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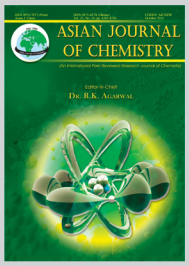

Synthesis of 2-Amino-5-aryl-1,3,4-thiadiazolopeptides as Potent Antitubercular Agents

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A novel series of 2-amino-5-aryl-thiadiazole analogs of amino acids and dipeptides were synthesized using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide as coupling agent and N-methyl morpholine (NMM) as base. Structure of all the newly synthesized compounds was confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. All the synthesized compounds were screened for their antitubercular, antibacterial and antifungal activity.

Key Words: Thiadiazolopeptides, Solution phase technique, Antitubercular activity, Antibacterial activity and antifungal activity.

INTRODUCTION

Despite the ready availability of effective treatments, tuberculosis remains a major public health threat worldwide. The emergence of drug resistant strains of *Mycobacterium tuberculosis*, particularly multiple drug resistant strains[1-4] has complicated treatment protocols and raises the concern that tuberculosis may once again become an incurable disease. For this reason, it is critical to discover new drugs acting with a mechanism different from those of presently used antitubercular drugs. Thiadiazoles have proved their potential in the development of pharmaceutically important organic compounds both of natural and synthetic origin[5]. Thiadiazole analogs deal with a variety of bioactivities *viz.*, antitumor[6,7], anti HIV[8], antimicrobial[9,10], anticonvulsant[11], antitubercular[12], antiinflammatory[13]. Literature is enriched with lot of work on synthesis of potent substituted thiadiazole derivatives with diverse pharmacological activities[14,15] but only few reports have been received on peptide coupling of thiadiazoles. Thus keeping in view the biological potency of thiadiazole derivatives as well as taking advantage of biodegradability and biocompatibility of amino acids/peptides and further, in continuation of our research work on synthesizing peptide derivatives of heterocyclic and aromatic compounds[16-18], a novel series of 2-aryl-5-amino thiadiazole analogs of amino acids and peptides with an anticipation to get potent agents of more therapeutic efficacy with lesser adverse effects. Each of the prepared analogues has been tested for their antitubercular, antibacterial and antifungal activity activities and the results are reported in this paper.

EXPERIMENTAL

Analytical grade solvents and commercially available reagents were used without further purification. The column chromatography was carried out over silica gel (60-120 mesh), purchased from Sisco Research Laboratories Pvt. Ltd. Melting points were determined in DBK, Prog, melting point apparatus Servewell Instruments Pvt. Ltd. IR spectra in KBr disk were recorded from 4000-400 cm^{-1} on Shimadzu FT-IR spectrometer. ^{1}H NMR spectra were recorded on 400 and 500 MHz Bruker spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in δ relative to TMS, the coupling constants are given in Hz. Mass spectra were recorded using Agilent 1100 MSD spectrometer in electro spray mode.

Preparation of 2-amino-5-aryl-1,3,4-thiadiazoles (1): 2-Amino-1,3,4-thiadiazoles are prepared according to literature procedure[19]. A second C-atom was introduced into thiosemicarbazide with a suitable cyclizing agent. A mixture of thiosemicarbazide (0.01 mol), arylcarboxylic acid (0.01 mol) and concentrated H_2SO_4 (5 drops) was refluxed for 1 h and poured onto crushed ice. The solid separated was filtered, washed with water and treated with $NaHCO_3$ (10 %) to remove the acidic impurities and finally recrystallized from distilled alcohol to get pure compounds.

Preparation of dipeptides: Boc-amino acids and amino acid methyl esters were prepared according to literature procedure[20]. Amino acid methyl ester hydrochloride (10 mmol) was dissolved in chloroform (20 mL). To this, N-methyl

morpholine (10 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 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) (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. Petroleum ether was added to the crude product at 0 ºC to recrystallize the pure product. Different dipeptides were prepared in this manner.

