CHAPTER 5

Synthesis and Characterization of 4-Hydroxybiphenyl Based Semicarbazide Derivatives as Urea Analogue for Recognition of Fluoride

ABSTRACT

4-Hydroxybiphenyl derived semicarbazide and phenyl semicarbazide ligands, 4-BOH-S and 4-BOH-P, have been synthesized and characterized. The fluoride recognition ability of 4-BOH-S and 4-BOH-P was examined through UV-visible, fluorescence and NMR spectroscopic analysis. Despite the weakly emissive nature of the ligands in the solution state, both 4-BOH-S and 4-BOH-P forms fluorescent aggregates in DMSO/H$_2$O which could be established from fluorescence and DLS studies. DFT calculations suggested that aggregation-induced enhanced emission could originate from the increased planarity of the luminogens and suppression of the non-radiative vibrational/rotations relaxation processes. The fluorescent aggregates of 4-BOH-S and 4-BOH-P in DMSO/H$_2$O could recognise HPO$_4^{2-}$ anion irrespective of the pH of the solution. Further, we recognised the ligands to have strong anion receptor ability for fluoride both in solution and in the solid state.
5.1 Introduction

The condensation of primary amines with carbonyl compounds produce Schiff bases (imine derivatives), which have been studied extensively because of their synthetic simplicity, clean reaction profile and reasonably high yields.[187,188] A large number of Schiff base/imine derivatives have been designed and synthesised as ligands for metal ions, particularly in the formation of coordination complexes and metal chelates.[189-191] Several metal-Schiff base complexes have been developed as active catalysts for polymerization [192], oxidation [193], epoxidation [194] and reduction reactions [195]. Recent studies have shown that Schiff base complexes of transition metal ions can catalyse C-C bond formation (e.g. Aldol condensation, Diels Alder reaction) [196] and C-H bond activation.[197]

Schiff bases containing ortho-hydroxyl groups often show intramolecular hydrogen bonding between the phenolic-OH and the imine group.[189,198-200] In many cases, the presence of a ortho-hydroxyl group play crucial role in metal ion binding and complex formation.[198] Again, the formation of metal-Schiff base complexes could result in perceptible changes in chromogenic/luminogenic behaviour, which can pave the way for the design of metal ion selective chemosensors.[201-205]

For instance, pyrimidine-based imine derivatives 87 and 88 were reported as sensitive fluorescent receptors for Al^{3+},[205] where the presence of a phenolic–OH group plays effective role on the inhibition of imine (C=N) isomerisation process and thereby generate high fluorescence upon binding of Al^{3+} (Scheme 5.1).
Scheme 5.1 (a) Proposed binding mode for interaction of Al$^{3+}$ with receptor 87 (b) Non-fluorescent nature of receptor 88 even after binding with Al$^{3+}$

As shown in Scheme 5.2, the binding of Zn$^{2+}$ ions to a tripodal Schiff base receptor 89, containing salicylaldehyde moiety, led to significant enhancement in fluorescence emission at 463 nm. The fluorescence enhancement of 89 in the presence of Zn$^{2+}$ ions was also attributed to chelation-induced inhibition of C=N isomerisation due to metal binding. In contrast, the binding of Cu$^{2+}$ and Fe$^{2+}$ ions to the same receptor led to distinct colour changes with no changes in the fluorescent emission. [202]

Scheme 5.2

In another example, a salicylaldehyde acylhydrazone 90 exhibited $>$ 200-fold enhancement in fluorescence at 436 nm upon binding of Mg$^{2+}$. [204] The fluorescence responses in such cases was scribed to the inhibition of C=N isomerisation, which was
the predominant decay process of the excited state. Similarly, the acylhydrazone-based ligand 91 could be utilised as turn-on luminescent chemosensor for Mg$^{2+}$, wherein the sensing mechanism was assigned as the inhibition of ESIPT process due to the binding of metal ions [198].

![Diagram showing the predominant decay process of the excited state and the sensing mechanism of ligand 91 for Mg$^{2+}$](image)

Although imine derivatives have been used extensively for their metal coordination and complexation abilities, recent studies suggest that imine derivatives could potentially be used as anion receptors.[206-209] The ortho-hydroxyl group in Schiff base compounds can serve hydrogen bond donors and assist anion binding events.[206-209] The selectivity of such receptors towards a particular anion depends on the acidity of the hydroxyl group, and the nature of substituents on the benzene ring with respect to the hydroxyl group. For instance, the salicylaldehyde-derived Schiff base 92 could be utilised as colorimetric sensor for $F^-$, AcO$^-$ and $H_2PO_4^-$ anions in acetonitrile with substantial red-shifting from 342 nm to 450 nm. The interaction of basic anions with 92 was driven by higher acidity of the phenolic-OH (and presence of electron withdrawing NO$_2$ group), the changes in colour were attributed to the intramolecular charge transfer.[206,209] Receptors 93 and 94 were reported as highly colorimetric sensor for fluoride anion wherein NO$_2$ group acted as the signalling unit and –OH and –NH as the binding sites.[207]
In another instance, it was observed that the addition of HSO$_4^-$ anion to receptor 95 led to dramatic enhancement of fluorescence at 485 nm. Detailed UV-visible, fluorescence and $^1$H NMR studies of the anion-receptor interactions pointed to the anion mediated hydrolysis of the C=N bond [210]. The same ligand system was used for Hg(II) binding, which revealed similar enhancement of fluorescence at 485 nm, apparently triggered by metal ion promoted hydrolysis of the C=N bond [211].

