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Short chain linear and cyclic cationic peptide designed from cecropin B: Synthesis and anticancer activity

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ABSTRACT

In the present work, we have designed short chain α-helical linear and cyclic peptide from cecropin B having same charge, hydrophobicity, and helicity. The designed compounds were synthesized by using solution phase method. Elucidation of structure of newly synthesized peptides was done by proton nuclear magnetic resonance, Carbon-13 nuclear magnetic resonance, Fourier-transform infrared spectroscopy, Fast atom bombardment mass spectrometry, and elemental analysis. Furthermore, the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltratrazolium bromide assay was performed for cytotoxicity of synthesized compounds against *Dalton's lymphoma ascites* (DLA), *Ehrlich's ascites carcinoma* (EAC), and Michigan Cancer Foundation-7 cell lines using 5-FU as a reference compound. Biological evaluation showed that short chain cyclicpeptides were more potent than linear peptides against EAC and DLA cell lines.

INTRODUCTION

At the global level, now the third leading cause of demise is cancer (Harris *et al*., 2013), and it has been estimated that within 20 years there will be approximately 26 million new cases of cancer and 17 million deaths will occur per annum (Thun *et al*., 2010). A series of cumulative genetic and epigenetic modification that occur in healthy cells initiates cancer and characterize by many distinct behaviors (Kreeger and Lauffenburger, 2010). Cancer cells generate signals of their own growth (Marusyk and Polyak, 2010), insensitive to growth suppressive signals, stave off cell death, rampant replication (Brooks *et al*., 2010), capability to stimulate angiogenesis and ability to invade tissues via basement membrane and walls of capillary (Sung *et al*., 2007).

Most widely used treatment for metastatic cancer is chemotherapy which works by targeting rapidly dividing cells (Donnelly, 2004). As a result, chemotherapy fails to differentiate cancer cells from normal cells and is unable to target dormant cancer (Naumov *et al*., 2003). In order to overcome this problem, valuable efforts have been made to develop a new class of anticancer drugs which have potential to selectively kill cancer cells while sparing healthy cells, regardless of their growth rate (Bush and Li, 2002). Various natural or synthetic cationic peptides alternative to conventional chemotherapy have been studied that exhibit anticancer activity with ability to kill target cells rapidly and specifically (Dennison *et al*., 2006).

In hunting for new anticancer agents, host defense peptides are promising alternative anticancer therapeutics that can contribute various benefits over other treatments (Hoskin and Ramamoorthy, 2008). Due to their activity and exclusivity on cancer cell membrane, there are less chance of toxicity to normal cells (Riedl *et al*., 2011).

Cationic antimicrobial peptides are small proteins, usually less than 40 amino acids in length, which are mainly made of basic (e.g., lysine and arginine) and hydrophobic (e.g., tryptophan) amino acids (Mader and Hoskin, 2006). A class of antimicrobial peptide Cecropins was first described in insects and the giant silk moth *Hyalophora cecropia* but later also reported in mammals. Cecropin A (KWKLFKKIEK VGQNIRDGIIKAGPAVAVVGQATQIAK**)** and Cecropin B (KWKVFKKIEKMGRNIRNGIVKAGPAIAVLGEAKAL) exhibit anticancer activity against various human cancer cell lines

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like lymphoma and leukemia cells without affecting the normal cells at peptide concentrations that are fatal to the transformed cells (Hui *et al*., 2002; Srisailam *et al*., 2000).

In the present work, we have designed, synthesized, and evaluated short chain α-helical linear and cyclic peptide from cecropin B having same charge, hydrophobicity, and helicity.

DESIGNING

It is clear from the fact that anticancer peptides (ACPs) obtained from different sources have very low similarity; nevertheless, they have some common characteristics, such as an amphipathic nature, a positive charge, the ability to possesses secondary structure (α-helical or β-sheet) in a hydrophobic environment and broad spectrum anticancer activity (Zelezetsky and Tossi, 2006).

The main class of cationic ACPs has α-helical structure and exhibit following properties:

(1) They retain basic amino acids, like arginine and lysine in their chain which revealed net positive charge of peptides from +2 to +9 in neutral pH; (2) Generally peptide chain is of 5–40 amino acid long with more than 30% hydrophobic residues; (3) They have amphipathic conformation (Liberio *et al*., 2013). Various physicochemical parameters of peptides, such as net charge, helicity, and hydrophobicity, have been signified to play principal roles in the selectivity and activity of α-helical ACPs against tumor cells (Huang *et al*., 2015).

In the present work, short chain peptides were designed by utilizing template-assisted approach based on structural parameters (charge, hydrophobicity, and helicity) from naturally occurring ACP cecropin B (KWKVFKKIEKMGRNI RNGIVKAGPAIAVLGEAK AL**)** (Chou *et al*., 2008) by removing amino acids in such a manner that it retains same or more cationic charge and hydrophobicity (Table 1). Hydrophobicity, hydrophilicity, and Charge of peptide were achieved by software package provided by Genscript. The amino acids sequence of Cecropin B, linear and cyclic compounds were designed as following:

Cecropin B: KWKVFKKIEKMGRNIRNGIVKAGPAIAVLGEAKAL
Designed Linear Compound: KKVKKIKRVKIAVLA
Designed Cyclic Compound: KKVKKIKRVKIAVLA

MATERIALS AND METHODS

Melting points were determined by open capillary method. Amino acids, di-tertbutylpyrocarbonate (Boc_2O), triethylamine (TEA), trifluoroacetic acid (TFA), dicyclohexylcarbodiimide (DCC), *N*-methylmorpholine (NMM), *p*-nitrophenol (PNP), tetrahydrofuran (THF), and pyridine were procured from Spectro Chem Limited (Mumbai, India). Determination of purity of synthesized peptide was accompanied by thin layer chromatography using chloroform & methanol as eluent. IR spectra were recorded on Perkin Elmer Spectrum RX-1 FTIR. ^{13}C NMR and Proton nuclear magnetic resonance (^1H NMR) spectra were recorded on Bruker AC NMR spectrometer (300 MHz), using $CDCl_3$ as a solvent and TMS as a internal reference. A fast atom bombardment mass spectrum was recorded on JMS-DX 303 Mass spectrometer. For elemental analysis of all the synthesized compounds, Vario EL III elemental analyzer was used.

Method for protection and deprotection of amino acid/peptide units (amino terminal)

For the protection of free α-amino group, 20 mmol of amino acid/peptide dissolved in 1N NaOH (40 ml) and isopropanol (30 ml) was added to 6 ml (2.6 mmol) Boc_2O with stirring for 2.5 hours at RT, followed by washing with 20 ml of light petroleum ether (b.p. 40ºC–60ºC). The above solution was acidified to pH 3.0 using 2N H_2SO_4 and then finally extracted by 60 ml of chloroform. Anhydrous Na_2SO_4 was used for drying the organic layer and resulting solution was evaporated under reduced pressure to obtain the crude product. The crude product was crystallized from petroleum ether and chloroform mixture (3:7).

For amino terminal deprotection, the solution of 10 mmol Boc peptide methyl ester and 2.28 g (20 mmol) of CF_3COOH in 15 ml $CHCl_3$ was stirred for 1 hour at room temperature. The solution was washed with 25 ml of saturated $NaHCO_3$ solution. The organic layer was dried by using anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was crystallized with petroleum ether and $CHCl_3$ mixture (1:9) producing deprotected peptide units (Dahiya and Pathak, 2007).

Method for protection and deprotection of amino acid/peptide units at carboxyl terminal

For protection of carboxyl group, 20 mmol of amino acid was dissolved in mixture of 100 ml methanol having 1.4 ml (20 mmol) thionyl chloride and then refluxed for 10 hours at 65°C. After evaporating the solvent, the residue was triturated with ether at 0ºC followed by crystallization of the crude product for purification from methanol/ether mixture (8:2).

For carboxyl terminal deprotection, 10 mmol of Boc-peptide methyl ester was dissolved in 36 ml of THF: H_2O (1:1),), and then 0.36 g (15 mmol) lithium hydroxide was added. The resulting mixture was stirred for 1 hour at room temperature followed by acidification with 1 N H_2SO_4 to pH 3.5. The aqueous layer was extracted with 100 ml of diethyl ether and then combined organic extract was dried over anhydrous Na_2SO_4. The organic layer was concentrated under reduced pressure and crystallized with methanol/ether mixture (8:2) (Dahiya and Sharma, 2008).