Preparation of 2-amino-5-aryl-1,3,4-thiadiazolopeptides (2a-2l): 2-Amino-5-aryl-l,3,4-thiadiazoles (10 mmol) was dissolved in chloroform (20 mL). To this, N-methyl morpholine (10 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 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (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. Petroleum ether was added to the filtrate at 0 ºC to recrystallize the pure product. Finally, compounds (**2a-2l**) was deprotected at amino terminals using trifluoroacetic acid (TFA) to get the corresponding derivatives.

2-(Alanyl)-amino-5-(4-chlorophenyl)-1,3,4-thiadiazole (2a): IR spectrum (ν_{max}, cm^{-1}): 3325 (-NH_2); 3124 (-CH aromatic); 2928 (-CH aliphatic); 2890 (-CH); 1691 (-CO). ^{1}H NMR spectrum (δ, ppm): 9.006 (s, b, amide NH); 7.99-7.38 (m, Ph); 4.69-4.68 (s, b, NH_2); 2.81 (s, 3H, Me); MS spectrum, m/z: 284 $[M + 1]^+$, (brown semi-solid, yield 81 %).

2-(Prolyl)-amino-5-(4-chlorophenyl)-1,3,4-thiadiazole (2b): IR spectrum (ν_{max}, cm^{-1}): 3390 (-NH_2); 3005 (-CH aromatic); 2935 (-CH aliphatic); 2885 (-CH of thiazole); 1705 (-CO). ^{1}H NMR spectrum (δ, ppm): 11.14 (s, CH); 7.98 (s, CH); 7.90 (s, amide NH); 7.36- 7.76 (m, Ph); 4.8(m, H) 2.48 (s, Me); 1.8 (m 6H, Me). MS spectrum, m/z: 350 $[M + 1]^+$, (yellow solid, yield 77 %).

2-(Phenylalanyl)-amino-5-(2,4-dichlorophenyl)-1,3,4-thiadiazole (2c): IR spectrum (ν_{max}, cm^{-1}): 3440 (-NH_2); 3115 (-CH aromatic); 2960 (-CH aliphatic); 2880 (-CH of thiazole); 1690 (-CO). ^{1}H NMR spectrum (δ, ppm): 11.14 (s, CH); 8.14 (s, amide NH); 7.98 (s, CH); 7.30-7.68 (m, Ph); 3.68 (m, 2H, CH_2). MS spectrum, m/z: 293 $[M + 1]^+$, (yellow semi-solid, yield 69 %).

2-(Threonyl)-amino-5-(2.4-dichlorophenyl)-1,3,4-thiadiazole (2d): IR spectrum (ν_{max}, cm^{-1}): 3445 (-NH_2); 3110 (-CH aromatic); 2960 (-CH aliphatic); 2880 (-CH of thiazole); 1690 (-CO). ^{1}H NMR spectrum (δ, ppm): 10.90 (s, CH); 8.14 (s, amide NH); 7.98 (s, CH); 7.30-7.68 (m, Ph); 4.8 (m, H); 3.68 (m, 2H, CH_2 of phenylalanine). MS spectrum, m/z: 427 $[M + 1]^+$, (yellow semi-solid, yield 71 %).

2-(Tyrosyl)-amino-5-(4-methoxyphenyl)-1,3,4-thiadiazole (2e): IR spectrum (ν_{max}, cm^{-1}): 3445 (-NH_2); 3110 (-CH aromatic); 2960 (-CH aliphatic); 2880 (-CH of thiazole); 1690 (-CO). ^{1}H NMR spectrum (δ, ppm): 10.16 (s, CH); 8.39 (s, amide NH); 7.86 (s, CH); 7.25-7.63 (m, Ph); 4.2 (m, H); 3.30 (m, 2H, CH_2 of tyrosine). MS spectrum, MS spectrum, m/z: 400 $[M + 1]^+$, (brown solid, yield 78 %).

2-(Leucyl)-amino-5-(4-methoxyphenyl)-1,3,4-thiadiazole (2f): IR spectrum (ν_{max}, cm^{-1}): 3390 (-NH_2); 3005 (-CH aromatic); 2935 (-CH aliphatic); 2885 (-CH of thiazole); 1705 (-CO). ^{1}H NMR spectrum (δ, ppm): 10.05 (s, CH); 7.87 (s, CH); 8.20 (s, amide NH); 7.21- 7.25 (m, Ph); 4.16 (m, H); 2.81 (s, Me); 1.41-1.45 (m 6H, Me). 5.55(s, OH). MS spectrum, m/z: 366 $[M + 1]^+$, (brown semi-solid, yield 76 %).