In recent years, Schiff base compounds possessing interesting luminogenic properties such as aggregation induced emission (AIE), aggregation induced enhanced emission (AIEE), aggregation induced phosphorescent emission (AIPE), mechanochromism, and piezofluorochromism have been developed.[212-219] Such materials could potentially be applicable as optical memories, photodetectors, photostabilizers, dyes for solar collectors, solar filters and in bioimaging.[216-219]

Salicylaldehyde azines (SAs) have been considered as good candidates to show AIE behaviour, wherein the photophysical properties of the aggregates changes with the nature of substituents.[220, 221] AIE behaviour in such compounds originated from the
restriction of rotations of the N–N bond due to the presence of intramolecular hydrogen bond. Such intramolecular hydrogen bonding can promote keto-enol tautomerism through excited-state intramolecular-proton transfer (ESIPT) process. Some salicylaldehyde-based salen Schiff bases also exhibit AIE properties, although most of such ligands show ACQ. Salen ligands connected with non-conjugated long alkyl bridges exhibit AIE property.[215] Restriction of intramolecular rotation was recognised as the cause of aggregation induced emission in such molecules since this restriction could suppress the non-radiative pathways.

Biphenyl derivatives are a class of compounds which can undergo intramolecular rotation of the two rings (Scheme 5.3). In gas-phase biphenyl (BP) exhibits a twisted conformation with a dihedral angle (ϕ) of about 45°, which was explained as arising from repulsions between the ortho-hydrogens.[216]

Scheme 5.3 Possible orientation of biphenyl group in gaseous state

In the crystalline state biphenyl tend to adopt a planar conformation at room temperature (and still at 110°K), which however has been shown in a double minimum
potential caused by rotation of the two rings.[216,222] Thus, an important challenge in this regard will be to develop AIEE materials by restricting the intramolecular rotation of a biphenyl group.

5.2 Results and Discussions

5.2.1 Synthesis and characterization

As shown in Scheme 5.4, formylation of 4-hydroxy-biphenyl with hexamethylenetetramine and TFA afforded aldehyde 1 in moderate yields (Scheme 5.4). Subsequently, the reaction of 1 with semicarbazide and phenyl-semicarbazide afforded 4-BOH-S and 4-BOH-P in moderate yields. The products were recrystallised and characterized using FT-IR, UV-visible, fluorescence, NMR and mass spectrometric techniques.

Scheme 5.4 Synthesis of ligands 4-BOH-S and 4-BOH-P used for this study
5.2.2 X-ray structure of 4-BOH-S

During initial studies, we observed that 4-BOH-S could be crystallised from methanol as pale yellow blocks. Subsequent X-ray diffraction studies of the single crystal of 4-BOH-S revealed the presence of methanol in the lattice (Figure 5.1). The solvated methanol was hydrogen bonded to the phenolic-"OH [O1– H···O3 0.820(2) Å, 2.683(2) Å, 3.044(3) Å, 108.51(18), 108°] and the semicarbazide-NH2 groups [N3– H···O3 0.852(2) Å, 2.160(2) Å, 2.911(2) Å, 146.85°].

As shown in Figure 5.2, the crystal packing featured hydrogen bonded dimeric structures of the 4-BOH-S molecules, involving the C=O and NH group [N-H···O 0.915Å, 2.020Å, 170°]. The hydrogen bonded dimeric arrangements extended into a lamellar structure parallel to the (10-2) plane. The important hydrogen bonding parameters have been given in Table 5.1. As shown in Figure 5.3, the crystal packing diagrams also indicated aromatic- π interactions between the biphenyl groups wherein the aromatic groups adopted a head-to-tail arrangement with an average interplanar distance of 3.362 Å.

Figure 5.1 Crystal structure of 4-BOH-S as a solvate of MeOH (Triclinic, P-1).
Figure 5.2 (a), (b) Packing structures of 4-BOH-S.MeOH showing the formation of hydrogen bonded lamellar networks, parallel to (10-2) plane.

Figure 5.3 Aromatic \( \pi \)-interactions (interplanar distance 3.362 Å) between the biphenyl moieties of 4-BOH-S in the crystal lattice.