Method for coupling

To the solution of 10 mmol amino acid methyl ester/ peptide methyl ester dissolved in 20 ml chloroform, 2.3 ml (21 mmol) of N-methylmorpholine was added at 0°C followed by stirring for 15 minutes. Then after, a solution of 2.1 gm 10 mmol of dicyclohexylcarbodiimide and 10 mmol of Boc-amino acid/peptide dissolved in chloroform (20 ml) was added to the previous mixture with stirring for 36 hours and then filtered followed by washing with 30 ml of chloroform. The resulting filtrate was washed with 25 ml

Table 1. Physical characteristics of cecropin B and designed compounds.

S. No	Natural ACP (no. of amino acid)	Charge	Hydrophobicity (%)	Hydrophilicity (%)	Designed short chain peptide	Charge	Hydrophobicity (%)	Hydrophilicity (%)
1	Cecropin B (35)	$^+7$	51.43	31.42	ACP-5(15)	$^+7$	53.33	46.67

of sodium bicarbonate (5%) and 25 ml of saturated sodium chloride solution. Anhydrous Sodium sulfate was used for drying organic layer, filtered and evaporated under vacuum. Crystallization of the crude product was accomplished by a mixture of light petroleum ether and chloroform mixture (2:8) (Dahiya *et al*., 2010).

Procedure for Synthesis of KKVKKIKRVKIAVLA (10)

To the solution of 4.48 g (5 mmol) of compound **6** in 20 ml of chloroform, 1.1 ml (10.5 mmol) of N-methylmorpholine was added at 0°C, followed by stirring for 15 minutes. Then after, a solution of 1.06 g, 5 mmol of dicyclohexylcarbodiimide and 9.84 g, 5 mmol of compound **5** in 20 ml of chloroform was added into the previous solution with stirring for 36 hours and then filtered followed by washing the residue with 30 ml of chloroform. The resulting filtrate was washed with 25 ml of sodium bicarbonate (5%) and 25 ml of saturated sodium chloride solution and dried over anhydrous Na_2SO_4, filtered and evaporated under vacuum. The crude product was crystallized from a mixture of light petroleum ether and chloroform producing a pure Boc-K(2-Cl-Z)-K(2-Cl-Z)-V-K(2-Cl-Z)-K(2-Cl-Z)-I-K(2-Cl-Z)-R-V-K(2-Cl-Z)-I-A-V-L-AOCH$_3$ compound **7.**

To the solution of 7.10g, 2.5 mmol of compound **7** dissolved in 36 ml THF:H_2O (1:1), 0.09g, 3.75 mmol lithium hydroxide was added. The resulting mixture was stirred for 1h at room temperature followed by acidification with 1N H_2SO_4 to pH 3.5. The aqueous layer was extracted with 100 ml diethyl ether and dried over anhydrous Na_2SO_4, then concentrated under reduced pressure. The crude product was crystallized with methanol/ether (8:2) to get pure Boc-K(2-Cl-Z)- K(2-Cl-Z)-V-K(2-Cl-Z)-K(2-Cl-Z)-I-K(2-Cl-Z)-R-V-K(2-Cl-Z)-I-A-V-L-AOH compound **8**.

At room temperature, the solution of 2.83 g, 1 mmol of compound **8** and 0.228 g, 2 mmol CF_3COOH in 15 ml $CHCl_3$ was stirred for 1 hour. The previous solution was washed with saturated 25 ml of $NaHCO_3$ solution. The organic layer was dried by using anhydrous Na_2SO_4 and concentrated under reduced pressure. The crystallization of crude product was accomplished by petroleum ether and $CHCl_3$(2:8) producing a pure K(2-Cl-Z)- K(2-Cl-Z)-V-K(2-Cl-Z)-K(2-Cl-Z)-I-K(2-Cl-Z)-R-V-K(2-Cl-Z)-I-A-V-L-AOH compound **9** (Dahiya *et al*., 2009).

Deprotection of 2-Cl-benzyloxycarbonyl (2-Cl-Z) of peptide units

A mixture of 5 g, 36% solution of dry hydrogen bromide in glacial acetic acid (20 ml) and 2.46 g, 0.9 mmol of compound **9** in a flask protected with a calcium chloride was allowed to stand at room temperature with occasional shaking for 0.5 hours. Then 150 ml of dry ether was added to precipitate the desired compound. The produced solid was triturated with ether and filtered followed by washing with ether. The crude product was crystallized with methanol/ether mixture (8:2) yielding pure compound **10** (Ben-Ishai and Berger, 1952; Kosaku *et al*., 1970).

HPLC (125 mm Nucleodur 100-5 C-18, 4.0 mm, Methanol: Water = 70:30, 0.8 ml/minutes, 220 nm, 9.3 MPa, 308 K): t_R = 11.21 minutes (72.5% peak area).

IR (KBr), cm^{-1}: 3,410–3,230 (O–H stretching COOH), 3,288.55 (N–H stretching amide), 2,928.97 (C−H asym. stretching CH_2), 2,870.07 (C−H sym. stretching CH_2), 1,722.63 (C=O stretching acid), 1,677.32 (C=O stretching amide), 1,627.49 (N−H bend amine), 1,578.63 (N−H bend 2° amide), 1,308.67 (O−H bend COOH), and 703.18 (C−N bending 2° amide).

^{1}H NMR ($CDCl_3$), δ: 1.088 (t, 6H, J = 6.0 Hz, γ-H, Ile), 1.240 (d, 18H, J = 17.4 Hz, ββ′-H, Val), 1.346 (d, 18H, J = 12.3 Hz, α-H, Ala, γγ′-H, Leu, β′-H, Ile) 1.611–1.824 (m, 31H, β-H, γ-H, Lys, β-H, Ile, β-H Arg, β-H Leu), 1.905 (t, 16H, J= 11.1Hz, α-H, Lys, α-H Arg, α-H, Leu), 3.199 (s, 18H, NH_2, Lys, NH_2, NH, Arg), 3.450–3.476 (m, 5H, α-H, Ile, α-H, Val), 4.147 (t, 14H, J = 3.6Hz, γ-H, Arg, δ-H, Lys), 4.207–4.285 (m, 15H, NH-CH-CO), 8.418 (s, 14H, NH , amide), and 9.153 ppm (s, 1H, COOH, Ala).

Synthesis of Cyclic KKVKKIKRVKIAVLA (12)

The solution of 2.83 g, 1 mmol of compound **8,** 0.188 g, 1.34 mmol of *p*-nitrophenol and 0.212 g, 1 mmol dicyclohexylcarbodiimide dissolved in 45 ml chloroform at 0°C was stirred for 12 hours at room temperature and then filtered. The filtrate was washed with 45 ml $NaHCO_3$ (10 %) and 10 ml HCl (5 %). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to get the compound **8a**. To solution of 2.95 g, 1 mmol of compound 8a in 35 ml chloroform, 0.182 g, 1.6 mmol CF_3COOH was added and stirred for 1 hour at room temperature. The previous solution was washed with 25 ml of $NaHCO_3$ solution (10%). Anhydrous Na_2SO_4 was used for drying organic layer and concentrated under reduced pressure to get the compound **8b**. To the solution of 2.85 g, 1 mmol of compound **8b** in 25 ml chloroform, 0.56 ml, 4.2 mmol N-Methyylmorpholine was added. After that whole contents were allowed to stand at 0°C (7 days) followed by washing with 50 ml $NaHCO_3$ solution (10 %) and finally washed with 75 ml HCl (5 %). Anhydrous Na_2SO_4 was used for drying organic layer and concentrated under reduced pressure. Pure cyclicpeptide **11** was crystallized from chloroform/*n*-hexane (6:4) (Dahiya *et al*., 2009).

