Selective colorimetric sensors for cyanide and acetate ion in partially aqueous medium

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Abstract

4-(thiazol-2-yl)diazene-4-yl)-phenol (L1) and 2-((4-hydroxyphenyl)diazene-4-yl)-5-nitrophenol (L2) based on azo phenol were synthesised and used as selective colorimetric sensor for CN– and AcO– ion in DMSO/H2O-HEPES (v/v; 1:1, pH-7.3±0.2) and showed good sensitivity with large red shifts and nanomolar detection limit for CN– and AcO– ion. The stoichiometry of L1 with CN–/AcO– ion was found to be 1:1 and L2 with CN–/AcO– ion was found to be 1:2. Binding constant for L1 + CN–, L1 + AcO–, L2 + CN– and L2 + AcO– were calculated by B-H plot as 1.6 × 10³, 8.0 × 10², 8.4 × 10³ and 1.7 × 10² respectively. L2 showed high selectivity towards CN– ion with low detection limit of 81 nM and large binding constant. In addition, 1H NMR titration and DFT studies also supported the deprotonation mechanism of receptors in the presence of selective anions.

Keywords: Chemosensors, CN– selective sensor, Test strip sensor
1. Introduction

In supramolecular chemistry, the development of chromogenic sensor is of great interest in anion sensing due to their chemical and biological applications [1]. The most hazardous cyanide ion widely used in the various chemical industries (140,000 tons of cyanide per year) causes the severe pollution of water supplies [2-3]. Cyanide is extremely toxic, increases environmental pollution due to its industrial use mainly production of textiles, papers and plastics. Importantly, cyanide rigorously suppresses the transport of oxygen as it binds with cytochrome c oxidase and effects electron transfer from cytochrome c oxidase to oxygen in mitochondria [4]. Acetate ion is more important in living organisms as acetyl coenzyme [5].

There are many conventional detection methods for quantitative determination of cyanide and acetate ion mainly based on electrochemical and voltammetric techniques [6-10]. Due to their cost and sophisticated instruments, detection of cyanide and acetate ion by absorption or fluorescence or both studies are easier and growing very fast [11-15]. So the development of colorimetric anion sensor is more attractive due to no requirement of expensive equipment as color changes can be easily detected by the naked-eye. Generally, the chromogenic anion sensor consisted many pathways as: displacement of a metal complex (Fig. 1), anion receptor and chromophore, these sensors having chromogenic centre that is covalently bonded to the receptor unit. The receptor unit or binding sites are based on hydrogen bond donor group, generally –OH and –NH group of phenols, sulphonamide, urea, thiourea [16-19]. Compared to the above traditional chemosensor method, an alternative chemodosimeter approach based on an irreversible specific chemical reaction has emerged as an active research area of significant importance. However, considerable efforts have been devoted to the development of a chemodosimeter for anions. Many examples are available about colorimetric sensors for acetate and cyanide ion in the literatures [20-28].

In this paper, we described two phenol based azo dyes L₁ and L₂ which selectively sense biologically important CN⁻ and AcO⁻ ion in partially aqueous medium without any interference of different anions. Two azo dye based ligands were synthesised having phenolic group for better sensing ability towards anions, resulting in the enhancement of push-pull approach of intramolecular charge transfer (ICT), which reproduced red-shifted absorption. Azo dye ligand L₂ with –NO₂ units (electron-withdrawing substituent) performed high selective binding ability towards cyanide ion over other studied ion.
2. Experimental Section

2.1. Reagents and Instruments

Analytical grade anion salts are purchased from Merck. Phenol, 2-aminothiazole and 2-amino-5-nitrophenol were purchased from Sigma-Aldrich. CHNS analysis was recorded on an Elementar model Vario EL-III. IR spectra were obtained using a Perkin Elmer FT–IR 1000 spectrophotometer as films between KBr. $^1$H NMR spectra were recorded on Bruker AVANCE 500 MHz spectrometer and Zeol 400 MHz spectrometer. UV-vis spectra were recorded on Shimadzu, UV-3600 double beam spectrophotometer using 10 mm path length of silica cell. DFT computational studies were obtained with Gaussian 09 W programme in gas phase using a B3LYP function with 6-31G(d,p) basis set for L$_1$, L$_2$, L$_1$+CN$^-$ and L$_2$+CN$^-$. 

