Journal of Applied Sciences and Nanotechnology

Journal homepage: https://jasn.uotechnology.edu.iq/

Bioinformatical Prediction of G-quadruplex Aptamer for Detection of a Ligand in Practice

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Article information

Article history:
Received: August, 07, 2023
Accepted: October, 22, 2023
Available online: December, 10, 2023

Keywords:
G-Quadraplex nanostructure,
Aptamers,
Bioinformatics,
Aflatoxin M1,
Molecular docking

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Abstract

Considering the introduction of aptamers as a new generation of analyte identifiers, this class of materials can be used in diagnostic systems because aptamers are easier to produce, more sensitive, higher accuracy, less sensitive to environmental factors, easier to handle and can be used. A special type of aptamer that has sequence rich in guanine base can create a special nanostructure called G-quadraplex. The creation of this structure gives the aptamer an enzyme property so that it can act like an enzyme in the vicinity of it, oxidize a chromogenic substrate and produce a colored signal. The main way to produce aptamers is a laboratory technique called SELEX (Systematic evolution of ligands by exponential enrichment), in which a mixture of different oligo libraries in the vicinity of the target analyte creates aptamers in several consecutive cycles. The aim of this study was the introduction a novel approach for obtaining DNA aptamers for detection a ligand such as aflatoxin M1 in bioinformatically manner in replacing to SELEX for obtaining the specific oligoaptamers against aflatoxin M1. For this purpose, the structure of the selected oligoaptamers were predicted using some molecular simulators and bioinformatically techniques. The results of these molecular simulations suggested G-quadruplex aptamers with suitable affinity for binding to aflatoxin M1 in colorimetric assays.

DOI: 10.53293/jasn.2023.7058.1234, Department of Applied Sciences, University of Technology

1. Introduction

Aptamer has been introduced as a recognition element and capable for diagnostic and targeted therapeutic applications [1]. Indeed, aptamers are mainly DNA or RNA oligomers which can interact with difference targets such as micro and macro molecules with highly selectivity [2]. Aptamers versus others recognition elements

have lots of advantages; they have higher stability, low immunogenicity, portability, reusability and easy to use [3-5]. Generally, Aptamers obtained by an *in vitro* selection technique called systematic evolution of ligands by exponential enrichment (SELEX) **(Figure 1)** [6].

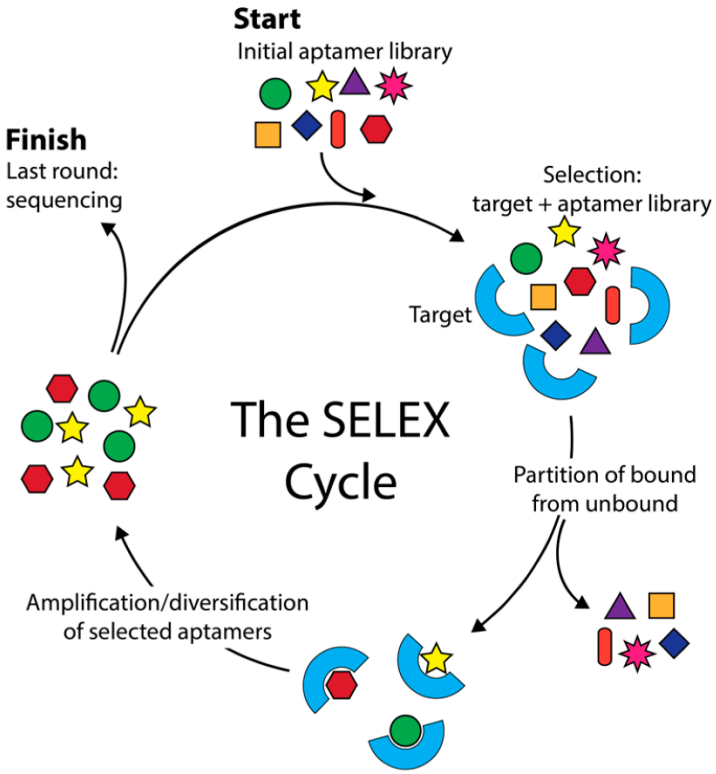


Figure 1. Schematic diagram of a SELEX process, the library of random oligomers is incubated with the target, in the next step the target is washed to remove non-linked oligomers and finally the linked oligomers (aptamers) are amplified and entered into the next cycle. A minimum of 12 cycles is required. DNA based aptamers are major subtype of aptamers which could form different structures like hairpin, stem loop, G-quadraplex and etc. [7]. G-quadraplex (G4) is a unique structure and it's formed when the oligomers had G-rich sequences. There are different structures of G4 conformations, such as unimolecular G4, bimolecular G4, and tetramolecular G4[8] (**Figure 2**).