Deprotection of 2-Cl-benzyloxycarbonyl(Z) of peptide units

A mixture of 5 g, 36% solution of dry hydrogen bromide in (20 ml) glacial acetic acid and 2.72 g, 1 mmol of compound **11** in a flask protected with calcium chloride at room temperature with occasional shaking for 0.5 hour. Nearly, 150 ml of dry ether was added for the formation of precipitate. After that, supernatant was decanted and solid was triturated with ether followed by filtration, and washing with ether. The crude product was crystallized with methanol/ether to get pure compound **12** (Ben-Ishai and Berger, 1952; Kosaku *et al*., 1970).

HPLC (125 mm Nucleodur 100-5 C-18, 4.0 mm, Methanol: Water = 70:30, 0.8 ml/minutes, 220 nm, 9.3 MPa, 308 K): t_R = 10.11 minutes (78.4% peak area).

IR (KBr), cm^{-1}: 3,320.25 (N−H asym stretching amide), 3,239.99 (N–H stretching amine), 2,975.82 (C−H asym. stretching CH_2), 2,936.17 (C−H sym. stretching CH_2), 1,661.30 (C=O stretching amide), 1,597.18 (N−H bend amide), 1,514.88 (C−C stretching skeletal bands), 1,267.45 (CH bend isopropyl), and 749.67 (C−N bending amide).

^{1}H NMR ($CDCl_3$), δ: 0.921 (t, 6H, J= 3.47Hz, γ-H, Ile), 1.107 (d, 18H, J = 4.3Hz, ββ′-H, Val) 1.218 (d, 6H, J = 9.54Hz, α-H, Ala), 1.270 (d, 6H, J = 6.6Hz, γ γ′-H, Leu), 1.312 (d, 6H, J = 6.6Hz, β′-H, Ile), 1.3427–1.6048 (m, 16H, β-H, Lys, β-H, Ile), 1.7218–1.7704 (m, 15H, γ-H, Lys, β-H Arg, β-H, Leu), 1.838 (t, 14H, J= 8.2 Hz, α-H, Lys, α-H Arg), 1.929 (t, 16H, J= 5.8 Hz, γ-H, Arg, δ-H, Lys, α- H, Leu), 2.0190–2.0659 (m, 5H, α-H, Ile, α-H,

Val), 2.1870 (s, 16H, NH_2, Lys, NH_2, NH, Arg), 3.2217–3.7426 (m, 15H, N-CH-CO), and 7.9844 ppm (s, 15H, NH , amide).

Carbon-13 nuclear magnetic resonance (^{13}C NMR) ($CDCl_3$), δ: 175.2 (2C, C=O, Ala-2), 174.5 (6C, C=O, Lys-6), 173.9 (C, C=O, Leu), 172.6 (C, C=O, Arg), 171.6 (2C, C=O, Ile-2), 168.7 (3C, C=O, Val-3), 160.2 (C, C=NH, Arg), 63.8 (3C, C-α, Val-3), 58.8 (2C, C-α, C-Ile-2), 56.8 (6C, C-α, Lys-6), 55.6 (C,C-α, C-Arg), 54.3 (C, C-α, Leu), 51.8 (2C, C-α, Ala-2) 44.4 (6C, C-ε, Lys-6), 43.7 (C, C-α, Leu), 39.7 (C, C-δ, C-Arg), 37.4 (2C, C-β, Ile-2), 35.6 (6C, C-ε, Lys-6), 33.1 (6C, C-β, Lys-6) 32.2 (3C, C-β, Val-3), 31.6 (C, C-β, Arg), 28.4 (2C, C-γ, Ile-2), 26.8 (C, C-γ, C-Arg), 24.8 (2C, C-γγ′, Leu), 23.6 (C, C-β, Leu), 22.7 (6C, C-γ, Lys-6), 19.8 (2C, C-β, Ala-2), 18.5 (6C, C-γγ′, Val-3), 17.2 (2C, C-γ, Ile-2), and 11.1 (2C, C-δ, Ile-2).

Fast atom bombardment mass spectrometry (*m/z*, rel. int.): 1704.2 [(M + H)+, 100], 1676.3 [(1704.2 – CO)+, 23], 1633.2 [(1704.2–A)+, 32], 1576.4 [(1704.2–K)+, 29], 1520.1 [(1704.2–LA)+, 17], 1448.0 [(1704.2–KK)+, 39], 1421.0 [(1704.2–VLA)+, 44], 1349.9 [(1704.2–AVLA)+, 52], 1348.9 [(1704.2–VKK)+, 67], 1236.8 [(1707.2– IAVLA)+, 79], 1220.8 [(1704.2–KVKK)+, 44], 1108.8 [(1704.2–KIAVLA)+, 32], 1092.8 [(1704.2–KKVKK)+, 47], 1009.7 [(1704.2–VKIAVLA)+, 41], 979.6 [(1704.1–IKKVKK)+, 19], 853.6 [(1707.2–RVKIAVLA)+, 41], 851.5 [(1704.2–KIKKVKK)+, 27], 725.5 [(1704.2– KRVKIAVLA)+, 26], 695.4 [(ALVAIKV)+, 24], 612.4 [(KKVKK)+, 46], 596.3 [(ALVAIK)+,38], 484.3 [(KKVK)+, 34], 468.3[(ALVAI)+, 46], 356.2 [(KKV)+, 51], 355.2 [(ALVA)+,38], 284.2 [(VLA)+, 34], 257.2 [(KK)+, 24], 185.1 [(VL)+, 46], 129.1 [(K)+, 34], 72.2 [(V)+, 51], 86.2 [($C_5H_{12}N$)+, 22], 44.1 [(C_2H_6N)+, 16], 31.0 [(CH_3O)+, 9], 30.1 [(CH_4N)+, 12], 29.1 [(C_2H_5)+, 11], and 15.0 [(CH_3)+, 10].

In-vitro cytotoxic activity for synthesized peptide

The cytotoxic activity of synthesized compound **10** and **12** was carried out using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltratrazolium bromide (MTT) assay *in vitro* against *Dalton'slymphoma ascites (DLA*), *Ehrlich's ascites carcinoma* (*EAC*), and Breast Cancer cell line Michigan Cancer Foundation-7 (*MCF-7*) by 1×10^4 cells in 96-well plates. The plates were seeded and incubated with serially fourfold diluted concentration (21.33–170.70 µg/ml) of different peptides for 48 hours at 37°C. Standard drug 5-fluorouracil was also used as positive control. 200 ml, 5 mg/ml of MTT solution in phosphate buffered saline was added to cells and left for 4 hours at 37°C followed by addition of 150 ml dimethyl sulfoxide, which was added for dissolving formazan crystals. The absorbance was determined at 490 nm. The results were expressed as IC50, representing the concentration at which cell viability was reduced by 50%. The cytotoxicity assays were repeated in quadruplicates.

