Mechanism of Interactions of dsDNA Binding with Apigenin and Its Sulfamate Derivatives Using Multispectroscopic, Voltammetric, and Molecular Docking Studies

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ABSTRACT: DNA binding investigations are critical for designing better pharmaceutical compounds since the binding of a compound to dsDNA in the minor groove is critical in drug discovery. Although only one in vitro study on the DNA binding mode of apigenin (APG) has been conducted, there have been no electrochemical and theoretical studies reported. We hereby report the mechanism of binding interaction of APG and a new class of sulfonamide-modified flavonoids, apigenin disulfonamide (ADSAM) and apigenin trisulfonamide (ATSAM), with deoxyribonucleic acid (DNA). This study was conducted using multispectroscopic instrumentation techniques, which include UV-vis absorption, thermal denaturation, fluorescence, and Fourier transform infrared (FTIR) spectroscopy, and electrochemical and viscosity measurement methods. Also, molecular docking studies were conducted at room temperature under physiological conditions (pH 7.4). The molecular docking studies showed that, in all cases, the lowest energy docking poses bind to the minor groove of DNA and the apigenin–DNA complex was stabilized by several hydrogen bonds. Also, π–sulfur interactions played a role in the stabilization of the ADSAM–DNA and ATSAM–DNA complexes. The binding affinities of the lowest energy docking pose (schematic diagram of Table of contents (TOC)) of APG–DNA, ADSAM–DNA, and ATSAM–DNA complexes were found to be −8.2, −8.5, and −8.4 kcal mol⁻¹, respectively. The electrochemical binding constants Kₘ were determined to be (1.05 × 10⁵) ± 0.04, (0.47 × 10⁵) ± 0.02, and (8.13 × 10⁵) ± 0.03 for APG, ADSAM, and ATSAM, respectively (all of the tests were run in triplicate and expressed as the mean and standard deviation (SD)). The Kₘ constants calculated for APG, ADSAM, and ATSAM are in harmony for all techniques. As a result of the incorporation of dimethylsulfamate groups into the APG structure, in the ADSAM–dsDNA and ATSAM–dsDNA complexes, in addition to hydrogen bonds, π–sulfur interactions have also contributed to the stabilization of the ligand–DNA complexes. This work provides new insights that could lead to the development of prospective drugs and vaccines.

1. INTRODUCTION

Flavonoids are ubiquitous in nature as glycosylated or esterified forms, consisting of a backbone C₆–C₃–C₆, namely, rings A and B linked by a three-carbon-ring C. A variety of classes exist such as flavones (e.g., apigenin (APG), luteolin, and chrysin), flavonols (e.g., quercetin, kaempferol, myricetin, and fisetin), and others. The various classes differ (Figure S1) in the level of oxidation of the C ring, while individual compounds within a class differ in the pattern of substitution of the A and B rings.

Although the antioxidant activity has been reported as the representative bioactivity of flavonoids, studies have demonstrated that flavonoid-plant extracts can alter cellular functions. For example, the A-ring (5-hydroxyl group) and C-ring (4-carbonyl functionality) moieties of flavonoids are believed to mimic the adenine moiety of adenosine triphosphate (ATP), and certain flavonoids are inhibitors of the ATP-binding proteins (e.g., protein kinases and ATPases) via ATP-competitive binding. Similarly, they are expected to bind to the ATP-binding site of the ATP-binding cassette (ABC) transporters to inhibit the efflux of the drugs and/or drug conjugates. It is not surprising to note that there have been many attempts to evaluate the multidrug resistance (MDR) activity of flavonoids. Much of these have led to the identification of quercetin, apigenin, kaempferol, chrysin, and dehydroalizarin as potential safe MDR modulators. Significant role of apigenin in suppressing tumor growth in human breast cancer cells and human bladder cancer T24 cells and...
inducing anticolon cancer effect has been reported.\textsuperscript{6} Polymer-conjugated flavonoids have been shown to increase the accumulation of chemotherapeutic drugs in resistant cancer cells with overexpressed drug efflux transporters, permeability-glycoprotein (P-GP) that belong to the ABC transporter superfamily.\textsuperscript{9,10} When administered together with paclitaxel, apigenin and rutin have been shown to increase the plasma concentration of the anticancer agents,\textsuperscript{11} which had been attributed to the interaction of the flavonoids with P-GP, a protein that is responsible for the efflux of xenobiotics.\textsuperscript{11}

Sulfonamides are important functional groups in medicinal chemistry. They have been used to develop novel drugs with advanced pharmacological characteristics that show a vast biological activity spectrum. The history of sulfa-drug development dates back to the 1930s with the synthesis of antibacterial prontosil.\textsuperscript{12} The patenting peak of these drugs was between 2008 and 2012;\textsuperscript{13,14} the trend in the latter period has been focused on compounds incorporating the sulfamoyl moiety. This work relies on the hypothesis that using sulfamoyl chloride reagents as the starting material could result in novel derivatives of quercetin and apigenin sulfonamide with unique biological properties. Undeniably, sulfonamides make up an important class of drugs, with several of the pharmacological agents having antibacterial,\textsuperscript{15} antitumor, anticonvulsant, antitrypanosomal, smooth muscle relaxation agents.\textsuperscript{16}

Furthermore, sulfonamides have been incorporated as potential enzyme inhibitors, among them are carbonic anhydrases (C\textsubscript{A}s), the majority of the aspartate proteases (HIV-1 protease), as well as protein tyrosine phosphatase inhibitors. In carbonic anhydrases and other metalloenzymes, they bind as anions to the Zn\textsuperscript{2+} ion in the active site. Zn\textsuperscript{2+} is an essential metal needed by hundreds of enzymes for enzymatic function.\textsuperscript{16} Similarly, sulfamates and sulfate analogues have been reported as inhibitors of steroid sulfatase (STS), an emerging drug target for endocrine therapy of hormone-dependent diseases that catalyzes the hydrolysis of estrone sulfate to estrone.\textsuperscript{7-25} In recent years, the discovery of new drugs in the oncological field continues to grow rapidly to design new drugs that can bind to cancerous DNA by irreversibly destroying the DNA structure.\textsuperscript{22} For this reason, since DNA is assumed to be the primary target in the cellular system for many therapeutic and cytotoxic drugs, it has become an active area of research to investigate the interaction of a potential drug candidate molecule with DNA.\textsuperscript{23} Although newly synthesized drug candidate molecules with small molecular weight can interact directly with DNA, the factors (mechanisms) that meet this need to be known clearly. The mechanisms of these interactions are mainly categorized into four groups: (i) covalent bonding of the drug directly with DNA; (ii) electrostatic interaction, electrostatic attractions with the anionic sugar–phosphate backbone of DNA; (iii) groove binding, interactions with the DNA groove; and (iv) intercalation between the base pairs.\textsuperscript{24} The effectiveness of such interactions depends on several factors, including the affinity of the outer groups of the candidate molecules and the nature of binding.

