Journal of Science-FAS-SEUSL (2020) 01(01) 01-11
ISSN:2738-2184

DNA binds to non-steroidal anti-inflammatory drugs (NSAIDs): Evidences through *In vitro* and *In silico* studies

M. I. S. Safeena

Department of Biology, Faculty of Applied Sciences, South Eastern University Sri Lanka, Sammanthurai, Sri Lanka

Abstract
The mechanism of interaction of drugs with potential target and off-target biomolecules such as with DNA enables the development of a rational drug designing system especially for therapeutic anticancer or anti-tumor drugs. Diclofenac, indomethacin and mefenamic acid are non-steroidal anti-inflammatory drugs (NSAIDs) which have been tested in this study. These NSAIDs have diverse biological and pharmacological activities. *In vitro* methods such as various biophysical techniques and *In silico* studies using molecular docking were applied to investigate the binding abilities and mode of binding of these drugs with calf thymus DNA (ct-DNA).
The UV-visible absorbance spectra and fluorescence emission profile of above NSAIDs upon addition of ct-DNA indicates the formation of a drug–DNA complex. The Wolf shimmer binding constant (Kb) of diclofenac, indomethacin and mefenamic acid from UV – visible experiment was found to be $2.05x10^4$ M^{-1}, $4.29x10^4M^{-1}$ and $2.73x10^4$ M^{-1} respectively. The results of fluorescence experiments revealed the binding constants as $8x10^{-3}$ µl ng^{-1}, $3x10^{-3}$ µl ng^{-1} and $6x10^{-3}$ µl ng^{-1} for diclofenac, indomethacin and mefenamic acid respectively and these values are consistent with those of well-known groove binders. The binding constants of all tested drugs showed the groove binding mode of interaction with ct-DNA. In addition, the testing of drug-DNA complex for relative specific viscosity and the resulted output images of the molecular docking experiments further confirmed the effective binding interactions between ct-DNA and diclofenac, indomethacin and mefenamic acid.

Keywords: Ct - DNA, NSAIDs, UV – visible absorbance spectra, Fluorescence spectroscopy, Viscosity, Molecular docking.

1. Introduction

The major activities in living cells of any organism such as gene expression, cell multiplication, transcription processes to synthesize RNAs, protein synthesis are controlled by DNA which is one of the main genetic materials DNA. The elucidated structure and function of the DNA enables the intervention of using it as a primary source to further therapeutic studies as a basis of interaction molecule with different classes of anticancer drugs to antibiotics [1]. These investigations were much involvement with the rising attention in the binding studies of small molecules with DNA and understanding the drug–DNA interactions [2-4]. The 'intercalation' between the DNA and drug complex is formed by weak bonds like π-stacking interactions caused among the nitrogen base pairs, hydrogen bonds and vander-waals forces without distorting the DNA helix [5,6]. Electrostatic interaction is also a type of non-covalent interaction which takes place out of the groove during drug–DNA binding [7]. Interestingly, the number of known drugs targeting DNA is still very limited compared to the drugs targeting proteins and a detailed study is needed to explore this field [8]. Understanding the nature of interaction of these drugs with off target biomolecules like DNA and protein can characterize

Corresponding Author: safeenim@seu.ac.lk

the potential of these drugs for other targets as well as to minimize the side effects of these drugs [9].

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medication in the world because of their demonstrated efficacy in reducing pain and inflammation. NSAIDs are used to cure inflammatory, analgesics, tumors [10], rheumatic diseases [11] and pyretic by interacting with cyclooxygenase (pro-inflammatory enzyme) and forming prostaglandins. The study of interaction of NSAIDs with DNA is very sensitive and significant not only in understanding the mechanism of interaction, but also in new drug synthesis [12].

Diclofenac is a class of phenylacetic acid derivative; composed of two aromatic chromophores (Fig.1). It has been approved to be the first NSAIDs due to more active than several other carboxylic acid containing derivatives. Diclofenac binds and chelates both isoforms of cyclooxygenase, thereby blocking the conversion of arachidonic acid to pro-inflammatory prostaglandins [13].

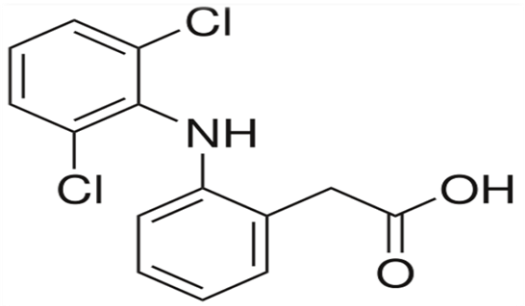


Fig. 1. The structure of Diclofenac

Indomethicin is an odorless pale yellow to yellow tan in color, complexes ring crystalline and lipid soluble substance (Fig.2). It is a kind of NSAIDs belongs to salicylate class, possess indole as a central chromophore ring system, which contribute to the antipyretic and analgesic properties of indomethacin capsules. It has a main functional group of (–COOH) which strongly contribute to the hydrogen bonding in between nitrogen bases. Commonly used in the treatment of active stages of moderate to severe rheumatoid arthritis by inhibiting the activity of both cyclooxygenases [14].

