

Research Article

Study of the Binding Interaction between Wortmannin and Calf Thymus DNA: Multispectroscopic and Molecular Docking Studies

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Introduction. Wortmannin (WTN) is a steroid metabolite that inhibits phosphatidylinositol 3-kinase and other signaling pathways. Structurally, the WTN consists of a cyclopentanophenanthrene-like structure with several oxygen-rich moieties which have the potential to interact with deoxyribonucleic acid (DNA) molecules. **Methods.** We aim to evaluate the WTN and calf thymus DNA (ct-DNA) interaction with molecular docking using the AutoDock 4.2 software. UV and fluorescence spectroscopy and viscosity techniques were performed to confirm the *in silico* analysis. **Results.** Molecular docking showed that the WTN interacted with ct-DNA via hydrogen bonds at guanine-rich sequences. The number of hydrogen bonds between the WTN and DNA was 1-2 bonds (average 1.2) per WTN molecule. The *in silico* binding constant was $2 \times 10^3 \text{ M}^{-1}$. UV spectroscopy showed that the WTN induced a hyperchromic feature without wavelength shifting. The WTN and DNA interaction led to quenching of DNA-emitted fluorescence. The different concentrations of WTN had no effect on DNA viscosity. Taken together, our results demonstrated WTN interacts with DNA in the nonintercalating mode, which is considered as a new mechanism of action. **Conclusion.** These results suggest that the WTN may exert its biological effects, at least in part, via interaction with DNA.

1. Introduction

In 1957, Brian et al. isolated the wortmannin (WTN) from filtrates of *Penicillium wortmannii* Klocker, and Millan et al. proposed the molecular structure based on chemical and spectroscopic evidence, which is illustrated in Figure 1 [1, 2]. This steroid metabolite contains a cyclopentanophenanthrene-like structure, in which A ring is modified to lactone ring and a heterocyclic furan is added to cyclopentanophenanthrene as the fifth ring. WTN belongs to furanosteroids which originate from lanosterol [3].

The subsequent studies confirmed this structure and reported several biologic functions of WTN including antifungal, cell arresting, proapoptotic, and anti-inflammatory activities [5–8]. Due to anti-inflammatory effects of WTN, it may be a therapeutic option to treat of inflammatory disorders such as gout and rheumatoid arthritis.

In 1993, Yano et al. reported that WTN interacts with phosphatidylinositol 3-kinase and inhibits its signaling pathway [9]. The inhibitory effects of WTN on NF-kB and MAPK signaling pathways were also reported [10–12].

Here, we hypothesized that WTN interacts with deoxyribonucleic acid (DNA) molecules that mediate some of its biological function. This hypothesis is based on the evidence indicating that most steroid metabolites modulate the DNA function such as gene expression by direct interaction with DNA and histones [13, 14]. A pioneer study by Cohen and Kidson demonstrated that steroids interact with guanine homoribopolynucleotides predominantly via hydrogen (H) bonds. Polyguanine has maximum hydrogen atoms for H bond formation, and progesterone presents maximum affinity to polynucleotides due to the oxygen-rich structure [15]. On the other hand, WTN contains eight oxygen atoms, which can contribute to the formation of H bonds. In

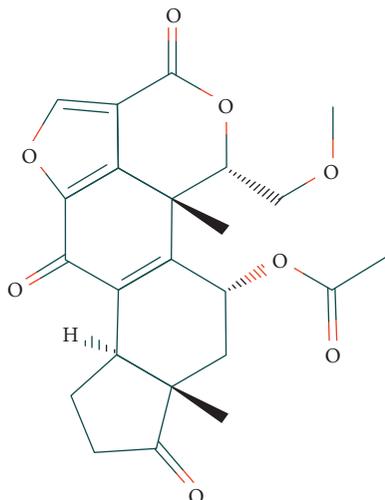


FIGURE 1: WTN structure (obtained from PubChem [4]).

addition, the WTN contains a bay region at the cyclopentanophenanthrene-like structure that may mediate the possible WTN-DNA interaction via the hydrophobic interaction [16].

Therefore, here we propose that WTN may exert its biological effects, at least in part, via interaction with DNA besides the putative inhibitory effects on signaling pathways. Nevertheless, to the best of our knowledge, there is no report regarding *in silico* and/or *in vitro* experiments about the WTN and DNA interaction. In this study, we evaluated WTN and calf thymus DNA (ct-DNA) interaction by molecular docking, viscosity, UV spectroscopy, and fluorescence spectroscopy.

