

Development of diphenylmethylpiperazinylpeptides conjugates as a novel class of antimicrobial agents.

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ABSTRACT

Novel diphenylmethylpiperazinylpeptide conjugates were prepared through the coupling of shorter peptides with diphenylmethylpiperazine using IBCF as the coupling agent, HOBt, and NMM as the base at ± 15 °C. Initially, the reaction involved stirring the reaction mixture at ± 15 °C for 2 hours, followed by an overnight reaction. The synthesized compounds were characterized by ¹H NMR and R_f values, and subsequently evaluated for antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, as well as antifungal activity against *Aspergillus Niger*, *Aspergillus flavus*, and *Fusarium moniliforme*. Among the synthesized compounds, XXIV demonstrated significant activity, while XXII and XXIII exhibited moderate activity.

Keywords: Antimicrobial resistance, Bac7 (Bactenecin7), diphenylmethylpiperazine, hydrophobicity and antimicrobial peptide.

1. INTRODUCTION

Antimicrobial resistance (AMR) poses a most significant challenge to public health on a global scale. AMR occurs when viruses, bacteria, fungi, and parasites evolve to resist the effects of antimicrobial medications, allowing them to survive and multiply within the host. The main driver behind this critical situation is the overuse and misuse of antimicrobials, particularly antibiotics, which is exacerbating the worldwide problem of antimicrobial resistance. As a result, the global consumption and distribution of antibiotics are closely monitored [1].

On the contrary, combining two or more compounds with different modes of action is an effective strategy to either halt or slow down the development of resistance to antimicrobials. Therefore, the fusion of an antimicrobial peptide (AMP) with other bioactive compounds presents an intriguing approach for the creation of novel antimicrobial agents [2]. It is widely recognized that peptides possess excellent solubility and selectivity in targeting specific sites, while heterocyclic compounds offer a wider range of drug actions. Consequently, the conjugation of peptides with heterocycles represents a

significant category of therapeutic agents in the modern drug development process. Functionalized heterocycles containing nitrogen and oxygen have been extensively utilized as frameworks in drug development due to their prominent role in medicinal chemistry.

In this context, by considering the crucial role played by Pro-Arg-Pro triplets in maintaining the antimicrobial potency of Bac7, as well as the structure-activity relationship studies conducted on shorter peptide analogues of Bac7. The studies of antimicrobial evaluations of various series of tetrapeptide fragments substituting different D and L-amino acid residues with varying hydrophobicity at the N-terminal of the Pro-Arg-Pro triplet [3-5]. It was reported that altering peptide parameters such as chain length, hydrophobicity, cationic charge, or the position of amino acid residues could optimize their antimicrobial activity. [6-8]

The conjugation of different amino acids/peptides with biologically active heterocycles has yielded remarkable results and has shown great potential as drug candidates [9]. Furthermore, amino acid/peptide-based drugs exhibit low toxicity, sufficient bioavailability and permeability,

moderate potency, and favourable metabolic and pharmacokinetic properties [10-11]. Based on the above facts, we decided to design and synthesize peptides conjugated heterocycles by coupling heterocyclic precursors such as diphenylmethylpiperazine with shorter analogues of Bac7 peptides such as RP, PRP and GPRP.

1.1 EXPERIMENTAL

1.1.2 Materials and methods:

All tert-butyloxycarbonyl (Boc) amino acid derivatives, 1-hydroxybenzotriazole (HOBT), Isobutylchloroformate (IBCF), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) and *N*-methyl morpholine (NMM) were purchased from Sigma Chemicals Co, diphenylmethylpiperazine or benzhydrylpiperazine (BHP) synthesized by using standard protocol. All solvents and reagents were of analytical grade or were purified according to standard procedure recommended for peptide synthesis. The melting points were determined with open capillary and are uncorrected. ¹H NMR spectra were obtained on a 300 MHz Bruker FT-NMR Spectrometer instrument by using CDCl₃/DMSO as solvent and TMS as an internal standard. The thin layer chromatography (TLC) was carried out on precoated silica gel plates prepared in laboratory with the following solvent systems: R_f¹:CHCl₃-MeOH-HOAc (95:5:3), R_f²:CHCl₃-MeOH-HOAc (90:10:3), R_f³:CHCl₃-MeOH-HOAc (85:15:3).

