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A fused pyridopyrimidine molecule based “on-off” fluorescence sensor for HSO_4^- anions

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Abstract: A new selective and sensitive HSO_4^- ion receptor based on fused pyridopyrimidine was developed. The detection of HSO_4^- over other anions such as Cl^- , F^- , Br^- , I^- , ClO_4^- , H_2PO_4^- , NO_3^- , and AcO^- was confirmed by fluorescent color changes, UV-vis and fluorescence spectroscopy. The HSO_4^- ion binding with the receptor was also investigated with a binding constant $1.09387 \times 10^5 \text{ M}^{-1}$, which revealed that the receptor showed a remarkable binding ability towards the HSO_4^- ion. The limit of detection was calculated to be $6.0378 \times 10^{-5} \text{ M}$ in $\text{ACN}:\text{H}_2\text{O}$ (1:3, v:v) solvent mixture.

Keywords: Anion sensor, pyridopyrimidine, UV-vis, Fluorescence

1. Introduction

The detection of anions is of significant importance due to its role in a wide range of industrial, agriculture and environmental problems [1-3]. Hydrogensulfate (HSO_4^-) anion is usually found in various agricultural fertilizers and industrial raw materials such as nuclear fuel waste [4-5]. Literature survey revealed that recognition and separation

of the HSO_4^- anion from an aqueous media is a challenging task because the strong hydration in aqueous phase stops the probe from recognising the anions [6]. Therefore, the developments of selective and sensitive detection techniques for tracing small amounts of HSO_4^- in the aqueous phase have attracted a growing interest. Recently, a few probes have been investigated that are able to recognise HSO_4^- ion in the aqueous

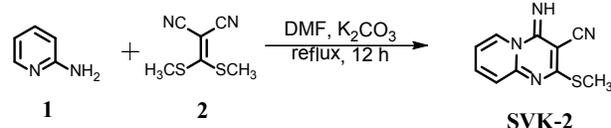
phase [7-13]. The design and synthesis of new HSO_4^- -selective receptor having both colorimetric and fluorescence response is more attractive and applicable. In our ongoing research, we are aiming to design simple chemosensor for selective HSO_4^- ion detection in aqueous medium [14].

In this manuscript, we report a HSO_4^- -sensing platform based on fused pyridopyrimidine molecule with simple structure as the colorimetric and fluorescent probe. The 4-imino-2-(methylthio)-4H-pyrido[1,2-a]pyrimidine-3-carbonitrile (**SVK-2**) and its derivatives have been used as a potentially bioactive heterocycles [15]. The **SVK-2** exhibiting several binding sites would prove effective probe for HSO_4^- ion sensor. The probe **SVK-2** was synthesized following known literature protocol [15-17].

2. Results and discussion

2.1. Synthesis

Typically, the reaction mixture of 2-amino pyridine **1** and bis(methylthio)methylene malononitrile **2** in the presence of a catalytic amount of anhydrous potassium carbonate in dry DMF was refluxed for 12 h. The analytically pure product of **SVK-2** (Scheme 1) was obtained by recrystallization from DMF: EtOH (1:1, v:v) solvent mixture.



Scheme 1 Synthetic pathway of receptor **SVK-2**

2.2. Colorimetric studies

Figure 1 represents naked-eye colour differences of receptor **SVK-2** in ACN:H₂O

(1:3, v:v) solvent mixture upon exposure to various anions such as Cl^- , F^- , Br^- , I^- , ClO_4^- , HSO_4^- , H_2PO_4^- , NO_3^- , and AcO^- (as its TBA salts) with the same concentration. **SVK-2** shows a spring-green fluorescence colour under UV light of 365 nm. The solution colour of receptor **SVK-2** converts from spring-green to fluorescence off in the presence of HSO_4^- anion. However, in the presence of other anions original colour of **SVK-2** remains intact. This colorimetric difference towards various anions allows the use of **SVK-2** as a colour tuneable HSO_4^- sensor for qualitative determination. These results clearly demonstrate probe **SVK-2** is selective for HSO_4^- ion.