2. Materials

Highly polymerized ct-DNA and WTN *Penicillium funiculosum* were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Tris (hydroxymethyl) aminomethane was obtained from Merck (Darmstadt, Germany). All experiments were performed in Tris buffer solution (10 mM pH 7.4). The stock solution of ct-DNA was prepared by dissolving an appropriate amount of ct-DNA in buffer solution by shaking gently. The stock solution was stored for 24 h at 4°C and utilized not more than 4 days. The concentration of ct-DNA in stock solution was 4.4×10^{-4} M that determined by spectrophotometry at 260 nm using an extinction coefficient (ϵ) of $6600 \text{ M}^{-1} \cdot \text{cm}^{-1}$. DNA purity was checked using absorption of DNA in 260 nm (A_{260}) and 280 nm (A_{280}). The A_{260}/A_{280} ratio was 1.85, indicating that the ct-DNA was sufficiently pure and protein free [17, 18]. Stock solution of WTN (5×10^{-3} M in ddH₂O) was prepared immediately before experiments due to instability.

3. UV Absorption Spectra Analysis

UV spectra analysis is a simple technique for ligand-DNA interaction. Absorbance in 230–300 nm wavelength range was measured, using a spectrophotometer (Analytikjena

spekol 2000) equipped with a 1.0 cm quartz cell. For quantification of the WTN-DNA binding constants, the absorption spectra measurements were performed at different concentrations of DNA ((0, 0.66, 1.1, 1.54, 2.2) $\times 10^{-5}$ M) and constant WTN concentration (1×10^{-5} M).

4. Fluorescence Emission Spectra Analysis

Fluorometric assay was performed, using a Shimadzu Spectrophotometer (RF-5301 PC) equipped with a quartz cell of 1.0 cm path length. WTN fluorescence emission spectra were recorded at 280–320 nm by excitation in 260 nm. Analysis was performed at constant concentration of WTN (7.5×10^{-6} M) in presence of different ct-DNA concentrations ((0, 0.22, 0.66, 1.1, 1.54, 1.98, 2.42, 2.86) $\times 10^{-5}$ M).

5. Viscosity Measurements

The effect of WTN on the viscosity of DNA was measured using a Lovis 2000 M digital micro viscometer. The temperature was kept constant at 25°C. The DNA concentration was 6.6×10^{-6} M while varying the WTN concentrations ((0, 0.25, 0.5, 0.75, 1) $\times 10^{-5}$ M). The data were presented as (η/η_0) 1/3 vs $[\text{WTN}]/[\text{DNA}]$, where η and η_0 are the viscosity of ct-DNA in the presence and absence of WTN, respectively [19].

6. Molecular Docking Study

Docking study was performed, using the AutoDock Tools 4.2 software. Structure of B-DNA dodecamer (PDB ID: 1BNA) was obtained from the protein data bank (<http://www.rcsb.org>) [20]. The WTN molecular structure was drawn, and energy was minimized using the HyperChem 8.0.6 software. The DNA file was prepared by deleting water molecules followed by addition of polar H atoms and Gasteiger charge. Grid box dimensions at grid points in $x \times y \times z$ directions were set to 56' 59' and 49 with a grid spacing of 0.375 Å. The coordinate centers of grid box were set to $x = 1.389'$ $y = 2.417'$ and $z = 13.472$. Lamarckian genetic algorithms were used to carry out molecular docking calculations. The number of runs was set to 100. All other parameters were assigned the default values [21, 22].

7. Results and Discussion

7.1. UV Absorption Spectroscopy. Figure 2(a) shows the UV spectra of the WTN-DNA complex. WTN showed an absorbance at 260 nm, and the peak intensities were enhanced with increasing the concentration of ct-DNA. A hyperchromic effect without red or blue shift was observed for all molar ratios, which demonstrated there is a nonintercalative binding interaction [23, 24]. The observed spectral changes proposed that WTN interacts with DNA at the groove-binding model [23]. The binding constant (K_b) was calculated using equation (1) from UV data [25].

$$\frac{A_0}{A - A_0} = \frac{\epsilon_G}{\epsilon_{H-G} - \epsilon_G} + \frac{\epsilon_G}{\epsilon_{H-G} - \epsilon_G} \times \frac{1}{K_b [\text{DNA}]}, \quad (1)$$

