IBUPROFEN BINDS DNA: EVIDENCED THROUGH FLUORESCENCE SPECTROSCOPIC AND MOLECULAR DOCKING

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ABSTRACT

Ibuprofen belongs to a class of drugs called non-steroidal anti-inflammatory drugs (NSAIDs) with various pharmacological effects. A set of spectroscopic (in-vitro) and molecular docking (in-silico) studies were accounted to illustrate the binding mode of ibuprofen with calf thymus DNA (ct-DNA), in order to predict its possibility to be a 'DNA binder' which is one of the features expected in anticancer and antitumor drug designing. Both the UV-Visible and Fluorescence spectroscopic results denote that, there is a drug-DNA complex formation with binding constants of 1.58 x 105 M-1 and 2 x 10-3 µl ng-1 respectively. These values suggest that ibuprofen having intercalative mode of interaction with ct-DNA. As a confirmation of these results, it was further validated through relative specific viscosity measurements of ct-DNA dug complex. It was revealed an increment while mixing DNA with the drug in ascending order. Molecular docking studies further complemented the experimental results.

Keywords: NSAIDs, DNA binding, Fluorescence spectroscopic, Molecular docking

1. INTRODUCTION

Deoxyribonucleic acid (DNA) is an universal genetically encoding material, which plays an important role in manipulating cells, protein synthesis and transcription of genetic information in living cells of an organism. Since the structure of DNA was found, it has been considering the DNA would be a prime target biomolecule to develop therapeutically valued small molecules such as varieties of anticancer or antitumor drugs or antibiotics (Mohammed Amir Husain et al. 2017). The study of interaction of drug with DNA is very sensitive and significant not only in understanding the mechanism of interaction, but also in new drug designing. The mechanism of interactions between drug molecules and DNA is still exactly little known. It is important to introduce more simple methods for investigating the mechanism of interaction. It may eventually lead to design the new DNA-targeted drugs and the screening of these in vitro can be achieved (Hajian et al. 2009). Primarily there are three different ways by which drugs interact with DNA, (i) by control of transcription factors and DNA polymerases, (ii) by forming a RNA and DNA complex to form nucleic acid triple helix structures or RNA hybridizations to exposed DNA single strand forming RNA-DNA hybrids that may interfere with transcriptional activity and finally (iii) by binding of small aromatic ligand molecules to DNA duplex. As explained in the third category the binding of drug like ibuprofen to DNA can be further differentiated using electrostatic interaction, intercalation between base pairs (in the presence of ethidium

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bromide) and minor or major DNA grooves binding interaction (Rauf et al.2005).

Non-steroidal anti-inflammatory drugs (NSAIDs) are used in the treatment of inflammatory and degenerative diseases by interacting with cyclooxygenase and forming prostaglandins (Yanrui Cui et al. 2013). NSAIDs are the kind of small molecules that interact with DNA. They are classified as the derivatives of salicylate, phenyl alkanoic acids, oxicams, anthranilic acids sulfonamides and furanones (Stella Fountoulaki et al. 2011). NSAIDs are among the most widely used medication in the world because of their demonstrated efficacy in reducing pain and inflammation. Even though, the basic mode of action is inhibition of the pro-inflammatory enzyme cyclooxygenase (Ong et al. 2007).

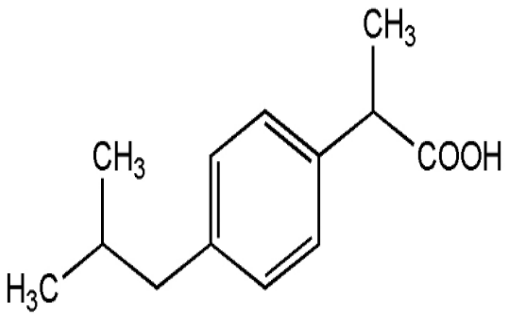

Figure 1: The structure of ibuprofen.

Ibuprofen is a benzene-acetic acid (fig.1), and one of the arylpropionic acid derivatives of NSAIDs with anti-inflammatory, analgesic and antipyretic activity. A common characteristic of propionic acid derivatives is their ability to generate reactive oxygen species (ROS) in the presence of light. The structure itself composed of an aromatic chromophore, also there is a (-COOH) which make the compound active for the hydrogen bonding (Mohammed Amir Husain et al. 2015). Many sophisticated and efficient biophysical techniques have been conducted for the investigation of the interaction mechanism of DNA with small molecules including UV-visible spectroscopy, fluorescence spectroscopy, Circular Dichromism (CD) spectroscopy, Isothermal Titration Calorimetry (ITC), Fourier Transform infrared (FT-IR) spectroscopy, Gel electrophoresis and Dynamics and Viscosity measurement studies. The software aided molecular docking plays an important role in the drug design as well as in the mechanistic study by placing the molecule into the binding site of a target macromolecule in a non-covalent fashion (Rescifina et al. 2014). This study evaluates about the pattern of interaction of Ibuprofen with DNA, *in vitro* by using biophysical techniques and *in silico* by exploiting molecular docking.