<table>
<thead>
<tr>
<th>Table 5.1: Selected hydrogen bond distances (Å), and angles (°)</th>
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<td>Interactions</td>
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5.2.3 UV-visible and fluorescence studies

As monitored by UV-visible spectroscopy, the absorption spectra of 4-BOH-S in methanol (MeOH) was characterised by broad absorption centred at 334 nm ($\lambda_{\text{max}}$). Similarly, the UV-visible spectra of 4-BOH-P in methanol was characterised by absorption at 336 nm, with a tail which extended ~ 430 nm (Figure 5.4). Following this, we compared the absorption spectra of 4-BOH-S and 4-BOH-P in solvents of different polarities, such as acetonitrile (MeCN), tetrahydrofuran (THF) and dimethyl sulfoxide (DMSO) and found that the absorption spectra were solvent dependent (Table 5.2). From the absorption spectra, two important observations could be made. Firstly, the $\lambda_{\text{max}}$ for 4-BOH-S and 4-BOH-P were both red-shifted from 331 nm in tetrahydrofuran to 337 nm in DMSO. This absorption was apparently caused by $\pi-\pi^*$ transitions, and expectedly solvatochromic.[222].

![Figure 5.4](image)

Figure 5.4 UV-visible spectra of (a) 4-BOH-S (b) 4-BOH-P in different solvents.

Secondly, the weak absorption bands at ~ 400 nm for 4-BOH-S and 4-BOH-P in DMSO possibly originated from $\pi-\pi^*$ transitions, but from a relatively planar
conformation of the fluorophore. This was understandable because the dipolar nature of DMSO can facilitate changes in the conformation and planarization of the molecule by interacting with the phenolic-OH and semicarbazide groups.[222]

The fluorescence spectra of 4-BOH-S in methanol was characterised by an emission at 399 nm, and a shoulder at 446 nm (Table 5.2). For comparison, we also recorded the fluorescent spectra of 4-BOH-S in acetonitrile, tetrahydrofuran and DMSO. As shown in Figure 5.5a, 4-BOH-S exhibited intense emissions at 387 nm and 440 nm in DMSO, while being only weakly emissive in acetonitrile and tetrahydrofuran. As shown in Figure 5.5b, the fluorescence spectra of 4-BOH-P in methanol also revealed two emissions at 391 nm and 440 nm. In DMSO, the emissions were observed at 392 nm and 442 nm, whereas in tetrahydrofuran and acetonitrile, the compound was weakly emissive.

Figure 5.5 Emission spectra of (a) 4-BOH-S (b) 4-BOH-P in solvents of different polarity, $\lambda_{ex}$ 300nm
From Table 5.2, it was apparent that changes in the emission maxima ($\lambda_{\text{em}}$) of both 4-BOH-S and 4-BOH-P could be related to relative polarity of solvent. This was possibly due to selective stabilization of the excited state leading to reduction in energy gap ($\Delta E$).[223] Since the excited state, i.e. charge separated state, becomes more polar relative to the ground state, increasing solvent polarity will stabilize the former. This could lead to lowering of the energy gap, so that the emission shifts towards higher wavelength.[222,223] The higher intensity emission of 4-BOH-S and 4-BOH-P in methanol could be attributed to the solvent induced disruption of photoinduced electron transfer (PET) from the semicarbazide N to the phenolic fluorophore moiety.[224] Alternatively, the observation of dual emission in methanol and DMSO with increasing relative solvent polarity could be indicative of the formation of the phenolic tautomers in the excited states.[224]

We attributed the weak emissions of 4-BOH-S and 4-BOH-P in solution to relatively flexible molecular structures, which facilitated non-radiative decay of the
excited states. In such cases, the non-radiative decay processes involving intramolecular rotations and/or vibrations could be suppressed via controlled aggregation in poor solvents.[225-227] To probe this aspect, we sought to induce controlled aggregation by adding water, as a non-solvent, to a solution of 4-BOH-S (or 4-BOH-P) in DMSO and study the effects through UV-visible and fluorescence spectroscopy.

Figure 5.6 Changes observed in UV-visible spectra of (a) 4-BOH-S [conc. = 1.1 nmol]; (b) 4-BOH-P [0.9 nmol] in different composition of DMSO/H$_2$O

Increasing the water content from 10-50% (v/v) to a solution of 4 BOH-S in DMSO produced distinct changes in its absorption spectra. As shown in Figure 5.6a, when the water content in DMSO was ~ 20% (v/v), the absorption spectra of 4-BOH-S was red shift by 40nm, from 337 nm to 378 nm. Further increase of water/DMSO composition to 50% (v/v) caused the absorption spectrum to become broad. Similarly, red shifted absorptions were observed in the UV-visible spectra of 4-BOH-P in DMSO-water mixtures (Figure 5.6b). In this case, the absorption spectra 4-BOH-P was red shifted by ~ 60 nm, when the solvent composition was 30% H$_2$O in DMSO (v/v).
Figure 5.7 (a) Changes in the fluorescence spectra of (a) 4-BOH-S [conc. = 1.1 nmol], (b) 4-BOH-P [0.9 nmol] in DMSO/H\textsubscript{2}O mixtures (\(\lambda\text{ex} 300\text{nm}\)); (c) Variation in the emission intensity at 471nm for 4-BOH-S as function of DMSO/H\textsubscript{2}O fraction; (d) Photographs showing the emissions of 4-BOH-S in DMSO/H\textsubscript{2}O mixtures with different water fractions (\(f_w\)) when irradiated with 365 nm UV illumination.