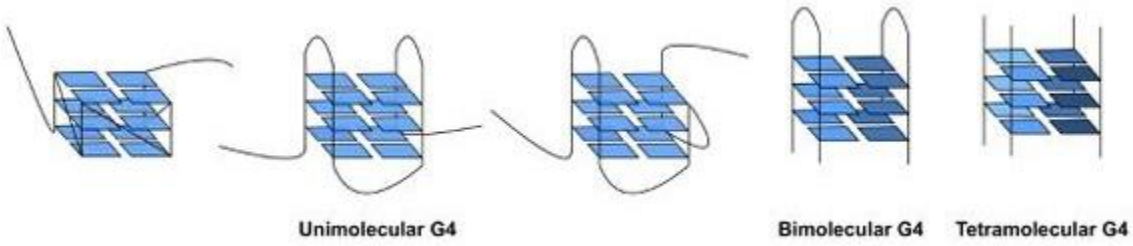


Figure 2. different G-quadruplex structure. unimolecular G4s with different backbone arrangements (parallel, anti-parallel and mixed); Bimolecular quadruplex; Tetramolecular quadruplex [8]

G4 conformation is the predictable structure based on the number of guanines in the oligomer sequence and their secondary structure [8]. Online webserver, QGRS[1] Mapper calculate G-Score of each oligomer sequence and a higher G-score equals a higher probability of G4 conformation [9]. In

[1] Quadruplex forming G-Rich Sequences

addition, two online webservers, M-fold and RNA structure, predict secondary structures of oligomers that give the ability to estimate the number of loops or stem-loops structures likely to exist in the oligomers [10, 11]. There are some literatures indicated the application of in silico methods to optimize DNA based aptamer and affinity predication such as artificial intelligent or Auto Dock vina softwares [12-15]. Meanwhile some other studies suggested the use of in-silico methods with caution due to several errors probably occur during predictions and optimizations [16, 17]. As mentioned before, aptamer could be used in therapeutic and diagnostic approaches, like a biosensor for detection of a specific ligand. Several platforms have been developed for the use of aptamers in biosensors [18]. In colorimetric approach, signals detectable with a naked eye and it could be quantifying with an OD[2] measurement by a microplate reader or a UV-visible spectrophotometer [19]. For example, in a colorimetric aptasensor based on gold nanoparticles, its color can be appearance when the aptamer bind to its analyte due to their high affinity, after that gold nanoparticles aggregated and then it produced a purple color [20], In another approach, a G-quadruplex aptamer could be produced a color in a single step, in which the aptamers bind to their specific ligands and then they change to G4 conformations which have peroxidase activity such as horseradish peroxidase enzyme. In this case aptamers could produce colors if there is any chromogenic substrate such as tetramethylbenzidine (TMB) in reactions[19, 20]. Sometimes the oligomer aptamers which obtained by SELEX have low affinity and need post-SELEX optimizations [13]. Because of time consuming of SELEX to obtain ideal aptamers we can used in-silico methods to achieve the best oligomers [22]. There is different strategy for obtaining and optimizing oligomers as aptamers using an in-silico method; however, this study describe a methodology to choose aptamers could be formed to G-quadruplex conformations which they have introduced by former studies and predicted the secondary structure and estimated G-score with different online web server for obtaining of a former step to increase the affinity of aptamer and G-score for predicting a new oligoaptamer for aflatoxin M1 as a ligand by Autodock Vina software.

2. Experimental Procedure

2.1. Oligonucleotides

To obtained original oligoaptamers which used to as a recognition element to detect the aflatoxin M1, different publications [23-37]. For obtaining the articles, the search query was the same including phrases such as aptamer and aflatoxin M1 and aptasensor and aflatoxin M1 and aptamer-based biosensor and aflatoxin M1.

2.2. Online Webservers and Softwares

2.2.1. UNAFold Web Server and RNA Secondary Structure

UNAFold[3] web server (Mfold[4])[5] and RNA secondary structure were used to predict the secondary structure of the oligonucleotides which obtained by searching the publications. For M. fold, the FASTA[6] format of sequences were used with default condition of predicted situation. In RNA secondary structure, the sequence type changed from RNA to DNA mode and default condition of predicted situation was used, too. From M. fold, the JPG and Vienna file of the structure which had the least ΔG was saved and the result of RNA secondary structure was saved, too.

