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# Aspects of DNA interaction with the natural heterocyclic compounds

**Abstract.** Cancer is now one of the major challenges in the medical world. An unhealthy lifestyle, and abuse or inappropriate use of drugs stimulate this disease. The drug resistance and imperfections of drugs used in cancer therapy have enforced the development of new drugs with therapeutic potential. Despite the introduction of synthetic drugs, the discovery of new drugs with natural origin has increasing attention in the treatment of diseases, recently. As natural compounds show a considerable diversity of chemical structures, they are liable for diverse mechanisms of action and interaction with target molecules. Heterocyclic natural compounds are a substantial part of natural compounds with excellent properties. These compounds possess various interaction modes with DNA. This review focus on the heterocyclic natural compounds with therapeutic potential and their binding mode with DNA.
**Key words**: Natural drugs, heterocyclic compounds, cancer, DNA binding.

## Introduction

Cancer is the third main reason of death all over the world due to poor treatments and drug resistance [1]. It is estimated that deaths caused by cancer will grow dramatically in the following decades. The DNA damage induced by chemical and physical agents plays a critical role in cancer induction. The need for the protection of DNA against damage has led to much attention on the natural compounds with antimutagenic and antioxidant properties, which have minimal or no side effects for use in cancer treatments. Natural compounds or phytochemicals are important resources of bioactive compounds for developing new potent drugs due to their anticancer activity and clinical potential [2, 3]. These promising compounds are found in plants, bacteria, mushrooms, or marine organisms [4]. From ancient civilizations until today, the use of natural compounds has been considered medicinal agents. An impressive number of FDA-approved drugs are organic compounds derived from natural sources [5-7]. Many phytochemicals have a chemopreventive effect on DNA. Most natural biologically active compounds are heterocyclic ring-containing compounds that include hetero atoms such as oxygen, sulfur, or nitrogen as a part of the carbonic ring. Heterocycles have a special place among drug molecules owing to their comprehensive properties [8, 9]. Thanks to the presence of hetero atoms in their structure, heterocycles are able to effectively interact with receptors and enzymes through hydrogen bonding [10, 11]. These compounds can improve the pharmacokinetic and pharmaceutical properties of drug molecules through moderation of their lipophilicity [12]. It should be pointed out that the antioxidant and anti-mutagenic properties are the most important characteristics of heterocyclic natural compounds. As antioxidants can prevent DNA damage, their chronic consumption is increasing. Our survey of curcumin-derived heterocycles [13] motivated us to conduct a systematic literature review on the modes of DNA binding of natural heterocyclic compounds used as drugs.

## Major considerations

*Drug-DNA interactions.* The DNA strand is known to be the cellular target of many anti-cancer compounds. Since DNA contains genetic information, knowing the interaction mechanism of drug molecules with DNA strands is very important in biological systems. Drug interaction with the DNA strand can control cell function by regulating transcription (gene expression and protein synthesis) or by interfering with replication (the main stage in cell growth and division) [14, 15]. This aspect of DNA-interacting drugs has fascinated the field and expanded its applications. Small ligands bind

to DNA strands to mutate or inhibit their functions. Modified functions in transcription or replication can be used to treat various diseases. Anticancer drugs can interact with DNA in three ways: I) control of polymerases and transcription factors, where the drug molecules interact with proteins attached to DNA, and II) RNA-DNA binding. The structure of the triple helix or hybrid with RNA plays a critical role in the transcription of the DNA strand, and III) sticking of aromatic molecules to double-stranded DNA, which includes electrostatic interactions, intercalation between base pairs, and minor and major grooves binding [16].

*Binding modes of DNA and drug molecules.* The interaction between DNA and drug molecules can be classified into covalent and non-covalent interactions. Many drugs bind to DNA strands by forming covalent bonds within or between strands. Covalent binding of the drug to the DNA strand is irreversible, leading to complete prevention of processes related to DNA and, finally, cell death. These covalent binders are also called alkylating agents because of adding an alkyl group to the DNA chain. The most important advantage of alkylating agents is their high binding strength to base pairs in DNA [17]. Since alkylating agents kill cancer cells by inducing significant DNA damage, they have been used as anticancer drugs [18]. Alkylating agents can bind to the N and O atoms of the DNA bases, but O6 and N7 of guanine, N1, and N3 of adenine, and N3 of cytosine are preferential sites for intercalation [19].

*Non-covalent mode of binding.* This binding mode is classified into three types, including intercalation, groove binding, and backbone binding [20]. Figure 1 displays the schematic diagram of the non-covalent mode of binding. Non-covalent interacting agents possess less cytotoxic than alkylating agents. These interactions interfere with protein-DNA interactions, and also, affect the DNA conformational, mitochondrial DNA, and its function [21].