2.2. Results and Discussion

2.2.1. Synthesis of Azo dyes:

Synthesis of 4-(thiazol-2-yl diazenyl)phenol (L$_1$)

4-(thiazol-2-yl diazenyl)phenol (L$_1$) was synthesised based on the previously reported literature [29]. 1 gm of 2-aminothiazole was dissolved in 12 ml of H$_2$SO$_4$ and then add 0.3 gm of sodium nitrite (NaNO$_2$) in 10 ml water in dropwise manner with continuous stirring. The mixture was stirred for half an hour at 0ºC (Solution 1). For the coupling reaction, the mixture of 0.9 gm of phenol and 10 ml of 0.1 M NaOH were taken in a separate flask and maintain the temperature at 0ºC (Solution 2). Solution 2 was added in solution 1 while stirring at 0ºC. The formed precipitate washed with distilled water and recrystallised using ethanol and collect the orange powdered product (Scheme 1).

Yield-60 %; C$_9$H$_7$N$_3$OS; C, 52.67; H, 3.44; N, 20.47; O, 7.80; S, 15.62; found C, 52.26; H, 3.4; N, 20.40; O, 8.0; S, 15.94; FTIR (KBr, $\nu_{\text{max}}$, cm$^{-1}$) –OH: 3436, -N-N, 1591, C-O: 1242, Ar-H: 1143, $^1$H NMR (500 MHz, DMSO-d$_6$, ppm); $\delta$ 10.796 (s, 1H), 8.046 (d, 1H), 7.862 (d, 2H), 7.818 (d, 1H), 6.983 (d,2H), $^{13}$C NMR (100 MHz, DMSO-d$_6$): 117.1, 122.3, 126.7, 131.0, 131.6, 144.1, 144.8, 163.4, 177.2 ppm; UV-vis (DMSO, $\lambda_{\text{max}}$, nm); 253, 393. [ESI Fig.1 – ESI Fig.7]

2-((4-hydroxyphenyl)diazenyl)-5-nitrophenol (L$_2$)

Took, 2 gm of 2-amino-5-nitrophenol and dissolved in 24 ml of H$_2$SO$_4$ and then add 0.6 gm of sodium nitrite (NaNO$_2$) in 10 ml water in dropwise manner on continuous stirring. The mixture was stirred for half hour at 0ºC (Solution 1). For the coupling reaction, the mixture of
1.8 gm of phenol and 10 ml of 0.2 M NaOH were taken in a separate flask and maintain the temperature of this solution at 0ºC (Solution 2). Solution 2 was added slowly on stirring at 0ºC to the solution 1. The occurred precipitate washed with distilled water and recrystallised by ethanol and collect yellow-orange powder (Scheme 1).

Yield - 51 %; C_{12}H_{9}N_{3}O_{4}; C, 55.60; H, 3.50; N, 16.21; O, 24.69; found C, 56.30; H, 3.47; N, 16.27; O, 24.69; FTIR (KBr, ν_{max}, cm^{-1}) –OH; 3429, -N-N; 1585, 1401, Ar-H; 1186; ^1H NMR (500 MHz, DMSO-d_6, ppm); δ 10.884 (s, 1H), 8.973 (s, 1H), 7.445 (m, 2H), 7.051 (d, 1H), 6.988 (s, 1H), 6.883 (d, 1H), 6.688 (d, 2H); ^13C NMR (100 MHz, DMSO-d_6, ppm) 120.6, 121.9, 122.6, 127.0, 128.9, 129.6, 130.1, 132.1, 141.3, 143.3, 165.3, 172.4; UV-vis (DMSO, λ_{max}, nm); 213, 262, 374.

2.2.2. Naked Eye Experiments:
The recognition and chromogenic sensing ability of azo dye L_1 and L_2 (20 µM) were checked with the sodium salt of a series of anions F^-, Cl^-, Br^-, CN^-, H_2PO_4^-, HPO_4^{2-}, SO_4^{2-}, SO_3^{2-}, AcO^-, N_3^- and SCN^- (200 µM) in DMSO/H_2O-HEPES (v/v; 1:1, pH = 7.3±0.2) solution. By examine the chromogenic properties of azo dyes L_1 and L_2, it was found that both ligands showed instant color changes from yellow to violet with more basic anion, CN^-/AcO^- and other anions showed no such color changes (Fig. 2). Basicity of anions is known to be in order of CN^- > AcO^- > N_3^- > F^- > H_2PO_4^- > Cl^- > Br^- > I^- [30] and the ability to form hydrogen bond in order of F^- > AcO^- > H_2PO_4^- > CN^- > N_3^- > SO_4^2- so that both anions with more basic and least H-bonding character might deprotonate the phenolic hydrogen rather than forming H-bonding. Further the sensitivity of azo dyes with CN^- and AcO^- ion was analysed by absorption spectra.