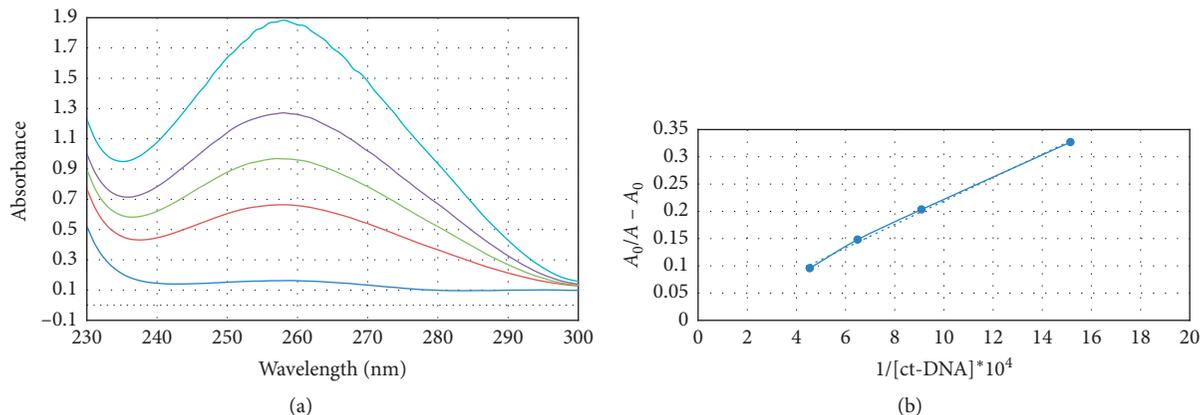


FIGURE 2: (a) UV spectra of WTN in the presence of different concentrations of ct-DNA (pH 7.4 and room temperature). (b) Plot $A_0/A - A_0$ versus $1/[DNA]$ for K_b calculation.

where A_0 and A are the absorbance of WTN in the absence and presence of ct-DNA, respectively. The ε_G and ε_{H-G} are the absorption coefficients of WTN and WTN-DNA complex, respectively. The K_b is $2 \times 10^3 \text{ M}^{-1}$ obtained by plotting $A_0/A - A_0$ versus $1/[DNA]$ (Figure 2(b)). This value for K_b suggested the groove binding mode that is similar to previous studied agents (e.g., Cu-Sn₂ complex, $1.67 \times 10^4 \text{ M}^{-1}$ [26], metformin complex, $8.3 \times 10^4 \text{ M}^{-1}$ [27], Ho(phen)²Cl³-H₂O ($1.36 \times 10^4 \text{ M}^{-1}$ [28], adefovir dipivoxil, $3.33 \pm 0.2 \times 10^4 \text{ M}^{-1}$ [20], and sorafenib complex, $5.6 \times 10^3 \text{ M}^{-1}$ [29]).

7.2. Fluorescence Spectra. The fluorescence emission spectra of WTN alone and in the presence of ct-DNA are shown in Figure 3(a). WTN has emission spectra with maximum emission at about 291 nm when excited at 260 nm. The fluorescence of WTN was quenched by ct-DNA without any shift in maximum emission in a concentration-dependent manner. Reduced emission intensities (quenching) confirmed WTN-DNA interaction. Fluorescence quenching is described by the Stern–Volmer equation (equation (2)) [30, 31]:

$$\frac{F_0}{F} = 1 + K_q \tau_0 [Q] = 1 + K_{sv} [Q], \quad (2)$$

where F_0 and F denote the steady state fluorescence intensities in the absence and presence of the quencher Q , respectively, K_q is the quenching rate constant of the biomolecule, τ_0 is the average lifetime of the molecule without the quencher, $[Q]$ is the quencher concentration, and K_{sv} is the Stern–Volmer quenching constant. Figure 3(b) shows the Stern–Volmer plots of F_0/F versus $[Q]$.

The calculated K_{sv} value was 3.5×10^5 , which is higher than the classical groove binding small molecule [32–34]. This severe quenching phenomenon indicated that WTN interacts with DNA strongly and probably via the intercalation model.

Apparent binding constant (K_b) and the binding stoichiometry (n) were calculated according to Zhang et al.'s equation (3) [35]:

$$\log \left[\frac{(F_0 - F)}{F} \right] = \log K_b + n \log [Q], \quad (3)$$

where F_0 and F are the fluorescence intensity in the absence and presence of a quencher at various concentrations $[Q]$, respectively. Figure 3(c) shows the plot of $\log [(F_0 - F)/F]$ versus $\log [DNA]$. The intercept is about 1.47, which indicates WTN binds to ct-DNA in more than one position. This observation is consistent with *in silico* docking analysis, which indicates in some positions WTN binds to ct-DNA via two H bonds.

