Biotechnology Research and Innovation, 2024, 8(1): e2024010. https://doi.org/10.4322/biori.00102024

SBBiotec
SOCIEDADE BRASILEIRA DE BIOTECNOLOGIA

Biotechnology
Research & Innovation

www.biori.periodikos.com.br

REVIEW ARTICLE

Naphthoxazole and benzoxazole as fluorescent DNA probes - a systematic review

Délis Galvão Guimarães[a,*], Victória Laysna dos Anjos Santos[a], Sidney Silva Simplicio[b], Larissa Araújo Rolim[a], Arlan de Assis Gonsalves[b], Cleônia Roberta Melo Araújo[c]

[a]*Program in Biotechnology - Northeast Network of Biotechnology - RENORBIO, Federal Rural University of Pernambuco - UFRPE, Rua Dom Manoel de Medeiros, s/n - Dois Irmãos, CEP 52171-900, Recife, PE, Brazil*
[b]*Graduate Program in Materials Science, Federal University of Vale do São Francisco, Av. Antônio Carlos Magalhães, 510 - Country Club, CEP 48902-300, Juazeiro, BA, Brazil*
[c]*Graduate Program in Health and Biological Sciences, Federal University of Vale do São Francisco, Av. José de Sá Maniçoba, s/n - Centro, CEP 56304-917, Petrolina, PE, Brazil*

Highlights

- Changes in fluorescence emission are dependent on the DNA concentration
- A primary mode of interaction, dependent on DNA concentration, was identified
- The feasibility of exploring this field of study was revealed

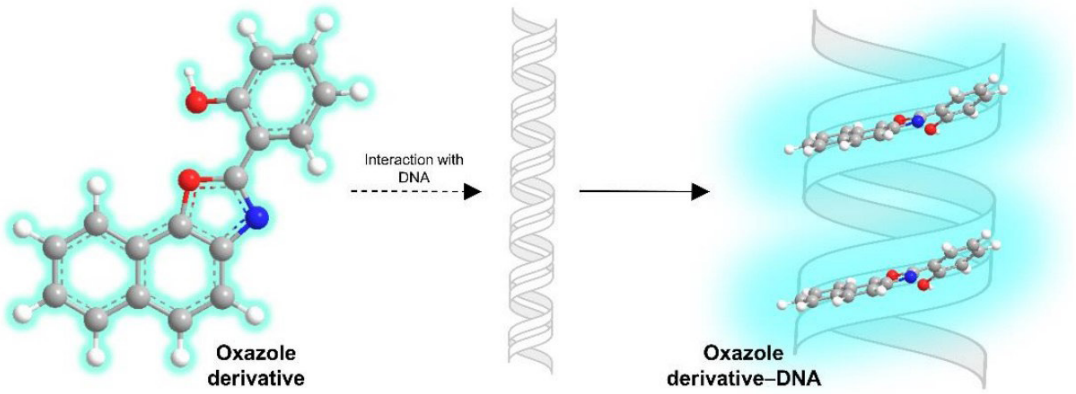
Received Apr-24-2024; Accepted Aug-04-2024

KEYWORDS
Fluorescence emission;
Nucleic acid;
Extended aromatic system;
Oxazole derivative;
Small molecule-DNA interaction.

Abstract: Considering the mutagenic effects and environmental concerns associated with commonly used fluorescent DNA probes, benzoxazoles and naphthoxazoles emerge as sensitive and safer alternatives. Benzoxazoles and naphthoxazoles are attractive from both chemical and biological perspectives. In addition to their multiple biological activities, these compounds exhibit promising photoluminescent properties and other characteristics that are favorable for use as fluorescent DNA probes. This systematic review investigates the potential of benzoxazole and naphthoxazole derivatives to bind to DNA and emit fluorescence. Scientific articles published in the last decade (2012-2023), selected based on pre-established inclusion criteria, indicate the potential of these compounds to bind to DNA and exhibit enhanced fluorescence emission. More than 70% of the compounds evaluated for DNA interaction were naphthoxazole derivatives. All seven assessed compounds showed DNA binding behavior, with intercalation being the predominant mode of interaction, particularly at increasing DNA concentrations. Furthermore, all reported benzoxazoles and naphthoxazoles exhibited increased fluorescence emission intensity with higher DNA concentrations. The results of this study indicate that benzoxazole and naphthoxazole derivatives have significant potential for use as fluorescent DNA probes and suggest considerable opportunities for further research and development in this area.

*Corresponding author.
E-mail: delisgalvao@gmail.com (D. G. Guimarães).

Graphical Abstract



Introduction

Benzoxazoles and naphthoxazoles are versatile heterocycles featuring the oxazole nucleus. These compounds can be applied across various fields, ranging from biology and pharmacology to chemistry and materials science (Kurutos et al., 2022; Prabhala et al., 2022). While oxazoles exhibit chemical characteristics favorable for their application as fluorescent DNA probes, such as binding to receptors in biological systems through non-covalent interactions and attractive photoluminescent properties (Deng et al., 2022; Guerrero-Pepinosa et al., 2021; Phatangare et al., 2013; Rubio & Zanocco, 2016), studies on the interaction of benzoxazole and naphthoxazole derivatives with DNA have been sparsely reported in the literature.

Considering this context and the need to develop effective and less toxic DNA fluorescent probes, this systematic review provides insight into the potential of benzoxazole and naphthoxazole derivatives to bind to DNA and emit enhanced fluorescence. Additionally, the scarcity of peer-reviewed literature on the fundamentals of fluorescence emission and its biotechnological applications has encouraged us to introduce such concepts at the beginning of this study.

The principle of fluorescence: from light absorption to light emission

Luminescence is the phenomenon of emitting ultraviolet (UV), visible (UV-Vis), or infrared (IR) photons by an electronically excited species. The stimuli, which represent different modes of excitation, determine the classification of the various types of luminescence. Luminescence can be divided, for example, into electroluminescence, thermoluminescence, chemiluminescence, bioluminescence, and photoluminescence, according to whether the stimuli are electrical, thermal, chemical, biochemical, or luminous, respectively (Valeur, 2001).

Therefore, the emission of photons, accompanied by the relaxation of an absorbing species previously brought to an excited electronic state by the absorption of a photon, is referred to as photoluminescence. Within this classification, the concepts of fluorescence and phosphorescence are encompassed, distinguished by the nature of the excited state (Valeur, 2001).

Fluorescence is the emission of light from excited singlet electronic states (S_1, S_2). In singlet excited states, the electron in the excited orbital remains paired, that is, with the opposite spin to the second electron in the orbital of the ground state (S_0) (Figure 1a). Consequently, the return to the ground state is allowed and occurs rapidly through the emission of a photon. Fluorescence emission rates are typically on the order of 10^{-8} s. This transition is referred to as a singlet-singlet transition since the spin remains unchanged after the promotion of the electron from the ground molecular orbital to the higher energy molecular orbital. In other words, the emission of photons, accompanied by the relaxation $S_1 \rightarrow S_0$, is termed fluorescence (Figure 1b) (Lakowicz, 2006; Valeur, 2001).

Fluorescence typically occurs in aromatic organic molecules. Some examples include fluorescein, rhodamine B, 1,4-bis(5-phenyloxazol-2-yl)benzene (POPOP), and quinine (Figure 2). Fluorescent substances, such as those mentioned earlier, are referred to as fluorophores. Quinine, found in tonic water, was the first known and studied fluorophore, and it was from the study of this fluorophore that the first spectrofluorometers were developed in the 1950s (Lakowicz, 2006; Valeur, 2001). Except for lanthanides, atoms generally do not fluoresce in condensed phases, unlike aromatic organic molecules (Lakowicz, 2006).

When excited by photon absorption, fluorophores can return to the ground state with fluorescence emission. However, other relaxation pathways are also possible, such as direct return to the ground state without fluorescence emission, referred to as internal conversion; intersystem crossing, possibly followed by phosphorescence emission; intramolecular charge transfer; and conformational changes

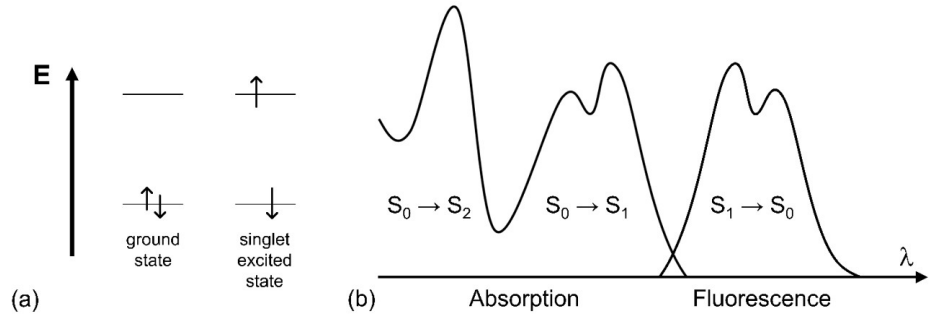


Figure 1. (a) Description of the excited singlet state; (b) Representation of the relative positions of absorption and fluorescence spectra.

