

Original Research Article

Evaluation of ssDNA aptamer binding to Aurora Kinase A and its cytotoxic effects on HeLa and MCF-7 cancer cell lines

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Abstract

Purpose: To investigate the binding potential of ssDNA aptamer to Aurora kinase A (AURKA) and assess its inhibitory effect on HeLa and MCF-7 cell lines.

Methods: A detailed analysis was conducted on the binding between aptamer and AURKA protein using HDOCK and HADDOCK, with 2D interaction obtained by LIGPLOT+ v1.4. Binding of FAM-labelled ssDNA aptamer to AURKA was analyzed by EVOS-5000 imaging and the inhibitory effects of aptamer and aptamer-drug complexes (Bleomycin Sulfate, Metformin, and Mitomycin C) at different concentrations and times were evaluated by PrestoBlue assay.

Results: Docking results showed a HADDOCK score of -82.0 ± 6.4 and a Z-score of -1.5. The HDOCK result showed best docking result (-327.13) at a confidence interval of 0.9719. Free aptamer and its drug complexes effectively inhibited HeLa cell growth, with the highest cytotoxic effect observed at higher concentrations. Free aptamer achieved the highest drug sensitivity score (DSS: 40.7) in HeLa, followed by its bleomycin sulfate and metformin complex (DSS: 31.6). However, MCF-7 exhibited lower sensitivity and DSS score.

Conclusion: ssDNA aptamer shows potential in cancer detection and therapy. In vitro and in vivo studies are needed to ascertain the potential of ssDNA aptamers in cancer therapy.

Keywords: Aurora kinase A, Aptamer-drug complex, Cancer treatment, Drug Sensitivity Score, ssDNA Aptamer, Targeted therapy

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INTRODUCTION

Chemotherapy's severe side effects, including organ damage and drug resistance, remain a significant challenge. As a result, previous studies focused on novel biomarkers, targeted

therapies and advanced drug delivery systems [1,2]. Since the late 20th century, extensive research has focused on the biomedical applications of aptamers, demonstrating their potential in precision medicine [2,3].

Aptamers, developed through Systematic Evolution of Ligands by EXponential enrichment (SELEX), offer high specificity and affinity, interacting with targets via hydrogen bonding and electrostatic forces. Their adaptability allows conjugation with therapeutic agents or nanocarriers, improving targeted drug delivery and reducing toxicity. Optimized aptamers enhance nuclease resistance, tumor targeting and internalization, making them promising candidates for cancer therapy, including triple-negative breast cancer (TNBC) [3,4].

Aptamers and aptamer-drug conjugates (ApDCs) offer promising targeted cancer therapies by improving precision and effectiveness. They regulate cellular functions by blocking receptors, inducing apoptosis and inhibiting survival pathways. Additionally, ApDCs enhance drug delivery by conjugating with drugs, siRNA, or nanoparticles, enabling selective tumor targeting while minimizing toxicity to healthy cells [5,6].

This study explores an innovative approach for treating cancer by incorporating three drugs into aptamer to make aptamer complexes. The drugs include bleomycin sulfate, mitomycin C (antibiotics with anticancer properties) and metformin (an anti-diabetic drug with synergistic cancer effects) [7,9]. While previous studies focused on conventional chemotherapy drugs like doxorubicin and cisplatin, this strategy expands therapeutic options, potentially enhancing efficacy and reducing side effects [1,10]. However, SELEX is effective but time-consuming and costly, prompting researchers to develop High Throughput-SELEX (HT-SELEX) and High Throughput Sequencing SELEX (HTS) to enhance efficiency. These methods accelerate aptamer selection, reduce bias and leverage bioinformatics for better characterization [11].

Aurora kinase A (AURKA) is a key regulator of mitosis and its overexpression is associated with cancers like gastric, breast and glioblastoma. Its amplification disrupts cell division and promotes tumor growth. Aptamer-based inhibition of AURKA could suppress the PI3K/AKT/mTOR pathway, restore p53 activity and degrade β -catenin, ultimately reducing cancer cell proliferation and inducing apoptosis [12,13].

EXPERIMENTAL

The ssDNA aptamer was selected and screened using the *in silico* method according to a previous study [14] and manufactured using Integrated DNA Technology (IDT). All reagents and chemicals were commercially purchased. The cell lines were obtained from the Council for

Scientific and Industrial Research (CSIR), SNB laboratory. The *in silico*/computational materials used in the study were Protein data bank (PDB), PDBsum, H++ server, Primer-BLAST (<https://www.ncbi.nlm.nih.gov/tools/primer-blast>), RNAfold web server (www.ViennaRNAweb services-rna.tbi.univie.ac.at), RNA composer (www.rnacomposer.cs.put.poznan.pl), HADDOCK (<https://www.bonvinlab.org/education/HADDOCK-protein-protein-basic>), HDCK (<http://hdock.phys.hust.edu.cn/>), Ligand Interaction Profiler (PLIP; <https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index>), LigPlot v2.2 and PyMOL v.2.5 (www.pymol.org). For the wet lab experiments, polylysine, phosphate buffer saline (PBS), TE buffer, SELEX buffer (comprising 1xDPBS and MgCl₂), a thermocycler (PCR machine), 12 well-plate, EVOS-M5000 Imaging system with 20X objective lens, hemocytometer, Tecan infinite F500 microplate reader machine, laminar flow hood and inverted microscopes were used.