RESULTS AND DISCUSSION

Chemistry

Disconnection strategy was used to carry out the synthesis of designed compounds. The designed compounds were split into three tetrapeptide units **Boc-K(2-Cl-Z)-K(2-Cl-Z)-V-K(2-Cl-Z)-Q-OH** (**1**) **K(2-Cl-Z)-I-K(2-Cl-Z)-R-OCH_3** (**2**), **Boc-V- K(2-Cl-Z)-I-A-OH (3),** and a tripeptide unit **V-L-A–OCH_3** (**4**). To obtain tetrapeptide **Boc-K(2-Cl-Z)-K(2-Cl-Z)-V-K(2-Cl-Z)-Q-OH**, Boc-V- OH was coupled with K(2-Cl-Z)-OCH_3.HCl to give Boc-V-K(2-Cl-Z)-OCH_3 by employing DCC as coupling agents and TEA/NMM as bases. Trifluoroacetic acid was used for removing Boc group of Boc-V-K(2-Cl-Z)-OCH_3 to give V-K(2-Cl-Z)-OCH_3. Dipeptide V-K(2-Cl-Z)-OCH_3 was coupled with Boc-K(2-Cl-Z)-OH to give Boc-K(2-Cl-Z)-V-K(2-Cl-Z)-OCH_3 by employing DCC as coupling agents and TEA/NMM as bases. Trifluoroacetic acid was used for removing Boc group of Boc-K(2-Cl-Z)-V-K(2-Cl-Z)-OCH_3 to give K(2-Cl-Z)-V-K(2-Cl-Z)-OCH_3. Tripeptide K(2-Cl-Z)-V-K(2-Cl-Z)-OCH_3 was coupled with Boc-K(2-Cl-Z)-OH to give Boc-K(2-Cl-Z)-K(2-Cl-Z)-V-K(2-Cl-Z)-OCH_3 by employing DCC/DIPC as coupling agents and TEA/NMM as bases. Lithium hydroxide (LiOH) was used for removing ester group of Boc-K(2-Cl-Z)-K(2-Cl-Z)-V-K(2-Cl-Z)-OCH_3 to give **Boc-K(2-Cl-Z)-K(2-Cl-Z)-V-K(2-Cl-Z)-Q-OH** (**1**). Other tetrapeptide units **K(2-Cl-Z)-I-K(2-Cl-Z)-R-OCH_3** (**2**), **Boc-V- K(2-Cl-Z)-I-A-OH (3)** and tripeptide **V-L-A –OCH_3** (**4**) were prepared by coupling of Boc-amino acids with corresponding amino acid methyl ester hydrochlorides as above. Tetrapeptide **Boc-K(2-Cl-Z)-K(2-Cl-Z)-V-K(2-Cl-Z)-Q-OH** (**1**) and **K(2-Cl-Z)-I-K(2-Cl-Z)-R-OCH_3** (**2**) were coupled to get the octapeptide unit **Boc-K(2-Cl-Z)-K(2-Cl-Z)-V-K(2-Cl-Z)-Q-K(2-Cl-Z)-I-K(2-Cl-Z)-R-OCH_3**. Lithium hydroxide (LiOH) was used for removing ester group of **Boc-K(2-Cl-Z)-K(2-Cl-Z)-V-K(2-Cl-Z)-Q-K(2-Cl-Z)-I-K(2-Cl-Z)-R-OCH_3** to give **Boc-K(2-Cl-Z)-K(2-Cl-Z)-V-K(2-Cl-Z)-Q-K(2-Cl-Z)-I-K(2-Cl-Z)-R-OH** (**5**). Tetrapeptide **Boc-V- K(2-Cl-Z)-I-A-OH (3)** and tripeptide **V-L-A –OCH_3** (**4)** were then coupled to get the heptapeptide **Boc-V-K(2-Cl-Z)-I-A-V-L-A –OCH_3** . Trifluoroacetic acid was used for removing Boc group of **Boc-V-K(2-Cl-Z)-I-A-V-L-A –OCH_3** to give **V-K(2-Cl-Z)-I-A-V-L-A-OCH_3 (6)**. Octaapeptide **Boc-K(2-Cl-Z)-K(2-Cl-Z)-V-K(2-Cl-Z)-Q-K(2-Cl-Z)-I-K(2-Cl-Z)-R-OH** (**5)** and heptapeptide **V-K(2-Cl-Z)-I-A-V-L-A-OCH_3 (6)** units were coupled to get the linear pentadecapeptide unit **Boc-K(2-Cl-Z)-K(2-Cl-Z)-V-K(2-Cl-Z)-Q-K(2-Cl-Z)-I-K(2-Cl-Z)-R-V-K(2-Cl-Z)-I-A-V-L-A-OCH_3 (7)**. The Compound **Boc-K(2-Cl-Z)-K(2-Cl-Z)-V-K(2-Cl-Z)-Q-K(2-Cl-Z)-I-K(2-Cl-Z)-R-V-K(2-Cl-Z)-I-A-V-L-A-OCH3 (7)** was deprotected at carboxyl end to give **Boc-K(2-Cl-Z)-K(2-Cl-Z)-V-K(2-Cl-Z)-Q-K(2-Cl-Z)-I-K(2-Cl-Z)-R-V-K(2-Cl-Z)-I-A-V-L-A-OH (8)** by using lithium hydroxide (LiOH) and amino terminal by using CF_3COOH to give **K(2-Cl-Z)-K(2-Cl-Z)-K(2-Cl-Z)-Q-R-I-K(2-Cl-`Z)-A-A-V-A-A-T-I-K(2-Cl-Z)-A-OH (9).** Hydrogen bromide in glacial acetic acid **(36%)** was used for deprotection of 2-chloro-Benzyloxycarbonyl of peptide **K(2-Cl-Z)-K(2-Cl-Z)-K(2-Cl-Z)-Q-R-I-K(2-Cl-`Z)-A-A-V-A-A-T-I-K(2-Cl-Z)-A-OH (9)** at amino terminal of side chain to get **KKVKKIKRVKIAVLA (10)** peptide (Fig. 1). The linear peptide **Boc-K(2-Cl-Z)-K(2-Cl-Z)-V-K(2-Cl-Z)-Q-K(2-Cl-Z)-I-K(2-Cl-Z)-R-V-K(2-Cl-Z)-I-A-V-L-A-OH (8)** was coupled with *p*-nitrophenyl to give **BocK(2-Cl-Z)K(2-Cl-Z)VK(2-Cl-Z)K(2-Cl-Z)IK(2-Cl-Z)RVK(2-Cl-Z)IAVLAOPNP (8a)** followed by deprotection of Boc group by using TFA to give **K(2-Cl-Z)K(2-Cl-Z)VK(2-Cl-Z)K(2-Cl-Z)IK(2-Cl-Z)RVK(2-Cl-Z)IAVLAOPNP (8b)**. Linear peptide **K(2-Cl-Z)K(2-Cl-Z)VK(2-Cl-Z)K(2-Cl-Z)IK(2-Cl-Z)RVK(2-Cl-Z)IAVLAOPNP (8b)** was allowed to stand with catalytic amount of NMM at 0°C for 7 days to get cyclicpeptide **K(2-Cl-Z)K(2-Cl-Z)VK(2-Cl-Z)K(2-Cl-Z)IK(2-Cl-Z)RVK(2-Cl-Z)IAVLA (11)**. Hydrogen bromide in glacial acetic acid (36%) was used for deprotection of 2-chloro-Benzyloxycarbonyl of Cylic-**K(2-Cl-Z)K(2-Cl-Z)VK(2-Cl-Z)K(2-Cl-Z)IK(2-Cl-Z)RVK(2-Cl-Z)IAVLA (11)** at amino terminal of side chain to get compound **KKVKKIKRVKIAVLA (12)** (Fig. 2).

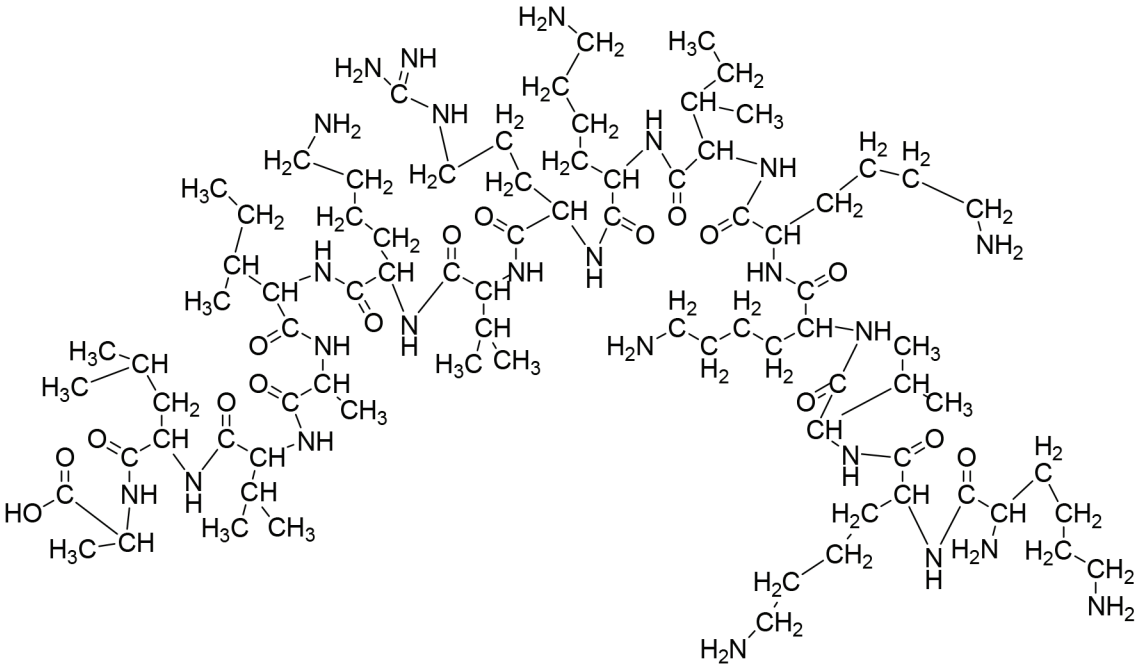

Figure 1. Structure of compound 10.

