Designing a Temporin-1CEa Analog with Improved in Vitro Selectivity towards Prostate Cancerous Cell Line (LNCaP)

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ABSTRACT. Temporins are small antimicrobial peptides which have cytotoxic effects against different cancerous cell lines. However, their cytotoxicity against normal cells has been reported in some studies. Designing new temporin analogs with selective cytotoxicity against cancerous cell lines is considered a goal in drug development. In this regard, four new temporin-1CEa analogs (either C-terminally amidated or not) with increased net positive charge, as well as the potential of hydrogen bond formation were designed and their selective cytotoxicity against prostate carcinoma cell line (LNCaP) was evaluated. MTT assay was employed to test the simultaneous effects of the modifications on the cytotoxicity of the analogs against both normal (HFFi) and cancerous (LNCaP) cell lines. Then, the in vitro selectivity index of each analog was determined using the equation of selectivity index (SI= IC50 of peptide against normal cell line / IC50 of peptide against cancerous cell line). The structural modifications of temporin-1CEa (increasing the net positive charge and removing C-terminally amidated group, as well as the potential of hydrogen bond formation on the non-polar face of α-helical structure) reduced the cytotoxicity of K3K6W13-COOH analog against both cancerous (LNCaP) and normal (HFFi) cell lines. However, the modifications resulted in improving the in vitro selectivity index of K3K6W13-COOH compared to the temporin-1CEa (1.67 vs. 1.53, respectively). The results of the present study indicated that some structural modifications may lead to a diminution in the cytotoxicity of new analogs against cancerous cells. However, the same modifications can be associated with better selectivity indexes of new analogs due to a further decrease in their cytotoxicity against normal cells.

Keywords: analog designing; antimicrobial peptide; cancerous cell line; cytotoxicity; in vitro selectivity; normal cell line; temporin.

Introduction

Prostate cancer is considered the second most common cancer among men with more than 1.4 million new cases worldwide in 2020 (Prostate Cancer Statistics [Internet]. World Cancer Research Fund International. Available from https://www.wcrf.org/cancer-trends/prostate-cancer-statistics/). The disease is predicted to be one of the top four cancers in the United States by 2050 (Weir, Thompson, Stewart, & White, 2021). Despite various therapeutic strategies, many patients have developed therapeutic resistance and experience disease recurrence, both of which cause cancer-related mortality among men (Teo, Rathkopf, & Kantoff, 2019). In addition, the cytotoxicity of chemotherapy agents towards healthy cells due to non-specific targeting is associated with deleterious side effects such as hair loss, mouth sores, nausea and vomiting, diarrhea, easy bruising or bleeding, and fatigue, as well as the loss of appetite and increased chance of infections (Prostate Cancer [Internet]. American Cancer Society. Available from: https://www.cancer.org/cancer/prostate-cancer.html). Thus, it seems urgent to find new therapeutic agents with more effectiveness and selectivity.

Further, new therapeutic agents with anti-tumor effects have been discovered during last decades. Among the agents, antimicrobial peptides (AMPs), as natural defenders in the immune systems of organisms, exhibit hopeful anti-tumor effects in in vitro and in vivo models (Araki et al., 2019; de Azevedo et al., 2015; Eliassen et al., 2006; Jäkel, Meschenmoser, Kim, Weiher, & Schmidt-Wolf, 2012; S. Liu et al.,...
2011; Switchenbank et al., 2020). The models proposed for their mechanism of action (Tornesello, Borrelli, Buonaguro, Buonaguro, & Tornesello, 2020) make AMPs promising templates for developing novel anti-tumor agents with a low potential of resistance although it is not yet well-defined (Deslouches & Peter Di, 2017). Considering one of their unique mechanisms of action (initiating with the electrostatic interaction between the positive charges of AMPs and negative charges of cancerous cell membrane), AMPs can be categorized as drug candidates with almost specific effects against cancerous cells. However, some AMPs have cytotoxicity against normal cells (Hoskin & Ramamoorthy, 2008) due to other mechanisms of action (Tornesello et al., 2020). Therefore, a need is still felt to design the new analogs of AMPs with the improved selectivity towards cancerous cells.