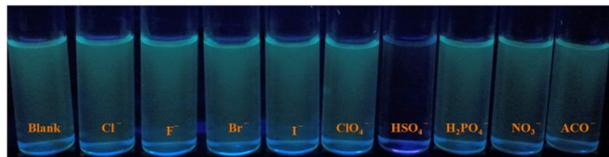


Figure 1. Naked-eye colour changes of receptor **SVK-2** (3×10^{-5} M) in ACN: H₂O (1:3, v:v) solvent mixture under 365 nm UV light with an addition of 10 equiv. of Cl^- , F^- , Br^- , I^- , ClO_4^- , HSO_4^- , H_2PO_4^- , NO_3^- , and AcO^- (as its TBA salts).

2.3. Absorption studies

Furthermore, the physical properties of probe **SVK-2** were investigated in a ACN: H₂O (1:3, v:v) solvent mixture. The UV-vis absorption measurements were employed to investigate the selectivity and sensitivity of the receptor **SVK-2** against different anions such as Cl^- , F^- , Br^- , I^- , ClO_4^- , HSO_4^- , H_2PO_4^- , NO_3^- , and AcO^- (as its TBA salts) as shown in Figure 2 (a). The UV-vis absorption spectra of **SVK-2** showed three peaks at 268 nm, 310 nm and 395 nm. Among the tested anions a significant absorption change is recorded for receptor **SVK-2** solution only in the presence of HSO_4^- ion (Figure 2 (a)).

The titration of UV-vis absorption changes of **SVK-2** solutions containing HSO_4^- with various concentrations (0-10 equiv.) was investigated (Figure 2(b)). Upon addition of (0-10 equiv.) of HSO_4^- ion to the **SVK-2** showed gradual decrease in peak intensity at 268 nm, 310 nm and 395 nm with appearance of new peaks at 283 nm, 354 nm and 370 nm, along with formation of three isosbestic points at 300 nm, 322 nm and 373 nm, respectively. This clearly demonstrates complex formation between **SVK-2** and HSO_4^- ion. Interestingly, for peak at 283 nm, there was a significant absorption enhancement (≈ 5 fold) upon addition of HSO_4^- (10 equiv.) was observed (Figure 2c). The binding constant was calculated using the Benesi-Hildebrand method for **SVK-2** with HSO_4^- ion (Figure 2d). The binding constant value was determined to be $1.09387 \times 10^5 \text{ M}^{-1}$.

High selectivity of receptor is necessary for the practical applicability of **SVK-2** as HSO_4^- sensor. To investigate the competitive sensing of HSO_4^- using **SVK-2**, the UV-vis absorption spectroscopy was employed. The selectivity experiments were carried out by the addition of HSO_4^- (10 equiv.) ion to the receptor **SVK-2** in the presence of other anions such as Cl^- , F^- , Br^- , I^- , ClO_4^- , H_2PO_4^- , NO_3^- , and AcO^- (as its TBA salts) in $\text{ACN}:\text{H}_2\text{O}$ (1:3, v:v) solvent mixture. In the presence of other anions, the UV-vis spectra of **SVK-2** showed no significant changes in comparison as obtained by the addition of only HSO_4^- to the probe **SVK-2** (Figure 3a and 3b). These results confirm that the receptor **SVK-2** detect HSO_4^- selectively in the presence of other interfering anions in water.