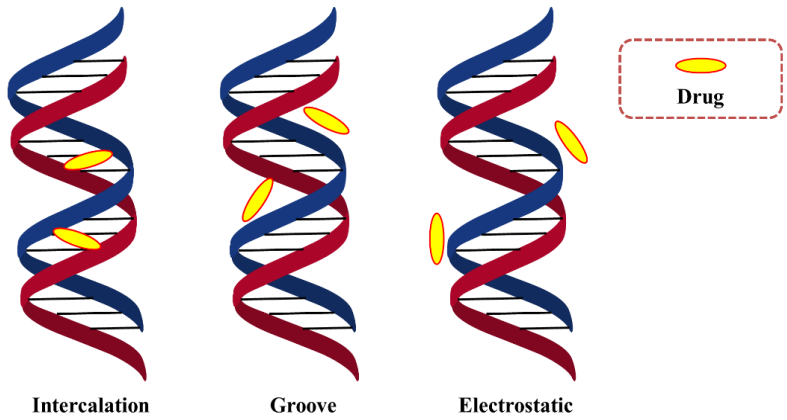



**Figure 1 –** Schematic diagram of different interaction modes

DNA intercalation is defined as the insertion of planar aromatic compounds, especially planar heterocyclic compounds, between adjacent base pairs in the DNA strand through a combination of π–π stacking, hydrogen bonding, and hydrophobic interactions, leading to significant changes in the DNA structure [22]. As intercalators disturb biological functions, such as DNA replication, they are used as antitumor drugs [23].

Various compounds can interact noncovalently with the minor or major groove of DNA by hydrogen bonding, and van der Waals interactions [24]. Major groove binders are often molecules with high molecular weights, such as proteins. Minor groove binders are aromatic or hetero-aromatic compounds with concave shapes to match the groove shape [25]. These compounds often bind to adenine N3 and O2 thymidine via hydrogen binding. Because of their ability to turn on/off gene expression, and disrupt protein–DNA interactions, minor groove binders are used in anticancer and anti-infective therapy as well as the treatment of viral, parasitic and bacterial infections [26, 27]. Backbone binding is the interaction of some molecules with phosphate groups of DNA. Metal complexes are a model of backbone binders with a positive charge and can bind with the DNA phosphate backbone with a negative charge [28].

*Methods of study of the mechanism of drug-DNA interactions.* Various analytical techniques, including UV-vis, infrared (IR), fluorescence, nuclear magnetic resonance (NMR) and circular dichroism spectroscopies, DNA melting studies, viscosity measurements, molecular docking, and electrochemical methods are employed to study the interaction nature of small molecules with DNA. This review focuses on UV-vis and fluorescence spectroscopies as the most commonly used methods.

*UV–Vis absorption spectroscopy.* UV-Vis absorption spectroscopy is one of the most commonly used techniques for studying DNA interactions with molecules because of its low cost, and simplicity. In these spectrometric studies, the changes in the absorption properties of the DNA or the molecules binding to DNA are monitored. DNA molecule shows maximum absorption at 250 nm [29]. As DNA interacts with the molecules, its structure changes, and it results in the decreasing or increasing absorption band along with the bathochromic shift or hypsochromic shift (Figure 2A).

However, DNA-binding molecules exhibit an absorption band in the visible region, which shifts to longer or shorter wavelengths. The magnitude of these shifting is considered an indicator of the interaction strength [29-32]. Intercalators usually induce hypochromism or hyperchromism effects, which mean a decrease or increase in the intensity of the absorbance band, respectively [33]. Hyperchromism arises from the damage to the double helix structure of the DNA, which leads to forming its single-stranded.[34-36] Hypochromism occurs because of the contraction of DNA in the helix axis. It also arises from the conformational changes of DNA [37, 38]. These compounds also result in bathochromic shifts of over 15 nm [39, 40]. In the case of groove binders, a decrease or increase in the absorption band occurs along with a small shifting or no shifting because the structural alteration of DNA or the binder is minimal (Figure 2B) [41]. An increase in the absorption intensity (hyperchromism) is observed in the case of the electrostatic mode of binding [16].