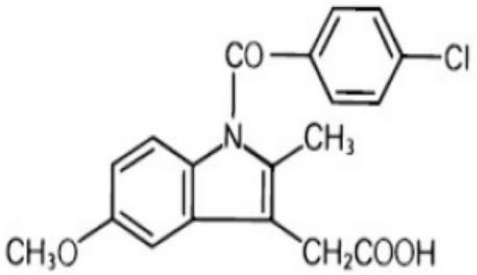


Fig. 2. The structure of indomethacin

An Anthranilic acid derivative of fenamate group of NSAIDs is named as Mefenamic acid (Fig.3). It is considered as a powerful painkiller for many physiological disorders such as menstrual pain, migraines associated with menstruation etc. [15]. Mefenamic acid also inhibits both isoforms of COX and prevents formation of prostaglandins. The structure itself inherently

possess a (-NH) group and a (-COOH) group, obviously having two aromatic benzene rings as the central chromophore [16].

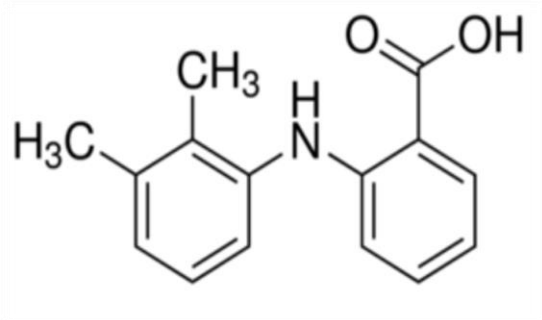


Fig. 3. The structure of Mefenamic acid

The mechanism of interactions between drug molecules and DNA is still little known. It is appropriate to present more simple methods for investigating the mechanism of interaction in order to make some convenient ways to designing drugs. This study reports the molecular aspects and energetics of indomethacin complexation to DNA. The interaction study of NSAIDs and DNA is much needed to reveal how thiscompounds may be further modified to enhance its biological properties. Therefore, this study includes a set of biophysical and molecular docking experiments. The experiments were conducted to investigate the interaction mechanism of DNA with small drug molecules of Diclofenac, Indomethacin and Mefenamic acid using UV-visible spectroscopy, Fluorescence spectroscopy 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 [15-17].

2. Materials and methods

2.1. Reagents and stock solutions preparations

Highly polymerized and purified calf thymus DNA (ct-DNA), Diclofenac, Indomethacin and Mefenamic acid purchased from Sigma-Aldrich. The solvents and chemicals were of reagent grade. The buffer of 10mM Tris-HCl (pH 7.2) was used as a reagent to carry out the reaction. The stock solutions of drugs (300 ng/µl) were prepared in 5% DMSO. The DNA stock solution was prepared by dilution of ct-DNA into buffer and stored at 4^0C. ct-DNA was dissolved in 10 mM Tris–HCl buffer (pH 7.2) at room temperature with occasional stirring to ensure the formation of a homogeneous solution. The purity of the ct-DNA was checked using UV-Visible spectrophotometer by measuring a certain concentration of the DNA at 260 nm and 280 nm. It was decided that the ct-DNA was not needed further purification since the absorbance ratio between 260/ 280 was nearly 1.8.

2.2. UV - visible spectroscopy

The UV-VIS spectrum was recorded using Genesys 10S UV-VIS spectrophotometer using a 1 cm × 1cm quartz cuvette. The absorbance range of wavelength was in between 250-350 nm. The absorbance was by maintaining a fixed concentration of drugs while titrating each of them separately using six aliquots (0 - 85 µM) of ct-DNA. The final volume of the reaction mixture was made to 3 ml by adding 10 mM Tris–HCl buffer (pH 7.2). The same concentrations of DNA solutions without indomethacin were used as the blank to observe the UV-spectra specific to the drug–DNA complex.

2.3. Fluorescence Spectroscopy

The fluorescence emission spectra were obtained using Hitachi F-7000 spectrofluorophotometer (Japan) equipped with a xenon flash lamp using 1.0 cm quartz cells. The excitation wavelength was set to be 288 nm and the emission spectra were recorded in the range of 280-500 nm for fixed concentration of drugs (50 µM) with increasing concentration of ct-DNA (0-125 ng).

2.4. Viscosity Measurements

The relative specific viscosity data of fixed concentration of ct-DNA (50 ng/µl) and increasing concentration of drugs were obtained using thermally maintained (25±1^{o}C) Ostwald viscometer. The flow times of ct-DNA recorded for increasing concentration of both NSAIDs to give certain R (R= [HA]/ [DNA]) whereas the DNA concentration was kept constant. The 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.

2.5. Molecular docking studies

The docking studies were carried out using "Vina" based "Autodock 1.5.6" software. It is an interactive molecular graphics program used for calculating and displaying feasible docking modes of DNA [18]. The molecular structures of Diclofenac, Indomethacin and Mefenamic acid were drawn using Gaussian software and confirmed the drawn structure by rechecking them with the molecular structure files of those drugs from http://pubchem.ncbi.nlm.nih.gov/. Gauss views were optimized into PDB file using Avogadro. The ct-DNA dodecamer receptor was accepted from Protein Data Bank (1d66.pdb, http://www.rcsb.org./pdb), with 12 base pair sequence of B - DNA d(CGCGAATTCGCG)$_2$. 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 complexes of all three drugs were visualized using RasMol viewer software.