2.2.3. UV-Vis Analysis:
Upon addition of different anion like F^-, Cl^-, Br^-, AcO^-, HPO_4^{2-}, H_2PO_4^-, CN^- and N_3^- (200 µM), changes in UV-vis absorption spectra of L_1 and L_2 (20 µM) with CN^-/AcO^- ions were found. Azo dye L_1 exhibited two absorption peak at 253 (π-π* transition), 393 nm (n-π* transition) and L_2 exhibited three absorption peak at 213 (σ-σ* transition), 262 (π-π* transition) and 374 nm (n-π* transition). L_1 showed new absorption band with CN^- and AcO^- ion at 486 and 483 nm respectively hence L_2 also showed same behaviour with CN^- and AcO^- ion and found a new absorption band at 435 and 437 nm respectively. With this absorption studies, it was confirmed that both azo dye ligands selectively detect cyanide and acetate ion. This new absorption band may occur because of the abstraction of proton with cyanide and acetate ion. The titrations of L_1 (20 µM) were performed in DMSO/H_2O-HEPES (v/v; 1:1,
pH = 7.3±0.2) solution with CN\(^-\) and AcO\(^-\) ion. For the titration, a series of spectra were taken and started by adding the little amount of the anion solution with the help of microsyringe in the quartz cuvettes containing the solution of azo dye (Fig. 3a-3b). The series of UV–vis spectra were taken after each addition and absorbance values were recorded. L\(_1\) exhibits changes in absorption spectra upon addition of CN\(^-\) ion, the band at 393 nm were quenched with consequently increases in the absorption band at 486 nm due to interaction of CN\(^-\) ion with ligand. Titration experiments for L\(_2\) with CN\(^-\) and AcO\(^-\) ion was performed in the same way (Fig. 4a-4b). The absorption spectra of L\(_2\) shown the changes upon addition of CN\(^-\) ion, the band at 374 nm were quenched with consequently increases in absorbance at 435 nm due to interaction of CN\(^-\) ion with ligand. Three isosbestic point were found and showed that free azo dye and adduct (dyne + anion) was in equilibrium (L\(_1\)+CN\(^-\); 417, 301, 258; L\(_1\)+AcO\(^-\); 433, 298, 267; L\(_2\)+CN\(^-\); 400, 300, 270; L\(_2\)+AcO\(^-\); 399, 301, 273). The binding constant of CN\(^-\) and AcO\(^-\) ion with L\(_1\) and L\(_2\) was calculated using BH-plot (Inset; Fig. 3 and 4) and found to be 1.6 × 10\(^3\), 8.0 × 10\(^2\), 8.4 × 10\(^3\), and 1.7 × 10\(^2\) respectively.

Table 1 lists the detection limit of CN\(^-\) and AcO\(^-\) ions presented by L\(_1\) and L\(_2\). The detection limit in DMSO–H\(_2\)O (1:1, v/v) was determined to be 87 nM (L\(_1\) + CN\(^-\)), 83 nM (L\(_1\) + AcO\(^-\)), 81 nM (L\(_2\) + CN\(^-\)), 89 nM (L\(_2\) + AcO\(^-\)) respectively. 1:1 stoichiometry of the formation of adducts between anion (CN\(^-\) and AcO\(^-\)) and azo dye L\(_1\). 1:2 stoichiometry between anions (CN\(^-\) and AcO\(^-\)) and azo dye L\(_2\) was found by Job’s plot analysis. A plot was obtained by recording the absorbance of equimolar solution (50 μM in DMSO/H\(_2\)O, 1:1, v/v solution) of azo dyes and anion with different mole fraction which show 1:1 stoichiometry of L\(_1\)+CN\(^-\), L\(_1\)+AcO\(^-\), L\(_2\)+CN\(^-\) and L\(_2\)+AcO\(^-\) (ESI Fig. 9-10).

