Chapter 12

Artificial Intelligence and Quantum Computing for the Design of Novel Kisspeptin-10 Analogues and Their Experimental Evaluation in Cervical Cancer Cells

Deisy Yurley Rodríguez-Sarmiento

Faculty of Health Sciences, Universidad Autónoma de Bucaramanga, Bucaramanga, 680003, Colombia

Abstract

Cervical cancer remains a leading cause of morbidity and mortality in women, underscoring the need for innovative therapies. Kisspeptin-10 (KP10), a decapeptide that activates the G-protein-coupled receptor (GPCR) Kiss1R, shows antitumour potential. Using artificial intelligence (AI) based modelling, a focused library of KP10 analogues was designed; 10 top candidates were synthesised and evaluated. Molecular docking predicted enhanced Kiss1R affinity, which was confirmed in vitro by MTT and wound-healing assays in cervical cancer cells. Several analogues showed superior cytotoxicity and migration inhibition versus KP10. These results highlight AI-assisted peptide design as an efficient approach for developing GPCR-targeted therapeutics in cervical cancer.

Keywords: *Kisspeptin-10, Cervical cancer, GPCR, Artificial intelligence, Peptide design, Antitumour activity*

Introduction

Cervical cancer remains a major global health concern and one of the leading causes of cancer-related death among women, particularly in low- and middle-income countries. Despite progress in screening, vaccination, and treatment, many cases are diagnosed at advanced stages with poor outcomes, highlighting the need for new targeted therapies[1,2].

The kisspeptin system has recently gained attention beyond its reproductive role[3]. Kisspeptins, derived from the *KISS1* gene, include the potent fragment Kisspeptin-10 (KP10), which binds the G-protein-coupled receptor (GPCR) Kiss1R[4]. Initially identified for its antimetastatic properties in melanoma and

[1]Marc Arbyn et al., "Estimates of Incidence and Mortality of Cervical Cancer in 2018: A Worldwide Analysis," *The Lancet Global Health* 8, no. 2 (2020): e191–203, https://doi.org/10.1016/s2214-109x(19)30482-6.
[2]World Health Organization: WHO, "Cervical Cancer," December 2, 2025, https://www.who.int/news-room/fact-sheets/detail/cervical-cancer.
[3]Simina M. Popa, Donald K. Clifton, and Robert A. Steiner, "The Role of Kisspeptins and GPR54 in the Neuroendocrine Regulation of Reproduction," *Annual Review of Physiology* 70 (2008): 213–38, https://doi.org/10.1146/annurev.physiol.70.113006.100540.
[4]Masato Kotani et al., "The Metastasis Suppressor Gene KiSS-1 Encodes Kisspeptins, the Natural Ligands of the Orphan G Protein-coupled Receptor GPR54," *Journal of Biological Chemistry* 276, no. 37 (2001): 34631–6, https://doi.org/10.1074/jbc.m104847200.

breast cancer[5], kisspeptin signalling is now linked to key oncogenic processes such as proliferation, migration, angiogenesis, and apoptosis[6]. These findings support the exploration of kisspeptin analogues as therapeutic modulators, particularly in hormone-responsive and reproductive cancers like cervical cancer.

Artificial intelligence (AI) and machine learning have transformed peptide-based drug discovery by enabling rational design, predictive modelling, and efficient screening of large peptide libraries[7]. These tools accelerate the identification of bioactive sequences with favourable pharmacological profiles and guide experimental validation[8].

This study applies AI-driven prediction and molecular docking to design and evaluate KP10 analogues with potential antitumour activity. Ten top-scoring peptides were synthesised and tested for cytotoxic and anti-migratory effects in cervical cancer cells, establishing a framework for AI guided GPCR targeted peptide discovery in oncology.