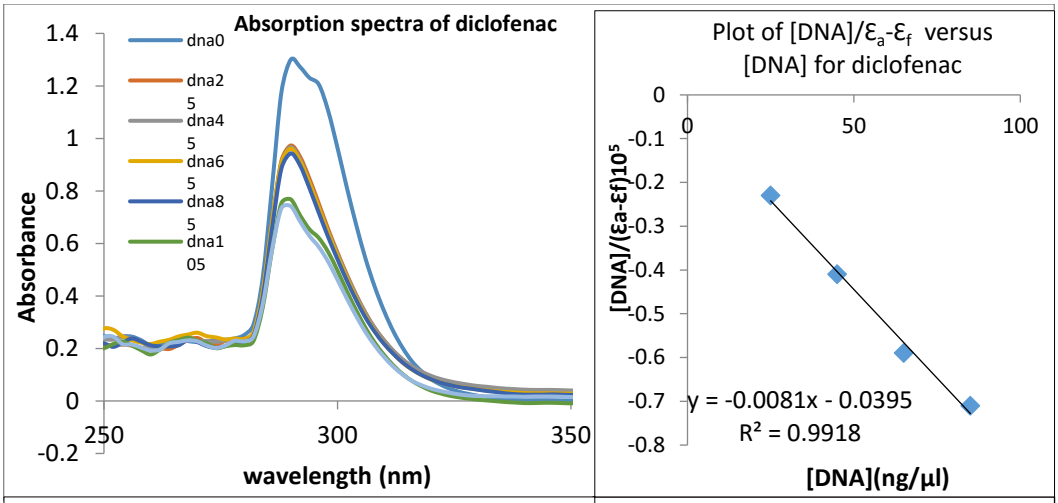
3. Results and Discussion

3.1. UV visible absorption spectroscopy

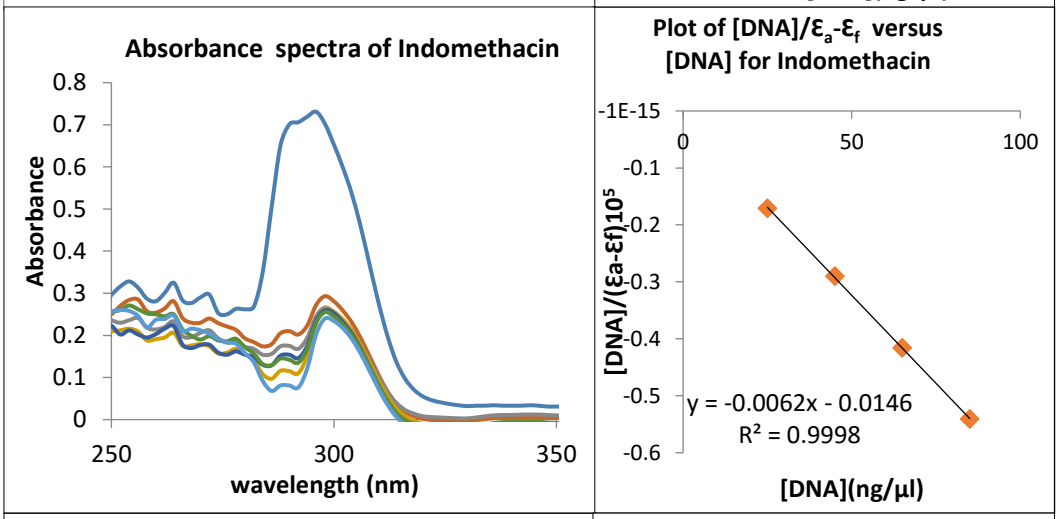
It is the most extensively used and effective method to investigate the structural changes of bio macromolecules in the presence of small molecules. Generally, when the DNA is bound or interact with a small molecule results a complex which makes changes in the magnitude of absorbance and in the position of the absorption peak [19]. The transferal position of the peak and the magnitude of change of absorbance are associated with the strength of the interaction [20, 21].

Hyperchromism ((i.e., increase in band intensity) and hypochromism (i.e., decrease in band intensity) are the two main effects that can be observed in addition of increasing concentration of DNA and measuring the effect at particular wavelength. As it is shown in figures (4a, 4b and 4c) the drugs diclofenac, Indomethacin and mefenamic acid resulting in the tendency of hypochromism. The extent of the shift in the graph (Fig. 4a, 4b and 4c) indicated the forte of the interaction of the DNA with drug or the ligand molecules. This could be derived using the Wolfe-Shimmer equation,

4a.



4b.



4c.