Red shifting of the absorption maxima was indication of aggregate formation from 4-BOH-S and 4-BOH-P in DMSO-water. The addition of water caused the molecules to pack into aggregates which in turn restricted the intramolecular
vibration/rotations and increased the rigidity of the molecule. We anticipated that aggregation process could induce changes in the interplanar angle of the biphenyl motif thereby leading to red-shifting of the absorption maxima.[222] This aspect was substantiated later from DFT calculations (Section 5.2.4)

The formation of solvent induced aggregates of 4-BOH-S and 4-BOH-P could also be monitored by fluorescence spectroscopy. As shown in Figure 5.7a, the emission of 4-BOH-S increased 3-fold at 446 nm 9:1 in DMSO/water (v/v) as compared that in DMSO. Increasing the water content to 1:1 DMSO-water (v/v) was accompanied by gradual red shifting of the emission maxima to 471nm, with maximum enhancement of intensity. At higher water content, the fluorescence intensity of 4-BOH-S decreased with gradual red shifting.

In case of 4-BOH-P, maximum enhancement of emissions at 444 nm was observed for 9:1 DMSO/H2O (v/v) composition. Further increase in the water content in DMSO led to decrease in the emission intensity albeit with red shifting (Figure 5.7b). On the basis of these results, the enhancement of emissions observed for 4-BOH-S and 4-BOH-P in DMSO/water mixtures was attributed to aggregates formation. The formation of aggregates and the variation of size with solvent composition were examined using Dynamic Light Scattering (DLS) experiments (Figure 5.8).

For instance, DLS measurements of the aggregates formed by 4-BOH-S in 8:2 DMSO/H2O mixtures (v/v) provided an average particle size (108 ± 41) nm, which were found to increased to (385 ± 152) nm in 1:1 DMSO/water (v/v) mixtures.
Considering the results obtained from UV-visible, fluorescence and DLS experiments, we inferred that the aggregation of 4-BOH-S and 4-BOH-P was solvent dependent, and that the emission behaviour of the aggregated species was directly dependent upon the size of the particle. Whereas, both 4-BOH-S and 4-BOH-P were weakly-emissive in pure solution and as larger aggregates, both compounds became emissive within a specific limit of size of particle.

5.2.4 Computational studies

To gain insight into the effect of molecular geometry on the HOMO-LUMO energy gap, we performed DFT calculations of 4-BOH-S for three different geometries corresponding to the relative changes in interplanar torsion angle.

As shown in Figure 5.9, the gradual reduction in the interplanar angle between the two aromatic groups of biphenyl moiety was accompanied by lowering of the $\Delta E$ value. The calculations suggested that the energy difference ($\Delta E$) for the geometry having torsion angle 4° was 0.1499 Hartree. On the other hand when the torsion angle
increased to ~85°, the ΔE became 0.1594 Hartree. The trends indicated by these DFT calculations were in agreement with the red shifting of the absorption bands, which were ascribed to planarization of biphenyl in the aggregated states.

![Figure 5.9](image)

Figure 5.9 Frontier molecular orbital amplitude plots and energy levels of the HOMOs and the LUMOs of 4-BOH-S when the interplanar angle between the two aromatic groups of biphenyl moiety was 85° (G1), 37° (G2) and 4° (G3)

5.2.5 Anion recognition studies

Due to the presence of polar groups such as semicarbazide and phenolic –OH, it was expected that 4-BOH-S and 4-BOH-P could potentially exhibit anion binding and recognition ability. In this context, we investigated the fluoride recognition ability of 4-BOH-S and 4-BOH-P in DMSO using UV-visible and fluorescence spectroscopy.
Figure 5.10a shows the changes in the UV-visible spectra of 4-BOH-S in DMSO following the addition of fluoride (as TBAF). Gradual addition of fluoride to 4-BOH-S produced a new absorption at 428 nm, besides its original absorption at 337 nm, both attaining saturation with 5 equivalents of fluoride. Similarly, the addition of fluoride anions to 4-BOH-P in DMSO also produced two new absorption at 384 nm and 435 nm respectively apart from its initial absorption. In this case, the binding isotherms revealed saturation with ~12 equivalents of the anion (Figure 5.10b).

![Figure 5.10](image)

Figure 5.10 Changes in the UV-visible spectra and the corresponding binding isotherms for (a) 4-BOH-S (conc. = 1.1 nmol) and (b) 4-BOH-P (conc. =0.9 nmol) following the addition of fluoride (as TBAF) in DMSO

The interactions of 4-BOH-S and 4-BOH-P with fluoride in DMSO were also monitored using steady state fluorescence spectra. As shown in Figure 5.11a, addition of 2 equiv of fluoride to 4-BOH-S, initially increased its emission at 440 nm. Further addition of fluoride (>2 equiv) led to red shifting of its emission maxima to 480 nm with sharp increase in emission intensity. Plot of fluorescence intensity vs [TBAF] at 480 nm shows saturation with ~ 5 equiv. of the fluoride concentration. Similar changes
were also observed in the emission spectra 4-BOH-P during the addition of fluoride (Figure 5.11b). In this case, the addition of ~ 1 equiv. of fluoride triggered shifting of its initial emission at 444 nm to 491 nm and saturation with ~ 10 equiv. of fluoride.