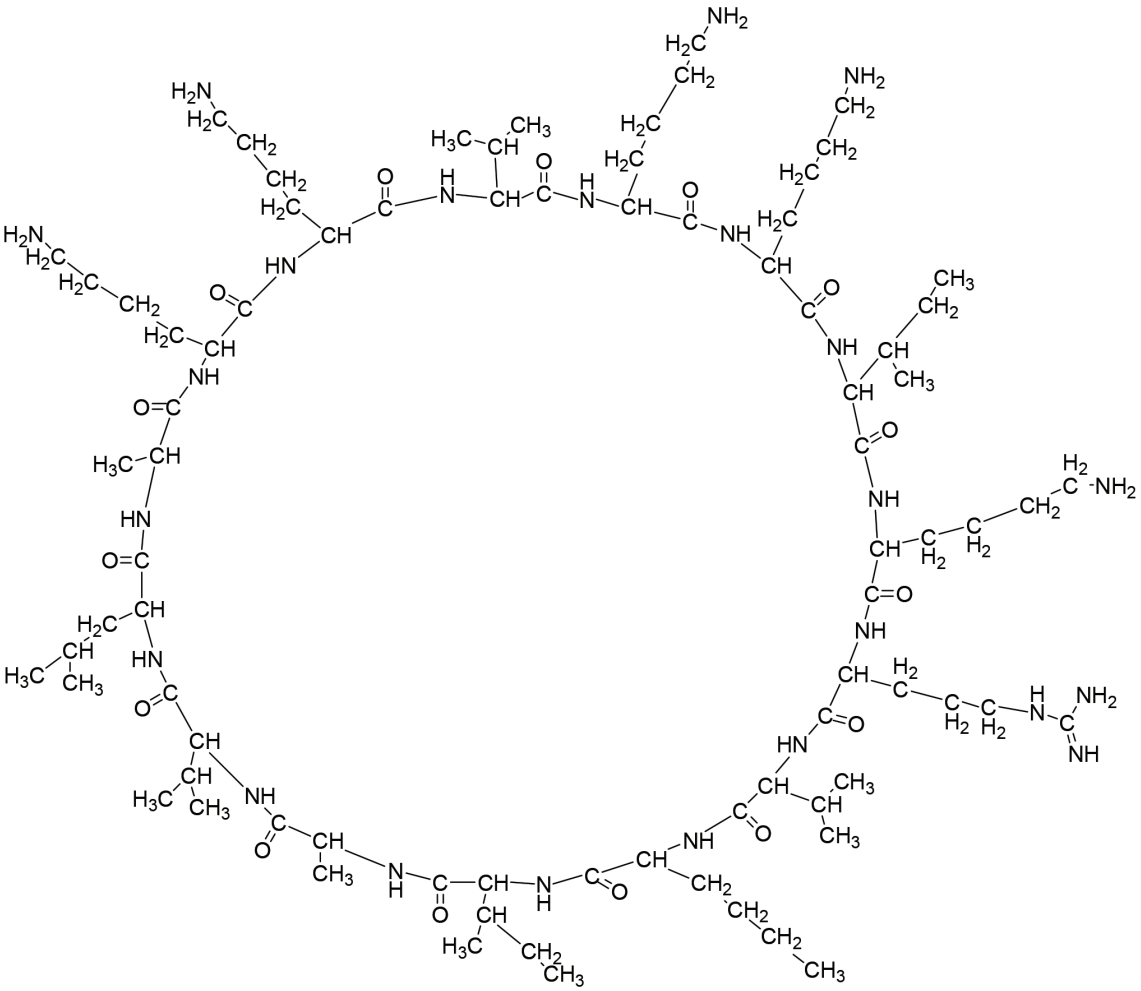

Figure 2. Structure of compound 12.

Table 2. Physical Characterization for Synthesized Compounds 1–12.

Compound	Physical state	Mol. formula (Mol. Weight)	M.P.(°C)	% Yield	Rf[a] (cm)	Elemental analysiscalculated/found		
						%C	%H	%N
1	Light brown solid	$C_{52}H_{70}Cl_3N_7O_{13}$ (1105.11)	132–134	75 (8.28g)	0.37	56.39/56.27	5.91/5.97	6.37/6.12
2	White Solid	$C_{41}H_{61}Cl_2N_9O_9$ (893.4)	151–153	74 (6.60g)	0.61	55.03/54.97	7.92/7.78	14.09/14.01
3	Brown solid	$C_{33}H_{52}ClN_5O_9$ (697.35)	132–134	82 (5.71g)	0.45	56.76/56.52	7.51/7.65	10.03/09.92
4	Yellowish brown solid	$C_{15}H_{29}N_3O_4$ (315.22)	119–121	92 (2.90g)	0.41	57.12/57.04	9.27/9.05	13.32/13.14
5	White solid	$C_{92}H_{127}Cl_5N_{16}O_{21}$ (1966.78)	201–203	58 (11.40g)	0.29	56.08/56.02	6.50/6.38	11.37/11.19
6	White solid	$C_{43}H_{71}ClN_8O_{10}$ (894.52)	122–124	68 (6.07g)	0.72	57.67/57.43	7.99/7.78	12.51/12.37
7	White crystalline solid	$C_{135}H_{196}Cl_6N_{24}O_{30}$ (2843.27)	211–213	71 (10.09g)	0.36	55.94/55.71	6.94/6.84	11.80/11.72
8	Dark brown solid	$C_{134}H_{186}Cl_6N_{24}O_{30}$ (2829.25)	183–185	86 (6.08g)	0.42	56.79/56.56	6.90/6.64	11.86/11.66
9	Dark yellow solid	$C_{129}H_{186}Cl_6N_{24}O_{28}$ (2729.2)	197–199	90 (2.45g)	0.57	56.68/56.33	6.86/6.76	12.30/12.11
10	Dark brown solid	$C_{81}H_{155}N_{23}O_{17}$ (1722.2)	121–123	76 (1.17g)	0.71[b]	56.46/56.32	9.07/8.98	18.69/18.44
11	Brown solid	$C_{129}H_{184}Cl_6N_{24}O_{27}$ (2711.19)	200–202	89 (2.41g)	0.27	57.05/56.87	6.83/6.75	12.38/12.18
12	Brown solid	$C_{81}H_{153}N_{23}O_{16}$ (1704.19)	151–153	77 (1.31g)	0.57[b]	57.05/56.88	9.04/8.88	18.89/18.64

[a]($CHCl_3$:CH_3OH/7:3).
[b]($CHCl_3$:CH_3OH/8:2).

Characterization of newly synthesized peptide was accomplished by elemental analysis as well as spectral analysis. Solution phase technique (Bodanzsky and Bodanzsky, 1984) was used for synthesis of designed peptides. For this DCC was used as a coupling agents and TEA/NMM as bases. Compound **12** was synthesized with 77 % yield. Absence of absorption bands at 1722.63 cm^{-1} (C=O stretching of acid) in IR spectra of compound 12 proved the cyclization of linear peptide **10**. Cyclization was further confirmed by absence of singlets at 9.153 ppm for one protons of free carboxylic acid group in 1H NMR of compound **12** and M^+1 peak at m/z 1704.2 in mass spectrum is compatible with the moecular formula $C_{77}H_{143}N_{25}O_{18}$. Physical characterization of synthesized compounds is shown in Table 2.