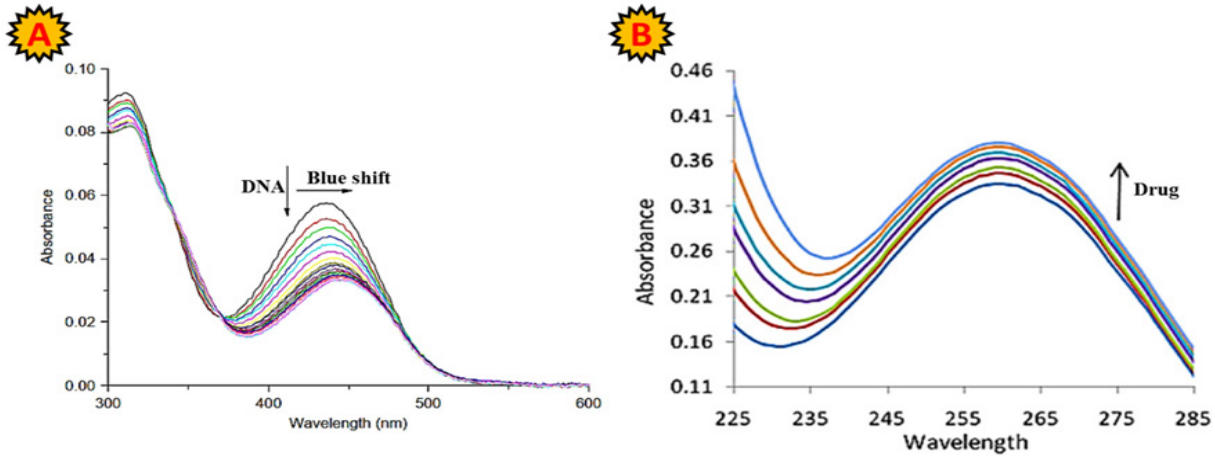



**Figure 2 –** Investigating the interaction between DNA and drug using UV-vis spectroscopy related to (A) an intercalator, (B) a groove binder. It is reproduced from References [42], [43]

Concerning the absorbance changes, the binding constant (K) of the molecule with DNA can be calculated based on the following equation, [44]:

$$\frac{A_0}{A-A_0} = \frac{\varepsilon_M}{\varepsilon_{Com}-\varepsilon_M} + \frac{\varepsilon_M}{\varepsilon_{Com}-\varepsilon_M} \times \frac{1}{K[DNA]}$$

Where K, $A_0$, and A are the binding constant, molecule absorbance, and absorbance of molecule-DNA complex, respectively. $\varepsilon_M$ and $\varepsilon_{Com}$ represent the absorption coefficients of the molecule and its complex with DNA. K can be obtained in two ways: i) from the intercept-to-slope ratios of $A_0$/(A-$A_0$) vs. 1/[DNA] plot, ii) from the intercept-to-slope ratios of the plot of [DNA] vs. [DNA]/$\varepsilon_M$–$\varepsilon_{Com}$.

*Fluorescence spectroscopy.* The other most common method to study interactions between DNA and molecules is fluorescence spectroscopy. This method has advantages over other techniques

due to its high selectivity, sensitivity, and wide linear dynamic range. Compounds containing aromatic rings possess intense fluorescence [31, 45]. The fluorescence spectroscopy and techniques based on fluorescence, such as fluorescence quenching, can be used to determine the binding mode of interaction between DNA and small molecules. Commonly, the change in the intensity of drug fluorescence in the presence of different concentrations of DNA is investigated due to the lack of fluorescence in DNA.

Fluorescence emission strongly depends on the environment, and so, the spectral shifts of about 10 to 20 nm occur in the excitation and emission spectra of binding molecules because of fluorophore transfer from high polar environments to non-polar environments [46]. Moreover, a strong interaction between DNA and binding molecules can usually lead to an increase in fluorescence intensity significantly. Thus, the fluorescence intensity enhances usually, whereas molecules bind to DNA through intercalative mode (Figure 3A) [16].

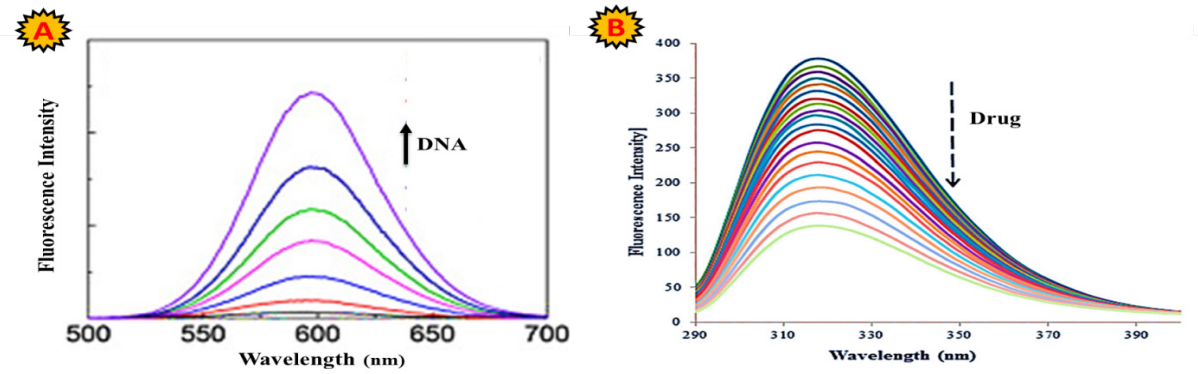



**Figure 3 –** Investigating the interaction between DNA and drug using fluorescence spectroscopy related to (A) an intercalator, (B) a groove binder. It is reproduced from References [43] and [49]

It is noteworthy that when a molecule binds to the DNA groove, its fluorescence intensity decreases normally compared to before the binding to DNA (Figure 3B) [46-48].

Generally, the rotation of the molecule leads to fluorescence quenching. As intercalators incorporate into the DNA base stack, they induce a significant enhancement in the fluorescence emission.

