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Enhanced Luminescent Probe Utilizing Schiff Base Ligand for 2,4,6-Trinitrophenol Detection in Aqueous Media

Ayhan ALTUN[1], Kenan BULDURUN[2]*, Nevin TURAN[3]

Highlights:

- Both photophysical and fluorescence sensor properties of Schiff base ligand were evaluated
- Fluorescent sensors have shown excellent sensitivity and selectivity for TNP
- Detecting TNP with an impressively low LOD of 0.253 µM covering a linear working range of 2.50–30.00 µM
- This finding underlines the potential utility of this ligand as a promising tool in environmental monitoring

Keywords:

- Fluorescent sensor
- Schiff base
- Nitroaromatic compounds
- TNP

ABSTRACT:

This research explored the fluorescence properties of a Schiff base ligand which was synthesized and characterized used by standard spectroscopic methods. The examination into its photophysical and fluorescent sensor properties involved UV-Vis spectroscopy, as well as fluorescence spectroscopy, time-resolved and steady-state. Fluorescent sensors were found to exhibit excellent sensitivity and selectivity for 1,3,5-trinitrophenol (TNP), over testing with other nitroaromatic (dinitrobenzene (DNB), 2,4,6-trinitrotoluene (TNT), 2,4-dinitrotoluene (DNT), 2-nitrophenol (2-NP), 4-nitrophenol (4-NP), and 2,4-dinitrophenol (DNP)), explosives with significant fluorescence "off" responses, resulting in immediate fluorescence color change. The binding mechanisms between Schiff base and TNP were assessed using Job's drawing. The compound exhibited exceptional sensitivity in detecting TNP with an impressively low LOD of 0.253 µM covering a linear working range of 2.50-30.00 µM. The study revealed that the compound exhibited robust fluorescent properties, proved to be effective in fluorescence quenching-based TNP detection in water solutions, and demonstrated both high selectivity and sensitivity. This finding underlines the potential utility of this ligand as a promising tool in environmental monitoring or related fields where the detection of TNP is crucial.

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INTRODUCTION

Recently, the sensitive detection of high-energy nitroaromatic organic compounds (NACs) containing nitro groups such as TNP, DNT and TNT has become increasingly important owing to concerns about health, safety and environmental impacts (Verbitskiy et al., 2020). Among these compounds, TNP is widely used in various industries, including leather, pharmaceuticals and dyes. They also play an important role in the production of explosives, fireworks and other applications. The significant solubility of TNP in water facilitates its dispersion into soil and water bodies, leading to substantial environmental pollution (Zhao et al., 2021). The persistence of TNP in the environment is exacerbated by its resistance to biodegradation, attributed to its electron-attracting nitro groups, which heightens the severity of the contamination issue (Wyman et al., 1992; Zheng et al., 2021). Additionally, the documented toxicity and mutagenicity of TNP present serious risks to both human health and wildlife (Wyman et al., 1979; Trinh et al., 2019). Exposure to TNP, whether through inhalation or skin contact, can result in a range of health complications, from dermatitis and bronchitis to severe conditions such as chronic liver and kidney toxicity, and in extreme cases, death. Therefore, the sensitive detection of TNP is crucial for protecting human health and maintaining ecofriendly integrity.

Various techniques are employed for the detection of explosives, including mass spectrometry (Forbes & Sisco, 2018) and ion mobility spectrometry (Caygill et al., 2012), as well as gas chromatography (Grate et al., 2012) and surface-enhanced Raman scattering (Gillibert et al., 2018). Infrared spectroscopy (Wen et al., 2018), fluorescence spectroscopy (Sun et al., 2015; Wang et al., 2024), colorimetric analysis (Salinas et al., 2012), electrochemical analysis (Yu et al., 2017), and electrophoresis (Calcerrada et al., 2016) are also utilized. Among these methods, fluorescence-based sensors have stood out owing to their exceptional sensitivity, fast response times, real-time monitoring capabilities, precise selectivity, and user-friendliness. In recent years, there has been a significant increase in the development and research of fluorescent sensors specifically designed for the detection of nitro explosives. These sensors use a variety of materials, such as conjugated polymers (McQuade et al., 2000; Rose et al., 2005: Tsai et al., 2018; Kumar et al., 2019; Altun et al., 2021), porous metal–organic cages (MOFs) (Kreno et al., 2012; Hu et al., 2014; Lustig et al., 2017; Chhatwal et al., 2018; Altun et al., 2023), luminescent gels (Kartha et al., 2015), organic–inorganic hybrid materials (Akhgari et al., 2015), and Schiff bases (Kose et al., 2023; Altun, 2024) covers.

Schiff bases are compounds containing a wide variety of aliphatic and aromatic groups that are widely studied in coordination chemistry. Schiff base ligands are obtained by a basic condensation reaction between a carbonyl group (aldehyde/ketone) and a primary amine. Schiff base ligands with CH=N structure have attracted great interest of researchers due to their outstanding catalytic properties, biological activities, industrial uses and medical benefits. The choice of appropriate amines and substituted aromatic carbonyls in the synthesis of Schiff bases significantly affects their steric, electronic and biological properties (Mahadevi & Sumathi, 2023). Many of them are still used to form transition metal complexes with different structural, electronic, magnetic and pharmacological properties and their use is increasing day by day (Buldurun et al., 2022; Nayab et al., 2023).

