JCRS

Journal of Chemical Reactivity and Synthesis 2022: 12(1) 29-37

Research Article

Investigation of the interaction between Aripiprazole drug with calf thymus DNA: spectroscopic and molecular docking studies

Saba Hadidi

Department of Inorganic Chemistry, Faculty of Chemistry, Razi University, Kermanshah, Iran

ARTICLE INFO:

Received:
27 December 2021

Accepted:
4 March 2022

Available online:
18 March 2022

✉: S. Hadidi
s.hadidi@razi.ac.ir

ABSTRACT

Studies on the binding mechanism of small molecules with DNA is of great help to understand the action mechanism of some drugs, and therefore, to design new and more efficient DNA targeted drugs. In this research paper, the interaction of native calf thymus DNA with the drug Aripiprazole (ARI) has been investigated by absorption, emission, and molecular docking studies. The remarkable hyperchromism as obtained from UV–Visible spectra indicated a high binding affinity of ARI for DNA, which is in accordance with groove binding mode. The results of competitive fluorescence experiments with methylene blue dye along with molecular docking simulation proved that ARI is located in the minor groove of DNA.

Keywords: Aripiprazole drug, In vitro DNA interaction, Spectroscopic methods, Molecular docking, Groove binder

Introduction

Numerous biological experiments have demonstrated that DNA is the primary intracellular target of anticancer drugs; interaction between small molecules and DNA can cause damage in cancer cells, blocking the division and resulting in cell death [1]. Understanding the binding of small molecules to DNA is potentially useful in developing principles to guide the synthesis of new improved drugs which can recognize a specific site or conformation of DNA

[2-4]. Small molecules can react with DNA via covalent or non-covalent interactions. There are several sites in the DNA molecule where such binding can occur: (i) between two base pairs (full intercalation), (ii) in the minor groove, (iii) in the major groove, (iv) on the outside of the helix and (v) electrostatics binding [5, 6]. The development of cancer drugs is slow and costly. One approach to accelerate the availability of new drugs is to reposition drugs approved for other indications as anticancer agents. Understanding how drugs interact with DNA and how induce DNA damage can be helpful to design more efficient and specifically targeted therapeutics, with lower side effects. Hence, with knowledge of the importance of the subject, we planned to carry out the binding studies of Aripiprazole drug (Fig. 1) with calf thymus DNA to know interactive details of its binding with DNA using the combination of spectroscopic and computational methods.

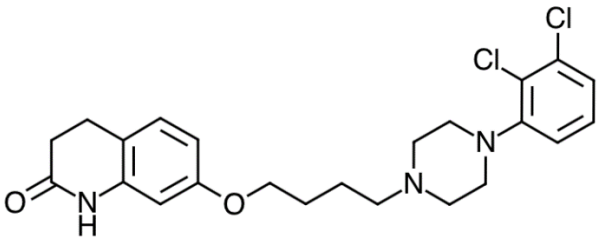

Fig. 1. The molecular structure of Aripiprazole drug

Experimental

Reagents

All materials and solvents were purchased commercially and used without further purification. A stock solution (1×10^{-3} M) of ARI was prepared by dissolving its crystals in 1:1 DMSO/Ethanol. DNA stock solution was prepared by dissolving an appropriate amount of DNA in 50 mM of the Tris–HCl buffer at pH 7.4 and stored at 4 °C in the dark for about a week. The concentration of DNA in stock solution was determined by UV absorption at 260 nm using a molar absorption coefficient 6600 L mol^{-1} cm^{-1}. Purity of the DNA was checked

by monitoring the ratio of the absorbance at 260 nm to that at 280 nm. The solution gave a ratio of >1.8 at A_{260}/A_{280}, indicating that DNA was sufficiently free from protein [7]. All solutions were prepared using double distilled water.

Apparatus

UV–Vis absorption spectra were measured on a Nordantec T80 UV–Vis spectrophotometer using a 1.0 cm cell. Fluorescence measurements were performed with a JASCO spectrofluorimeter Model FP-6200 equipped with a thermostated bath, using a 1.0 cm quartz cell. pH measurements were carried out with a digital pH-meter with a combined glass–calomel electrode.