2.2.4. Interference study on sensor performance:

As we use the both azo dyes for the detection of cyanide and acetate ion, we also tested the absorption response of L\(_1\) and L\(_2\) towards various anions (N\(_3\)\(^-\), HPO\(_4\)\(^2-\), H\(_2\)PO\(_4\)\(^-\), SCN\(^-\), AsO\(_2\)\(^-\), Cl\(^-\), Br\(^-\), SO\(_4\)\(^2-\), SO\(_3\)\(^2-\), NO\(_2\)\(^-\), NO\(_3\)\(^-\) and F\(^-\)) and we found that there was no significant changes in the absorption spectra in the presence of various anions under the same conditions. L\(_1\) and L\(_2\) were found to be more selective towards cyanide and acetate ion even in presence of
various other anions (Fig. 5). We have done competitive experiments [33] in the presence of detecting anion (100 µM) and other anions (100 µM) with azo dyes (20 µM) and we found that the absorption spectra of azo dyes with detecting anions remained unaffected with the addition of other anions. This demonstrated that both azo dyes could be used to quantitative detection of cyanide and acetate ion concentration with great selectivity. In the figure 5-a and 5-b, the blue bar indicates the absorption intensity of CN\(^{-}\) with azo dye L\(_{1}\) and L\(_{2}\) and the red bar indicates the absorption intensity of azo dyes with CN\(^{-}\) ion in the presence of interfering ions.

### 2.2.5. \(^{1}\)H-NMR titration:

To know the mechanism of interaction between azo dye and cyanide ion, \(^{1}\)H NMR spectra of L\(_{1}\) and L\(_{2}\) were recorded in absence and presence of cyanide ion. L\(_{1}\) shows NMR peak at \(\delta\) 10.796 ppm for –OH proton and azo dye L\(_{2}\) shows NMR peak at 10.884 ppm and 8.973 ppm for hydroxy protons and the rest peak of NMR assigned for aromatic region. After adding CN\(^{-}\) ion solution to L\(_{1}\) and L\(_{2}\), shake the solution for a while and \(^{1}\)H NMR at room temperature were taken which showed all the peaks of NMR were shifted to the upfield region in both azo dye due to the abstraction of phenolic proton or formation of phenolate ion which increased the electronegativity on π-e\(^{-}\) cloud of aromatic region. The peak of –OH proton of (10.796 ppm) L\(_{1}\) and (10.884 and 8.973 ppm) L\(_{2}\) was found to be completely disappeared and aromatic proton signals of L\(_{1}\) and L\(_{2}\) slightly shifted to upfield region (\(\Delta\delta = 0.1\) ppm and 0.08 ppm respectively). When adding more amount of CN\(^{-}\) ion solution, aromatic protons of L\(_{1}\) and L\(_{2}\) were dramatically shifted into a high field region (\(\Delta\delta = 0.2\) ppm and 0.17 ppm) due to the possible phenolate ion formation. The \(^{1}\)H NMR studies, clearly illustrated that the both L\(_{1}\) and L\(_{2}\) were interacted cyanide ion via. deprotonation of –OH group (Fig. 6 and 7). From the careful analysis of \(^{1}\)H NMR spectra, the colorimetric change could take place partly through proton abstraction. We reasoned that the basic cyanide anions (pKa<9.4) are expected to readily abstract the acidic proton of OH group (pKa<4).

