INDIAN JOURNAL OF SCIENCE AND TECHNOLOGY

RESEARCH ARTICLE

*Corresponding author.

drkdbhatt@outlook.com

Competing Interests: None

Oxacalix[4]arene Based "Turn-off Florescence Sensor" for Selective and Sensitive Screening of 4-Nitrotoluene: Insight from Spectrophotometry and Spectrofluorometry

Mansi Panchal[1], Keyur Bhatt[1]*

1 Department of Chemistry, Mehsana Urban Institute of Sciences, Ganpat University, Mehsana, 384001, Gujarat, India

Abstract

Objective: A chemosensor of novel and highly promising nature, namely 5,17-di(2-naphthoylsulphonyl)tetranitrooxacalix[4]arene (DNSTNOC), has been synthesized. This innovative compound exhibits exceptional potential in the domain of molecular screening, particularly in the realm of selective detection of 4-nitrotoluene (4-NT) in comparison to a variety of other explosive compounds. **Methods:** The receptor that was synthesized underwent characterization through the utilization of H1 NMR, ESI-MS, and IR spectroscopy. In order to detect explosives, spectroscopic and spectrofluorometric techniques were employed. **Findings:** A novel chemosensor has been successfully synthesized and investigated for its efficacy in detecting 4-nitrotoluene (4-NT) in the presence of various explosives. In order to monitor the host-guest complexation, absorption and emission studies were conducted with different explosives. The electron-withdrawing nitro group of 4-NT readily interacts with the highly conjugated naphthoyl sulphonyl ring, resulting in a significant quenching of the fluorescence intensity of DNSTNOC through photo induced electron transfer (PET). **Novelty:** The aforementioned innovative chemosensor, namely 5,17-di(2-naphthoylsulphonyl)tetranitrooxacalix[4]arene (DNSTNOC), presents a pragmatic resolution for real-life predicaments, such as the identification of explosive materials. Its exceptional capability to detect 4-nitrotoluene (4-NT) within a linear concentration range of 50 μM to 1 mM is noteworthy.

Keywords: Oxacalix[4]arene; Turnoff fluorescent sensor; 4nitrotoluene; Quenching; Explosives

1 Introduction

In this emerging world sensing of explosives is a real-world problem which need to be addressed. Various functionalized Calix[4]arene are designed and explored for the sensing of explosives[1,2]. Oxacalixarene a versatile class of hetera calixarene, can proficiently be synthesized by nucleophilic aromatic substitution (SNAr)[3,4]. Oxacalixarene

is regarded as an alluring host due to tunable cavity, ease of preparation and exceptional complexation proficiency towards various analytes[5].

"Explosives are very reactive organic materials that contain large amount of energy that can lead to an explosion by engendering heat or increasing an abrupt pressure effect"[6]. It is extremely crucial to detect life-threatening explosives for national security, endeavors related to military, criminal numeration and for environmental and human health[7]. Owing to the growing needs of counter terrorism and protection activities, the recognition of munitions particularly the excessively used nitro aromatics has become an immediate concern demanding certain methods that are comparatively more miniature and sensitive coupled with being cost-effective[8–10].

In the field of analyte detection, a diverse range of chemosensors has been introduced by researchers. However, the development of novel chemosensors for the selective and sensitive detection of explosives takes precedence due to the critical need for enhanced techniques in this domain. While several electrochemical methods are available for the detection of explosives, their cost and limited mobility pose significant drawbacks, impeding their widespread utilization. When evaluating various analytical approaches, fluorescence-based detection emerges as a standout choice, offering a host of compelling advantages. Firstly, it exhibits remarkable sensitivity, enabling the detection of even minute quantities of target analytes. Additionally, it boasts specificity, ensuring that the identified substances are accurately differentiated from others. Real-time monitoring capabilities further enhance its appeal, enabling continuous tracking of analyte presence and concentration.