2.2.2. QGPRS Mapper

For enrolling QGPRS Mapper[7], the input sequences were the queries in FASTA format and the highest G score and overlaps were recorded for each sequence.

2.2.3. RNA Compressor

[2] Optical density
[3] Unified Nucleic Acid Folding
[4] Multiple fold
[5] http://www.unafold.org/mfold/applications/rna-folding-form.php
[6] Fast-All
[7] https://bioinformatics.ramapo.edu/QGRS/index.php

RNA compressor[8] is used for predicted 3D structures of oligomers. RNA compressor is used for RNA sequences so all DNA sequences were mutated to RNA. In the first step used Vienna file of secondary structure of Mfold and all thymine bases were replaced with uracil by Notepad^{++} software. Input sequences were put in the query form and saved as the files of each Oligomers.

2.2.4. Discovery Studio Software

After receiving the results from RNA compressor, Discovery Studio Software[9] was used for changing the RNA sequences to DNAs. All uracil bases substituted by thymine bases and changed to sugar of all bases from ribose to deoxyribose.

2.2.5 PubChem and Open Bable

To achieved to 3D structure of aflatoxin M1 (as a target) PubChem online webserver[10] was used and saved the SDF[11] file. In the next step, SDF file was converted to PDB[12] file by Open Bable[13] software.

2.2.6. Autodock Tools

The aflatoxin M1 was put as the target in Autodock tools software[14] and merged the nonpolar hydrogens and edited its surface charge (kollman and gasteger) and then it saved as a PDBQT[15] file as the output. In addition, Autodock Tools software was used for oligomers as a macromolecule and merged nonpolar its hydrogens and added its surface charges and then saved as PDBQT file. In the other hand, it determined the binding grid as a binding site.

2.2.7. Autodock Vina

Autodock Vina[16] was used to evaluate the docking interaction between different oligoaptamers and the target. The score of interactions were recorded.

3. Results and Discussion

Thirty-nine distinct nucleotide sequences were selected from the papers including aptamers against aflatoxin M1 (Table 1) and they used as primarily aflatoxin-specific aptamers for analysing with the softwares have been described above.

Table1. Sequences introduced in different studies to be used as aflatoxin M1 specific aptamer

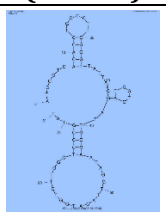
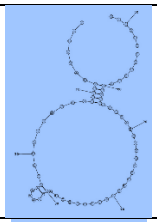
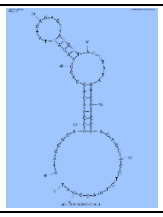
No.	Sequence (5'→3')	Ref. no.
1	ATCCGTCACACCTGCTCTCGAATAGAGTACTCGATACTGACTTTATTTACCGTCTGGTGTTGGCTCCCGTAT	[23]
2	ATCCGTCACACCTGCTCTCCTCGAACTAGTTAGACCCTCCTCCAAGTCAACTTGTGGTGTTGGCTCCCGTAT	[23]
3	ATCCGTCACACCTGCTCTTGGGGTTATTACTCGTGAGATTGGGAATAGGTTACATGGTGTTGGCTCCCGTAT	[23]
4	ATCCGTCACACCTGCTCTTGTAGGGTTCCCACCCAATTCAGTTCCGTTAAACCATGGTGTTGGCTCCCGTAT	[23]
5	ATACGGGAGCCAACACCAAAGTAAGATCATCACCCGGACGCGGACATAATAGGAGAGCAGGTGTGACGGAT	[23]
6	ATCCGTCACACCTGCTCTAACTTACACATAATTCTAGGTTACATCTTGCTCATATGGTGTTGGCTCCCGTAT	[23]
7	ATACGGGAGCCAACACCACCAACATTATCAGAGTATGTTACTTATAGTGGTGGCAGAGCAGGTGTGACGGAT	[23]
8	ATACGGGAGCCAACACCACGTCAACAATTATTCAATGAGAAGCGGCTTATGAGGAGAGCAGGTGTGACGGAT	[23]
9	ATACGGGAGCCAACACCACATCGACTTACGAATCAACGCGTTTATTATTGGTTCAGAGCAGGTGTGACGGAT	[23]
10	ATCCGTCACACCTGCTCTGTGTACGCCCGTATTTACGTTCCTAGCAATTGCTATGTGGTGTTGGCTCCCGTAT	[23]
11	ATCCGTCACACCTGCTCTACACTCCGCACGATCTTTTTTAGAACGCGTACCCGTTGGTGTTGGCTCCCGTAT	[23]