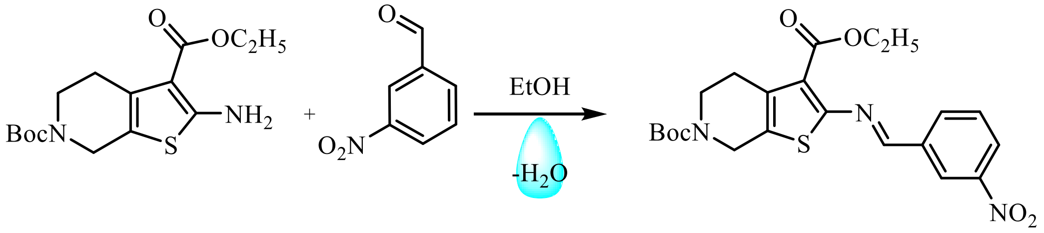
This study focuses on the in-depth investigation of the photophysical properties of a Schiff base ligand, building on the foundational work by Özdemir (Özdemir, 2019; Buldurun & Özdemir, 2020). Specifically, the research examines the fluorescence sensing capabilities of Schiff base ligand in relation to nitro-containing aromatic compounds (NACs). The findings indicate that ligand demonstrates unique fluorescence sensor characteristics, with a pronounced sensitivity to 2,4,6-trinitrophenol (TNP). This highlights the potential of ligand as a highly promising candidate for use as a selective sensor in the detection of TNP.

MATERIALS AND METHODS

Chemical reagents and solvents for synthesis were purchased from Sigma Aldrich. Solvents and reagents were of analytical purity and were used without drying or purification. Microanalyses (C, H, N and S) of the Schiff base ligand were performed using a PerkinElmer CHN analyzer (2400). The FT-IR spectra of the Schiff base was recorded on a PerkinElmer 65 FT-IR spectrophotometer in the 4000-400 cm^{-1} range using KBr pellets. Mass analysis was performed on a Bruker microflex LT MALDI-TOF MS mass spectrometer. Electronic spectra were measured in ethanol on a Shimadzu spectrophotometer UV-1800. The proton and carbon NMR spectra of the ligand were recorded on a Brucker FT-300 MHz, NMR spectrophotometer in DMSO-d_6 solvent and TMS as reference material.

Synthesis and characterization of Schiff base ligand

Schiff base ligand ((E)-6-tert-butyl 3-ethyl 2-(3-nitrobenzylideneamino)-4,5-dihydrothieno[2,3-*c*]pyridine-3,6(7H)-dicarboxylate) was synthesized using the previously published procedure (Özdemir, 2019). In 35 mL ethanol dissolved 0.65 g (2.0 mmol) 6-tert-butyl 3-ethyl 2-ethyl 2-amino-4,5-dihydrothieno[2,3-*c*]pyridine-3,6(7H)-dicarboxylate and 0.31 g (2.0 mmol) 3-nitrobenzaldehyde in the same molar ratios. The solution was refluxed for 4 hours. TLC was used to evaluate the occurrence of the reaction and the progress and purity of the ligand obtained. The resulting light-colored product precipitate was washed several times with diethyl ether and crystallized in chloroform/ethanol mixture (yield: 85%). The synthesis of the Schiff base ligand is shown in Scheme 1.



Scheme 1. Synthetic procedure for the Schiff base ligand

Yield: 85%, color: cream, mp: 238 °C. MS (MALDI): m/z $[M-H]^+$ 460.150 Calc., 460.358 Found. Elemental analysis: ($C_{22}H_{25}N_3O_6S$) Calc: C; 57.50, H; 5.48, N; 9.14, S; 6.96. Found: C; 57.49, H; 5.51 57.49; N; 9.19; S; 6.93. FT-IR (KBr, ν cm^{-1}): 1662 $(C=O)_{br}$, 1584 $(CH=N)_{br}$, 1528, 1486 (C=C, Ar.), 1384 (C-NO_2), 781 (C-S-C). ^{1}H-NMR (300 MHz, DMSO-d_6, δ, ppm): 8.74 (s, 1H, CH=N), 7.27-6.88 (m, 4H, Ar. -CH), 4.56-2.92 (m, 6H, CH_2 (Py)), 4.41 (q, 2H, CH_2CH_3), 1.68-1.40 (s, 9H, -$C(CH_3)$)), 1.38 (t, 3H, -CH_2CH_3).

Fluorescence study of TNP

Fluorescence titration investigation was carried out in an aqueous solution by incrementally increasing the concentration of TNP (2.5-90) µM within a micro quartz cuvette, while keeping the concentration of Schiff base ligand constant at 15 µM. The excitation wavelength was set at 393 nm, with a slit width of 5 nm, optimized for the ligand.

The correlation between fluorescence emission intensity (I_0/I) and increasing quencher concentration ([Q]) was calculated using the Stern-Volmer (S-V) equation: $I_0/I = 1 + K_{SV}[Q]$. In this equation, K_{SV} is the S-V quenching constant, calculated from the slope of the S-V graph.

