RJC

RASĀYAN *J. Chem.*
Vol. 15 | No. 2 |875-884| April - June | 2022
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

THE DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF THE PEPTIDE DERIVATIVES CONTAINING GUANIDINE MOIETY WITH 5-CHLORO-THIOPHENE-2-CARBOXYLIC ACID CONJUGATES

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ABSTRACT

A new series of dipeptide derivatives with 5-chloro-thiophene-2-carboxylic acid conjugates have been designed and synthesized using solid-phase peptide synthesis. The synthesized dipeptide derivatives were characterized by using spectroscopic tools, like ^{1}H and ^{13}C NMR, IR, and mass and elemental analysis. Additionally, the obtained target dipeptide derivatives were evaluated for *in vitro* antimicrobial activity. The antibacterial activity of the target compounds was evaluated against four bacteria (two Gram-positive; *Streptococcus pyogenes* and *Staphylococcus aureus*, two Gram-negative; *Escherichia coli* and *Pseudomonas aeruginosa*). The screened analogs showed good antibacterial activity. Noteworthily, among them, Thiophene-Tyr-Arg-OH derivative exhibited excellent activity against *Escherichia coli* (MIC=15 µg/mL) as compared to standard drug ampicillin. The antifungal activity was evaluated against two fungi (*Aspergillus niger* and *Candida albicans*). The screened analogs also showed good antifungal activity.

Keywords: Heterocyclic Hybrids, Thiophene Conjugates, Solid Phase Peptide Synthesis, Dipeptides, Antibacterial Activity, Antifungal Activity.

RASĀYAN *J. Chem.*, Vol. 15, No.2, 2022

INTRODUCTION

Heterocyclic compounds are ubiquitous in nature having several synthetic applicability and biological activity which encourage researchers to plan the discovery of novel drugs.[1-6] Among all the available heterocyclic analogs, thiophene, a five-membered heteroaromatic compound containing a Sulphur atom, is of high importance.[7-11] Thiophene derivatives have notable applications in many disciplines. The biological importance of thiophene derivatives can be described in the following different categories which are antioxidant[12], antiviral[13], antimicrobial[14], analgesic and anti-inflammatory[15], antihypertensive[16], and antitumor[17] activities, along with condrotoxicity[18], ototoxicity[19], and retinopathy.[20] Additionally, the thiophene derivatives can be employed as corrosion inhibitors of metals[21] or in material science for the synthesis of light-emitting diodes.[22] Antimicrobial peptides are the indispensable component of the living system found in various organisms like bacteria, fungi, plants and animals.[23] There are many drugs available in the pharmaceutical market with thiophene moieties like Suprofen[24], Tenidap[25], Tienilic acid[26], Ticlopidine[27], Tigabine[28], etc. (Fig.-1).

In drug discovery, it is highly critical to determine and fine-tune the intricate networks of protein-protein interactions (PPIs) that drive the mechanisms of disease. Peptides are model motifs to determine and fine-tune the PPIs. The resurgence in therapeutic peptide development over the past two decades is due to the number of benefits they offer over the therapeutics of other classes. Peptide therapeutics are adequately endured in the biological systems, easy to insert due to their stereochemistry, thus, offering target selectivity, and can offer high efficacy during interaction with large protein surfaces. Peptides can proficiently bind to often untenable surfaces on proteins that regulate PPIs.[29-32] Additionally, they offer

http://dx.doi.org/10.31788/RJC.2022.1526907

better cell membrane permeability than small molecule drugs, which is a critical criterion for passive absorption in the gastrointestinal tract.

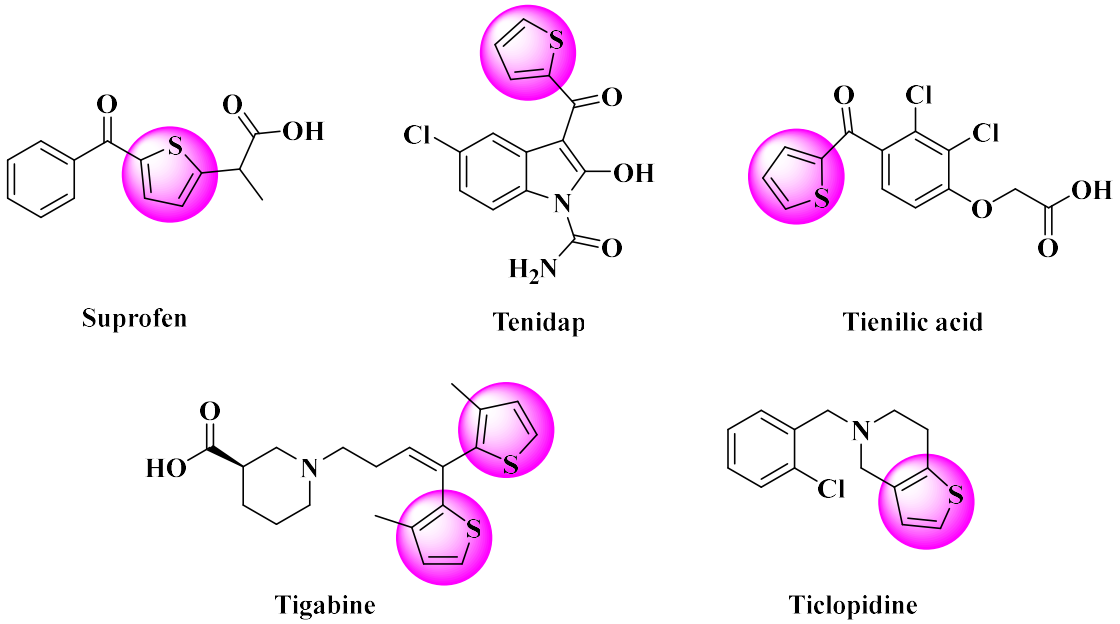


Fig.-1: Drugs containing thiophene moieties: Suprofen, Tenidap, Tienilic acid, Ticlopidine and Tiagabine.