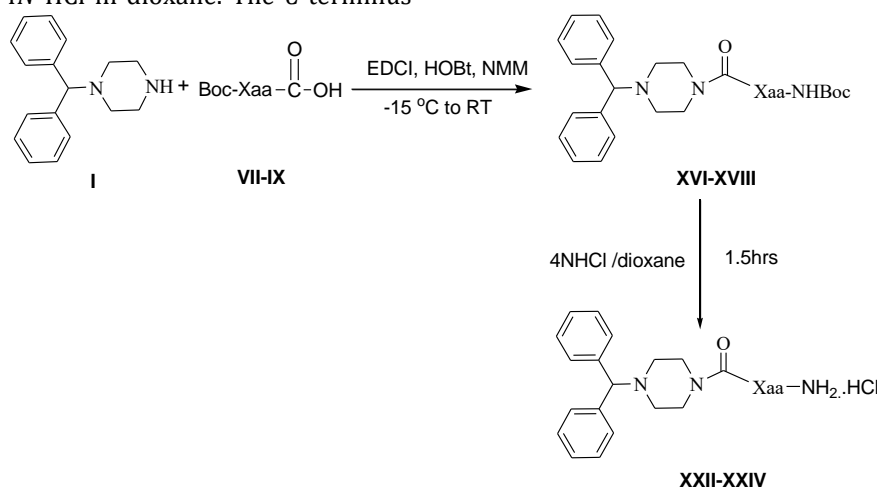
Peptide Synthesis:

The peptide synthesis was carried out by the stepwise solution phase method. The Boc group was chosen for *N*^α-protection and its removal was achieved with 4*N* HCl in dioxane. The *C*-terminus

carboxyl group was protected by the benzyl ester and its removal was achieved by hydrogenolysis using HCOONH₄/Pd-C (10%) or by saponification using 1*N* NaOH. The *N*^ε- of Arg was protected by nitro group and its removal was affected by hydrogenolysis using HCOONH₄/Pd-C (10%). All the coupling reactions were achieved with IBCF. The protected peptides were characterized by standard physical and analytical techniques.

The synthesis of the tetrapeptide Gly-Pro-Arg-Pro was carried out using a stepwise approach.⁷⁻⁹ In this method, Boc-Arg(NO₂)-Pro-OBzl was first synthesized through the mixed anhydride method in the presence of HOBT. The Boc protecting group was then removed, and the resulting compound was coupled with Boc-Pro to yield Boc-Pro-Arg(NO₂)-Pro-OBzl. Subsequently, the Boc protecting group was removed again, and the compound was coupled with Boc-Gly to obtain Boc-Gly-Pro-Arg(NO₂)-Pro-OBzl.

In the present study, the peptides Boc-Arg(NO₂)-Pro-OH, Boc-Pro-Arg(NO₂)-Pro-OH and Boc-Gly-Pro-Arg(NO₂)-Pro-OH are coupled to diphenylmethylpiperazine. The synthesized Boc protected peptide conjugates have been characterized by standard physical and analytical techniques. The synthesized peptide conjugated-heterocycle conjugates were deblocked with 4*N* HCl in dioxane to remove Boc group and the nitro group protection of *N*^α of Arg was removed by hydrogenolysis using HCOONH₄/Pd-C (10%). The deblocked compounds were used for antibacterial and antifungal activities. The stepwise synthesis of diphenylmethylpiperazinylpeptides conjugates is presented in **Scheme-1**.



Scheme- 1: Synthesis of diphenylmethylpiperazinylpeptide conjugates.