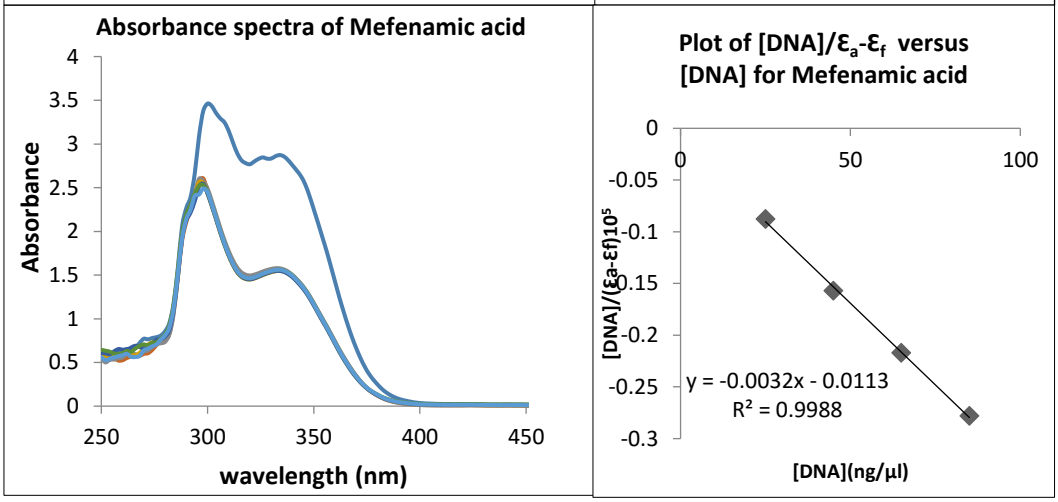


Fig. 4a, 4b and 4c (right) show the interaction of diclofenac, indomethacin and mefenamic acid respectively with Ct-DNA under UV-visible spectroscopy.
Fig. 4a, 4b and 4c (left) show the double reciprocal plot of 1/(Ɛa − Ɛf) versus 1/CDNA. It was found to be linear at 298 K and the value of the constant K was found to be 2.05×10^4 $M^{-1,}$ $4.29 \times 10^4 M^{-1}$ and 2.73×10^4 M^{-1} respectively.

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

Where, [DNA] is concentration of DNA, ε_b, ε_f are apparent absorption coefficients for bounded and free DNA. Intrinsic binding Kb can be obtained as the ratio of slope to intercept from a plot of [DNA] / (ε_a- ε_f) versus [DNA], a slope 1/ (ε_b- ε_f) and an intercept 1/ K_b (ε_b- ε_f) the 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 [22]

The Wolf shimmer binding constant (Kb) of diclofenac, indomethacin and mefenamic acid from UV – visible experiment was found to be $2.05x10^4$ M^{-1}, $4.29x10^4$ M^{-1} and $2.73x10^4$ M^{-1} respectively. This is because when drug molecule binds to DNA, the orbital of the binding ligand could couple with orbital of base pairs in the DNA. The coupling orbital will be partially filled by electrons, thus lead to decrease in the transition probabilities and leads to hypochromism [23]. Hence, it can be concluded that the binding mode of diclofenac, indomethacin and mefenamic acid with DNA might be a non-intercalation binding mode and probably would be a groove binding.

3.2. Fluorescence spectroscopy

The emission intensity of all three drugs increases while DNA concentration increases. Obviously, it has been concluded that diclofenac, indomethacin and mefenamic acid bind to ct-DNA. The effective interaction of small drug molecules with DNA usually results a significant enhancement of the fluorescence emission intensity as a consequence of various factors. In case of intercalators, the rotation of free molecules favour radiation less deactivation of the excited state due to the binding of particular drug to the DNA. Hence, the deactivation through fluorescence emission is favoured. Ultimately, a significant enhancement in emission intensity is observed. In case of groove binders, it is being possible to observe a decrease in the emission intensity [24].

The strength of fluorescence quenching is described by the Stern-Volmer equation,

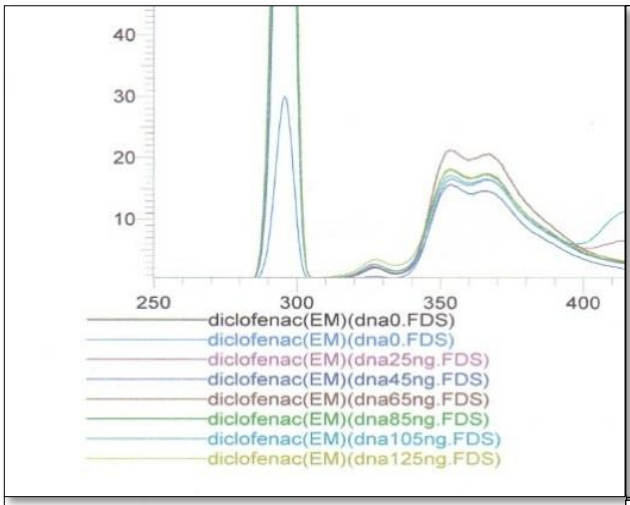
$$Fo / F = Ksv [Q] + 1$$

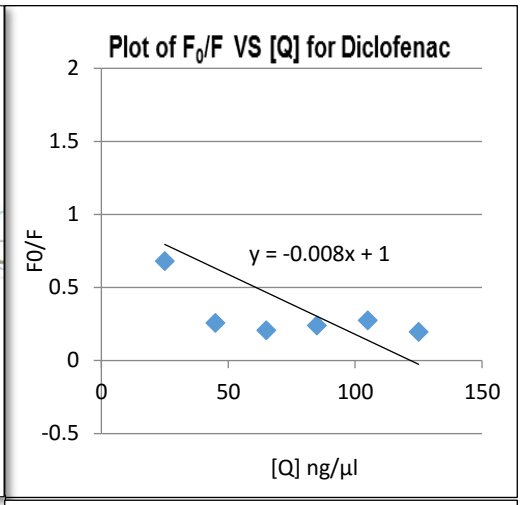
Additionally, the Stern-Volmer quenching constants (K_{SV}) obtained from the plots of figures (5a, 5b and 5c) were determined to be $8*10^{-3}$ µl ng^{-1}, $3*10^{-3}$ µl ng^{-1} and $6*10^{-3}$ µl ng^{-1} respectively. These values show there is a significant binding affinity. The higher affinity of the diclofenac was due to the two substitute molecules of chlorine in the aromatic ring which encourages the inter molecule hydrogen bonding with DNA backbone. UV-visible absorbance and steady state fluorescence experiments revealed a binding constant on the order of 10^3 L mol^{-1}, which is consistent with those of well-known groove binders.