In the light of the above facts, we have designed the synthesis of a new series of dipeptide derivatives with 5-chloro-thiophene-2-carboxylic acid at N terminus and Arginine at the C terminus (Fig.-2) and evaluated their antimicrobial activities. Moreover, we have compared the observed antibacterial and antifungal activities of the synthesized dipeptides with the standard drugs Ampicillin and Nystatin, respectively. We chose the solid phase peptide synthesis (SPPS) route for the synthesis purpose because it is simple, highly efficient, involves easy purification, rapidly generates the linear peptides, the synthesized intermediates do not require isolation and high-speed method as compared to the traditional solution-phase peptide synthesis.[33]

EXPERIMENTAL

Material and Methods

The resin was procured from Merck. All the amino acids used are L and Fmoc protected and were procured from Sichuan China. Melting points were obtained by an open capillary method and are uncorrected. The coupling and deprotection reactions were monitored by the Kaiser test. Compounds were purified by preparative HPLC or precipitation using ethyl acetate and hexane mixtures. IR spectra were recorded on Nicolet impact 400 FT/IR spectrometer using KBr pressed pellet technique. MASS spectra were recorded on Shimadzu LC-MS (at 70 eV) Mass Spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Neo 400 MHz NMR spectrometer using DMSO-d_6 solvent.

General Procedure for the Synthesis of Intermediates 1a-j

The 2-chlorotrityl chloride (2-CTC) resin (substitution 1.0 mmol/g) was added to the peptide synthesizer, washed with 10 volumes of dichloromethane (DCM) and drained. Further, added 10 volumes of DCM and stirred for 1h (swelling) then drained. Dissolved Fmoc-Arg(pbf)-OH (3 eq.) in dry DCM (10 ml/gm) and added diisopropylethylamine (DIPEA) (4 eq.). Added the amino acid solution to the resin. Agitated the reaction mixture vigorously for 2 h. Filtered and washed the peptidyl resin twice with DCM and then twice with dimethylformamide (DMF). Capped the unreacted functional group of resin by using DCM, methanol and DIPEA (85:10:5). Loading percentage monitored by UV absorption in the range of 300 nm.