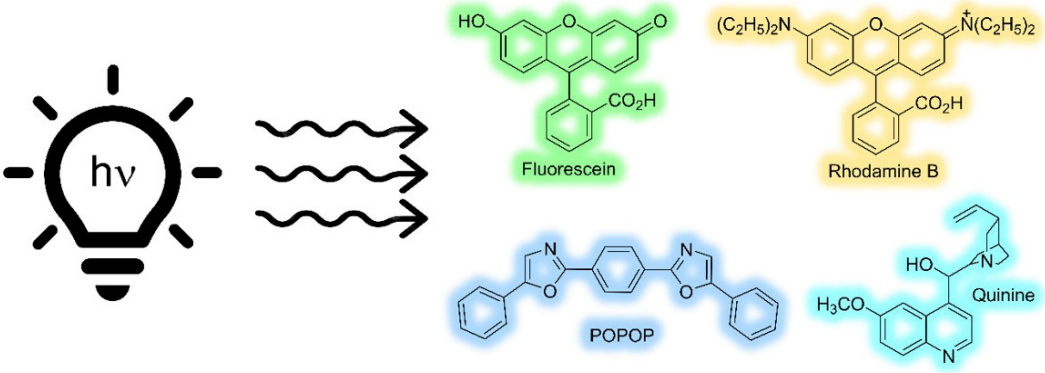


Figure 2. Molecular structures of fluorescein, rhodamine B, POPOP, and quinine, illustrating their respective fluorescence colors.

(Valeur, 2001). Additionally, intermolecular interactions in the excited state can also compete for the relaxation of fluorophores, such as electron, proton, and energy transfer processes. These relaxation pathways can compete with fluorescence emission if they occur on a timescale comparable to the lifetime of a fluorophore, which is the average time between excitation and return to the ground state (Lakowicz, 2006; Valeur, 2001). On the other hand, some of these excited state processes, such as conformational changes and electron, proton, and energy transfers, may lead to a fluorescent species whose emission can overlap with that of the initially excited molecule. This emission, therefore, must be distinguished from "primary" fluorescence (Valeur, 2001).

Molecules with excited-state intramolecular proton transfer (ESIPT) properties exemplify this characteristic well. ESIPT involves a very rapid phototautomerism, on the order of subpicoseconds, of organic molecules following photoexcitation (Benelhadj et al., 2014; Sun & Fang, 2023; Wang et al., 2023). During this mechanism, an acidic proton is transferred to a basic site within the same molecule following photoexcitation (Lukeman et al., 2015). Molecules possessing ESIPT properties exist as enol (E) structures in the ground state (S_0) and, upon light stimulation, transition from the ground state (S_0) to the excited state (E*; S_1). In this state, the molecule is energetically elevated and structurally unstable and can either revert to the ground state (S_0) by fluorescence emission or form a keto structure (K*) through an ultrafast ESIPT reaction, thereby releasing excess energy. This keto structure in the excited state (K*) is also unstable and reaches the ground state through fluorescence emission. Finally, the ketonic structure in the ground state (K) undergoes reverse proton transfer (RPT) back to the most stable state, the enol structure of the ground state (E) (Felouat et al., 2021; Wang et al., 2023).

Fluorophores are divided into two main classes: intrinsic (or natural) and extrinsic. Intrinsic fluorophores occur naturally and include aromatic amino acids (tryptophan, tyrosine, and phenylalanine), nicotinamide adenine dinucleotide (NADH), flavins, pyridoxal derivatives, and chlorophyll. Extrinsic fluorophores, on the other hand, are added to a sample when it lacks intrinsic fluorescence or to alter the spectral properties of the sample. Fluorescein and rhodamines, as mentioned earlier, are examples of widely used extrinsic fluorophores (Lakowicz, 2006).

After the term "fluorescence" was introduced by physicist and mathematician George Gabriel Stokes in 1832, fluorophores have been integrated into various aspects of daily life (Valeur, 2001). Examples include polycyclic aromatic hydrocarbons such as anthracene and perylene, whose fluorescence emission is utilized in environmental monitoring of oil pollution (Lakowicz, 2006); fluorescein, which is employed in various medical practices, such as diagnostics and surgical procedures (Rodrigues et al., 2022); and POPOP and acridine orange, used respectively in scintillation counting

and as DNA probe (Lakowicz, 2006). Additionally, fluorophores find applications across diverse research fields, ranging from hydrographic studies to their role as emergency markers for locating individuals at sea, such as during spacecraft capsule landings in the ocean (Lakowicz, 2006).

Properties of fluorescent DNA probes

Rarely does a species of interest exhibit fluorescence, or fluorescence in a convenient region of the UV-Vis spectrum. For example, DNA and lipids lack intrinsic fluorescence; in these cases, extrinsic fluorophores are utilized as markers (Lakowicz, 2006). Extrinsic fluorophores that bind to biomacromolecules, such as membranes, proteins, and DNA, are commonly referred to in the literature as fluorescent probes (Lakowicz, 2006; Liu et al., 2023; Santos-Junior et al., 2021).

Fluorescence lifetime (τ_f) and fluorescence quantum yield (Φ_f) are important properties of a fluorophore (Lakowicz, 2006; Valeur, 2001). The fluorescence lifetime of the excited state is defined as the average time the molecule spends in the excited state before returning to the ground state. Generally, fluorescence lifetimes are on the order of 10 nanoseconds (ns) (Lakowicz, 2006). The fluorescence quantum yield (Φ_f) is the ratio of the number of emitted photons to the number of absorbed photons (Brouwer, 2011; Lakowicz, 2006; Valeur, 2001).

Several probes spontaneously bind to DNA and then exhibit enhanced emission (Lakowicz, 2006). Ethidium bromide (EB) is one of the most commonly used fluorescent DNA probes (Lakowicz, 2006; O'Neil et al., 2018; Osadchii et al., 2011). Before binding to DNA, EB exhibits low fluorescence emission in water, but after binding to DNA, its emission intensity increases by 20 to 30 times (Lakowicz, 2006; Osadchii et al., 2011). The τ_f of EB is approximately 1.7 ns in water, and it increases to about 20 ns upon binding to DNA (Lakowicz, 2006).

Although τ_f and Φ_f are properties with straightforward definitions, their determination can be complex. According to Brouwer (2011), reliable measurements of the quantities of absorbed and emitted photons can be difficult to obtain. The author states that the simplest method for determining Φ_f for species in a dilute solution is to measure the fluorescence spectrum and compare its integrated intensity with the same quantity for a reference system with a known quantum yield from the equation: $\Phi_f^i = F^i f_s n_i^2 / F^s f_i n_s^2 \times \Phi_f^s$; where Φ_f^i and Φ_f^s are the quantum yield of the sample and the standard, respectively; F^i and F^s are the integrated intensities (in photon units) of the sample and standard spectra, respectively; f_x is the absorption factor, given by $f_x = 1 - 10^{-A_x}$, where A = absorbance; e n_i and n_s are the refractive indices of the sample and reference solution, respectively.

However, even when compared with a standard, determining Φ_f can be deceptively simple, according to Brouwer (2011). In his work, Brouwer (2011) discusses important and often underestimated sources of error and provides recommendations to minimize them. These include using the same absorbance at the excitation wavelength for both the sample and reference, utilizing as much as possible the same spectral range of emission and similar emission intensities for both the sample and reference, among others.

In addition to the recommendations above, Brouwer (2011) emphasizes the importance of selecting appropriate standards for determining Φ_f in solution. He highlights the need for more standards with reliable quantitative data, particularly for wavelength regions below 400 nm and above 650 nm. Furthermore, according to the author, reference materials are necessary for determining low Φ_f values, or clear procedures should be established for dealing with large differences in fluorescence intensity between the sample and the reference.

The most intense emissions are displayed by fluorophores with Φ_f approaching unity, such as rhodamines (Brouwer, 2011; Lakowicz, 2006). Although many fluorophores are selected for application due to their high Φ_f, it is important to note that these high Φ_f values often result from high emission rates of the fluorophore, which generally correspond to short lifetimes. However, the τ_f is also important as it determines the time available for the fluorophore to interact or diffuse in the environment and, therefore, the information available from its emission (Lakowicz, 2006).

Valeur (2001) reports that many parameters can affect Φ_f and τ_f in the condensed phase, such as temperature, pH, polarity, viscosity, hydrogen bonding, and the presence of quenchers (fluorescence inhibitors, such as molecular oxygen). Therefore, attention should be paid to potential misinterpretations caused by the simultaneous effects of several of these factors.

Rhodamines, which exhibit high Φ_f, display narrow absorption and emission spectra and small Stokes shifts (Valeur, 2001). The Stokes shift (ΔS) is the difference between the maximum of the absorption band and the maximum of the emission band, expressed in wavenumbers (Figure 3). This phenomenon, observed by G. G. Stokes, occurs because fluorescence typically happens at lower energies or longer wavelengths than absorption (Lakowicz, 2006; Valeur, 2001).

According to Brouwer (2011), broad absorption and emission bands and high ΔS values are favorable properties. Additionally, Valeur (2001) suggests that, from a practical standpoint, detecting a fluorescent species is easier when the ΔS is larger. This is because high ΔS values contribute to increasing the clear fluorescence signal and reducing background interference (Le et al., 2018). Another important property of a fluorescent probe is its photostability, as nearly all fluorophores are photobleached under continuous

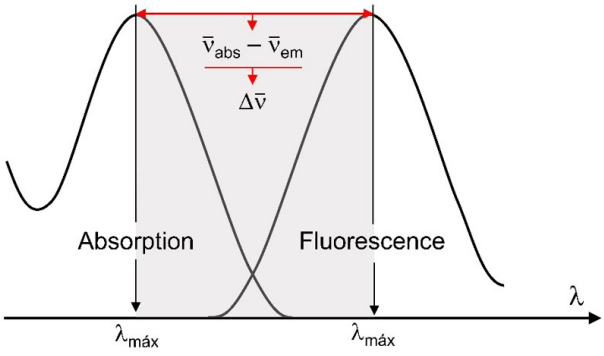


Figure 3. Definition of the Stokes shift.

illumination (Lakowicz, 2006). According to Burrows et al. (2007), photobleaching limits the total number of photons a compound can absorb and convert into fluorescent light. However, although the environment may influence photostability, Lakowicz (2006) indicates that there do not appear to be general principles that can be used to predict it.