In-vitro cytotoxic activity

IC50 values of synthesized linear peptide **10** were **59.52, 76.25,** and **94.24** µg, respectively, and IC50 values of synthesized cyclopeptide **12** were **52.19**, **62.27,** and **86.88** µg, respectively, in comparison to standard drug (5-FU) which exhibited IC50 values of **51.77, 61.31, and 81.72** µg against *DLA* (Fig. 3), *EAC* (Fig. 4) and *MCF-7* (Fig. 5) cell lines, respectively. The IC50 values of synthesized compound **12** were found much lower than compound **10** but almost equal potency to that of standard drug 5-FU against *DLA* and *EAC* cell lines. The Comparison of IC50 values of synthesized compound and 5-FU against all cell lines is shown in Figure 6.

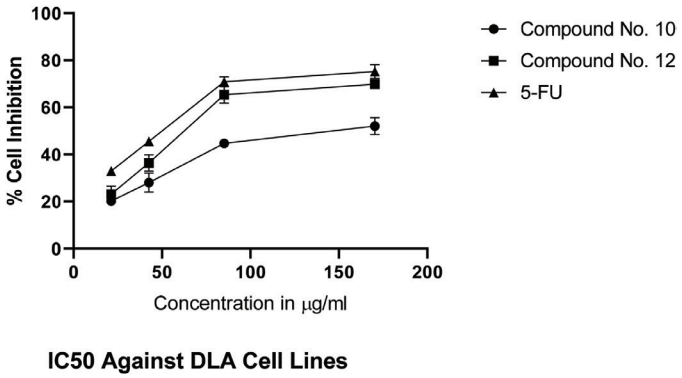

Figure 3. IC50 against DLA cell lines.

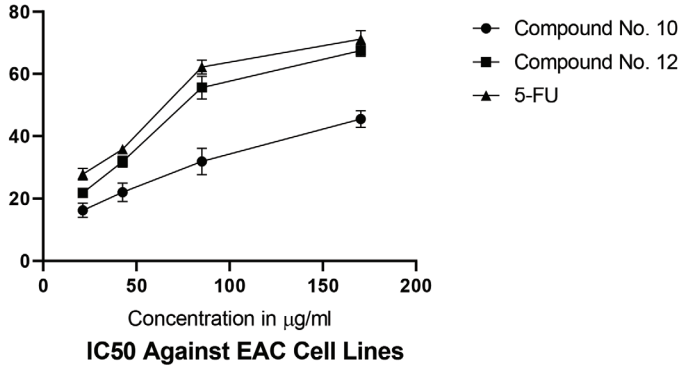

Figure 4. IC50 against EAC cell lines.

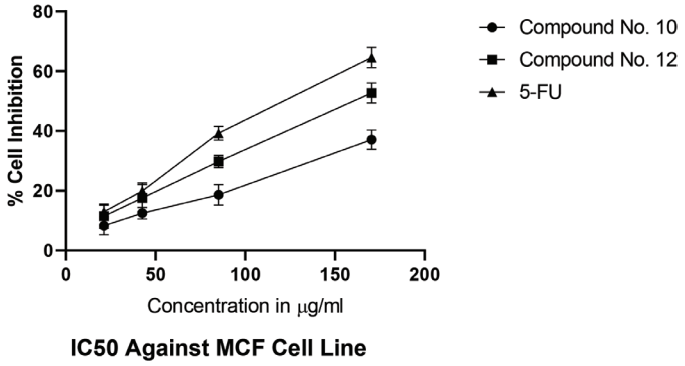

Figure 5. IC50 against MCF-7 cell lines.

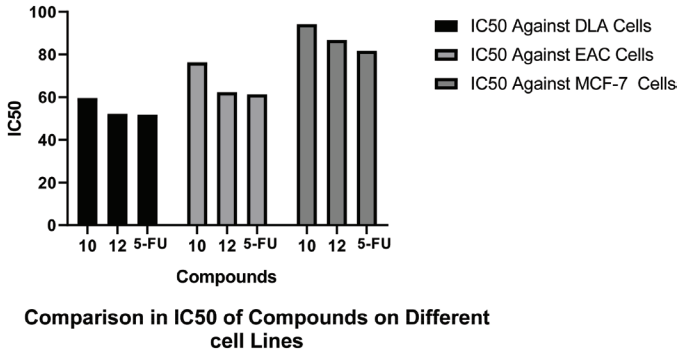

Figure 6. Comparison of IC50 of compounds on different cell lines.

CONCLUSION

We were able to evolve short chain linear and cyclic peptides having same charge and hydrophobicity/hydrophilicity with improved activity by utilizing a structure-based rational approach. Based on removing of amino acid of the peptide at non-polar/polar face of the amphipathic α-helical cationic anticancer peptide cecropin B, the desired peptides were designed. We were able to retain peptide charge and hydrophobicity/hydrophilicity in linear and cyclic peptide by systematically removing amino acids which resulted in optimize anticancer activity against *DLA* and *EAC* cell lines.

FINANCIAL SUPPORT

None.

CONFLICT OF INTEREST

Authors declare that they have no conflicts of interest.

REFERENCES

Ben-Ishai D, Berger A. Cleavage of N-Carbobenzoxy groups by dry hydrogen bromide and hydrogen chloride. J Org Chem, 1952; 17(12):1564–70.

Bodanzsky M, Bodanzsky A. The practice of peptide synthesis. Springer-Verlag, New York, NY, 1984.

Brooks SA, Lomax-Browne HJ, Carter TM, Kinch CE, Hall DM. Molecular interactions in cancer cell metastasis. Acta Histochem, 2010; 112(1):3–25.

Bush JA, Li G. Cancer chemoresistance: the relationship between p53 and multidrug transporters. Int J Cancer, 2002; 98(3):323–30.

Chou HT, Kuo TY, Chiang JC, Pei MJ, Yang WT, Yu HC, Lin SB, Chen SW. Design and synthesis of cationic antimicrobial peptides with improved activity and selectivity against *Vibrio* spp. Int J Antimicrob Agents, 2008; 32(2):130–38.

Dahiya R, Kumar A, Gupta R. Synthesis, cytotoxic and antimicrobial screening of a proline-rich cyclopolypeptide. Chem Pharm Bull, 2009; 57(2):214–17.

Dahiya R, Mourya R, Agrawal SC. Synthesis and antimicrobial screening of peptidyl derivatives of bromocoumarins/methylimidazoles. Afr J Pharm Pharmacol, 2010; 4(5):214–25.

Dahiya R, Pathak D. First total synthesis and biological evaluation of halolitoralin A. J Serb Chem Soc, 2007; 72(2):101–07.

Dahiya R, Sharma RD. Synthesis and bioactivity of a novel cyclic hexapeptide from *Stellaria Delavayi.* Eur J Sci Res, 2008; 21(2):277–87.

Dennison SR, Whittaker M, Harris F, Phoenix DA. Anticancer alpha-helical peptides and structure/function relationships underpinning their interactions with tumour cell membranes. Curr Protein Pept Sci, 2006; 7(6):487–99.

Donnelly JG. Pharmacogenetics in cancer chemotherapy: balancing toxicity and response. Ther Drug Monit, 2004; 26(2):231–35.

Harris F, Dennison SR, Singh J, Phoenix DA. On the selectivity and efficacy of defense peptides with respect to cancer cells. Med Res Rev, 2013; 33(1):190–234.

Hoskin DW, Ramamoorthy A. Studies on anticancer activities of antimicrobial peptides. Biochim Biophys Acta Biomembr, 2008; 1778(2):357–75.

Huang Y, Feng Q, Yan Q, Hao X, Chen Y. Alpha-helical cationic anticancer peptides: a promising candidate for novel anticancer drugs. Mini Rev Med Chem, 2015; 15(1):73–81.

Hui L, Leung K, Chen HM. The combined effects of antibacterial peptide cecropin A and anti-cancer agents on leukemia cells. Anticancer Res, 2002; 22(5):2811–16.

Kosaku N, Shigeyuki T, Nobuo I. Modified benzyloxycarbonyl group for protection of ε-amino groups of lysine. Bull Chem Soc Jpn, 1970; 43(6):1883–85.

Kreeger PK, Lauffenburger DA. Cancer systems biology: a network modeling perspective. Carcinogenesis, 2009; 31(1):2–8.