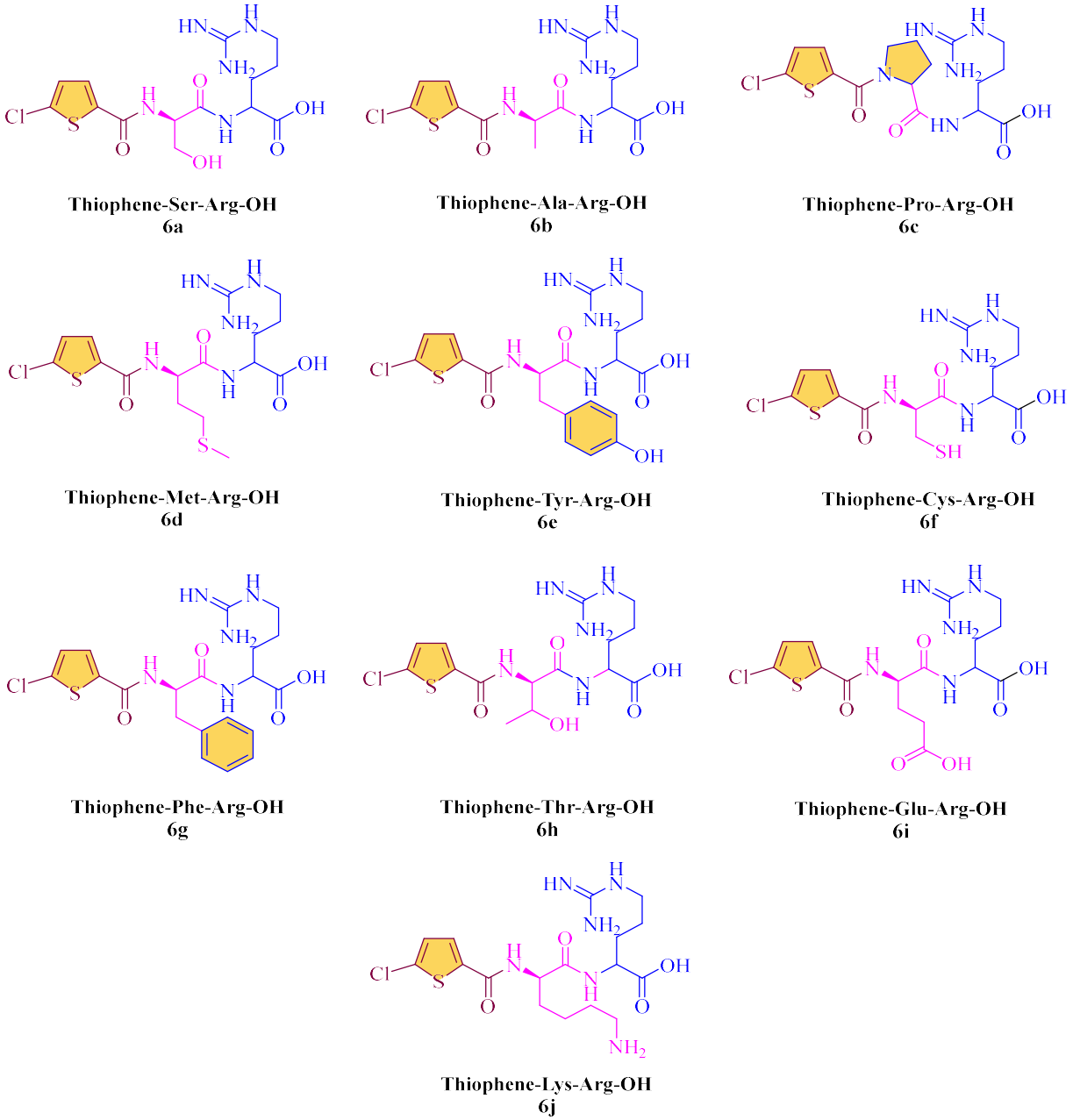


Fig.-2: Peptide Library of titled dipeptide derivatives **6a-j**.

General Procedure for the Synthesis of Intermediate 5a-j

The standard SPPS method was applied with Fmoc/*t*Bu/boc protocol. Fmoc-Arg(Pbf)-2-CTC resin (loading 1 mmol/g) was placed into a reaction vessel and allowed to swell in DMF (20 v with respect to resin weight) for 30 min. After swelling, the peptides were synthesized using CSBio automated peptide synthesizer by repetition of the following steps: (1) Fmoc deprotection performed (wash) with a solution of 20% piperidine in DMF (v/v) two times; 5 minutes and 10 minutes (10 v with respect to the initial weight of resin). The reaction completion was monitored by the Kaiser test (the blue color solution was observed, complies). After completion of the synthesis, the peptidyl resin was filtered and then washed with DMF (×2), IPA (×1) and DMF (×3). (2) Coupling reactions performed with 3.0 eq. (according to resin initial loading) of Fmoc amino acid (3 mmol, DMF 6 v with respect to initial weight of resin), 3 eq. of HBTU (3 mmol) and 6 eq. of DIPEA (6 mmol). Each coupling step was stirred for 1 h.[34] The reaction was monitored using Kaiser test (colorless beads and solution were observed, complies)[35] and the peptidyl resin was filtered and then washed with DMF (×5).

General Procedure for the Synthesis of Intermediate 6a-j

The protected peptidyl resin was treated with a mixture of TFA:TIS:H_2O (90:5:5) (10 mL/gm) at 20 °C for 3 h.[36] The mixture was precipitated with DIPE (50 volume), filtered and washed with 3x10 volume diisopropylether (DIPE). The filter cake was dried under a vacuum at room temperature to obtain the crude.

Compound-6a (5-chlorothiophene-2-carbonyl)-D-serylarginine

79.0% yield; mp 186-192 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.64 (s, 1H), 8.62 (s, 1H), 8.27 (s, 1H), 8.02, (d, 1H), 7.76 (s, 1H),6.80 (d, 1H), 6.62 (s, 2H), 4.91 (s, 1H), 4.54 (t, 1H), 4.20 (t, 1H), 3.91 (d, 2H), 3.35 (m, 2H), 1.77 (m, 2H), 1.55 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 173.8, 170.2, 160.7, 157.2, 139.2, 133.5, 129.2, 128.5, 72.5, 61.9, 56.5, 40.7, 28.6, 25.5; LCMS (ESI): *m/z* = 406.85 [M^+ 100%]. Anal. Calcd. for $C_{14}H_{20}ClN_5O_5S$: C, 41.43; H, 4.97; Cl, 8.73; N, 17.26; O, 19.71; S, 7.90; Found C, 41.40; H, 4.95; Cl, 8.77; N, 17.20; O, 19.76; S, 7.93 %.

Compound-6b (5-chlorothiophene-2-carbonyl)-D-methionylarginine

77.0% yield; mp 202-206 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm):12.65(s, 1H), 8.76 (s, 1H), 8.31 (s, 1H), 8.07 (d, 1H), 7.85 (s, 1H), 6.85 (d, 1H), 6.64(s, 2H), 4.53 (t, 1H), 4.49 (t, 1H), 3.33 (t, 2H), 2.49 (s, 1H), 2.02-2.60 (m, 4H), 2.05 (s, 3H), 1.75 (t, 2H), 1.50 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 174.6, 171.0, 161.2, 158.0, 140.6, 138.2, 135.8, 129.7, 56.3, 56.0, 41.5, 31.5, 29.6, 29.2, 24.5, 15.3; LCMS (ESI): *m/z* = 450.91 [M^+ 100%]. Anal. Calcd. for $C_{16}H_{24}ClN_5O_4S_2$: C, 42.71; H, 5.38; Cl, 7.88; N, 15.56; O, 14.22; S, 14.25; Found C, 42.70; H, 5.39; Cl, 7.89; N, 15.55; O, 14.21; S, 14.26%.