Protein prediction, aptamer selection and preparation

The X-ray crystal structure of the AURKA protein was obtained from PDB and PDBsum, followed by cleaning and validation using Discovery Studio V and Verify 3D. The pH was analyzed using the H++ server. The ssDNA aptamer sequence was processed through RNAfold for 2D structure prediction, and its 3D structure was modeled using RNAcomposer based on dot-bracket notation. Binding analysis between the aptamer and AURKA protein was conducted using HADDOCK v2.4 and HDCK, and the best complex was selected based on docking scores and visualized using PyMOL. Interaction analysis was performed with PLIP and LIGPLOT+ v2.2.

In vivo assessment of aptamer cytotoxicity

A stock solution (100 μ M) of the aptamer was prepared in 10X Tris-EDTA (TE) buffer. Various concentrations of aptamer solution were prepared using the dilution equation including low concentrations of 1, 1.5, 2, 2.5 and 3 μ M, and high concentrations of 20, 40, 60, 80 and 100 μ M. The aptamer was then diluted in a folding buffer (1X DPBS with 5 mM MgCl₂), denatured at 95 °C for 5 minutes, gradually cooled to 22 °C for 10 minutes and maintained at 37 °C for 15 minutes to ensure optimal folding. Thereafter, the aptamer was applied to MCF-7 and HeLa cancer cell lines at varying concentrations and incubation periods, with cytotoxicity assessed using the Presto Blue assay. For the aptamer-drug complex, bleomycin

sulfate, metformin and mitomycin C were mixed with the aptamer at different molar ratios (Aptamer/Drug = 1.0, 1.2, 1.4, 1.6 and 1.8) using a conjugation buffer (comprising 0.1 M CH_3COONa , 0.05 M NaCl and 5 mM MgCl_2) [10]. The combination, containing a fixed drug concentration of 100 nM, was incubated at 37 °C for 1 h to facilitate drug intercalation [1,10]. Data were normalized relative to DMSO (set as 100) and BzCl (set as 0), and dose-response curves were plotted using ggplot2 (Version 4.3.2) in R.

Cell culture and Presto Blue assay test

HeLa (cervical cancer) and MCF-7 (breast cancer) cell lines were cultured in DMEM supplemented with 10 % heat-inactivated FBS and 1 % antibiotics (penicillin, streptomycin, and fungizone). Cells were maintained in T75 flasks at 37 °C in a humidified atmosphere containing 5 % CO_2 , seeded in 96-well plates and incubated overnight before treatment with free aptamer or aptamer-drug complexes. After incubation, 10 μL of PrestoBlue solution was added per well and incubated for 10 minutes. Fluorescence intensity was measured using a Tecan Infinite F500 plate reader with λEx = 535 nm and λEm = 612 nm.

Preparation of protein (AURKA) solution

Aurora kinase A protein was purchased from Thermo Fisher Scientific, South Africa. The protein sample was diluted in 500 μL of PBS solution. To make the coating solution, 5 mg of polylysine powder was dissolved in 50 mL of sterile tissue-culture grade water and mixed thoroughly. A volume (5 μL) of the resulting

polylysine solution was used to coat each well of the 12-well plate, which was then left to dry overnight. After the incubation, 50 μL of protein and 50 μL of aptamer were added to the wells. Phosphate-buffered saline (PBS) served as a negative control, while AURKA protein was used as positive control. Both experimental samples and controls were immobilized using polylysine. An EVOS M5000 Cell imaging system with a 200 μM scale bar was used to capture the images.

Drug Sensitivity Score (DSS)

Drug Sensitivity Score (DSS) was analyzed using the Breeze online platform, a web-based tool designed for dose-response data analysis. For the analysis, the dataset containing information from both the treatment and control groups was uploaded to the system, and the analysis was executed.

Statistical analysis

A two-sample unpaired T-test was used to analyze the differences between two groups, while one-way ANOVA compared multiple groups. Results were expressed as mean $\pm$ SEM (n = 3) and statistical significance was set at p < 0.05.

RESULTS

The aptamer used in this study had a GC content of 60 % and 54 nucleotides. Two-dimensional (2D) and three-dimensional (3D) structures of the aptamer are displayed in Figure 1.