Different research groups have reported the interaction between APG and DNA using different spectrophotometric methods.\textsuperscript{25-27} In particular, although Zhang et al.\textsuperscript{26} have conducted a detailed spectrophotometric in vitro study of the APG’s DNA binding mode, without any electrochemical, viscosimetric, and molecular docking studies. DNA binding studies of N-benzylated derivatives of sulfonamide have also been reported.\textsuperscript{28-31}

In this study, the mechanism of APG to DNA and a novel class of apigenin sulfamate derivatives, apigenin disulfonamide (ADSAM) and apigenin trisulfonamide (ATSAM) (Figure 1), have been investigated using in vitro and in silico methods. These include UV absorption spectroscopy, thermal denaturation, fluorescence, and electrochemical methods, together with the determination of a series of thermodynamic parameters and binding constants, as well as DNA melting techniques and viscosity measurements.

Apigenin has been extensively used in anticancer studies with promising results for cancer prevention. However, apigenin bioavailability is affected by its low solubility in most solvents. We modified apigenin by installing sulfonamides to the apigenin structure to investigate the physicochemical properties of the compounds. The DNA interaction studies with apigenin and its derivatives are very fundamental in drug discovery. In molecular docking calculations, we observed that, in all cases, the lowest energy docking poses bind to the minor groove of DNA and several hydrogen bonds have stabilized the APG–DNA complex. In addition to hydrogen bonds, \(\pi\)–sulfur interactions were discovered to have a part in the stabilization of the ADSAM and ATSAM–DNA complexes.

2. MATERIALS AND METHODS

2.1. Reagents and Solutions. The following chemicals were purchased from Sigma-Aldrich Company (St. Luis, MO) and used without further purification: tetrahydrofuran (THF), potassium carbonate (K\textsubscript{2}CO\textsubscript{3}), triethylamine, N,N-dimethyl-sulfamoyl chloride (DMsCl), p-toluenesulfonyl chloride (TsCl), and methanesulfonyl chloride (MsCl), anhydrous methanol, ethyl acetate, hexane, dichloromethane (DCM), anhydrous sodium sulfate (Na\textsubscript{2}SO\textsubscript{4}), NaCl, double-helix fish sperm DNA (dsDNA), tris(hydroxymethyl)aminomethane hydrochloride (Tris–HCl), acetic acid, sodium hydride, and ethidium bromide (EtBr). APG was purchased from Indofine Chemical Company (Hillsborough, NJ). In dsDNA binding studies, a 400 \(\mu\)M dsDNA stock solution was prepared by dilution of dsDNA to buffer solution (containing 0.2 M Tris–HCl and 150 mM NaCl at pH 7.4) followed by robust stirring for 2 days at room temperature and kept at 4°C for no longer than 7 days. The dsDNA solution thus prepared yielded a UV absorbance ratio of 1.85 at 260 and 280 nm (\(A_{260}/A_{280}\)), indicating that dsDNA was sufficiently purified from protein contamination. In this way, the molar concentration of DNA was determined using the molar absorption coefficient (\(\epsilon = 6600\text{ M}^{-1}\text{ cm}^{-1}\)).\textsuperscript{32} In all dsDNA binding experimental studies,
1 × 10^-3 M APG, 9 × 10^-4 M ADSAM, and 1.07 × 10^-4 M ATSAM stock solutions prepared using buffer solution were used. In addition, all binding experiments for all techniques were performed in triplicate, and standard deviations (SDs) from the mean in the calculated binding constants were calculated.

### 2.2. Physical Measurements and Instrumentation

Thin-layer chromatography (TLC) was performed on Millipore plastic-baked silica gel 60/UV254 plates. Bruker TopSpin NMR software was used for 1H NMR and 13C NMR spectral analysis with CDC13, or dimethyl sulfoxide (DMSO) as a solvent, while infrared (IR) spectra analysis was done on a Shimadzu ITTracer-100 at 0.25 cm⁻¹ resolution. NMR spectra were recorded on a Bruker Avance III HD 400 MHz spectrometer, and chemical shifts were reported in parts per million (ppm) relative to tetramethylsilane (TMS) at δ = 0.00 as an internal reference. The absorption spectra were recorded in the range of 200–400 nm on a T80+ UV–vis spectrophotometer using cells of 1 cm light path, and samples that contain dsDNA and the compound (individually for each compound) were mixed by vortexing before the spectra were recorded.

Absorbance measurement experiments were performed as follows: the dsDNA solution was added at increasing concentrations from 5 to 140 μM (rᵢ = [DNA]/[APG] = 0.17, 0.67, 1.17, 1.67, 2.17, 2.67, 3.17, 3.67, 4.17, and 4.67) to a 30 μM APG solution. The absorption spectra of the solutions thus prepared were taken against the blank solution (Tris–HCl, pH 7.4). The concentration range for ADSAM and ATSAM is 20–95 μM (rᵢ = [DNA]/[ADSAM] = 0.67, 1.17, 1.67, 2.17, 2.67, and 3.17; rᵢ = [DNA]/[ATSAM] = 1, 1.5, 1.75, 2.5, and 4.75). The wavelength range in the graphs was made for all three items (in the range of 245–445 nm). The values on the y-axis are multiplied by 10³ and shifted by 8 units, and the values on the x-axis by 10⁴ and shifted by 4 units. All of this is done to get clearer graphics. Original values were used in the preparation of all graphics. The denaturation profile of the dsDNA solution (120 μM) has been obtained by incrementally increasing the temperature from 10 to 100 °C while measuring the absorbance values at 260 nm wavelength. Then, in this dsDNA solution, separately APG solution (10 μM) and EtBr solution (10 μM) were added to obtain the new denaturation profile of dsDNA under the same increasing temperature conditions. The same concentrations were used in denaturation studies of substances ADSAM and ATSAM.

All fluorescence measurements were performed on an Agilent Technologies spectrofluorometer using a quartz cell of 1 cm light path by keeping the concentration of the 2.5 μM EtBr + 25 μM dsDNA solution constant. The varying concentrations of the APG solution from 12.5 to 100 μM (rᵢ = [APG]/[dsDNA] = 0.5, 1, 1.5, 1.75, 2, 2.25, 2.5, 3, 3.5, and 4) were added to this solution at room temperature (298 K). Samples were excited at 294 nm, and emission spectra were recorded in the range of 450–750 nm. The competitive spectrofluorometric dsDNA binding studies of APG with EtBr-bound dsDNA in the Tris–HCl buffer solution have been conducted to find whether APG could replace EtBr from the EtBr–dsDNA complex. Fluorescence experiments were performed separately in items ADSAM and ATSAM using the same procedure for ADSAM solution from 12.5 to 75 μM (rᵢ = [ADSAM]/[DNA] = 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0), for ATSAM solution from 12.5 to 75 μM (rᵢ = [ATSAM]/[DNA] = 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0).