7.3. Viscosity Study. Viscosity experiment is an effective tool to determine the binding mode between small molecules and ct-DNA [36]. In the intercalative binding mode, the DNA double helix separation and conformational change result in an increase in DNA viscosity while groove binding interaction has no significant effect on the DNA viscosity [37, 38]. The effect of WTN on the viscosity of ct-DNA at 25°C is shown in Figure 4. There is no significant change in the ct-DNA viscosity by increasing concentration of WTN. This observation suggests that the groove binding is the mode of interaction between WTN and ct-DNA.

7.4. Molecular Docking Study. Molecular docking study is an appealing method to understand the interaction between ligands and DNA [39]. Molecular models were made to discuss the binding modes using the AutoDock software for the interactions of WTN with ct-DNA (PDB ID: 1BNA). The structure of WTN was drawn and subjected to energy optimization [40]. 100 docking runs were successfully carried out and the obtained runs data for the conformers are listed in Table 1. The first conformation was taken from one of the lowest binding energy docking conformation. Our results showed WTN incorporated to DNA and the H bond formed between amino groups at C-2 on guanine and oxygen atom of the acetoxy group on C-11. In some conformations, the oxygen atom of furan and lactone rings was involved in H bond formation. There are 63 groups in which H bonds formed, including 39 groups with single H bond with

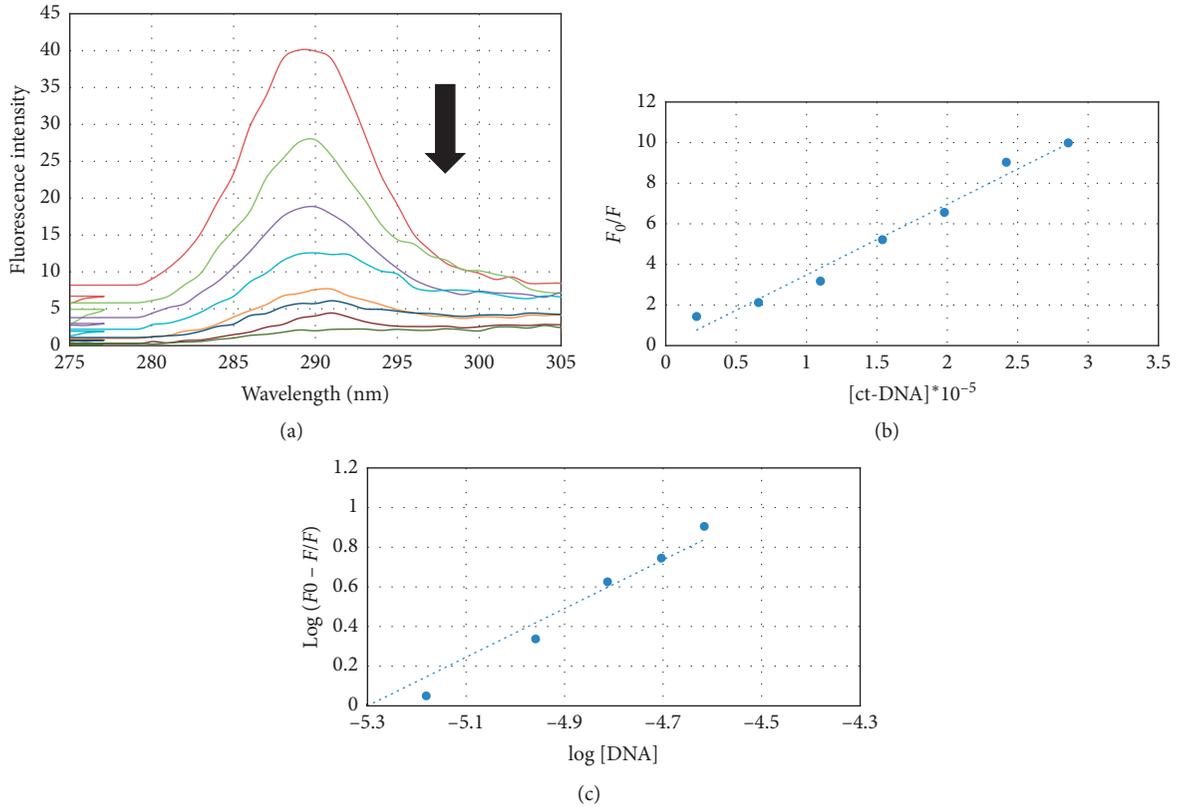


FIGURE 3: (a) Fluorescence emission spectra of WTN in the presence of different concentrations of ct-DNA. (b) Stern-Volmer plot of F_0/F versus $[Q]$. (c) Plot of $\log [(F_0 - F)/F]$ versus $\log [DNA]$.