Temporins are one of the most investigated families of AMPs, as anti-tumor agents (Diao et al., 2012; Khan et al., 2022; Shaheen et al., 2018; Switchenbank et al., 2020; C. Wang et al., 2016; C. Wang, Li, Li, Tian, & Shang, 2012; C. Wang et al., 2013; Yang et al., 2013). They were initially isolated from the skin of the European red frog Rana temporaria. Temporins are hydrophobic, C-terminally α-amidated peptides, which can adopt α-helical structures in hydrophobic environments. Given the presence of 8-17 amino acids in their sequences, they are considered the smallest members of AMPs (Rinaldi & Conlon, 2013). The results of different studies have demonstrated the effect of modifying the structural characteristics (e.g., net positive charge, helicity, and hydrophobicity) of AMPs on their selective cytotoxicity against cancerous cells (Diao et al., 2012; Guo, Zhang, Dong, Guan, & Shang, 2022; Y. Liu et al., 2019; Tan et al., 2018; K. Wang et al., 2008; Yang et al., 2013). Furthermore, the α-helical conformation of peptide is necessary for its anti-tumor activity (Wang et al., 2008). Both increasing hydrophobicity and net positive charge can enhance peptide selectivity as long as there is a balance between them (Guo et al., 2022; Y. Liu et al., 2019; Tan et al., 2018; Yang et al., 2015).

In the present study, four peptide analogs of temporin-1CEa with elevated net positive charge, as well as the potential of hydrogen bond formation with cell membrane, were designed. Then, the study focused on examining the simultaneous effect of the structural modifications on the cytotoxic potency of designed analogs, as well as their selectivity towards cancerous (LNCaP) cell line. After treating normal and cancerous cell lines with the various concentrations of the analogs for 24h, the percentage of viable cells was assessed using MTT assay. Additionally, the IC50 value against normal and cancerous cell lines was determined for each peptide. The selectivity index (SI) of each analog was obtained by dividing peptide IC50 against normal cell line by its IC50 against cancerous cell line. All of the SIs were compared with the SI of parent peptide, temporin 1CEa.

**Material and method**

**Ethical approval**

This study was approved by the Ethics Committee of Guilan University of Medical Sciences (ethics code: IR.GUMS.REC.1396.458).

**Peptide analog design**

To design peptide analogs, temporin-1CEa, a C-terminally α-amidated AMP, was used as parent peptide. Table 1 shows the amino acid sequence of temporin-1CEa. The design strategy was based on the previous studies, as well as the main mechanism of action of AMPs. Some studies (Diao et al., 2012; Yang et al., 2013) have reported more cytotoxicity following a rise in the net positive charge of AMPs. The main mechanism of action of the peptides initiates with an electrostatic interaction between the positive charges of AMPs and negative charges of cancerous cell membrane, and consequently the cell membrane is disrupted (Tornesello et al., 2020). Thus, it was hypothesized that the potential of hydrogen bond formation between the designed analogs and cancerous cell membranes may potentiate their interaction, and subsequently leads to an improved cytotoxic effect of the peptides against cancerous cells. In this regard, C-terminally α-amidated (-CONH₂) and non-α-amidated (-COOH) analogs were designed (Table 1). It is worth noting that the amino acid modification of the sequence of temporin-1CEa for increasing its net positive charge is based on the results of a study by Yang et al. (Yang et al., 2013).

There were two analogs of K₂K₃W₁₅-CONH₂ (FKKLKKIANIINSIWGK-CONH₂) and K₂K₃Y₁₅-CONH₂ (FKKLKKIANIINSIFYK-CONH₂) in the C-terminally α-amidated series, both of which had Val and Asp.
substitutions with Lys. Further, Phe$_{15}$ substitution with Trp (as a hydrogen-donor amino acid (Pommié, Levadoux, Sabatier, Lefranc, & Lefranc, 2004)) was observed in K$_2$K$_3$W$_{15}$-CONH$_2$, while K$_2$K$_3$Y$_{15}$-CONH$_2$ had Gly$_{16}$ substitution with Tyr (as both hydrogen-donor and -acceptor amino acid (Pommié et al., 2004)).

Along with the structural modifications of the first series, the C-terminally α-amide group was removed in the second series (C-terminally non-α-amidated series) to examine the effect of this removal on the cytotoxic potency of peptide against both normal and cancerous cell lines. This series included K$_2$K$_3$W$_{15}$-COOH (FKKLKKIANIINSIWGK-CONH$_2$) and K$_2$K$_3$Y$_{15}$-COOH (FKKLKKIANINSIFYK-CONH$_2$).