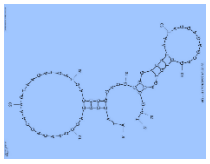
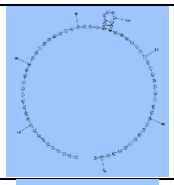
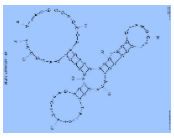
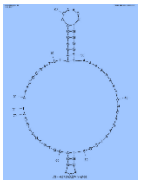
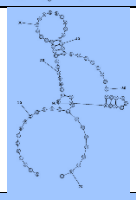
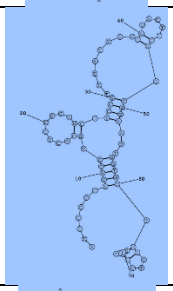
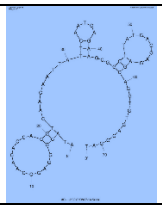
[8] https://rnacomposer.cs.put.poznan.pl/
[9] https://discover.3ds.com/discovery-studio-visualizer-download
[10] http://pubchem.ncbi.nlm.nih.gov/
[11] Spatial Data File
[12] Protein data bank
[13] http://openbabel.org/wiki/Main_Page
[14] https://autodock.scripps.edu/
[15] Protein Data Bank, Partial Charge (Q), & Atom Type (T) format
[16] https://vina.scripps.edu/

12	ATACGGGAGCCAACACCAGGTGCGGTAAGGTTCGCCCTAAAGCTTATATACTCAAGAGCAGGTGTGACGGAT	[23]
13	ATACGGGAGCCAACACCACTTGGTTAACCAATCAAACCGGACAACGAAGGGTCCAGAGCAGGTGTGACGGAT	[23]
14	ATACGGGAGCCAACACCAACAACCTAGATGTTCTGATAACACGAATCGCTTCGAAGAGCAGGTGTGACGGAT	[23]
15	ATACGGGATCTAACACCAAGAACCTGGGGTTAAAAAAACAGGAGTATGGATGCAGAGCAGGTGTGACGGAT	[23]
16	ATACGGGAGCCAACACCAAGTAACACACGCGGACCAGAAATACATCCCCCCGTAGAGCAGGTGTGACGGAT	[23]
17	ATCCGTCACACCTGCTCTCAATCTGAAATATTGCAAGCAGTGCTCACAATTTGTTGGTGTTGGCTCCCGTAT	[23]
18	ATCCGTCACACCTGCTCTCCCCGGCGTCCGTTTATTAGCAGACTTTGGCGGAATTGGTGTTGGCTCCCGTAT	[23]
19	ATCCGTCACACCTGCTCTGGCATTAGTATTCCATAGCCGGCCAAGTCTATGTAGTGGTGTTGGCTCCCGTAT	[23]
20	ATCCGTCACACCTGCTCTGACGCTGGGGTCGACCCGGAGAAATGCATTCCCCTGTGGTGTTGGCTCCCGTAT	[23]
21	ACTGCTAGAGATTTTCCACAT	[23]
22	ATCCGTCACACCTGCTCTGACGCTGGGGTCGACCGGAGAAATGCATTCCCCTGGTGTTGGCTCCCGTAT	[24]
23	TAACACGAGACACTGCTAGAGATTTTCCACATTTCTCTTGTTCC	[25]
24	GTTGGGCACGTGTTGTCTCTCTGTGTCTCGTGCCCTTCCTAGGCCCACA	[26]
25	ACTGCTAGAGATTTTCCACATGCTGAGGCCGCTCTCTAGCAGTAAAA	[27]
26	ACTGCTAGAGATTTTCCACA	[28]
27	ATCCGTCACACCTGCTCTGACGCTGGGGTCGACCCG GAGAAATGCATTCCCCTGTGGTGTTGGCTCCCG TAT	[29]
28	ATCCG CAACCTGCTCTGACGCTGGGGTCGACCCGGAGAAATGCATTCCCCTGTGGTGTTGGCTCCCGTAT	[30]
29	TCTCACTGCTAGAGATTTTCCACAT	[31]
30	ATCCGTCACACCTGCTCTGACGCTGGGGTCGACCCG	[32]
31	ACTGCTAGAGATTTTCCA	[33]
32	ATCCGTCACACCTGCTCTGACGCTGGGGTCGACCCGGAGAAATGCATTCCCCTGGGTGTTGGCTCCCGTAT	[34]
33	ATCCGTCACACCTGCTCTGACGCTGGGGTCGACCCGGAGA	[35]
34	CAACGCCAGTCAGTATCTTATATGCTATACTGGCTGGTGTTG	[36]
35	CCGGCGGATGCTAATTGCAGAGCAGGTGTGCCGG	[35]
36	AAAA-ACACTATGTAGTGGTGT	[35]
37	GTTGGGCACGTGTTGTCTCTCTGTGTCTCGTGCCCTTCGCTAGGCCCACA	[36]
38	GGGATGTGAGGTGGCTCTCGT	[37]