Additionally, the fluorescence spectra of ligand were examined across various solvents. To prepare the samples, 15 µM solutions of ligand were obtained by finely grinding the compound and dispersing it in 2.0 mL of each solvent (H_2O, EtAc, MeOH, ACN, DCM, and acetone). The resulting suspensions were then placed into quartz cuvettes for spectral analysis.

The fluorescence response to nitro-containing aromatic compounds (NACs) was further investigated using ligand at a fixed concentration of 15 μM in a designated solvent system. The photophysical properties and fluorescent sensor performance of ligand were evaluated through a combination of fluorescence and UV-Vis absorption spectroscopy.

RESULTS AND DISCUSSION

This research presents a novel fluorescent sensor derived from the water-soluble Schiff base ligand, designed for the selective and sensitive spectrofluorometric detection of TNP in aqueous solutions. To assess its effectiveness, we conducted an in-depth investigation of the photophysical properties and fluorescence sensing capabilities using UV-Vis absorption and fluorescence spectroscopy.

Additionally, experimental parameters were carefully optimized to improve the spectrofluorimetric detection of TNP in completely aqueous medium. This optimization was intended to enhance the sensor's precision and efficiency in detecting TNP.

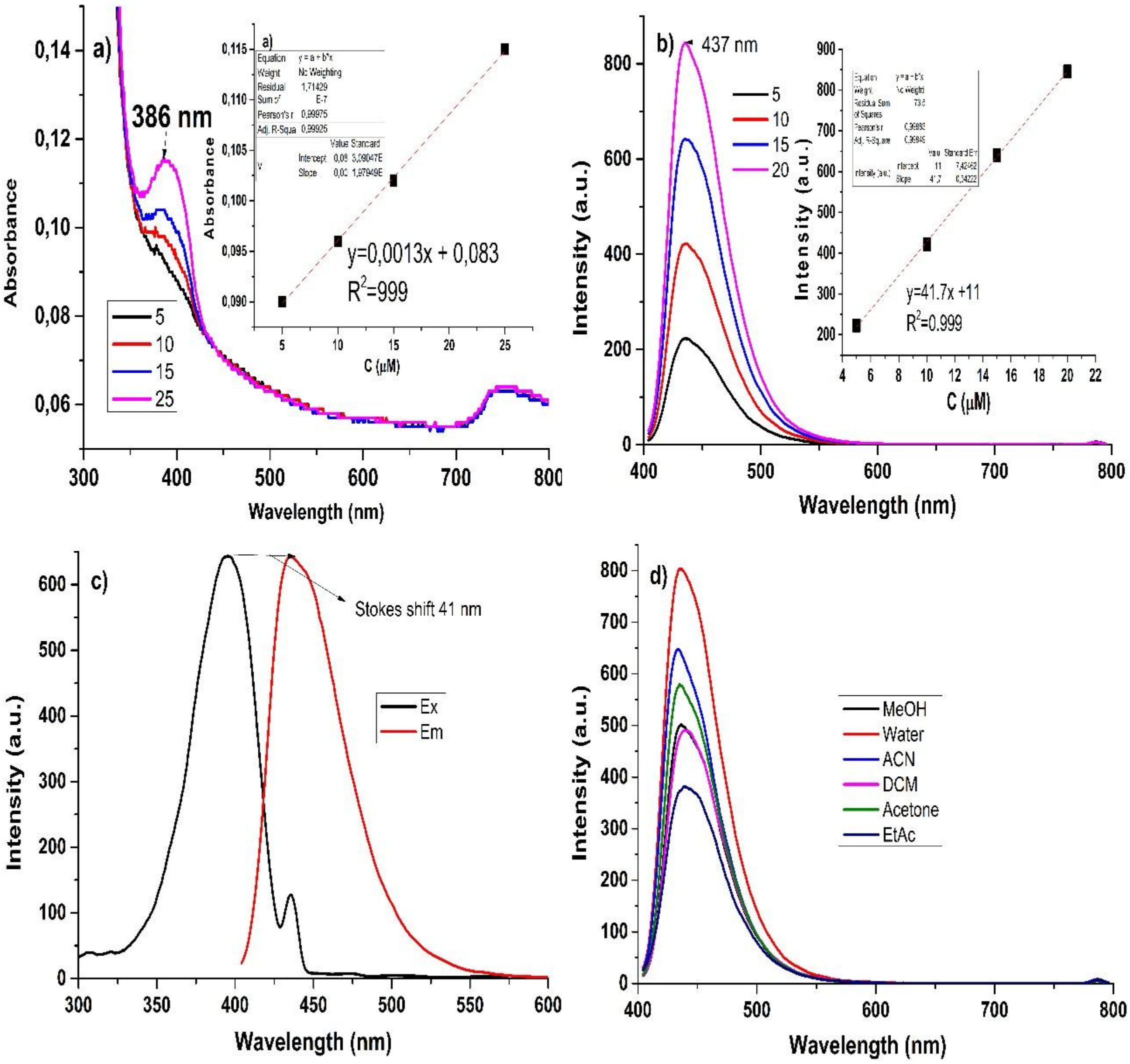


Figure 1. a) UV-Vis absorption, b) Fluorescence spectrum, c) Stokes shift in water, d) Fluorescence spectrum of ligand in different solvent systems (λex = 393 nm, max emission 437 nm)

We further explored the analytical performance of Schiff base ligand, focusing on its fluorescence response in the 'turn-off' mode upon exposure to TNP. This analysis offered valuable insights into the sensor's effectiveness and its potential applications in practical detection scenarios.