UV–Vis absorption measurements

The absorbance measurements were performed by keeping the DNA concentration constant (4.3×10^{-5} M) while varying the ARI concentration. The spectra were recorded in the range of 200–350 nm. Equal small aliquots of ARI stock solution was added to both DNA and reference solutions to eliminate the effect of ARI absorbance.

Competitive fluorescence measurement

The competitive interaction between methylene blue dye and ARI with DNA was carried out as follows: fixed amounts of MB (5×10^{-6} M) and DNA (1.4×10^{-3} M) were titrated by successive additions of stock solutions of ARI. These samples were excited at 630 nm and emission was observed between 630 and 730 nm.

Molecular docking simulation

The docking calculation has been conducted by the open-source AutoDock Vina (version 1.1.2) with MGL tools 1.5.6. The known crystal structure of DNA (PDB ID: 1BNA) was obtained from the Protein Data Bank. Receptor (DNA) and ligand (ARI) files were provided using AutoDock Tools. The selected DNA model was enclosed in a box with the number of points in x, y and z dimensions of 26, 26 and 42 and center grid box of 17.347, 21.346 and 8.414 with a grid spacing of 1.00 Å. Lamarckian genetic algorithm was employed to perform docking calculations. All other parameters were default settings. Visualization of the docked pose has been carried out by using BIOVIA Discovery Studio Visualizer 2021.

Result and discussion

UV–Vis absorption spectroscopy

The preliminary in vitro evaluation of binding mode of ARI to DNA helix has been performed through spectrophotometric titrations by following the changes in absorbance values and the positions of the absorption band of DNA. In general, hyperchromism and hypochromism are the spectral features of DNA concerning of its double helix structure; hyperchromism means the breakage of the secondary structure of DNA, and hypochromism means that the DNA-binding mode of molecule is electrostatic effect or intercalation which can stabilize the DNA duplex, while the existence of a red-shift is indicative of the stabilization of DNA duplex [8]. By increasing ARI concentration, the maximum absorption of DNA helix at 260 nm represents a hyperchromism with a blue wavelength shift, demonstrating that ARI has strong interaction with DNA (Fig. 2). The resulted hyperchromism might come from a non-intercalation binding mode [8].

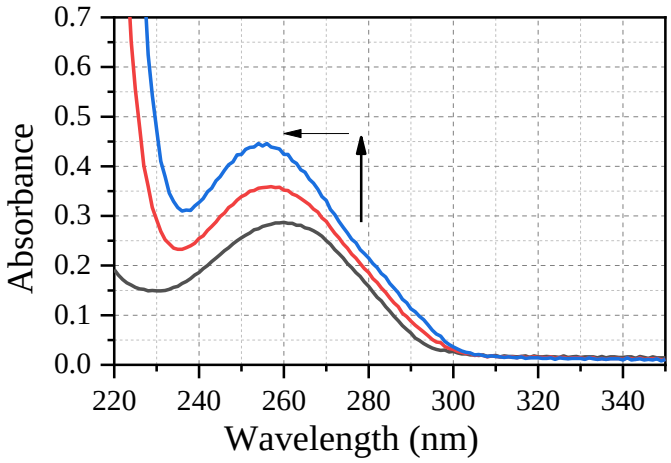

Fig. 2. Absorption spectra of DNA (4.3×10^{-5} M) in the absence and presence of increasing amounts of ARI (2.1 and 4.2×10^{-8} M)

Fluorescence studies (competitive binding studies with methylene blue)

Phenothiazinium dyes, such as methylene blue that can interact with DNA by intercalation have been used in several spectroscopic studies. Upon binding to DNA the fluorescence probe is efficiently quenched by the DNA bases with no apparent shifts in the emission maximum. This emission-quenching phenomenon also reflects the changes in the excited-state electronic structure in consequence of the electronic interactions in the MB–DNA complex [9, 10]. The emission-quenching phenomenon, the hypochromic and red-shift effects in the absorption spectra fit the intercalative mode of binding of MB to DNA. There isn't any clear increase in the fluorescence intensity of the probe molecule upon adding ARI (Fig. 3). These results indicate that MB molecules are not released from the DNA helix after addition of ARI and are indicative of a non-intercalative mode of binding.