3.3. Viscosity measurements

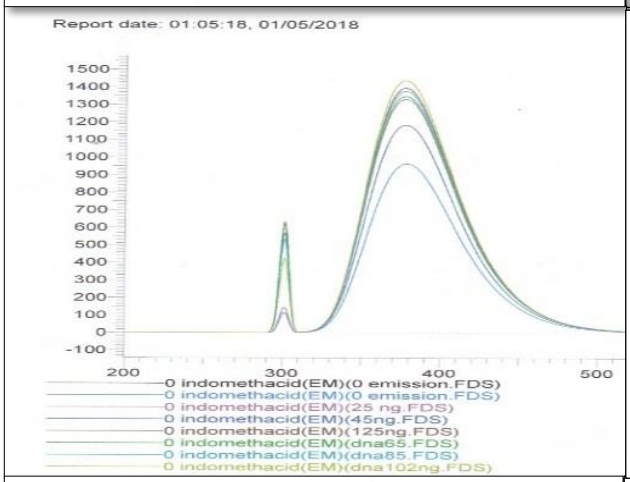
The classical intercalators often result in increased viscosity of DNA solution due to lengthening of DNA duplex as base pairs are unwounded to accommodate such ligands. Though, in case of groove binders there is no any noticeable increase in the viscosity of DNA solution. Relatively small changes in viscosity can be considered as for groove binders [25]. Since the plots of figures 6a, 6b and 6c show that the viscosity of ct-DNA increased upon increasing the concentration of diclofenac, indomethacin and mefenamic acid.