The ability of oligomers was examined by QGQRS Mapper and the aptamers which could form G-quadraplex configurations were separated. After that, the second structure of these aptamers were obtained using M-fold and RNA structure (Table 2).

Table2. The sequences with G-Score and their secondary (2D) structures via M-Fold and RNA structure.

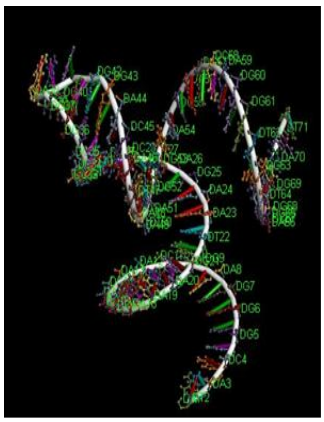
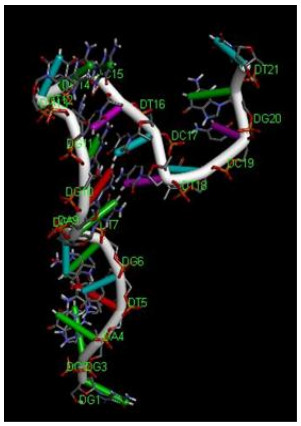
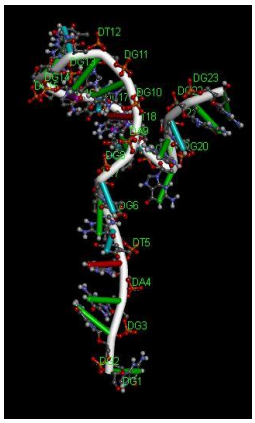
No	Sequence (5'→3')	G-Score	2D structure (M-Fold)	2D structure (RNA structure)
3	ATCCGTCACACCTGCTCTTGGGGTTATTACTCGTGAGA TTGGGAATAGGTTACATGGTGTTGGCTCCCGTAT	19		
7	ATACGGGAGCCAACACCACCAACATTATCAGAGTATGTT ACTTATAGTGGTGGCAGAGCAGGTGTGACGGAT	15		

5	ATACGGGAGCCAACACCAAAGTAAGATCATCAC CCGGACGCGGACATAATAGGAGAGCAGGTGTGACGGAT	19		
12	ATACGGGAGCCAACACCAGGTGCGGTAAGGT TCGCCCTAAAGCTTATATACTCAAGAGCAGGTGTGACGGAT	13		
15	ATACGGGATCTAACACCAAGAACCTGGGGTTAA AAAAACAGGAGTATGGATGCAGAGCAGGTGTGACGGAT	16		
18	ATCCGTCACACCTGCTCTCCCCGGCGTCCGTTTATTAGC AGACTTTGGCGGAATTGGTGTTGGCTCCCGTAT	18		
8	ATACGGGAGCCAACACCACGTCAACAATTATTCAATGAGA AGCGGCTTATGAGGAGAGCAGGTGTGACGGAT	20		