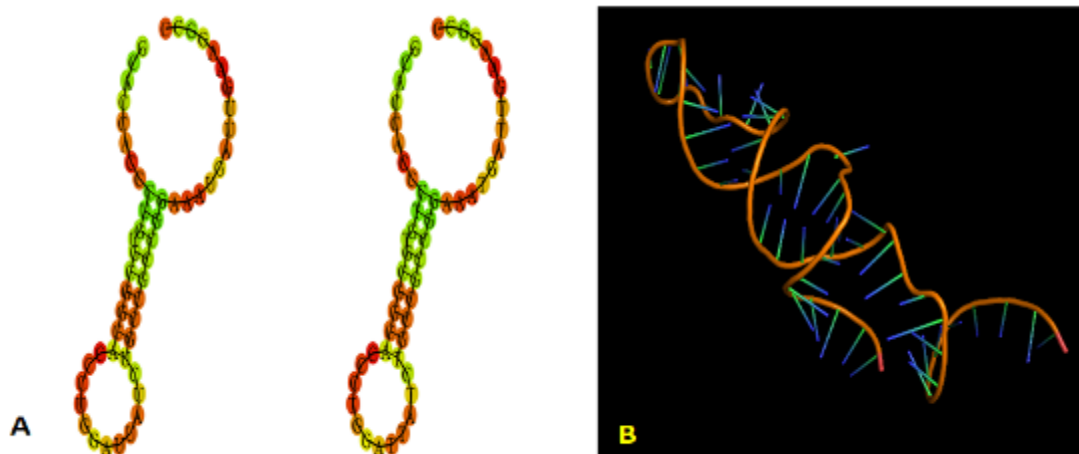


Figure 1: Predicted 2D structure of the ssDNA aptamer created by RNAfold (A) and 3D structure predicted and visualized by RNAcomposer and PyMOL (B), respectively

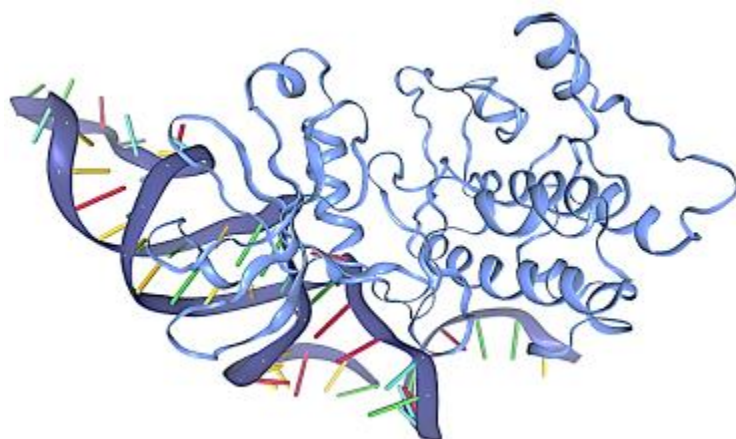


Figure 2: Binding structure of AURKA (4J8N) protein and ssDNA aptamer generated by using HADDOCK v. 2.4 web-based server

Docking of ssDNA aptamer-AURKA

Molecular docking and visualization of the ssDNA aptamer-AURKA protein complex were conducted using HADDOCK (Figure 2), HDOCK and PyMol. The HADDOCK docking score was -82.0 ± 6.4 with a Z-score of -1.5 , while HDOCK yielded the best docking score of -327.13 with a confidence interval of 0.9719 .

Protein-ligand interaction study

The interaction study was executed using the Protein Ligand Interaction Profiler (PLIP) web server tools. Amino acids (AA) involved in

hydrogen bonding between the aptamer and AURKA are presented in the supplementary Table 1.

Two-Dimensional (2D) Interaction Study

Using LIGPLOT, a 2D graphic representation of the protein-ligand complex was generated, illustrating hydrogen bonds, hydrophobic interactions and atom accessibility (Figure 3). This analysis revealed the main binding regions, hydrogen bonds and other intermolecular forces that enhanced the stability and specificity of aptamer-protein interaction. Interacting residues are listed in Tables 1 - 3.

Table 1: Hydrogen bonds between ssDNA aptamer and AURKA

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein Donor?	Side Chain
1	54A	GLN	3.25	3.65	106.46	✓	✓
2	54A	GLN	2.96	3.78	141.93	✓	X
3	70A	LYS	1.91	2.82	143.62	✓	✓
4	81A	ARG	2.96	3.78	136.23	✓	✓
5	82A	ALA	2.03	2.99	164.33	✓	X
6	111A	GLN	2.03	3.15	143.65	✓	✓
7	112A	GLY	3.46	3.81	103.63	✓	X
8	112A	GLY	3.20	3.72	115.30	x	X
9	112A	GLY	2.91	3.53	123.29	x	X
10	113A	THR	2.39	3.12	132.17	✓	✓
11	114A	SER	2.03	2.74	127.79	✓	X

Table 2: Interacting residues that form hydrogen bonds between AURKA and the aptamer

