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Synthesis of Quinazolinone Conjugated Shorter Analogues of Bactenecin7 as Potent Antimicrobials

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Abstract: A series of shorter peptide analogues of Bactenecin7 (RP, PRP, GPRP and RPRP) were synthesized and conjugated to 3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoic acid to study the effect of conjugation. All the peptides and their conjugates were characterized by analytical and spectroscopic techniques. The synthesized compounds *viz.*, peptides, heterocyclic conjugates and the hydrogenolyzed products were evaluated for antimicrobial activity against a panel of pathogens. The results revealed that all the conjugates have shown enhanced activity than their counterparts. Further, hydrogenolyzed tetrapeptide conjugates (**10** and **13**) have exerted highly potent activity nearly 3-4 times than the standard drugs used.

Keywords: Quinazolinone. bactenecin7. conjugation. potent antimicrobials.

INTRODUCTION

Despite a number of antibiotics available for the treatment of microbial infections, emergence of multi-drug resistant organisms has posed a great challenge to the scientists. There appears to be a need for the development of new drugs with improved efficacy and better toxicity [1]. Researchers however are continuing their efforts to develop new classes of antimicrobials. One of these research lines aims to exploit the therapeutic potential of a variety of natural cationic antimicrobial peptides and their synthetic analogues, for the development of antimicrobials with a mechanism of action different from those of conventional antibiotics [2].

Bactenecin7 (Bac7), a cathelicidin-derived antimicrobial peptide of bovine neutrophil granules [3], exhibits antibacterial activity primarily against gram negative bacteria and has been found to represent a new family of Pro/Arg-rich antibiotics [4]. Bac7 comprises 59 amino acid residues and includes three tandem repeats of a tetradecamer characterized by several Pro-Arg-Pro triplets spaced by single hydrophobic amino acid residues. Proline is known as a strong α-helix breaker, which can induce kinks in α-helix of proteins, suggesting that the repeating triplet may play an important role in antibacterial activity and/or in maintaining the peptide conformation [5]. In this connection, two peptide fragments were synthesized corresponding to residues 1-17 (an Arg-clustered region) and 46-59 (one of three tandem repeats) sequences of Bac7 [6]. While the fragment 1-17 showed weak antimicrobial activity against several bacteria, the fragment 46-59 was almost inactive. Therefore, a detailed study on the property and function of the repeating regions that extends from the near *N*-terminal to the *C*-terminal portions of the peptides were of interest. Hence, in our laboratory, a series of tetrapeptide fragments (X-Pro-Arg-Pro, where X = Gly, Arg, Leu, Ile and Phe) were synthesized and found that Gly and Arg containing tetrapeptide fragments showed more potent activity compared to rest of the analogues synthesized [7]. Therefore, in the current investigation the two potent analogues GPRP and RPRP were chosen for the conjugation.

On the other hand, the pharmacodynamic versatility of quinazolinone moiety has been documented not only in many of its synthetic derivatives but also in several naturally occurring alkaloids isolated from animals [8], families of plant kingdoms [9] and from microorganisms [10]. The quinazolinone derivatives are found to have wide range of biological properties including anti-cancer [11], antimicrobial [12], antihyperlipidemic [13] and anti-inflammatory [14] activities. In the light of the above and in continuation of our work [12, 15-19] the present work aims at the synthesis and biological studies of the quinazolinone conjugated shorter active analogues of Bac7.

MATERIALS AND METHODS

Reagents

All the amino acids used except glycine were of *L*-configuration unless otherwise mentioned. All Boc-amino acids, EDCI and HOBt were purchased from Advanced Chem. Tech. (Louisville, Kentucky, USA). NMM was purchased from Sigma Chemical Co. (St. Louis, MO). All solvents and reagents used for the synthesis were of analytical grade. The progress of the reaction was monitored by TLC using silica gel coated on glass plates with the solvent system comprising chloroform/methanol/acetic acid in the ratio 98:2:3. IR spectra were recorded on Shimadzu FTIR-8300 Spectrometer (USA). ^{1}H NMR spectra were obtained on VARIAN 400 MHz instrument (Palo Alto, USA) using DMSO and the chemical shifts are reported as parts per million (δ ppm) using TMS as an internal standard. Mass spectra were obtained on LCMSD-Trap-XCT instrument. Elemental analysis was performed by using VARIO EL III CHNS Elementar (Germany). The chemical/reagents used

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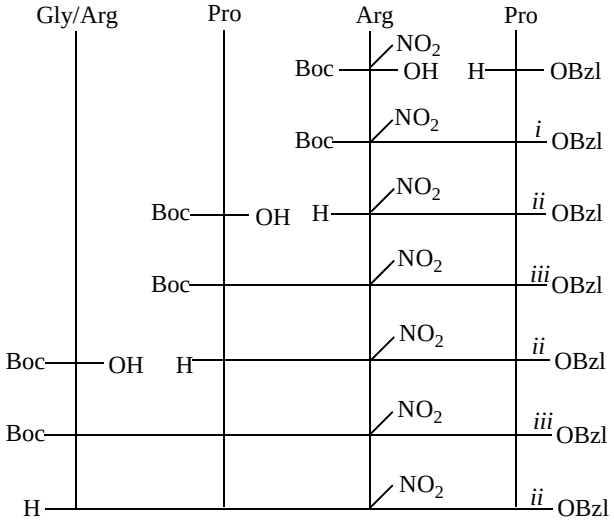
for the microbial studies were of bacteriological grade and purchased from Himedia (Mumbai, India). All the pathogens used for the assay were obtained from a local hospital.

Chemistry

The 3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoic acid **1** (QZN) was synthesized as previously reported using anthranilamide and succinic anhydride [20, 21]. The peptides **2, 5, 8** and **11** were synthesized by stepwise solution phase method using Boc chemistry as shown in scheme **1**. The Boc group used for temporary N^{α} protection was removed with 4N HCl in dioxane. Peptides were coupled to quinazolinone moiety **1** using EDCI/HOBt as coupling agent and NMM as base to get heterocyclic conjugated peptides **3**, **6**, **9** and **12**. The *C*-terminus carboxyl group protected by the benzyl ester and the guanidine function of arginine was masked by nitro group, and their removal was effected by hydrogenolysis using $HCOONH_4$ as hydrogen donor and 10% Pd on carbon as catalyst [22] to get the hydrolysed products **4**, **7**, **10** and **13** respectively. The procedure followed for the synthesis is as shown in the Scheme **2**.