**Peptide synthesis and preparation**

Temporin-1CEa and designed analogs were synthesized by KJ Ross-Petersen ApS (Klampenborg, Denmark). Furthermore, 10 mg of synthetic peptides with 95% purity were dissolved in 1 mL of 20% ethanol (Merck), divided into small aliquots (100 µL), and kept at -20°C.

**Peptide parameter calculations**

The helical wheel representations of temporin-1CEa and its analogs were obtained from Protein ORIGAMI program (http://www.ibg.kit.edu/protein_origami/?page=sequence) (Reißer, Prock, Heinzmann, & Ulrich, 2018). Regarding each peptide, normalized hydrophobic moment (μH), normalized hydrophobicity, and net charge were determined using the Database of Antimicrobial Activity and Structure of Peptides (DBAASP) (https://dbaasp.org/tools?page=property-calculation) (Pirtskhalava et al., 2021) based on the Kyte and Doolittle hydrophobicity scale (Kyte, Doolittle, Diego, & Jolla, 1982).

**Cell lines and culture**

The human prostate carcinoma cell line (LNCaP, research resource identifier (RRID): CVCL_W668) and human dermal normal cell line (HFFFH, RRID: CVCL_2489) were purchased from Pasteur Institute of Iran. Both cell lines were grown in the Dulbecco’s modified eagle medium (DMEM) (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Invitrogen) and 50 µg/ml gentamicin (Gibco). The cells were incubated at 37°C in a humidified atmosphere containing 5% CO$_2$ and the culture medium was refreshed every 2–3 days. In addition, the cultured cells were passaged at 80% confluency. Cell viability was assessed using dye exclusion method, in which trypan blue (Sigma) was applied as cell stain.

**In vitro cytotoxicity assay**

To determine the cytotoxic effects of temporin-1CEa and its analogs on the LNCaP and HFFFH cell lines, methyl thiazol tetrazolium (MTT) (Sigma) assay was performed 24h after incubating both cell lines with the various concentrations of designed peptides (Bahadori et al., 2021). As for each cell line, 10$^4$ cells per well were seeded in a 96-well plate in complete DMEM and incubated for 24h at 37°C in a humidified atmosphere containing 5% CO$_2$. After removing non-adherent cells, the adherent ones were treated with the different concentrations of each peptide (5–520 µM) and incubated more for 24h under the same condition. The cultured cells receiving an equal volume of peptide solvent were considered as control group. Both treated and control groups were washed with PBS. Further, 20 µL of MTT solution (5 mg mL in PBS) and 180 µL of fresh DMEM (without FBS) were added to each well and incubated for 4h. Following supernatant removal, 150 µL of DMSO (Sigma) was poured to each well and incubated for 10 min. The optical density (OD) of the solution was measured by using an absorbance microplate reader (BioTek) at 570 nm using a reference wavelength of 650 nm. Furthermore, MTT assay was performed in triplicate for each peptide. The percentage of viable cells (either normal or cancerous cells) in each of the treated groups was calculated as follows.

\[
\% \text{ viability} = \left( \frac{\text{OD}_{\text{treated group}}}{\text{OD}_{\text{control group}}} \right) \times 100
\]

$IC_{50}$ of peptides against each cell line was defined as the concentration at which only 50% of cells were alive, which was computed on GraphPad Prism (version 8, GraphPad Software, USA).

**Selectivity index (SI)**

As for temporin-1CEa and its designed analogs, $IC_{50}$ against normal cell line was divided by its $IC_{50}$ against cancerous one to calculate SI at their $IC_{50}$ (Yousef beyk et al., 2022).
Statistical analysis

GraphPad Prism (version 8, GraphPad Software, USA) was utilized to determine peptide IC_{50}. Additionally, statistical analyses were conducted in SPSS statistics software. One-way ANOVA and Tukey HSD post hoc tests were respectively employed to calculate differences. A *p*-value less than 0.05 was considered a statistically significant difference.

Results

Peptide analog design

As already mentioned, C-terminally α-amidated (−CONH₂) and non-α-amidated (−COOH) analogs were designed based on the amino acid sequence of temporin-1CEa (FVDLKIJIAINSIFGK−CONH₂) (Table 1).