## Results and discussion

A systematic review of articles about the interaction between natural heterocyclic compounds and DNA was conducted on the articles published from 2005 to 2022. Searches for related publications were carried out in Google scholar. This study aimed to provide practical guidance that helps researchers in the development of new drugs, especially drugs based on natural compounds. English-language original articles concerning heterocyclic compounds, natural compounds, and the evaluation of DNA interaction were chosen. After reviewing the articles based on the title and abstract, selected full-text articles were read, and those fitted with the exclusion criteria were eliminated (Table 1).

Cancer is one of the main causes of mortality worldwide. This disease is characterized by the uncontrolled proliferation of organ cells, leading to metastasis [50]. The main aim of cancer researchers is to identify new strategies to reduce cancer treatment costs and patient suffering. Chemotherapy shows severe adverse effects such as the death of healthy cells, anemia, hair loss, etc. Also, many used medications are not effective and safe [51, 52].

Natural products and their derivatives possess a critical role in the development of chemotherapeutics due to their wide structural diversity, and pharmacological and molecular characteristics. They also reduce the side effects of chemo-radiotherapies [53, 54].

**Table 1 –** Inclusion and exclusion criteria utilized in this study

| Parameter | Inclusion criteria | Exclusion criteria |
|---|---|---|
| Language | English | Any other language |
| Type of publication | Original Articles | Conference articles, letters, or any other type of publication |
| Origin of compounds | Natural compounds | Synthetic compounds |
| Chemical structure | Heterocyclic compounds consisted of the single rings and fused rings. | Cyclic compounds without heteroatom |

49% of all anticancer molecules approved between 1984 and 2014 were derived from natural products [55]. Note, plant-derived medicines are used in treating other degenerative diseases such as HIV/AIDS and diabetes [56].

One of the most important compounds in the innovation of new drugs is heterocyclic compounds, especially natural heterocyclic compounds. They possess unique properties, including antioxidant and anti-mutagenic properties. Also, heterocyclic compounds can effectively interact with DNA through different modes. This article has systematically reviewed literature data on binding modes of complexes formed between the natural heterocyclic compounds and DNA. In this study, natural heterocyclic compounds were classified based on the number of their interactions with DNA as follows: single binders, dual binders, and multi binders.

*Single binders.* These compounds interact with DNA through only one binding mode. Natural compounds included in the single binders are alkaloids, flavonoids, diterpene, and carotenoids.

Figure 4 shows the structure of some of the single binders.

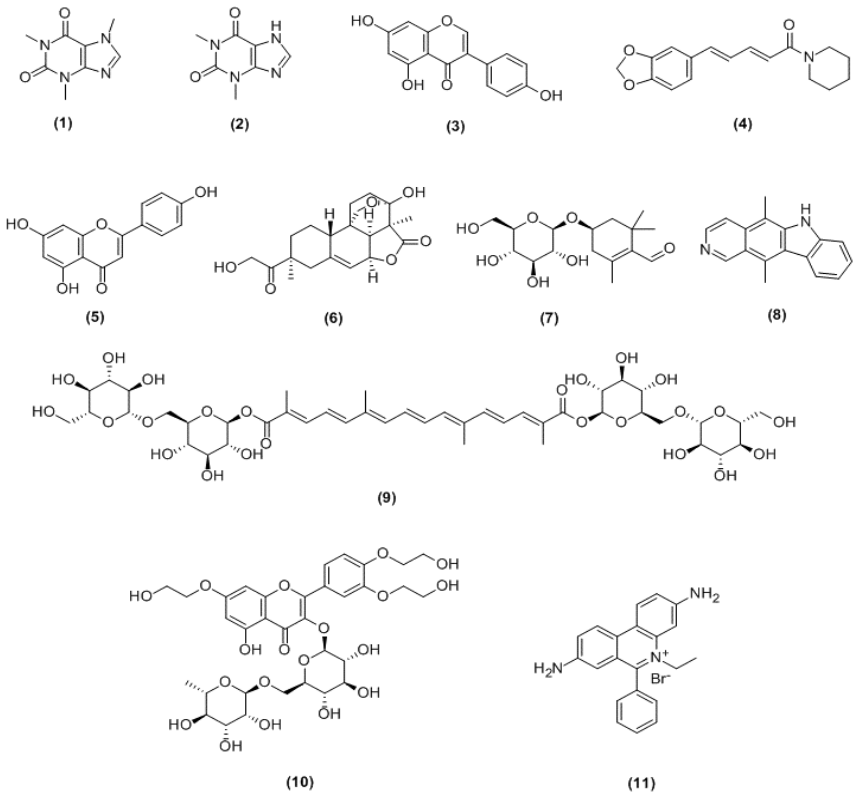



**Figure 4 –** Structures of single natural binders described in the text: (1) caffeine, (2) theophylline, (3) genistein, (4) piperine, (5) apigenin, (6) annonalide, (7) picrocrocin, (8) ellipticine, (9) crocin, (10) troxertuin, (11) ethidium bromide