Liberio MS, Joanitti GA, Fontes W, Castro M.S. Anticancer peptides and proteins: a panoramic view. Protein Pept Lett, 2013; 20(4):380–91.

Mader JS, Hoskin DW. Cationic antimicrobial peptides as novel cytotoxic agents for cancer treatment. Expert Opin Investig Drugs, 2006; 15(8):933–46.

Marusyk A, Polyak K. Tumor heterogeneity: causes and consequences. Biochim Biophys Acta Rev Cancer, 2010; 1805(1):105–17.

Naumov GN, Townson JL, MacDonald IC, Wilson SM, Bramwell VHC, Groom AC, Chambers AF. Ineffectiveness of doxorubicin treatment on solitary dormant mammary carcinoma cells or late—developing metastases. Breast Cancer Res Treat, 2003; 82(3): 199–206.

Riedl S, Zweytick D, Lohner K. Membrane-active host defense peptides—challenges and perspectives for the development of novel anticancer drugs. Chem Phys Lipids, 2011; 164(8):766–81.

Srisailam S, Arunkumar AI, Wang W, Yu C, Chen HM. Conformational study of a custom antibacterial peptide cecropin B1: implications of the lytic activity. Biochim Biophys Acta Protein Struct Mol Enzymol, 2000; 1479(1–2):275–85.

Sung SY, Hsieh CL, Wu D, Chung LW, Johnstone PA. Tumor microenvironment promotes cancer progression, metastasis, and therapeutic resistance. Curr Probl Cancer 2007; 31(2):36–100.

Thun MJ, DeLancey JO, Center MM, Jemal A, Ward EM. The global burden of cancer: priorities for prevention. Carcinogenesis 2010; 31(1):100–10.

Zelezetsky I, Tossi A. Alpha-helical antimicrobial peptides—using a sequence template to guide structure-activity relationship studies. Biochim Biophys Acta Biomembr, 2006; 1758(9):1436–49.

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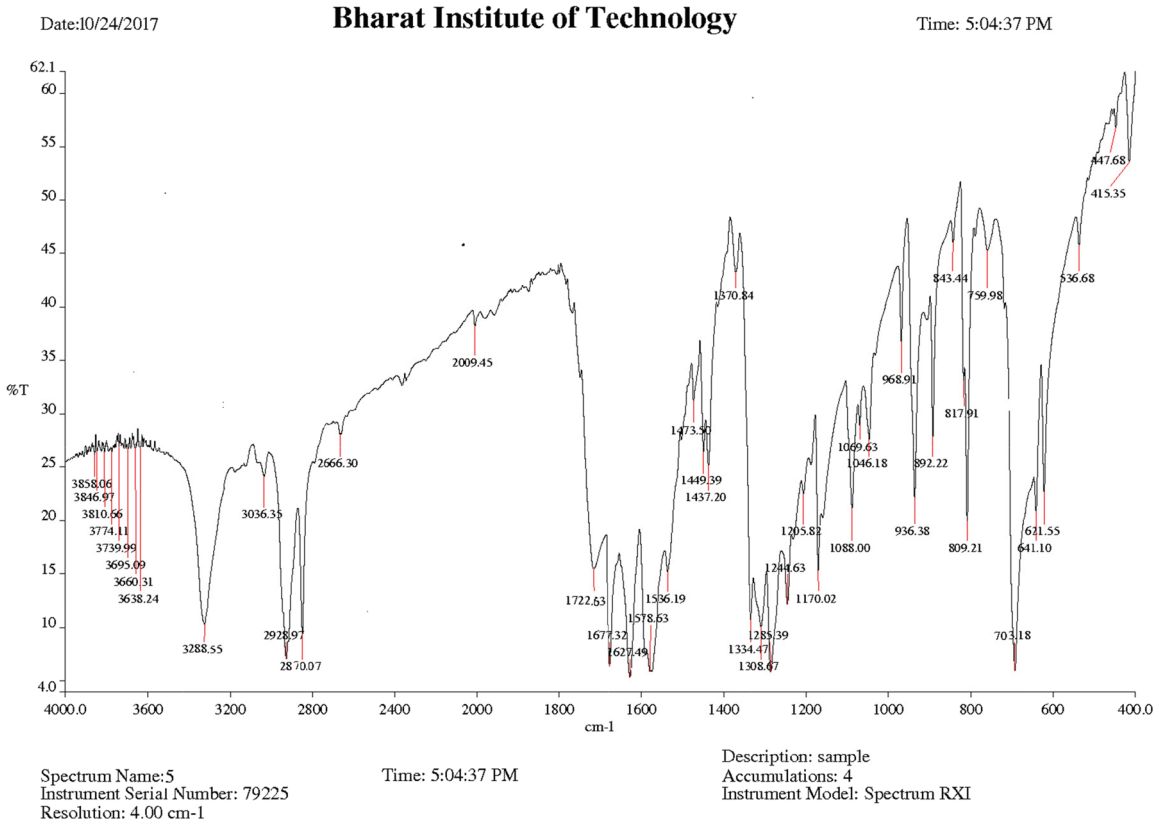
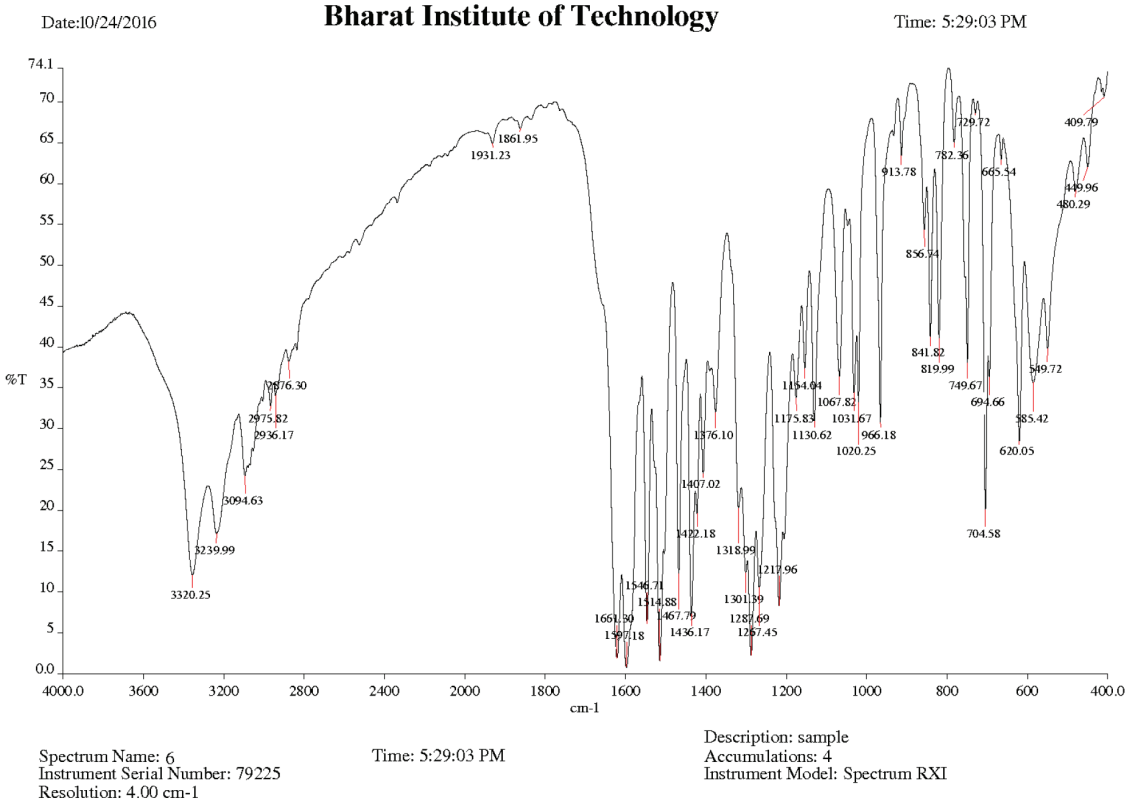
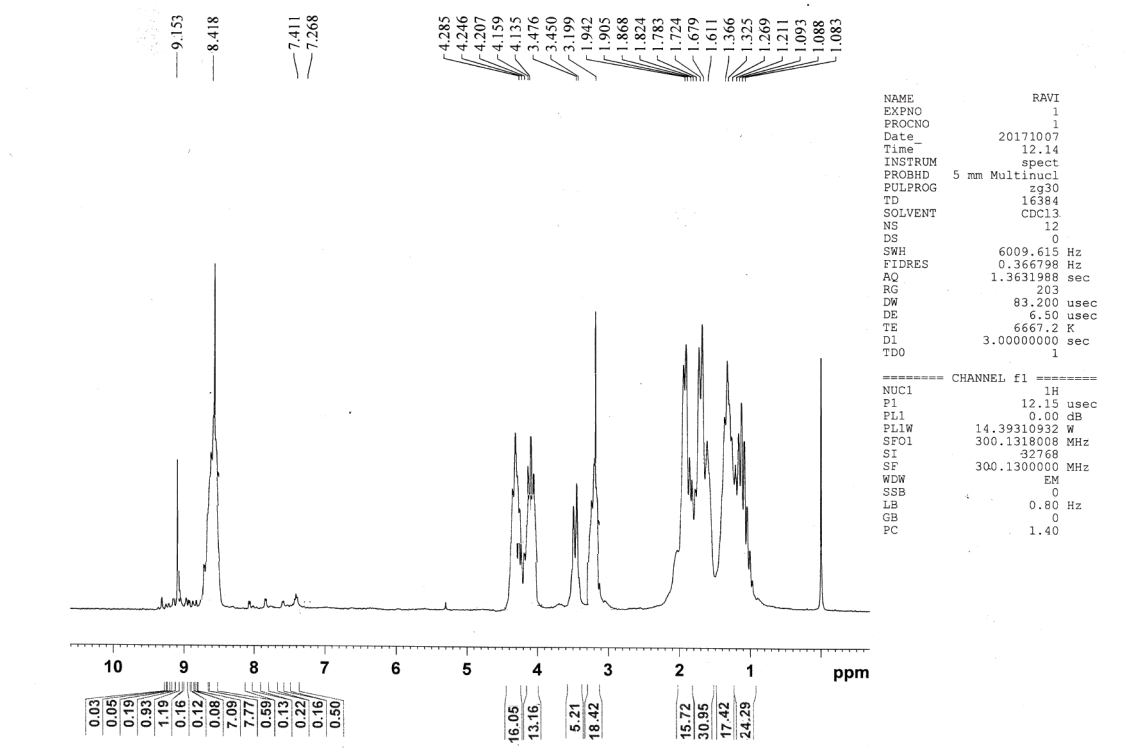
SUPPLEMENTARY DATA