In the C-terminally α-amidated series, both K₆K₈W₁₅−CONH₂ and K₆K₉Y₁₆−CONH₂ had Val₂ and Asp₃ substitutions with Lys on the polar face of α-helical structure (Figure 1). As shown in Table 1, substituting a hydrophobic amino acid (Val₃) with lysine (a positive-charged hydrophilic amino acid) decreased hydrophobicity, and consequently increased the amphipathicities of both analogs. Following the substitution of Phe₁₅ with Trp (a hydrogen-donor amino acid (Pommié et al., 2004)) and Y₁₆ with Tyr (both hydrogen-donor and -acceptor amino acid (Pommié et al., 2004)), K₆K₉W₁₅−CONH₂ and K₆K₉Y₁₆−CONH₂ exhibited the potential of hydrogen-bond formation on the non-polar face of α-helical structure, respectively (Figure 1).

Regarding the C-terminally non-α-amidated series, K₆K₉W₁₅−COOH and K₆K₉Y₁₆−COOH were the non-α-amidated isofoms of K₆K₈W₁₅−CONH₂ and K₆K₉Y₁₆−CONH₂, respectively.

Table 1 summarizes the structural characteristics (i.e., normalized hydrophobic moment (µH), normalized hydrophobicity (H), and net charge of peptides).

<table>
<thead>
<tr>
<th>NO.</th>
<th>PEPTIDE NAME</th>
<th>PEPTIDE SEQUENCE</th>
<th>NET CHARGE</th>
<th>µH</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TEMPORIN-1CEa</td>
<td>FVDLKIJIAINSIFGK−CONH₂</td>
<td>3</td>
<td>1.39</td>
<td>-0.39</td>
</tr>
<tr>
<td>2</td>
<td>K₆K₈W₁₅−CONH₂</td>
<td>FKKLKIJIAINSIFGK−CONH₂</td>
<td>6</td>
<td>1.71</td>
<td>0.13</td>
</tr>
<tr>
<td>3</td>
<td>K₆K₉Y₁₆−CONH₂</td>
<td>FKKLKIJIAINSIFGY−CONH₂</td>
<td>6</td>
<td>1.90</td>
<td>-0.04</td>
</tr>
<tr>
<td>4</td>
<td>K₆K₉W₁₅−COOH</td>
<td>FKKLKIJIAINSIWGK</td>
<td>5</td>
<td>1.71</td>
<td>0.13</td>
</tr>
<tr>
<td>5</td>
<td>K₆K₉Y₁₆−COOH</td>
<td>FKKLKIJIAINSIFYK</td>
<td>5</td>
<td>1.90</td>
<td>-0.04</td>
</tr>
</tbody>
</table>

Figure 1. Helical wheel representations of temporin-1CEa and its analogs, obtained from Protein ORIGAMI program at the website: http://www.ibg.kit.edu/protein_origami/?page=sequence (Reißler et al., 2018) (a) temporin-1CEa (b) K₆K₈W₁₅−CONH₂ and K₆K₉W₁₅−COOH, and (c) K₆K₉Y₁₆−CONH₂ and K₆K₉Y₁₆−COOH.

K₆K₉W₁₅−COOH represented an improved selectivity towards cancerous cell line

Regarding the SI of peptide, the cytotoxicity of temporin-1CEa and its analogs against normal (HFFF2) (Figure 2a−e) and cancerous cell lines (LNCaP) (Figure 3a−e) were evaluated. Then, the ratio of peptide IC_{50} against HFFF2 to its IC_{50} against LNCaP was calculated. Finally, all of the SIs were compared with the SI of parent peptide (temporin-1CEa). Compared to temporin-1CEa, K₆K₉W₁₅−COOH had an enhanced SI, while the SIs of others reduced (Table 2) (p-value < 0.05).
temporin analog with increased selective cytotoxicity

Figure 2. The cytotoxicity of temporin-1CEa and its analogs against HFFF2: (a) temporin-1CEa, (b) K2K3W15−CONH2, (c) K2K3Y16−CONH2, (d) K2K3W15−COOH, and (e) K2K3Y16−COOH.

Figure 3. The cytotoxicity of temporin-1CEa and its analogs against LNCaP: (a) temporin-1CEa, (b) K2K3W15−CONH2, (c) K2K3Y16−CONH2, (d) K2K3W15−COOH, and (e) K2K3Y16−COOH.