2-(Valyl)-amino-5-(2-methylphenyl)-5*H*-thiazolo[4,3-b]-1,3,4-thiadiazole (2g): IR spectrum (ν_{max}, cm^{-1}): 3390 (-NH_2); 3005 (-CH aromatic); 2935 (-CH aliphatic); 2885 (-CH of thiazole); 1705 (-CO). ^{1}H NMR spectrum (δ, ppm): 11.14 (s, CH); 7.98 (s, CH); 7.90 (s, amide NH); 7.36-7.76 (m, Ph); 4.8(m, H) 2.48 (s, Me); 1.8 (m 6H, Me). MS spectrum, m/z: 350 $[M + 1]^+$, (yellow semi-solid, yield 72 %).

2-(Glycyl)-amino-5-(2-methylphenyl)-1,3,4-thiadiazole (2h): IR spectrum (ν_{max}, cm^{-1}): 3405 (-NH_2); 3005 (-CH aromatic); 2955 (-CH aliphatic); 2875(-CH of thiazole); 1715 (-CO). ^{1}H NMR spectrum (δ, ppm): 9.63 (s, CH); 7.83 (s, CH); 8.19 (s, amide NH); 7.18-7.34 (m, Ph); 4.15(m, H); 1.28 (1H, mH); 1.42 (m 6H, Me). 5.28 (s, OH). MS spectrum, m/z: 351 $[M + 1]^+$, (brown solid, yield 75 %).

2-(Glycyl-alanyl)-amino-5-(4-chlorophenyl)-1,3,4-thiadiazole (2i): IR spectrum (ν_{max}, cm^{-1}): 3445 (-NH_2); 3110 (-CH aromatic); 2960 (-CH aliphatic); 2880 (-CH of thiazole); 1690 (-CO). ^{1}H NMR spectrum (δ, ppm): 9.90 (s, CH); 9.30 (s, amide NH); 6.27 (s, CH); 7.26-7.72 (m, Ph); 3.09 (m, H); 3.02 (m, 2H, CH_2); 1.45 (s, 3H, Me). MS spectrum, m/z: 408 $[M + 1]^+$, (brown semi-solid, yield 78 %).

2-(Glycyl-alanyl)-amino-5-(2,4-dichlorophenyl)-1,3,4-thiadiazole (2j): IR spectrum (ν_{max}, cm^{-1}): 3440 (-NH_2); 3115 (-CH aromatic); 2960 (-CH aliphatic); 2880 (-CH of thiazole); 1690 (-CO). ^{1}H NMR spectrum (δ, ppm): 10.30 (s, CH); 8.14 (s, amide NH); 7.97 (s, CH); 7.26-7.70 (m, Ph); 4.10 (m, H); 1.55 (1H, mH); 1.06-1.28 (m 12H, Me). MS spectrum, m/z: 432 $[M + 1]^+$, (brown semi-solid, yield 66 %).

2-(Valyl-valyl)-amino-5-(4-methoxyphenyl)-1,3,4-thiadiazole (2k): IR spectrum (ν_{max}, cm^{-1}): 3445 (-NH_2); 3110 (-CH aromatic); 2960 (-CH aliphatic); 2880 (-CH of thiazole); 1690 (-CO). ^{1}H NMR spectrum (δ, ppm): 9.90 (s, CH); 9.30 (s, amide NH); 6.27 (s, CH); 7.26-7.72 (m, Ph); 3.09 (m, H); 3.02 (m, 2H, CH_2); 1.45 (s, 3H, Me) . MS spectrum, m/z: 408 $[M + 1]^+$, (brown semi-solid, yield 73 %).