Compound-6c (5-chlorothiophene-2-carbonyl)-D-cysteinylarginine

65.2% yield; mp 186-192 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.60 (s, 1H), 8.79 (s 1H), 8.41 (s, 1H), 8.08 (d, 1H), 7.82 (s, 1H), 6.80 (d, 1H), 6.66 (s, 2H), 4.64 (t, 1H), 4.51 (t, 1H), 3.31 (t, 2H), 3.15 (d, 2H), 2.49 (s, 1H), 1.71 (m, 2H), 1.51 (m, 2H), 1.42 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 173.81, 171.74, 160.76, 157.32, 138.92, 133.78, 129.31, 128.62, 56.54, 52,35, 40.77, 31.22, 29.47, 28.47; LCMS (ESI): *m/z* = 422.90 [M^+ 100%]. Anal. Calcd. For $C_{14}H_{20}ClN_5O_4S_2$: C, 39.85; H, 4.78; Cl, 8.40; N, 16.60; O, 15.17; S, 15.20; Found: C, 39.74; H, 4.68; Cl, 8.39; N, 16.50; O, 15.11; S, 15.58%.

Compound-6d (5-chlorothiophene-2-carbonyl)-D-tyrosylarginine

69.2% yield; mp 201-205 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.65 (s, 1H), 9.09 (s, 1H), 8.82 (s, 1H), 8.59 (s, 1H), 8.12 (d, 1H), 7.85 (s, 1H), 6.92 (d, 1H), 6.72 (s, 2H), 6.30-6.90 (d, 4H), 4.93 (t, 1H), 4.62 (t, 1H), 3.42 (d, 2H), 3.37 (t, 2H), 2.54 (s, 1H), 1.74 (m, 2H), 1.50 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 174.6, 171.5, 161.3, 158.1, 155.6, 140.0, 138.3, 135.9, 130.2, 129.7, 129.1, 115.7, 56.9, 56.2, 41.5, 37.3, 29.6, 24.6; LCMS (ESI): *m/z* = 482.95 [M^+ 100%]. Anal. Calcd. for $C_{20}H_{24}ClN_5O_5S$: C, 49.84; H, 5.02; Cl, 7.36; N, 14.53; O, 16.60; S, 6.65; Found: C, 49.82; H, 5.04; Cl, 7.35; N, 14.54; O, 16.61; S, 6.64.

Compound-6e (4R)-5-((1-carboxy-4-guanidinobutyl)amino)-4-(5-chlorothiophene-2 carboxamido)-5-oxopentanoic acid

62.9% yield; mp 184-190 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.61-12.01 (s, 2H), 8.78 (s, 1H), 8.32 (s, 1H), 8.01 (d, 1H), 7.81 (s, 1H), 6.88 (d, 1H), 6.60 (s, 2H), 4.46(t, 1H), 4.22 (t, 1H), 3.30 (m, 2H), 2.49 (s.1H), 2.33 (t, 2H,), 2.06 (m, 2H), 1.71 (m, 2H), 1.51 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 174.43, 173.70, 171.76, 160.67, 157.33, 139.04, 133.68, 129.16, 128.50, 72.50, 53.01, 52.11, 49.13, 30.84, 28.41, 25.66; LCMS (ESI): *m/z* = 448.89 [M^+ 100%]. Anal. Calcd. for $C_{16}H_{22}ClN_5O_6S$: C, 42.91; H, 4.95; Cl, 7.91; N, 15.64; O, 21.43; S, 7.16; Found: C, 42.82; H, 4.91; Cl, 7.93; N, 15.55; O, 21.33; S, 7.46%.

Compound-6f (5-chlorothiophene-2-carbonyl)lysylarginine

66.7% yield; mp 192-196 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.11 (s, 1H), 8.67 (s, 1H), 8.32 (s, 1H), 8.81 (d, 2H), 7.81 (s, 1H), 6.80 (d, 1H), 6.62 (s, 2H), 4.55 (t, 1H), 4.21 (s, 1H), 3.43 (t, 2H), 2.49

(s, 1H), 1.87-1.75 (m, 4H), 1.52 (m, 2H), 1.50 (s, 2H), 1.20 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 174.5, 171.0, 161.1, 157.0, 140.1, 138.2, 135.2, 129.1, 57.1, 56.5, 42.1, 40.5, 31.3, 29.4, 28.4, 24.5, 22.2 ; LCMS (ESI): *m/z* = 447.9 [M^+ 100%]. Anal. Calcd. for $C_{17}H_{27}ClN_6O_4S$: C, 45.68; H, 6.09; Cl, 7.93; N, 18.80; O, 14.32; S, 7.17; Found: C, 45.58; H, 6.15; Cl, 7.89; N, 18.04; O, 14.42; S, 7.92%.