AURKA			ssDNA aptamer		
Amino acid residues	Residue number	Chain	Nucleotides	Residue number	Chain
Histidine (His)	20	A	Uracil(U)	10,31	B
Lysine (Lys)	125,250	A	Adenine(A)	40,41	B
Arginine (Arg)	189,195	A	Cytosine(C)	9,33	B
Serine (Ser)	186	A	Guanine(G)	13, 35	B
Glutamine (Gln)	127	A			

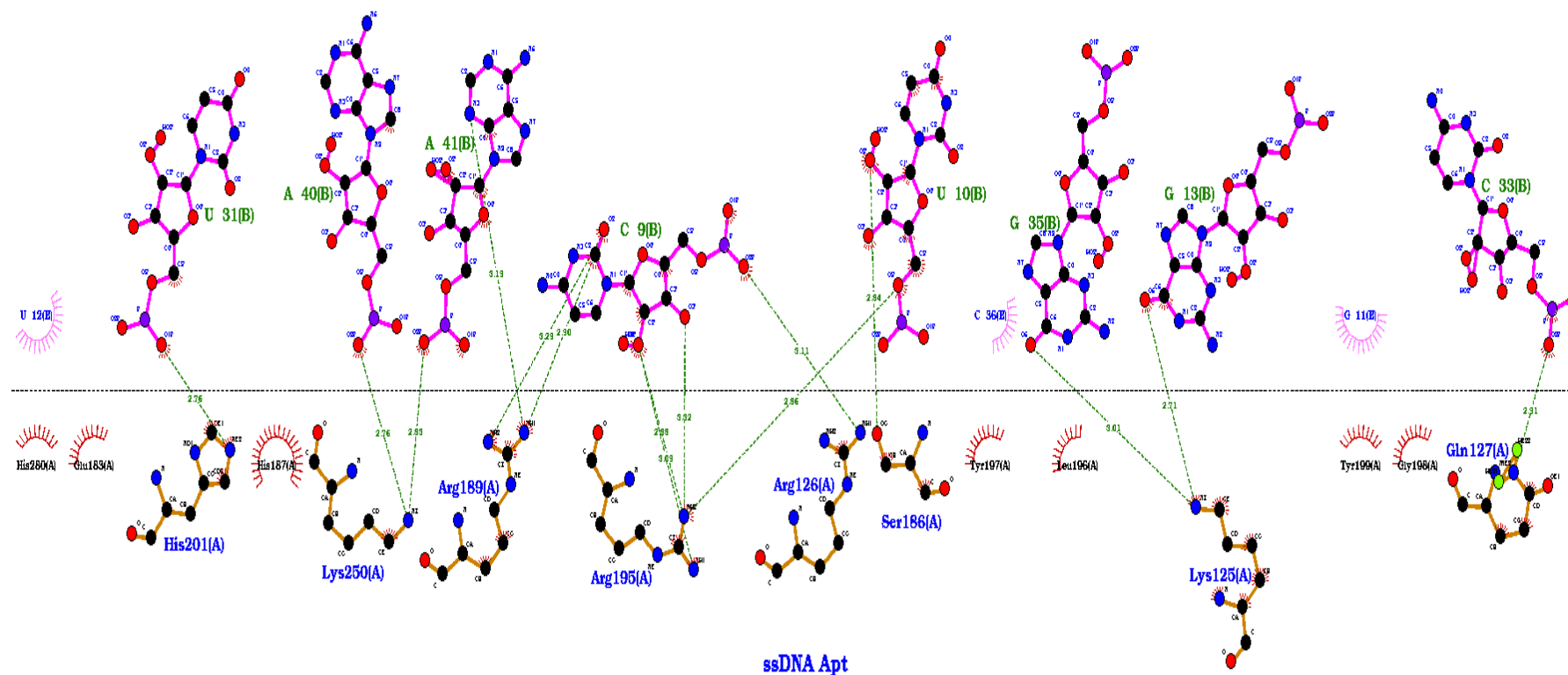


Figure 3: The 2D representation of the interactions between AURKA protein residues (A) and aptamer residues (B) generated using Ligplot. Hydrogen bonds are indicated with green dashed lines