2. MATERIALS AND METHODS

Highly polymerized calf thymus DNA (ct-DNA) and Ibuprofen were obtained from Sigma-Aldrich. Reactions were carried out by mixing ct-DNA with a buffer of 5mM Tris-HCl (pH 7.4). The UV absorbance ratio of ct-DNA at 260nm and 280nm was confirmed to be more than 1.8 to ensure the purity. The DNA stock solution was prepared by dilution of ct-DNA into buffer and stored at 4^0C. Ibuprofen (300 ng/µl) stock solution was prepared in 0.1M NaOH solution.

2.1 UV – Visible spectroscopy

The UV-VIS spectrum of ibuprofen was recorded by Genesys 10S UV-VIS spectrophotometer. The absorbance range of wavelength was in between 200-500 nm. The six aliquots (0-125 ng/µl) of ct-DNA stock solutions were prepared and fixed concentration of ibuprofen (300 ng/µl) was maintained in each of the sample. The 2 ml mixture of drug DNA solutions was prepared by mixing 1ml of DNA solution of the particular concentration of DNA with 1ml of drug solution. For each corresponding reaction mixture, exactly same concentration of DNA only was added without ibuprofen to designate as a blank.

2.2 Fluorescence spectroscopy

Fluorescence spectra were obtained using Hitachi F-7000 spectrophotometer, equipped with xenon flash lamp. The excitation wavelength was set to be 288nm and the emission spectra were recorded in the range of 280-500nm for fixed concentration of ibuprofen (300 ng/µl) with increasing concentration of ct-DNA (0-125 ng/µl).

2.3 Viscosity measurements

The relative specific viscosity data of fixed concentration of ct-DNA (50 ng/µl) and increasing concentration of ibuprofen (R= 0.06-0.66) were obtained using thermo stated (25±1^{o}C) Ostwald viscometer. The flow times of ct-DNA recorded for increasing concentration of ibuprofen to give certain R (R= [HA]/[DNA]) whereas the DNA concentration was kept constant. The experiment was carried out in three replicates and the mean data were plotted as $(\eta/\eta_0)^{1/3}$ versus R, where η and η_0 are the specific viscosity of DNA in the presence and absence of the drugs, respectively.

2.4 Molecular docking studies

The above experiment was carried out using “Vina” based “Autodock 1.5.6” software. The molecular structure of Ibuprofen was drawn using Gaussian and Gauss view and optimized into PDB file using Avogadro. The ct-DNA dodecamer receptor was accepted from Protein Data Bank (1d66.pdb), with 12 base pair sequence d 5’(CGCGAATTCGCG)3’. Both drug and DNA, PDB files were converted into PDBQT files to be accepted by Autodock 1.5.6 software. Docking was performed in between flexible drug molecule and DNA

molecule. The final DNA complex was visualized using RasMol viewer software.

3. RESULTS AND DISCUSSIONS

3.1 UV – Visible spectroscopy

UV-Visible absorption measurement is an effective method in detecting the binding strength and the mode of drug binding with ct-DNA. When a drug interacts with ct-DNA changes in the absorbance and in the position of the band should occur. As the molecules binds with ct-DNA, the shifts in absorption spectra such as hyperchromism and hypochromism are the special features of DNA. With reference of its double helix structure; hyperchromism reveals the breakage of the secondary structure of DNA and hypochromism and bathochromism (red shift) means that the DNA binding mode of molecule is intercalation which can stabilize the DNA duplex. (Sirajuddin et al. 2012).