Compound-6g (5-chlorothiophene-2-carbonyl)-D-alanylarginine
69.4% yield; mp 180-185 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.64 (s, 1H), 8.77 (s, 1H), 8.32 (s, 1H), 8.07 (d, 1H), 7.84 (s, 1H), 6.85 (d, 1H), 6.66 (s, 2H), 4.65 (m, 1H), 4.53 (m, 1H), 3.34 (t, 2H), 2.5 (s, 1H), 1.7 (m, 2H), 1.50 (m, 2H), 1.47 (d, 3H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 174.7, 171.7, 161.3, 158.0, 140.7, 138.3, 135.9, 129.8, 56.1, 53.6, 41.6, 29.7, 24.6, 17.9; LCMS (ESI): *m/z* = 390.86 [M^+ 100%]. Anal. Calcd. for $C_{14}H_{20}ClN_5O_4S$: C, 43.13; H, 5.17; Cl, 9.09; N, 17.96; O, 16.42; S, 8.22; Found: C, 43.12; H, 5.18; Cl, 9.08; N, 17.97; O, 16.42; S, 8.22%.

Compound-6h (5-chlorothiophene-2-carbonyl)prolylarginine
74.1% yield; mp 182-187 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.55 (s, 1H), 8.38 (s, 1H), 8.08 (d, 1H), 7.75 (s, 1H) 6.70 (d, 1H), 6.60 (s, 2H), 4.50 (t, 1H), 4.39 (t, 1H), 3.55 (t, 2H), 3.32 (t, 2H), 2.49 (s, 1H), 2.11 (m, 2H), 1.82 (m, 2H), 1.77 (m, 2H), 1.55 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 173.81, 171.74, 160.76, 157.32, 138.92, 133.78, 129.31,128.62, 56.54, 52,35, 40.77, 31.22, 29.47, 28.47; LCMS (ESI): *m/z* = 416.89 [M^+ 100%]. Anal. Calcd. For $C_{16}H_{22}ClN_5O_4S$: C, 46.21; H, 5.33; Cl, 8.52; N, 16.84; O, 15.39; S, 7.71; Found: C, 46.20; H, 5.34; Cl, 8.52; N, 16.84; O, 15.38; S, 7.72%.

Compound-6i ((2R)-2-(5-chlorothiophene-2-carboxamido)-3-hydroxybutanoyl)arginine 70.4% yield; mp 187-191 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.66 (s, 1H), 8.78 (s, 1H), 8.32 (s, 1H), 8.08 (d, 1H), 7.84 (s, 1H), 6.86 (d, 1H), 6.60 (s, 2H), 5.37 (d, 1H), 4.62 (m, 1H), 4.55 (t, 1H), 4.44 (d, 1H), 3.35(m, 2H), 2.50 (s, 1H), 1.70 (m, 2H), 1.51 (m, 2H), 1.16 (d, 3H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 174.7, 172.6, 161.3, 158.0, 140.7, 138.3, 135.9, 129.8, 67.6, 62.4, 56.1, 41.6, 29.7, 24.6, 19.0; LCMS (ESI): *m/z* = 420.88 [M^+100%]. Anal. Calcd. for $C_{15}H_{22}ClN_5O_5S$: C, 42.91; H, 5.28; Cl, 8.44; N, 16.68; O, 19.05; S, 7.64 Found: C, 42.95; H, 5.32; Cl, 8.45; N, 16.77; O, 19.02; S, 7.63%.

Compound-6j (5-chlorothiophene-2-carbonyl)-D-phenylalanylarginine
66.1% yield; mp 202-207 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.55 (s, 1H), 8.77 (s, 1H) 8.30 (s, 1H), 8.07 (d, 1H), 7.82 (s, 1H), 7.32-6.71 (d, 6H), 6.60 (s, 2H), 4.50 (t, 1H), 4.38 (t, 1H), 3.42 (t, 2H), 3.32 (d, 2H), 2.49 (s, 1H), 1.71 (m, 2H), 1.55 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 174.21, 171.59, 160.50, 158.79, 157.34, 139.03, 138.66, 133.56, 129.59, 128.98, 128.55, 126.75, 60.91, 55.15, 52.65, 37.55, 27.29, 25.74; LCMS (ESI): *m/z* = 466.95 [M^+ 100%]. Anal. Calcd. for $C_{20}H_{24}ClN_5O_4S$: C, 51.55; H, 5.19; Cl, 7.61; N, 15.03; O, 13.73; S, 6.88; Found: C, 51.45; H, 5.29; Cl, 7.52; N, 15.17; O, 13.65; S, 6.65%.

Biological Activity
Antibacterial Activity Assay
The strains used were newly procured and stored under appropriate conditions. The compounds were screened for their antibacterial activity in triplicates against the bacteria at different concentrations of 1000, 500, 250, 200 µg/mL. The compounds which were found to be effective in inhibition were further diluted and tested. Muller Hinton Broth dilution method was used for the antibacterial assay. 10 µg/mL suspension of respective bacteria were inoculated on appropriate media and growth was noted after incubation at 37 °C for a period of one or two days. The test mixture should contain 10^8 cells/mL. In this study, ampicillin was used as a standard drug for evaluating the antibacterial activity.

Antifungal Activity Assay
The strains used were newly procured and stored under appropriate conditions. The compounds were screened for their antifungal activity in triplicates against the bacteria at different concentrations of 1000, 500, 250, 200 µg/mL. The compounds which were found to be effective in inhibition were further diluted

and tested. Muller Hinton Broth dilution method was used for the antifungal assay. In this study, for fungal growth, Sabouraud's dextrose broth was used at 28 °C in aerobic conditions for 48 hours. Nystatin was used as a standard for comparison purposes.