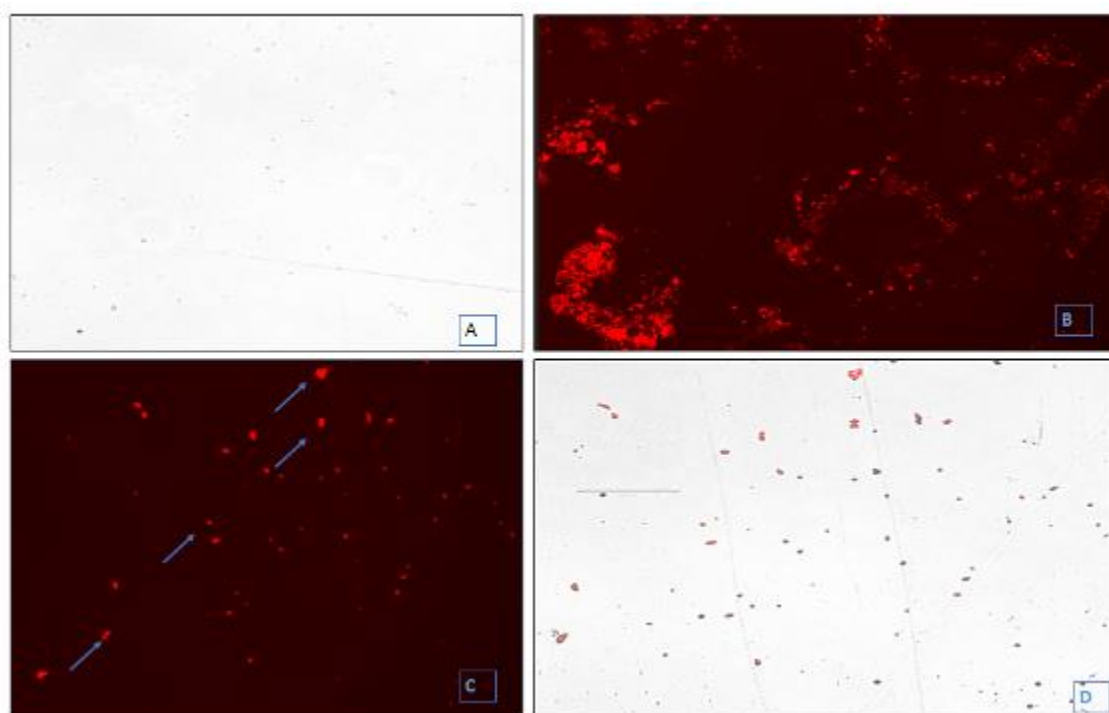


Figure 4: A FAM-labelled ssDNA aptamer was used to detect and image the AURKA protein. The negative control (PBS) and positive control (free AURKA protein; A) did not produce any images due to the absence of fluorescence labelling. However, fluorescence signals were observed for the FAM-labelled free ssDNA aptamer (B) and the localization of the FAM-ssDNA aptamer with AURKA protein (C & D) captured under TX RED and transmitted light. The images were captured using the EVOS M-5000 Imaging System

Detection of AURKA protein with FAM-labelled aptamer

The *in silico* study suggested an interaction between AURKA protein and the aptamer. A FAM-labelled ssDNA aptamer was used to assess its binding with AURKA (Figure 4). The signal change between SF1B and SF1C suggests aptamer-protein co-localization, indicating possible interaction. However, *in vitro* analysis is needed to confirm aptamer-protein conjugation.

Effect of low concentrations of aptamer on HeLa cell line

At low concentrations (1 - 3 μM), the aptamer showed minimal inhibition of HeLa cell growth after 1 and 24 h of incubation. However, a more potent inhibitory effect was observed at 48 and 72 hours (Figure 5 A and B), with statistical significance achieved after 72 hours ($p < 0.05$).

Effect of high concentrations of aptamer on HeLa and MCF-7 cell lines

To evaluate the cytotoxic effects, HeLa (Figure 5 C) and MCF-7 (Figure 7 A) cells were treated with higher concentrations of the aptamer (20 – 100 μM) for 24, 48 and 72 hours. Results

showed a significant cytotoxic effect on HeLa cells, with the most substantial impact observed at 48 hours. A statistically significant decrease in cell viability ($p < 0.05$) was noted across all treatment groups. In contrast, the free aptamer exhibited lower cytotoxicity on MCF-7 cells than on HeLa cells.

Efficacy of aptamer-drug complexes

The cytotoxic effects of the aptamer-drug complexes on HeLa (Figure 6 A - C) and MCF-7 (Figure 7 B - D) cells were evaluated at different incubation times. The outcomes demonstrated strong cytotoxic effects on HeLa cells, with statistically significant inhibition of cell growth ($p < 0.05$), indicating that the aptamer-drug complex effectively induced cell death. However, the efficacy of the complexes was lower on MCF-7 cells than on HeLa cells.

Drug Sensitivity Score (DSS)

Drug sensitivity score (DSS) integrates multiple dose-response parameters, including IC_{50} and AUC. The EC_{50} represents the concentration required for a 50 % maximal response. In HeLa cells, the free aptamer exhibited the highest DSS (40.7), followed by aptamer-bleomycin and aptamer-metformin (31.6 each), while aptamer-

mitomycin c had the lowest DSS (12.8). However, in MCF-7 cells, the free aptamer showed no DSS score, and the highest DSS score was observed for aptamer-metformin.