Therefore, the shifting of the position of the maximum of this band was examined from when the ligand is free in solution to when it is bound to DNA. It is considered that the magnitude of the shifting could be interpreted as an indication of the strength of the interaction between the DNA structure and ligand molecules using Wolfe-Shimmer equation,

$$[DNA] / (\varepsilon_a - \varepsilon_f) = [DNA]/ (\varepsilon_b - \varepsilon_f) + 1/K_b (\varepsilon_b - \varepsilon_f)$$

Where, [DNA] is concentration of DNA, ε_b, ε_f are apparent absorption coefficients for bounded and free. DNA. intrinsic binding constant (K_b) can be measured by monitoring the changes of absorbance in the absorption band with increasing concentration of DNA as mentioned above (Manish Pardhi et al, 2016). Whereas as shown in figure 2, Ibuprofen resulting in the tendency of hyperchromism. It might be resulted due to the intercalative binding mode.

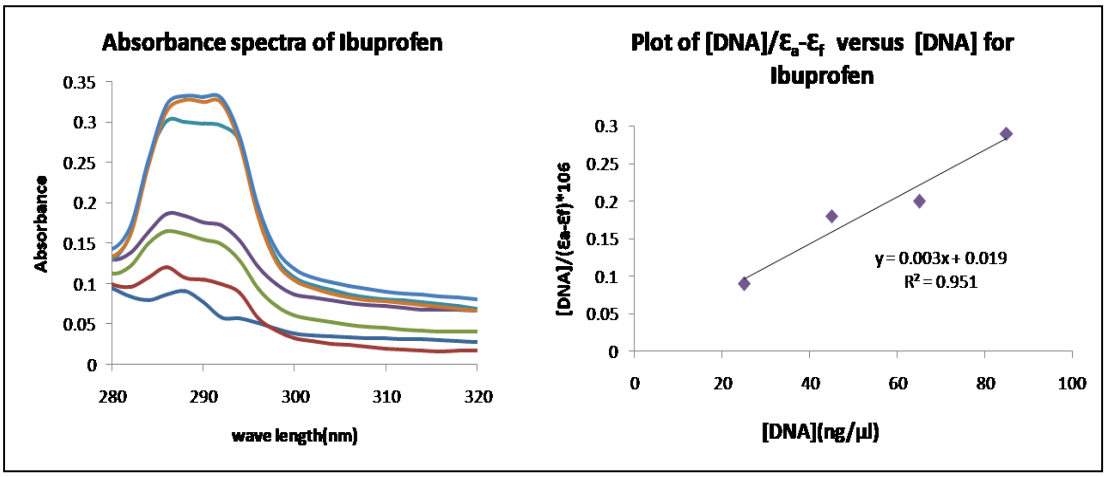

Figure 2. Trends in absorption Changes with increasing concentration of DNA

The hyperchromic effect is the outstanding increase in absorbance of DNA, when the interaction force holding the double helical structure is disrupted and DNA duplex unwinded to some extent. Consequently, the interaction between nitrogen bases will be reduced and UV absorption of DNA solution increases (Arjmand and Jamsheera, 2011; Sirajuddin et al. 2013). The observed binding constant was smaller than the classical intercalators where, binding constant is reported to be in the maximum order up to 10^7 M^{-1}. The K_b for Ibuprofen was found to be $1.58*10^5 M^{-1}$, as discussed the order of $10^5 M^{-1}$ indicates that there is an intercalative binding mode of interaction (Ghosh et al. 2011).

3.2 Fluorescence spectroscopy

Fluorescence spectroscopy is most commonly used method to study interactions between small ligand molecules and DNA. Fluorescence is found in compounds which have aromatic functional groups with low energy transitions. Fluorescence quenching experimental analysis gives additional information concerning the localization of the drugs and their mode of interaction with DNA. In the case of intercalations, the molecules are inserted into the base stack of the helix [Sirajuddin et al. 2012]. The rotation of the free molecules favours the radiation less deactivation of the excited states, but if the drugs are bound to DNA the deactivation through fluorescence emission is favoured, and a significant increase in the fluorescence emission is normally observed. In case of groove binding agents, electrostatic, hydrogen bonding or hydrophobic interactions are involved and the molecules are close to the sugar-phosphate backbone, being possible to observe decrease in fluorescence intensity in the presence of DNA (Lakowicz, 2006). A quantitative estimation of fluorescence studies in terms of the Stern-Volmer constant calculation can be obtain from Stern Volmer equation,

$$F_0/F = Ksv\,[Q] + 1$$

Where, Ksv - Stern Volmer Constant , Q- Concentration of DNA, F_0- Fluorescence intensity in absence of DNA and F -Fluorescence intensity in presence of DNA (Li et al. 2005). As shown on the figure 3, the fluorescence emission intensity increases upon increasing the concentration of ct-DNA. The effective interaction of small drug molecules with DNA are usually resulting in a significant enhancement of the fluorescence emission intensity as a consequence of various factors. In case of intercalations, the rotation of free molecules favour radiation and less deactivation of the excited state due to the binding of particular drug to the DNA. The deactivation through fluorescence emission is favoured. Ultimately, a significant enhancement in emission intensity is observed (Liet al. 1997). Additionally, the Stern-Volmer quenching constant (K_{SV}) obtained from the plot of figure (3) was determined to be $2*10^{-3}$ µl ng^{-1}. The value shows there is a significant binding affinity, reveals that ibuprofen binds to ct-DNA.