Scheme 1. Schematic representation of the synthesis of tetrapeptides. HCl.H.Gly/Arg-Pro-Arg-Pro-OBzl by stepwise approach
i. IBCF/HOBt, NMM
ii. HCl/dioxane
iii. IBCF, NMM

Peptide Synthesis

Boc-Arg(NO_2)-Pro-OBzl (2)

To Boc-Arg(NO_2)-OH (15.95 g, 0.05 mol) dissolved in acetonitrile (160 mL) and cooled to 0 °C was added NMM (5.5 mL, 0.05 mol). The solution was cooled to -15 °C ± 1°C and IBCF (6.85 mL, 0.05 mol) was added under stirring while maintaining the temperature at -15 °C. After stirring the reaction mixture for 10 minutes at this temperature, a pre-cooled solution of HOBt (6.80 g, 0.05 mol) in DMF (70 mL) was added. The reaction mixture was stirred for an additional 10 minutes and a pre-cooled solution of HCl.H-Pro-OBzl (12.10 g, 0.05 mol) and NMM (5.5 mL, 0.05 mol) in DMF (120 mL) was added slowly. After 20 minutes, pH of the solution was adjusted to 8 by the addition of NMM and the reaction mixture was stirred overnight at room temperature. Acetonitrile was removed under reduced pressure and the residual DMF solution was poured into about 2000 mL ice-cold 90% saturated $KHCO_3$ solution and stirred for 30 minutes. The precipitated peptide was filtered, washed with water, 1N HCl, water and dried. The crude peptide was recrystallized from ether and petroleum ether to obtain **2**. Yield: 88.0%, M.P. 44-46 °C (Lit. 45-46 °C [7]).

Boc-Pro-Arg(NO_2)-Pro-OBzl (5)

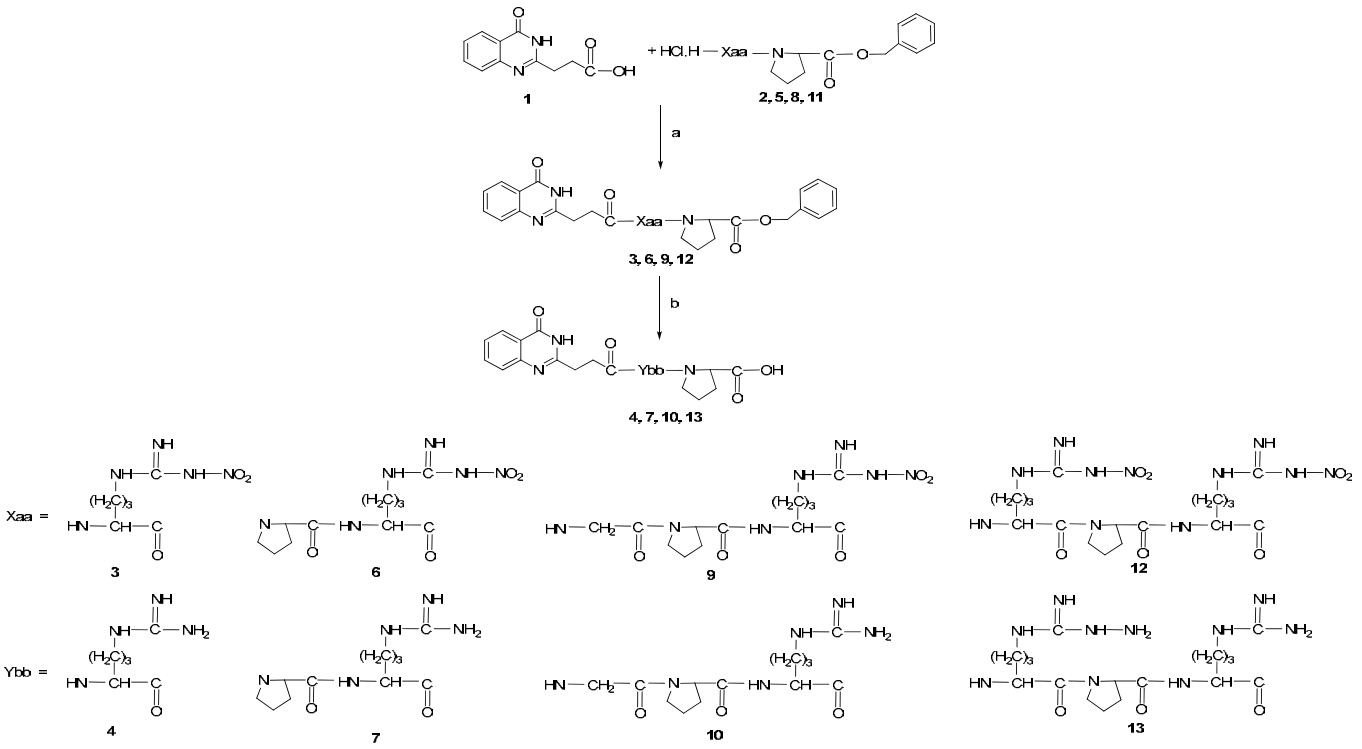
Compound **2** (20.25 g) was deblocked with 4N HCl/dioxane (200 mL) for 1.5 hr. Excess HCl and dioxane were removed under reduced pressure, triturated with ether, filtered, washed with ether and dried (yield: 100%). HCl.H-Arg(NO_2)-Pro-OBzl (17.72 g, 0.04 mol) in DMF (180 mL) was neutralized with NMM (4.4 mL, 0.04 mol) and coupled to Boc-Pro-OH (8.2 g, 0.04 mol) in acetonitrile (80 mL) and NMM (4.4 mL) using IBCF (5.5 mL, 0.04 mol) and worked up the same as **2** to obtain **5**. The sample was recrystallized from ether/petroleum ether. M.P. 46-48 °C (Lit. 47.24 °C [7]).

Boc-X-Pro-Arg(NO_2)-Pro-OBzl (X is Gly for 8 and Arg (NO_2) for 11)

Compound **5** (9.9 g) was deblocked with 4N HCl/dioxane (100 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%). HCl.H-Pro-Arg(NO_2)-Pro-OBzl was divided in to two equal parts and each part in DMF was neutralized with NMM (0.76 mL, 0.007 mol) and coupled to Boc-X-OH (0.007 mol) in acetonitrile and NMM (0.77 mL, 0.007 mol) using IBCF (0.95 mL, 0.007 mol) and worked up the same as **2** to obtain **8** and **11**. The samples were recrystallized from ether/petroleum ether. Compound **8**: M.P. 47-49 °C (Lit. 48.97 °C [7]) and Compound **11**: M.P. 58-60 °C (Lit. 59-61 °C [7]).