Synthesis of Boc-Arg-(NO₂)-Pro-OBzl (IV):

In acetonitrile (120 mL) cooled to 0°C, Boc-Arg(NO₂)-OH (12.72 g, 40 mmol) was dissolved,

followed by the addition of NMM (4.4 mL, 40 mmol). The solution was further cooled to -15 ± 1°C, and under stirring, IBCF (5.45 mL, 40 mmol)

was added while maintaining the temperature at $-15^{\circ}\text{C} \pm 1^{\circ}\text{C}$. After stirring for 10 minutes at this temperature, a pre-cooled solution of HOBt (6.12 g, 40 mmol) in DMF (60 mL) was added. The reaction mixture was then stirred for an additional 10 minutes, and a pre-cooled solution of HCl.H₂N-Pro-OBzl (9.7 g, 40 mmol) and NMM (4.4 mL, 40 mmol) in DMF (100 mL) was slowly added. After 20 minutes, the pH of the solution was adjusted to 8 by adding NMM, and the reaction mixture was stirred overnight at room temperature. The acetonitrile was removed under reduced pressure, and the remaining DMF solution was poured into approximately 600 mL of ice-cold 90% saturated KHCO₃ solution and stirred for 30 minutes. The resulting peptide precipitate was filtered, washed with water, 0.1N cold HCl, water, and dried. The crude peptide was recrystallized from ether and petroleum ether to obtain the desired product, with a yield of 88% and a melting point of 44-45°C (literature: 45-46°C).

Boc-Pro-Arg(NO₂)-Pro-OBzl (V):

11.5 g of **IV** was deblocked with 4N HCl/dioxane (115 mL) for 1.5 hr. Excess HCl and dioxane were removed under reduced pressure, triturated with ether, filtered, washed with ether and dried (Yield, 100%). The HCl.H₂N-Arg(NO₂)-Pro-OBzl (10 g, 22.5 mmol) in DMF (100 mL) was neutralized with NMM (2.5 mL, 22.5 mmol) and coupled to Boc-Pro-OH (4.85 g, 22.5 mmol) in acetonitrile (50 mL) and NMM (3 mL, 22.5 mmol) using IBCF (2.93 mL, 22.5 mmol) and HOBt (3.44 g, 22.5 mmol) worked up the same as **IV** to obtain **V**. The sample was recrystallized from ether/petroleum ether, Yield (87%), M. P. 46-47 °C (lit. 47 °C).⁵

Boc-Gly-Pro-Arg(NO₂)-Pro-OBzl (VI):

8.0 g of **V** was deblocked with 4N HCl/dioxane (80 mL) for 1.5 hr. Excess HCl and dioxane were removed under reduced pressure, triturated with ether, filtered, washed with ether and dried (Yield, 100%). The HCl.H₂N-Pro-Arg(NO₂)-Pro-OBzl (7.0 g, 13.0 mmol) in DMF (70 mL) was neutralized with NMM (1.4 mL, 13.0 mmol) and coupled to Boc-Gly-OH (2.27 g, 13.0 mmol) in acetonitrile (30 mL) and NMM (1.4 mL, 13.0 mmol) using IBCF (1.7 mL, 13.0 mmol) and worked up the same as **V** to obtain **VI**. The sample was recrystallized from ether/petroleum ether, Yield (90%), M.P. 47-48 °C (lit. 48-49 °C).⁵

Boc-Arg(NO₂)-Pro-OH (VII):

VI (6.10 g, 12.0 mmol) was dissolved in methanol (60 mL) and cooled to 0 °C. Then added a cooled solution of 1N NaOH (30 mL, 30 mmol) slowly and stirred the solution for about 1.5 hours. When the reaction get completed (monitored by TLC),

evaporated the methanol, cooled and neutralized with 1N HCl (cold). Then, extracted with chloroform (3 X 25 mL) and washed with 1N HCl (1 X 25 mL), water (1 X 25 mL) and dried over Na₂SO₄. The solvent was removed under reduced pressure to get Boc-Arg(NO₂)-Pro-OH (**VII**), Yield (85%).