2-(Valyl-valyl)-amino-5-(2-methylphenyl)-1,3,4-thiadiazole (2l): IR spectrum (ν_{max}, cm^{-1}): 3440 (-NH_2); 3115 (-CH aromatic); 2960 (-CH aliphatic); 2880 (-CH of thiazole); 1690 (-CO). ^{1}H NMR spectrum (δ, ppm): 10.30 (s, CH); 8.14 (s, amide NH); 7.97(s, CH); 7.26-7.70 (m, Ph); 4.10 (m, H); 1.55 (1H, mH); 1.06-1.28 (m 12H, Me). MS spectrum, m/z: 432 $[M + 1]^+$, (brown semi-solid, yield 75 %).

Pharmacological studies

Antitubercular activity: The antimycobacterial activity of compounds (**2a-2l**) was assessed against *M. tuberculosis* $H_{37}Rv$ (ATCC 2729411) using the microplate Alamar blue assay (MABA)[21,22]. This methodology is nontoxic, uses thermally stable reagent and showed good correlation with proportional and BACTEC radiometric methods[23] and the activity is expressed as the minimum inhibitory concentration (MIC) in μg/mL. The final drug concentration tested was 0.01-100 μg/mL. A blue

colour in the well was interpreted as no bacterial growth and pink colour was scored as growth. The MIC (minimal inhibition concentration) was defined as the lowest drug concentration, which prevented a colour change from blue to pink. The MICs of the compounds were given in Table-1. Streptomycin and pyrazinamide were used as standards. All the tested compounds showed better *in vitro* activity against streptomycin and pyrazinamide as standard drugs. A closer look into the biological results of the above compounds revealed that in a given thiadiazolopeptides, increasing the substitution on nitrogen gave better activity, perhaps due to increase in hydrophobicity, resulting into better penetration of the Mtb cell wall.

TABLE-1
ANTITUBERCULAR ACTIVITY OF 2-AMINO-5-ARYI-5*H*-THIAZOLO[4,3-B]-L,3,4-THIADIAZOLOPEPTIDES

Compound	R	Amino acids/peptides	MIC (µg/mL)
2a	4-Chloro	-Ala	25
2b	4-Chloro	-Pro	3.12
2c	2,4-Dichloro	-Phe	3.12
2d	2,4-Dichloro	-Thr	6.25
2e	4-Methoxy	-Tyr	6.25
2f	4-Methoxy	-Leu	6.25
2g	2-Methyl	-Val	0.8
2h	2-Methyl	-Gly	3.12
2i	4-Chloro	-Gly-Ala	1.6
2j	2,4-Dichloro	-Gly-Ala	1.6
2k	4-Methoxy	-Val-Val	3.12
2l	2-Methyl	-Val-Val	0.2
Streptomycin	–	–	6.25
Pyrazinamide	–	–	3.12

The MIC values were evaluated at concentration range, 0.1-100 µg/mL. The figure in the table showed the value in µg/mL.

Antibacterial and antifungal activity: Compounds (**2a-2l**) were screened for antibacterial and antifungal activities using the disc diffusion method[24,25] by measuring the zone of inhibition. A 24 h culture of bacterial strains of *S. aureus* ATCC 12598, *E. fecalis* ATCC 35550, *K. pneumonia* ATCC 29665 and *E. coli* ATCC 25922 were cultivated in brain heart infusion agar medium and the fungal strains of *A. niger* ATCC 9029 and *C. albicans* ATCC 2091 were cultivated in Sabouraud agar medium, respectively. All the compounds were tested at different concentration level. Dimethyl formamide was used as a solvent and as control. Ciprofloxacin and fluconazole were used as standards for comparison of the results. The diameter of zone of inhibition was measured in millimeter (mm) after 24 h incubation at 37 ºC. The antibacterial activity results are tabulated in Table-2 and antifungal in Table-3.