### 2.2.6. Theoretical Calculations:

To understand the recognition behaviour of phenol based azo dyes L\(_{1}\) and L\(_{2}\) with cyanide ion, theoretical studies have been done. The optimizied geometry of L\(_{1}\), L\(_{2}\), L\(_{1}\)+CN\(^{-}\) and L\(_{2}\)+CN\(^{-}\) was obtained in gas phase on Gaussian 09 W computational program [34]. HOMO (Highest Occupied Molecular Orbital) and LUMO (Lowest Unoccupied Molecular Orbital) of L\(_{1}\), L\(_{2}\), L\(_{1}\)+CN\(^{-}\) and L\(_{2}\)+CN\(^{-}\) obtained by optimised geometry and found that the free ligands L\(_{1}\) and L\(_{2}\) have higher energy gap (\(\Delta E = 2.362\) and 2.236 eV respectively) between
HOMO and LUMO then the adduct \( \text{L}_1+\text{CN}^- \) and \( \text{L}_2+\text{CN}^- \) (\( \Delta E = 1.865 \) and 1.773 eV respectively, Table 2). These computational calculations were performed using Gaussian 09 with B3LYP/6-31G (d,p) basis set. The deprotonation of OH proton of \( \text{L}_1 \) and \( \text{L}_2 \) concludes the decrease in energy gap of \( \text{L}_1+\text{CN}^- \) and \( \text{L}_2+\text{CN}^- \). The theoretical calculation leads to the success of experimental results. Fig. 8 shows the optimized structure and Fig. 9 shows HOMO-LUMO energy level diagram of \( \text{L}_1 \), \( \text{L}_2 \) and their cyanide adduct.

**2.2.7. Measurement of Anions with Test strips:**

The test strips were prepared by immersing the whatman filter paper in DMSO solution of azo dye \( \text{L}_1 \) and \( \text{L}_2 \). The paper strips were subsequently dried in air to figure out the “dip-stick” method suitability for the detection of both anions [35]. The coated test strips were further immersed in the aqueous solution of \( \text{CN}^- \) and \( \text{AcO}^- \) ion solution of different concentration and suddenly a fast color change was found from light yellow to violet red (Fig. 10). The development of such a “dip-stick” approach is extremely attractive for “in-the-field” measurements as it does not require any additional equipment.

**2.2.8. Fast Response**

To know the fast response time of chemosensor \( \text{L}_1 \) and \( \text{L}_2 \) towards cyanide ion, changes in absorption spectra was monitored with time. In this sensor, detection of \( \text{CN}^- \) ion with \( \text{L}_1 \) and \( \text{L}_2 \) was found to be very fast. New absorption band of \( \text{L}_1 \) and \( \text{L}_2 \) with \( \text{CN}^- \) ion was found at the higher intensity (plateau region) within 10 s and remains stable for 8-11 min. A curve between time v/s ratiometric absorption intensity reveals that reaction was completed in 10 s (Fig. 11).

**2.2.9. Analytical Application**

To know the applicability of the sensor \( \text{L}_1 \) and \( \text{L}_2 \), it was further used for the quantitative determination of cyanide ion in drinking water. The sample of drinking water was prepared by adding a known amount of cyanide ion (2 µM). The experiments were performed using absorption spectra of 2 ml volume of sensor sample after adding a known concentration of cyanide ion which found within the linear calibration range. The experiment was repeated three times and the average concentration of cyanide ion in drinking water was found to be 1.9 µM. Then, the recovery percentage performed well with relative standard deviation lower than 2%.
3. Conclusion
Highly selective chromogenic azo dyes L1 and L2 was designed that can detect both CN⁻ and AcO⁻ ion in 50% aqueous medium via proton abstraction. The stoichiometry of L1 and L2 with CN⁻/AcO⁻ ion was confirmed in account of Job’s plot. *i.e.* 1:1 and 1:2 respectively. The more basic cyanide and acetate ion abstract the more acidic proton of the molecule and allow the formation of phenolate ion. Thus the accumulation of the negative charge on the whole molecule exhibited a large significant red shift in CT band and major changes in color. LOD for CN⁻ ion with L1 and L2 was found to be 87 and 81 nM and for AcO⁻ ion with L1 and L2 was found to be 83 and 89 nM respectively. Therefore, the system used to detect the WHO suggested maximum allowed cyanide concentration in drinking water (1.9 mM).

Acknowledgement
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References:


Figure Captions

Scheme 1. Synthesis of 4-(thiazol-2-yldiazenyl)phenol $L_1$ and 2-((4-hydroxyphenyl)diazenyl)-5-nitrophenol $L_2$.

Fig.1. Illustration of “Anion Sensing”. $A, A^1, A^2 = $ Anions, $M= $Metal, $P, P_1, P_2= $ Product, $MA= $ ion pair.