The use of extrinsic fluorophores is one of the most widely employed analytical approaches for DNA studies (Bertozo et al., 2023; Osadchii et al., 2011). Given that nucleic acids are ubiquitous components of cells, investigations into DNA, including its structural alterations, sequencing, and interactions with micro- and macromolecules, are crucial for advancing numerous research fields such as biotechnology, pharmaceutical development, and diagnostics (Bertozo et al., 2023; Mohammadi et al., 2023). Regarding the binding modes of fluorescent probes with DNA, they can interact through three main mechanisms: intercalation between base pairs, binding to the major or minor grooves, or electrostatic interactions between phosphate groups and charged probes (Felouat et al., 2021; Li et al., 2012; Mallick et al., 2018; Sirajuddin et al., 2013). According to Sirajuddin et al. (2013), intercalation is generally favored by the presence of fused aromatic rings in the molecular structure of the fluorescent probe and prevented in probes with less extended aromatic systems. Meanwhile, probes that bind to grooves typically have aromatic rings connected by bonds that allow free rotation. Furthermore, according to Sirajuddin et al. (2013), in their review of interactions between DNA and small molecules, UV–Vis absorption and fluorescence emission spectroscopies are the most commonly used techniques for studying these modes of molecule–DNA binding, along with cyclic voltammetry. While several other techniques are also employed, such as viscosity experiments, electrophoresis, circular dichroism, Nuclear Magnetic Resonance (NMR), thermal denaturation studies, mass spectrometry, infrared spectroscopy, Raman spectroscopy, differential pulse voltammetry (Dumat et al., 2016; Hackl et al., 1997; Lindberg & Esbjorner, 2016; Mudasir et al., 2010; Pérez-Flores et al., 2005; Santos-Junior et al., 2021; Sirajuddin et al., 2013; Swathi et al., 2023; Tisoco et al., 2023; Xie et al., 2023; Yang et al., 2017).

EB binds to DNA by intercalating its planar aromatic ring between the DNA base pairs (Bertozo et al., 2023; Lakowicz, 2006). Many DNA fluorescent probes, such as acridine orange, also bind through intercalation (Bertozo et al., 2023; Lakowicz, 2006; Sirajuddin et al., 2013). Other probes bind to DNA grooves, such as 4',6-diamidino-2-phenylindole (DAPI) and Hoechst dyes, which bind to the minor groove, Figure 4 (Bertozo et al., 2023; Lakowicz, 2006).

Despite the favorable properties of commercially available DNA fluorescent probes, they may pose potentially toxic effects. For example, although EB is cost-effective and highly sensitive, it presents significant biological risks, including mutagenicity, carcinogenicity, and teratogenicity, depending on the organism and exposure conditions (El-Din et al., 2021; O'Neil et al., 2018; Soriano et al., 2016). Additionally, acridine compounds can inhibit topoisomerases, leading to DNA damage, disruption of DNA repair and replication, and ultimately, cell death (Sirajuddin et al., 2013).

Benzoxazoles and naphthoxazoles

Oxazoles constitute a class of aromatic heterocycles featuring five-membered rings containing nitrogen and oxygen atoms (Deng et al., 2022; Guerrero-Pepinosa et al., 2021). The oxazole ring is present in many naturally occurring compounds, as reported by Thakur et al. (2022). Oxazole derivatives can readily bind to various receptors in biological systems through non-covalent interactions (Deng et al., 2022; Guerrero-Pepinosa et al., 2021). This property makes these compounds attractive for the design of novel molecules

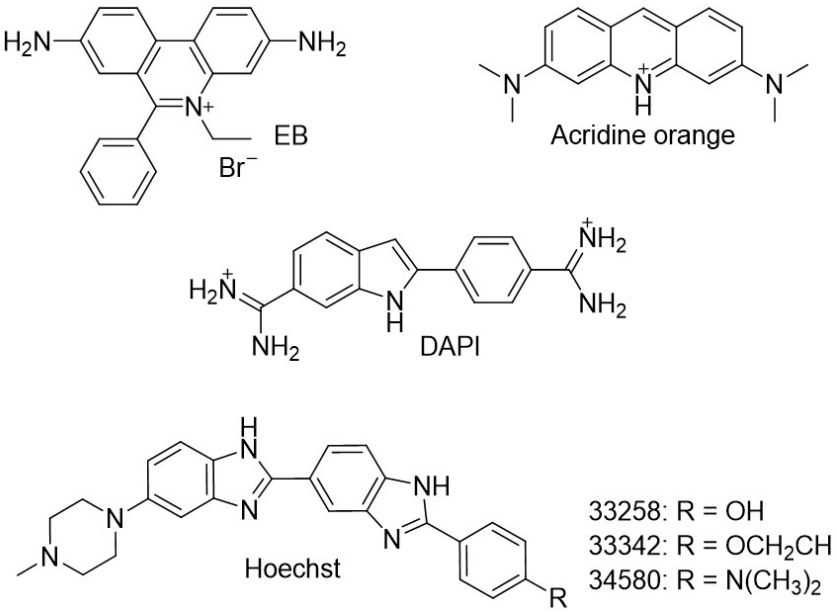


Figure 4. Molecular structures of fluorescent DNA probes: EB, acridine orange, DAPI, and Hoechst dyes.

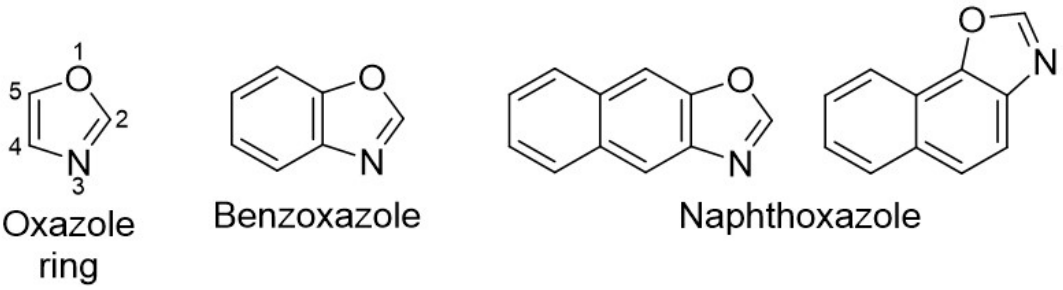


Figure 5. Classification of oxazole derivatives based on substitution at positions 4 and 5 of the oxazole ring.

of biotechnological interest (Deng et al., 2022; Guerrero-Pepinosa et al., 2021; Ooyama et al., 2011).

When the oxazole ring is substituted at positions 4 and 5 by benzene and naphthalene rings, the resulting derivatives are referred to as benzoxazole and naphthoxazole (linear or angular), respectively (Figure 5) (Santos et al., 2022).

Benzoxazole and naphthoxazole derivatives exhibit promising photophysical properties, including a broad spectral range, intense absorption and emission, fluorescence in the crystalline state, and significant enhancement of fluorescence upon binding to biological targets. An example of this is oxazole yellow (YO) (Figure 6), a DNA fluorescent probe (Ghodbane et al., 2015; Inoue et al., 1999; Murade et al., 2009; Ooyama et al., 2011; Phatangare et al., 2013; Zhang et al., 2013). These properties have facilitated their use in important applications, such as scintillators, organic light-emitting materials, and fluorescent probes for biological systems (Ghodbane et al., 2015; Ooyama et al., 2011; Phatangare et al., 2013; Rubio & Zanocco, 2016; Santos et al., 2022; Zhang et al., 2013).

Among the various synthetic methodologies described in the literature, benzoxazoles and naphthoxazoles can be readily obtained through one-pot multicomponent reactions (Santos et al., 2022; Thakur et al., 2022). These methodologies facilitate complex syntheses and are environmentally friendly and offer advantages such as decreased formation of byproducts, increased atom economy, energy efficiency, and a reduction in the number of reaction steps (Thakur et al., 2022). In addition to their attractive properties, ease of synthesis and molecular modification, which provide structural diversity, benzoxazole and naphthoxazole derivatives are photostable and exhibit low toxicity (Deng et al., 2022; Kurutos et al., 2022; Liu et al., 2019; Rubio & Zanocco, 2016; Santos et al., 2022).

Methodology

The systematic review was conducted through a bibliographic search carried out in March 2023, encompassing articles published in the last 10 years (2012-2023). The search was performed across three research databases: ScienceDirect, SciFinder, and Web of Science. Descriptors and Boolean operators used in the search: naphthoxazole OR benzoxazole AND DNA probe AND fluorescent. Articles were selected based on the following inclusion criteria: original articles published in scientific journals; articles containing the descriptors (naphthoxazole OR benzoxazole AND DNA probe AND fluorescent) in the title, abstract, or keywords;

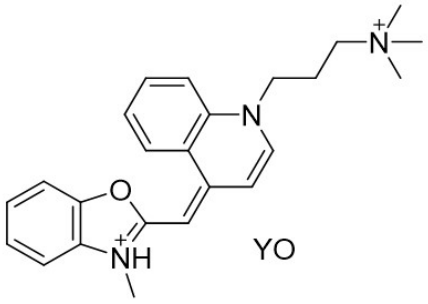


Figure 6. Oxazole yellow (YO), a benzoxazole derivative that acts as a fluorescent DNA probe.

and articles evaluating the interaction between oxazole derivatives and DNA. The selection process was independently conducted by three researchers, adhering strictly to the established inclusion criteria. Any disagreements were resolved through consensus among the investigators.