General Procedure for the Coupling of Benzyl Esters of Peptides 2, 5, 8 and 11 with 3-(4-Oxo-3,4-dihydroquinazolin-2-yl)Propanoic Acid 1

To 3-(4-oxo-3,4-dihydroquinazolin-2-yl)propanoic acid (0.436 g, 2 mmol) dissolved in DMF (5 mL) and cooled to 0 °C was added NMM (0.21 mL, 2 mmol) and EDCI (0.380 g, 2 mmol) under stirring while maintaining the temperature at 0 °C. The reaction mixture was stirred for 10 minutes and HOBt (0.765 g, 2 mmol) in DMF (8 mL) was added and stirred the reaction mixture for further 10 minutes. To this a pre-cooled solution of HCl.H-Zcc-OBzl [Zcc = RP (**2**), PRP (**5**), GPRP (**8**) and RPRP (**11**), 2 mmol] and NMM (0.21 mL, 2 mmol) in DMF was added slowly. After 20 minutes, pH of the solution was adjusted to 8 by the addition of NMM and the reaction mixture was stirred over night at room temperature. DMF was removed under reduced pressure and the residue was poured into about 100 mL ice-cold 90% saturated $KHCO_3$ solution and stirred for 30 minutes. The precipitated product was dissolved in $CHCl_3$ (15 mL) and the organic layer was washed with 0.1N HCl (2 x 20 mL), water (2 x 20 mL), 5% $NaHCO_3$ solution (2 x 20 mL) and finally with brine solution (2 x 20 mL). The $CHCl_3$ layer was dried over anhydrous Na_2SO_4 and the solvent was removed under



Scheme 2. Synthesis of quinazolinone conjugated peptides. Reagents and conditions: **a:** EDCI/HOBt/NMM, 0 °C
b: $HCOONH_4$/10% Pd-C, 30-45 min, rt

reduced pressure. The product obtained was triturated with ether/petroleum ether to obtain QZN-Zcc-OBzl [Zcc = RP (**3**), PRP (**6**), GPRP (**9**) and RPRP (**12**)] as hygroscopic foam.

General Procedure for the Hydrogenolysis of Benzyl Ester and Nitro Group of Quinazolinone Conjugated Peptides

Each conjugate QZN-Zcc-OBzl [Zcc = $R(NO_2)P$ (**3**), $PR(NO_2)P$ (**6**), $GPR(NO_2)P$ (**9**) and $R(NO_2)PR(NO_2)P$ (**12**), 1 mmol] was hydrogenolysed in methanol (10 mL/g of compound) using ammonium formate (2.0 equi.) and 10% Pd-C (0.1g/g of peptide) for 30-45 minutes at room temperature. The completion of the reaction was monitored by TLC. The catalyst was filtered and washed with methanol. The combined washings and filtrate were evaporated *in vacuo* and the residue taken in to $CHCl_3$, washed with water and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and triturated with ether, filtered, washed with ether and dried to obtain final products QZN-Zcc-OH [Zcc = RP (**4**), PRP (**7**), GPRP (**10**) and RPRP (**13**)]. Yield of the compounds obtained were 85.2%, 87.6%, 88.5% and 83.0% for **4**, **7**, **10** and **13** respectively.

Antibacterial Activity

In vitro antibacterial activity was evaluated against human pathogens of both gram positive organisms namely *K. pneumoniae* and *P. putida* and gram negative organisms namely *S. faecalis, S. pyogenes* and *E. coli* by agar well diffusion method [23] (Fig. **1**) as well as microdilution method [23] (Fig. **2**).

Agar Well Diffusion Method

The microorganisms were inoculated in to the sterilized nutrient broth and maintained at 37 °C for 24 hours. On the day of testing, bacteria were subcultured separately into 25 mL of sterilized nutrient broth. Inoculated subcultured broths were kept at room temperature for the growth of inoculums. Each test compounds (**1-13**) and standard drug (ciprofloxacin) of 10 mg was dissolved in 10 mL of DMSO to get a concentration of 1 mg/mL and further diluted to get a final concentration of 30 μg/mL. About 15-20 mL of molten nutrient agar was poured into each of the sterile plates. With the help of cork borer of 6 mm diameter, the cups were punched and scooped out of the set agar and the plates were inoculated with the suspension of particular organism by spread plate technique. The cups of inoculated plates were then filled with 0.1 mL of the test solution, ciprofloxacin solution and DMSO (negative control). The plates were allowed to stay for 24 hours at 37 °C and zone of inhibition (mm) was then measured.

Microdilution Method

All the microorganisms were grown in Muller-Hinton broth. After cultivation for 16-18 hours at 37 °C, the bacteria were harvested and their density was determined by measuring O.D at A_{600}. MIC of the compounds was determined by agar dilution method. Suspension of each microorganism was prepared to contain approximately ($1X10^4$ – $2X10^4$ CFU/mL) and applied to the plates with serially diluted compounds (dissolved in DMSO) to be tested and also refer-

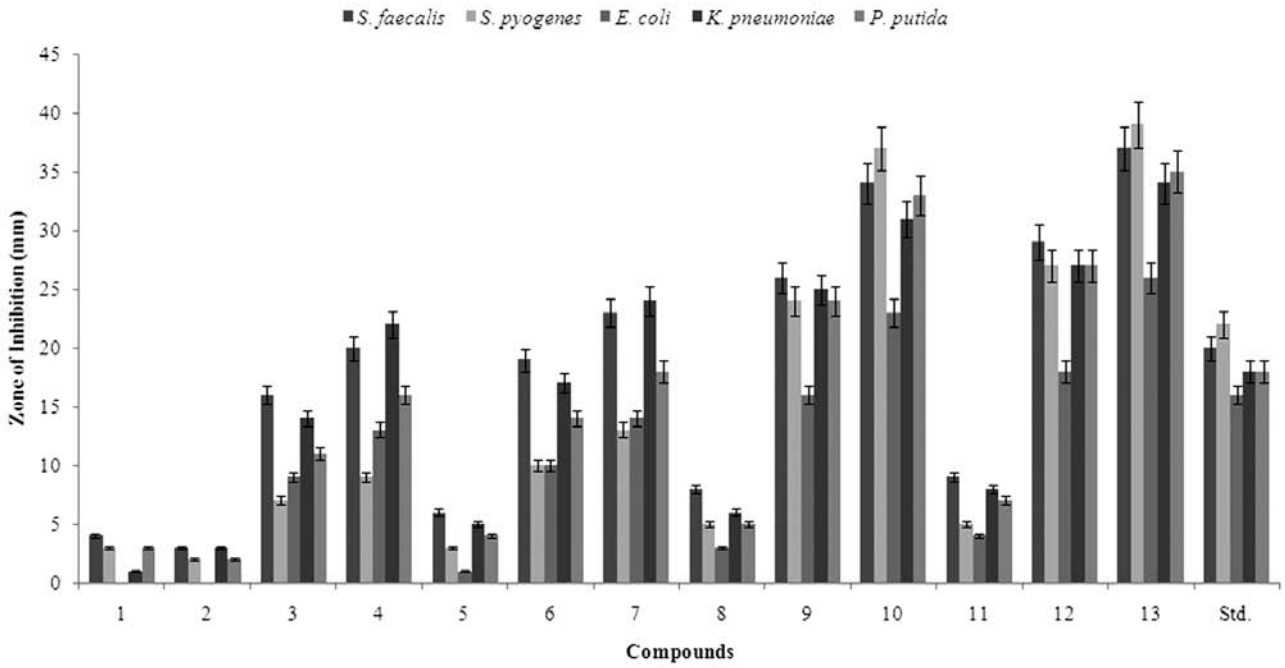


Figure 1. Diagramatic representation of antibacterial activity of the synthesized compounds by agar well diffusion method.