Fig.2. Absorption spectra of $L_1$ (20 µM, DMSO/H$_2$O-HEPES (v/v; 1:1, pH = 7.3±0.2) solution) with a series of anions and sudden color change of $L_1$ with CN$^-$ and AcO$^-$ ion, (b) Absorption spectra of $L_2$ (20 µM, DMSO/H$_2$O-HEPES (v/v; 1:1, pH = 7.3±0.2) solution) with a series of anions and sudden color change of $L_2$ with CN$^-$ and AcO$^-$ ion.

Fig.3. (a) Absorption spectra of $L_1$ (20 µM, DMSO/H$_2$O-HEPES (v/v; 1:1, pH = 7.3±0.2) solution) changes with increasing amount of CN$^-$ ion (0-100 µL), Inset shows BH-plot of $L_1$ with CN$^-$ ion, (b) Absorption spectra of $L_1$ (20 µM, DMSO/H$_2$O-HEPES (v/v; 1:1, pH = 7.3±0.2) solution) changes with increasing amount of AcO$^-$ ion (0-100 µL), Inset shows BH-plot of $L_1$ with AcO$^-$ ion.

Fig.4. (a) Absorption spectra of $L_2$ (20 µM, DMSO/H$_2$O-HEPES (v/v; 1:1, pH = 7.3±0.2) solution) changes with increasing amount of CN$^-$ ion (0-100 µL), Inset shows BH-plot of $L_2$ with CN$^-$ ion, (b) Absorption spectra of $L_2$ (20 µM, DMSO/H$_2$O-HEPES (v/v; 1:1, pH = 7.3±0.2) solution) changes with increasing amount of AcO$^-$ ion (0-100 µL), Inset shows BH-plot of $L_2$ with AcO$^-$ ion.

Fig.5. (a) Interference study of different anions with the selectivity of $L_1$ towards CN$^-$ ion (b) Interference study of different anions with the selectivity of $L_2$ towards CN$^-$ ion.

Fig.6. $^1$H-NMR titration of $L_1$ with CN$^-$ ion in DMSO-$d_6$.

Fig.7. $^1$H-NMR titration of $L_2$ with CN$^-$ ion in DMSO-$d_6$.

Fig.8. Optimized structure of $L_1$ and $L_2$ and their cyanide adduct.

Fig.9. HOMO-LUMO energy diagram of $L_1, L_1+CN^-, L_2$ and $L_2+CN^-$. 

Fig.10. Photographs of test strips of $L_1$ and $L_2$ with CN$^-$ and AcO$^-$ ion.

Fig.11. (a) Absorbance changes at 485 nm for $L_1$ (20 µM, 25°C) in a mixture of DMSO–H$_2$O (1:1, v/v) after addition of NaCN (1mM) (b) Absorbance
changes at 432 nm for $L_2$ (20 µM, 25°C) in a mixture of DMSO–H$_2$O (1:1, v/v) after addition of NaCN (1mM).

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Fig.3. (a) Absorption spectra of L₁ (20 µM, DMSO/H₂O-HEPES (v/v; 1:1, pH = 7.3±0.2) solution) changes with increasing amount of CN⁻ ion (0-100 µL). Inset shows BH-plot of L₁ with CN⁻ ion. (b) Absorption spectra of L₁ (20 µM, DMSO/H₂O-HEPES (v/v; 1:1, pH = 7.3±0.2) solution) changes with increasing amount of AcO⁻ ion (0-100 µL). Inset shows BH-plot of L₁ with AcO⁻ ion.
Fig. 4. (a) Absorption spectra of $L_2$ (20 $\mu$M, DMSO/H$_2$O-HEPES (v/v; 1:1, pH = 7.3±0.2) solution) changes with increasing amount of CN$^-$ ion (0-100 $\mu$L), Inset shows BH-plot of $L_2$ with CN$^-$ ion, (b) Absorption spectra of $L_2$ (20 $\mu$M, DMSO/H$_2$O-HEPES (v/v; 1:1, pH = 7.3±0.2) solution) changes with increasing amount of AcO$^-$ ion (0-100 $\mu$L), Inset shows BH-plot of $L_2$ with AcO$^-$ ion.

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Tables

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<th>S. No.</th>
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<th>Binding Constant</th>
<th>Stoichiometry</th>
<th>Limit of Detection (nM)</th>
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Table 2. Energy of HOMO-LUMO of L$_1$, L$_2$ and Complexes.

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<td>-8.441</td>
<td>-6.68</td>
<td>1.773</td>
</tr>
</tbody>
</table>