Results and discussion

The search across the three defined databases yielded 51 articles: 41 from the Web of Science, 9 from ScienceDirect, and 1 from SciFinder. After applying the pre-established inclusion criteria and removing duplicates, 4 articles remained. One of these was excluded because, although it investigating a benzoxazole derivative, DNA binding studies were conducted using a benzothiazole derivative as the representative compound. Consequently, 3 articles were selected for discussion in this systematic review. A flowchart illustrating the progressive selection of studies is presented in Figure 7.

Of the three selected articles, two investigated the interactions of a total of five naphthoxazoles with DNA, published in 2013 and 2014. The third and most recent study, from 2021, conducted similar investigations with two benzoxazoles (Figure 8).

The studies reported in the last decade

Molecules with ESIPT properties constitute a class of fluorophores of interest due to their attractive

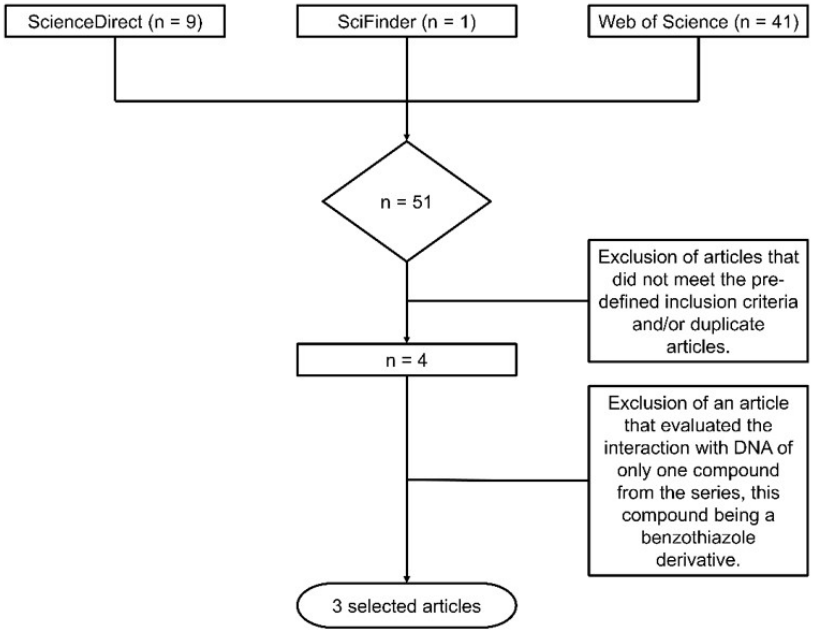


Figure 7. Flowchart illustrating the studies included in the systematic review.

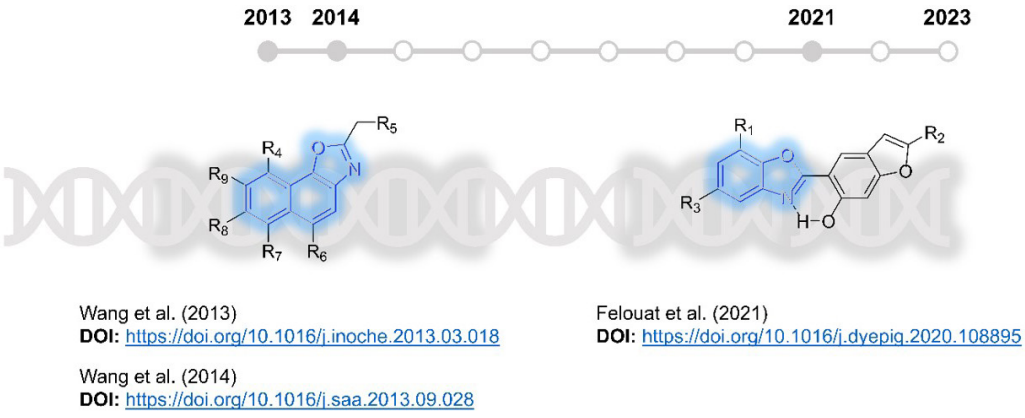


Figure 8. Representation of the generic structures of naphthoxazole and benzoxazole derivatives investigated for their interaction with DNA in 2013, 2014, and 2021, respectively.

photoluminescent features, such as dual fluorescence emission, large ΔS, and environment-sensitive emission (Durko-Maciag et al., 2023; Ma et al., 2023; Sun & Fang, 2023; Wang et al., 2023; Yin & Fang, 2023). To explore these properties, Felouat, Massue, and Ulrich synthesized and assessed the photophysical properties of two benzoxazole derivatives (**1** and **2**) and investigated their interactions with DNA (Figure 9) (Felouat et al., 2021).

The dual emission of organic compounds with ESIPT properties is a critical feature, for applications such as Organic Light-Emitting Diodes (OLEDs) or their application in studies involving interactions with biomacromolecules such as DNA (Benelhadj et al., 2014; Felouat et al., 2021). Thus, Felouat et al. (2021) investigated the influence of electron-donating and electron-withdrawing substituents on the compounds, as well as the effect of functionalizing the benzoxazole nucleus with long oligo(ethylene glycol) (OEG) chains, on the dual emission.

The photophysical properties of benzoxazoles **1** and **2** were evaluated in solvents of varying polarities as well as in polymer films. They exhibited similar absorption profiles with minimal dependence on solvent nature. Furthermore, benzoxazoles **1** and **2** displayed broad and high-intensity main absorption bands, with maximum absorption wavelengths (λ_{abs}) in the ranges of 378/390 nm and 385/392 nm, and molar absorptivity (ε) values spanning from 18500 to 35100 M^{-1} cm^{-1} and 16700 to 24100 M^{-1} cm^{-1}, respectively.

Analyzing the structural differences between benzoxazoles **1** and **2**, Felouat et al. (2021) observed that the introduction of two OEG chains into the benzoxazole ring resulted in a slight bathochromic shift (red shift) and hypochromism of the main absorption band. In contrast, protonation of

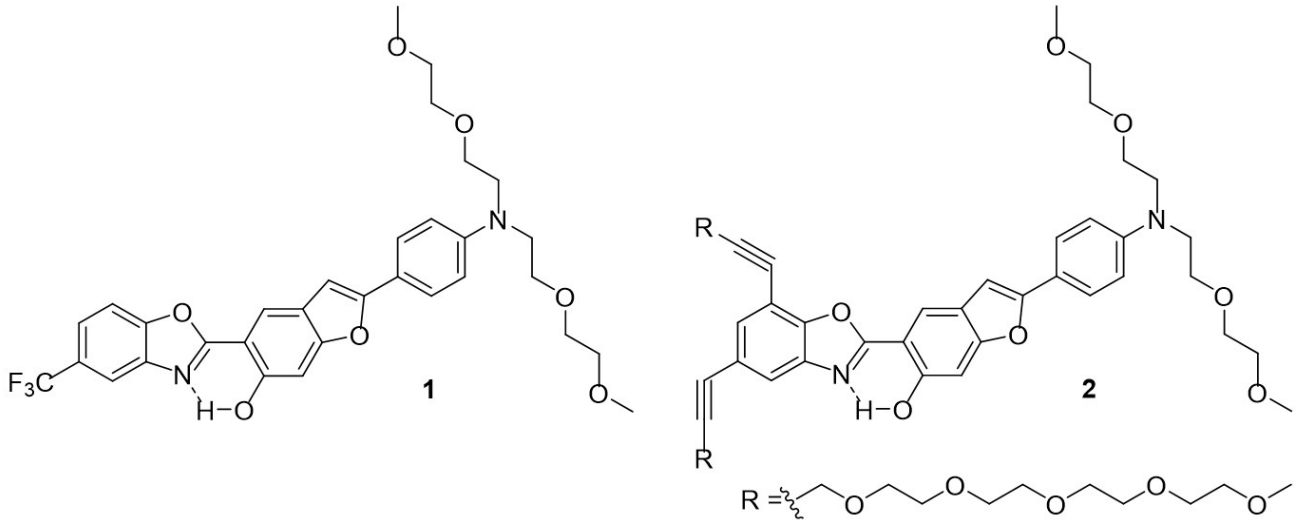


Figure 9. Benzoxazole derivatives **1** and **2** synthesized by Felouat et al. (2021).

the aromatic amine by bubbling gaseous HCl into solutions of benzoxazoles **1** and **2** in dichloromethane (protonated dichloromethane) induced a pronounced hypsochromic shift (blue shift) for both compounds, a characteristic that is consistent with previous studies (Benelhadj et al., 2014).

As a general practice, the excitation wavelength (λ_{ex}) used to record the emission spectrum of a fluorescent compound typically corresponds to the λ_{abs} (Rangel & Merçon, 2012). Based on the absorption profiles of benzoxazoles **1** and **2**, λ_{ex} was set at 390 nm for both compounds. Upon excitation at 390 nm, benzoxazoles **1** and **2** exhibited one or two intense emission bands attributed to the radiative decay of excited states K* or E*/K*, with emission wavelengths (λ_{em}) ranging from 470-582 nm and 471-599 nm, respectively.