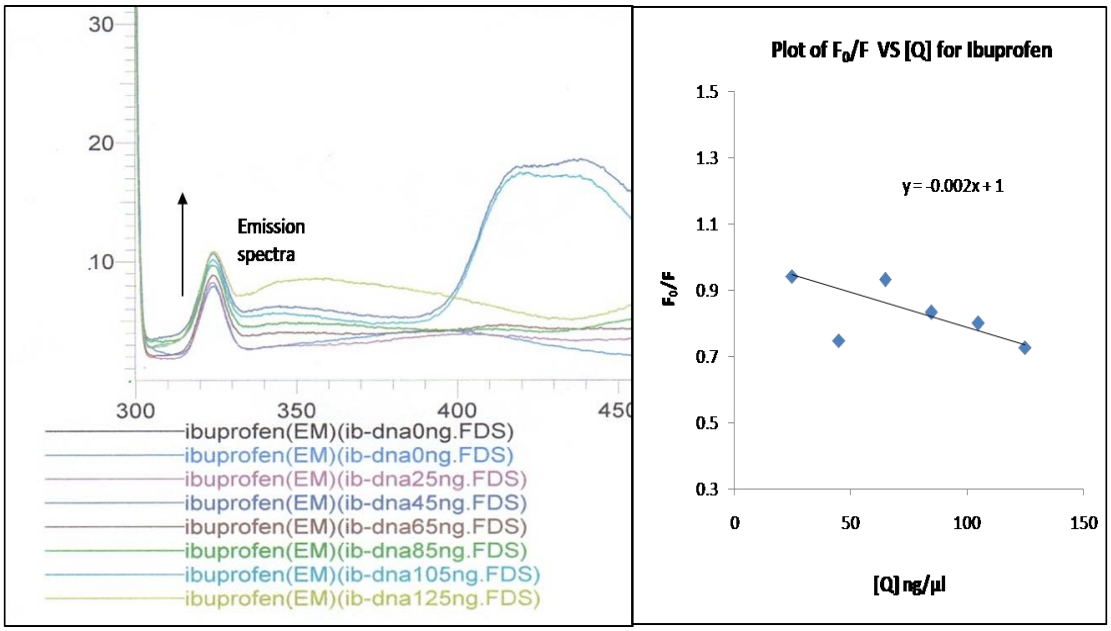

Figure 3. Fluorescence emission spectra of ibuprofen with increasing concentration of DNA

3.3 Viscosity measurements

Viscosity measurements were carried out for further confirmation of the binding mode of Ibuprofen with ct-DNA. It is also considered as the most consistent method to identify the binding mode of small drug molecules with DNA when ligand molecules are bound to DNA and make the DNA to be sensitive to alter its length (Ling et al. 2008). The classical intercalators often results in increased viscosity of DNA solution due to lengthening of DNA duplex as base pairs are unwinded to accommodate such ligands. However, in case of groove binders there is no any noticeable increase in the viscosity of DNA solution. Relatively small changes in viscosity can be considered as for groove binders (Satyanarayana et al. 1992). The Figure (4) shows that the viscosity of ct-DNA increased upon increasing the concentration of Ibuprofen while ct-DNA concentration kept in constant. It indicates that ibuprofen binds to DNA in intercalative mode.

3.4 Molecular docking studies

Molecular docking technique is an attractive scaffold to understand the drug-DNA interactions for the rational drug design and discovery. As well as in the mechanistic study, by placing a small molecule into the binding site of the target specific region of the DNA mainly in a non-covalent fashion, this can substantiate the spectroscopic results.