The optical properties of the ligand were assessed at room temperature using fluorescence and UV-vis spectroscopy. To thoroughly investigate its absorption behavior, 15 µM solutions of the ligand were prepared in several solvents, including water, acetone, ethyl acetate, methanol, acetonitrile, and dichloromethane (see **Figure 1d**). As depicted in **Figure 1a**, the ligand displayed distinct absorption peaks around 386 nm, attributed to the π—π* transition (Tümay & Yeşilot, 2019), indicating consistent absorption characteristics across different solvents. Furthermore, we examined the ligand's absorption (**Figure 1a**) and fluorescence (**Figure 1b**) in aqueous solutions at concentrations ranging from 5 to 20 µM, offering additional insights into how its absorption properties vary with concentration in a relevant solvent environment.

The fluorescence emission properties of the Schiff base ligand were determined used by fluorescence spectroscopy with an excitation wavelength of 393 nm. As shown in **Figure 1b**, ligand exhibited intense blue fluorescence, with a peak emission at approximately 437 nm and a calculated Stokes shift of 41 nm (**Figure 1c**).

As a result of the evaluation its photophysical properties, additional parameters related to Schiff base ligand's behavior were determined, including molar absorptivity (Ɛ) of 1.3 × 10^3 L·mol^{-1}·cm^{-1} and fluorescence lifetimes (τ_0), which are summarized in **Table 1.**

Fluorescent sensor characteristics investigation

A key factor in the development of novel fluorescence sensors is reach high selectivity for the accurate analysis of fluorescence signals within ligand samples. To evaluate the selectivity of the sensor under investigation, UV-Vis and fluorescence measurements were performed in an aqueous medium.

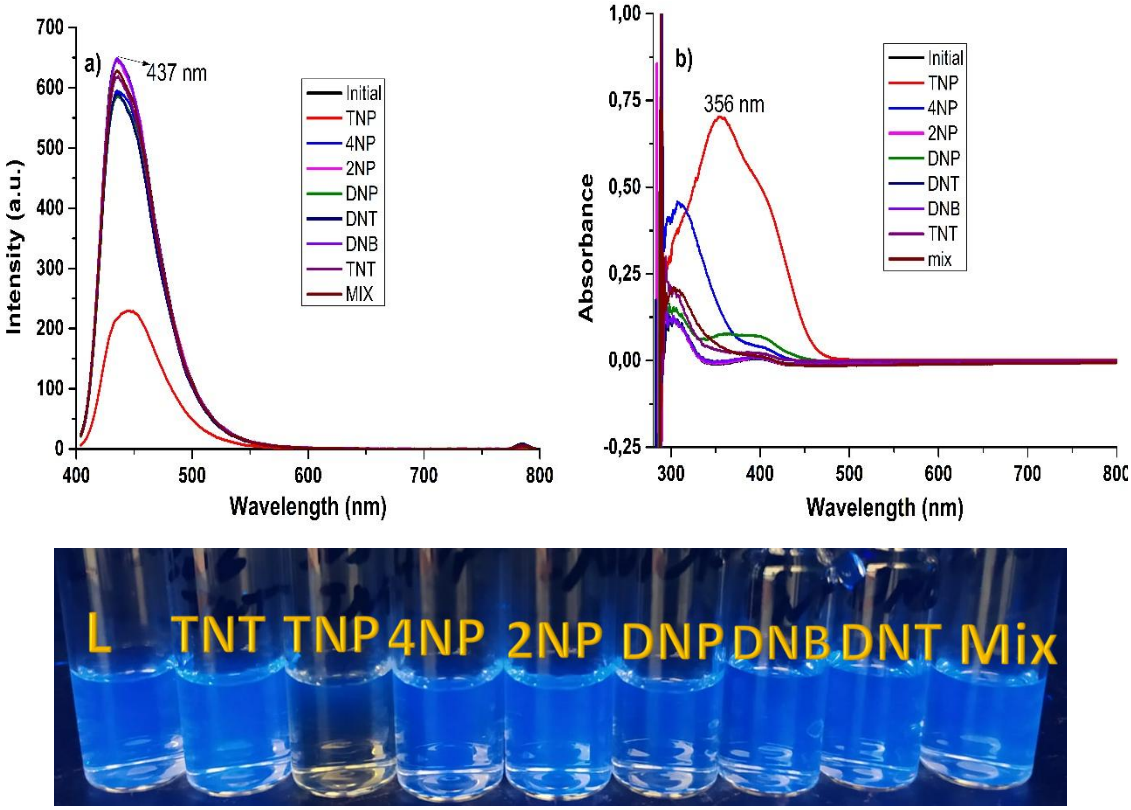


Figure 2. a) Fluorescence signal (λex = 393 nm) b) UV–Vis electronic absorption spectra c) Color changes under fluorescence irradiation of 15 µM ligand upon addition of 3 equivalents of different NACs analysts in water