The rapid response times inherent to fluorescence-based detection contribute to its efficiency in addressing time-sensitive scenarios. Given these advantageous qualities, it becomes clear that the development of methods that merge the desirable traits of miniaturization, sensitivity, and cost-effectiveness is essential. By creating techniques that are compact in design, yet incredibly sensitive in their ability to detect explosives, researchers can significantly enhance detection capabilities while minimizing the financial burden of employing these methods. This pursuit of innovation aligns with the requirements of real-world applications where efficient and reliable explosive detection plays a pivotal role in ensuring security and safety. Consequently, the ongoing exploration and establishment of such advanced, versatile, and economically viable detection methods remain crucial for addressing the challenges posed by the detection of explosives[11,12]. In order to detect explosives using "nitroaromatics, "the novel fluorophore 5,17-di(naphthalene sulphonyl)tetranitrooxacalix[4]arene has been synthesized and thoroughly examined.

Nitroaromatic compounds are extensively used as explosives. 4-nitrotoluene (4-NT), a nitro aromatic explosive besides being used as a marking agent/taggant is also used as an industrial raw material for the production of several dyes, synthetic fiber and pharmaceutical auxiliaries[13,14]. In the context of the commercial usage, this nitro aromatic explosives are used in the production of some explosives, dyes, rubber and agricultural chemical[5]. Oxacalix substituted arenes were successfully synthesized and underwent a thorough biological analysis. The aim of the study was to determine the compounds effects and their therapeutic potential. The biological profiles of the compounds were examined in order to determine their efficacy in the fight against cancer, fungal infection and bacterial growth. Molecular design for biomedical applications is a growing field that offers promising insights into developing new treatments. The diverse range of activities studied highlights the multifunctional character of the synthesised oxacalix[4]arenes. It may pave the way for a novel class of therapeutic agents.

The growing usage of 4-NT has also led to some serious toxicological effects[15,16]. It has been found that 4-NT is a degradation product of trinitrotoluene (TNT) in the form of reduced product in the soil environment making it a soil pollutant. Certain adverse effects on the human body have also been observed due to being carcinogenic in humans. Hence, in order to eliminate harmful aromatic pollutant from the environment and waste, it is inevitable to develop some efficacious methods.

2 Methodology

2.1 Chemical and reagents

1,5-difluoro-2,4-dinitrobenzene, phloroglucinol and 2-naphthalene sulphonyl chloride of analytical grade were purchased from sigma-Aldrich, SD-Fine and TCL. Additional solvents and reagents employed directly during the synthesis and were bought from commercial sources.

2.2 Instrumentation

Prior to use, all glassware was dried overnight. The melting points were attained from a VEEGO melting point apparatus (Model; VMO-DS). Using E-Merck silica gel 60 E_{254} precoated plates, the reaction's progress was scrutinized with TLC and seen with UV light. The Jasco V-570 UV-Vis recording spectrophotometer is used to investigate the ultraviolet absorption spectra in the 200–800 nm range (Tokyo, Japan). A BBFO probe was used to record NMR data on a Bruker AV (III)-400 MHz HORIBA

FluoroMax-4 spectrofluorometer was used to record fluorescence spectra.