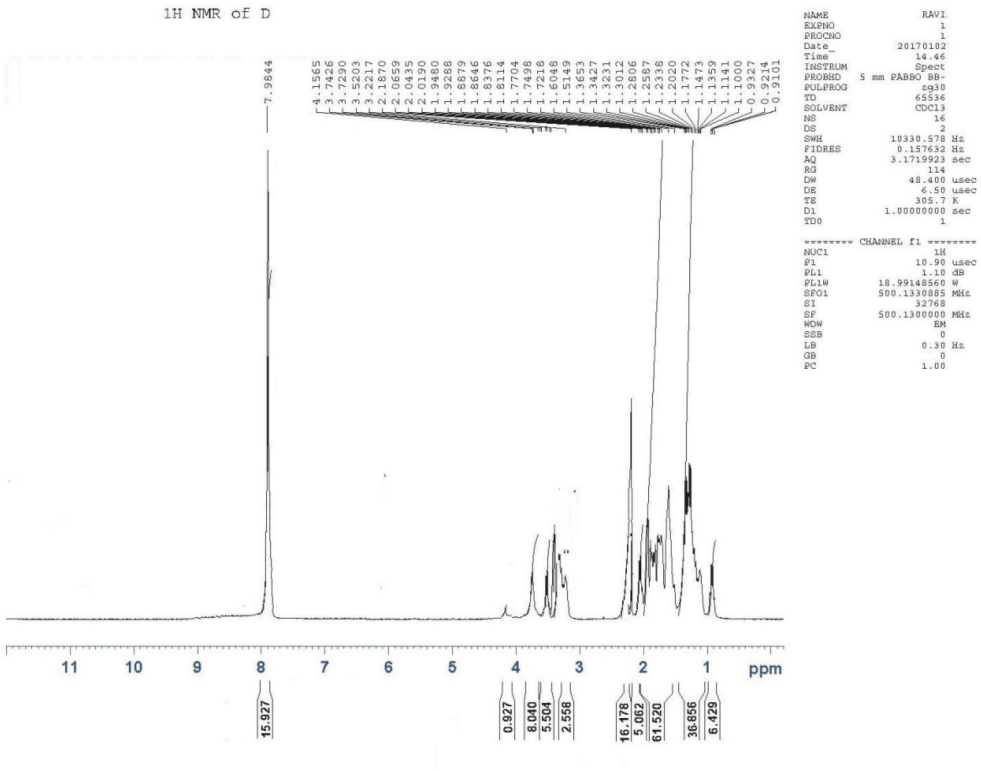
Supplementary Figure 1. FTIR SPECTRA OF COMPOUND 10



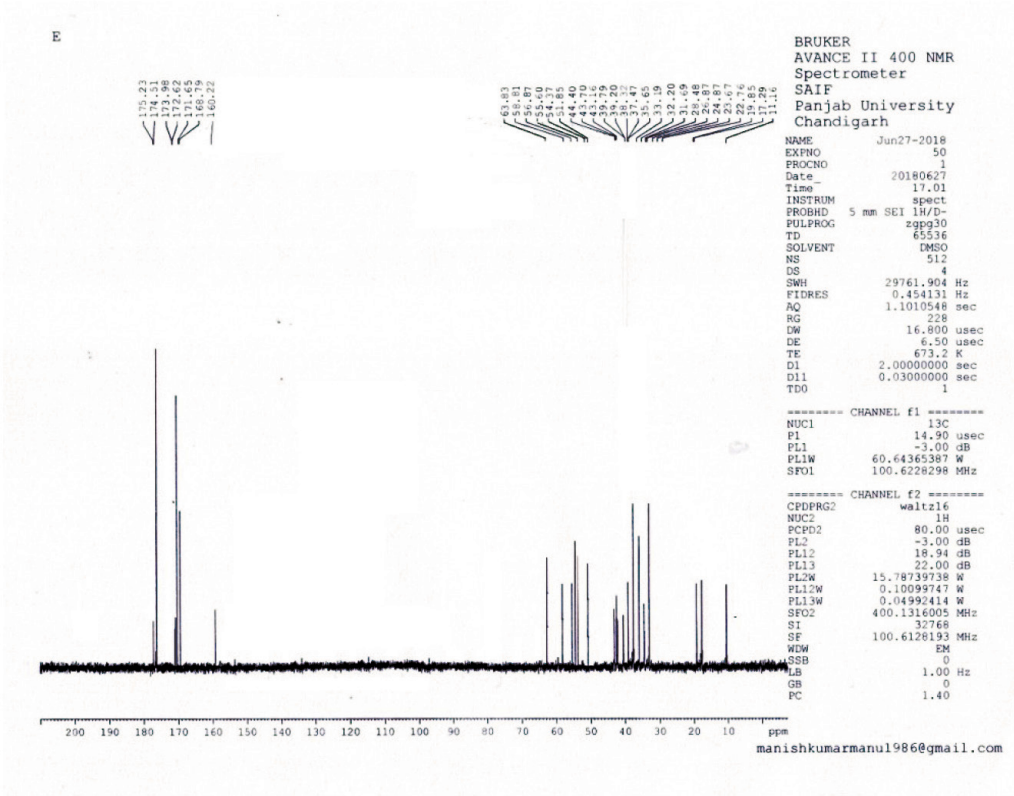
Supplementary Figure 2. FTIR SPECTRA OF COMPOUND 12



Supplementary Figure 3. ^{1}H NMR OF COMPOUND 10



Supplementary Figure 4. [1]H NMR OF COMPOUND 12



Supplementary Figure 5. [13]C-NMR OF COMPOUND 12