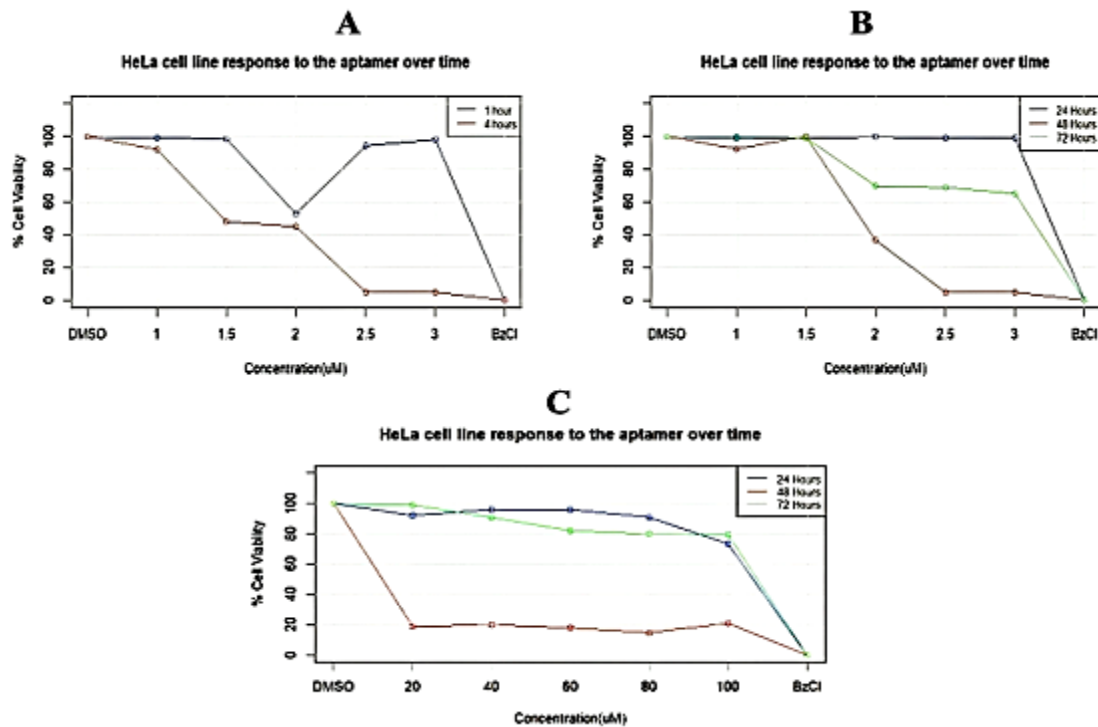


Figure 5: The effect of free ssDNA aptamer on HeLa cancer cells. Treatments included low concentrations for short durations (A), low concentrations for extended durations (B), and higher concentrations for prolonged periods (C). Data were normalized and compared against DMSO (100) and BzCl (0)