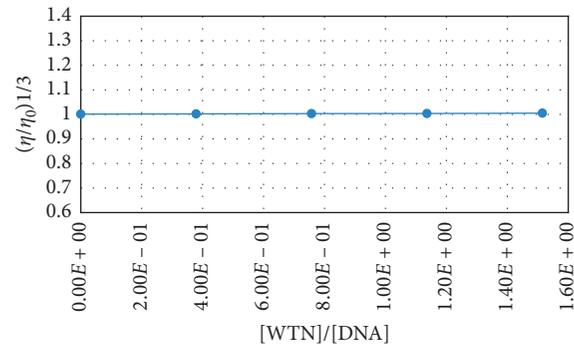


FIGURE 4: Viscosity of ct-DNA in constant concentration (6.6×10^{-6}) at 25°C in presence of WTN at different concentrations ($(0, 2.5, 5, 7.5, 10) \times 10^{-6}$).

TABLE 1: The data on DNA and WTN docking obtained from the AutoDock software.

Rank	Run	Lowest binding energy (kcalM^{-1})	Mean binding energy (kcalM^{-1})	Number of cluster	Ki (μM)	Cluster RMSD	Reference RMSD
1	8	-6.83	-6.61	28	9.84	0.00	30.84
2	63	-6.28	-6.23	29	24.88	0.00	32.52
3	37	-6.20	-6.08	13	28.35	0.00	31.86
4	31	-6.19	-6.04	4	29.09	0.00	29.51
5	23	-6.11	-6.01	12	33.43	0.00	31.40
6	4	-5.99	-5.96	3	40.75	0.00	34.94
7	80	-5.91	-5.86	11	46.82	0.00	31.71

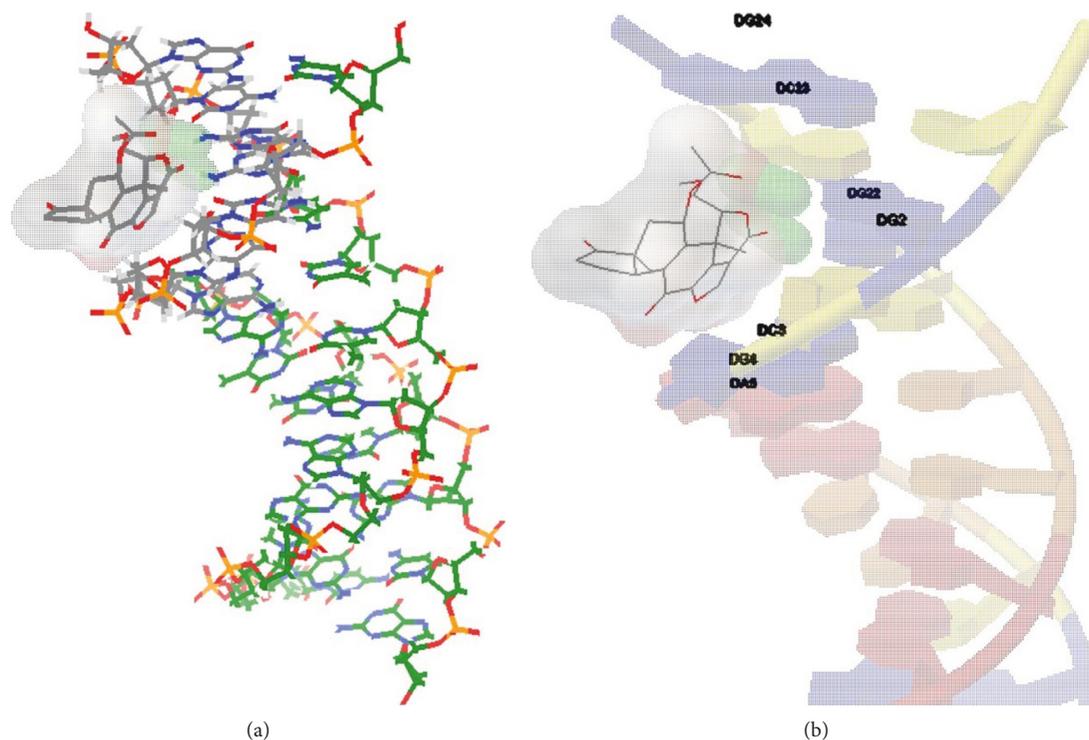


FIGURE 5: (a) *In silico* groove binding of WTN and DNA. (b) H bond (green) between WTN and guanine.

guanine, 12 groups with two H bonds simultaneously with guanine and cytosine, 11 groups with cytosine, and 1 group with two H bonds between one WNT and two guanines. These results proposed that WTN have interaction predominantly with guanine. The energetically most desirable conformation of the docked pose is shown in Figure 5.