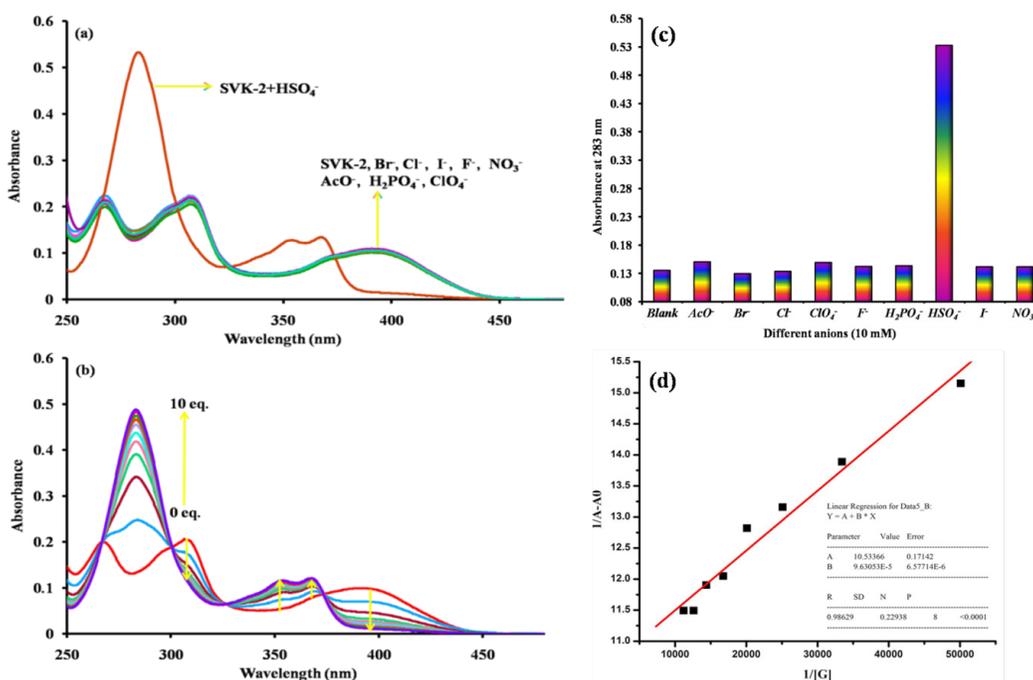


Figure 2. The UV-vis absorption of **SVK-2** ($1 \times 10^{-5} \text{ M}$): (a) with an addition of various anions in $\text{ACN}:\text{H}_2\text{O}$ (1:3, v:v) solvent mixture; (b) with an incremental amount of HSO_4^- ion (0-10 equiv.; 1 mM) in $\text{ACN}:\text{H}_2\text{O}$ (1:3, v:v); (c) absorbance responses of **SVK-2** at 283 nm upon addition of various anions (10 equiv.) in $\text{ACN}:\text{H}_2\text{O}$ (2.5:7.5, v:v) solvent mixture; (d) Benesi-Hildebrand plot of receptor **SVK-2** for HSO_4^- ion.

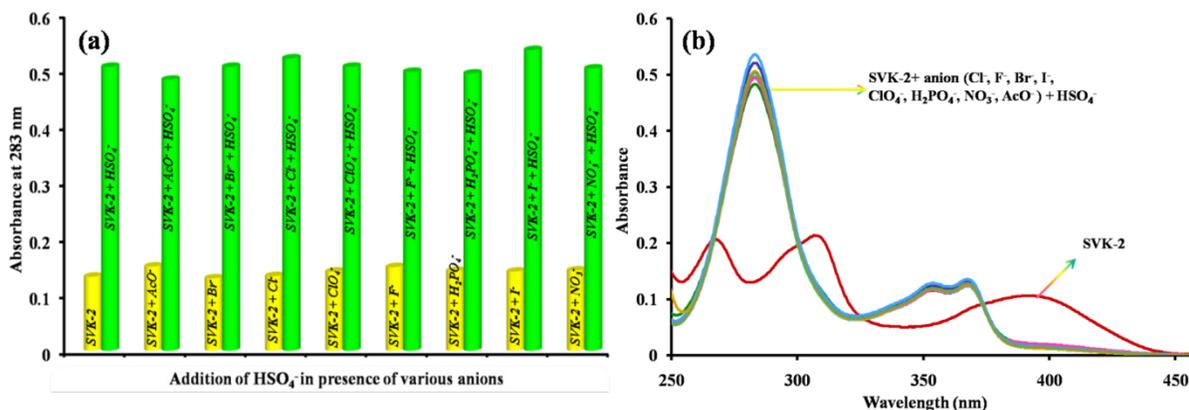


Figure 3. (a) Selectivity of receptor **SVK-2** (1×10^{-5} M) towards HSO_4^- in the presence of other anions (10 equiv.) in ACN: H_2O (1:3, v:v) solvent mixture; (b) Competitive selectivity of receptor **SVK-2** (1×10^{-5} M) towards HSO_4^- in the presence of other anions (10 equiv.) in ACN: H_2O (2.5:7.5, v:v) solvent mixture.