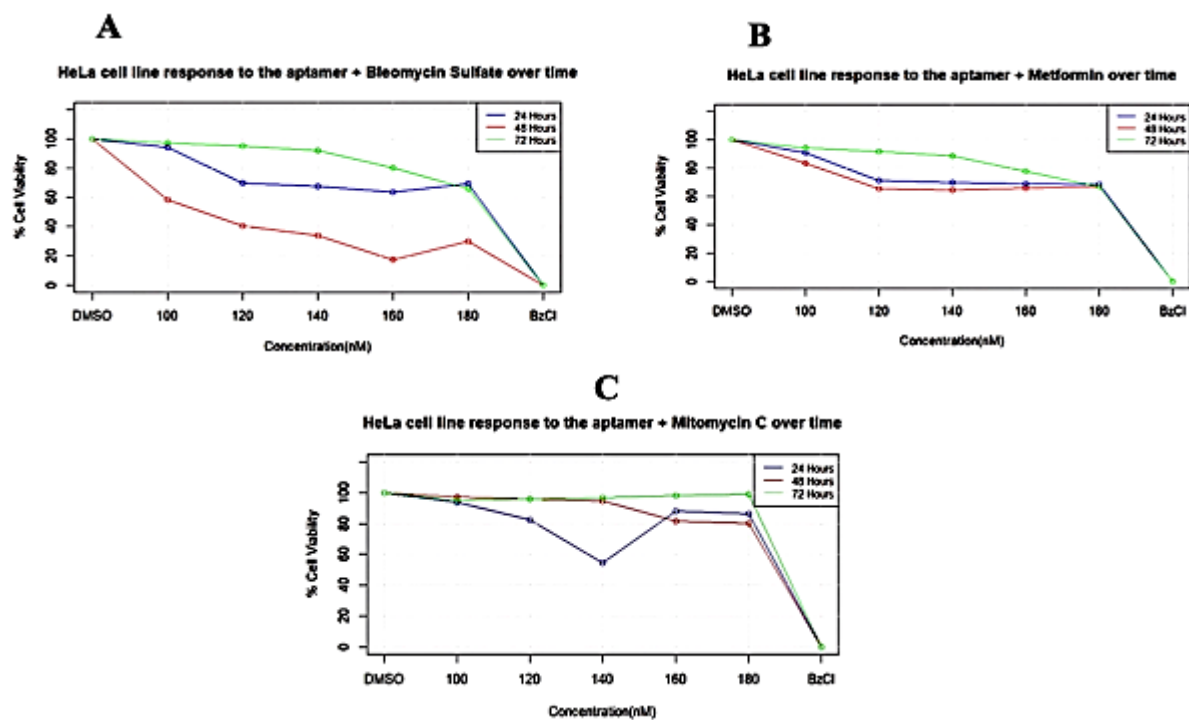


Figure 6: The effects of aptamer-drug complexes on HeLa cells were assessed for aptamer-bleomycin sulfate (A), aptamer-metformin (B), and aptamer-mitomycin C (C)

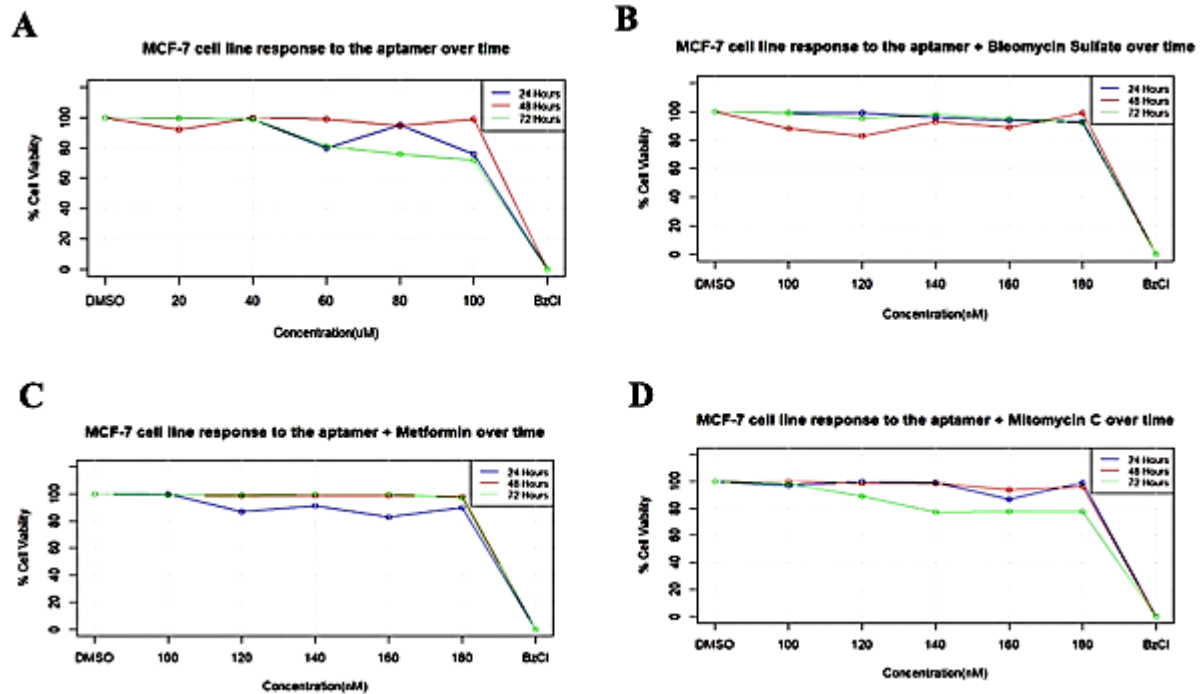


Figure 7: Effects of the free aptamer and aptamer-drug complexes on MCF-7 cells. Free aptamer (A), aptamer-bleomycin sulfate (B), aptamer-metformin (C), and aptamer-mitomycin C (D)