Boc-Xaa-Arg(NO₂)-Pro-OH (Xaa is Pro for VIII, Gly-Pro for IX):

Each peptide (**V & VI**, 10 mmol) was dissolved in methanol (10 mL/g of peptide) and cooled to 0 °C. Then, added a cooled solution of 1N NaOH (20 mL, 20 mmol) slowly and stirred the solution for about 1.5 hours. When the reaction get completed (monitored by TLC), evaporated the methanol, cooled and neutralized with 1N HCl (cold). Then, extract with chloroform (3 X 25 mL) and washed with 1N HCl (1 X 25 mL), water (1 X 25 mL) and dried over Na₂SO₄. The solvent was removed under reduced pressure and dried over vacuum to get debenzylated peptide (**VIII**, yield (88%) & **IX**, yield (88%)).

The above synthesized peptidic precursors (**VII to IX**) were divided into two parts. One part of which is used for further coupling with heterocycles and other part was used for the antimicrobial studies after removing side chain protections completely.

Boc-Arg-Pro-OH (X):

The guanidine nitro group of **VII** (0.6g, 1.4 mmol) underwent hydrogenolysis in methanol (5 mL) with the use of ammonium formate (2 eq.) and 10% Pd/C (0.1 g) for 30 minutes at room temperature. The catalyst was then filtered and rinsed with methanol. The combined filtrate was concentrated under reduced pressure, and the resulting residue was dissolved in chloroform, washed with water, and dried over Na₂SO₄. The solvent was evaporated under reduced pressure, and the product was triturated with ether, filtered, washed with ether, and dried to yield (**X**, 90%).

Boc-Xaa-Arg-Pro-OH (Xaa is Pro for XI, Gly-Pro for XII):

The guanidine nitro group of each peptide (**VIII & IX**, 1.5 mmol) underwent hydrogenolysis in methanol (5 mL) utilizing ammonium formate (2 eq) and 10% Pd/C (0.1 g) for a duration of 30 minutes at room temperature. The catalyst was separated through filtration and subsequently rinsed with methanol. The combined filtrate was subjected to evaporation under reduced pressure, and the resulting residue was dissolved in chloroform, washed with water, and dried using Na₂SO₄. The solvent was then removed under reduced pressure, and the resulting product was triturated with ether, filtered, washed with ether, and dried, resulting in a yield of (**XI & XII**, 90%).

HCl.NH₂-Arg-Pro-OH (XIII):

0.5 g of **X** was deblocked by stirring with 5 mL of 4*N* HCl/dioxane for 1.5 hr. Excess HCl and dioxane were removed under reduced pressure, triturated with ether, filtered, washed with ether and dried, Yield (**XIII**, 100%).

HCl.NH₂-Xaa-Arg-Pro-OH (Xaa is Pro for XIV, Gly-Pro for XV):

0.5 g of each peptide (**XI & XII**) was deblocked by stirring with 5 mL of 4*N* HCl/dioxane for 1.5 hr. Excess HCl and dioxane were removed under reduced pressure, triturated with ether, filtered, washed with ether and dried, Yield (**XIV & XV**, 100%).

Synthesis of diphenylmethylpiperazinylpeptide conjugates:

To the stirred solution of Boc-peptide (**VII-IX**, 2 mmol) and HOBT (0.31 g, 2 mmol) in DMF (10 mL) cooled to 0 °C, added NMM (0.22 mL, 2 mmol). The reaction mixture was further cooled to -15 °C ± 1 °C and added EDCI (0.39 g, 2 mmol) and diphenylmethylpiperazine (2 mmol). After 20 minutes, the pH of solution was adjusted to 8 by the addition of NMM and the reaction mixture was stirred overnight while slowly warming to rt. The reaction mixture was quenched with water (2 mL) and the solvent was condensed. The residue was dissolved in chloroform (25 mL), washed with 5% NaHCO₃ (3x20 mL), H₂O (1x20 mL) followed by 0.1*N* cold HCl (3x20 mL) and brine solution (3x20 mL), dried over anhydrous Na₂SO₄. The chloroform was removed under reduced pressure to obtain the desired products (**XVI - XVIII**). Yield, melting point and ¹H NMR data of these compounds are presented below.