2.3 Synthesis and characterization of receptor (DNSTNOC)

Oxacalixarene was synthesized by previously reported method. Oxacalixarene (1mmol) was taken in acetone to which Triethylamine (2.5 mmol) was, which was then stirred for 30 min at ambient temperature. 2-napthalene sulfonyl chloride (1.0 mmol) were added to reaction mixture followed by stirring for 24h at room temperature. After the completion of the reaction mixture was washed with dil. HCl and organic layer was extracted using ethyl acetate (100 ml). The organic layers were washed with brine solution, dried over sodium sulphate. Solvent was removed in vacuo, precipitation was filtered, washed with water and dried in vacuo to obtain the product. Pale yellow colour precipitates; yield 60 %.; (Scheme 1) Melting point: 210 to 220 °C; ^{1}H NMR (400 MHz, CDCL$_3$); δ 7.0-8.6(m,24 H, Ar-H); ESI-MS (m+1= 190.1).
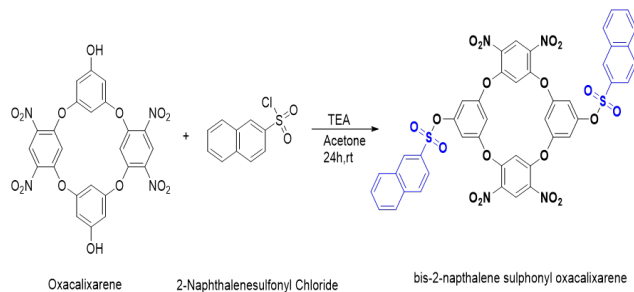
Scheme 1: **Synthesis of DNSTNOC**

2.4 Method of explosive detection using spectrophotometry and spectrofluorometry

The stock solution of DNSTNOC (1 mM) and various NACs (1 mM) were prepared using acetonitrile. 2.5ml stock solution of receptor and 2.5 ml solution of explosive were taken in 5ml volumetric flask. The absorption spectra of DNSTNOC and solutions of various NACs were recorded. The same stock solutions were used for emission spectra. Moreover, the receptor was stimulated at 342 nm and upon addition of 4-NT the change in emission maxima were observed.

3 Result and Discussion

3.1 Absorption study

Absorption studies were performed in the presence of different NACs to ensure the selectivity. The wavelength of DNSTNOC at 279 nm was shifted at 289 nm after the addition of 4-NT. Merely 4-NT exhibit a significant change in absorption spectra. Thus, with no interference, DNSTNOC was found to be selective for 4-NT. Spectra shows the absorption spectra of ligand and solution of 4-NT. With the addition of 4-nitrotoluene, the absorption band (279 nm) was shifted to 289 nm. The other NACs remain unaffected Figure 1.
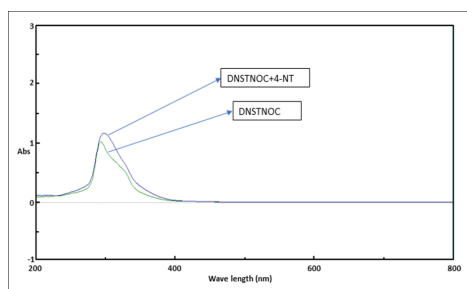

Fig 1. Absorption spectra of DNSTNOC with 4-NT

3.2 Fluorescence study

An emission studied were performed in order to evaluate the ability of DNSTNOC to act as a Fluorescence chemosensor. DNSTNOC shows an emission at wavelength of 375nm. However, strong quenching in fluorescence intensity was observed in the presence of 4-NT even at a very low concentration range of 10^{-4} M in aqueous solution of explosives, while the remainder of the NACs are unaffected. Represents the fluorescence spectra of ligand with 4-NT. Spectra shows the strong quenching in intensity of ligand with the addition of 4-nitrotoluene Figure 2.
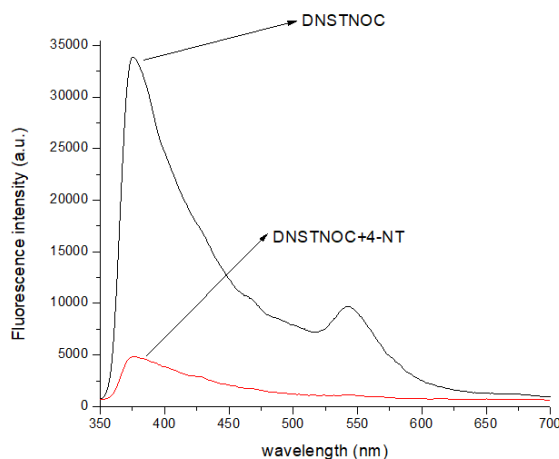