The viscosity values of the samples were measured using a rheometer (Haake RheoStress 1, Germany) equipped with a parallel plate sensor (d = 35 mm, gap = 1 mm). The measurements were conducted twice for each sample at room temperature. The shear rate ramp was applied in the range of 0.01 and 200 s⁻¹ in 60 s. The measured data were modeled from the flow curves (shear stress versus shear rate) using a software (RheoWin3 Data Manager, Germany) according to the Herschel–Bulkley model for all samples

\[ \tau = \tau_0 + Ky^n \]  

(1)

where \( \tau_0 \) is the yield stress (Pa), K is the consistency index (Pa·s^n), y is the shear rate (s⁻¹), and n is the flow behavior index. For Herschel–Bulkley fluids, \( K > 0, 0 < n < \infty, \) and \( \tau_0 > 0 \). After obtaining \( \tau_0, K, n \) values from the modeling, the apparent viscosity (\( \eta \)) values calculated at 20 s⁻¹ using the following equation

\[ \eta = \tau_0 \frac{y}{\gamma} + Ky^{n-1} \]  

(2)

The data is reported as (\( \eta/\eta_0 \))^{1/3} versus \( r (r = [\text{APG}]/[\text{dsDNA}]) = 0.75–3.0 \) (the same \( r \) ratio was used in the other two derivatives), where \( \eta_0 \) is the viscosity of the dsDNA solution alone and \( \eta \) is the viscosity of the dsDNA + APG (or DNA + ADSAM or DNA + ATSAM) solution (samples 1–10). The electrochemical measurements were executed with an Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, Netherlands). The experimental conditions were managed with General Purpose Electrochemical System (GPES) and Nova 2.2 software packages. Glassy carbon electrode (GCE) (BAS; U, 3 mm diameter) was used in voltammetry studies, and all measurements were performed using a BAS 100 W (Bioanalytical System) potentiometer. In all studies, APG (or ADSAM or ATSAM) solutions with increasing amounts were added to the dsDNA solution, and the changes in the voltammogram of the dsDNA solution were examined using the cyclic voltammetric (CV) technique in an acetic acid/sodium acetate buffer solution (pH 4.8). For the triple-electrode system, a Ag/AgCl reference electrode (BAS; 3 M KCl in all experiments), a platinum wire counter electrode, and a 10 mL standard single-compartment three-electrode cell were used. Before each measurement, the glassy carbon electrode surface is manually polished and cleaned with a slurry of alumina powder (U, 0.01 cm) with regular circular motions on a specific suede soft polishing cloth (BAS suede polishing pad). The CV studies were performed by keeping temperatures at 288.15, 298.15, and 308.15 K for all APG, ADSAM, and ATSAM to observe the behavior of interaction at different temperatures and derive thermodynamic parameters.

### 2.3. Molecular Docking Studies

Molecular docking studies have been performed using AutoDock Tools and AutoDock Vina software packages. Discovery Studio Visualizer has been used for the representation of the docking results and the interactions between ligands and DNA. The crystal structure of DNA has been obtained from the RCSB Protein Data Bank (PDB accession number 1BNA). Before docking calculations, water molecules were removed and hydrogens and Gasteiger charges were added. Three-dimensional (3D) molecular structures of the APG, ADSAM, and ATSAM molecules have been obtained from geometry optimizations. In geometry optimizations, Gaussian 09, rev. D.01, and GaussView software packages have been used. Before geometry optimizations, a conformational search has
of APG (bu) with nano pure water and dried over anhydrous Na2SO4. The reaction mixture was extracted in ethyl acetate and washed monitored by TLC every 4 h. The resulting yellowish crude reaction mixture was brought to room temperature and stirred to completion for 12 h while being monitored by TLC every 4 h. The reaction was stirred for 2 h. The reaction was placed on an ice bath and stirred for 5 min. Triethylamine (2 equiv per hydroxyl) was added dropwise, and the reaction was stirred for an additional 2 min; excess N,N-dimethanesulfamoyl chloride was added dropwise, and the reaction was stirred for 2 h. The reaction was brought to room temperature and stirred to completion for 12 h while being monitored by TLC every 4 h. The resulting yellowish crude reaction mixture was extracted in ethyl acetate and washed with nanopure water and dried over anhydrous Na2SO4. The organic layer was concentrated under pressure to yield an oily yellow compound, which was purified by flash chromatography (gradient separation) using ethyl acetate and hexane as solvents.

2.5. Procedure for the Synthesis of 2-(4-((N,N-Dimethylsulfamoyl)oxy)phenyl)-5-hydroxy-4-oxo-4H-chromen-7-yl Dimethylsulfamate (ATSAM). The synthesis of the novel apigenin sulfonamide derivative (ATSAM) has been reported elsewhere.41 APG (1 mmol, 270 mg) was transferred into an oven-dried round-bottomed flask charged with a magnetic stir bar. To the flask, 3 mL of anhydrous THF was added under nitrogen and the solution was placed on an ice bath and stirred for 5 min. Triethylamine (2 equiv per hydroxyl) was added dropwise, and the reaction was stirred for an additional 2 min; excess N,N-dimethanesulfamoyl chloride was added dropwise, and the reaction was stirred for 2 h. The reaction was brought to room temperature and stirred to completion for 12 h while being monitored by TLC every 4 h. The resulting yellowish crude reaction mixture was extracted in ethyl acetate and washed with nanopure water and dried over anhydrous Na2SO4. The organic layer was concentrated under pressure to yield an oily yellow compound, which was purified by flash chromatography (gradient separation) using ethyl acetate and hexane as solvents.

3. RESULTS AND DISCUSSION

3.1. Characterization of ADSAM and ATSAM. The structures of the two compounds (Figure S1) were elucidated based on 1H, 13C NMR, and infrared spectroscopy.

3.2. DNA Binding Studies. 3.2.1. Absorption Spectra Measurements. Based on our previous studies and scientific literature reports, the ultraviolet absorption titration technique is one of the most common and valid methods used to investigate DNA interactions.42,43 The structural changes of DNA in the presence of any small compound, along with the affinity and binding mode of small molecules, are effectively seen from the UV electronic absorption spectrum. The absorption of DNA occurs due to the chromophoric groups (conjugated double bonds) of the pyrimidine and purine ring system within the structure. When compounds interact with DNA in the presence of any small compound, alongside the UV absorption of DNA occurs due to the chromophoric groups (conjugated double bonds) of the pyrimidine and purine ring system within the structure. When compounds interact with DNA complex occurs,
hypochromic and hyperchromic effects are observed in the spectral properties of DNA. The complex formation of DNA and small molecules vary with the magnitude of the peak position and absorbance associated with the interaction strength. Generally, the ultraviolet–visible absorption spectra of small molecules that bind to DNA in the groove-binding mode exhibit a significant hypochromic effect, while the position of the maximum wavelength is almost unchanged; this can be explained by the fact that the electronic states of the chromophore of the complex overlap with the \(-N\) groups within the grooves of DNA.