8. Conclusion

The results of this study indicated that WTN interacts with DNA molecules. Our results did not provide strong evidence about the mode of interaction but based on *in silico* molecular docking, UV absorption spectroscopy, fluorescence emission spectroscopy, and viscosity measurement, a fluctuation between intercalation and groove binding model. The H bond may be an important force in this interaction where DNA is a hydrogen donor and the WTN oxygen is considered as a H acceptor. To the best of our knowledge, this is the first report that revealed a new and nontraditional mechanism for the biologic effects of WTN.

Taken together lack of a red-blue shift in UV spectra and the value of obtained K_b , it is suggested the groove-binding mode. The UV spectra results and groove-binding mode were confirmed by *in silico* analysis. However, the high K_{sv} value suggests the intercalation mode of interaction.

As a third mechanism, it is possible that the interaction may occur through both groove binding and intercalation, which was reported for some molecules previously [41, 42].

Circular dichroism, isothermal titration calorimetry, and ionic strength and melting temperature experiments could be performed to elucidate the precise interaction mode.

However, they were not carried out due to WNT instability. In addition, it should be determined whether the WTN-DNA interaction influences the gene expression and other biologic functions. Taken together, these results suggest that the WTN may exert its biological effects, at least partly, by interaction with DNA besides the putative signaling pathway inhibitory properties.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

- [1] P. W. Brian, P. J. Curtis, H. G. Hemming, and G. L. F. Norris, "Wortmannin, an antibiotic produced by *Penicillium wortmanni*," *Transactions of the British Mycological Society*, vol. 40, no. 3, pp. 365-373, 1957.
- [2] J. MacMillan, A. E. Vanstone, and S. K. Yeboah, "The structure of wortmannin, a steroidal fungal metabolite," *Chemical Communications (London)*, no. 11, pp. 613-614, 1968.