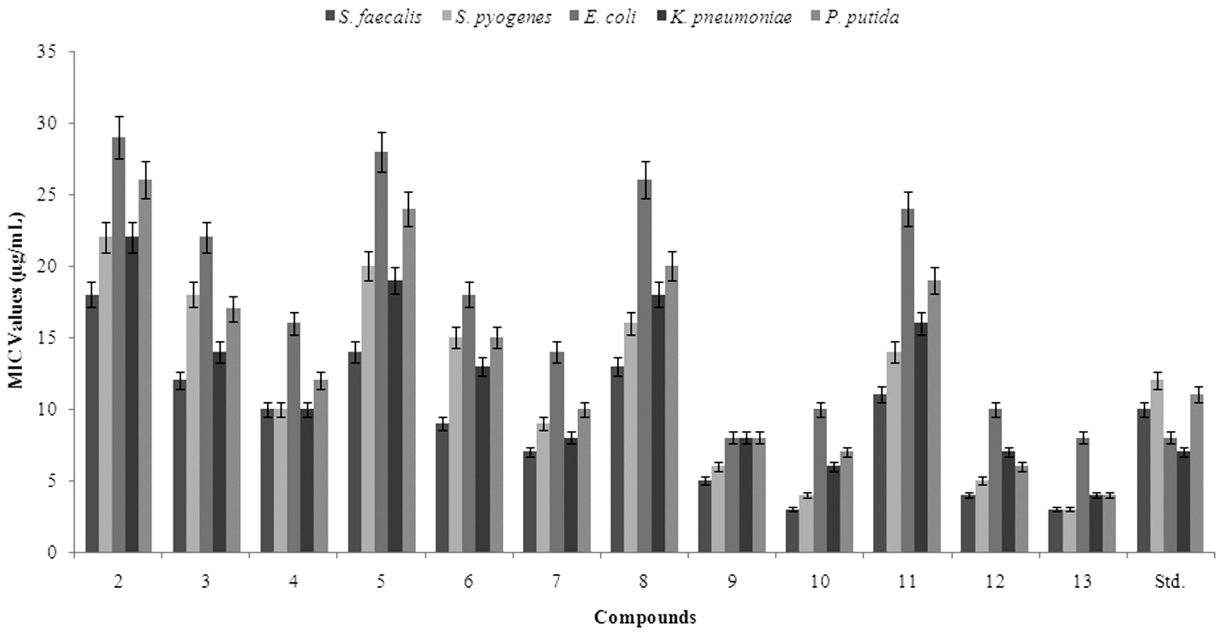


Figure 2. Diagramatic representation of antibacterial activity of the synthesized compounds by microdilution method.

ence drug and incubated at 37 °C overnight. The minimum inhibitory concentration was considered to be the lowest concentration that completely inhibited the growth of microorganisms on the plates. Zone of inhibition (mm) was measured after 24 hours and MIC values were determined.

Antifungal Activity

In vitro antifungal activity was evaluated against five fungal species namely *A. flavus, C. capsisi*, *F. oxysporum, F. verticellartar* and *A. alternata* by agar well diffusion method (Fig. **3**) as well as microdilution method (Fig. **4**).

Agar Well Diffusion Method [24]

The fungal strains were subcultured separately into 25 mL of sterilized nutrient broth and incubated for one day to obtain the inoculums. Each test compounds (**1-13**) and standard drug (griseofulvin) of 10 mg was dissolved in 10 mL of DMSO to get a concentration of 1 mg/mL and further diluted to get a final concentration of 30 µg/mL. Molten media of

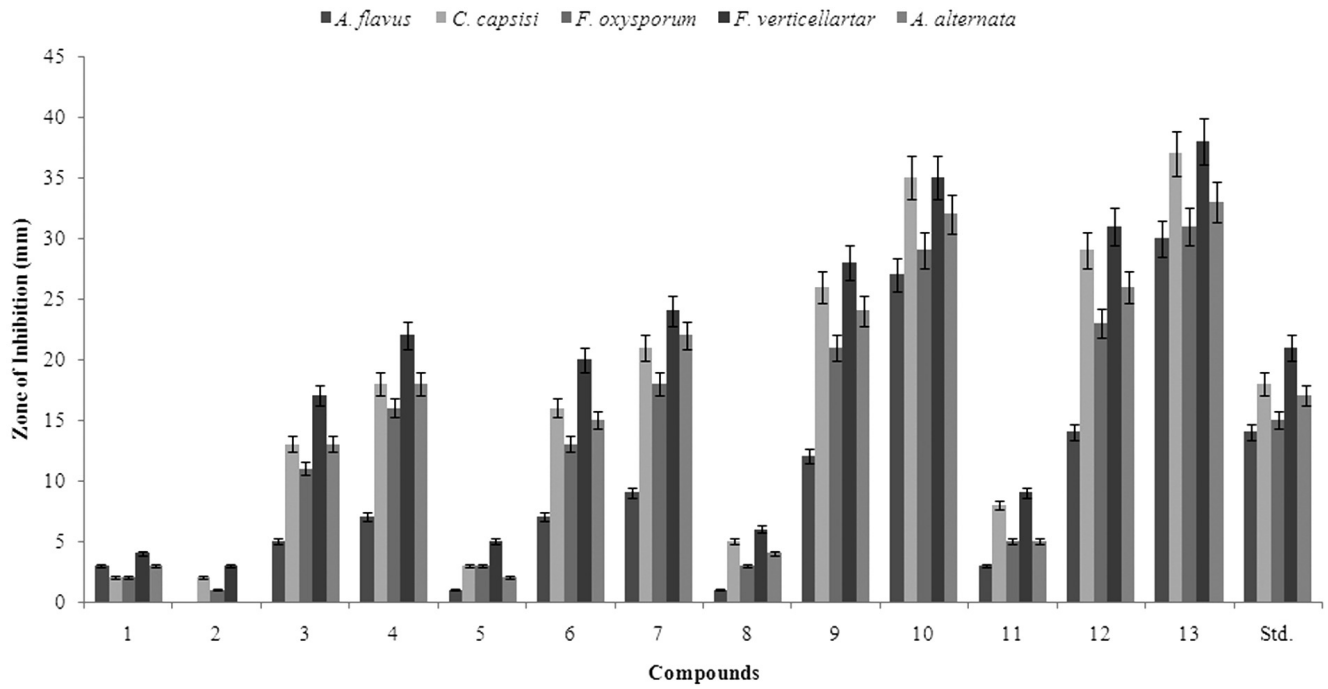


Figure 3. Diagramatic representation of antifungal activity of the synthesized compounds by agar well diffusion method.