2.4 Emission studies

To get detail insight for the anion binding affinity of the receptor **SVK-2**, we evaluated the changes in fluorescence emission spectra with the addition of 10 equiv. of various anions (Cl^- , F^- , Br^- , I^- , ClO_4^- , HSO_4^- , H_2PO_4^- , NO_3^- , and AcO^-) in ACN: H_2O (1:3, v:v). Receptor **SVK-2** showed fluorescence emission band at 487 nm upon excitation at 360 nm. Upon addition of anions such as Cl^- , F^- , Br^- , I^- , ClO_4^- , HSO_4^- , H_2PO_4^- , NO_3^- , and AcO^- to the solution of receptor **SVK-2** resulted in very negligible change of its emission spectra, while significant change was observed by addition of 10 equiv. of HSO_4^- ion (Figure 4 (a)). Furthermore, incremental addition of HSO_4^- (0-10 equiv.) to the receptor **SVK-2** showed quenching of fluorescence at 487 nm ($\lambda_{\text{ex}}=360$ nm). These fluorescence emission observations indicated that receptor **SVK-2** showed a high selectivity towards HSO_4^- ion through complex formation.

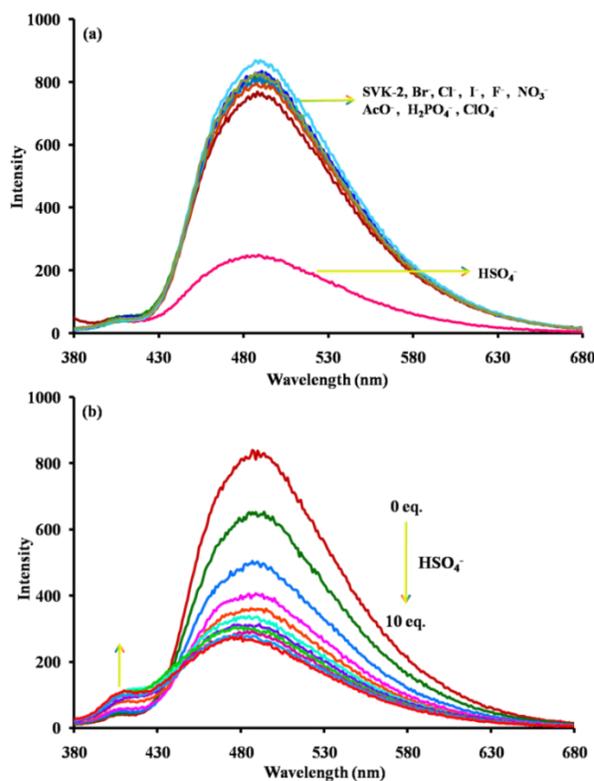


Fig. 4 Fluorescence emission spectra ($\lambda_{\text{ex}} = 360$ nm) of **SVK-2** (1×10^{-5} M) in ACN: H_2O (1:3; v:v) solvent mixture: (a) upon addition of 10 equiv. of various anions and (b) upon an incremental amount of HSO_4^- ion (0-10 equiv., 1×10^{-3} M).

2.5. Computational studies

The naked-eye, UV-vis and fluorescence study indicated that the **SVK-2** showed selective recognition of HSO_4^- ion over other tested anions such as Cl^- , F^- , Br^- , I^- , ClO_4^- , H_2PO_4^- , NO_3^- , and AcO^- . We presume that this is due to the complex formation (**YMN-2**) between **SVK-2** and HSO_4^- ions *via* hydrogen bonding (Figure 5 (a)). The DFT calculations were performed to support our assumption, for which the Gaussian 09 ab initio/DFT quantum chemical simulation package was used [18]. The geometries of molecules **SVK-2**, HSO_4^- , and complex **YMN-2** were optimized at B3LYP/6-311++G (d,p) level of theory. The polarisable continuum model (PCM) is employed to investigate the effect of solvent (water) on geometries of the receptor **SVK-2** and complex **YMN-2**. At the same level frequency calculations were carried out to ensure structures to be real. As shown in Fig.5b, the frontier molecular orbitals (HOMO and LUMO) of **SVK-2** and **YMN-2** are calculated at the B3LYP/6-311++G (d,p) level of theory and generated by using Avogadro[19-20]. The calculated results showed that the energy difference between molecular orbitals HOMO and LUMO for **SVK-2** is 3.803 eV whereas energy gap between HOMO and LUMO of **YMN-2** is 3.795 eV, indicating in this state PET is significant as compare to **SVK-2**. The quenching of the fluorescence emission intensity (Figure 4) of the **SVK-2** with the addition of the HSO_4^- ions can be rationalized based on the decrease in energy gap (Figure 5 (b)).