The observed dual emission (E*/K*) for benzoxazole **1** in all solvents, except protonated dichloromethane, highlights the importance of the electron-donating group in the benzofuranol moiety and the electron-withdrawing group in the benzoxazole nucleus for increasing the pKa of benzofuranol and the pKb of benzoxazole. This stabilization of the enol isomer in the excited state (E*) enhances the likelihood of observing dual emission, as previously reported by Benelhadj et al. (2014). The exclusive observation of the K* band, accompanied by a blue shift in λ_{abs}, for benzoxazole **1** in protonated dichloromethane underscores the significance of the structural characteristics of this compound class. This is attributed to the protonation of the tertiary amine, which lowers the pKa of benzofuranol (Benelhadj et al., 2014).

Furthermore, it was observed that the spectral separation between the E* and K* bands was particularly pronounced in the nonpolar solvent toluene, where a broad emission was recorded, as indicated by the white emission observed for benzoxazoles **1** and **2**. It is noteworthy that organic compounds emitting white light have various practical applications in optical devices and materials (Wei et al., 2023). The emission spectra of benzoxazoles **1** and **2** in the solid state exhibited a similar trend to that observed in the solution. The nature of the polymeric matrix did not show a significant influence on the E*/K* intensity ratio.

Regarding the relative Φ_f of benzoxazoles **1** and **2**, Felouat et al. (2021) observed low values in solution (Φ_f = 0.01 – 0.17) using rhodamine 6G as a reference (Φ_f = 0.88 in ethanol, λ_{ex} = 488 nm). These values are consistent with those observed for similar derivatives in previous studies (Heyer et al., 2017a; Heyer et al., 2017b). However, it is important to consider the potential differences in emission intensity between the sample and the reference, as discussed in earlier sections. Furthermore, the Φ_f reported for rhodamine 6G in ethanol (Φ_f = 0.88, λ_{ex} = 488 nm) differs from that cataloged by Brouwer (2011) in his review on the choice of reference standards for determining the Φ_f in solution (Φ_f = 0.94 in ethanol, λ_{ex} = 488 nm). This discrepancy underscores the challenges associated with determining the Φ_f of photoluminescent species.

Regarding the absolute Φ_f of benzoxazoles **1** and **2**, determined in polymer films using an integrating sphere, higher values were observed (Φ_f = 0.07-0.33). The average lifetime was typical for fluorescent organic molecules, falling within the nanosecond range.

The environment-dependent fluorescence profile and the presence of the 2-(2-methoxyethoxy)ethylamino group in the benzoxazole, which enhances solubility in aqueous media and serves acts as a DNA anchoring group through hydrogen bonding with base pairs, prompted the authors to investigate the interaction of these derivatives with DNA (Shi et al., 2016). Consequently, Felouat et al. (2021) studied the interaction of benzoxazole derivatives with DNA, focusing on benzoxazole **1** as the model.

DNA binding studies were conducted by monitoring changes in the absorption and emission properties of benzoxazole **1** (12 μM) upon the addition of increasing amounts of calf thymus DNA (CT-DNA) in a 1:1 mixture of CH_3CN/Tris(hydroxymethyl)aminomethane-HCl buffer (pH 7.4).

The addition of aliquots of CT-DNA (0-100 μM) to benzoxazole **1** in solution resulted in significant changes to its absorption profile. Initially, with the incorporation of 10-60 μM of CT-DNA, there was a gradual increase in ε. However,

the elevation of CT-DNA concentration (60-100 μM) triggered a pronounced decrease in ε, reaching a plateau at 90 μM. This substantial change at higher DNA concentrations may, according to the authors, indicate a second mode of binding occurring after an initial mode of interaction. Despite the changes in ε, no shift in λ_{abs} was observed.

According to Ressler et al. (2023), changes in the absorption intensity of a compound in the presence of DNA can indicate its mode of interaction with this biomacromolecule. Ressler et al. (2023) propose that intercalation leads to conformational changes in DNA, resulting in hypochromism. This observation is supported by previous studies, such as that of Manna and Chakravorti (2012), who assert that spectral changes, including hypochromism, bathochromic shift, and the presence of an isosbestic point, are indicative of the intercalation mode of interaction. Conversely, binding to the DNA grooves causes the unwinding of the DNA helix. Hydrogen bonds between base pairs decrease with the unwinding of the DNA helix, consequently an increase in the absorption of DNA bases. This unwinding results in a hyperchromic effect (Ressler et al., 2023).

In the absence of CT-DNA, excitation at 390 nm resulted in a broad emission band with λ_{em} at 604 nm, attributed to the K* decay. Upon the addition of initial aliquots of CT-DNA (0-60 μM), a gradual decrease in emission intensity at 604 nm was observed. This fluorescence quenching is likely due to a photoinduced electron transfer (PET) process from purine bases to the K* state of benzoxazole **1**, indicating an initial mode of interaction with DNA. Increasing the concentration of CT-DNA (60-100 μM) led to an enhancement in emission intensity and the emergence of a primary emission band with a blue shift (λ_{em} = 579 nm), accompanied by the appearance of a second band at higher energies (λ_{em} = 475 nm). The observation of the typical E*/K* dual emission is consistent with a sudden change in the polarity of the environment. Hydrogen bonds are known to stabilize the E* tautomer, suggesting that an intercalation mode of binding may prevail at higher DNA concentrations. Additionally, the full width at half maximum (FWHM) was also reduced after DNA binding (FWHM = 3540 cm^{-1} for 0 μM CT-DNA; FWHM = 2740 cm^{-1} for 100 μM CT-DNA), suggesting a restriction of molecular motions consistent with an intercalation mode of binding, according to Felouat et al. (2021).

In 2013 and 2014, Wang and his research team published two studies investigating the interactions of naphthoxazoles with DNA, using methodologies similar to those employed in the recent work by Felouat et al. (2021). Due to the limited number of studies on the interaction of naphthoxazole derivatives interactions with DNA, Wang et al. (2013) synthesized and evaluated four naphthoxazoles (**3**–**6**) (Figure 10) as DNA ligands. In this study, the interaction between naphthoxazoles and DNA was assessed using absorption and emission titrations, to analyze changes in absorption and emission properties as well as viscosity.

Changes in the absorption and emission properties of the four synthesized naphthoxazoles (**3**–**6**) were analyzed following the addition of increasing amounts of CT-DNA in Tris-HCl buffer. Wang et al. (2013) observed a gradual decrease in the absorption intensity of naphthoxazoles **3**–**6** (40 μM) gradually decreased with the addition of increasing concentrations of CT-DNA, with the hypochromism being most pronounced for naphthoxazole **4**. A slight bathochromic shift of the main absorption band was observed for each of the four naphthoxazoles.

The spectral characteristics of naphthoxazoles **3**–**6** are summarized in Table 1. The DNA binding constants (K), derived from absorption spectra, ranged from 3.58 x 10^3 M^{-1} to 5.29 x 10^4 M^{-1}, with the highest value corresponding to naphthoxazole **4**. Wang et al. (2013) compared the K values of naphthoxazoles **3**–**6** with that of the complex $[Ru(bpy)_2(dppz)]^{2+}$ (4.90 x 10^6 M^{-1}), with that of the intercalating complex, but all were lower than this reference value (Deshpande et al., 2009; Wang et al., 2021).

As previously discussed, changes in absorption properties suggest that naphthoxazoles **3**–**6** interact with DNA through intercalation. The emission spectra of naphthoxazoles **3**–**6** exhibited increased emission intensity with rising concentrations of CT-DNA. This increase in emission intensity ranged from 1.53 times (for **6**) to 1.84 times (for **3**) in the

Table 1. Spectroscopic properties of naphthoxazoles **3**–**6** in the presence of CT-DNA.

Naphthoxazoles	λ_{abs}	K (M^{-1})	Hypochromism H (%)[a]
3	219	5.06 x 10^3	42.13
4	220	5.29 x 10^4	56.04
5	221	3.58 x 10^3	44.21
6	221	7.75 x 10^3	38.08

[a]H% = 100 (A_{free} – A_{bound}) / A_{free}

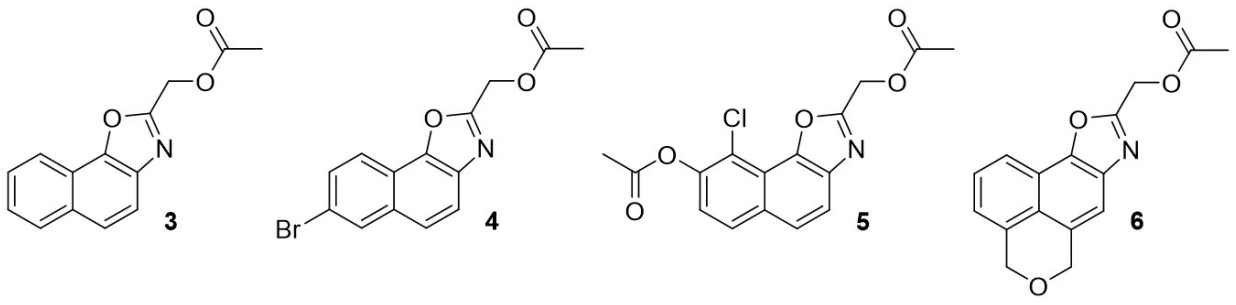


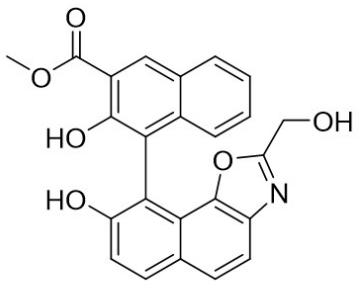
Figure 10. Naphthoxazoles **3**–**6** synthesized by Wang et al. (2013).

presence of DNA. According to the authors, supported by previous studies, the restricted mobility of naphthoxazoles **3**–**6** within the hydrophobic environment of the DNA helix results in decreased vibrational relaxation modes, which accounts for the observed enhancement in emission intensity (Tan et al., 2005). The DNA binding constants, derived from emission spectra, were determined and ranged from 4.18 x 10^3 (for **5**) to 5.29 x 10^4 M^{-1} (for **4**). Wang et al. (2013) noted that the variation in values compared to those obtained from absorption spectra can be attributed to the use of different spectroscopic methods. Furthermore, employing the continuous variation method with emission data, Wang et al. (2013) determined the binding stoichiometries of these derivatives with DNA. They observed that naphthoxazoles **3**, **4**, and **6** each exhibited a naphthoxazole/DNA binding ratio of approximately 1:1, whereas naphthoxazole **3** displayed a ratio of 1.5:1.