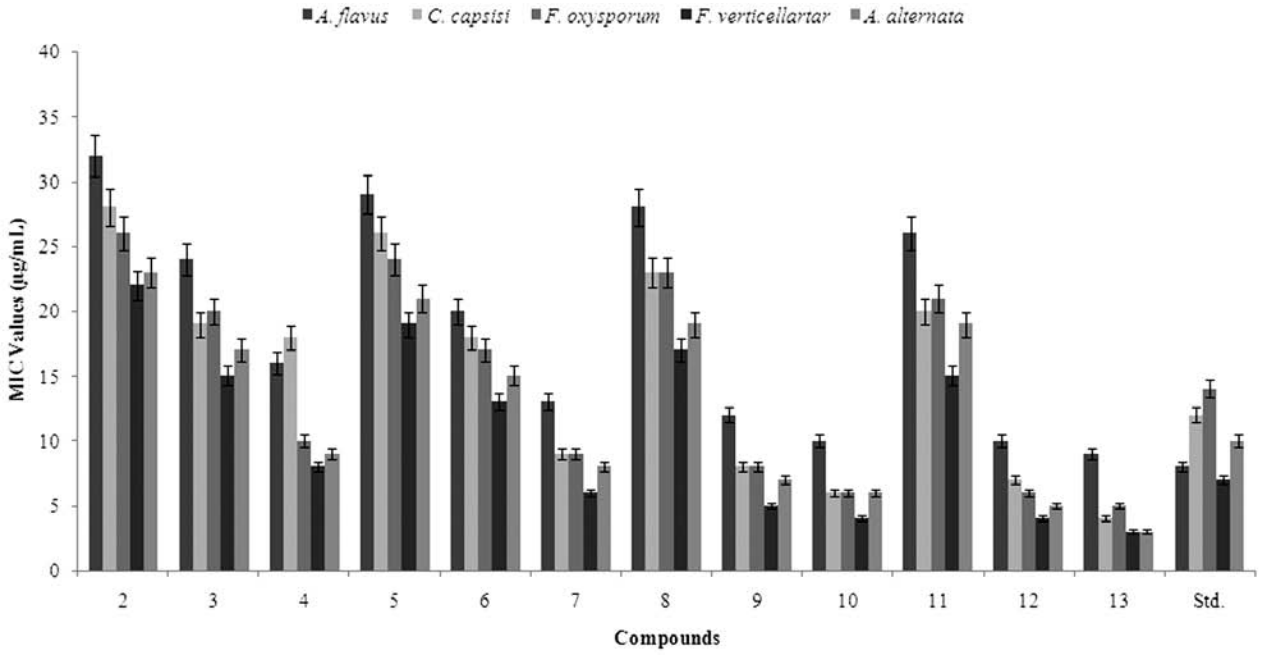


Figure 4. Diagramatic representation of antifungal activity of the synthesized compounds by microdilution method.

Sabouraud agar of 10-15 mL was poured into the petriplates and allowed to solidify. Fungal subculture was inoculated on the solidified media. With the help of 6 mm cork borer, the cups were punched and scooped out of the set agar. The cups of inoculated plates were then filled with 0.1 mL of the test solution, griseofulvin solution and DMSO (negative control). The plates were allowed to stay for 3 days at room temperature and zone of inhibition (mm) was then measured.

Microdilution Method [24]

Sabouraud agar was used for the preparation of plates. Suspension of each microorganism was prepared to contain 10^5 CFU/mL. The agar plates were inoculated with fungal strains and serially diluted test compounds and reference drug dissolved in DMSO. The plates were incubated at 25 °C for 48-72 hours. The minimum inhibitory concentration was

considered to be the lowest concentration that completely inhibited the growth of microorganisms on the plates. Zone of inhibition (mm) was measured after 72 hours and MIC values were determined.

RESULTS AND DISCUSSION

Previous work has shown that it is possible to improve the activities, or reduce the toxicities of naturally occurring peptide antibiotics by producing synthetic analogues with modified primary and/or secondary structures [25]. The present work was primarily aimed to synthesize some heterocyclic conjugated shorter active analogues of Bac7 to obtain some information on the importance of conjugation. The type of modification included attachment of bioactive quinazolinone moiety at the *N*-terminal of the peptides to study the change in the biological activity.

Chemistry

The peptide synthesis was performed by step-wise solution phase method using Boc chemistry. The synthesized peptides were conjugated with the precursor **1**. The protected peptides and heterocycle conjugated peptides were characterized by TLC, IR, ^{1}H NMR, mass spectroscopic techniques and also elemental analysis. The ^{1}H NMR; analytical and physical data of the synthesized compounds are provided in Table **1** and **2** respectively. The ^{1}H NMR, mass and elemental analysis data were found to be in good agreement with the structures assigned.

Biology

The efficacy of the synthesized compounds **1-13** were evaluated for antibacterial activities against different strains of gram negative bacteria like *S. faecalis, S. pyogenes* and *E. coli* and gram positive bacteria like *K. pneumoniae* and *P. putida* and antifungal activities against *A. flavus, C. capsisi, F. oxysporum, F. verticellartar and A. alternata*. The results obtained as zone of inhibition (mm) and MIC values (μg/mL) are presented in Table **3** and **4** respectively.

