.39(60.41), 5.10 (5.07), 11.28(11.27), 6.45(6.45).

***tert*-Butyl-3-(1-(benzyloxymethyl)-1H-imidazol-4-yl)-1-oxo-1-(4-phenylthiazol-2-ylamino)propan-2-ylcarbamate (4e):**

Yield: 80%; mp: 108°C; IR (KBr, cm^{-1}): 1515, 1467, 1055, 1649; ^{1}HNMR($CDCl_3$) δ: 12.49 (1H, s, NH thiazole), 7.33-7.80 (13H, m, CH, Ar-H), 7.10 (1H, d, Boc-NH), 5.80 (2H, s, CH$_2$-Bom), 4.81 (2H, s, O-CH$_2$-,Bom), 4.73 (1H, t, α CH-His), 3.43 (2H, d, β CH$_2$-His), 1.39 (9H, s, 3CH$_3$-Boc); Elemental Analysis (C, H, N, S in %) found (calculated) for $C_{28}H_{31}N_5O_4S$: 63.04(63.02) 5.89(5.86) 13.13(13.12) 6.00(6.01).

***tert*-Butyl-3-(1-(benzyloxymethyl)-1H-imidazol-4-yl)-1-(4-(4-chlorophenyl)thiazol-2-ylamino)-1-oxopropan-2-ylcarbamate (5e):**

Yield: 84%; mp: 101°C; IR(KBr, cm^{-1}): 1510, 1460, 1045, 1640; ^{1}HNMR($CDCl_3$) δ: 12.49 (1H, s, NH thiazole), 7.44-7.81 (12H, m, CH, Ar-H), 7.41 (1H, d, Boc-NH), 5.80 (2H, s, CH$_2$-Bom), 4.80 (2H, s, O-CH$_2$-,Bom), 4.74 (1H, t, α CH-His), 3.45 (2H, d, β CH$_2$-His), 1.40 (9H, s, 3CH$_3$-Boc); Elemental Analysis (C, H, N, S in %) found (calculated) for $C_{28}H_{30}ClN_5O_4S$: 59.19(59.20), 5.36 (5.32), 12.33(12.33), 5.64(5.64).

***tert*-Butyl-3-(benzyloxy)-1-oxo-1-(4-phenylthiazol-2-ylamino)propan-2-ylcarbamate (4f):**

Yield 82%; mp:101°C; IR(KBr, cm^{-1}): 1510, 1460, 1045, 1640; ^{1}HNMR($CDCl_3$) δ: 12.48 (1H, s, CONH-thiazole), 7.33-7.85 (11H, m, Ar-H), 7.10 (1H, d, Boc-NH), 5.23 (2H, s, CH$_2$-OBzl), 4.93 (1H, t, αCH-Ser), 3.43 (2H, d, βCH$_2$-Ser), 1.38 (9H, s, 3CH$_3$-Boc); Elemental Analysis (C, H, N, S in %) found (calculated) for $C_{24}H_{27}N_3O_4S$: 63.56(63.56), 6.04(6.00), 9.27(9.26), 7.07(7.07).

***tert*-butyl-3-(benzyloxy)-1-(4-(4-chlorophenyl)thiazol-2-ylamino)-1-oxopropan-2-ylcarbamate (5f):**

Yield: 84%; mp: 120°C; IR(KBr, cm^{-1}): 1513, 1465, 1049, 1643; ^{1}HNMR($CDCl_3$) δ: 12.48 (1H, s, CONH-thiazole), 7.44-7.88 (10H, m, Ar-H), 7.10 (1H, d, Boc-NH), 5.24 (2H, s, CH$_2$-OBzl), 4.95 (1H, t, αCH-Ser), 3.46 (2H, d, βCH$_2$-Ser), 1.40 (9H, s, 3CH$_3$-Boc); Elemental Analysis (C, H, N, S in %) found (calculated) for $C_{24}H_{26}ClN_3O_4S$: 59.10(59.07), 5.40(5.37) 8.63(8.61), 6.57(6.57).

Benzyl-5-(tert-butylamino)-6-oxo-6-(4-phenylthiazol-2-ylamino)hexyldicarbamate (4g):

Yield: 86%; mp: 126°C; IR(KBr, cm^{-1}): 1510, 1460, 1045, 3300, 1220, 1640; ^{1}HNMR($CDCl_3$) δ: 12.49 (1H, s, CONH-thiazole, 7.38-7.87 (11H, m, Ar-H), 7.09 (1H, d, Boc-NH), 5.09 (2H, d, CH$_2$, Z), 4.48 (1H, m, αCH, Lys), 3.18 (2H, m, εCH, Lys), 2.00 (1H, t, NH, Lys), 1.79 (2H, m, βCH, Lys), 1.41 (2H, m, δCH, Lys), 1.38 (9H, s, CH$_3$, Boc), 1.25 (2H, m, γCH, Lys); Elemental Analysis (C, H, N, S in %) found (calculated) for $C_{28}H_{34}N_4O_5S$): 62.44(62.43), 6.40(6.36), 10.40(10.40), 5.95(5.95).