RESULTS AND DISCUSSION

Chemistry

A new series of dipeptides derivatives with 5-chloro-thiophene-2-carboxylic acid conjugates **6a-j** was synthesized using the solid-phase peptide synthesis method. The SPPS route was employed for the synthesis purpose as it is simple, highly efficient, involves easy purification, rapidly generates the linear peptides, the synthesized intermediates do not require isolation and high-speed method as compared to traditional solution-phase peptide synthesis. The first step involves an attachment of Fmoc-Arg(pbf)-OH to the CTC resin using DIPEA in DCM (Scheme-1). Loading percentage monitored by UV absorption in the range of 300 nm. The second step involved a Fmoc deprotection using 20% piperidine in DMF. The reaction completion was monitored by the Kaiser test (the blue color solution was observed, complies). The third step was the coupling of the second amino acid using HBTU, HOBt, DIPEA in DMF. The reaction completion was monitored by the Kaiser test (the colorless solution was observed, complies). In the fourth step, a Fmoc deprotection was carried out using 20% piperidine in DMF which was followed by the fifth step which is a coupling of 5-Chloro-thiophene-2-carboxylic acid using HBTU, HOBt and DIPEA in DMF. Further, the final step was global cleavage using a cleavage cocktail to give the final deprotected product. The protected peptidyl resin was treated with a mixture of TFA:TIS:H_2O (90:5:5) (10 mL/gm) at 20 °C for 3 h. DIPE was used for the precipitation purpose to obtain the crude.

The structures of **6a-j** were characterized by ^{1}H NMR, ^{13}C NMR, IR and mass spectroscopic techniques. The IR spectra displayed the characteristic absorption bands at 3540-3598, 1610-1690 and 3303-3404 cm^{-1} for -COOH, -CONH-, and -N-H stretching, respectively. ^{1}H NMR spectrum of compounds **6a-j** showed the predictable aromatic signals, i.e., two singlets at 1.31-1.60 and 8.65-9.80 δ ppm for protons of N-H and -COOH, respectively. The complete physical date of the dipeptide derivatives **6a-j** is incorporated in Table-1.

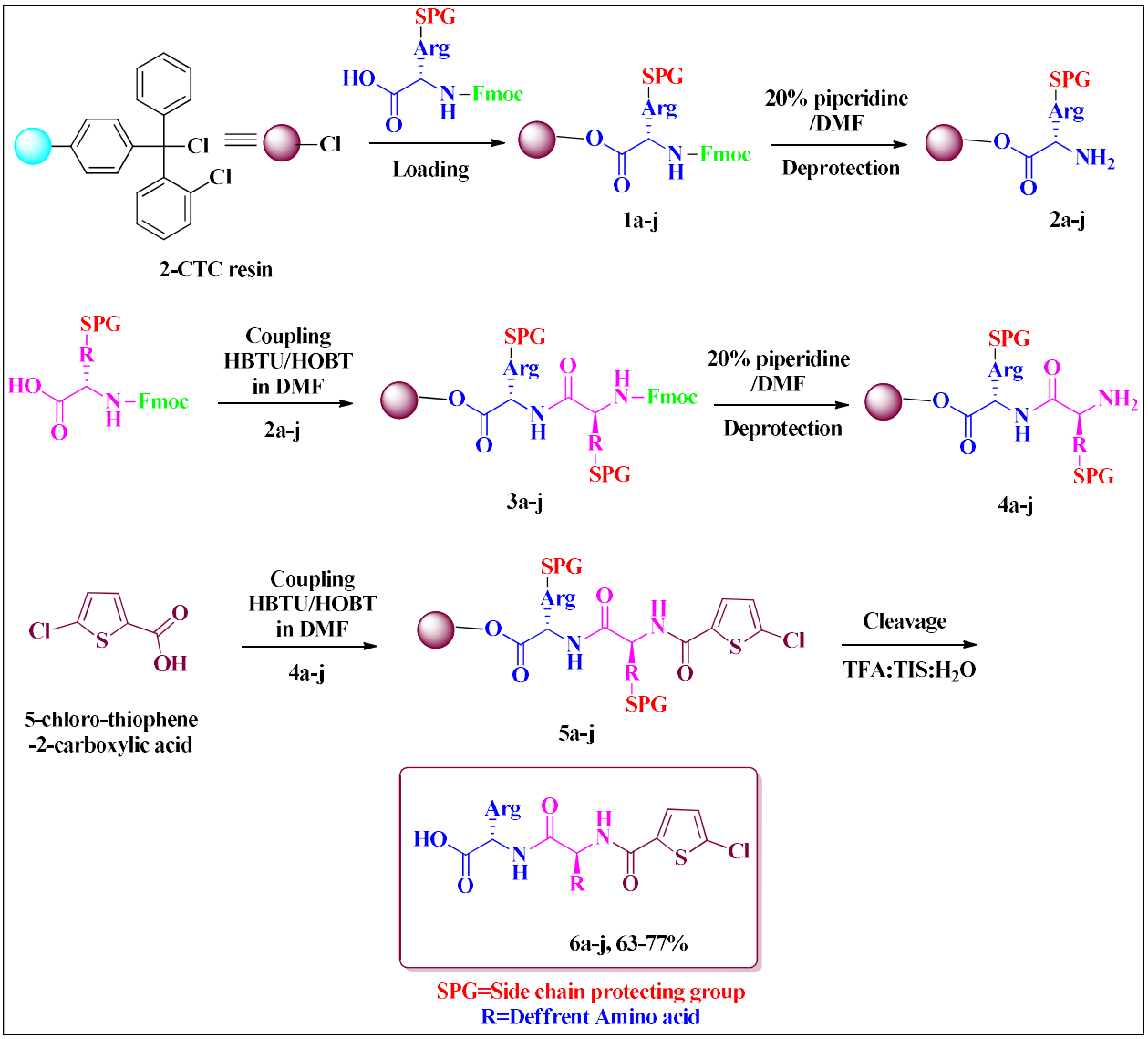
Table-1: The Complete Physical Data of Compounds 6a-j