Table 2. Peptide IC50 against HFFF2 and LNCaP and their SIs (The SIs of all designed analogs were statistically different from the SI of their parent peptide (p-value < 0.05.).

<table>
<thead>
<tr>
<th>No.</th>
<th>Peptide name</th>
<th>IC50 (µM) against HFFF2</th>
<th>IC50 (µM) against LNCaP</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Temporin-1CEa</td>
<td>145.31</td>
<td>108.34</td>
<td>1.33</td>
</tr>
<tr>
<td>2</td>
<td>K2K3W15−CONH2</td>
<td>48.16</td>
<td>45.70</td>
<td>1.05</td>
</tr>
<tr>
<td>3</td>
<td>K2K3Y16−CONH2</td>
<td>85.17</td>
<td>84.82</td>
<td>1.00</td>
</tr>
<tr>
<td>4</td>
<td>K2K3W15−COOH</td>
<td>205.15</td>
<td>122.85</td>
<td>1.67</td>
</tr>
<tr>
<td>5</td>
<td>K2K3Y16−COOH</td>
<td>153.9</td>
<td>164.45</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Despite a diminution in the cytotoxicity of K2K3W15−COOH against LNCaP, a better SI was found in comparison with the parent peptide due to a higher reduction in the cytotoxicity of the analog against normal cells (p-value < 0.05). Further, both K2K3W15−CONH2 and K2K3Y16−CONH2 exhibited an elevated cytotoxicity against LNCaP although their more increased cytotoxicity against normal cells led to a significant reduction in their SIs compared to temporin-1CEa (p-value < 0.05).
It is worth noting that there was no statistically significant difference between the SIs of $K_{K_a}W_{15}-CONH_2$, $K_{K_a}Y_{16}-CONH_2$, and $K_{K_a}Y_{16}-COOH$ (p-value > 0.05).

**Discussion**

In the present study, four peptide analogs of temporin-1CEa were designed to improve the in vitro selectivity of peptide. Compared to parent peptide, all of the designed analogs had greater net positive charge, as well as the potential of hydrogen bond formation with cell membrane. Among the analogs, two were C-terminally α-amidated ($K_{K_a}W_{15}-CONH_2$ and $K_{K_a}Y_{16}-CONH_2$) and the others were C-terminally non-α-amidated ($K_{K_a}W_{15}-COOH$ and $K_{K_a}Y_{16}-COOH$). The LNCaP and HFFF2 cell lines were treated with the designed analogs to examine the effect of the structural modifications on the cytotoxic potency and SIs of the analogs.

Electrostatic interactions play effective roles in peptide-cell membrane interaction and peptide internalization (Kalafatovic & Giralt, 2017). Along with elevating the net positive charge of designed analogs, substituting a hydrophobic amino acid (Val) with lysine (a positive-charged hydrophilic amino acid) results in decreasing hydrophobicity, and consequently improving the amphiphaticities of both analogs as a strategic parameter for the membrane binding of peptides (Kauffman, Fuselier, He, & Wimley, 2015). A rise in net positive charge and amphiphaticities increased the cytotoxicity of both C-terminally α-amidated analogs ($K_{K_a}W_{15}-CONH_2$ and $K_{K_a}Y_{16}-CONH_2$) against LNCaP and HFFF2 cell lines compared to that of temporin-1CEa (p-value < 0.05), leading to a reduction in their SIs. Based on the results of different studies, an enhancement in the positive charge of AMPs increases potency against cancerous cell lines (i.e., human lung adenocarcinoma epithelial cell (A549), human colorectal carcinoma (SW1116), human gastric carcinoma (BGC-823), human epithelial carcinoma (Hela), human hepatocellular liver carcinoma (HepG2), human hepatocellular carcinoma (SMMC-7721), and human breast cancer (MCF-7, MDA-MB-231 and Bcap-37) ones) (Diao et al., 2012; Yang et al., 2015) and mammalian erythrocytes as normal cell (Dathe, Nikolenko, Meyer, Beyermann, & Bienert, 2001; Jiang et al., 2008; Pál, Sonnevend, Galadari, & Conlon, 2005; Tan et al., 2018). The results are correlated with those of the present study. In addition, the formation of hydrogen bonds between peptide and cell membrane is considered one of the key interactions for internalization (Kalafatovic & Giralt, 2017; Rothbard, Jessop, Lewis, Murray, & Wender, 2004). As already mentioned, Phe$_{15}$ and Gly$_{16}$ were respectively substituted with Trp (a hydrogen-donor amino acid (Pommie et al., 2004)) in $K_{K_a}W_{15}-CONH_2$, analog and Tyr (both hydrogen-donor and -acceptor amino acid (Pommie et al., 2004)) in $K_{K_a}Y_{16}-CONH_2$. It was hypothesized that the potential of hydrogen-bond formation between the designed analogs and cell membrane be caused by this substitution. Further, Trp is known as a strategic amino acid in designing new cell-penetrating peptides, which facilitates peptide insertion into cell membrane (Wimley & White, 1996). The presence of Trp in peptide structure destabilizes cell membrane (Chen et al., 2011). Trp, like Phe, is a hydrophobic amino acid with an aromatic residue and has the same volume as Phe (Dathe et al., 2001; Kauffman et al., 2015). Unlike Gly, Tyr possesses a very large volume, which may promote the possibility of its interaction with lipid bilayer. Both Tyr and Gly are neutral amino acids (Pommie et al., 2004).