Exactly, a 15 µM solution of ligand was exposed to a range of NACs, including DNP, DNB, 2-NP, DNT, 4-NP, TNT, and TNP. The UV-Vis and fluorescence responses of ligand in the presence of these compounds were carefully recorded, as illustrated in **Figure 2**. In addition, the fluorescence effect was investigated by adding various metals in the presence of 15 μM ligand. It was found that the metals added in the same amount as TNP had no effect (**Fig. S4**).

Figure 2b illustrates the UV–Vis spectroscopic analysis conducted after the incorporation of TNP into the solution, showing a marked increase in the absorbance of the ligand, initially centered at 356 nm. This significant change highlights the exceptional selectivity of ligand, which exhibited a distinct response to TNP compared to other aromatic compounds tested. It is important to note that this selectivity is closely related to the number of nitro groups present in the compounds, as has been found in previous studies (Carrillo-Carrión et al., 2013). After the introduction of TNP, the shifts observed in the UV-Vis absorption spectra are likely a result of electronic structural reconfigurations within ligand. These modifications are thought to be driven by efficient charge transfer interactions between the electron-withdrawing NACs and the electron-providing ligand (Zhang et al., 2015).

Following the completion of UV-Vis absorption measurements, the selectivity of ligand for TNP was further assessed using fluorescence spectroscopy within the same experimental parameters (**Figure 2b**). At a concentration of 15 μmol/L, negligible effects on the fluorescence response of the ligand were observed when various nitroaromatic compounds were added. However, the introduction of Trinitrophenol induced an important decrease in fluorescence intensity, demonstrating a 64.5% fluorescent "turn-off" response at 437 nm for the ligand (**Figure 2a**). The results highlight the exceptional selectivity of the ligand for TNP, even in the absence of other competing nitroaromatic compounds in the sample matrix. In addition, the data showed that the magnitude of the fluorescence quenching effect increased in direct ratio to the degree of nitro group containing in the molecules.

In the present research, we performed interfering experiments using a 15 μmol/L ligand solution in water, adding diverse nitroaromatic compounds, for example, TNT, DNB, DNP, 2-NP, DNT and 4-NP, each at a concentration of 45.00 μM. The assessment was conducted by examining the relative changes in fluorescence signals. The strong selectivity of ligand, particularly towards TNP, remained evident. The observed selectivity in the fluorescent sensor's response can be attributed to the efficient electron transfer processes that occur between the sensor and 2,4,6-trinitrophenol (TNP). This interaction is selectively induced, resulting in a marked "turn-off" response in fluorescence at a wavelength of 437 nm when measured in an aqueous medium. This selectivity highlights the potential of the fluorescent sensor as a sensitive detection platform for TNP, with implications for environmental monitoring and analytical chemistry applications. In conclusion, even in the presence of other competing types, spectrofluorometric detection of low concentrations of TNP can be reliably performed using the fluorescent sensor ligand in 100% aqueous solution.

To further emphasize the selectivity of Schiff base ligand, a visible detection test was performed under 393 nm irradiation (**Figure 2a**). Upon the addition of TNP, the color of the ligand solution changed from a vibrant blue to colorless, whereas other competing species did not cause any noticeable color change (**Figure 2c**). These findings clearly demonstrate that ligand is a highly promising applicant for the efficiently, sensitive and selective detection of TNP using spectrofluorometric. The findings clearly showed that the fluorescence signals of both the ligand and the ligand+TNP complex remained steady over the 100 min. observation period (**Figure 3**). This consistency underscores the exceptional photostability of both the Schiff base and its complex with TNP, highlighting their resistance to photodegradation stable under extended light exposure.

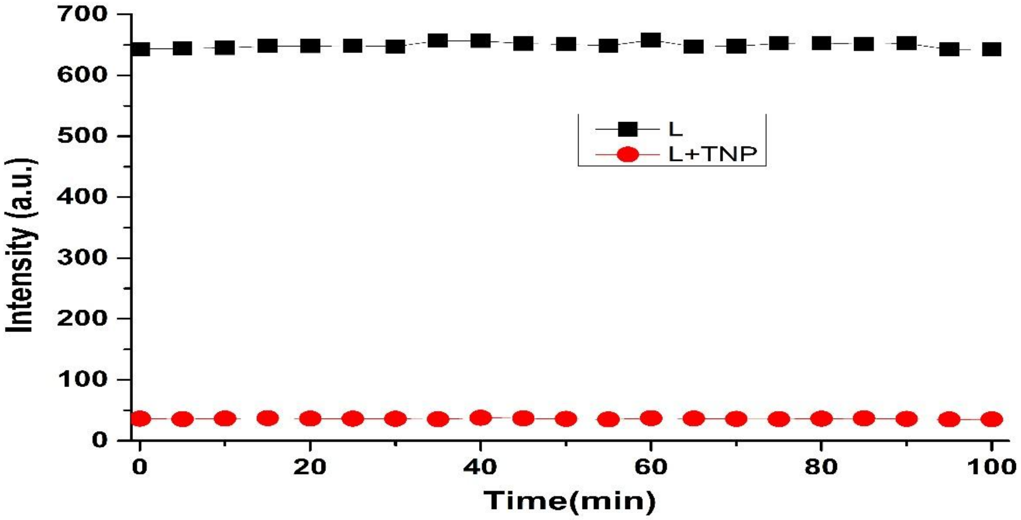


Figure 3. Photostability of ligand and ligand + TNP in water (λex = 393 nm, λem = 437 nm)