RESULTS AND DISCUSSION

All thiadiazolopeptide derivatives **2a-2l** were synthesized in good yields using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide as coupling agents and N-methyl morpholine as base and the structures were confirmed by IR, ^{1}H NMR and mass spectral data. The synthesis of compounds **3a-3j** is mentioned in **Scheme-I** and their physicochemical data is described in Table-4. IR spectra of peptide derivatives **2a-2l** showed amide I and amide II bands at 1639-1662 and 1538-1531 cm^{-1} indicating formation of peptide bonds and successfulness of coupling

TABLE-2
ANTIBACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS

Compounds	MIC in µg/mL and zone of inhibition in mm gram +ve bacteria		gram –ve bacteria	
	S. aureus	*E. fecalis*	*K. pneumonia*	*E. coli*
2a	50 (12)	25 (<5)	75 (<5)	75 (<5)
2b	50 (22)	25 (10)	50 (5)	75 (<5)
2c	25 (<5)	25 (<5)	75 (5)	75 (<5)
2d	50 (12)	5 (11)	50 (8)	75 (<5)
2e	50 (18)	25 (8)	75 (<5)	75 (<5)
2f	25 (20)	25 (<5)	50 (15)	75 (<5)
2g	25 (<5)	25 (<5)	75 (10)	75 (<5)
2h	25 (<5)	25 (12)	75 (<5)	75 (<5)
2i	25 (<5)	50 (5)	50 (5)	50 (8)
2j	5 (12)	5 (11)	50 (5)	50 (5)
2k	25 (12)	25 (12)	50 (5)	50 (7)
2l	5 (12)	5 (10)	25 (8)	50 (6)
DMF (control)	–	–	–	–
Ciprofloxacin (standard)	10 (32)	10 (32)	10 (32)	10 (32)

The MIC values were evaluated at concentration range, 5-75 µg/mL. The figure in the table showed the value in µg/ml and the corresponding zone of inhibition in millimeter (mm).

TABLE-3
ANTIFUNGAL ACTIVITY OF SYNTHESIZED COMPOUNDS

Compounds	MIC in µg/mL and zone of inhibition in mm	
	Aspergillus niger	*Candida albicans*
2a	75 (<5)	75 (10)
2b	75 (<5)	50 (7)
2c	75 (<5)	75 (<5)
2d	50 (9)	75 (<5)
2e	75 (9)	75 (<5)
2f	75 (<5)	75 (<5)
2g	25 (8)	25 (9)
2h	50 (8)	50 (8)
2i	10 (10)	25 (10)
2j	50 (12)	25 (8)
2k	10 (10)	25 (6)
2l	50 (10)	50 (11)
DMF (control)	–	–
Fluconazole (standard)	25 (26)	25 (26)

The MIC values were evaluated at concentration range, 5-75 µg/mL. The figure in the table showed the value in µg/mL and the corresponding zone of inhibition in millimeter (mm).

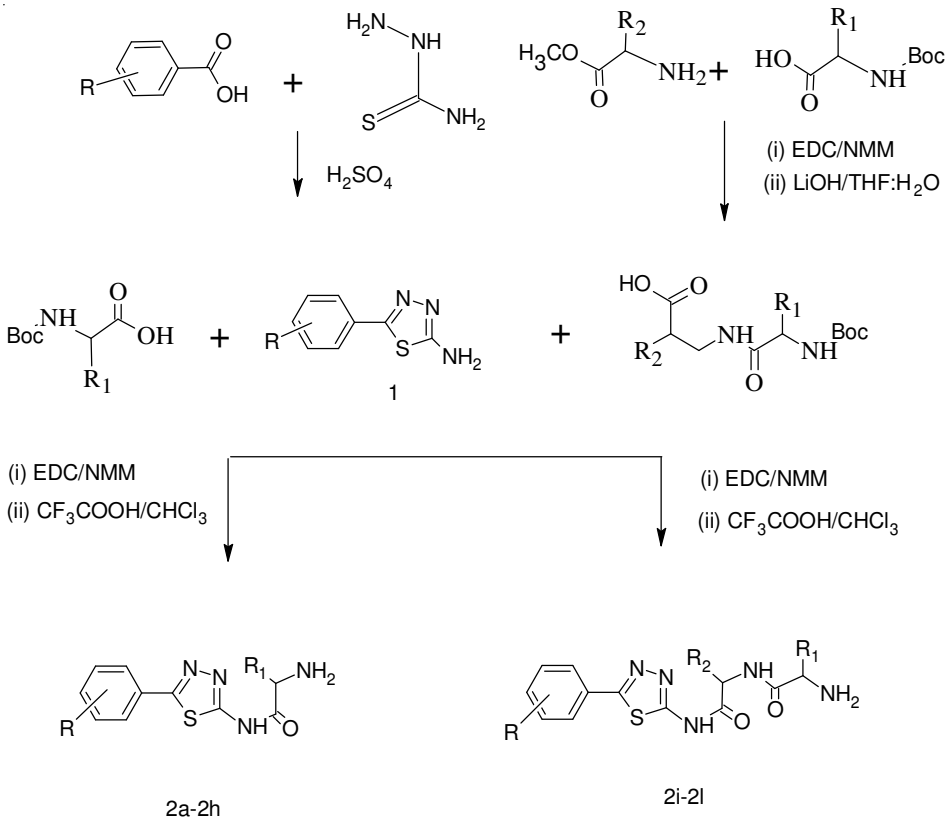
reaction. This fact was further confirmed by appearance of broad singlets at 9.32-6.96 ppm (for imino proton of CO-NH moiety) in ^{1}H NMR spectra of compounds **2a-2l**. Mass spectra of peptide ester derivatives showed molecular ion peaks along with isotopic peaks at m/z values, consistent with their respective molecular formulas.