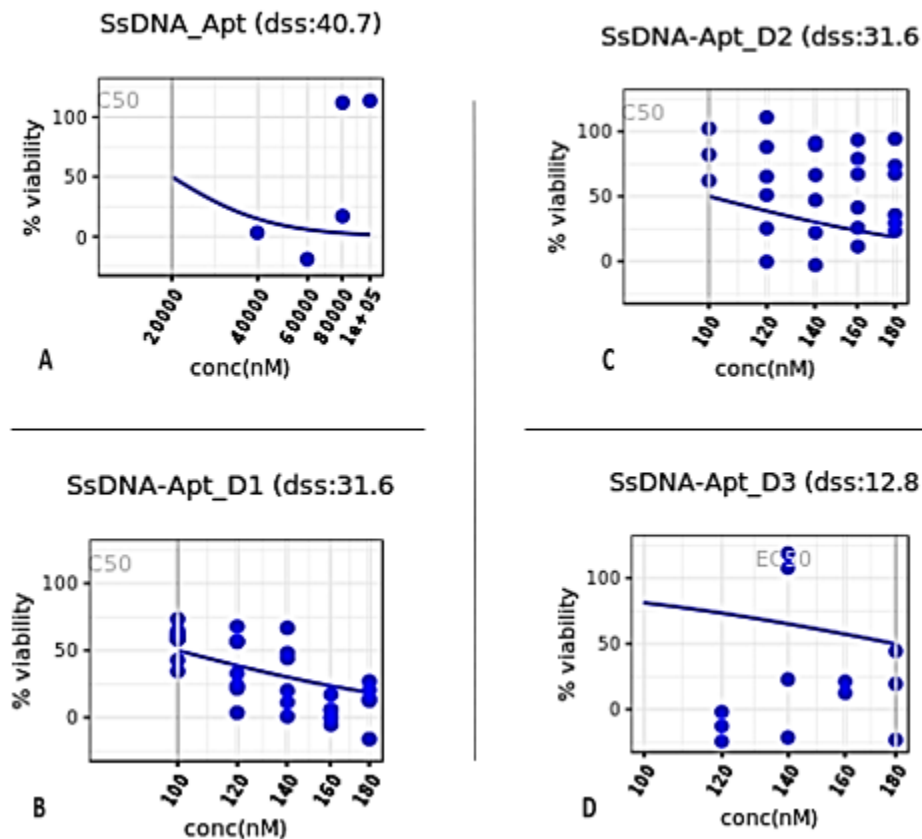


Figure 8: Illustration of dose response curve (% viability Vs concentration) and EC₅₀ values for each drug. Effect of free aptamer (SsDNA-Apt; A), Aptamer-Bleomycin Sulfate conjugate (ssDNA-Apt_D1; B), Aptamer-Metformin (SsDNA-Apt_D2) and Aptamer-Mitomycin C conjugate (ssDNA-Apt-D3) on HeLa cell line

DISCUSSION

The ssDNA aptamer likely forms a G-quadruplex structure, enhancing its binding to cancer-related proteins like AURKA. Docking results from HADDOCK and HDock suggest strong, reliable interactions, supported by LIGPLOT analysis showing optimal hydrogen bond distances (2.7 – 3.3 Å). Key interacting residues support its potential in protein targeting and drug delivery [15]. Previous studies reported aptamers with strong anticancer effects and high specificity, sometimes outperforming antibodies [6]. In this study, the designed aptamer showed strong cytotoxicity in HeLa cells but was less effective in MCF-7 cells. PrestoBlue assays over 72 hours confirmed a dose- and time-dependent cytotoxic effect, especially at higher concentrations [16].

G-quadruplex aptamers, such as 26-mer AS1411 and EpCAM-targeting aptamers, are similar to the aptamer used in this study with 60 % GC content. They have shown promise in customizable cancer therapies [10], supporting the possibility that the aptamer used in this study could be optimized for specific applications through rational modifications to demonstrate cytotoxicity in various *in vitro* tumor cell assays. Aptamer complexes with bleomycin sulfate, mitomycin C and metformin were assessed for targeted delivery, aiming to reduce the side effects and improve efficacy over traditional chemotherapy. The complexes showed promising cytotoxicity at 24, 48 and 72 hours [6,7].

Unlike traditional chemotherapy agents like doxorubicin and cisplatin, this approach expands potential anti-tumor strategies, offering more effective and versatile cancer treatments with fewer side effects [1,16]. These findings support the viability of aptamer-drug conjugates for precise drug delivery, enhancing chemotherapy efficacy while minimizing systemic toxicity [17].

The result offers a promising strategy to improve treatment precision and overcome challenges in targeted cancer therapy [18]. Drug Sensitivity Scoring (DSS) revealed that the aptamer-metformin complex had the highest DSS (7.2) on MCF-7 cells, though overall responses in this cell line were limited. Heatmap data confirmed the concentration-dependent effect, but the therapeutic impact on MCF-7 remained modest. The overall DSS scores and heatmap results for MCF-7 cells were insignificant, suggesting that the aptamer lacked a significant therapeutic impact on this cell line.

CONCLUSION

The ssDNA aptamer exhibits strong specificity for cancer markers, making it promising for diagnostics, drug delivery, and therapy. This study highlights the incubation time- and concentration-dependent cytotoxicity of aptamers, supporting their potential in targeted cancer treatment. Further studies are recommended to validate the antitumor efficacy and selective uptake of these aptamers by cancer cells.

DECLARATIONS

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Data availability statement

The data used or analyzed during this study are available from the corresponding author on request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Haregewoin B. Woldekidan: Conceptualization, Methodology, performed the analysis, and the main writer of the article; Deepak B. Thimiri Govinda Raj: funding acquisition, Writing-Review & editing, and supervision; Adugna A. Woldesemayat: Conceptualization, Methodology, Writing, Reviewing & editing, and supervision; Mutsa Takundwa: funding acquisition, reviewing, and editing.

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