Catalytic transfer hydrogenation of diphenylmethylpiperazinylpeptide conjugates:

The guanidine nitro group of each diphenylmethylpiperazinylpeptides conjugates (**XVI-XVIII**, 1.5 mmol) underwent hydrogenolysis in methanol (10 mL/ g of peptide) with the use of ammonium formate (2 eq.) and 10% Pd/C (0.1 g/1.0 g of compound) for a duration of 30 minutes at room temperature. Following this, the catalyst was separated by filtration and rinsed with methanol. The combined filtrate was then evaporated under vacuum, and the resulting residue was dissolved in chloroform, washed with water, and dried using Na₂SO₄. The solvent was subsequently removed under reduced pressure, and the remaining material was triturated with ether, filtered, washed with ether, and dried to obtain the desired compounds (**XIX- XXI**) with a yield of 90%.

Deprotection of N^α-Boc of diphenylmethylpiperazinylpeptide conjugates:

0.5 g of each diphenylmethylpiperazinylpeptides conjugates (**XIX-XXI**) was deblocked by stirring with 5 mL of 4*N* HCl/dioxane for 1.5 hr. Excess HCl and dioxane were removed under reduced pressure, triturated with ether, filtered, washed with ether and dried get hydrochloride salts of peptide conjugated heterocycles (**XXII-XIV**), Yield (100%). These compounds were used for antimicrobial study (**table-1 & 2**).

Boc-RP-BHP

Yield (%): 88; Arg² 4.50[1H, t, ^αCH], 2.17[2H, m, ^βCH₂], 1.84[2H, m, ^γCH₂], 3.49[2H, m, ^δCH₂], 8.18-8.28 [2H, s, NH], 1.45[9H, s, Boc]; Pro¹ 4.32[1H, t, ^αCH], 3.22[2H, m, ^βCH₂], 2.62[2H, m, ^γCH₂], 3.55[2H, t, ^δCH₂]; BHP 7.10-7.35[10H, m, ArH], 3.24[4H, t, -CH₂-piperazine], 2.32[4H, t, -CH₂-piperazine], 4.68[1H, s, ^αCH].

Boc-PRP-BHP

Yield (%): 90; Pro³ 4.23[1H, t, ^αCH], 3.17[2H, m, ^βCH₂], 2.59[2H, m, ^γCH₂], 3.50[2H, t, ^δCH₂], 1.45[9H, s, Boc]; Arg² 4.47[1H, t, ^αCH], 2.13[2H, m, ^βCH₂], 1.82[2H, m, ^γCH₂], 3.43[2H, m, ^δCH₂], 8.14-8.25 [2H, s, NH]; Pro¹ 4.34[1H, t, ^αCH], 3.23[2H, m, ^βCH₂], 2.64[2H, m, ^γCH₂], 3.58 [2H, t, ^δCH₂]; BHP 7.10-7.30 [10H, m, ArH], 3.24 [4H, t, -CH₂-piperazine], 2.28[4H, t, -CH₂-piperazine], 4.70[1H, s, ^αCH].

Boc-GPRP-BHP

Yield (%): 85; Gly⁴ 4.15[2H, s, ^αCH₂], 8.0[1H, s, NH], 1.43 [9H, s, Boc]; Pro³ 4.33[1H, t, ^αCH], 3.19 [2H, m, ^βCH₂], 2.63[2H, m, ^γCH₂], 3.56[2H, t, ^δCH₂]; Arg² 4.49[1H, t, ^αCH], 2.14[2H, m, ^βCH₂], 1.80 [2H, m, ^γCH₂], 3.45[2H, m, ^δCH₂], 8.10-8.20 [2H, s, NH]; Pro¹ 4.34[1H, t, ^αCH], 3.23 [2H, m, ^βCH₂], 2.64[2H, m, ^γCH₂], 3.58[2H, t, ^δCH₂]; BHP 7.1-7.32 [10H, m, ArH], 3.24 [4H, t, -CH₂-piperazine], 2.30[4H, t, -CH₂-piperazine], 4.60[1H, s, ^αCH].

b) General method for antibacterial assay:

In order to assess their effectiveness against various bacteria, antibacterial assays were carried out invitro using the agar well diffusion system.¹² The bacterial strains, including Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa, were cultivated in Muller-Hinton broth. The inoculum concentration was adjusted using the mid-logarithmic phase system (OD 600 = 0.5). To prepare the agar media, sterile distilled water was added to Muller-Hinton agar and autoclaved for one hour. The autoclaved media was then poured into pre-sterilized 90 mm petriplates and allowed to solidify. Using an 8 mm sterile cork borer, the media was removed from the centre, creating a well for the assay. The inoculum was spread evenly over the media. Additionally, 50 μL of a stock solution of compounds (10 μg/well) was added to the wells created in the petriplates. The petriplates were

then incubated at 37 °C for 3-4 days. All synthesized compounds were tested in triplicate, with streptomycin serving as the positive control

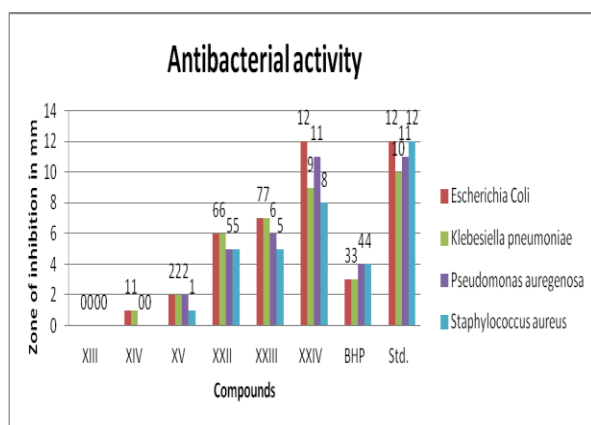
and water as the negative control. The zone of inhibition, measured in millimetres, was recorded and presented in **table-1** and **graph-1**.

Table-1: Antibacterial Activity of diphenylmethylpiperazinylpeptide conjugates:

Entry	Compounds ^a	Zone of Inhibiton (diameter) mm ^b			
		Escherichia coli	Klebesiella pneumoniae	Pseudomonas auregenosa	Staphylococcus aureus
XIII	RP	00	00	00	00
XIV	PRP	01	01	00	00
XV	GPRP	02	02	02	01
XXII	BHP-RP	06	06	05	05
XXIII	BHP-PRP	07	07	06	05
XXIV	BHP-GPRP	12	09	11	08
BHP	BHP	03	03	04	04
Std.	Streptomycin	12	10	11	12

^a Concentration of compounds and reference drug:10 µg/ml.

^b Values are means of three determinations, the ranges of which are less than 5% of the mean in all cases.



Graph-1: Antibacterial Activity of diphenylmethylpiperazinylpeptide conjugates.

a) General method of antifungal assay:

In order to assess their effectiveness against *Aspergillus Niger*, *Aspergillus flavus*, and *Fusarium monoliforme*, antifungal tests were conducted in vitro using the agar well diffusion technique ¹³. The fungal cultures were grown on

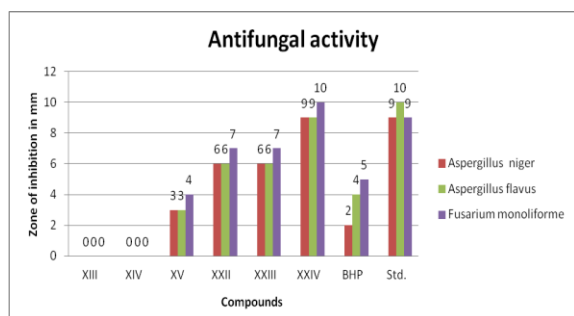
PDA media with a pH of 7.4 for six days at a temperature of 25°C. The spores were collected in sterilized normal saline solution and adjusted to a concentration of 1x10⁶/ml using a Haemocytometer. Each sterilized 90 mm petriplate was filled with 20mL of autoclaved molten media and allowed to solidify. To evaluate the growth response of the fungal species, 0.4 mL of the synthesized compounds (10 µg/mL) was evenly spread over the agar media in each plate. Then, 10µL of spore suspension was added to the small depression created at the center of each plate, and the plates were incubated for six days at 25°C. After the incubation period, the plates were examined and compared to their respective control plates. The control plates contained only distilled water, representing 100% fungal growth with no inhibition. The fungicidal activity of the synthesized compounds was determined by comparing the zone of fungal growth in the treated plates to that of the control plates in millimetres. The results can be found in **table-2** and **graph-2**.