Fig 2. Fluorescence spectra of DNSTNOC upon the addition 4-NT

The spectrofluorometric titration of DNSTNOC in the presence of different explosives. It is worth noting that a distinct decrease in fluorescence intensity was only observed when 4-nitrotoluene (4-NT) was introduced. Interestingly, the fluorescence behavior of the other explosives remained unchanged throughout the titration process. This exceptional selectivity, where only 4-NT causes significant quenching, highlights the potential of DNSTNOC as a highly specific chemosensor for detecting 4-NT among various explosives. This finding demonstrates the considerable promise of DNSTNOC for precise and sensitive detection applications, especially in situations where accurate identification of 4-NT is crucial. The absence of quenching in response to other explosives underscores the unique molecular interaction between DNSTNOC and 4-NT, which serves as the foundation for a reliable and discerning detection method in the field of explosives screening Figure 3.
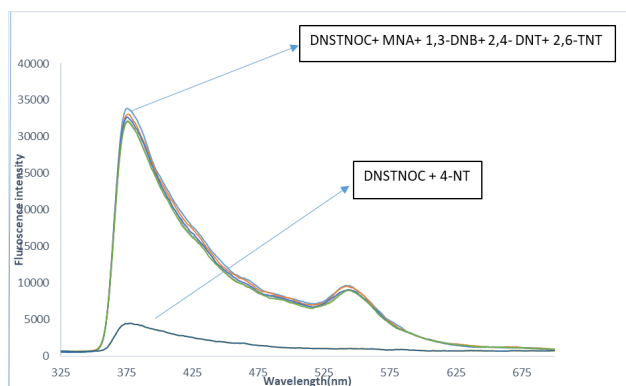

Fig 3. Fluorescence spectra of DNSTNOC upon the addition of different explosives

3.3 Sensing Mechanism

DNSTNOC consists of two distinct structural components: a naphthalene ring with an abundance of electrons and a benzene ring with a substitution of nitro group (–NO2), which creates an electron deficiency. Due to its electron deficiency, the 4-NT

portion of the molecule draws electrons through interactions with the electron-rich naphthalene ring, primarily through π-π stacking and π-sigma interactions. Consequently, this interaction disrupts the electronic conjugation within the receptor, leading to a decrease in the emission intensity. Figure 4 represents the change in emission intensities of DNSTNOC upon the addition of increasing concentration of 4-NT. The fluorescence intensity gradually decreases with the concentration of 4-NT.[17,18]
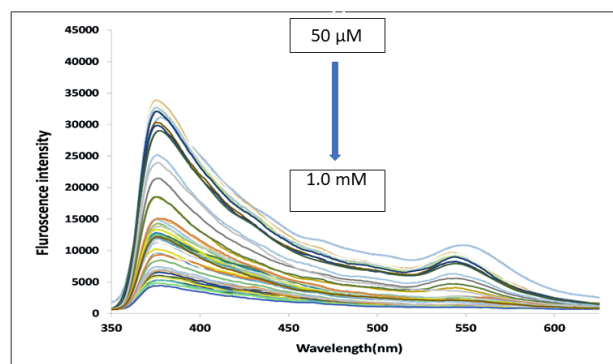

Fig 4. Fluorescence spectra of DNSTNOC upon the addition of different explosives

3.4 Binding constant and quantum yield

The fluorophore binding constant was determined using the previously described method and the provided equation.

$$\frac{(Io - I)}{(I - I1)} = \frac{[C]}{(Kdiss)^n} \qquad (1)$$

Here, I_o and I_1 are the fluorescence intensities of the complex without the addition of 4-NT and at the highest concentration of the 4-NT, respectively. I is the complex's fluorescence intensity at any given 4-NT concentration. Thus, the binding constant Ks is obtained by plotting log $(I_0\text{-}I/I\text{-}I_1)$ versus log [C] The value of log [C] at log $[(I_0\text{-}I)/(I\text{-}I_1)]$ = 0 gives the value of log (K_{diss}), the reciprocal of which is the binding constant (Ks).