Table 1. ^{1}H NMR Data of the Synthesized Compounds

Compounds	Component	^{1}H NMR (DMSO-d_6, δ ppm)
Boc-RP-OBzl	Boc	1.42 (s, 9H, Me_3)
	Arg^1	8.41 (s, 1H, NH), 4.53 (m, 1H, $^{\alpha}$CH), 1.53 (m, 2H, $^{\beta}CH_2$), 1.45 (m, 2H, $^{\gamma}CH_2$), 3.07 (m, 2H, $^{\delta}CH_2$)
	Pro^2	4.35 (m, 1H, $^{\alpha}$CH), 3.06 (m, 2H, $^{\beta}CH_2$), 2.20-2.25 (m, 2H, $^{\gamma}CH_2$), 3.52-3.64(t, 2H, $^{\delta}CH_2$)
	OBzl	5.07 (m, 2H, CH_2), 7.30-7.34 (m, 5H, Ar-H)
Boc-PRP-OBzl	Boc	1.36 (s, 9H, Me_3)
	Pro^1	4.10 (m, 1H, $^{\alpha}$CH), 1.82-1.90 (m, 2H, $^{\beta}CH_2$), 1.80-1.81 (m, 2H, $^{\gamma}CH_2$), 3.67-3.76 (t, 2H, $^{\delta}CH_2$)
	Arg^2	8.00 (s, 1H, NH), 4.48 (m, 1H, $^{\alpha}$CH), 1.55 (m, 2H, $^{\beta}CH_2$), 1.47 (m, 2H, $^{\gamma}CH_2$), 3.09 (m, 2H, $^{\delta}CH_2$)
	Pro^3	4.46 (m, 1H, $^{\alpha}$CH), 3.09 (m, 2H, $^{\beta}CH_2$), 2.15-2.30 (m, 2H, $^{\gamma}CH_2$), 3.52-3.67 (t, 2H, $^{\delta}CH_2$)
	OBzl	5.08 (m, 2H, CH_2), 7.29-7.34 (m, 5H, Ar-H)
Boc-GPRP-OBzl	Boc	1.40 (s, 9H, Me_3)
	Gly^1	8.10 (s, 1H, NH), 4.13 (s, 2H, $^{\alpha}CH_2$)
	Pro^2	4.29 (m, 1H, $^{\alpha}$CH), 2.31-2.48 (m, 2H, $^{\beta}CH_2$), 2.16-2.31 (m, 2H, $^{\gamma}CH_2$), 3.77-3.84 (t, 2H, $^{\delta}CH_2$)
	Arg^3	7.95 (s, 1H, NH), 4.50 (m, 1H, $^{\alpha}$CH), 1.52 (m, 2H, $^{\beta}CH_2$), 1.47 (m, 2H, $^{\gamma}CH_2$), 3.10 (m, 2H, $^{\delta}CH_2$)
	Pro^4	4.44 (m, 1H, $^{\alpha}$CH), 3.12 (m, 2H, $^{\beta}CH_2$), 2.16-2.30 (m, 2H, $^{\gamma}CH_2$), 3.53-3.64 (t, 2H, $^{\delta}CH_2$)
	OBzl	5.08 (m, 2H, CH_2), 7.29-7.34 (m, 5H, Ar-H)
Boc-RPRP-OBzl	Boc	1.39 (s, 9H, Me_3)
	Arg^1	8.22 (s, 1H, NH), 4.60 (m, 1H, $^{\alpha}$CH), 1.61 (m, 2H, $^{\beta}CH_2$), 1.52 (m, 2H, $^{\gamma}CH_2$), 3.15 (m, 2H, $^{\delta}CH_2$)
	Pro^2	4.30 (m, 1H, $^{\alpha}$CH), 2.35-2.51 (m, 2H, $^{\beta}CH_2$), 2.19-2.33 (m, 2H, $^{\gamma}CH_2$), 3.78-3.81 (t, 2H, $^{\delta}CH_2$)
	Arg^3	8.34 (s, 1H, NH), 4.51 (m, 1H, $^{\alpha}$CH), 1.59 (m, 2H, $^{\beta}CH_2$), 1.50 (m, 2H, $^{\gamma}CH_2$), 3.13 (m, 2H, $^{\delta}CH_2$)
	Pro^4	4.46 (m, 1H, $^{\alpha}$CH), 3.03 (m, 2H, $^{\beta}CH_2$), 2.21-2.24 (m, 2H, $^{\gamma}CH_2$), 3.53-3.66 (t, 2H, $^{\delta}CH_2$)
	OBzl	5.11 (m, 2H, CH_2), 7.30-7.35 (m, 5H, Ar-H)
QZN	-	12.11 (s, 1H, NH), 11.69 (s, 1H, COOH), 7.06-8.43 (m, 4H, Ar-H), 2.48-2.53 (t, 4H, $(CH_2)_2$)
QZN-RP-OBzl	QZN	12.11 (s, 1H, NH), 7.07-8.45 (m, 4H, Ar-H), 2.46-2.55 (t, 4H, $(CH_2)_2$)
	Arg^1	7.88 (s, 1H, NH), 4.40 (m, 1H, $^{\alpha}$CH), 1.53 (m, 2H, $^{\beta}CH_2$), 1.45 (m, 2H, $^{\gamma}CH_2$), 3.07 (m, 2H, $^{\delta}CH_2$)
	Pro^2	4.37 (m, 1H, $^{\alpha}$CH), 3.07 (m, 2H, $^{\beta}CH_2$), 2.20-2.25 (m, 2H, $^{\gamma}CH_2$), 3.52-3.64(t, 2H, $^{\delta}CH_2$)
	OBzl	5.08 (m, 2H, CH_2), 7.30-7.34 (m, 5H, Ar-H)

(Table 1) Contd….