In this bioanalytical study, the interaction of APG, ADSAM, and ATSAM with dsDNA (separately) was investigated by examining the absorption spectra of the mixture solutions prepared at the appropriate concentrations. Thus, several solutions were prepared by adding increasing amounts of the dsDNA solution (from 5 to until reaching 140 μM for APG and 20–95 μM for others) to APG (or ADSAM or ATSAM) solutions at a certain concentration (30 μM) in each case prepared from the stock solutions. The UV–vis spectra of APG (ADSAM and ATSAM) in the absence and presence of dsDNA are given in Figure 2a–c. The addition of different amounts of dsDNA to the APG solution exhibited that the intensity of absorption decreased gradually (hypochromic effect) for about 22.70% and was not shifted in terms of wavelength. The same situation was observed in ADSAM, and the ATSAM solution and percent (%) hypo values were found as 27.60 and 39.30, respectively. The spectrum analysis and the changes in absorption peaks are shown in Figure 2a–c clearly. These room-temperature experiments and results are helpful in providing a clear explanation of the mode of the molecule–DNA interaction.

The decrease in the absorbance values and the absence of significant wavelength shifts in the experiments at the constant concentrations of APG (or ADSAM or ATSAM) showed that these compounds were binding to dsDNA via the groove-binding mode. In some important research studies, the same binding mode has been proposed in a significant number of compounds, for instance; anticancer drugs 6-thioguanine44 and fludarabine45 with similar spectral properties, i.e., hypochromic in the presence of dsDNA and no shift in the wavelength. In addition, the DNA equilibrium binding constants (\(K_b\)) of these compounds were calculated based on titration data. The intrinsic binding constant \(K_b \) of these compounds with dsDNA represents the binding constant per DNA base pair, can be obtained by monitoring the changes in absorbance between 200 and 400 nm with extended concentrations of dsDNA from plots \([DNA]/(\varepsilon_a - \varepsilon_l)\) versus \([DNA]\), and is given by the ratio of the slope to the \(y\)-intercept, according to the Benesi–Hildebrand equation (eq 3).46

\[
[DNA] = \frac{[DNA]}{(\varepsilon_a - \varepsilon_l)} + \frac{1}{K_b \times (\varepsilon_a - \varepsilon_l)}
\]

where \(\varepsilon_a\) is the apparent extinction coefficient obtained by calculating \(A_{\text{obsd}}/[\text{compound}]\), \(\varepsilon_l\) is the extinction coefficient of the compound free in solution, \(\varepsilon_b\) is the extinction coefficient for the compound in the fully bound form, and \([\text{DNA}]\) is the concentration of dsDNA in terms of base pairs, and all \(K_b\) (±SD) values are given in Table 1.

The \(K_b\) values were between (2.95 × 10^5) and (9.12 × 10^5) ± 0.03–0.08 L mol\(^{-1}\) for each compound (from the graphics in the upper right corner of Figure 2a–c) and are lower than the known value for a basic intercalator such as EtBr (10^7 L mol\(^{-1}\)), but these \(K_b\) values were coherent with one previously reported for groove-binding drugs such as moxifloxacin (9.4 × 10^5 L mol\(^{-1}\))44, methotrexate (1 × 10^5 L mol\(^{-1}\)),45 and spermidine (8.22 × 10^4 L mol\(^{-1}\)).46 It has been found from these results that APG, ADSAM, and ATSAM were bound to dsDNA via the groove-binding mode. However, this cannot be the only proving method, and some other experimental results were further needed obviously, and this explains why the thermal denaturation studies, fluorescence spectrum measurements, and viscosity and voltammetric measurements were also employed in this study as efficient tools to obtain information regarding the binding mode.

### 3.2.2. Thermal Denaturation Studies

In the context of our study, the stability of the secondary structure of dsDNA resulting from binding of all three compounds (APG, ADSAM, and ATSAM) to the dsDNA helix is mostly determined by the thermal denaturation technique. The melting temperature of a biological polymer structure such as DNA is an important consideration for the interaction of molecules such as APG, ADSAM, and ATSAM with nucleic acids. When a certain concentration of the dsDNA solution is prepared, and the temperature of this solution is gradually increased, the hydrogen bonds between the base pairs in the double-stranded dsDNA structure are broken, and the dsDNA begins to decompose slowly into single strands. The melting temperature (\(T_m\)) of dsDNA is defined as the temperature at which half of the total base pairs are separated.47 \(T_m\) is an extremely important parameter for the balance of the dsDNA helical structure. Any small compound changes their \(T_m\) values depending on their binding affinity to dsDNA.

In thermal denaturation studies, samples were performed by preparing compound–dsDNA solutions containing 120 μM dsDNA solution and 10 μM APG (10 μM ADSAM or 5 μM ATSAM) in Tris–HCl buffer. The thermal denaturation data (°C) of APG, ADSAM, and ATSAM are given in Table 2.

While the prepared dsDNA solutions were heated from 23 to 100 °C with the help of a temperature-programmed heater, the absorption values of the solutions at 260 nm were recorded simultaneously. The denaturation process for APG, ADSAM, and ATSAM was investigated by thermal denaturation studies, fluorescence spectrum measurements, and viscosity and voltammetric measurements.

| Compound | \(K_b \pm \text{SD}^a\) | \(\log K_b \pm \text{SD}^a\) | \(\lambda_{\text{max}}\) (nm) | \%hypo \pm \text{SD}^a\) |
|----------|-----------------|------------------|-----------------|-----------------|
| APG      | (9.12 × 10^5) ± 0.08 | 4.96 ± 0.06 | 340              | 22.70 ± 0.05   |
| ADSAM    | (3.10 × 10^5) ± 0.05 | 5.49 ± 0.03 | 278              | 27.60 ± 0.04   |
| ATSAM    | (2.95 × 10^5) ± 0.03 | 5.47 ± 0.02 | 302              | 39.30 ± 0.07   |