Compound	R	M.F	M.W.	M.P.(°C)	Yield (%)
6a	$-CH_2-OH$	$C_{14}H_{20}ClN_5O_5S$	405.85	186-192 °C	70.10%
6b	$-(CH_2)_2-S-CH_3$	$C_{16}H_{24}ClN_5O_4S_2$	449.91	182-189 °C	77.00%
6c	$-CH_2-SH$	$C_{14}H_{20}ClN_5O_4S_2$	421.9	186-192 °C	65.20%
6d	$-CH_2-C_6H_5-OH$	$C_{20}H_{24}ClN_5O_5S$	481.95	201-205 °C	69.20%
6e	$-(CH_2)_2-COOH$	$C_{16}H_{22}ClN_5O_6S$	447.89	184-190 °C	62.90%
6f	$-(CH_2)_4-NH_2$	$C_{17}H_{27}ClN_6O_4S$	447.9	192-196 °C	66.70%
6g	$-CH_3$	$C_{14}H_{20}ClN_5O_4S$	389.86	180-185 °C	69.40%
6h	$-(CH_2)_3-$	$C_{16}H_{22}ClN_5O_4S$	415.89	182-187 °C	74.10%
6i	$-CH_2(-OH)-CH_3$	$C_{15}H_{22}ClN_5O_5S$	419.88	187-191 °C	70.40%
6j	$-CH_2-C_6H_5$	$C_{20}H_{24}ClN_5O_4S$	465.95	202-207 °C	66.10%

Biological Evaluation (*In vitro* Antimicrobial activity)

In vitro antimicrobial activities (MIC) of all the newly synthesized dipeptides **6a-j** were evaluated by the conventional Broth microdilution method. The antibacterial activity was evaluated against four bacteria (two Gram-positive; *Staphylococcus aureus (S. a.) MTCC 96* and *Streptococcus pyogenes (S. p.) MTCC 442*, two Gram-negative; *Escherichia coli MTCC 443 (E. c.)* and *Pseudomonas aeruginosa (P. a.) MTCC 1688*) and the results were tabulated in Table-2. Among the screened analogs, dipeptides **6c, 6d, 6f** and **6i** showed good antibacterial activity compared with the standard drug ampicillin. Additionally, the derivative **6e** showed excellent antibacterial activity against *Escherichia coli* (MIC=15 µg/mL) compared with the standard drug ampicillin (MIC=100 µg/mL) (Fig.-3).

Additionally, *in vitro* antifungal activity of all the newly synthesized dipeptides **6a-j** was evaluated against two fungal strains, *Candida albicans (C. a.) MTCC 227* and *Aspergillus niger (A. n.) MTCC 282* and the results were tabulated in Table-2. The dipeptide derivatives **6a** and **6e-j** showed good antifungal activity compared with the standard Nystatin (Fig.-4).



Scheme-1: Synthetic route for the preparation of dipeptide derivatives **6a-j**

Table-2: The Results of *In-vitro* Antibacterial and Antifungal Screenings of Compounds 6a-j

Compounds	MIC (Anti-bacterial Activity) in µg/mL				MIC (Anti-fungicidal Activity) in µg/mL	
	E. c.	*P. a.*	*S. a.*	*S. p.*	*C. a.*	*A. n.*
6a	125	150	200	250	150	200
6b	150	200	500	500	200	200
6c	50	150	250	250	250	500
6d	25	100	200	250	200	200
6e	15	25	500	500	150	500
6f	30	50	250	250	150	500
6g	150	175	200	125	150	200
6h	250	150	250	250	150	500
6i	125	100	200	250	125	200
6j	150	200	500	500	250	500
Ampicillin (std-1)	100	100	250	100	-	-
Nystatin (std-2)	-	-	-	-	100	100