Compounds	Component	^{1}H NMR (DMSO-d_6, δ ppm)
QZN-PRP-OBzl	QZN	12.11 (s, 1H, NH), 7.07-8.45 (m, 4H, Ar-H), 2.46-2.55 (t, 4H, $(CH_2)_2$)
	Pro[1]	4.26 (m, 1H, $^{\alpha}CH$), 1.84-1.91 (m, 2H, $^{\beta}CH_2$), 1.80-1.83 (m, 2H, $^{\gamma}CH_2$), 3.66-3.76 (t, 2H, $^{\delta}CH_2$)
	Arg[2]	8.21 (s, 1H, NH), 4.49 (m, 1H, $^{\alpha}CH$), 1.55 (m, 2H, $^{\beta}CH_2$), 1.49 (m, 2H, $^{\gamma}CH_2$), 3.08 (m, 2H, $^{\delta}CH_2$)
	Pro[3]	4.48 (m, 1H, $^{\alpha}CH$), 3.10 (m, 2H, $^{\beta}CH_2$), 2.15-2.30 (m, 2H, $^{\gamma}CH_2$), 3.52-3.67 (t, 2H, $^{\delta}CH_2$)
	OBzl	5.08 (m, 2H, CH_2), 7.29-7.33 (m, 5H, Ar-H)
QZN-GPRP-OBzl	QZN	12.11 (s, 1H, NH), 7.10-8.44 (m, 4H, Ar-H), 2.45-2.57 (t, 4H, $(CH_2)_2$)
	Gly[1]	8.12 (s, 1H, NH), 4.15 (s, 2H, $^{\alpha}CH_2$)
	Pro[2]	4.30 (m, 1H, $^{\alpha}CH$), 2.31-2.48 (m, 2H, $^{\beta}CH_2$), 2.16-2.32 (m, 2H, $^{\gamma}CH_2$), 3.77-3.86 (t, 2H, $^{\delta}CH_2$)
	Arg[3]	7.97 (s, 1H, NH), 4.50 (m, 1H, $^{\alpha}CH$), 1.52 (m, 2H, $^{\beta}CH_2$), 1.46 (m, 2H, $^{\gamma}CH_2$), 3.09 (m, 2H, $^{\delta}CH_2$)
	Pro[4]	4.43 (m, 1H, $^{\alpha}CH$), 3.13 (m, 2H, $^{\beta}CH_2$), 2.17-2.31 (m, 2H, $^{\gamma}CH_2$), 3.14-3.53 (t, 2H, $^{\delta}CH_2$)
	OBzl	5.10 (m, 2H, CH_2), 7.30-7.32 (m, 5H, Ar-H)
QZN-RPRP-OBzl	QZN	12.11 (s, 1H, NH), 7.11-8.43 (m, 4H, Ar-H), 2.44-2.56 (t, 4H, $(CH_2)_2$)
	Arg[1]	8.23 (s, 1H, NH), 4.62 (m, 1H, $^{\alpha}CH$), 1.61 (m, 2H, $^{\beta}CH_2$), 1.52 (m, 2H, $^{\gamma}CH_2$), 3.15 (m, 2H, $^{\delta}CH_2$)
	Pro[2]	4.31 (m, 1H, $^{\alpha}CH$), 2.35-2.51 (m, 2H, $^{\beta}CH_2$), 2.19-2.33 (m, 2H, $^{\gamma}CH_2$), 3.78-3.81 (t, 2H, $^{\delta}CH_2$)
	Arg[3]	8.3 (s, 1H, NH), 4.52 (m, 1H, $^{\alpha}CH$), 1.60 (m, 2H, $^{\beta}CH_2$), 1.51 (m, 2H, $^{\gamma}CH_2$), 3.13 (m, 2H, $^{\delta}CH_2$)
	Pro[4]	4.48 (m, 1H, $^{\alpha}CH$), 3.04 (m, 2H, $^{\beta}CH_2$), 2.21-2.24 (m, 2H, $^{\gamma}CH_2$), 3.53-3.67 (t, 2H, $^{\delta}CH_2$)
	OBzl	5.09 (m, 2H, CH_2), 7.30-7.33 (m, 5H, Ar-H)

Table 2. Physical and Analytical Data of the Synthesized Compounds

Entry[a]	Molecular Formula	Theoretical Mol.Wt.	Actual MS Values (M^+)	Yield (%)	IR cm^{-1} (KBr)	Elemental analysis[b]		
						% C	% H	% N
Boc-RP-OBzl	$C_{23}H_{34}N_6O_7$	506	507	88.3	1655 (C=O), 3176 (NH)	54.51 (54.53)	6.74 (6.77)	16.60 (16.59)
QZN-RP-OBzl	$C_{29}H_{34}N_8O_7$	606	607	85.0	1689 (C=O), 3125 (NH)	57.40 (57.42)	5.66 (5.65)	18.49 (18.47)
Boc-PRP-OBzl	$C_{28}H_{41}N_7O_8$	603	604	88.0	1642 (C=O), 3120 (NH)	55.68 (55.71)	6.84 (6.85)	16.26 (16.24)
QZN-PRP-OBzl	$C_{34}H_{41}N_9O_8$	703	704	84.2	1712 (C=O), 3182 (NH)	58.06 (58.03)	5.88 (5.87)	17.93 (17.91)
Boc-GPRP-OBzl	$C_{30}H_{44}N_8O_9$	660	661	89.6	1676 (C=O), 3136 (NH)	54.50 (54.43)	6.70 (6.71)	16.98 (16.96)
QZN-GPRP-OBzl	$C_{36}H_{44}N_{10}O_9$	760	761	85.8	1646 (C=O), 3193 (NH)	56.81 (56.83)	5.85 (5.83)	18.44 (18.41)
Boc-RPRP-OBzl	$C_{34}H_{52}N_{12}O_{11}$	804	805	89.1	1710 (C=O), 3148 (NH)	50.72 (50.74)	6.52 (6.51)	20.86 (20.88)
QZN-RPRP-OBzl	$C_{40}H_{52}N_{14}O_{11}$	904	905	86.7	1688 (C=O), 3175 (NH)	53.11 (53.09)	5.82 (5.79)	21.69 (21.67)

[a]G-Glycine, P-Proline, R-Arginine
[b]Values given in the parentheses are the calculated for elemental analysis

It is observed from the results that all the heterocyclic conjugated peptides (**3, 6, 9** and **12**) have shown enhanced activity than either heterocycle (**1**) or peptides (**2, 5, 8** and **11**) which are moderately active. Among the conjugated compounds, tetrapeptide conjugates **9** and **12** (5 and 4 µg/mL respectively) have exerted high activity which is attributed to the increase in charge, hydrophobicity as well as the length of the peptide chain [15]. Whereas the dipeptide conjugate **3**

Table 3. Inhibitory Zone (diameter) mm of the Synthesized Conjugates (1-13) Against Tested Bacterial and Fungal Strains by Agar Well Diffusion Method