*Alkaloids.* These compounds are a group of nitrogen-containing compounds, naturally occurring organic compounds used as a rich source for drug discovery [57]. Most single natural binders are small alkaloids, including two to four rings, such as caffeine, theophylline, and ellipticine. Caffeine, and theophylline are alkaloids with two fused rings containing oxy and hydroxyl groups, as well as nitrogen atoms in heterocyclic rings. Nafisi et al. [58], studied the DNA interaction with theophylline and caffeine using UV-visible and FT-IR spectroscopies. The results showed that both compounds could form hydrogen binding to the A-T and G-C bases through their NH and C=O functional groups, and the groove binding mode was revealed for them. Piperine is the major alkaloid present in black pepper (Figure 4).

It possesses wide pharmacological activities, such as antimutagenic, anti-inflammatory, antioxidant, antitumor, antiapoptotic, antiarthritic antigenotoxic, antimicrobial, anti-HBV activities, etc. [59-61]. Haris et al. [62] studied the interaction mechanism between piperine and ctDNA using spectroscopy, DSC, melting, and simulation methods, for the first time. Since the UV-vis spectrum of ctDNA in the presence of piperine shows a hypochromic effect without any significant shift in the maximum wavelength (Figure 5A), it can be inferred that the DNA conformation remains unchanged. This indicates the groove binding mode between this natural alkaloid and ctDNA. Piperine exhibits a strong emission peak at 486 nm, which decayed in the presence of DNA (Figure 5B).

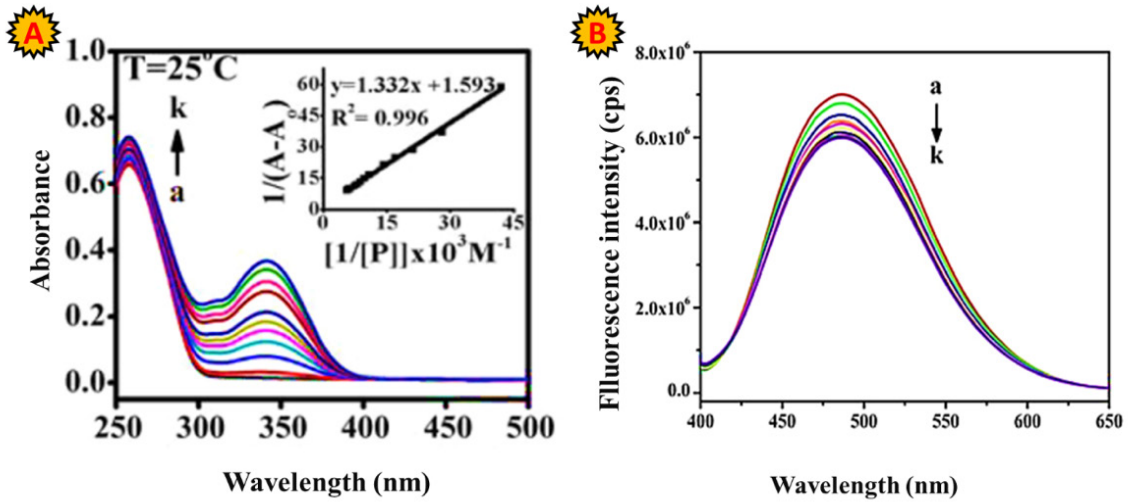



**Figure 5 –** (A) UV-vis absorption spectra of ctDNA (100 μmol $L^{-1}$) in the absence and presence of piperine (0-180.45 μmol $L^{-1}$) in phosphate buffer solution (pH 7.0, 100 mmol $L^{-1}$ NaCl) at 25 °C, (B) Fluorescence emission spectra of piperine (50 × $10^{-5}$ mol $L^{-1}$) in the presence of different concentrations of ctDNA (0-45.33 ×$10^{-6}$ μmol $L^{-1}$) on excitation at 342 nm. Reproduced from the reference [62]

The emission quenching is an indication of the groove binding mode, and it confirms the results obtained by the UV-vis study. In this study, the results obtained from spectroscopy methods were agreement with those obtained from melting and DSC analysis. It was evident from the simulation studies that Piperine interacts with ctDNA through wan der Waals and H-bonding. Ellipticine, a plant-derived alkaloid, consists of four fused rings (Figure 4). Owing to its planar structure, ellipticine acts as the intercalator and it stacks the DNA helix through interaction between its methyl groups and the thymine bases of DNA [63, 64].

*Flavonoids.* These compounds are the most abundant polyphenols found in plant-based products. Flavonoids are known to possess diverse pharmacological activities [65, 66]. They generally refer to a class of compounds formed by two phenyl rings and a heterocyclic containing oxygen atom. Most flavonoid compounds, such as genistein apigenin, and troxerutin, belong to the single binder group. Genistein is an important polyphenolic compound with anticancer property existed in our diet [67]. The interaction between genistein and DNA was investigated by Bocian et al. [68]. Their findings indicated that the phenyl ring A in the

genistein structure is not coplanar with other rings. Consequently, genistein is a DNA groove-binding molecule due to its non-planar structure. A hydrogen bond between hydroxyl groups of genistein and protons of cytidine NH is suggested (Figure 6).