![Figure 5.11 Changes in the fluorescence spectra, and the corresponding binding isotherm of (a) 4-BOH-S (conc. = 1.1 nmol) and (b) 4-BOH-P (conc. = 0.9 nmol) following the addition of fluoride in DMSO (λex 300nm).](image)

Figure 5.11 Changes in the fluorescence spectra, and the corresponding binding isotherm of (a) 4-BOH-S (conc. = 1.1 nmol) and (b) 4-BOH-P (conc. = 0.9 nmol) following the addition of fluoride in DMSO (λex 300nm).

![Figure 5.12 Visual changes observed for 4-BOH-S following the addition of fluoride in DMSO: (a) In day light, (b) Under 365nm UV- irradiation](image)

Figure 5.12 Visual changes observed for 4-BOH-S following the addition of fluoride in DMSO: (a) In day light, (b) Under 365nm UV- irradiation

The addition of fluoride to a solution of 4-BOH-S and 4-BOH-P in DMSO changes the color of the solution from colorless to yellow and also led to the appearance of green fluorescence which could be observed visually under 365 nm UV illumination (Figure 5.12).
Figure 5.13 Comparing the changes observed in the (a) UV-visible and (b) fluorescence spectra of 4-BOH-S (conc. = 1.1 nmol) following the addition of different anions (approx. 12 equiv.).

We investigated the interactions of 4-BOH-S and 4-BOH-P with other anions such as Cl⁻, Br⁻, SO₄²⁻, AcO⁻, NO₃⁻, PO₄³⁻ and compared the result with fluoride. But unlike fluoride, addition of equivalent amount of other anions did not cause any significant changes to the UV-visible and fluorescence spectra of 4-BOH-S and 4-BOH-P. However, changes observed in UV-visible and fluorescence spectra following the addition of a large concentration of OH⁻ (~ 33 equiv.) were comparable with fluoride. Figure 5.12 shows the changes observed in the UV-visible and fluorescence spectra of 4-BOH-S following the addition of different anions.

The interaction of 4-BOH-S and 4-BOH-P with fluoride were substantiated using ¹H NMR titration analysis which revealed deprotonation and consequent charge transfer as the aspect of fluoride recognition.
5.2.5.1 NMR investigations

$^1$H NMR titration spectra of 4-BOH-S (and 4-BOH-P) with TBAF gave valuable information about the nature of receptor-fluoride interactions. As shown in Figure 5.14, the semicarbazide urea NH connected to the imine group of 4-BOH-S resonated at 10.12 ppm, whereas the other urea-NH$_2$ resonate at 6.5ppm.

Initial addition of fluoride (0.1 equiv) caused disappearance of the of the urea NH proton linked to the imine group at 10.12 ppm, and concomitantly, the phenolic (-OH) proton shifted upfield from 10.23 ppm to 9.8 ppm. This was possibly a reflection of the increasing electron density due to deprotonation of urea NH. Scheme 5.5 shows the possible interactions of fluoride with 4-BOH-S at low anion concentration, and the possible intramolecular hydrogen bonding between the imine and phenolic-OH.

![Scheme 5.5 Plausible hydrogen bonding interactions of 4-BOH-S with fluoride anion in DMSO (the intramolecular hydrogen bond between the imine N and OH group is highlighted)](image)

Further, we noticed gradual downfield shifting of the aldimine (–CH=N) proton, from 8.17 ppm to 8.31 ppm suggesting participation in the fluoride binding event. It appeared that deprotonaation event was accompanied by delocalization of the charge over the semicarbazide moiety which led to a overall shielding effect for the free -NH$_2$ and other aromatic protons including the meta-CH of phenyl. However, at high
concentration of fluoride (> 2 equiv), the phenolic-OH resonance at 9.8 ppm was shifted downfield to 10.47 ppm indicating multiple fluoride binding events.

Figure 5.14  (a) Partial $^1$H NMR spectra of 4-BOH-S in DMSO-­d$_6$ (1.5 mg in 0.4 mL) showing the fluoride complexation-induced chemical shifts for the –OH (●), urea NH
linked to imine (▼), -CH of imine (▲) and the aromatic meta -CH (♦) and -NH₂(■) at 25°C (b) Changes in the chemical shifts of –OH, imine CH and aromatic meta CH with TBAF concentration.

On the basis of the NMR spectral data, we proposed that 4-BOH-S could interact with fluoride anions through the carbamide NH group and the imine CH. This was supported by the fact that the aldimine proton at 8.17 ppm was gradually shifted downfield during the anion binding process.