All of the tests were conducted in triplicate. (%hypo = % hypochromism = \(A - A_0/A_b\)).
On the other hand, the diminishment of these compounds (APG, ADSAM, or ATSAM) does not diminish the stability of DNA dsDNA melting temperature, suggesting that the introduction of dsDNA binding studies of APG’s fluorescence spectroscopy technique were investigated by Zhang et al. 5 years ago. However, there are no experiments to replace the studies with EtBr. However, another important section that examines the interactions of small molecules with dsDNA is the replacement studies with EtBr using fluorescence spectroscopy. EtBr is a planar molecule, and dsDNA intercalates between base pairs. While EtBr alone shows weak fluorescence, emission intensities increase significantly when bound to DNA. All scientific data on this subject show that small molecules with intercalation features are added to DNA solutions in increasing amounts, resulting in a significant reduction in emission intensity. If APG or synthesized compounds are intercalated between the base pairs of DNA, as in the case of EtBr, these compounds may compete with EtBr, and in this case, emission intensities of EtBr—dsDNA solutions will be observed in fluorescence measurements. The amount of reductions in this emission intensity clearly indicates the intercalation strength of the compounds with dsDNA. In fluorescence studies, decreases in emission intensities caused by replacement with EtBr are a result of the compound’s intercalation, removing EtBr molecules from the dsDNA helix and its replacement. Such compounds are called emission extinguishers. Figure 4a—c shows the reductions in APG, ADSAM, and ATSAM emission intensities of EtBr—DNA solutions. In the EtBr displacement experiment, the compounds were treated with solutions containing 25 μM dsDNA and 2.5 μM EtBr previously prepared at increasing concentrations. To ensure thermal equilibrium in prepared solutions, it was kept at 25 °C for half an hour before measurements and fluorescence spectra of solutions containing EtBr were stimulated at 294 nm and recorded in the range of 450—750 nm. Fluorescence quenching abilities of compounds Stern—Volmer constant (KSV) values are calculated.

\[ I_0/I = 1 + K_{SV}[\text{compounds}] \]  

While \( I_0 \) shows only the emission intensity of the EtBr—dsDNA solution, \( I \) shows the emission intensity of EtBr—dsDNA solutions in the presence of compounds, and it is called the \( K_{SV} \) quenching coefficient. Stern—Volmer graphs were obtained using data obtained from fluorescence experiments, and \( K_{SV} \) values of the compounds calculated from the slopes of the lines of these graphics are given in Table 3.

However, the apparent DNA binding constant (\( K_{app} \)) values of the compounds can be calculated from the changes in emission intensity in response to increasing compound concentration with the help of the following equation:

\[ K_{EtBr}[\text{EtBr}] = K_{app}[\text{compounds}] \]

In this equation, [compounds] is the compound concentration of EtBr—dsDNA that reduces the fluorescence intensity to 50%. EtBr concentration was taken as 2.5 μM (since the \( K_{EtBr} \) value is \( 1.0 \times 10^7 \) M\(^{-1}\)). The \( K_{app} \) values could not be determined for ADSAM and ATSAM because the corresponding compounds did not cause a 50% reduction in the fluorescence intensity of the EtBr—dsDNA solutions (Figure 4b,c). Nevertheless, the \( K_{SV} \) values calculated by adding compounds to EtBr—dsDNA solutions are in the range of \((3.70 \times 10^3) - (1.15 \times 10^5)\) M\(^{-1}\), and these values show that

| compound       | \( T_m \pm SD \) * | \( \Delta T_m \)  |
|----------------|-------------------|------------------|
| dsDNA          | 68.60 ± 0.06      |                  |
| dsDNA + EtBr   | 80.20 ± 0.03      | 11.60            |
| dsDNA + APG    | 72.80 ± 0.04      | 4.20             |
| dsDNA + ADSAM  | 74.10 ± 0.06      | 5.50             |
| dsDNA + ATSAM  | 75.05 ± 0.07      | 6.90             |

*All of the tests were run in triplicates and expressed as the mean and standard deviation.
Because one of the hydrodynamic measurements that are sensitive to changes in the length of DNA is viscosity.\textsuperscript{52} Viscosity experiments were carried out using a rheometer. Viscosity was done by measuring the relative viscosities of the solutions prepared by adding increasing amounts of compound to the dsDNA solution (120 μM dsDNA for APG and ADSAM; 30 μM dsDNA for ATSAM) in a constant concentration in the Tris–HCl medium (pH 7.4). Thus, changes in the relative viscosity of dsDNA could be monitored.

A series of solutions were made, which contained a fixed concentration of dsDNA and increasing concentrations of compounds (samples 1–10), and the viscosity measurements were conducted at room temperature. The Herschel–Bulkley model parameters and calculated apparent viscosities at 20 s \(^{-1}\) of samples 1–10 (\(\eta_s\)), only dsDNA (\(\eta_0\)), only APG (or ADSAM or ATSAM), and buffer solution are given in Tables S1–S3. For each sample, the measurement was repeated three times and average flow times were calculated. Figure 5 shows the changes in relative viscosity of dsDNA solution with increasing concentrations of APG, ADSAM, and ATSAM.

![Figure 4](https://dx.doi.org/10.1021/acsomega.0c02612) Effect of the increasing amount of APG (yellow line), ADSAM (blue line), and ATSAM (green line) on the relative viscosity of dsDNA at room temperature.

The change in viscosity upon molecular intercalation with dsDNA requires the condition that the distance of the adjacent base pairs should be large enough to be able to bind the small molecules (ligand or drug) within the double-stranded chain. The electrostatic or groove surface binders tend to make quite insignificant changes in the relative viscosity suggested that the mode of binding of the compounds with dsDNA was groove binding. This situation has made it necessary to do further studies since it is partially in contradiction with the intercalation explanation of APG’s fluorescence results. For

![Figure 5](https://dx.doi.org/10.1021/acsomega.0c02612) Illustration of emission spectra variations when increasing amounts of APG (a), ADSAM (b), and ATSAM (c) are added to EtBr–dsDNA solutions; \([\text{EtBr}] = 2.5 \text{ μM}, [\text{dsDNA}] = 25.0 \text{ μM}\). The arrow shows the changes in intensity upon increasing amounts of APG (a), ADSAM (b), and ATSAM (c). Insets: Stern–Volmer plots of the fluorescence data. The \(r\) values on the graph show the ratios of \([\text{APG}]/[\text{dsDNA}]\) (a), \([\text{ADSAM}]/[\text{dsDNA}]\) (b), and \([\text{ATSAM}]/[\text{dsDNA}]\) (c). The binding constants shown are the mean of three measurements and the uncertainty shown in the standard deviation from the mean derived from these measurements (in the top right corner).

Because one of the hydrodynamic measurements that are sensitive to changes in the length of DNA is viscosity.\textsuperscript{52} Viscosity experiments were carried out using a rheometer. Viscosity was done by measuring the relative viscosities of the solutions prepared by adding increasing amounts of compound to the dsDNA solution (120 μM dsDNA for APG and ADSAM; 30 μM dsDNA for ATSAM) in a constant concentration in the Tris–HCl medium (pH 7.4). Thus, changes in the relative viscosity of dsDNA could be monitored.