- [3] G.-Q. Wang, G.-D. Chen, S.-Y. Qin et al., "Biosynthetic pathway for furanosteroid demethoxyviridin and identification of an unusual pregnane side-chain cleavage," *Nature Communications*, vol. 9, no. 1, p. 1838, 2018.
- [4] National Center for Biotechnology Information, *PubChem Database. Wortmannin*, CID=312145, National Center for Biotechnology Information, Bethesda, MA, USA, 2019, <https://pubchem.ncbi.nlm.nih.gov/compound/Wortmannin>.
- [5] O. Hazeki, K. Hazeki, T. Katada, and M. Ui, "Inhibitory effect of wortmannin on phosphatidylinositol 3-kinase-mediated cellular events," *Journal of Lipid Mediators and Cell Signalling*, vol. 14, no. 1-3, pp. 259-261, 1996.
- [6] V. Singh, V. Praveen, D. Tripathi et al., "Isolation, characterization and antifungal docking studies of wortmannin isolated from *Penicillium radicum*," *Scientific Reports*, vol. 5, no. 1, p. 11948, 2015.
- [7] J. Wang, C. Zhang, P. Xu, Z. W. Yang, C. Z. Weng, and Y. X. Lai, "Phosphoinositide 3-kinase/protein kinase B regulates inflammation severity via signaling of Toll-like receptor 4 in severe acute pancreatitis," *Molecular Medicine Reports*, vol. 17, no. 6, pp. 7835-7844, 2018.
- [8] J. Yun, Y. G. Lv, Q. Yao, L. Wang, Y. P. Li, and J. Yi, "Wortmannin inhibits proliferation and induces apoptosis of MCF-7 breast cancer cells," *European Journal of Gynaecological Oncology*, vol. 33, no. 4, pp. 367-369, 2012.
- [9] H. Yano, S. Nakanishi, K. Kimura et al., "Inhibition of histamine secretion by wortmannin through the blockade of phosphatidylinositol 3-kinase in RBL-2H3 cells," *Journal of Biological Chemistry*, vol. 268, no. 34, pp. 25846-25856, 1993.
- [10] I. M. Ferby, I. Waga, C. Sakanaka, K. Kume, and T. Shimizu, "Wortmannin inhibits mitogen-activated protein kinase activation induced by platelet-activating factor in Guinea pig neutrophils," *Journal of Biological Chemistry*, vol. 269, no. 48, pp. 30485-30488, 1994.
- [11] S. K. Manna and B. B. Aggarwal, "Wortmannin inhibits activation of nuclear transcription factors NF- κ B and activated protein-1 induced by lipopolysaccharide and phorbol ester," *FEBS Letters*, vol. 473, no. 1, pp. 113-118, 2000.
- [12] S. S. Mendes, A. Candi, M. Vansteenbrugge et al., "Microarray analyses of the effects of NF- κ B or PI3K pathway inhibitors on the LPS-induced gene expression profile in RAW264.7 cells: synergistic effects of rapamycin on LPS-induced MMP9-overexpression," *Cellular Signalling*, vol. 21, no. 7, pp. 1109-1122, 2009.
- [13] K. Sunaga and S. S. Koide, "Interaction of calf thymus histones and DNA with steroids," *Steroids*, vol. 9, no. 4, pp. 451-456, 1967.
- [14] M. Beato, "Gene regulation by steroid hormones," *Cell*, vol. 56, no. 3, pp. 335-344, 1989.
- [15] P. Cohen and C. Kidson, "Interactions of hormonal steroids with nucleic acids. I. A specific requirement for guanine," *Proceedings of the National Academy of Sciences*, vol. 63, no. 2, pp. 458-464, 1969.
- [16] S. Kashino, D. E. Zacharias, R. M. Peck, J. P. Glusker, T. S. Bhatt, and M. M. Coombs, "Bay region distortions in cyclopenta[*a*]phenanthrenes," *Cancer Research*, vol. 46, no. 4, pp. 1817-1829, 1986.
- [17] N. Shahabadi and S. Bagheri, "Spectroscopic and molecular docking studies on the interaction of the drug olanzapine with calf thymus DNA," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 136, pp. 1454-1459, 2015.
- [18] Y. Zhang, G. Zhang, P. Fu, Y. Ma, and J. Zhou, "Study on the interaction of triadimenol with calf thymus DNA by multi-spectroscopic methods and molecular modeling," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 96, pp. 1012-1019, 2012.
- [19] Y. Sun, S. Bi, D. Song, C. Qiao, D. Mu, and H. Zhang, "Study on the interaction mechanism between DNA and the main active components in *Scutellaria baicalensis* Georgi," *Sensors and Actuators B: Chemical*, vol. 129, no. 2, pp. 799-810, 2008.
- [20] N. Shahabadi and M. Falsafi, "Experimental and molecular docking studies on DNA binding interaction of adefovir dipivoxil: advances toward treatment of hepatitis B virus infections," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 125, pp. 154-159, 2014.
- [21] N. Shahabadi and S. Amiri, "Spectroscopic and computational studies on the interaction of DNA with pregabalin drug," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 138, pp. 840-845, 2015.
- [22] P. Zhu, G. Zhang, Y. Ma, Y. Zhang, H. Miao, and Y. Wu, "Study of DNA interactions with bifenthrin by spectroscopic techniques and molecular modeling," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 112, pp. 7-14, 2013.
- [23] Z.-Q. Liu, Y.-T. Li, Z.-Y. Wu, and S.-F. Zhang, "[Cu₄(H₂O)₄(dmapox)₂(btc)]_n·10nH₂O: the first two-dimensional polymeric copper(II) complex with bridging μ -trans-oxamidate and μ_4 -1,2,4,5-benzotetracarboxylate ligands: synthesis, crystal structure and DNA binding studies," *Inorganica Chimica Acta*, vol. 362, no. 1, pp. 71-77, 2009.
- [24] D. K. Jangir, S. Charak, R. Mehrotra, and S. Kundu, "FTIR and circular dichroism spectroscopic study of interaction of 5-fluorouracil with DNA," *Journal of Photochemistry and Photobiology B: Biology*, vol. 105, no. 2, pp. 143-148, 2011.
- [25] G. Wang, C. Yan, D. Wang, D. Li, and Y. Lu, "Specific binding of a dihydropyrimidinone derivative with DNA: spectroscopic, calorimetric and modeling investigations," *Journal of Luminescence*, vol. 132, no. 7, pp. 1656-1662, 2012.
- [26] F. Arjmand and M. Muddassir, "Design and synthesis of heterobimetallic topoisomerase I and II inhibitor complexes: in vitro DNA binding, interaction with 5'-GMP and 5'-TMP and cleavage studies," *Journal of Photochemistry and Photobiology B: Biology*, vol. 101, no. 1, pp. 37-46, 2010.
- [27] N. Shahabadi and L. Heidari, "Binding studies of the anti-diabetic drug, metformin to calf thymus DNA using multi-spectroscopic methods," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 97, pp. 406-410, 2012.
- [28] S. Niroomand, M. Khorasani-Motlagh, M. Noroozifar, and A. Moodi, "Spectroscopic studies on the binding of holmium-1,10-phenanthroline complex with DNA," *Journal of Photochemistry and Photobiology B: Biology*, vol. 117, pp. 132-139, 2012.
- [29] J.-H. Shi, J. Chen, J. Wang, and Y.-Y. Zhu, "Binding interaction between sorafenib and calf thymus DNA: spectroscopic methodology, viscosity measurement and molecular docking," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 136, pp. 443-450, 2015.
- [30] J. R. Lakowicz and B. R. Masters, "Principles of fluorescence spectroscopy," *Journal of Biomedical Optics*, vol. 13, no. 2, p. 9901, 2008.
- [31] F.-L. Cui, J. Fan, J.-P. Li, and Z.-D. Hu, "Interactions between 1-benzoyl-4-p-chlorophenyl thiosemicarbazide and serum albumin: investigation by fluorescence spectroscopy," *Bioorganic & Medicinal Chemistry*, vol. 12, no. 1, pp. 151-157, 2004.
- [32] A. Usman and M. Ahmad, "Binding of bisphenol-F, a bisphenol analogue, to calf thymus DNA by multi-