We also performed the ⁴H NMR titration experiment for 4-BOH-P with TBAF in DMSO in order to examine the receptor-anion interactions. As shown in Figure 5.15, addition of only 0.5 equivalents of fluoride led to disappearance of urea NH resonance at 10.26 ppm and simultaneously caused broadening of the hydroxyl proton at 10.67 ppm. Again, a downfield shifting and broadening was noticed for the aldimine (–CH=N) proton suggesting the participation in the anion binding event. Moreover, fluoride/ receptor interaction produced a shielding effect for the meta aromatic –CH from 8.2 ppm to 7.89 ppm, which reflected increasing electron density due to deprotonation of the NH protons of semicarbazide moiety. The possible interactions of 4-BOH-P with fluoride have been shown in Scheme 5.6.

Scheme 5.6 Plausible hydrogen bonding interactions of 4-BOH-P with fluoride anion in DMSO
Figure 5.15 Partial $^1$H NMR of 4-BOH-P (1.5 mg in 0.4 mL) showing the effect of TBAF on the –OH resonance(●), urea NH resonances linked to imine (▼), other urea NH resonance (▲), -CH of imine (♦) and the aromatic meta -CH resonances(■), in DMSO at 25°C.
Figure 5.16 Changes in the chemical shifts of aldimine CH, aromatic meta-CH and urea NH₂ with fluoride anion concentration.

5.2.5.2 Anion recognition studies in aggregated state

As already described in section 5.2.3, the receptor 4-BOH-S and 4-BOH-P could produce fluorescent aggregates in DMSO/water and thereby, we anticipated the fluorescent aggregates of 4-BOH-S and 4-BOH-P to have anion responsive ability. Accordingly, we performed the UV-visible and fluorescence titration of 4-BOH-S with various anions (F⁻, Cl⁻, Br⁻, SO₄²⁻, HSO₄⁻, H₂PO₄⁻, HPO₄²⁻, PO₄³⁻, OAc and OH⁻) and found the ligand responsive to HPO₄²⁻.

Figure 5.17 (a) shows the changes observed in the UV-visible spectra of 4-BOH-S aggregates during titration with HPO₄²⁻ in DMSO/water (4:1). A new red-shifted absorption band was produced at 394 nm with two clear isobestic points at 334 nm and 350 nm respectively. Plot of Abs vs [HPO₄²⁻] at 387 nm was sigmoidal and achieved saturation with 9 nmol of the anion.
Again, the addition of HPO$_4^{2-}$ to the nano aggregates of 4-BOH-S caused perceptible changes in their fluorescence emission spectra. As shown in Figure 5.17(b) addition of HPO$_4^{2-}$ enhanced the fluorescence intensity 483 nm in 4:1(v/v) DMSO/water and this was accompanied by the appearance of green fluorescence. The plot of fluorescence intensity Vs [HPO$_4^{2-}$] at 483nm was also sigmoidal in nature. The sigmoidal nature of the binding isotherm could be attributed to a cooperative anion binding process where the initial anion binding event makes subsequent binding more favorable.

Similar to 4-BOH-S, significant changes were also observed in the UV-visible and fluorescence spectra for the aggregates of 4-BOH-P in DMSO/H$_2$O following the addition of HPO$_4^{2-}$. As shown in Figure 5.18a, initial addition of 2.2 nmol of [HPO$_4^{2-}$] caused the appearance of a new absorption at 397nm in the
UV-visible spectra for the nano aggregates of 4-BOH-P which attain saturation with 8.8 nmol of this anion.

Figure 5.18 Changes observed in the (c) UV-visible spectra and (d) fluorescence spectra for the aggregates of 4-BOH-P following the addition of hydrogen phosphate anions in DMSO-water at 25°C [4-BOH-P] = 1.1 nmol).

Figure 5.18b shows the changes observed in the fluorescence spectra for nano aggregates of 4-BOH-P following the addition of HPO$_4^{2-}$. Initial addition of 3.3 nmol HPO$_4^{2-}$ decreased the initial emission of the aggregates of 4-BOH-P at 454 nm to reach a minimal. Beyond that, increasing the anion concentration was accompanied by increase in fluorescence emission at 492 nm, and this emission itself was red-shifted by 32 nm from its initial emission.

Addition of equimolar amount of other anions (acetate, nitrate, sulphate, hydrogen sulphate, and phosphate and dihydrogen phosphate anion) led to quenching of the fluorescence of aggregates of 4-BOH-S and 4-BOH-P. We also examined the effects
of $\text{PO}_4^{3-}$ and $\text{H}_2\text{PO}_4^-$ on the fluorescence emission of the aggregates. Addition of both the anions quenched the fluorescence of the aggregates, while the addition of fluoride, chloride and bromide anions had no perceptible effect on the fluorescence emissions of aggregates. Figure 5.19 shows the fluorescence spectral changes for aggregates of 4-BOH-P following the addition of different anions in DMSO/H$_2$O.

![Fluorescence Spectra](image)

Figure 5.19 Changes observed in the fluorescent spectra for the aggregates of 4-BOH-P (0.9 nmol) following the addition of 10 nmol of different anions.