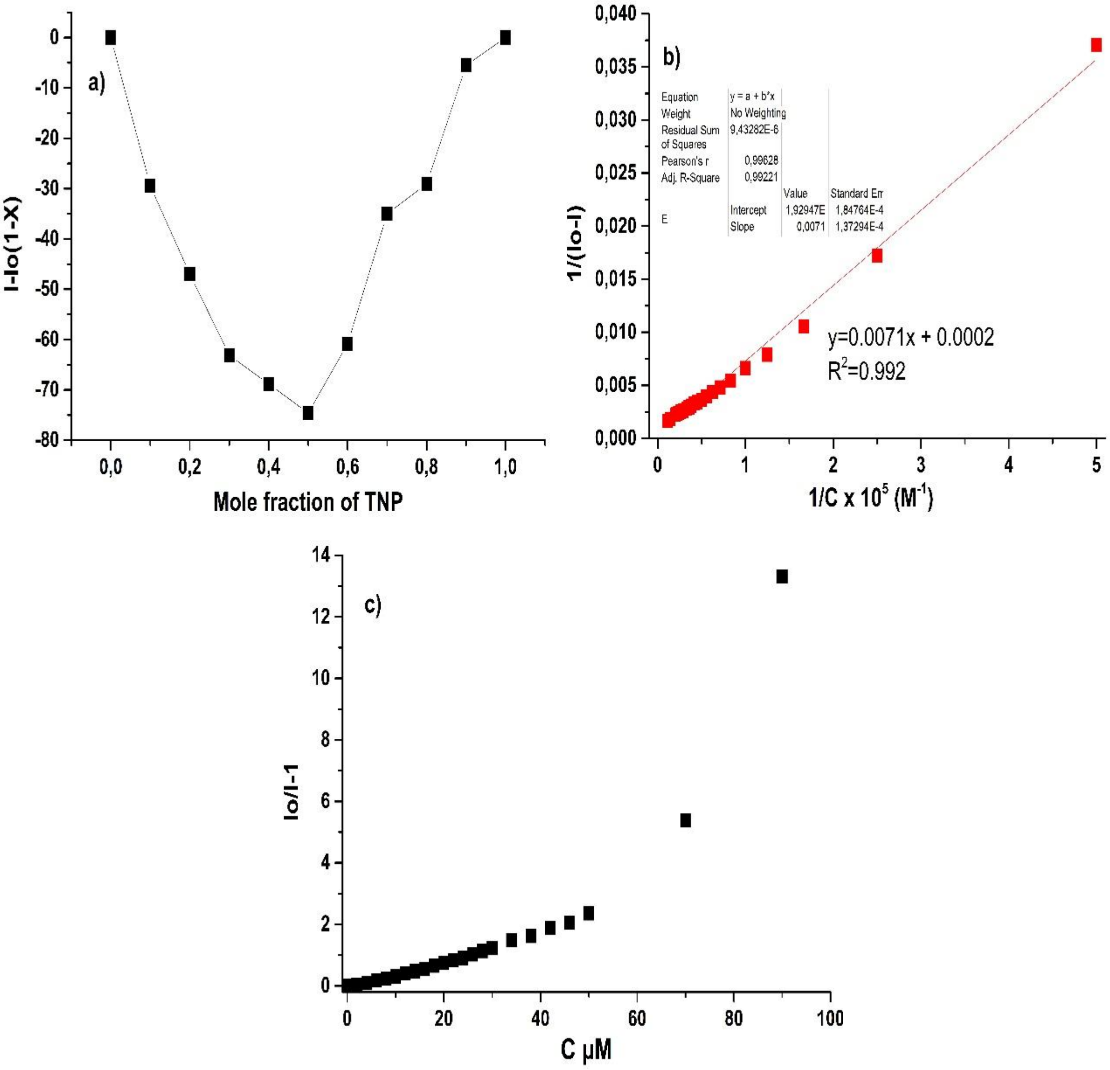


Figure 4. a) Job’s plot, b) Benesi Hildebrand graph, c) S-V non-linear graph for ligand+ TNP in water (λex = 393 nm, λem=437 nm)

To elucidate the stoichiometry of the interaction between the chemosensor ligand and 2,4,6-trinitrophenol (TNP), a series of experiments were conducted utilizing the Permanent Variation method, commonly known as Job's method. In these experiments, a fixed concentration of 15 μmol/L of the ligand was maintained while systematically varying the mole fraction of TNP in the resulting ligand-TNP complex within an aqueous medium. The resulting Job plot revealed that the maximum mole fraction indicative of selective TNP detection was achieved at a value of 0.5 for the ligand, as illustrated in **Figure 4a**. This finding supports the conclusion that the ligand forms a 1:1 complex with TNP, confirming a stoichiometric ratio of ligand to TNP of 1:1.