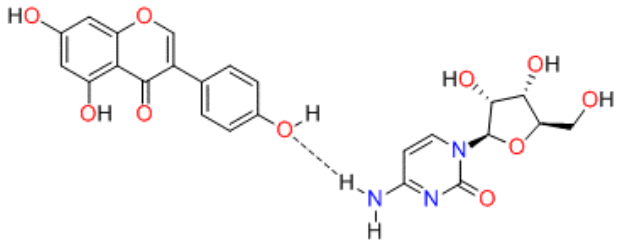


**Figure 6 –** The interaction between genistein and cytidine

Apigenin (Figure 4) is a flavonoid that has aroused great interest because of its medicinal properties, including antioxidant, anticancer, and anti-inflammatory [69]. For the first time, Zhang et al. [70] explored the interaction between apigenin and ctDNA using fluorescence spectroscopy, UV-vis spectroscopy, DNA melting, and viscosity measurements. The isoabsorptive point, hypochromism, and bathochromic shift were observed at the UV-vis spectrum of the apigenin-DNA complex, confirming the intercalation of apigenin into the DNA structure (Figure 7A). In the presence of DNA, the intensity of apigenin emission enhanced along with a bathochromic shift in the emission maximum (Figure 7B).

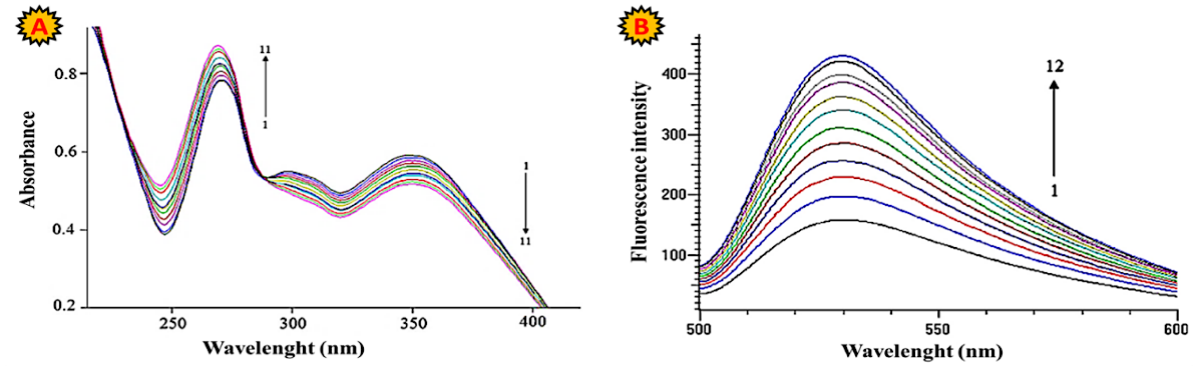
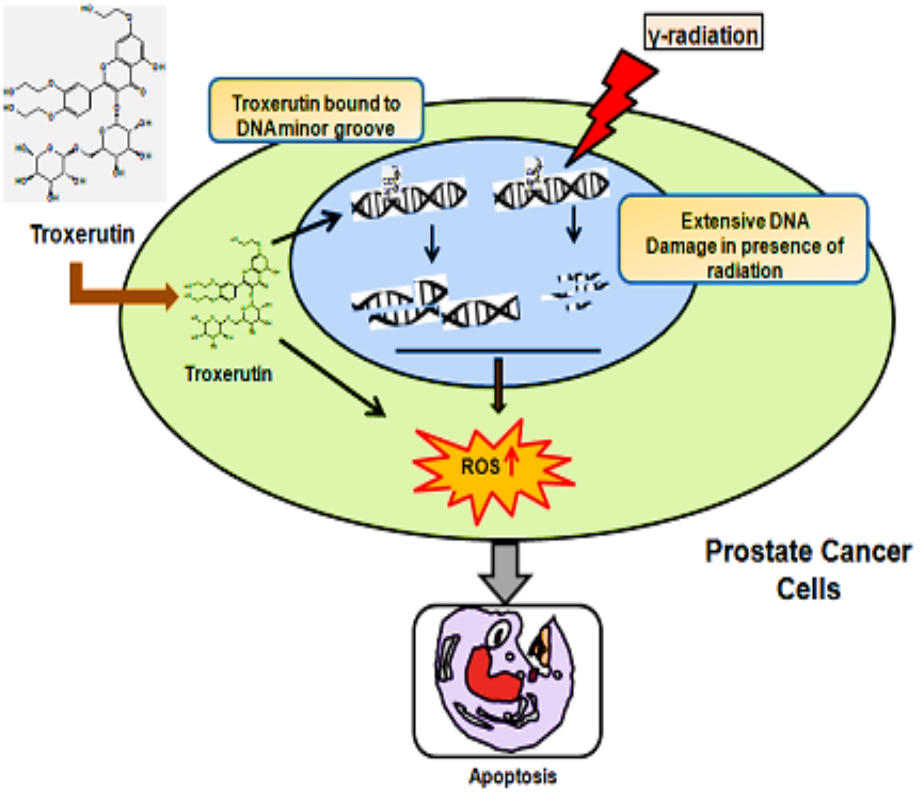