Further information about the effect of $\text{HPO}_4^{2-}$ on the fluorescence aggregates of 4-BOH-S and 4-BOH-P was obtained from fluorescence lifetime measurement (Figure 5.18). In this case, we observed that the average fluorescence lifetimes $<\tau>$ of aggregates of 4-BOH-S changed from 0.60 ns to 5.66 ns after addition of $\text{HPO}_4^{2-}$. The dramatic increase in fluorescence lifetime was indicative of the formation of a stable aggregate, particularly after the binding of the anions. Since the aggregates remained solvated, initial binding of $\text{HPO}_4^{2-}$ anions could trigger reorganisation and desolvation of the aggregated molecules. Further addition $\text{HPO}_4^{2-}$ anions caused more binding sites
to be occupied, and this induced possible planarization of the receptor molecules. As a result of the anion binding process, the aggregates became more rigid and hence non-radiative decay due to vibrational and rotational motion were suppressed, and hence the enhanced fluorescence emissions. The possible modes of interaction of 4-BOH-P with HPO$_4^{2-}$ anions in the aggregate state have been shown in Scheme 5.7.

Figure 5.20  Comparison of fluorescence lifetime of aggregates of 4-BOH-S (a) before and (b) after addition of HPO$_4^{2-}$.  

- a) 

Data and Fitted Curve

- b) 

Data and Fitted Curve
5.2.6 Anion recognition studies in solid state

Anion recognition properties of 4-BOH-S and 4-BOH-P were also examined in solid state. We observed that both the ligands were weakly emissive in solid state. The weak emissions were attributed to the non-radiative vibrational relaxation pathways originating from lack of planarity in the molecules.

Another factor that could contribute to the weak emission of 4-BOH-S and 4-BOH-P in the solid state was a possible PET effect from the NH group of urea. Addition of a protic solvent (methanol) could block the PET process through the formation of intermolecular hydrogen bond with the NH group and thus blue emission can be generated in the solid state. The blue turn on fluorescence by the addition of protic solvent were reported earlier in a non fluorescent Schiff base compound. The turn on fluorescence was attributed to the suppression of PET process which in turn opened the possibility for excited state intramolecular proton transfer (ESIPT)[224].

The crystal structure of 4-BOH-S suggested the presence of an intramolecular hydrogen bond in the molecule which was the prerequisite for an ESIPT to occur.
Therefore, we reasoned that the fluorescence turn on in 4-BOH-S was because of an incipient ESIPT process through the suppression of PET (Scheme 5.6).

Scheme 5.6 Fluorescent turn on mechanism for 4-BOH-S via ESIPT on addition of methanol

Figure 5.21 Solid state emission of 4-BOH-S with $\lambda_{\text{max}}$ at 398 nm, which increased almost 12 fold after grinding with fluoride with emission maximum at 476nm. A small increased in the emission intensity was observed at 450nm while adding fluoride to 4-BOH-S in presence of water.
As shown in Figure 5.21, the pristine sample of 4-BOH-S was characterized by a weak solid-state emission with emission maxima ($\lambda_{em,max}$) at 398 nm. Mixing a powdery sample of 4-BOH-S with fluoride (as TBAF) produced a material with bluish-green emission with emission maximum at 476 nm. Furthermore, the fluoride induced emission of solid 4-BOH-S was sustained even after exposing the bluish-green emissive material to water vapour.

Figure 5.22 (a) Preparation of test paper for fluoride detection (b) Nanomole detection of fluoride by 2-BOH-S on paper strip

We reasoned that the fluoride induced bluish-green emission of 4-BOH-S was characteristic of increased rigidity in the molecules due to fluoride binding. Enhanced
rigidity attributed to the suppression of nonradiative relaxation pathways, which can be correlated with the enhanced emissions upon aggregation.

In order to investigate the practical applicability of 2-BOH-S, test paper sensor coated with 2-BOH-S were prepared. The test papers were utilized for sensing of fluoride at different concentration and variation in emission observed under 365nm UV-illumination (figure 5.22). The minimum detection limit of fluoride was found to be less than 1.2nmole.
5.3 Conclusions

In conclusions, we have synthesized and characterized two hydroxyl biphenyl derived imine ligands 2-NOH-S and 2-NOH-P from semicarbazide and phenyl-semicarbazide and studied their photophysics and anion recognition properties. Both the ligands produced fluorescent aggregates in DMSO/water, which could be recognised from the enhanced fluorescence and appearance of red shifted absorptions upon gradual addition of water in DMSO. DFT calculations revealed that aggregation induced enhanced emission could be correlated with the increased planarity of the molecule which restricted the intramolecular rotations and thereby suppressed the nonradiative relaxation processes. Furthermore, both the ligands highly recognize fluoride both in solution and in the solid state. In solution, addition of fluoride led to the appearance of red-shifted absorptions in the UV-visible spectra and caused 10-12 fold enhancement of fluorescence in the emission spectra. $^1$H NMR titrations revealed fluoride induced deprotonation of the hydroxyl group and one of the urea NH. Although the ligands exhibited very weak emission in the solid state addition of fluoride produced a blue emissive materials, which was characterised by a solid state emission with $\lambda_{em,max}$ at 476nm. The enhancement of fluorescence in the solid state was possibly because of the increased rigidity in the molecule due to fluoride binding. Further, we observed that the fluorescent aggregates of 4-BOH-S and 4-BOH-P were responsive to HPO$_4^{2-}$, which enhanced the fluorescence of the aggregates. We reasoned that fluorescence enhancement was because of further increase in the planarity of the molecules of aggregates due to binding with HPO$_4^{2-}$. 
5.4 Experimental Section