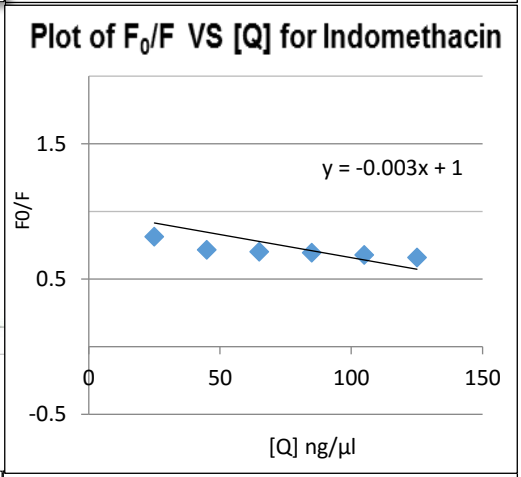
5a



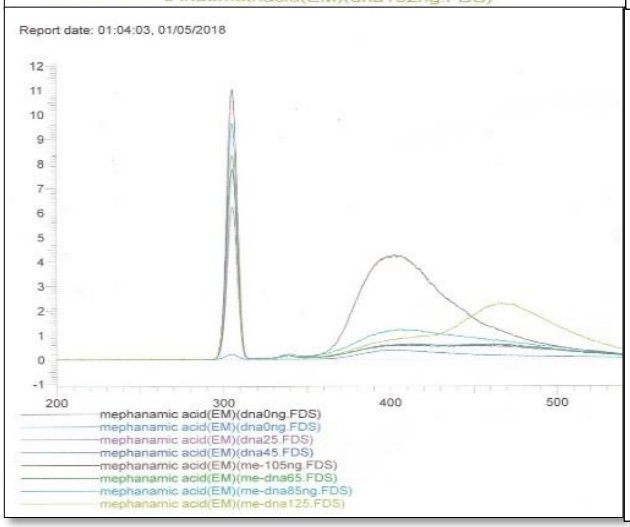


5b





5c



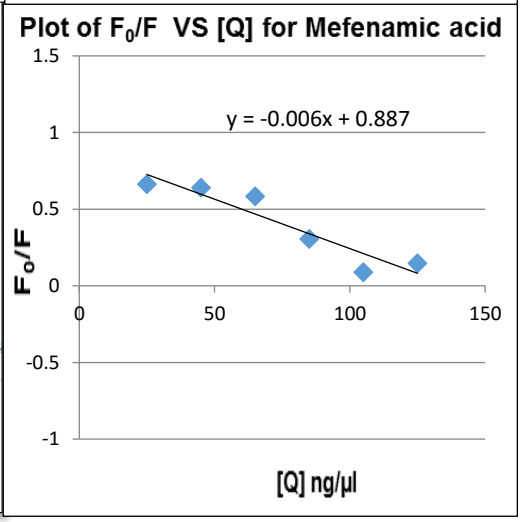
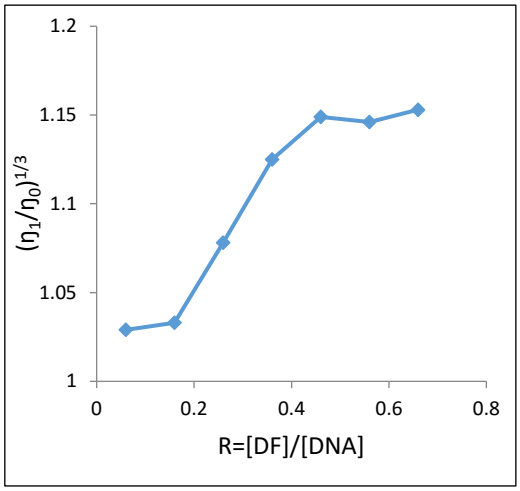
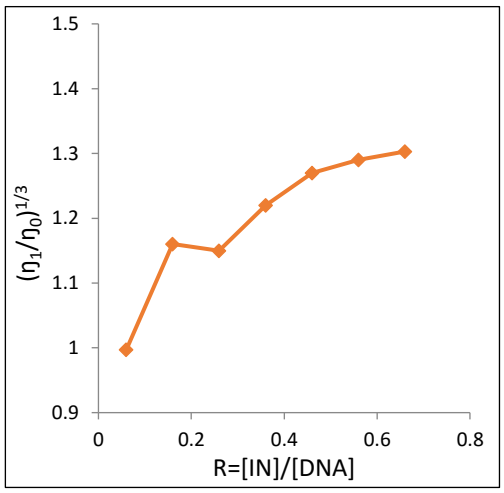


Fig. 5a, 5b and 5c show the emission spectra and Stern- Volmer Binding constant of diclofenac, indomethacin and mefenamic acid.

6a



6b



6c

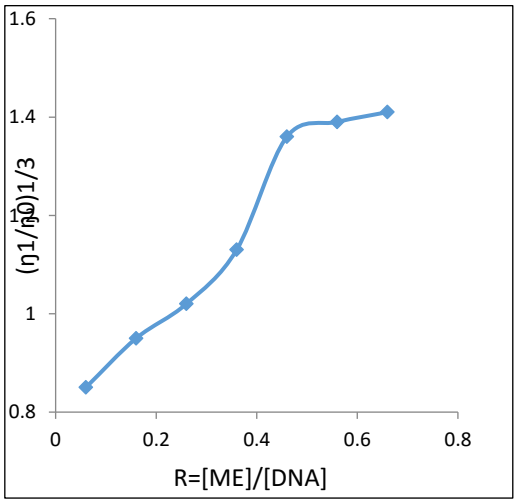
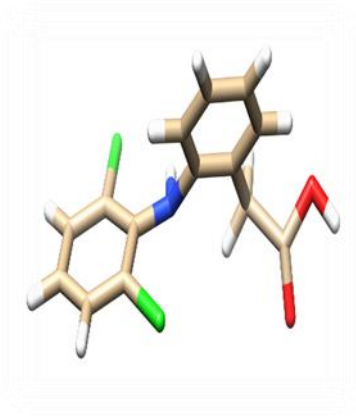


Fig. 6a, 6b and 6c show the Relative specific viscosity $(\eta 1/\eta_0)^{1/3}$ versus R graphs of diclofenac, indomethacin and mefenamic acid respectively.

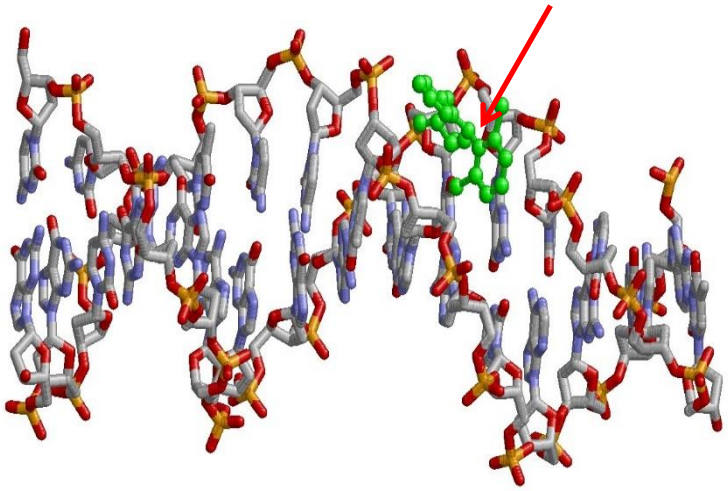
3.4. Molecular docking studies

As shown in the figures (7a, 7b and 7c), diclofenac, indomethacin and mefenamic acid interact with DNA via groove binding mode. The resulting relative binding energy of respective docked complexes (diclofenac, indomethacin and mefenamic acid) was found to be -6.0 kcal M^{-1}, -6.5kcal M^{-1} and -5.8 kcal M^{-1} respectively. Indomethacin reveals that it binds on major groove of DNA whereas diclofenac and mefenamic acid has resulted minor groove binding mode. It may be due to electrostatic potential and steric effects, because of the narrow pocket area, small molecules interact with minor groove when large molecules tend to be bound at the major groove [18]. It was confirmed that the molecular docking results are in approximate correlation with studied experimental results.