Conclusion

Present investigation describes successful synthesis of title compounds *via* coupling reaction in good yields. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide proved to be effective coupling agent both economically and yield wise, in comparison to DIPC. All the compounds showed potent antitubercular activity as compared to standard drugs. The Antitubercular activity study revealed that increasing the substitution at nitrogen showed good activity than standard streptomycin and pyrazinamide. They were effective in inhibiting *M. tuberculosis* infection at 0.2, 0.8, 1.6 and 3.12 µg/mL concentrations and could be a

TABLE-4
PHYSICAL DATA OF SYNTHESIZED COMPOUNDS

Compound	R	Amino acids/peptides	Physical state	m.p. (°C)	Yield (%)
2a	4-Chloro	-Ala	Brown semi-solid	–	81
2b	4-Chloro	-Pro	Yellow solid	136	77
2c	2,4-Dichloro	-Phe	Yellow semi-solid	–	69
2d	2,4-Dichloro	-Thr	Yellow semi-solid	–	71
2e	4-Methoxy	-Tyr	Brown solid	86	78
2f	4-Methoxy	-Leu	Brown semi-solid	–	76
2g	2-Methyl	-Val	Yellow semi-solid	–	72
2h	2-Methyl	-Gly	Brown solid	150	75
2i	4- Chloro	-Gly-Ala	Brown semi-solid	–	78
2j	2,4-Dichloro	-Gly-Ala	Brown semi-solid	–	66
2k	4-Methoxy	-Val-Val	Brown semi-solid	–	73
2l	2-Methyl	-Val-Val	Brown semi-solid	–	75



where R_1 and R_2 are the side chains of amino acids; EDC = 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, NMM = N-methyl morpholine

Scheme-I: R=4-Chloro, R_1 = Ala (**2a**), R = 4-Chloro, R_1 = Pro (**2b**), R = 2,4-dichloro, R_1 = Phe (**2c**), R = 2,4-dichloro, R_1 = Thr (**2d**), R = 4-methoxy, R_1 = Tyr (**2e**), R = 4-methoxy, R_1 = Leu (**2f**), R = 2-methyl, R_1 = Val (**2g**), R = 2-methyl, R_1 = Gly (**2h**),R = -4-Chloro, R_1 = Gly, R_2 = Ala (**2i**), R = -2,4-dichloro, R_1 = Gly, R_2 = Ala (**2j**), R = -4-methoxy, R_1 = Val, R_2 = Val (**2k**), R = -2-methyl, R_1 = Val, R_2 = Val (**2l**)

good start point for further studies, as well as to find new lead compounds with different framework which are also not cytotoxic to host cells at the same concentration. Greater anti-fungal activity was found in many derivatives. Gram negative bacteria were found to be more sensitive in comparison to gram positive bacteria towards the newly synthesized peptide derivatives.

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