- spectroscopic and molecular docking studies," *Chemosphere*, vol. 181, pp. 536–543, 2017.
- [33] I. Ahmad and M. Ahmad, "Dacarbazine as a minor groove binder of DNA: spectroscopic, biophysical and molecular docking studies," *International Journal of Biological Macromolecules*, vol. 79, pp. 193–200, 2015.
- [34] A. Nowicka, S. Hafner, and M. Hepel, "Antineoplastic drug interactions with DNA modified gold piezoelectrodes," *ECS Transactions*, vol. 19, no. 28, pp. 1–13, 2009.
- [35] G. Zhang, X. Hu, and P. Fu, "Spectroscopic studies on the interaction between carbaryl and calf thymus DNA with the use of ethidium bromide as a fluorescence probe," *Journal of Photochemistry and Photobiology B: Biology*, vol. 108, pp. 53–61, 2012.
- [36] N. Shahabadi and N. H. Moghadam, "Determining the mode of interaction of calf thymus DNA with the drug sumatriptan using voltammetric and spectroscopic techniques," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 99, pp. 18–22, 2012.
- [37] G. Zhang, L. Wang, X. Zhou, Y. Li, and D. Gong, "Binding characteristics of sodium saccharin with calf thymus DNA in vitro," *Journal of Agricultural and Food Chemistry*, vol. 62, no. 4, pp. 991–1000, 2014.
- [38] Z.-C. Liu, B.-D. Wang, Z.-Y. Yang, Y. Li, D.-D. Qin, and T.-R. Li, "Synthesis, crystal structure, DNA interaction and antioxidant activities of two novel water-soluble Cu(2+) complexes derivated from 2-oxo-quinoline-3-carbaldehyde Schiff-bases," *European Journal of Medicinal Chemistry*, vol. 44, no. 11, pp. 4477–4484, 2009.
- [39] I. Haq and J. Ladbury, "Drug-DNA recognition: energetics and implications for design," *Journal of Molecular Recognition*, vol. 13, no. 4, pp. 188–197, 2000.
- [40] E. M. Proudfoot, J. P. Mackay, and P. Karuso, "Probing site specificity of DNA binding metallointercalators by NMR spectroscopy and molecular modeling," *Biochemistry*, vol. 40, no. 15, pp. 4867–4878, 2001.
- [41] D. Banerjee and S. K. Pal, "Simultaneous binding of minor groove binder and intercalator to dodecamer DNA: importance of relative orientation of donor and acceptor in FRET," *The Journal of Physical Chemistry B*, vol. 111, no. 19, pp. 5047–5052, 2007.
- [42] E. Trotta, E. D'Ambrosio, G. Ravagnan, and M. Paci, "Simultaneous and different binding mechanisms of 4',6-Diamidino-2-phenylindole to DNA hexamer (d(CGATCG))₂: a ¹H NMR study," *Journal of Biological Chemistry*, vol. 271, no. 44, pp. 27608–27614, 1996.



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