7a

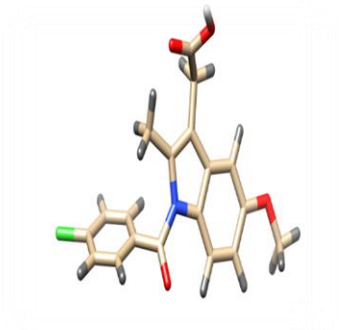


Optimized structure of diclofenac

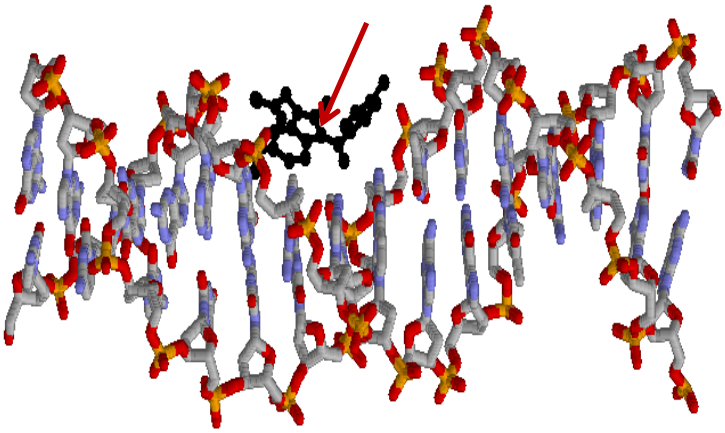


Diclofenac – DNA docked structure: Minor groove binding

7b

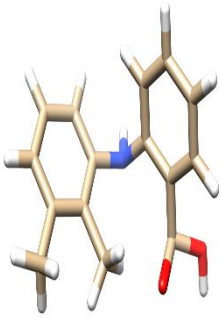


Optimized structure of indomethacin

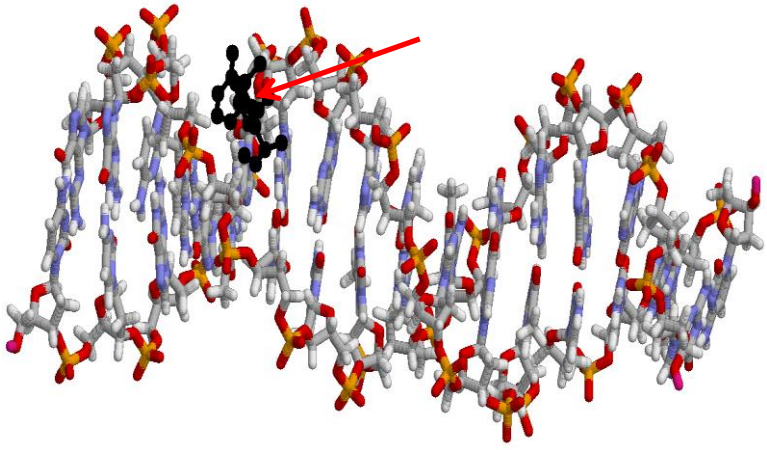


Indomethacin – DNA docked structure: Major groove binding

7c



Optimized structure of mefenamic acid



Mefenamic acid – DNA docked structure: Minor groove binding

Fig. 7a, 7b and 7c - Molecular docking structure of diclofenac, indomethacin and mefenamic acid respectively.

4. Conclusion

The tested NSAIDs; diclofenac, indomethacin and mefenamic acid showed a high affinity of binding with DNA. The k_b values of diclofenac, indomethacin and mefenamic acid were found to be $2.05*10^4M^{-1}$, $4.29*10^4M^{-1}$ and $2.73*10^4M^{-1}$ and also as a conclusion from emission spectra the k_{sv} values were found to be $8*10^{-3}$ µl ng^{-1}, $3*10^{-3}$ µl ng^{-1} and $6*10^{-3}$ µl ng^{-1} respectively. Experimental values suggest that all three NSAIDs show groove binding mode of interaction. In addition, the remarkable increase in the relative specific viscosity was observed, which confirms that there has been a binding interaction between ct-DNA and diclofenac, indomethacin, ibuprofen and mefenamic acid. Altogether, the results confirmed the prospective probability of using diclofenac, indomethacin and mefenamic acid probably used as groove binding DNA probe.