To further elucidate the interaction between the synthesized naphthoxazoles (**3**–**6**) and DNA, Wang et al. (2013) also conducted viscosity tests. These tests are important because, as noted by Wang et al. (2014), hydrodynamic evaluations — sensitive to change in molecular length — are considered among the most definitive methods for determining the binding model in solution when crystallographic data is not available.

According to Deshpande et al. (2009), classical intercalation of a ligand into DNA typically increases the viscosity of a DNA solution due to the increased separation of base pairs at the intercalation site, which consequently increases the overall molecular length of the DNA. In contrast, partial intercalation can cause DNA helix to twist or bend, leading to a reduction in its effective length and viscosity. Therefore, Wang et al. (2013) observed a consistent increase in the relative viscosity of DNA (0.30 mM CT-DNA at 25 ºC) with increasing concentrations of naphthoxazoles **3**–**6**, suggesting that these compounds likely intercalate between the base pairs of DNA. One year later, Wang et al. (2014) synthesized a new naphthoxazole (**7**) (Figure 11) and, consistent with their earlier study, evaluated its interaction with DNA through absorption and emission titration studies, as well as viscosity measurements.

In this study, Wang et al. (2014) observed similar behavior for the new naphthoxazole (**7**) compared to the previously studied naphthoxazoles **3**–**6** (Wang et al., 2013). Specifically, upon addition of increasing concentrations of CT-DNA (Tris-HCl buffer, pH 7.2), Wang et al. (2014) noted a decrease in absorption intensity (hypochromism) of the main absorption band (λ_{abs} = 232 nm) of naphthoxazole **7** (40 µM). This observation suggests that naphthoxazole **7** interacts with DNA through intercalation, similar to naphthoxazoles **3**–**6**. According to Wang et al. (2014), naphthoxazole **7** exhibited a hypochromic effect of 23.58%, which is lower than that observed for naphthoxazoles **3**–**6**. From the absorption spectra, Wang et al. (2014) determined the DNA binding constant (K) for naphthoxazole **7**, which was 6.16 x 10^3 M^{-1}. This value is close to the K value for naphthoxazole **3** (5.06 x 10^3 M^{-1}) but is lower than that of the $[Ru(bpy)_2(dppz)]^{2+}$ complex (4.90 x 10^6 M^{-1}), corroborating what was observed for naphthoxazoles **3**–**6**.



7

Figure 11. Naphthoxazole **7** synthesized by Wang et al. (2014).

The emission spectra of naphthoxazole **7** were recorded at the λ_{ex} of 300 nm, and similarly to naphthoxazoles **3**–**6**, naphthoxazole **7** demonstrated an increase in emission intensity with higher concentrations of CT-DNA. According to the authors, the emission intensity of naphthoxazole **7** was 1.86 times higher in the presence of DNA compared to its fluorescence in the absence of DNA. Thus, naphthoxazole **7** exhibited a greater emission intensity when interacting with DNA compared to naphthoxazoles **3**–**6**. Furthermore, using the continuous variation method applied to emission data, Wang et al. (2014) determined that naphthoxazole **7** showed a 1:1 binding stoichiometry with DNA. This is the binding ratio is consistent with naphthoxazoles **3**, **4**, and **6**, as reported earlier. Wang et al. (2014) also conducted viscosity tests with naphthoxazole **7**. Consistent with observed for naphthoxazoles **3**–**6**, the authors reported a steady increase in the relative viscosity of CT-DNA (200 µM) with a rising concentration of naphthoxazole **7**. Wang et al. (2014) performed viscosity tests whith naphthoxazole **7** and naphthoxazole **3** for comparison purposes. It was observed that the increase in relative DNA viscosity was more pronounced with the addition of increasing concentrations of naphthoxazole **7**.

Conclusion

The literature search conducted in this systematic review revealed few studies focused on the evaluation of the interaction between benzoxazoles and naphthoxazoles with DNA. However, the works published in the last decade and selected based on the systematic search demonstrate the potential of these compounds to interact with DNA and exhibit enhanced fluorescence emission. All benzoxazole and naphthoxazole derivatives synthesized and evaluated in the studies discussed in this review displayed DNA binding behavior, with the mode of interaction predominantly being intercalation at increasing DNA concentrations. Furthermore, they all exhibited an increase in fluorescence emission intensity with rising DNA concentrations. These results suggest that benzoxazole and naphthoxazole derivatives have promising uses as fluorescent DNA probes. Additionally, given their broad spectrum of biological activities reported in the literature, these derivatives may aid in understanding the chemical basis of mechanisms of action of substances targeting DNA and in the design of targeted drugs. In conclusion, the encouraging results described in this review, together with the limited number of studies on the interaction of this compound

class with DNA, highlight this is an area of research that has significant potential for further exploration and development.

Conflict of interests

The authors declare no competing interests.

Funding

This work was supported by the FACEPE (Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco) - Funding Code APQ-0788-1.06/22.

Acknowledgements

The authors would like to thank the FACEPE (Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco) grant IBPG-1482-4.03/19 and APQ-0788-1.06/22.

References

Benelhadj, K., Muzuzu, W., Massue, J., Retailleau, P., Charaf-Eddin, A., Laurent, A. D., Jacquemin, D., Ulrich, G., & Ziessel, R. (2014). White emitters by tuning the excited-state intramolecular proton-transfer fluorescence emission in 2-(2′-hydroxybenzofuran) benzoxazole dyes. *Chemistry*, *20*(40), 12843-12857. http://doi.org/10.1002/chem.201402717. PMid:25145709.

Bertozo, L. C., Tutone, M., Pastrello, B., Silva-Filho, L. C., Culletta, G., Almerico, A. M., & Ximenes, V. F. (2023). Aminoquinolines: fluorescent sensors to DNA: a minor groove probe. Experimental and in silico studies. *Journal of Photochemistry and Photobiology A Chemistry*, *444*, 114944. http://doi.org/10.1016/j.jphotochem.2023.114944.

Brouwer, A. M. (2011). Standards for photoluminescence quantum yield measurements in solution (IUPAC technical report). *Pure and Applied Chemistry*, *83*(12), 2213-2228. http://doi.org/10.1351/PAC-REP-10-09-31.

Burrows, S. M., Reif, R. D., & Pappas, D. (2007). Investigation of photobleaching and saturation of single molecules by fluorophore recrossing events. *Analytica Chimica Acta*, *598*(1), 135-142. http://doi.org/10.1016/j.aca.2007.07.026. PMid:17693317.

Deng, C., Yan, H., Wang, J., Liu, B.-S., Liu, K., & Shi, Y.-M. (2022). The anti-HIV potential of imidazole, oxazole and thiazole hybrids: a mini-review. *Arabian Journal of Chemistry*, *15*(11), 104242. http://doi.org/10.1016/j.arabjc.2022.104242.

Deshpande, M. S., Kumbhar, A. A., Kumbhar, A. S., Kumbhakar, M., Pal, H., Sonawane, U. B., & Joshi, R. R. (2009). Ruthenium(II) complexes of bipyridine-glycoluril and their interactions with DNA. *Bioconjugate Chemistry*, *20*(3), 447-459. http://doi.org/10.1021/bc800298t. PMid:19236020.

Dumat, B., Faurel-Paul, E., Fornarelli, P., Saettel, N., Metgé, G., Fiorini-Debuisschert, C., Charra, F., Mahuteau-Betzer, F., & Teulade-Fichou, M.-P. (2016). Influence of the oxazole ring connection on the fluorescence of oxazoyl-triphenylamine biphotonic DNA probes. *Organic & Biomolecular Chemistry*, *14*(1), 358-370. http://doi.org/10.1039/C5OB02225H. PMid:26599863.

Durko-Maciag, M., Ulrich, G., Massue, J., Mysliwiec, J., & Cyprych, K. (2023). Two is better than one: ESIPT dyes as photoinitiators in two-photon polymerization. *European Polymer Journal*, *195*, 112235. http://doi.org/10.1016/j.eurpolymj.2023.112235.

El-Din, A. E.-D. S., Abdullah, S., & Sayed, A. E.-D. H. (2021). Antioxidant capacity and DNA damage in Nile tilapia (*Oreochromis niloticus*) exposed to ethidium bromide: a protective role for *Spirulina Platensis*. *Scientific American*, *13*, e00961. http://doi.org/10.1016/j.sciaf.2021.e00961.

Felouat, A., Massue, J., & Ulrich, G. (2021). Oligo(ethylene glycol) (OEG) functionalized 2-(2′-hydroxy benzofuranyl)benzoxazole (HBBO) derivatives: Synthesis, photophysical properties and biomolecules binding studies. *Dyes and Pigments*, *185*, 108895. http://doi.org/10.1016/j.dyepig.2020.108895.