Results and Discussion

AI-based prediction of KP10 analogues

KP10 analogues were rationally designed through an AI-driven workflow to identify variants with improved antitumour potential. Starting from the native sequence (YNWNSFGLRF), over 5000 analogues were generated via Python-based systematic mutations and extensions (10–12 amino acids). Each sequence was encoded with 1477 physicochemical descriptors from Propy 1.0[9], and filtered by charge, hydrophobicity, molecular weight, and complexity, yielding 70 biologically plausible candidates. These were evaluated using three machine-learning classifiers: SVM, Random Forest, and ANN, trained on balanced anticancer peptide datasets. Consensus predictions identified the most robust candidates for subsequent structural modelling and biological testing.

In silico prediction of antitumour potential

The antitumour potential of 70 KP10 analogues was assessed using the MLACP 2.0 server[10], which applies physicochemical descriptors and machine learning algorithms (LightGBM, Extra Trees) trained

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[5]Jeong-Hying Lee et al., "KiSS-1, a Novel Human Malignant Melanoma Metastasis-Suppressor Gene," *JNCI Journal of the National Cancer Institute* 88, no. 23 (1996): 1731–7, https://doi.org/10.1093/jnci/88.23.1731.

[6]Saima Jabeen et al., "Kisspeptin Mediated Signaling in Cancer," *Current Topics in Medicinal Chemistry* 16, no. 22 (2016): 2471–6, https://doi.org/10.2174/1568026616666160212123309.

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[8]Kathy Sharon Isaac et al., "Machine Learning Tools for Peptide Bioactivity Evaluation – Implications for Cell Culture Media Optimization and the Broader Cultivated Meat Industry," *Current Research in Food Science* 9 (2024): 100842, https://doi.org/10.1016/j.crfs.2024.100842.

[9]Dong-Sheng Cao, Qing-Song Xu, and Yi-Zeng Liang, "Propy: A Tool to Generate Various Modes of Chou's PseAAC," *Bioinformatics* 29, no. 7 (2013): 960–2, https://doi.org/10.1093/bioinformatics/btt072.

[10]Le Thi Phan et al., "MLACP 2.0: An Updated Machine Learning Tool for Anticancer Peptide Prediction," *Computational and Structural Biotechnology Journal* 20 (2022): 4473–80, https://doi.org/10.1016/j.csbj.2022.07.043.

on validated anticancer peptide datasets. Peptides with high-confidence anticancer predictions were prioritised. This two-tiered strategy (AI classifiers optimised for HeLa cytotoxicity followed by external ACP validation) strengthened candidate selection for molecular docking and experimental evaluation. See Table **1**.

Table 1: List of designed analogues, including MLACP 2.0 prediction scores and consensus annotations.

Peptide	Sequence	Length (aa)	Net Charge	MW (Da)	MCLAP Score
KP10	YNWNSFGLRF	10	+2.0	1302.45	0.833
KP10-01	YNWNTFGLRF	10	+2.0	1316.48	0.848
KP10-02	YNWNSFSLRF	10	+2.0	1332.48	0.893
KP10-03	YNWNSFGIRF	10	+2.0	1302.48	0.887
KP10-04	YNFWNSFGLRF	11	+2.0	1449.63	0.880
KP10-05	YDWNSFGLRF	10	+1.0	1303.44	0.877
KP10-06	YNWNSWGLRF	10	+2.0	1341.49	0.843
KP10-07	YNFNSFGLRF	10	+2.0	1263.42	0.836
KP10-08	YNWNSFGLRW	10	+2.0	1341.49	0.828
KP10-09	YNWNSFGQLRF	11	+2.0	1430.58	0.819
KP10-10	YNWNSFGVRF	10	+2.0	1288.43	0.815

Molecular docking simulations and binding affinity estimations

Molecular docking simulations were conducted using HPEPDOCK 2.0[11] to assess receptor binding of KP10 analogues. All peptides showed negative docking scores, indicating favourable interactions. Native KP10 exhibited a score of –298.2, while most analogues ranged from –257.1 to –287.7; KP10-01 and KP10-06 showed comparable affinities. Binding free energies (ΔG) and dissociation constants (Kd) estimated with PRODIGY[12] supported these results, with KP10-05, KP10-06, KP10-09, and KP10-10 displaying lower ΔG values (–9.3 to –8.8 kcal/mol) and submicromolar Kd (≈10^{-7} M) versus KP10 (ΔG = –7.5 kcal/mol; Kd = 4.8×10^{-6} M) (See Table **2**). These findings indicate that specific sequence modifications improve peptide receptor interactions and guided analogue prioritisation for synthesis and biological testing.