A series of solutions were made, which contained a fixed concentration of dsDNA and increasing concentrations of compounds (samples 1–10), and the viscosity measurements were conducted at room temperature. The Herschel–Bulkley model parameters and calculated apparent viscosities at 20 s \(^{-1}\) of samples 1–10 (\(\eta_s\)), only dsDNA (\(\eta_0\)), only APG (or ADSAM or ATSAM), and buffer solution are given in Tables S1–S3. For each sample, the measurement was repeated three times and average flow times were calculated. Figure 5 shows the changes in relative viscosity of dsDNA solution with increasing concentrations of APG, ADSAM, and ATSAM.

![Figure 4](https://dx.doi.org/10.1021/acsomega.0c02612) Illustration of emission spectra variations when increasing amounts of APG (a), ADSAM (b), and ATSAM (c) are added to EtBr–dsDNA solutions; \([\text{EtBr}] = 2.5 \text{ μM}, [\text{dsDNA}] = 25.0 \text{ μM}\). The arrow shows the changes in intensity upon increasing amounts of APG (a), ADSAM (b), and ATSAM (c). Insets: Stern–Volmer plots of the fluorescence data. The \(r\) values on the graph show the ratios of \([\text{APG}]/[\text{dsDNA}]\) (a), \([\text{ADSAM}]/[\text{dsDNA}]\) (b), and \([\text{ATSAM}]/[\text{dsDNA}]\) (c). The binding constants shown are the mean of three measurements and the uncertainty shown in the standard deviation from the mean derived from these measurements (in the top right corner).

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### Table 3. Binding Constants (\(K_{\text{app}}\)) and Quenching Constants (\(K_{SV}\)) for Binding of APG, ADSAM, and ATSAM with dsDNA

| compound | \(K_{SV} \pm \text{SD}^a\) | \(\log K_{SV} \pm \text{SD}^a\) | \(K_{\text{app}} \pm \text{SD}^b\) | \(\log K_{\text{app}} \pm \text{SD}^b\) |
|----------|----------------|----------------|
| APG      | \(1.15 \times 10^4\) | \(4.05 \pm 0.04\) | \((6.53 \times 10^3) \pm 0.013\) | \(5.81 \pm 0.006\) |
| ADSAM    | \((3.70 \times 10^3) \pm 0.02\) | \(3.54 \pm 0.03\) | \(-^b\) | \(-^b\) |
| ATSAM    | \((3.60 \times 10^3) \pm 0.03\) | \(3.56 \pm 0.02\) | \(-^b\) | \(-^b\) |

\(^a\)All of the tests were run in triplicate and were expressed as the mean and standard deviation. \(^b\)\(K_{\text{app}}\) is called the apparent binding constant, which corresponds to a value of 50% reduction in the fluorescence intensity of the EtBr–dsDNA solutions. \(K_{SV}\) is called as the Stern–Volmer constant or the quenching constant obtained from fluorescence measurements with EtBr.
this reason, voltammetric and theoretical studies have been designed.

3.2.5. Electrochemical Methods. The primary use of cyclic voltammetric (CV) is for fundamental and diagnostic studies that provide qualitative information about electrochemical processes under various conditions. It also requires a small number of samples, so it is widely used in biological and chemical analyses. The electrochemical responses of DNA before and after the interaction with small molecules can be used to understand the interaction mechanism between small molecules (drugs or drug candidate molecules) and DNA because of (i) a dramatic decrease/increase in the oxidation/reduction peak current of small molecules (drugs or drug candidate molecules) when they selectively bind to dsDNA, (ii) a significant decrease/increase in the oxidation/reduction peak current of the electroactive DNA bases such as guanine or adenine, (iii) potential shifts to the more positive or negative side by the intercalation of nucleic acid-binding molecules into dsDNA. In this study, the APG (or ADSAM or ATSAM) solution with increasing amounts was added to the dsDNA solution, and the changes in the peak current and potential of the dsDNA solution were examined.

3.2.5.1. Scan Rate Studies. The scan rate studies are the most commonly used electrochemical studies to investigate electrode reactions in electrochemical studies. Furthermore, the electrochemical processes of molecular species in CV scan rate experiments can be investigated. The scan rate is controlled according to how fast the potential applied is scanned with scan rates. Useful information involving electrochemical mechanisms generally can be acquired from the relationship between the peak current and scan rate. Therefore, the voltammetric behavior of APG and ADSAM was studied using CV at different scan rates for clarifying the transfer of the compound under a diffusion- or adsorption-controlled process. The scan rate experiments varied from 5 to 1000 mV s\(^{-1}\) for \(5 \times 10^{-4}\) M APG and ADSAM. The equations are noted below in acetate buffer at pH 4.80

\[
\text{log } I_p (A) = 0.26 \log v (\text{mV s}^{-1}) - 0.2521 \quad , \quad r^2 = 0.9566, \quad n = 5 \quad \text{for APG}
\]

\[
\text{log } I_p (A) = 0.39 \log v (\text{mV s}^{-1}) - 0.923, \quad r^2 = 0.9735, \quad n = 5 \quad \text{for ADSAM}
\]

A plot of the logarithm of \(I_p\) versus the logarithm of the scan rate gave a straight line with slopes of 0.26 and 0.39 for APG and ADSAM, respectively, showing the diffusion-controlled process. Since a repeatable peak current at pH 4.8 for ATSAM could not be obtained, and no equation was given at this pH because ATSAM has no oxidizable groups like -OH in its chemical structure. The peak potential shifted to more negative potential values with an increase in the scan rates. As seen in the equation, \(E_p\) shifted to more anodic values with increasing scan rate, confirming and supporting the irreversibility. The linear relationship between \(E_p\) and log \(v\) can be expressed by the following equations

\[
E_p (V) = 0.27 \log v (\text{V s}^{-1}) + 0.551, \quad r^2 = 0.9795, \quad n = 5 \quad \text{for APG}
\]

\[
E_p (V) = 0.53 \log v (\text{V s}^{-1}) + 0.742, \quad r^2 = 0.9871, \quad n = 5 \quad \text{for ADSAM}
\]