Fluorescence quantum yield was determined by using following equation [16].

$$\phi = \phi std \frac{FA_{Std}\eta 2}{FStdA\eta 2Std} \qquad (2)$$

Where F and F_{Std} are the regions under the fluorescence emission curves of complex and standard DNSTNOC. A and A_{stds} are relative absorbance of sample and standard at the absorption wavelength respectively. n and η_{std} are refractive standard of solvent.

Figure 5, Plot of log B versus Log C is selected for the calculation of binding constant. From the calculation binding constant of complex is 2.3 $\pm$ 0.20.
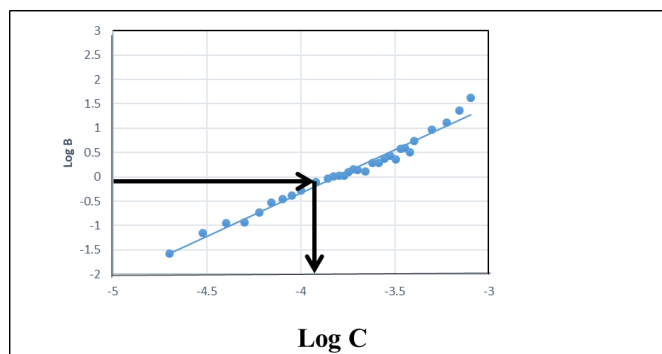
Fig 5. Binding Constant Plot of log B versus Log C

3.5 Stern-Volmer analysis

Stern-Volmer plots are helpful for comprehending how quenching works and were used to examine the nature of the quenching process during the complexation of 4-NT with receptor.

By graphing relative emission intensities (I_0/I) versus quencher concentration [Q], it is possible to identify quenching.

$$\frac{I_0}{I} = 1 + K_{sv}[Q] \tag{3}$$

According to the concentration of quencher, if the evolution of I_0/I is linear fluorescence quenching can be either static or dynamic and if the ratio is not linear quenching can be simultaneous dynamic and static. The plot of I_0/I-1 versus concentration of 4-NT. Linear plot was observed for 4-NT with DNSTNOC, which indicate that quenching is purely static Figure 6.
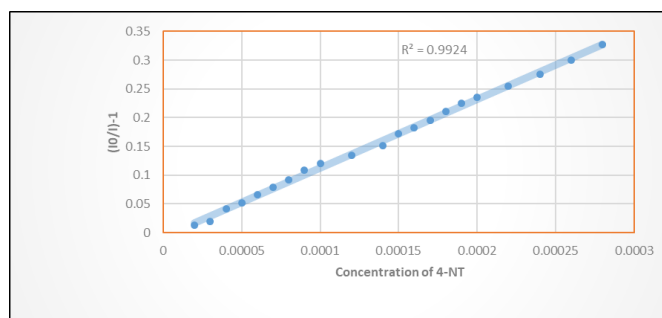

Fig 6. Stern-Volmer plot of fluorescence quenching of DNSTNOC by 4-NT

The graph shows the relationship between "ΔA" and the mole fraction of 4-nitrotoluene (4-NT). Employment plots indicate that single-person complexes have formed, as indicated by the observed specific values. This indicates a highly selective interaction between the analyzed components, indicating that only one characteristic complex was formed during the experiment. This result enhances the specificity of the system's interaction with 4-NT and shows its potential as a robust and sophisticated method to detect this specific compound among other substances Figure 7.