References

1. Kashanian, S., Shariati, Z., Roshanfekr, H. and Ghobadi, S. (2012) DNA binding studies of 3, 5, 6 trichloro-2-pyridinol pesticide metabolites. DNA Cell Biol., 31, 1341–1348.
2. Ding, Y., Zhang, L., Xie, J. and Guo, R. (2010) Binding characteristics and molecular mechanism of interaction between ionic liquid and DNA. J. Phys. Chem. B., 114, 2033–2043.
3. Elder, R. M., Emrick, T. and Jayaraman, A. (2011) Understanding the effect of polylysine architecture on DNA binding using molecular dynamics simulations. Biomacromolecules., 12, 3870–3879.
4. Shi, Y., Guo, C., Sun, Y., Liu, Z., Xu, F., Zhang, Y., Wen,Z. and Li, Z. (2011) Interaction between DNA and microcystin-LR studied by spectra analysis and atomic force microscopy. Biomacromolecules., 12, 797–803.
5. Barone, G., Terenzi, A., Lauria, A., Almerico, A.M., Leal, J.M., Busto, N. and Garcia, B. (2013) DNA binding of nickelIJII), copper (II) and zinc (II) complexes: Structure-affinity relationships. Coord. Chem. Rev., 257, 2848–2862.
6. Zhou, X., Zhang, G. and Pan, J. (2015) Groove binding interaction between daphnetin and calf thymus DNA. Int. J. Biol. Macromol., 74, 185–194.
7. Zhang, Y., Wang, X. M. and Ding, S. (2010) Interaction between tryptophan vanillin schiff base and herring sperm DNA. J. Serb. Chem. Soc., 75, 1191–1201.
8. Berman, H. M., Westbrook, J., Feng, Z., Gilliland,G., Bhat, T.N., Weissig, H., Shindyalov, I. N. and Bourne, P. E. (2000) The protein data bank. Nucleic Acids Res., 28, 235–242.
9. Husain, M. A., Ishqi, H. M., Sarwar, T., Rehman, S. U. and Tabish, M. (2017) Interaction of indomethacin with calf thymus DNA: a multi-spectroscopic, thermodynamic and molecular modeling approach. Med. Chem. Commun., 8, 1283.
10. Tarushi, A., Raptopoulou, C. P., Psycharis, V., Kessissoglou, D. P., Papadopoulos, A. N. and Psomas, G. (2014) Structure and biological perspectives of CuIJII)-indomethacin complexes. J. Inorg. Biochem., 140, 185–198.
11. Vishal Gupta, N., Gowda, D. V., Balamuralidhara, V. and Khan, M. S. (2013) Preparation and comparative bioavailability studies of indomethacin-loaded Cetyl Alcohol microspheres. Journal of Pharmaceutics., ID 109837:9.
12. Muhammad Sirajuddin., Saqib Ali. and Amin Badshah. (2013) Drug-DNA interactions and their study by UV-Visible, fluorescence spectroscopies and cyclic voltammetry. Journal of Photochemistry and Photobiology B: Biology., 124, 1-19.

13. Rauf, S., Gooding, J. J., Akhtar, K., Ghauri, M.A., Rahman, M., Anwer, M.A. and Khalid, A. M. (2005) Electrochemical approach of anticancer drugs-DNA interaction. J. Pharmaceut. Biomed, Anal., 37, 205-217.
14. Ong, C. K. S., Lirk, P., Tan, C. H. and Seymour, R. A. (2007) An evidence-based update on Non-Steroidal Anti-Inflammatory drugs. Clinical medicine and research., 5(1), 19-34.
15 Yanrui cui., Erjun Hao., Guangquan Hui., Wei Guo. and Fengling Cui. (2013) Investigation of interaction on the diclofenac sodium with HSA and ctDNA using molecular modeling and multispctroscopic method. Molecular and Biomolecular spectroscopy., 110, 92-99.
16. Hajian, R. and Tavakol, M. (2012) Interaction of anticancer drug methotrexate with DS-DNA analyzed by spectroscopic and electrochemical methods. J. Chem., 9(1), 471-480.
17. Christine A. Frederic., Loren Dean Williams., Giovanni Ughetto., Gijs A. Van der Marel., Jacques H. Van Boom., Alexander Rich. and Andrew H. J. Wang. (1990) Structural comparisons of anticancer drug-DNA complexes: Adriamycin and Daunomycin. Biochemistry., 29, 2538-2549.
18. Rehman, S. U., Sarwar, T., Ishqi, H. M., Husain, M. A., Hasan, Z. and Tabish, M. (2015) Deciphering the interactions between chlorambucil and calf thymus DNA: a multi-spectroscopic and molecular docking study. Arch. Biochem. Biophys., 15, 7–14.
19. Tabassum, S., Al-Asbahy, W. M., Afzal, M., Arjmand, F. and Bagch, V. (2012) Molecular drug design, synthesis and structure elucidation of a new specific target peptide based metallo drug for cancer chemotherapy as topoisomerase I inhibitor, Dalton Trans., 41, 4955–4964.
20. Jaumot, J. and Gargallo, R. (2012) Experimental methods for studying the interactions between G-quadruplex structures and ligands. Curr. Pharm. Des., 18, 1900–1916.
21. Sun, H., Xiang, J., Liu, Y., Li, L., Li, Q., Xu, G. and Tang, Y. (2011) A stabilizing and denaturing dual-effect for natural polyamines interacting with G-quadruplexes depending on concentration. Biochimie., 93, 1351–1356.
22. Manish Pardhi., Yamini Thakur., Bharati Verma., Rubi Khilari., Mamta Tirpathi. and Rama Pande. (2016) In vitro screening of Napthyl hydroxamic acids as binding agents. Asian journal of chemistry., 28 (12), 2605-2612.
23. Christianah, M. A. and Li, P. K. (1998) Analytical profiles of drug substances, Academic press Inc., Pp. 123-140.
24. Arjmand, F., and Jamsheera, A. (2011) DNA binding studies of new valine derived chiral complexes of Tin (iv) and Zirconium (iv). Spectrochim. Acta., 78, 45-51.
25. Pringsheim, T., Davenport, W.J. and Dodick, D. (2008) Acute treatment and prevention of menstrually related migraine headache. Evidence-Based Review, Neurology., 70(17), 1555-1563.