**Figure 7 –** (A) UV−vis absorption spectra of apigenin ($9.10 \times 10^{-5}$ mol $L^{-1}$) in the absence and presence of DNA (0-$8.33 \times 10^{-5}$ mol $L^{-1}$) at 25 °C, (B) Fluorescence emission spectra of piperine ($9.10 \times 10^{-5}$ mol $L^{-1}$) in the presence of different concentrations of ctDNA (0−$4.76 \times 10^{-5}$ mol $L^{-1}$) at 25 °C. It is reproduced from Ref. [70]

The intensity enhancement and bathochromic shift in the fluorescence emission upon molecule binding to DNA are the indicators of intercalative binding mode. The results obtained from DNA melting and viscosity measurements were consistent with the findings of fluorescence and UV-vis spectroscopies. Troxerutin is known to exhibit the radio-protective property. Panata et al. [71] reported that this flavonoid interacts with DNA through minor groove binding. They conducted competing experiments using EtBr and 4′,6-diamidino-2-phenylindole (DAPI). A significant decrease in DPA fluorescence with increasing concentration of troxerutin confirmed troxerutin as a groove binder. The results of the MTT assay showed that this flavonoid could penetrate in the nucleus of cancer cells, break the DNA strand by binding to the minor grooves of DNA and induce cell death. The cytotoxicity effect of troxerutin was enhanced in combination with gamma radiation (Scheme 1).

*Diterpene*. Annonalide (Figure 4), a pimarane-type diterpene, is also included in the class of single binders. This natural compound has attracted considerable interest because of its potential cytotoxic activity. Marques et al. [72] investigated the interaction of annonalide with DNA through fluorescence and computational studies. In this research, ethidium bromide (EB) (Figure 4) and Hoechst (HO) were used as the probes for fluorescent competition assays. EB is known as an intercalator that is widely used in competition assays. The free molecule of EB presents low emission, and when it intercalates DNA, a considerable enhancement occurs in its fluorescence emission. The presence of an intercalator in the medium can result in the displacement of EB, leading to a decrease in the

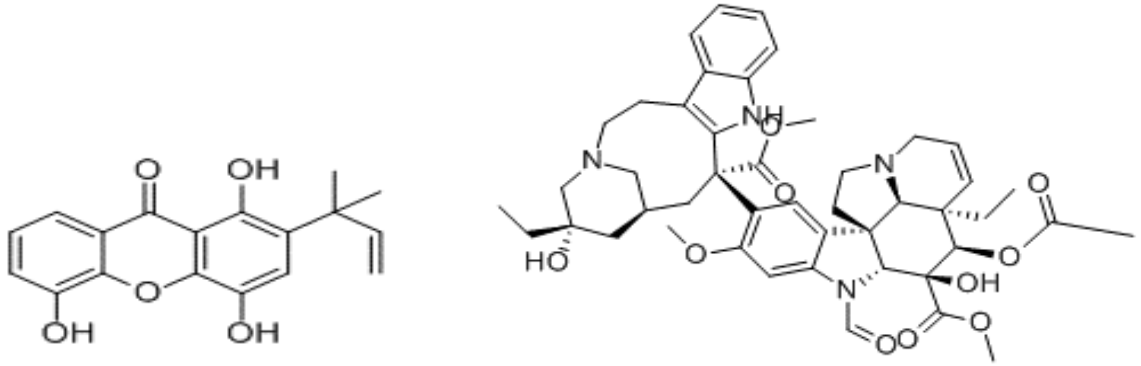
emission intensity of EB. HO is a groove binder, and the principle of its function in the competition assay is similar to EB. The spectrofluorometric titration of EB with DNA in the presence of annonalide indicated that this natural compound binds to DNA through intercalation mode. The spectroscopic results were confirmed by computational studies. So, annonalide is a typical DNA intercalator because it does not have the structure of general intercalators, including planarity. According to docking results, van der Walls is the main interaction between DNA and annonalide.




**Scheme 1 –** The cytotoxicity effect of troxerutin in prostate cancer cells alone and in combination with γ-radiation. Reproduced from the reference [71]

*Carotenoids and other bioactive compounds*. Carotenoids are a large group of pigments found in animals, plants, and microorganisms [73]. They are identified with several biological functions [74]. Crocin, and picrocrocin are the studied-DNA binding molecules found in saffron (Figure 4). The studies indicated that these compounds are DNA groove binders [6, 75]. Crocin is one of the important natural carotenoids of saffron that are responsible for the anticancer of property of this plant-based product [6]. Picrocrocin is a glycoside that plays a critical role in the taste formation of saffron [76]. Bathaie et al., [6, 75] studied the interaction between important saffron carotenoids, such as crocin and picrocrocin, using spectroscopies methods. The results obtained in this study revealed that these carotenoids bind to DNA through minor groove binding due to the lack of a planar ring.