Entry	Antibacterial Activity					Antifungal Activity				
	Zone of Inhibition[a] (mm) ± SD (n = 3)									
	S. faecalis	*S. pyo-genes*	*E. coli*	*K. pneumoniae*	*P. putida*	*A. flavus*	*C. capsisi*	*F. ox-ysporum*	*F. verticel-lartar*	*A. alternata*
QZN (**1**)	04 ± 0.31	03 ± 0.26	00 ± 0.10	01 ± 0.30	03 ± 0.15	03 ± 0.20	02 ± 0.15	02 ± 0.25	04 ± 0.35	03 ± 0.26
Boc-RP-OBzl (**2**)	03 ± 0.46	02 ± 0.42	00 ± 0.10	03 ± 0.35	02 ± 0.20	00 ± 0.06	02 ± 0.38	01 ± 0.17	03 ± 0.20	00 ± 0.12
QZN-RP-OBzl (**3**)	16 ± 0.49	07 ± 0.42	09 ± 0.26	14 ± 0.35	11 ± 0.57	05 ± 0.25	13 ± 0.32	11 ± 0.36	17 ± 0.17	13 ± 0.36
QZN-RP-OH (**4**)	20 ± 0.78	09 ± 0.50	13 ± 0.45	22 ± 0.31	16 ± 0.82	07 ± 0.20	18 ± 0.35	16 ± 0.36	22 ± 0.56	18 ± 0.46
Boc-PRP-OBzl (**5**)	06 ± 0.36	03 ± 0.40	01 ± 0.25	05 ± 0.58	04 ± 0.20	01 ± 0.35	03 ± 0.31	03 ± 0.32	05 ± 0.26	02 ± 0.20
QZN-PRP-OBzl (**6**)	19 ± 0.49	10 ± 0.57	10 ± 0.36	17 ± 0.56	14 ± 0.35	07 ± 0.47	16 ± 0.25	13 ± 0.46	20 ± 0.47	15 ± 0.55
QZN-PRP-OH (**7**)	23 ± 0.35	13 ± 0.61	14 ± 0.38	24 ± 0.52	18 ± 0.25	09 ± 0.21	21 ± 0.40	18 ± 0.35	24 ± 0.56	22 ± 0.61
Boc-GPRP-OBzl (**8**)	08 ± 0.35	05 ± 0.40	03 ± 0.35	06 ± 0.40	05 ± 0.29	01 ± 0.38	05 ± 0.45	03 ± 0.30	06 ± 0.29	04 ± 0.35
QZN-GPRP-OBzl (**9**)	26 ± 0.32	24 ± 0.51	16 ± 0.31	25 ± 0.75	24 ± 0.50	12 ± 0.45	26 ± 0.38	21 ± 0.30	28 ± 0.50	24 ± 0.36
QZN-GPRP-OH (**10**)	34 ± 0.53	37 ± 0.23	23 ± 0.62	31 ± 0.38	33 ± 0.47	27 ± 0.38	35 ± 0.21	29 ± 0.23	35 ± 0.64	32 ± 0.57
Boc-RPRP-OBzl (**11**)	09 ± 0.36	05 ± 0.42	04 ± 0.30	08 ± 0.30	07 ± 0.35	03 ± 0.49	08 ± 0.40	05 ± 0.25	09 ± 0.36	05 ± 0.21
QZN-RPRP-OBzl (**12**)	29 ± 0.10	27 ± 0.46	18 ± 0.36	27 ± 0.66	27 ± 0.67	14 ± 0.23	29 ± 0.40	23 ± 0.42	31 ± 0.32	26 ± 0.50
QZN-RPRP-OH (**13**)	37 ± 0.56	39 ± 0.59	26 ± 0.32	34 ± 0.47	35 ± 0.70	30 ± 0.42	37 ± 0.55	31 ± 0.55	38 ± 0.61	33 ± 0.57
Ciprofloxacin	20 ± 0.59	22 ± 0.42	16 ± 0.42	18 ± 0.46	18 ± 0.38	-	-	-	-	-
Griseofulvin	-	-	-	-	-	14 ± 0.17	18 ± 0.35	15 ± 0.60	21 ± 0.30	17 ± 0.51

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

and tripeptide conjugate **6** have shown comparatively moderate activity (12 and 9 μg/mL respectively). Thus, as the length of the peptide chain increases the activity also increases [12]. All the quinazolinone conjugated analogues are more effective in arresting or killing the growth of gram negative microorganisms when compared to gram positive microorganisms since the cationic antimicrobial peptides are likely to first be attracted to the net negative charges that exist on the outer envelope of gram negative bacteria [12]. One exception to this is *E. coli* and hence it may be inferred that compounds exhibit strain specificity particularly against *E. coli.*

When the *C*-terminal benzyl ester and nitro group of the conjugates were removed, all the free acid containing compounds (**4, 7, 10** and **13**) have exerted high potency in arresting the growth of microorganisms which is nearly 3-4 times greater than the antibiotics used. This fact is due to the increase in the polarity of these compounds [12, 16] which would help the molecules to interact or penetrate more through the cell membranes of microbes and there by inactivating them [6]. This indicates that increase in the polarity has an impact on the activity.

Among the tetrapeptide conjugates, RPRP containing analogue has exhibited slight enhancement in the activity over GPRP conjugate. This may be due to the presence of more bulky and charged groups in the former which interacts more with the anionic phosphate groups of the microbial cell membranes.

CONCLUSION

In the present study, novel potent antimicrobial agents were synthesized by conjugating quinazolinone motif to shorter active analogues of Bac7. Our studies revealed that the heterocyclic conjugated peptides showed enhanced activity against all the pathogens tested compared to peptides and quinazolinone tested alone. Further, the hydrogenolysed compounds have shown high potency than their counterparts in arresting the growth of microbes. Also, as the length of the peptide chain increases, activity also increases. Thus, it can be inferred that conjugation plays a vital role in biological studies.

In conclusion, the shorter quinazolinone conjugated peptides with small size, easy to synthesize, simple amino acid composition, high antimicrobial activity and low toxicity, makes them highly potent as novel templates for the design of pharmaceutical compounds and peptidomimetics for topical and systemic treatment of the antibiotic resistant strains of bacteria and fungi.

Table 4. Minimum Inhibitory Concentration (MIC) in µg/mL of the Synthesized Conjugates (1-13) Against Tested Bacterial and Fungal Strains by Microdilution Method

Entry	Antibacterial activity					Antifungal activity				
	Minimum Inhibitory Concentration (MIC) in µg/mL[a]									
	S. faecalis	*S. pyogenes*	*E. coli*	*K. pneumoniae*	*P. putida*	*A. flavus*	*C. capsisi*	*F. oxysporum*	*F. verticellartar*	*A. alternata*
QZN (**1**)	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30
Boc-RP-OBzl (**2**)	18	22	29	22	26	32	28	26	22	23
QZN-RP-OBzl (**3**)	12	18	22	14	17	24	19	20	15	17
QZN-RP-OH (**4**)	10	10	16	10	12	16	18	10	08	09
Boc-PRP-OBzl (**5**)	14	20	28	19	24	29	26	24	19	21
QZN-PRP-OBzl (**6**)	09	15	18	13	15	20	18	17	13	15
QZN-PRP-OH (**7**)	07	09	14	08	10	13	09	09	06	08
Boc-GPRP-OBzl (**8**)	13	16	26	18	20	28	23	23	17	19
QZN-GPRP-OBzl (**9**)	05	06	08	08	08	12	08	08	05	07
QZN-GPRP-OH (**10**)	03	04	10	06	07	10	06	06	04	06
Boc-RPRP-OBzl (**11**)	11	14	24	16	19	26	20	21	15	19
QZN-RPRP-OBzl (**12**)	04	05	10	07	06	10	07	06	04	05
QZN-RPRP-OH (**13**)	03	03	08	04	04	09	04	05	03	03
Ciprofloxacin	10	12	08	07	11	-	-	-	-	-
Griseofulvin	-	-	-	-	-	08	12	14	07	10

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

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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ABBREVIATIONS

AMP = Antimicrobial peptide

Boc = *t*-Butoxycarbonyl

EDCI = 1-(3-Dimethylaminopropyl)-3-ethyl-carbodiimide.HCl

HOBt = 1-Hydroxybenzotriazole

IBCF = Isobutyl chloroformate

NMM = *N*-Methylmorpholine

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