3.6 Colorimetric analysis

Oxacalixarene derivative that exhibits a remarkable colour change upon exposure to 4-nitrotoluene explosive. The compound changes from colourless to a distinct yellow colour in the presence of 4-NT. This colour change provides a simple and efficient way to detect the presence of 4-NT, as it can be observed with the naked eye. This characteristic is particularly useful in field applications where rapid and easy identification of explosives is crucial. It is important to note that the observed colour change is highly specific to 4-NT and is not observed when the compound is exposed to other explosives. Overall, the observed colour change in our Oxacalixarene derivative presents a promising development in the field of explosive detection Figure 8. It offers a simple and efficient detection method, which could potentially be used in various security and defense applications.
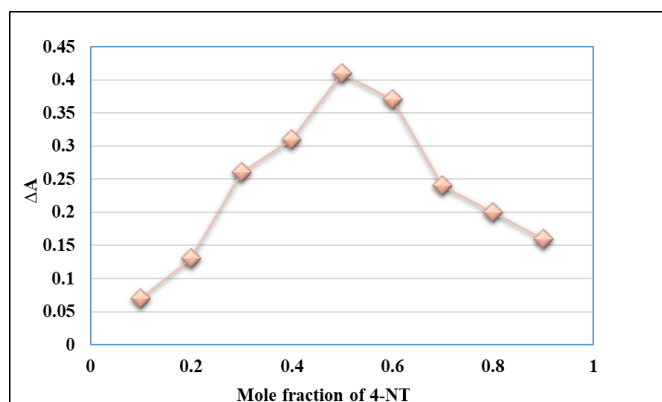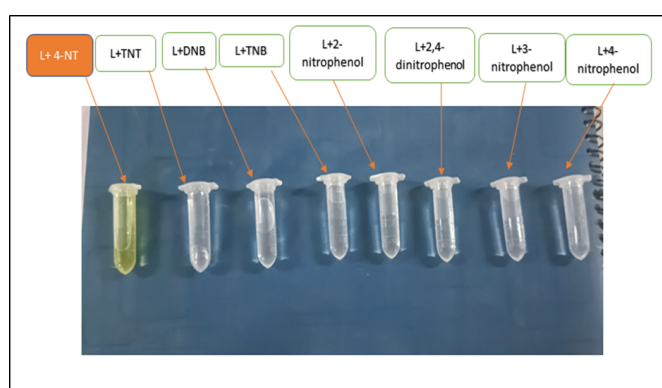
Fig 7. Jobs plot of DNSTNOC with 4-NT

Fig 8. Colorimetric study of DNSTNOC with different explosives

3.7 Comparison with existing systems

Previously reported research on explosive sensing has focused on either UV or fluorescence detection. However, novelty of our research highlights the ability to detect explosives in both UV and fluorescence, as well as visually via colour change. This makes our research a significant improvement over previous work, as it provides a wider range of detection capabilities and makes it easier to use in real-world applications.

Table 1.

Receptors	Analytes	Binding Mechanism	Detection Method	References
FON$_3$ PPh	4-NT, 2,6-DNT	DFT	Fluorescence	[19]
PON$_3$ PPh	4-NT, 2,6-DNT	DFT	Fluorescence	[20]
DNQTNOC	4-NT, MNA	DFT	Fluorescence	[21]
ABuCP	1,3-DNB	π-cation	Absorption	[22]
BNOC	4-NT and 2,3-DNT	π- π interaction	Fluorescence	[22]
DNSTNOC	4-NT	π- π stacking, charge transfer	Absorption, Fluorescence and Colorimetric detection	Present work

4 Conclusion

5,17-di(2-naphthoyl sulphonyl)tetranitrooxacalix[4]arene based novel chemo sensor was successfully synthesized and used as a recognition tool for 4-NT. This synthesized chemosensor can be used as a recognition tool for real sample analysis in the future. The spectrofluorimetric method of detection showed quenching in the fluorescence intensity of DNSTNOC on the addition of

4-NT. Quenching in fluorescence intensity suggest that receptor could act as 'Turn-off' fluorescent probe for the detection of 4-NT. The DNSTNOC emission in the 340-700nm region was sensitive to 4-nitrotoluene, while the emission of DNSTNOC beyond 700nm was unaffected. The selectivity is due to the electron rich nature of naphthalene ring, making it suitable towards electron deficient nitroaromatic compounds. Novelty of this work highlights the 4-NT detection via UV, fluorescence method as well as visual color change, making it more effective than previous methods.