*Dual binders*. Large alkaloids, such as vincristine, are included in the category of Dual binders. The structure of dual binders studied in this review is displayed on Figure 8.



(1) (2)

**Figure 8 –** Structures of natural dual binders described in the text:
(1) 12b-hydroxy-des-D-garcigerin, (2) vincristine

Vincristine is a natural plant alkaloid used widely for cancer therapy [77-79]. Hence, studying the mechanism of its interaction with DNA is biologically important. Tyagi et al. [80] studied the binding mode of vincristine to DNA using spectroscopic methods. The binding of vincristine to DNA caused a hypochromic effect accompanied by a bathochromic shift in the absorption of vincristine, indicating that it intercalates DNA. The results of FT-IR proposed that vincristine can also bind to DNA through guanine and cytosine, as well as phosphate backbone. Consequently, vincristine interacts with the DNA helix via external and intercalative binding modes. A few years later, Mohammadgholi et al. [81] confirmed these results.

*Xanthones* are a vast group of natural heterocyclic compounds known to have a wide range of pharmacological activities [82-84]. Wu et al., [85] investigated the interaction between 12b-hydroxy-des-D-Garcigerin (GA) and DNA using the displacement assay. In this study, acridine orange was used as the intercalator probe in the spectroscopic methods. The titration of DNA with acridine orange in the presence of GA led to changes in the absorption spectrum of GA-DNA. As these changes were similar to that of acridine orange-DNA, it could be concluded that GA acted as an intercalator. The results obtained by spectrofluorimetry confirmed the spectrophotometric result. Scatchard analysis implied the interaction of GA with DNA through electrostatic interaction (backbone binding). Consequently, GA binds to DNA through dual binding modes, including intercalation and backbone binding.

*Multiple binders.* Quercetin (Figure 9) is a natural phenolic compound with promising bioactive effects, including anticancer, antioxidant, antidiabetic, antimicrobial, etc. [86, 87]. Study on its interaction with DNA has gained prominence in recent years. Some researchers have regarded quercetin as the intercalator [88, 89]. With regard to their results, the planar structure of quercetin plays a critical role in its binding to DNA via intercalation. Others insist on groove binders or backbone binders [90, 91]. Hence, it can be concluded that quercetin can probably bind to DNA in several ways. It is proposed that the ability of this compound as the intercalator and groove binder is concentration-dependent [88]. It is worth highlighting that this compound preferably binds to the G base [92]. It strongly interacts with the protonated form of DNA through π-stacking and electrostatic interactions [91].

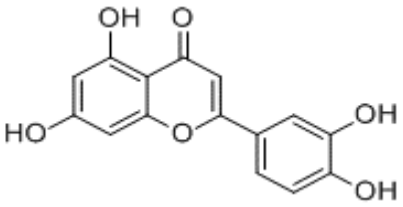


**Figure 9 –** The structure of quercetin

Finally, the results of this study demonstrated that almost of natural heterocyclic compounds contain nitrogen, oxygen, or both heteroatoms. The non-planar heterocyclic compounds can form hydrogen bonding with DNA bases and act as groove binders. In the case of intercalation mode, it can be stated that this binding mode is stronger influenced by the planarity compared to the heteroatom type. It is worth noting that in all investigated studies, there was no information about the type effect of hetero atom on the interaction mode.

## Conclusion

Natural heterocyclic compounds have increasing interest in the biomedical field, especially cancer therapy, owing to their wide range of pharmaceutical properties. They exhibit different DNA binding modes. Basically, small natural heterocyclic molecules interact with DNA through two different modes: intercalation or groove binding. All studies included in this review revealed that planarity is the most important characteristic of heterocyclic compounds acting as the intercalator. The studies on large natural molecules have provided promising results about the interaction between DNA and these large molecules. Large molecules containing planar and non-planar parts in their structure can interact with DNA in more than one way. To investigate the binding modes, different techniques, including spectrometry methods, are employed. In the case of UV-vis spectroscopy, a significant increase in the absorption intensity (hyperchromism) and redshift is typically observed for intercalation mode. In contrast, the decrease in the absorption intensity (hypochromism) is an indication of the electrostatic mode of interaction. In fluorescence spectroscopy, increasing the emission intensity is an indicator of intercalators, while decreasing the emission intensity is the indicative of groove binding and electrostatic modes. This systematic review revealed the great importance of natural heterocyclic compounds in the field of drug discovery, as well as their therapeutic potential and the mechanism of their interaction with DNA. This article and references can be helpful for researchers starting in this field.

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