5.4.1 Materials and Methods

All chemicals were commercially available from Sigma-Aldrich or Merck (India) and used as received. Solvents for spectroscopic experiments were distilled under nitrogen atmosphere before use. All $^1$H and $^{13}$C NMR were measured on a 300 MHz Bruker spectrometer, and reported in $\delta$/ppm. The absorption spectra were recorded on a Shimadzu UV-vis spectrophotometer (Model UV-1800), and fluorescence spectra were recorded using a Hitachi F2500 fluorimeter.

5.4.2 Synthetic procedures

**Formylation of 4-hydroxy-biphenyl:** A mixture of 4-hydroxy biphenyl (10 mmol), hexamethylene-tetramine (10 mmol) and TFA (15ml) were mixed. The reaction mixture was heated at 80-90°C for 12 hours. The products were concentrated and diluted with 60ml ice-cold water. The mixture was stirred for 15 min, and made alkaline with NaHCO$_3$. The solution was extracted with diethylether and subsequent evaporation of the solvent gave a yellow solid which was purified through column chromatography using 5% DCM/Hexane mixture.

**Synthesis of 4-BOH-S:** To a stirred solution of semicarbazide hydrochloride(2, 1 mmol) and sodium acetate (1 mmol) in H$_2$O (2 ml) was added a solution of the aldehyde (1, 1 mmol) in methanol (2 ml). After being stirred at room temperature for 10 min, the solvent was evaporated under reduced pressure. The solid residue was rinsed with chloroform and product was obtained as white powder.
Synthesis of 4-BOH-P: Same procedure as above provided

**4-BOH-S:** $^1$H NMR (300 MHz, DMSO) $\delta_{H} = 10.24$ (1H, s, -OH), 10.14 (1H, s, urea NH), 8.18 (1H, s, imine-CH), 8.07 (1H, s, aromatic CH), 7.66 (2H, d, J=6.9, aromatic CH), 7.49 (2H, d, J=8.1, aromatic CH), 7.39 (1H, m, J=7.2, aromatic CH), 7.2 (1H, d, J=6.9, aromatic CH), 6.9 (1H, d, J=8.4, aromatic CH), 6.5 (2H, s, -NH$_2$); $^{13}$C NMR (300 MHz, DMSO) $\delta_{C}$ = 156.78, 156.52, 139.80, 136.95, 131.36, 128.79, 128.49, 126.67, 126.27, 124.25, 121.10, 116.52 ppm; ES-MS: m/z 256.107 calc. For (M+H$^+$)

**4-BOH-P:** $^1$H NMR (300 MHz, DMSO) $\delta_{H} = 10.68$ (1H, s, -OH), 10.26 (1H, s, urea NH), 9.00 (1H, s, other urea NH), 8.318 (1H, s, aldimine-CH), 8.197 (1H, s, aromatic CH), 7.688 (2H, d, J=7.2, aromatic CH), 7.626 (2H, d, J=7.8, aromatic CH), 7.538 (2H, J=8.4, aromatic CH), 7.448 (2H, J=6.9, r aromatic CH) ES-MS: m/z 331.13 calc. For (M+H$^+$)
Figure 5.23 $^1$H NMR spectra of 4-BOH-S in DMSO-d$_6$

Figure 5.24 $^{13}$C NMR spectra of 4-BOH-S in DMSO-d$_6$
Figure 5.25 ES-MS spectra of 4-BOH-S, m/z 256.10 calc. for (M+H$^+$)

Figure 5.26 $^1$H NMR spectra of 4-BOH-P in DMSO-d$_6$
Figure 5.27 $^{13}$C NMR spectra of 4-BOH-P in DMSO-d$_6$.

Figure 5.28 ES-MS spectra of 4-BOH-P, m/z 332.13 calc. for (M+H)$^+$.
### Fitting range: \([150; 1500]\) channels

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\chi^2 = 0.988
\]

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**Shift**: 0.012 ns \((\pm 0.542 \text{ ns})\)

**Decay Background**: 0.378 \((\pm 0.142)\)

**IRF background**: 0.100

### Fitting range: \([210; 4096]\) channels

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</table>

**Shift**: 0.073 ns \((\pm 6.956 \text{ ns})\)

**Decay Background**: 0.670 \((\pm 0.081)\)

**IRF background**: 0.100

Figure 5.29 Fluorescent lifetime measurement data for the aggregates of 4-BOH-S (a) before (b) after addition of \(\text{HPO}_4^{2-}\).