3.2.5.2. Electrochemical Interaction Studies. There are several studies in the literature on the voltammetry of APG using different electrodes. For example, the electrochemical behavior of the APG was studied in 0.1 mol L\(^{-1}\) B-R buffer solutions (50% ethanol, pH 3.0) using CV at a glassy carbon electrode by Xing et al. Wang et al. exploited a simple electrochemical method to activate a bare screen-printed carbon electrode, and a novel amperometric APG sensor with high sensitivity was developed. However, electrochemical detection of the interaction between dsDNA and APG, ADSAM, and ATSAM based on the changes in guanine and adenine signals has not been studied before on any electrode. The APG molecule contains both the resorcinol and phenol moieties and the conjugated double bond between them. APG, which is a planar molecule due to functional groups bound to ring structures, is an electroactive substance. The cyclic voltammetry technique provides general information about the electroactivity and possible surface activities of such compounds. Figure 6a shows CV for a 10 \(\mu M\) APG solution in acetate buffer solutions (pH = 4.80) at GCE in the potential range of 0.60–1.40 V versus Ag/AgCl. Cyclic voltammogram of the 10 \(\mu M\) APG solution at a scan rate of 100 mV s\(^{-1}\) exhibited two well-defined oxidation peaks at 0.937 V (2.718 \(\times\) 10\(^{-7}\)) (first peak) and 1.107 V (1.581 \(\times\) 10\(^{-6}\) A) (second peak). The oxidation peaks are not accompanied by reduction peaks. The oxidation peaks are accompanied by reduction peaks.
peaks or waves, which indicate that the oxidation process is totally irreversible. That is, under these conditions, APG can only be oxidized on this electrode surface but not reduced on the electrode surface. These anodic peak potential values were in perfect agreement with the results obtained by Xing and his group.57

In this paper, to evaluate the interaction process, the changes were studied with gradual decreases (with the addition of the compound) in the peak current of dsDNA with CV based on the changes of guanine and adenine signals. In our study, the interaction between dsDNA and three compounds (separately from each other) was investigated in the bulk incubated solution based on the changes of guanine and adenine signals. The CV voltammograms of dsDNA showed two peaks24 corresponding to the oxidation of deoxyguanosine (dGuo) at 1.09 V and deoxyadenosine (dAdo) at 1.36 V, as shown in Figure 6a,b. As can be observed from Figure 6a,b, the oxidation peak of dGuo and the first peak of APG have overlapped at almost 1.10 V. Therefore, we only based the dAdo signal to evaluate the interaction.

The electrochemical study of the APG–dsDNA interaction by CV was carried out incubating 10 μM APG with 10 μM dsDNA in pH 4.80 acetate buffer with 1 min incubation period. After 1 min incubation time, the peak current of dAdo significantly decreased (3.64 × 10⁻⁷ A → 1.31 × 10⁻⁷ A). Also, it was observed that the oxidation peak of APG was shifting to a more positive potential (0.937 V → 0.966 V) with a smooth decrease in the first peak current.

In ADSAM, which we synthesized based on APG, we obtained an anodic peak current well defined only at 1.192 V (1.898 × 10⁻⁷ A) in the same buffer medium (pH 4.8 acetate buffer) of 5 μM ADSAM (Figure 7a,b). We think that this anodic peak originates from the –OH group in the para position of the ADSAM chemical structure. Naturally, no peak current was observed at this pH for the ATSAM solution. As seen in Figure 7a,b, when increasing amounts of ADSAM (0.625–5 μM) solutions were added to the 50 μM dsDNA solution, smooth decreases in anodic peak currents of dGuo (1.276 × 10⁻⁷ A → 4.038 × 10⁻⁸ A) and dAdo (3.644 × 10⁻⁷ A → 1.458 × 10⁻⁷ A) were recorded. In these conditions, where the currents decrease, the anodic peak potential has shifted to a more negative peak potential as in APG. Similar results were obtained in experimental studies with the ATSAM solution (Figure 8).

Voltammetric studies with dsDNA are crucial to explaining any biochemical mechanisms.58,59 Especially, experimental and theoretical explanation of the interaction mechanisms of small molecules with ssDNA/dsDNA is among the important research topics of the past 20 years. The most important reason for this is that these interactions are closely related to the toxicological, carcinogenic, and/or pharmacological activities of the substances studied.60 A decrease in the peak current of the small molecule by adding some ssDNA or dsDNA solution could be used for the determination of the binding constant and binding site size, while the shift in the peak potential of the small molecule could be used to determine the mode of interaction. In the literature, there are highly explanatory studies based on the interaction of different small molecules with dsDNA.61–63 The possible interaction mechanism between calf thymus dsDNA and three calcium antagonists, nifedipine, lercanidipine, and amiodipine, has published by Shahzad et al.64 In their study, the decrease in the peak current of guanine and adenine was used as an indicator for confirmation of the interaction event in acetate buffer of pH 4.70. Dogan-Topal et al.65 investigated the interaction of efavirenz with fish sperm dsDNA immobilized onto the pencil graphite electrode using the differential pulse voltammetric technique by an electrochemical DNA biosensor. They recorded a decrease in the guanine signal with the addition of the drug. Our another published work has been dedicated to

Figure 7. CV voltammograms of 50 μM dsDNA (black), 5.0 μM ADSAM (red), and dsDNA incubated with ADSAM (blue) in pH 4.8 acetate buffer (a). Cyclic voltammograms of 50 μM dsDNA (black) with increasing amounts of ADSAM (0.625–5 μM) in pH 4.8 acetate buffer (b). The arrow shows the decreases in peak current with respect to increases in the ADSAM concentration. The binding constants shown are the mean of three measurements and the uncertainty shown in the standard deviation from the mean derived from these measurements (in the top left corner).

Figure 8. Cyclic voltammograms of 50 μM dsDNA (black) with increasing amounts of ATSAM (0.3125–2.5 μM) in pH 4.8 acetate buffer. The arrow shows the decreases in peak current with respect to increases in the ATSAM concentration. The binding constants shown are the mean of three measurements and the uncertainty shown in the standard deviation from the mean derived from these measurements (in the top left corner).
deciphering the experimental and theoretical investigation of anticancer drug fludarabine-binding mechanism via multi-spectroscopic techniques, including UV absorption spectroscopy and thermal denaturation, fluorescence, and Fourier transform infrared (FTIR) spectroscopy, and electrochemical and viscosity measurement methods as well as molecular docking studies under physiological conditions. Our studies and literature search have shown that the binding mode between three compounds (APG, ADSAM, and ATSAM) and dsDNA is groove-binding as the dA-dO peak current significantly decreases regularly with increasing compound (APG, ADSAM, or ATSAM) concentration and the anodic peak potential shifts to a more negative region.

Based on our experimental results, the dsDNA binding constants ($K_b$) of APG, ADSAM, and ATSAM were calculated by the equation:

$$\log(1/[dsDNA]) = \log K_b + \log(S_{compound-dsDNA}/S_{compound})$$

The terms used in the equation are as follows: [dsDNA], dsDNA concentration alone; $S_{compound-dsDNA}$, current signal received from the compound after the interaction with dsDNA; and $S_{compound}$, current signal received from the compound alone. The $K_b$ and log $K_b$ values were calculated for each compound using this equation at room temperature (Table 4).

Table 4. Electrochemical Binding Constants $K_b$ for APG, ADSAM, and ATSAM

| compound | $K_b \pm SD^a$ | log $K_b \pm SD^a$ |
|----------|-----------------|-------------------|
| APG      | $(1.05 \times 10^9) \pm 0.04$ | $5.02 \pm 0.03$ |
| ADSAM    | $(3.47 \times 10^9) \pm 0.02$ | $5.54 \pm 0.03$ |
| ATSAM    | $(8.13 \times 10^9) \pm 0.03$ | $5.91 \pm 0.03$ |

$^a$All of the tests were run in triplicate and were expressed as the mean and standard deviation.