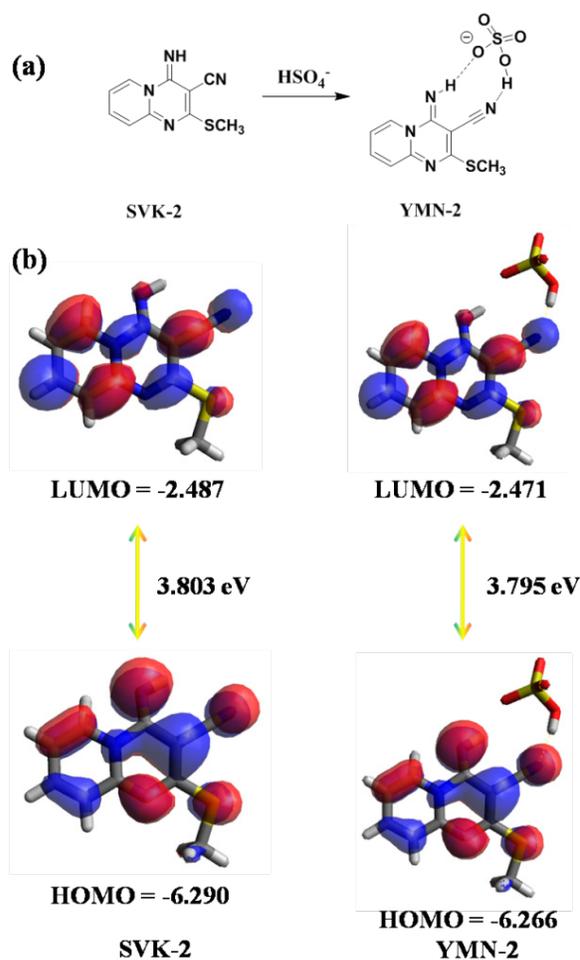


Fig. 5 (a) The plausible structure of the complex formation mechanism resulted between receptor **SVK-2** and HSO_4^- ion; (b) frontier molecular orbitals of **SVK-2** and its complex with HSO_4^- anion (**YMN-2**).

2.6. Limit of detection

To determine the practical applicability of the probe the detection limit of **SVK-2** towards HSO_4^- ion was calculated. At first the calibration curve was plotted between the absorbance intensity changes at 283 nm of **SVK-2** versus concentration of HSO_4^- ions added. The plot of **SVK-2** and $[\text{HSO}_4^-]$ anions showed sigmoidal relationship with r^2 value of 0.814 (Figure 6). Furthermore, the limit of detection was calculated by using an equation $3S/\rho$, where S is the standard

deviation of three blank measurements and ρ is the slope between absorbance intensity versus sample concentration. The **SVK-2** has detection limit to be 6.0378×10^{-5} M for HSO_4^- anions. The obtained value for limit of detection is comparable to the literature reported method (14). The beauty of the present work is **SVK-2** is synthesised in one step and employed to detect HSO_4^- ions selectively in presence of interfering anions e.g. Cl^- , F^- , Br^- , I^- , ClO_4^- , H_2PO_4^- , NO_3^- , and AcO^- .

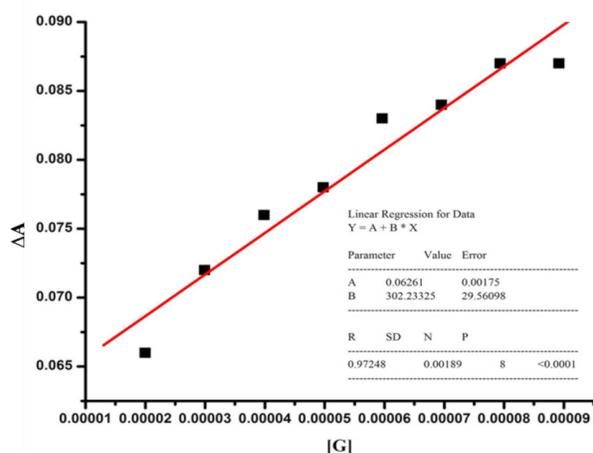


Figure 6. Calibration plot for successive addition of HSO_4^- ion to **SVK-2** solution in ACN:H₂O (2.5:7.5; v:v) solvent mixture.