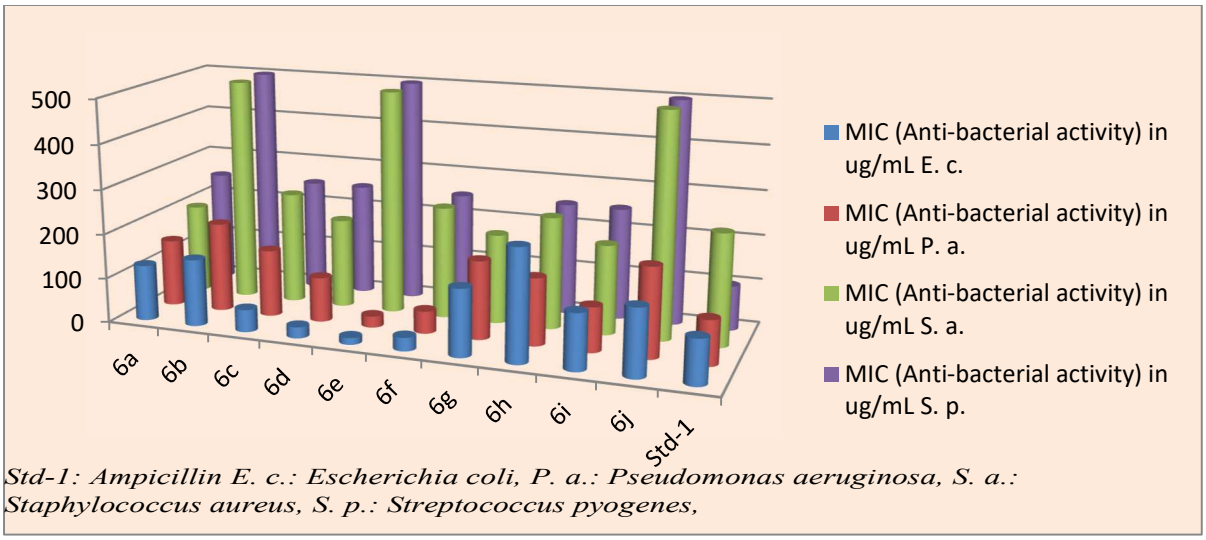


Fig.-3: The *In vitro* Antibacterial Activities of dipeptides **6a-j** against the Bacteria Strains *E. c.*, *P. a.*, *S. a.* and *S. p.* and their comparison with the Standard Drug Ampicillin

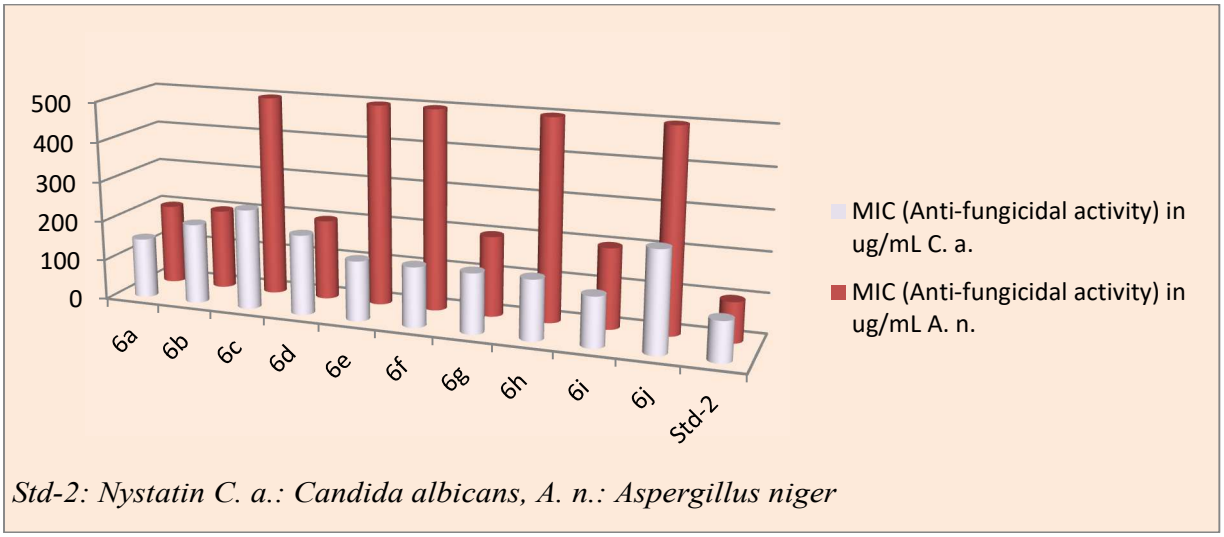


Fig.-4: The *In vitro* Antifungal Activities of dipeptides **6a-j** against the Fungal Strains *C. a.* and *A. n.* and their comparison with the Standard Drug Nystatin

CONCLUSION

In this research, we have designed and synthesized a new series of dipeptide derivatives with 5-chloro-thiophene-2-carboxylic acid conjugates **6a-j**. The characterization of the synthesized derivatives was carried out by spectroscopic techniques (^{1}H & ^{13}C NMR, IR and mass) and elemental analysis. Furthermore, with the anticipation of creating novel therapeutics aiding as effective antimicrobial agents, the obtained dipeptides were evaluated for their *in vitro* antimicrobial activities. The dipeptide derivatives **6c, 6d, 6f** and **6i** exhibited good antibacterial activity, while **6e** showed an excellent activity compared to the standard drug ampicillin. Additionally, the dipeptide derivatives **6a** and **6e-j** showed good antifungal activity compared with the standard Nystatin. The research work described in this article lays out a vision for the evolution of novel antimicrobial agents which are efficacious over a diversified span of pathogenic strains. Moreover, this research work sheds a light on the future progress of hybrids of heterocycles and peptides with antimicrobial properties.

ACKNOWLEDGEMENT

The authors are thankful to the University Grants Commission, New Delhi and Marwadi University for technical and academic support.

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[RJC-6907/2021]