Table 2: Predicted binding affinity of KP10 analogues to the receptor target based on molecular docking simulations.

Peptide	Docking Score	ΔG (kcal/mol)	Kd (M)
KP10	-298.2	-7.5	4.8x10-6
KP10-01	-287.7	-6.2	4.2x10-5
KP10-02	-275.1	-6.8	1.6x10-5
KP10-03	-265.9	-7.8	3.1x10-6
KP10-04	-257.1	-8.3	1.3x10-6
KP10-05	-264.6	-9.3	2.9x10-7

[11]Pei Zhou et al., "HPEPDOCK: A Web Server for Blind Peptide–protein Docking Based on a Hierarchical Algorithm," *Nucleic Acids Research* 46, no. W1 (2018): W443–50, https://doi.org/10.1093/nar/gky357.
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Table 2: Continued.

Peptide	Docking Score	ΔG (kcal/mol)	Kd (M)
KP10-06	-286.3	-9.3	2.8x10-7
KP10-07	-273.1	-5.8	8.7x10-5
KP10-08	-257.6	-6.2	4.1x10-5
KP10-09	-267.2	-8.8	6.4x10-7
KP10-10	-282.1	-8.8	6.4x10-7

Chemical synthesis of selected peptides

The 10 selected KP10 analogues were synthesised by solid-phase peptide synthesis (SPPS) using standard Fmoc chemistry on Rink amide resin[13]. Coupling was performed with HBTU/HOBt and DIPEA, and peptides were cleaved from the resin with a TFA-based cocktail. Crude products were purified on Sep-Pak C18 cartridges and confirmed by electrospray ionisation mass spectrometry (ESI-MS). Purified peptides were lyophilised and stored at –20 °C until biological testing.

Cytotoxicity and migration assays in cervical cancer cells

The antiproliferative activity of KP10 and its 10 analogues were evaluated in HeLa cells by MTT assay[14] after 48 hours of treatment at 10–500 nM. Most peptides induced a dose-dependent decrease in cell viability. KP10-09 and KP10-10 showed the strongest effects, reducing viability to 1.6% and 7.7% at 500 nM, compared with 74.1% for KP10. KP10-05 also showed high cytotoxicity (23.7% viability) (Figure **1**). Some analogues displayed non-linear profiles, suggesting compensatory cellular mechanisms. These results demonstrate that sequence modifications can enhance KP10 cytotoxicity against cervical cancer cells. Calculated IC_{50} values were 1618 nM for KP10, 0.2466 nM for KP10-09, and 0.5364 nM for KP10-10, confirming their markedly greater potency relative to the native peptide.

To further assess functional activity, KP10-09 and KP10-10 were selected for migration assays based on their strong cytotoxic effects. A wound healing assay at 100 nM evaluated their influence on HeLa cell migration. The mean open wound area was quantified at 0, 24, 48, and 72 hours for untreated controls, KP10, and the analogues (Figure **2**). At 24 hours, KP10-09 and KP10-10 maintained larger wound areas (63.37% and 64.01%) than the control (40.29%) and KP10 (55.26%), a trend persisting through 72 hours (18.71% and 14.62% vs. 1.12% for control). Both analogues significantly delayed wound closure compared with native KP10, demonstrating sustained inhibition of cell migration and supporting their potential to limit metastatic progression in cervical cancer.