In this study, the coupling constant for the formation of TNP with ligand in water was found to be 7.1 $\times 10^3$ M^{-1} using the Benesi-Hildebrand equation. This result was achieved by adding a ligand concentration of 15 μmol/L and a gradually increasing amount of TNP, as shown in **Figures 4b** and **4c**. The results obtained from both the Job plot and the non-linear curve fitting analysis agree and support each other.

The mechanism of fluorescence quenching of the ligand was studied by following its interaction with TNP. This investigation was significant as sensor systems are known to exhibit either static or dynamic quenching processes, which are fundamentally different. Dynamic quenching occurs through collisions between the quencher and the excited fluorophores, while static quenching involves the formation of non-radiative complexes (Albani, 2007). The conventional S−V equation ($I_0/I = K_{sv}[Q]+1$) was used to analyze the quenching effects resulting from the intensities monitored at 437 nm at low concentrations, and the Ksv value was calculated as 4.16×10^4 M^{-1}. When plotting I_0/I against [Q], the resulting graph was linear with a y-axis intercept of 1, which indicates the occurrence of static quenching (Hung et al., 2009). Schiff base ligands generally have an electronically rich structure, while the nitro groups of TNP act as strong electron acceptors. This donor-acceptor interaction leads to quenching of fluorescence. In particular, the interaction between the electron donor groups of the Schiff base and the nitro groups of TNP inhibits the fluorescence emission and triggers the turn-off mechanism (Dey et al., 2019).

The fluorescence lifetimes were found to be 4.590 ± 0.006 (τ_0) ns for ligand and 4.490 ± 0.006 (τ) ns for ligand+TNP (**Figure 5**). It was clearly found that the lifetime of the compound did not change with the addition of TNP and τ_0/τ for ligand was almost equal to 1. The measured lifetimes of the fluorescence signal may suggest the presence of static quenching, which is likely a consequence of the formation of non-fluorescent complexes in the ground state. This phenomenon occurs when the chemo sensor interacts with the analyte, leading to the establishment of a stable complex that does not exhibit fluorescence (Sharma & Mehata, 2021).

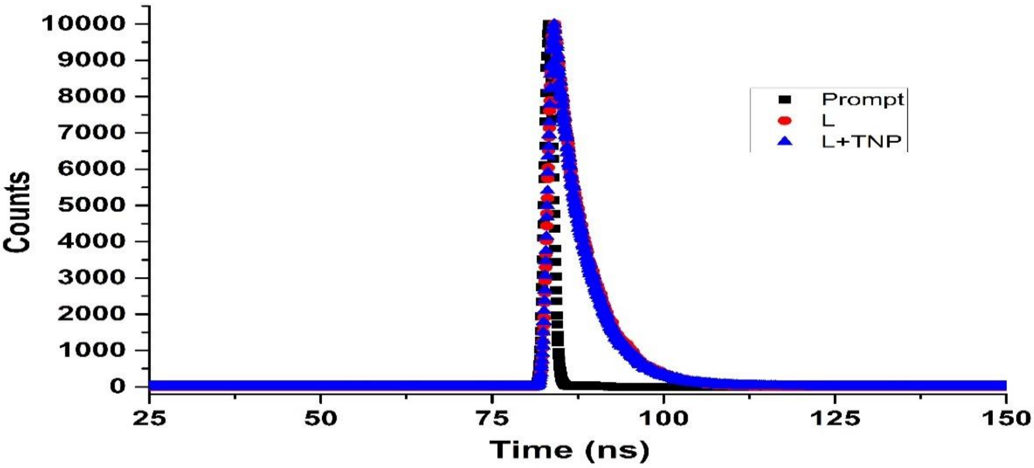


Figure 5. The fluorescence decay profiles of the ligand were examined in the presence of TNP, employing a laser excitation source emitting at 390 nm

Analytical studies for TNP detection

To illustrate the functional application of our fluorescent sensor (ligand), we performed spectrofluorometric measurements of TNP in aqueous solutions. This involved conducting fluorescence titration experiments in which the concentration of TNP was systematically increased while monitoring the fluorescence of ligand in optimized conditions, as depicted in **Figure 6a**. The Schiff base ligand exhibited a pronounced 'turn-off' fluorescence response, with its emission peak at 437 nm, when tested in aqueous media. Notably, this quenching effect was particularly significant as the TNP concentration increased, with a marked decrease in fluorescence intensity observed up to a concentration of 20.62 mg/L. This response highlights the ligand's potential as a sensitive fluorescent sensor for TNP detection in aqueous environments.