Ghodbane, A., Saffon, N., Blanc, S., & Fery-Forgues, S. (2015). Influence of the halogen atom on the solid-state fluorescence properties of 2-phenyl-benzoxazole derivatives. *Dyes and Pigments*, *113*, 219-226. http://doi.org/10.1016/j.dyepig.2014.08.011.

Guerrero-Pepinosa, N. Y., Cardona-Trujillo, M. C., Garzon-Castaño, S. C., Veloza, L. A., & Sepúlveda-Arias, J. C. (2021). Antiproliferative activity of thiazole and oxazole derivatives: a systematic review of *in vitro* and *in vivo* studies. *Biomedicine and Pharmacotherapy*, *138*, 111495. http://doi.org/10.1016/j.biopha.2021.111495. PMid:33765586.

Hackl, E. V., Kornilova, S. V., Kapinos, L. E., Andrushchenko, V. V., Galkin, V. L., Grigoriev, D. N., & Blagoi, Y. P. (1997). Study of Ca^{2+}, Mn^{2+} and Cu^{2+} binding to DNA in solution by means of IR spectroscopy. *Journal of Molecular Structure*, *408-409*, 229-232. http://doi.org/10.1016/S0022-2860(96)09534-8.

Heyer, E., Benelhadj, K., Budzák, S., Jacquemin, D., Massue, J., & Ulrich, G. (2017a). On the fine-tuning of the excited-state intramolecular proton transfer (ESIPT) process in 2-(2′-hydroxybenzofuran)benzazole (HBBX) dyes. *Chemistry*, *23*(30), 7324-7336. http://doi.org/10.1002/chem.201700299. PMid:28390153.

Heyer, E., Massue, J., & Ulrich, G. (2017b). Modulation of the excited-state intramolecular proton transfer (ESIPT) process in 2-(20-hydroxybenzofuran)benzoxazole (HBBO) dimers. *Dyes and Pigments*, *143*, 18-24. http://doi.org/10.1016/j.dyepig.2017.04.020.

Inoue, T., Sugiura, Y., Saitoh, J., Ishiguro, T., & Otsuka, M. (1999). Fluorescence property of oxazole yellow-linked oligonucleotide. Triple helix formation and photocleavage of double-stranded DNA in the presence of spermine. *Bioorganic & Medicinal Chemistry*, *7*(6), 1207-1211. http://doi.org/10.1016/S0968-0896(99)00035-8. PMid:10428393.

Kurutos, A., Minkovska, S., Nedialkov, P. T., & Fedorov, Y. V. (2022). Facile and environmentally benign synthetic approach to the selective mono-chlorination and mono-bromination of benzo[*d*] oxazol-2(3*H*)-ones. *Journal of Heterocyclic Chemistry*, *59*(10), 1824-1831. http://doi.org/10.1002/jhet.4522.

Lakowicz, J. R. (2006). *Principles of fluorescence spectroscopy* (3rd ed.). Cham: Springer. http://doi.org/10.1007/978-0-387-46312-4.

Le, B. H., Nguyen, T.-V. T., Joo, H. N., & Seo, Y. J. (2018). Large-stokes-shift-based folded DNA probing systems targeting DNA and miRNA 21 with signal amplification. *Bioorganic & Medicinal Chemistry*, *26*(17), 4881-4885. http://doi.org/10.1016/j.bmc.2018.08.027. PMid:30170926.

Li, X.-L., Hu, Y.-J., Wang, H., Yu, B.-Q., & Yue, H.-L. (2012). Molecular spectroscopy evidence of Berberine binding to DNA: comparative binding and thermodynamic profile of intercalation. *Biomacromolecules*, *13*(3), 873-880. http://doi.org/10.1021/bm2017959. PMid:22316074.

Lindberg, D. J., & Esbjorner, E. K. (2016). Detection of amyloid-ß fibrils using the DNA-intercalating dye YOYO-1: binding mode and fibril formation kinetics. *Biochemical and Biophysical Research Communications*, *469*(2), 313-318. http://doi.org/10.1016/j.bbrc.2015.11.051. PMid:26612254.

Liu, Q.-Q., Lu, K., Zhu, H.-M., Kong, S.-L., Yuan, J.-M., Zhang, G.-H., Chen, N.-Y., Gu, C.-X., Pan, C.-X., Mo, D.-L., & Su, G.-F. (2019). Identification of 3-(benzazol-2-yl)quinoxaline derivatives

as potent anticancer compounds: Privileged structure-based design, synthesis, and bioactive evaluation *in vitro* and *in vivo*. *European Journal of Medicinal Chemistry*, *165*, 293-308. http://doi.org/10.1016/j.ejmech.2019.01.004. PMid:30685528.

Liu, S., Xia, J., Jiang, D., Li, Q., Pan, X., Dong, X., Wang, J., Liu, Y., & Dong, Y. (2023). Detection of human serum albumin using a rare-earth nanosheet fluorescent probe based on intensity and lifetime signals. *Spectrochimica Acta. Part A: Molecular and Biomolecular Spectroscopy*, *302*, 123046. http://doi.org/10.1016/j.saa.2023.123046. PMid:37390718.

Lukeman, M., Simon, H., Wan, P., & Wang, Y.-H. (2015). Photocyclization and photoaddition reactions of arylphenols via intermediate quinone methides. *The Journal of Organic Chemistry*, *80*(22), 11281-11293. http://doi.org/10.1021/acs.joc.5b01580. PMid:26496171.

Ma, M., Zhang, Y., & Sun, C. (2023). Regulating the ESIPT processes and photophysical properties of indole derivates by introducing the appropriate functional groups. *Journal of Molecular Liquids*, *384*, 122263. http://doi.org/10.1016/j.molliq.2023.122263.

Mallick, T., Karmakar, A., Batuta, S., Ahamed, G., Das, S., Alam, M. N., Mukherjee, M., Das, N., Mandal, D., & Begum, N. A. (2018). Fluorescent small molecules are big enough to sense biomacromolecule: synthesis of aromatic thioesters and understanding their interactions with ctDNA. *ACS Omega*, *3*(1), 334-348. http://doi.org/10.1021/acsomega.7b01933. PMid:30023778.

Manna, A., & Chakravorti, S. (2012). Modification of a styryl dye binding mode with calf thymus DNA in vesicular medium: from minor groove to intercalative. *The Journal of Physical Chemistry B*, *116*(17), 5226-5233. http://doi.org/10.1021/jp301211m. PMid:22506549.

Mohammadi, M. M., Bavi, O., & Jamali, Y. (2023). DNA sequencing via molecular dynamics simulation with functionalized graphene nanopore. *Journal of Molecular Graphics & Modelling*, *122*, 108467. http://doi.org/10.1016/j.jmgm.2023.108467. PMid:37028198.

Mudasir, W., Wahyuni, E. T., Tjahjono, D. H., Yoshioka, N., & Inoue, H. (2010). Spectroscopic studies on the thermodynamic and thermal denaturation of the ct-DNA binding of methylene blue. *Spectrochimica Acta. Part A: Molecular and Biomolecular Spectroscopy*, *77*(2), 528-534. http://doi.org/10.1016/j.saa.2010.06.032. PMid:20637684.

Murade, C. U., Subramaniam, V., Otto, C., & Bennink, M. L. (2009). Interaction of oxazole yellow dyes with DNA studied with hybrid optical tweezers and fluorescence microscopy. *Biophysical Journal*, *97*(3), 835-843. http://doi.org/10.1016/j.bpj.2009.05.024. PMid:19651041.

O'Neil, C. S., Beach, J. L., & Gruber, T. D. (2018). Thiazole orange as an everyday replacement for ethidium bromide and costly DNA dyes for electrophoresis. *Electrophoresis*, *39*(12), 1474-1477. http://doi.org/10.1002/elps.201700489. PMid:29645293.

Ooyama, H. E., Ooyama, Y., Hino, T., Sakamoto, T., Yamaguchi, T., & Yoshida, K. (2011). Synthesis and photophysical properties of structural isomers of novel 2,10-disubstituted benzofuro[2,3-*e*] naphthoxazole-type fluorescent dyes. *Dyes and Pigments*, *91*(3), 481-488. http://doi.org/10.1016/j.dyepig.2011.03.031.

Osadchii, S. A., Shubin, V. G., Kozlova, L. P., Varlamenko, V. S., Filipenko, M. L., & Boyarskikh, U. A. (2011). Improvement of ways to obtain ethidium bromide and synthesis of ethidium ethyl sulfate, a new fluorescent dye for detection of nucleic acids. *Russian Journal of Applied Chemistry*, *84*(9), 1541-1548. http://doi.org/10.1134/S107042721109014X.

Pérez-Flores, L., Ruiz-Chica, A. J., Delcros, J. G., Sánchez-Jiménez, F., & Ramírez, F. J. (2005). Intercalation and groove binding of an acridine–spermine conjugate on DNA sequences: an FT-Raman and UV-visible absorption study. *Journal of Molecular Structure*, *744-747*, 699-704. http://doi.org/10.1016/j.molstruc.2004.10.086.

Phatangare, K. R., Borse, B. N., Padalkar, V. S., Patil, V. S., Gupta, V. D., Umape, P. G., & Sekar, N. (2013). Synthesis, photophysical property study of novel fluorescent 4-(1,3-benzoxazol-2-yl)-2-phenylnaphtho[1,2-*d*][1,3]oxazole derivatives and their antimicrobial activity. *Journal of Chemical Sciences*, *125*(1), 141-151. http://doi.org/10.1007/s12039-012-0324-3.