As can be seen in Table 4, the binding constant values calculated by electrochemical methods are in very good agreement with the binding constant values we obtained with other methods. These values indicate that the binding is in the form of groove binding. While the binding constants of the intercalated substances are around $\times 10^7$, the binding constants of the substances bound by groove binding are approximately $\times 10^9$ or $\times 10^{10}$.

3.2.5.3. Determination of Thermodynamic Parameters. The calculated $K_b$ values (Table 4) indicated that the three compounds formed at the 4.8 pH level were stable, such that there was a very strong interaction of all compounds with dsDNA. The following equations are used to determine the thermodynamic parameters ($\Delta H$, $\Delta S$, and $\Delta G$) from the $K_b$ constant values calculated at different temperatures.

$$\Delta G = -RT \ln K$$

$$\Delta G = \Delta H - T \Delta S$$

$$\ln K = \frac{-\Delta H}{R} (1/T) + \frac{\Delta S}{R}$$

$\Delta G$ values calculated at different temperatures against 1/T values were plotted using eq 13. It can be found that the correct $\Delta H$ values obtained were constant at different temperatures. In the graphs drawn for all items, $\Delta H$ values from the slope of this line ($-\Delta H/R$) and $\Delta S$ values from the cutting points ($\Delta S/R$) were calculated. All thermodynamic results are given in Table 5.

Table 5. Thermodynamic Parameters for APG–dsDNA, ADSAM–dsDNA, and ATSAM–dsDNA

| temperature (K) | $\Delta G$ (kJ mol$^{-1}$) | $\Delta H$ (kJ mol$^{-1}$) | $\Delta S$ (J K$^{-1}$ mol$^{-1}$) |
|-----------------|--------------------------|--------------------------|-------------------------------|
| APG–dsDNA       |                          |                          |                               |
| 288.15          | $-28.19$                 | $-11.70$                 | $+57.15$                      |
| 298.15          | $-28.65$                 | $-11.70$                 | $+57.15$                      |
| 308.15          | $-29.32$                 | $-11.70$                 | $+57.15$                      |
| ADSAM–dsDNA     |                          |                          |                               |
| 288.15          | $-31.62$                 | $-7.50$                  | $+81.36$                      |
| 298.15          | $-32.56$                 | $-7.50$                  | $+81.36$                      |
| 308.15          | $-32.93$                 | $-7.50$                  | $+81.36$                      |
| ATSAM–dsDNA     |                          |                          |                               |
| 288.15          | $-33.73$                 | $-10.80$                 | $+76.86$                      |
| 298.15          | $-34.45$                 | $-10.80$                 | $+76.86$                      |
| 308.15          | $-34.85$                 | $-10.80$                 | $+76.86$                      |

The obtained $\Delta G$ values proved that the interaction process was spontaneous and favorable for all three compounds. In addition, the results we have obtained from thermodynamic studies are in agreement with the literature. That is, if $\Delta H < 0$ or $\Delta H \approx 0$ and $\Delta S > 0$, the interaction type is electrostatic. When $\Delta H < 0$ and $\Delta S < 0$, van der Waals interactions or hydrogen bonds dominate the reaction, and when $\Delta H > 0$ and $\Delta S > 0$, hydrophobic interactions dominate the bonding process. The negative value of $\Delta G$ reveals that the interaction process is spontaneous, and the negative $\Delta H$ and positive $\Delta S$ values indicate that the electrostatic force plays the main role in binding the compounds to DNA.

3.2.6. Molecular Docking Results. Molecular docking calculations have been performed for APG, ADSAM, and ATSAM to determine the docking poses and to reveal the possible interactions between the investigated ligands and DNA. In molecular docking studies, for ADSAM and ATSAM, it was observed that the ligand molecules prefer minor groove binding. In addition to several hydrogen bonds, $\pi$–sulfur interactions were also involved in the stabilization of ADSAM–DNA and ATSAM–DNA complexes. These results were found to be consistent with the experimentally obtained data (Figure 9).

On the other hand, in fluorescence experimental studies, evidence has been obtained for APG, which indicates that ligand–DNA binding occurs probably via intercalation. Although docking results showed that APG also prefers minor groove binding, in contrast to ADSAM and ATSAM, it was observed that APG appears to be partially oriented toward the center of DNA on the vertical axis, probably due to its relatively small molecular structure (Figure 10). This orientation may cause elongation even if not causing unwinding in the DNA structure and can explain the experimental results obtained for APG, albeit partially.

Furthermore, it was observed that hydrogen bonds were involved in the stabilization of the APG–DNA complex. Binding affinities of the lowest energy docking poses of APG–DNA, ADSAM–DNA, and ATSAM–DNA complexes were found to be $-8.2$, $-8.5$, and $-8.4$ kcal mol$^{-1}$, respectively.
4. CONCLUSIONS AND FUTURE ASPECTS

We have reported the mechanism of dsDNA binding with apigenin and two novel apigenin sulfonamide derivatives (ADSAM and ATSAM). The $K_b$ constants calculated for APG, ADSAM, and ATSAM are in harmony for all techniques. The dsDNA binding studies revealed that APG performs partial intercalation between the base pairs as well as minor groove binding (partially causing elongation, even if not causing unwinding). The molecular docking studies of the investigated compounds (APG, ADSAM, and ATSAM) prefer minor groove binding. Furthermore, it was observed that the stabilization of the ligand–DNA complexes is achieved mainly by hydrogen bonds. As a result of the incorporation of dimethylsulfamate groups into the structure, in the ADSAM–dsDNA and ATSAM–dsDNA complexes, in addition to hydrogen bonds, $\pi$–sulfur interactions have also contributed to the stabilization of the ligand–DNA complexes.

The binding of three compounds to dsDNA in the minor groove promises that these compounds can be used in the medical field for further in vivo studies. Future work will include enzyme inhibition activity of the apigenin sulfonamide derivatives and investigate whether there are a direct correlation between enzyme inhibition activity and the concentration of apigenin and its sulfonamide derivatives. Consequently, further studies will be conducted to determine the active site of the sulfonamide derivative and the mechanisms of apigenin sulfonamide compounds to determine active pharmaceutical ingredients and food additives.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c02612.

- Flavonoid structural backbone of the main flavonoid groups (anthocyanidin, flavanone, flavone, isoflavone, chalcone, flavanidol, flavanonol, and flavonol) and Herschel–Bulkley parameters and calculated apparent viscosities for APG, ADSAM, and ATSAM at 20 s$^{-1}$ (PDF)

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Notes

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