Benzyl-5-(tert-butylamino)-6-oxo-6-(4-(4-chlorophenyl)thiazol-2-ylamino)hexyldi carbamate (5g):

Yield: 86%; mp: 120°C; IR(KBr, cm^{-1}): 1513, 1466, 1048, 3305, 1224, 1642; ^{1}HNMR($CDCl_3$) δ 12.50 (1H, s, CONH-thiazole), 7.48-7.88 (10H, m, Ar-H), 7.08 (1H, d, Boc-NH), 5.09 (2H, d, CH$_2$, Z), 4.49 (1H, m, αCH, Lys), 3.19 (2H, m, εCH, Lys), 2.02 (1H, t, NH, Lys), 1.78 (2H, m, βCH, Lys), 1.42 (2H, m, δCH, Lys), 1.39 (9H, s, CH$_3$, Boc), 1.27 (2H, m, γCH, Lys);); Elemental Analysis (C, H, N, S in %) found (calculated) for $C_{28}H_{33}ClN_4O_5S$): 58.69(58.68) 5.83(5.80), 9.78(9.78), 5.60(5.59).

***tert*-Butyl-5-(3-nitroguanidino)-1-oxo-1-(4-phenylthiazol-2-ylamino)pentan-2-ylcarbamate (4h):**

Yield: 86%; mp: 82°C; IR(KBr, cm^{-1}): 1510, 1460, 1045, 1570, 1360, 1640, 3400, 1250; ^{1}HNMR($CDCl_3$) δ: 12.50 (1H, s, CONH-thiazole), 7.33-7.82 (6H, m, Ar-H), 7.08 (1H, d, Boc-NH), 6.58 (1H, s, NH, Arg), 4.52 (1H, m, αCH, Arg), 2.65 (2H, m, δCH, Arg), 2.00 (2H, t, 2NH, Arg), 1.76 (2H, m, βCH, Arg), 1.62 (2H, m, γCH, Arg), 1.38 (9H, s, 3CH$_3$, Boc); Elemental Analysis (C, H, N, S in %) found (calculated) for $C_{20}H_{27}N_7O_5S$: 50.31(50.30), 5.74(5.70), 20.55(20.53), 6.71(6.71).

***tert*-Butyl-1-(4-(4-chlorophenyl)thiazol-2-ylamino)-5-(3-nitroguanidino)-1-oxopentan-2-ylcarbamate (5h):**

Yield: 86%; mp: 80°C; IR(KBr, cm^{-1}): 1514, 1465, 1040, 1574, 1362, 1643, 3406, 1255; ^{1}HNMR($CDCl_3$) δ: 12.52 (1H, s, CONH-thiazole), 7.42-7.88 (5H, m, Ar-H), 7.08 (1H, d, Boc-NH), 6.59 (1H, s, NH, Arg), 4.53 (1H, m, αCH, Arg), 2.66 (2H, m, δCH, Arg), 2.03 (2H, t, 2NH, Arg), 1.78 (2H, m, βCH, Arg), 1.60 (2H, m, γCH, Arg), 1.39 (9H, s, 3CH$_3$, Boc Elemental Analysis (C, H, N, S in %) found (calculated) for $C_{20}H_{26}ClN_7O_5S$: 46.93(46.92), 5.15(5.12), 19.16(19.15), 6.27(6.26).

Table 1: Antibacterial activity of amino acid conjugated 2-amino-4-phenylthiazole and 2-amino-4-(4'-cholorophenyl)thiazole derivatives

Sl. No.	Inhibitory zone (diameter) mm [a]				
	Gram negative bacteria			Gram positive bacteria	
	E - coli	Klebesiella pnemoniae	Pseudomonas auregenosa	Staphylococcus aureus	Bacillus Substilis
4	02	01	01	01	01
5	03	01	02	01	02
6a	04	03	02	02	02
6b	05	03	03	02	02
6c	05	04	03	03	02
6d	06	06	05	04	03
6e	03	03	04	02	02
6f	03	02	02	02	02
6g	04	03	02	03	02
6h	04	03	03	02	02

7a	04	03	05	02	04
7b	06	04	04	03	06
7c	07	08	05	04	07
7d	08	09	08	06	08
7e	04	03	04	04	04
7f	04	04	03	02	04
7g	05	04	03	04	03
7h	05	06	05	04	05
Streptomycin	12	10	11	12	10

[a] Values are means of three determinations, the ranges of which are less than 5% of the mean in all cases.

Table 2: Antifungal activity of amino acid conjugated 2-amino-4-phenylthiazole and 2-amino-4-(4'-cholorophenyl)thiazole derivatives

Sl. No.	Inhibitory zone (diameter) mm[a]		
	Aspergillus niger	Aspergillus flavus	Fusarium monoliforme
4	00	00	00
5	00	00	00
6a	01	01	02
6b	02	01	03
6c	02	02	03
6d	03	04	03
6e	03	03	05
6f	00	00	00
6g	04	04	05
6h	05	05	08
7a	02	01	02
7b	04	02	04
7c	04	03	03
7d	04	04	03
7e	05	04	07
7f	00	00	00
7g	06	04	08
7h	06	06	11
Bavistin	09	10	09

[a] Values are means of three determinations, the ranges of which are less than 5% of the mean in all cases

CONCLUSION

Overall, all the amino acid analogs showed better antibacterial and antifungal activities when compared with that of 2-amino-4-phenylthiazole and 2-amino-4-(4'-cholorophenyl) thiazole. The enhancement of antimicrobial activity of amino acids conjugated thiazole derivative might be due to the effect of amino acid residue which can interact with the cell wall serving easy passage of conjugate in to the cytosol of bacteria and fungi, where as the function of thiazole moiety might be to disrupt the metabolic function of the bacteria and fungi.

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