Prabhala, P., Sutar, S. M., Manjunatha, M. R., Pawashe, G. M., Gupta, V. K., Naik, L., & Kalkhambkar, R. G. (2022). Synthesis, in vitro and theoretical studies on newly synthesized deep blue emitting 4-(p-methylphenylsulfonyl-5-aryl/alkyl)oxazole analogues for biological and optoelectronic applications. *Journal of Molecular Liquids*, *360*, 119520. http://doi.org/10.1016/j.molliq.2022.119520.

Rangel, H. A., & Merçon, F. (2012). Estudo da emissão por fluorescência de ultravioleta na quantificação de um inibidor de corrosão do tipo sal de amônio quaternário em água. *Quimica Nova*, *35*(7), 1287-1293. http://doi.org/10.1590/S0100-40422012000700002.

Ressler, A. J., Frate, M., Hontoria, A., Ream, A., Timms, E., Li, H., Stettler, L. D., Bollinger, A., Poor, J. E., Parra, M. A., Ma, H., Seeram, N. P., Meschwitz, S. M., & Henry, G. E. (2023). Synthesis, anti-ferroptosis, anti-quorum sensing, antibacterial and DNA interaction studies of chromene-hydrazone derivatives. *Bioorganic & Medicinal Chemistry*, *90*, 117369. http://doi.org/10.1016/j.bmc.2023.117369. PMid:37320993.

Rodrigues, A. O., Motta, F. M. L., Carvalho, R. G., Ferraz, R. R. N., & Rodrigues, F. S. M. (2022). Usos clínicos da fluoresceína na prática médica: revisão da literatura. *International Journal of Health Management Review*, *8*(1), e0314. http://doi.org/10.37497/ijhmreview.v8i1.314.

Rubio, N., & Zanocco, A. L. (2016). Thermodynamics of the inclusion of naphthoxazole derivatives in cucurbit[7]uril. *Afinidad. Journal of Chemical Engineering Theoretical and Applied Chemistry*, *73*(573), 69-74. https://raco.cat/index.php/afinidad/article/view/308446

Santos-Junior, P. F. S., Nascimento, I. J. S., Silva, E. C. D., Monteiro, K. L. C., Freitas, J. D., Lins, S. L., Maciel, T. M. S., Cavalcanti, B. C., Vieira Neto, J. B., Abreu, F. C., Figueiredo, I. M., Santos, J. C. C., Pessoa, C. O., Silva-Júnior, E. F., Araújo-Júnior, J. X., & Aquino, T. M. (2021). Synthesis of hybrids thiazole-quinoline, thiazole-indole and their analogs: *in vitro* anti-proliferative effects on cancer cell lines, DNA binding properties and molecular modeling. *New Journal of Chemistry*, *45*, 13847. http://doi.org/10.1039/D1NJ02105B.

Santos, V. L. A., Guimarães, D. G., Nishimura, R. H. V., Rolim, L. A., Gonsalves, A. A., & Araújo, C. R. M. (2022). Naftoimidazóis e naftoxazóis - Promissores componentes de sistemas teranósticos. *Quimica Nova*, *45*(5), 560-577. http://doi.org/10.1590/S0100-40421998000500005.

Shi, P., Jiang, Q., Zhang, Q., & Tian, Y. (2016). Synthesis, characterization, emission and DNA binding properties of four alkynylplatinum(II) terpyridine complexes. *Journal of Organometallic Chemistry*, *804*, 66-72. http://doi.org/10.1016/j.jorganchem.2015.12.017.

Sirajuddin, M., Ali, S., & Badshah, A. (2013). Drug-DNA interactions and their study by UV-visible, fluorescence spectroscopies and cyclic voltametry. *Journal of Photochemistry and Photobiology. B, Biology*, *124*, 1-19. http://doi.org/10.1016/j.jphotobiol.2013.03.013. PMid:23648795.

Soriano, E., Holder, C., Levitz, A., & Henary, M. (2016). Benz[*c*,*d*] indolium-containing monomethine cyanine dyes: synthesis and photophysical properties. *Molecules*, *21*(1), 23. http://doi.org/10.3390/molecules21010023. PMid:26712725.

Sun, G., & Fang, H. (2023). Fluorescent properties based on ESIPT and TICT of novel acylhydrazone-based probe and its sensing mechanism for Al^{3+}: a TD-DFT investigation. *Journal of Molecular Liquids*, *379*, 121639. http://doi.org/10.1016/j.molliq.2023.121639.

Swathi, M., Shankar, D. S., Daravath, S., Ganji, N., & Lakshmi, P. V. A. (2023). Computational studies, cytotoxicity, DNA interactions of bioactive novel 2-methoxy 5-trifluoromethyl benzenamine Schiff base metal complexes. *Inorganic Chemistry Communications*, *153*, 110826. http://doi.org/10.1016/j.inoche.2023.110826.

Tan, L.-F., Chao, H., Li, H., Liu, Y.-J., Sun, B., Wei, W., & Ji, L.-N. (2005). Synthesis, characterization, DNA-binding and photocleavage studies of $[Ru(bpy)_2(PPIP)]^{2+}$ and $[Ru(phen)_2(PPIP)]^{2+}$. *Journal of Inorganic Biochemistry*, *99*(2), 513-520. http://doi.org/10.1016/j.jinorgbio.2004.10.028. PMid:15621284.

Thakur, A., Verma, M., Bharti, R., & Sharma, R. (2022). Oxazole and isoxazole: from one-pot synthesis to medical applications. *Tetrahedron*, *119*, 132813. http://doi.org/10.1016/j.tet.2022.132813.

Tisoco, I., Köhler, M. H., Nogara, P. A., Rocha, J. B. T., Nascimento, C. S., Garcia, R. Q., Boni, L., & Iglesias, B. A. (2023). Study on the photophysical property of meso-tetra(2-fluorenyl) porphyrin derivatives and their interactions with DNA/HSA biomacromolecules. *Inorganica Chimica Acta*, *556*, 121639. http://doi.org/10.1016/j.ica.2023.121639.

Valeur, B. (2001). *Molecular fluorescence: principles and applications*. Weinheim: Wiley-VCH. http://doi.org/10.1002/3527600248.

Wang, Q., Li, X., Song, L., Zhao, J., & Tang, Z. (2023). Theoretical study on ESIPT mechanism and negative solvatochromism effect of 3-(4,5-Diphenyl-1H-imidazol-2-yl)-9-phenyl-9H-carbazol-4-ol compound in different solvents. *Journal of Molecular Liquids*, *382*, 122000. http://doi.org/10.1016/j.molliq.2023.122000.

Wang, X., Liu, X., & Tan, L. (2021). Substituent effects on the interactions of ruthenium(II) polypyridyl complexes $[Ru(bpy)_2(6\text{-}R\text{-}dppz)]^{2+}$ (R = hydroxy and fluorine) with the RNA triplex poly(rU).poly(rA)xpoly(rU). *Polyhedron*, *202*, 115196. http://doi.org/10.1016/j.poly.2021.115196.

Wang, X.-Z., Yao, J.-H., Xie, Y.-Y., Lin, G.-J., Huang, H.-L., & Liu, Y.-J. (2013). Synthesis, DNA-binding and antioxidant activity studies of naphthoxazole compounds. *Inorganic Chemistry Communications*, *32*, 82-88. http://doi.org/10.1016/j.inoche.2013.03.018.

Wang, X.-Z., Jiang, G.-B., Xie, Y.-Y., & Liu, Y.-J. (2014). Synthesis, molecular structure, DNA interaction and antioxidant activity of novel naphthoxazole compound. *Spectrochimica Acta. Part A: Molecular and Biomolecular Spectroscopy*, *118*, 448-453. http://doi.org/10.1016/j.saa.2013.09.028. PMid:24080575.

Wei, Z., Zhang, Y., & Zhang, X. (2023). Controlled reversible single-molecule white-light emission from keto-enol interconversion of ß-diketone dyes. *Dyes and Pigments*, *216*, 111347. http://doi.org/10.1016/j.dyepig.2023.111347.

Xie, Z., Zhang, S., Wu, Y., Liang, J., Yao, W., Qu, R., Tong, X., Zhang, G., & Yang, H. (2023). Interaction of isoquinoline alkaloids with pyrimidine motif triplex DNA by mass spectrometry and spectroscopies reveals diverse mechanisms. *Heliyon*, *9*(4), e14954. http://doi.org/10.1016/j.heliyon.2023.e14954. PMid:37082631.

Yang, H., Tang, P., Tang, B., Huang, Y., He, J., Li, S., & Li, H. (2017). Studies of DNA-binding properties of lafutidine as adjuvant anticanceragent to calf thymus DNA using multi-spectroscopic approaches, NMR relaxation data, molecular docking and dynamical simulation. *International Journal of Biological Macromolecules*, *99*, 79-87. http://doi.org/10.1016/j.ijbiomac.2017.02.062. PMid:28235605.

Yin, F., & Fang, H. (2023). Solvent controlled excited state intramolecular proton transfer (ESIPT) behavior and luminescent property of a novel phthalimide-based fluorophore: a TD-DFT Study *Journal of Molecular Structure*, *1272*, 134123. http://doi.org/10.1016/j.molstruc.2022.134123.

Zhang, C., Li, L., Wu, H., Liu, Z., Li, J., Zhang, G., Wen, G., Shuang, S., Dong, C., & Choi, M. M. F. (2013). Synthesis and photophysical studies of oxazole rings containing compounds as electron accepting units. *Spectrochimica Acta. Part A: Molecular and Biomolecular Spectroscopy*, *102*, 256-262. http://doi.org/10.1016/j.saa.2012.10.005. PMid:23220665.