3. Conclusion

In summary, we report the synthesis of fused pyridopyrimidine **SVK-2** and its application as a naked-eye fluorescent on-off sensor for the selective detection of HSO_4^- ion in aqueous medium. It was found that **SVK-2** is an excellent selective sensor for HSO_4^- in the presence of other interfering anions such as Cl^- , F^- , Br^- , I^- , ClO_4^- , H_2PO_4^- , NO_3^- , and AcO^- . The binding constant of **SVK-2** towards HSO_4^- was found to be 1.09387×10^5 M⁻¹. The detection limit of **SVK-2** for HSO_4^- was determined to be 6.0378×10^{-5} M.

4. Experimental section

4.1. Materials and Methods

2-amino pyridine, dimethylformamide (DMF) and tetrabutyl ammonium salts of anions were purchased from Sigma Aldrich (Bengaluru, Karnataka, India) and are used without further purification, unless otherwise specified. UV-vis absorption spectra were recorded by UV-vis-1800 Shimadzu spectrophotometer. Fluorescence emission spectra were measured on RF-6000 (Shimadzu, Japan) Spectrofluorophotometer.

4.2. Synthesis of SVK-2

2-Amino pyridine **1**, (3 mmol) and bis(methylthio)methylene malonitrile **2** (3 mmol) was dissolved in 10 mL anhydrous N-N dimethylformamide and stirred the reaction mixture for half an hour. Then the catalytic amount of K_2CO_3 was added and the reaction mixture was refluxed for 12 h. The reaction mixture was cooled to room temperature and then poured dropwise in crushed ice with continuous stirring. The obtained wine red precipitate was filtered, washed with water and recrystallised from solid DMF: EtOH to yield pure 4-imino-2-(methylthio)-4H-pyrido[1,2-a]pyrimidine-3-carbonitrile (**SVK-2**), 64%.

FT-IR (KBr, ν cm⁻¹): 779, 824, 981, 1027, 1093, 1126, 1268, 1470, 1504, 1613, 1641, 1711, 2203 (CN), 2926, 3287, 3438. **¹H NMR** (CDCl_3 , 500 MHz) δ : 2.62 (s, 3H) 7.14 (t, $J=7.9$ Hz, 1H) 7.47 (d, $J=8.6$ Hz, 1H) 7.83 (t, $J=7.1$ Hz, 1H) 9.35 (d, $J=6.8$ Hz, 1H); **¹³C NMR** (125 MHz, CDCl_3) δ : 12.9, 83.6, 115.7, 125.5, 129.2, 139.7, 150, 152.1, 168.4; **ESI-MS** (m/z %): 217 (100) $[\text{M}+\text{H}]^+$; **HRMS** calculated for $\text{C}_{10}\text{H}_9\text{N}_4$ $S = 217.05473$ Found $[\text{M}+\text{H}]^+ = 217.05424$.

4.3. UV-vis spectrophotometric titrations

The probe **SVK-2** was dissolved in acetonitrile:

water (2.5:7.5; v/v) solvent mixture with $c \approx 1.0 \times 10^{-5}$ M corresponding to the maximum of absorbance in the range ≈ 0.1 to 0.6 for the UV experiments. The solution of the probe **SVK-2** was placed in a quartz cuvette ($l = 1$ cm, $V_0 = 3$ mL) followed by addition of various anion solutions ($c \approx 1$ mM). Upon each addition, UV-vis spectrum was recorded at room temperature.

4.4. Fluorescence titrations

To a solution of 1.0×10^{-5} M of receptor **SVK-2** in acetonitrile: water (2.5:7.5; v/v) the various anion solutions were added. Emission spectrum was recorded with excitation wavelength of ($\lambda_{\text{ex}} = 360$ nm) with each addition at room temperature.

4.5. Naked-eye experiment

The anion solutions were added to a solution of **SVK-2** probe (3.0×10^{-5} M) of in acetonitrile: water (1:3; v/v) solvent mixture. With the addition of anion solutions (10 equiv., 1×10^{-3} M) to the receptor **SVK-2**, image was recorded with well resolved camera at room temperature.

4.6. Computational methods

Gaussian 09 ab initio/DFT quantum chemical simulation package were employed to perform the ground state calculations in the frame of density functional theory and were optimized at DFT/B3LYP/6-31G** level of theory.

Disclosure statement

No potential conflict of interest was reported by the authors

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