Considering IC$_{50}$S, a higher cytotoxicity against LNCaP was observed in $K_{K_a}W_{15}-CONH_2$ than $K_{K_a}Y_{16}-CONH_2$ (p-value < 0.001), reflecting that the potential of hydrogen-bond formation on the non-polar face of α-helical structure led to further increase in peptide potency compared to this potential on the polar face did.

According to previous studies, C-terminally α-amidated group stabilizes α-helical structure at the membrane interface (Dennisona, Mortonb, & Phoenixc, 2012; Strandberg et al., 2007) and this elevated structural stability potentiates peptide internalization (Irudayam & Berkowitz, 2012; Kalafatovic & Giralt, 2017). Based on the results of an unpublished study, the non-α-amidated analog of temporin-1CEa with the same amino acid sequence (FVDLKKIANISIFGK-COOH) has a decreased cytotoxicity against both normal (HFFF2) and cancerous (LNCaP) cell lines. Furthermore, the SIs of the analog and temporin-1CEa were not significantly different. It was reported that only a rise in positive charge does not compensate the lack of C-terminally amidated group in an amidated AMP (Dathe et al., 2001). Thus, two non-α-amidated analogs with the potential of hydrogen bond formation were designed in the present study to compensate the lack of C-terminally α-amidated group. Given the lack of a difference in the IC$_{50}$ of $K_{K_a}W_{15}-COOH$ and temporin-1CEa against LNCaP cell line (p-value > 0.05), the presence of Trp in the peptide sequence of $K_{K_a}W_{15}-COOH$ compensated the absence of C-terminally α-amidated group, giving the analog an equal potency to that of temporin-1CEa against cancerous cells. It is worth noting that the presence of Trp in the peptide sequence of $K_{K_a}W_{15}-COOH$ significantly reduced its cytotoxicity against normal cells, and consequently improved its
SI in comparison with temporin-1CEa (p-value < 0.05). Unlike Trp, Gly₁₆ substitution with Tyr was not associated with this compensation. The lack of C-terminally α-amidated group, and subsequently comparative structural instability of K₆K₆Y₁₆−COOH resulted in diminishing its cytotoxicity against cancerous cell line compared to temporin-1CEa (p-value < 0.001). It seems that the presence of Tyr, having a very larger volume than the Gly, in the polar face of α-helical structure played the role of an obstacle against forming multiple electrostatic bonds between the positive charges of peptide and negative surface charge of LNCaP, causing a decreased potency. This negative effect of Tyr in the polar face of α-helical structure was not found in α-amidated analog (K₆K₆Y₁₆−CONH₂), which may be ascribed to C-terminally α-amidated group, which stabilizes α-helical structure (Dennisona et al., 2012; Dos Santos Cabrera et al., 2008; Mura et al., 2016; Strandberg et al., 2007) and seems to cover the negative effect of the large volume of Tyr.

**Conclusion**

To design the new analogs of AMPs, SI is considered a more important issue along with peptide cytotoxicity against cancerous cells. However, the removal of C-terminally α-amidated group increased positive charge, as well as the potential of hydrogen-bond formation on the non-polar face of α-helical structure and reduced cytotoxicity against cancerous cells. All of the structural modifications led to a rise in the selectivity of peptide towards cancerous cells.

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