Table-2: Antifungal activity of diphenylmethylpiperazinylpeptide conjugates:

Entry	Compounds ^a	Zone of Inhibiton (diameter) mm ^b		
		<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Fusarium monoliforme</i>
XIII	RP	00	00	00
XIV	PRP	00	00	00
XV	GPRP	03	03	04
XXII	BHP-RP	06	06	07
XXIII	BHP-PRP	06	06	07
XXIV	BHP-GPRP	09	09	10
BHP	BHP	02	04	05
Std.	Bavistin	09	10	09

^a Concentration of compounds and reference drug:10 µg/mL

^b Values are means of three determinations, the ranges of which are less than 5% of the mean in all cases



Graph - 2: Antifungal activity diphenylmethylpiperazinylpeptide conjugates.

1.1.3 RESULTS AND DISCUSSION

Antibacterial studies

The antibacterial efficacy of the above said compounds were tested against both gram+ve and gram-ve bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas auregenosa* at the same concentration as that of the standard, streptomycin. The results are expressed as zone of inhibition measured in mm. The results are summarized in **Table-1**.

Among all the synthesized compounds, **GPRP-BHP (XXIV)** showed highest activity against all the bacterial strains tested which are equipotent with the standard drug. On the whole, **all the compounds showed good activity against the gram -ve bacteria when compared to gram +ve bacteria**. This may be due to the easy passage of tested compounds through thin peptidoglycan layer of gram-negative bacteria cell wall when compared to the gram-positive bacteria, which is very much thicker. In view of this, antibacterial activity of the synthesized compounds reveals that, in both heterocyclic conjugated peptides and peptides alone, activity increases from dipeptide to tetrapeptide *i.e.*, **as the chain length of the peptide increases the activity also increases**. Whereas peptides tested alone, the dipeptide does not show any activity, but, tri and tetrapeptides showed some activity against all the bacterial strains tested. On the contrary, heterocycle diphenylmethylpiperazine taken in isolation have also been subjected to antibacterial studies which fetched the negligible results. Thus, it can be emphasized that the conjugation of peptides with diphenylmethylpiperazine resulted in marked increase in the activity which can be viewed as lead forefront molecules for further investigations.

Antifungal activity:

Antifungal potency of the above said compounds were tested against various fungal strains such as *Aspergillus niger*, *Aspergillus flavus* and *Fusarium moniliforme* at the same concentration with the standard, Bavistin¹⁶ and the results are expressed as

zone of inhibition measured in mm. The results are summarized in **table-2**.

Among all the compounds tested the tetrapeptide conjugated diphenylmethylpiperazine derivatives (**XXIV**) showed maximum activity in the series equipotent with bavistin. The attributes discussed under antibacterial activity holds good for the antifungal studies also. The remaining di and tripeptide conjugated diphenylmethylpiperazine showed moderate to good activity compared to the peptide and heterocycle diphenylmethylpiperazine alone.

1.1.4 CONCLUSION

In an effort to explore peptide conjugated heterocycles as a new class of antimicrobial drugs, we found that compounds containing tetrapeptide fragments of diphenylmethylpiperazine showed good antibacterial activity as well as good antifungal activity and all other compounds showed a moderate activity. Hence, it can be inferred that conjugation of peptides of different chain length with heterocyclic motifs enhanced both the antibacterial and antifungal activities compared to their mother molecules. The results of the present study indicate that compounds **XXII**, **XXIII** and **XXIV** might be of great interest for the development of novel molecules for the better antimicrobial potency.

Conflict of interest

The author has no conflict of interest.

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