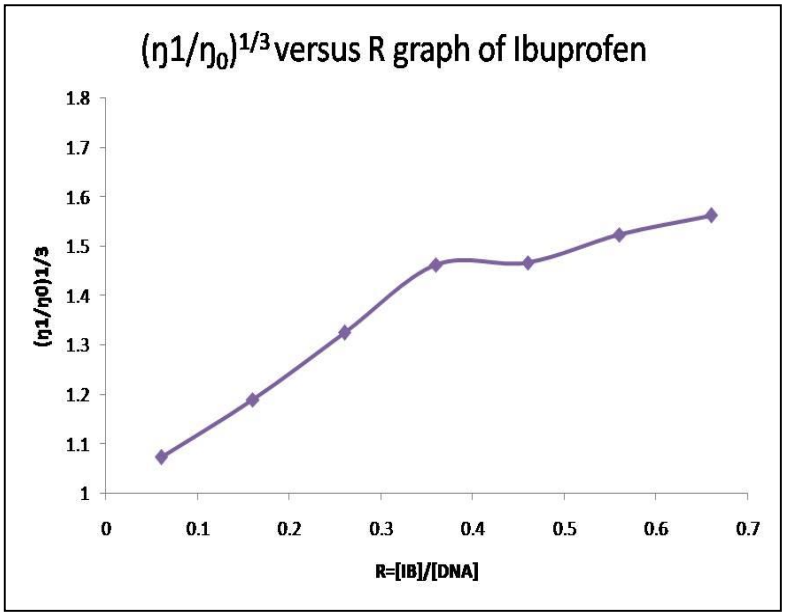

Figure 4. Increase in the viscosity of DNA solution in the presence of ibuprofen

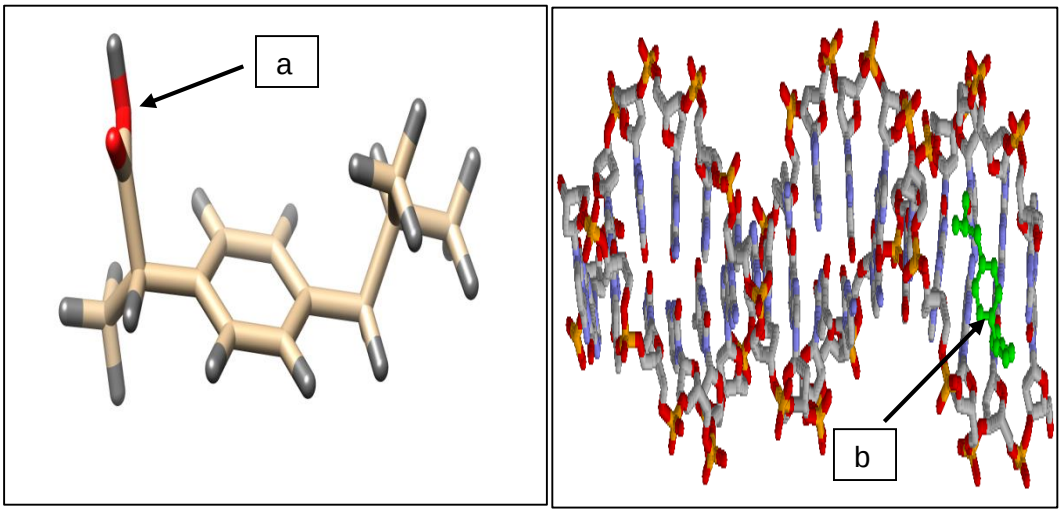

Figure 5. Ibuprofen interacts with DNA via intercalative binding mode (a and b)

In contrast, the drug has been made flexible to attain different conformations in order to obtain the best drug-DNA fit, and the energetically preferable best docked pose is analysed (Hajian and Tavakol, 2012). As shown in the figure 5, that reveals ibuprofen interacts with DNA via intercalative binding mode while having binding energy of -5.6 kc/mol. It may be due to its small molecular size and higher polarity. It showed that molecular docking result was conformity and had good correlation with other results of studies as mentioned in the above sections.

4. CONCLUSIONS

The performed research study concludes that ibuprofen can bind to the DNA and there is high possibility to retard the function of DNA. The strength of

ibuprofen-DNA binding is approximated by the determination of intrinsic binding constant (k_b) and Stern-Volmer constant (k_{sv}) by analyzing absorbance spectroscopy and fluorescence spectra. The k_b value of ibuprofen was found to be $1.58*10^5 M^{-1}$ and also as a conclusion from emission spectra the k_{sv} value was found to be $2*10^{-3}$ µl ng^{-1} respectively. These values suggest that ibuprofen might have an intercalative mode of interaction with ct-DNA. In additionally, the remarkable increase in the relative specific viscosity observed, which confirms that there have been a binding interaction between ct-DNA and ibuprofen as well.

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