5 Abbreviations

DNSTNOC: 5,17-di(2-naphthoylsulphonyl)tetranitrooxacalix[4]arene, 4-NT: 4-Nitrotoluene, PET: Photo induced electron transfer, TNT: Trinitrotoluene, NACs: Nitroaromatic compounds

References

1) Cowart A, Brük ML, Žoglo N, Roithmeyer H, Uudsemaa M, Trummal A, et al. Solution- and gas-phase study of binding of ammonium and bisammonium hydrocarbons to oxacalix[4]arene carboxylate. *RSC Advances*. 2023;13(2):1041–1048. Available from: DOIhttps://doi.org/10.1039/D2RA07614D.

2) Desai V, Panchal M, Dey S, Panjwani F, Jain VK. Recent Advancements for the Recognization of Nitroaromatic Explosives Using Calixarene Based Fluorescent Probes. *Journal of Fluorescence*. 2022;32(1):67–79. Available from: https://doi.org/10.1007/s10895-021-02832-y.

3) Sen S, Onder FC, Capan R, Ay M, Erdogan CO. Humidity effect on real-time response of tetranitro-oxacalix[4]arene-based surface plasmon resonance (SPR) acetone sensor at room temperature. *Optik*. 2023;272:170303. Available from: https://doi.org/10.1016/j.ijleo.2022.170303.

4) Wang Z, Cheng H, Zhai TL, Meng X, Zhang C. Altering synthetic fragments to tune the AIE properties and self-assemble grid-like structures of TPE-based oxacalixarenes. *RSC Advances*. 2015;5(94):76670–76674. Available from: https://doi.org/10.1039/C5RA15214C.

5) Şen S, Önder FC, Çapan R, Ay M. A room temperature acetone sensor based on synthesized tetranitro-oxacalix[4]arenes: Thin film fabrication and sensing properties. *Sensors and Actuators A: Physical*. 2020;315:112308. Available from: https://doi.org/10.1016/j.sna.2020.112308.

6) Panchal M, Bhatt KD, Modi K, Panchal M. A Review on Recognition of Explosives using Calixarene Framework. *Letters in Applied NanoBioScience*. 2022;11(1):3093–3101. Available from: https://nanobioletters.com/wp-content/uploads/2021/06/22846808111.30933101.pdf.

7) Sun R, Huo X, Lu H, Feng S, Wang D, Liu H. Recyclable fluorescent paper sensor for visual detection of nitroaromatic explosives. *Sensors and Actuators B: Chemical*. 2018;265:476–487. Available from: https://doi.org/10.1016/j.snb.2018.03.072.

8) Parikh J, Bhatt K, Patel N, Modi K, Parmar N. Host-guest interaction of tryptophane with acid-functionalized calix[4]pyrrole: a fluorescence-based study. *Journal of Biomolecular Structure and Dynamics*. 2023;p. 1–8. Available from: https://doi.org/10.1080/07391102.2023.2229448.

9) Martelo LM, Marques LF, Burrows HD, Berberan-Santos MN. Explosives Detection: From Sensing to Response. In: Fluorescence in Industry;vol. 18 of Springer Series on Fluorescence. Springer. 2019;p. 293–320. Available from: https://doi.org/10.1007/4243_2019_9.

10) Kumar R, Sharma A, Singh H, Suating P, Kim HS, Sunwoo K, et al. Revisiting Fluorescent Calixarenes: From Molecular Sensors to Smart Materials. *Chemical Reviews*. 2019;119(16):9657–9721. Available from: https://doi.org/10.1021/acs.chemrev.8b00605.