As shown in **Figure 6b**, the relative fluorescence signal of ligand showed a great linear correlation within the TNP concentration range of 2.5 to 30.0 μM. The linear regression equation determined for TNP was (y= 0.0416(TNP)-0.072) with an (R^2) value of 0.992, based on the change in the relative fluorescence signal of ligand. This approach underscores the sensor's effectiveness in quantifying TNP in aqueous samples.

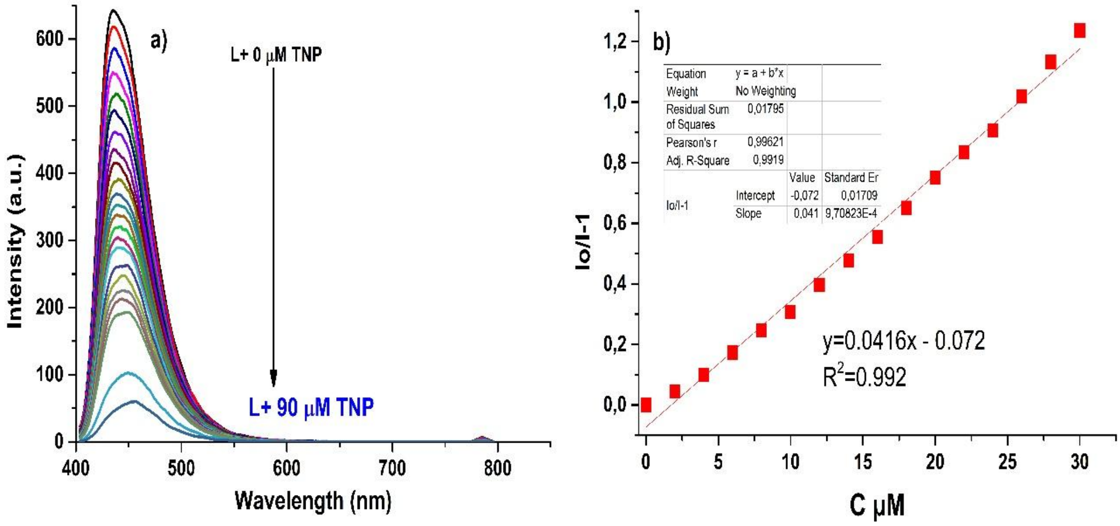


Figure 6 a) The fluorescence titration and b) shows the linear relationship (λex = 393 nm max emission 437 nm and 15 μM of ligand+TNP in aqua)

Table 1. Investigative parameters for Schiff base in water

Analytical parameters	Ligand
Ɛ (L/mol.cm) x10^3	1.3
Excitation wavelength (nm)	393
Emission wavelength (nm)	437
Linear range (μM)	2.5-30
Sensor concentration (μM)	15
Ksv (1/M)	41.6 10^3
R^2 of linear	0.992
STD	0.0032
LOD (μM)	0.253
LOQ (μM)	0.769

The analytical performance of our developed spectrofluorimetric sensor, based on a Schiff base ligand, was meticulously evaluated, with a particular focus on the limits of detection (LOD) and quantification (LOQ) for the detection of TNP. The LOD and LOQ were determined using the standard criteria of 3.3σ/K and 10σ/K, respectively. The calculated LOD of 0.253 μmol/L and LOQ of 0.769

μmol/L demonstrate a marked improvement over previously reported values for fluorescent sensors used in TNP detection (Kumari et al., 2016; Guo et al., 2021; Sharma & Mehata, 2021; Zheng et al., 2021; Desai et al., 2023; Köse et al., 2023; Li et al., 2023; Ture et al., 2023; Desai et al., 2024). These findings underscore the superior sensitivity, precision, and specificity of our method for detecting TNP in aqueous media. A comprehensive summary of the analytical parameters is provided in **Table 1**.

CONCLUSION

The synthesized Schiff base demonstrated exceptional sensor capabilities towards various nitroaromatic compounds, with a particular emphasis on its extraordinary sensitivity to TNP. The ligand exhibited a high Sterm-Volmer constant (Ksv: 4.16×10^4 M^{-1}) and maintained linearity in the range of 2.5 to 30 µM, with an impressive limit of detection (LOD) of 0.253 µM. Denoted as ligand, the Schiff base exhibited robust fluorescence properties, thereby establishing its potential for application in fluorescence quenching-based TNP detection in aqueous solutions. Significantly, the Schiff base displayed both high selectivity and sensitivity in detecting TNP.

Further insights from fluorescence titrations, particularly through Job's plot analysis, revealed a specific interaction stoichiometry between the ligand and TNP at a 1:1 ratio (ligand to TNP). This finding suggests that two molecules of the complex associate with one molecule of TNP during their interaction, providing critical information about the nature of the ligand-TNP interaction mechanism. The results highlight the promise of Schiff base-based fluorescent receptors as effective, selective, and sensitive tools for detecting nitroaromatic compounds. These receptors have potential applications in diverse areas, such as environmental monitoring and chemical analysis.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author’s Contributions

Ayhan ALTUN: Writing, Validation, Methodology, Research, Formal analysis, Conceptualization. **Kenan BULDURUN:** Writing – review and editing, Supervision. **Nevin TURAN:** Review and editing, and Supervision.

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