According to the results obtained from QGPRS Mapper, 2D structures of oligomers (Table2), according to the loop-stem structures and thermodynamically stability of the selected oligomers, sequence No. 5 seem to have a better ability to be selected as a G-quadraplex aptamer for aflatoxin M1 detection. These findings were also reported for selection of the suitable aptamers for several target ligands avoiding lengthy experimental SELEX and instead using computational modelling to predict aptamer sequences [5, 19, 20]. As a result, the third structure of No. 5 sequence was predicted and obtained by using online software (RNA compressor). In the other hand, the sequence of ACTGCTAGAGATTTTCCACAT was the most used oligomer as the aflatoxin M1 aptamer in different studies [23, 25, 27, 31]. In addition, the sequence of GGGATGTGAGGTGGCTCTCGT had multiple guanines and in the second structure had stem-loop configuration; hence it had potential get G-Score by edit the sequence, so we modified this sequence to GGGATGTGAGGTGGCTCTGGTGG and finally evaluated these sequences in docking interaction (Figure 3). The results from the simulations demonstrated that three oligomers could be selected as the G-quadruplex aptamers for targeting and of aflatoxin M1. For instance, oligomer no. 21 was the aptamer due to its most use in various studies and it had a short length. The oligomer no. 38 had the ideal 2D structure (stem-loop) and also it had multiple guanines. The modified sequence from the sequence no. 38 had a changing process done randomly in such a way that its 2D structure did not change and it get more G-Score than the original state. On the other hands, also the obtained results demonstrated that these changes were in a way that the modified sequence had a 2D structure similar to the sequence no. 38; therefore, it gained

a G-score equal to 18. In docking process, the modified oligomer had higher interaction score when compared to the sequence no. 38.

A:

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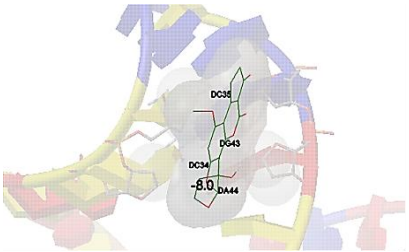
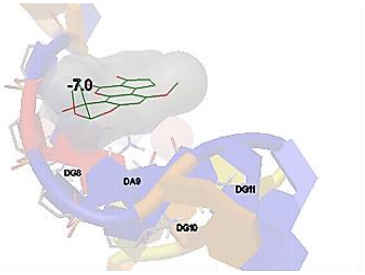
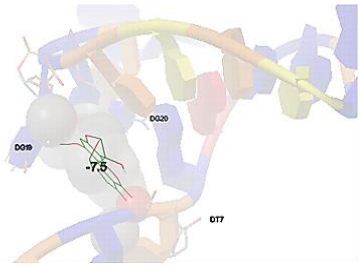

C:

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-8.0	0.000	0.000
2	-7.9	9.962	13.796
3	-7.9	8.789	13.197
4	-7.8	2.571	3.290
5	-7.6	1.178	5.689
6	-7.3	5.234	8.172
7	-7.3	3.803	6.308
8	-7.1	11.211	13.980
9	-7.1	5.440	9.141

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-7.0	0.000	0.000
2	-7.0	1.463	5.706
3	-7.0	1.866	6.656
4	-6.8	12.633	13.990
5	-6.7	2.311	3.518
6	-6.7	13.507	14.802
7	-6.6	12.456	13.418
8	-6.6	2.104	6.434
9	-6.6	11.327	12.364

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-7.5	0.000	0.000
2	-7.3	14.285	15.822
3	-7.1	13.283	15.626
4	-7.1	11.410	13.253
5	-7.1	12.951	15.056
6	-7.0	11.738	14.073
7	-6.7	12.323	13.891
8	-6.5	13.560	15.305
9	-6.4	11.491	13.699

Figure 3. Docking interaction from vina Autodock simulator; A: left, sequence no. 21, middle, sequence no. 38, and right, the modified sequence; B: 3D structures of oligomers; and C: virtual interaction of the aptamers and aflatoxin M1.

4. Conclusions

Selection of the specific aptamers particularly G-quadruplex aptamers (with duality in function after interaction to its ligand) was always one of the most important steps for the study that it aimed detecting colorimetrically a target molecule. Here, we introduced a novel approach to obtain the DNA aptamer for detection a ligand such as aflatoxin M1 in bioinformatically manner, because of time consuming and the cost of SELEX method. This study demonstrated that the specific oligoaptamers against aflatoxin M1 could be predicted theoretically by using some simulation softwares such as the ones described here and then their findings could be simplified in practice the selection of one suitable oligomer as the specific aptamer for design and development of colorimetric assays.

Acknowledgement

We really appreciate the financial support of Mazandaran University of Medical Sciences and the Center for Health Research in Plant and Livestock Products. This research was also funded by Vice‐Chancellor of Research and Technology, Mazandaran University of Medical Sciences, grant number 10384.

Conflict of Interest

The authors declare that they have no conflict of interest.

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