11) Patel N, Modi K, Bhatt K, Mohan B, Parikh J, Liska A, et al. Cyclotriveratrylene (CTV): Rise of an untapped supramolecular prodigy providing a new generation of sensors. *Journal of Molecular Structure*. 2023;1273:134330. Available from: https://doi.org/10.1016/j.molstruc.2022.134330.

12) Gruznov VM, Baldin MN, Efimenko AP, Maksimov EM, Naumenko II, Pronin VG. Rapid gas-chromatographic determination of marking agents added to the industrial plastic explosives in air. *Journal of Analytical Chemistry*. 2015;70:207–212. Available from: https://doi.org/10.1134/S1061934814120053.

13) Panchal U, Modi K, Dey S, Prajapati U, Patel C, Jain VK. A resorcinarene-based "turn-off" fluorescence sensor for 4-nitrotoluene: Insights from fluorescence and 1 H NMR titration with computational approach. *Journal of Luminescence*. 2017;184:74–82. Available from: https://doi.org/10.1016/j.jlumin.2016.11.066.

14) Ramalingam S, Periandy S, Govindarajan M, Mohan S. FTIR and FTRaman spectra, assignments, ab initio HF and DFT analysis of 4-nitrotoluene. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2010;75(4):1308–1314. Available from: https://doi.org/10.1016/j.saa.2009.12.072.

15) Yu Y, Lan W, Wang X, Gao M, Yang R, Wang D, et al. Photophysical Properties of Naphthalene-oxacalix[m]arene and Recognition of Fullerene C60. *ACS Omega*. 2022;7(18):15411–15422. Available from: https://doi.org/10.1021/acsomega.1c07068.

16) Desai AL, Bhatt KD, Modi KM, Patel C, Pillai SG. Novel edifice calix[4]pyrrole derivatives as a potential sensor for the detection of analytes and in silico computational behaviour. *Talanta Open*. 2022;5:1–12. Available from: https://doi.org/10.1016/j.talo.2022.100115.

17) Cottet K, Marcos PM, Cragg PJ. Fifty years of oxacalix[3]arenes: A review. *Beilstein Journal of Organic Chemistry*. 2012;8:201–226. Available from: https://doi.org/10.3762/bjoc.8.22.

18) Costa AI, Barata PD, Fialho CB, Prata JV. Highly Sensitive and Selective Fluorescent Probes for Cu(II) Detection Based on Calix[4]arene-Oxacyclophane Architectures. *Molecules*. 2020;25(10):1–17. Available from: https://doi.org/10.3390/molecules25102456.

19) Panchal M, Kongor A, Athar M, Modi K, Patel C, Dey S, et al. Structural motifs of oxacalix[4]arene for molecular recognition of nitroaromatic explosives: Experimental and computational investigations of host-guest complexes. *Journal of Molecular Liquids*. 2020;306:112809. Available from: https://doi.org/10.1016/j.molliq.2020.112809.

20) Mehta V, Athar M, Jha PC, Kongor A, Panchal M, Jain VK. A turn-off fluorescence sensor for insensitive munition using anthraquinone-appended oxacalix[4]arene and its computational studies. *New Journal of Chemistry*. 2017;41(12):5125–5132. Available from: https://doi.org/10.1039/C7NJ01111C.

21) Desai AL, Patel NP, Parikh JH, Modi KM, Bhatt KD. In Silico Studies and Design of Scrupulous Novel Sensor for Nitro Aromatics Compounds and Metal Ions Detection. *Journal of Fluorescence*. 2022;32:483–504. Available from: https://doi.org/10.1007/s10895-021-02866-2.

22) Vora M, Panchal M, Dey S, Pandya A, Athar M, Verma N, et al. Oxacalix[4]arene based dual-signalling fluorimetric and electrochemical chemosensor for the selective detection of nitroaromatic compounds. *Journal of Molecular Liquids*. 2022;362:119791. Available from: https://doi.org/10.1016/j.molliq.2022.119791.