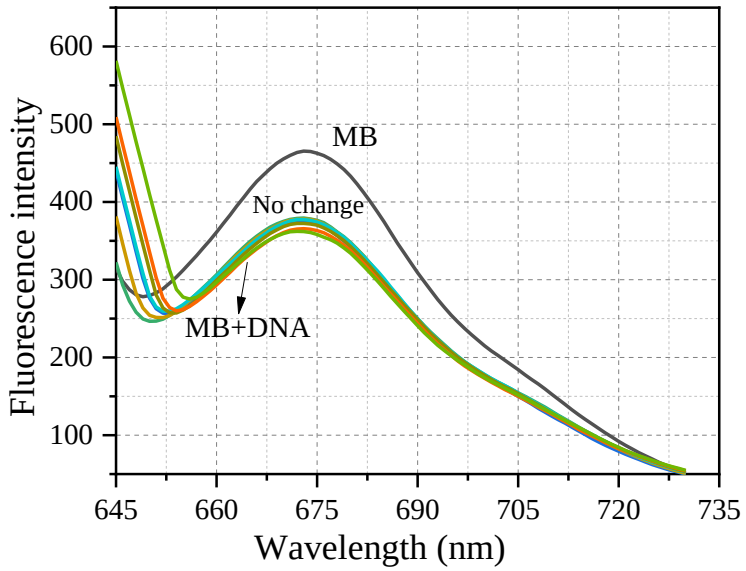

Fig. 3. Fluorescence spectra of the competition between ARI and MB. [MB]: 5×10^{-6} M, [DNA]: 1.4×10^{-3} M and [ARI]: 0 to 6.2×10^{-7} M

Molecular docking analysis

From the docking analysis, the best conformer with the lowest binding energy was picked from the 20 unique conformations among 500 runs (Fig. 4). The selected docking pose showed that ARI can bind to DNA model with high affinity (-8.2 kcal/mol, Table 1). From Fig. 4, it is clear that ARI was located in DNA minor groove. Dock results also show the presence of two conventional ant two carbon hydrogen bonds were formed between ARI with dG4, dG22 and dC21, respectively (Fig. 5).