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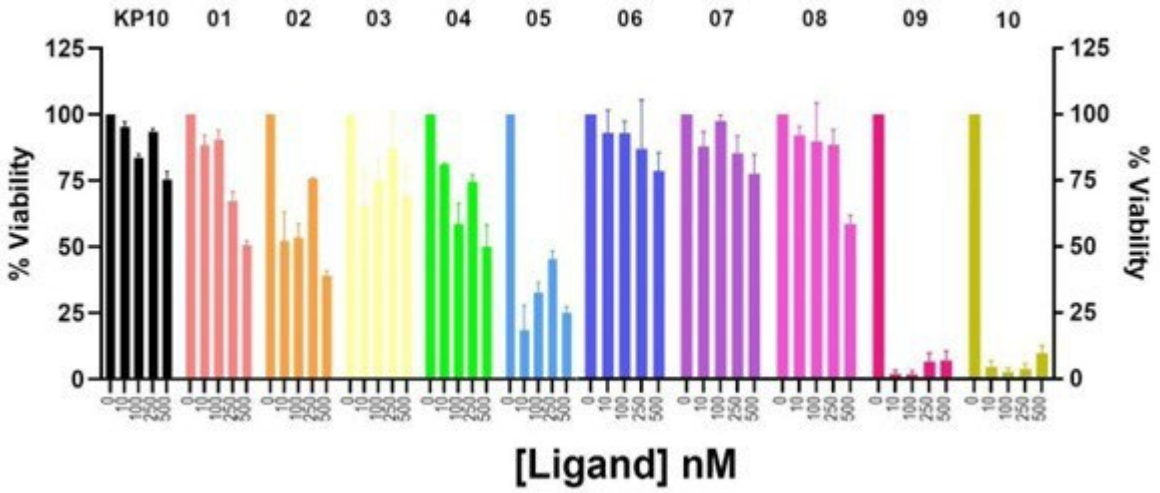

Figure 1: Cell viability of HeLa cells treated with KP10 and its analogues. HeLa cells were treated with increasing concentrations (10, 100, 250, and 500 nM) of KP10 and 10 synthesised analogues for 24 hours. Cell viability was measured using the MTT assay and is expressed as a percentage relative to untreated control cells (set at 100%). Data represent the mean ± standard deviation of three independent experiments. A dose-dependent reduction in viability was observed for most analogues, with KP10-09 and KP10-10 showing the strongest cytotoxic effects at 500 nM.

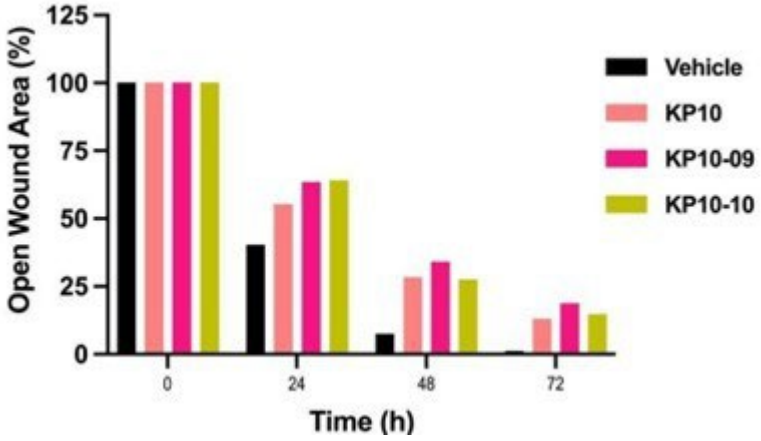

Figure 2: Inhibitory effects of KP10, KP10-09, and KP10-10 on HeLa cell migration assessed by wound healing assay. Cells were treated with 100 nM of each peptide, and the mean percentage of open wound area was measured at 0, 24, 48, and 72 hours for untreated controls, native KP10, and its analogues. Bars represent the average wound area (%) remaining over time, indicating delayed wound closure in cells exposed to the analogues.

Conclusion

This study demonstrates the value of integrating AI-driven peptide design, molecular modelling, and experimental validation to identify KP10 analogues with enhanced antitumour activity. From the computational library, 10 peptides were synthesised and tested in vitro; KP10-09 and KP10-10 showed the strongest cytotoxicity and migration inhibition in HeLa cells, consistent with in silico predictions. These results highlight the efficiency of computational pipelines for early peptide discovery and support the potential of rationally engineered kisspeptin analogues as leads for cervical cancer therapy.

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