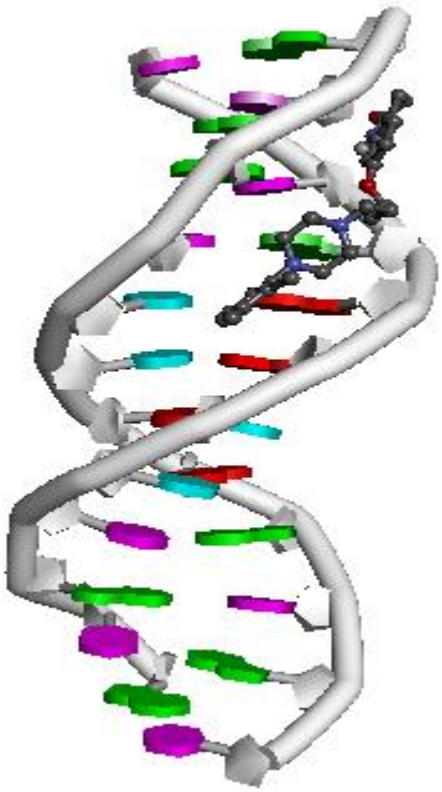

Fig. 4. Molecular modeling of the interaction between ARI and DNA dodecamer

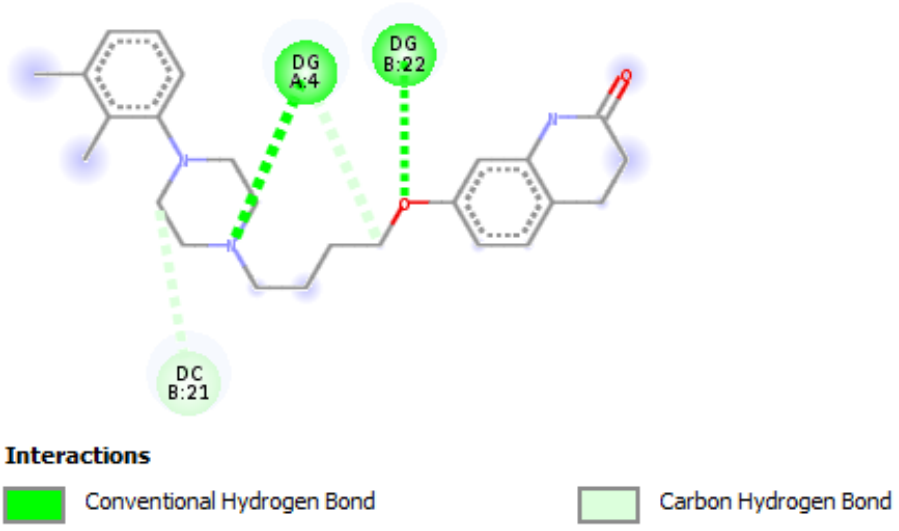

Fig. 5. The involvement of hydrogen bonds in the interaction of ARI with DNA model

Table 1: Docking results of DNA and ARI by using the AutoDock vina program generated different ligand conformers using Lamarkian Genetic Algorithm

Mode	Binding affinity (kcal/mol)	Dist. from rmsd 1.b.	Best mode rmsd u.b.
1	-8.2	0.000	0.000
2	-8.2	1.620	2.264
3	-8.2	20.945	25.014
4	-8.2	1.277	2.052
5	-8.1	4.752	11.734
6	-8.0	4.631	11.789
7	-7.7	22.385	25.781
8	-7.6	5.197	12.351
9	-7.5	4.796	11.637
10	-7.3	4.731	11.401
11	-7.3	24.356	28.165
12	-7.2	23.773	26.767
13	-7.2	22.373	25.241
14	-6.9	3.436	5.617
15	-6.8	24.227	25.999
16	-6.7	24.559	26.535
17	-6.7	2.748	4.679
18	-6.7	1.612	2.261
19	-6.6	2.633	3.460
20	-6.6	2.401	3.246

Conclusion

In this work instrumental and molecular simulation methods were used to finding the interaction mechanism of Aripiprazole drug with DNA. The results suggest that ARI binds to DNA via groove binding mode. The interaction occurrence is supported by the following findings:

1. The absorption spectrum of the DNA shows that as the concentration of ARI increases, a hyperchromism develops in the spectrum.

2. There isn’t any clear increase in the fluorescence intensity of the methylene blue (MB) molecule upon adding ARI.

3. Molecular docking studies proved that ARI fit in the minor groove of DNA.

We believe that the obtained results will provide useful information on the mechanism of drug binding to DNA and thus will be very helpful to the design of new drugs with low toxicity.

References

[1] B.C. Baguley, C.J. Drummond, Y.Y. Chen, G.J. Finlay, *Molecules* 26 (2021) 552.

[2] A.H. Alkaff, M. Saragih, S.N. Imana, M.A.F. Nasution, *Molecules* 26 (2021) 375.

[3] L.H. Hurley, *J. Med. Chem*. 32 (1989) 2027-2033.

[4] Y. Cho-Chung, *Curr. Pharm. Des*. 11 (2005) 2811-2823.

[5] L. Andrezálová, Z. Országhová, *J. Inorg. Biochem.* 225 (2021) 111624.

[6] A. Kellett, Z. Molphy, C. Slator, V. McKee, N.P. Farrell, *Chem. Soc. Rev*. 48 (2019) 971-988.

[7] N. Shahabadi, S. Hashempour, *Nucleosides Nucleotides Nucleic Acids* 38 (2019) 428-447.

[8] N. Shahabadi, S. Hadidi, *Spectrochim Acta A Mol Biomol Spectrosc.* 96 (2012) 278-283.

[9] P. Vardevanyan, A. Antonyan, M. Parsadanyan, M. Shahinyan, L. Hambardzumyan, *J. Appl. Spectrosc*. 80 (2013) 595-599.

[10] C. Tong, Z. Hu, J. Wu, *J. Fluoresc.* 20 (2010) 261-267.