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Thiourea recognition by 2,6-*bis*(2-benzimidazolyl)pyridine using spectroscopic techniques and DFT

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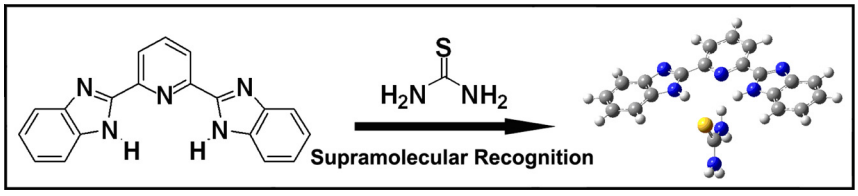
HIGHLIGHTS

- Thiourea can be recognized by 2,6-*bis*(2-benzimidazolyl)pyridine receptor.
- UV visible and florescence spectroscopy can be used to follow this recognition.
- Binding mode of thiourea is compared with urea.
- DFT calculations prove that binding is dependent on the bigger size of sulfur.

GRAPHICAL ABSTRACT



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ABSTRACT

Recognition of thiourea by 2,6-*bis*(2-benzimidazolyl)pyridine, **bbp**, a neutral tridentate ligand was studied by UV visible and fluorescence spectroscopic techniques. FTIR spectroscopy and supportive DFT calculations established that, thiourea molecule, while bound to the binding site of **bbp** took up a near perpendicular orientation to the plane of the receptor. While forming the complex, the two imidazole H atoms present in the binding site of **bbp** formed two weak interactions with S atom of thiourea, which has low electronegativity. Moreover, bigger size of S atom restricted approach of thiourea inside the binding site. Stability of the **bbp**:thiourea complex basically increased as one of the imine H atom of thiourea is involved in a hydrogen bond with the pyridine N atom of **bbp**, which forced the near perpendicular orientation of thiourea on the plane of **bbp**. This binding mode is significantly different from the binding mode of urea with **bbp** as reported earlier.

1. Introduction

Thiourea causes toxicity in physiological system including bone marrow toxicity and reduction in red blood cells, white blood cells, and platelets. Enlargement of the thyroid (goitre) and spleen has also been reported from exposure to thiourea. Moreover, it is confirmed to be a human carcinogen based on adequate evidence of carcinogenicity on experimental animals [1]. Thiourea inhibits lignin degradation by phanerochaete chrysosporium [2]. When administered in drinking water, it induces thyroid adenomas and carcinomas in rats. The presence of thiourea in urine was reported to be non-specific indicator of cancer and has been labeled as having carcinogenic activity. It also has adverse effect on carbohydrate metabolism [3] which increases the solubility of membrane proteins.

In spite of much concern on neutral receptors for urea developed so far [4], the design and synthesis of novel receptors capable of recognizing thiourea has not yet been studied. Fluorimetry [5], titrimetry [6], thin layer chromatography [7], colorimetric [8], etc. are the various methods that have been proposed for the determination of thiourea. Yet, efforts to develop viable receptor systems for thiourea recognition lie dormant. Major challenges that need attention are the development of structurally simple and stable receptors that would have better utility and much wider

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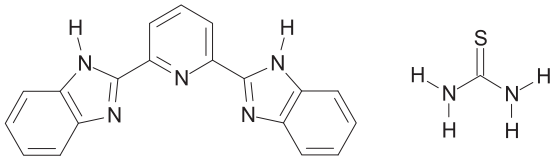

Fig. 1. 2,6-*Bis*(2-benzimidazolyl)pyridine (**bbp**) and thiourea.

applicability. Receptors capable of recognizing chemical and biological guest molecules by supramolecular interactions has contributed greatly in the development of host guest and senor chemistry [9–12]. We present here the use of a simple tridentate ligand 2,6-*bis*(2-benzimidazolyl)pyridine (**bbp**) for the recognition of thiourea (Fig. 1). Moreover, recognition of thiourea by **bbp** is compared with recognition of urea by the same receptor which was reported earlier by our group [13]. Easy economic synthetic method and use of simple spectroscopic techniques to follow the recognition process possess **bbp** as a novel effective supramolecular receptor for thiourea. This detail systematic study supported by structure optimization with DFT method, would significantly contribute to optimize future synthetic receptors for similar biological molecules.

Ligand **bbp** has two imine nitrogen lone pairs and two —NH fragments in addition to the pyridine nitrogen lone pair that can form hydrogen bonded adducts with guest molecules [14–16]. It is structurally very simple, stable to heat/light and can be synthesized in a single step from commercially cheap starting materials. Additionally due to its solubility in several laboratory solvents, **bbp** can be used as a host molecule for the recognition of thiourea by hydrogen bonding interactions.

2. Materials and methods

All reagents were used as received without further purification unless mentioned. These materials were of reagent grade or better. Acetonitrile was distilled from calcium hydride. UV/visible spectra were recorded on a Perkin Elmer Lambda-25 spectrophotometer. Fluorescence spectra were recorded on a Varian Carry 25 spectrophotometer. FT-IR spectra were taken on a Perkin Elmer spectrophotometer with samples prepared as KBr pellets.

To study the binding of thiourea by the **bbp** receptor, the gradual changes in the UV visible and fluorescence spectra of a solution of **bbp** in acetonitrile is followed upon addition of thiourea solution up to 1.0 equivalents in increments of 0.1 equivalents.

UV/visible titration is performed as follows: A solution of **bbp** (0.128 mM) in acetonitrile was titrated with 20 μL aliquots of a solution of thiourea (0.158 mM) in acetonitrile. The addition was done stepwise, and after each step, the formation of [**bbp**:thiourea] was monitored by UV/visible spectroscopy. Fluorescence titration experiments were also performed in a similar manner.

2.1. Synthesis of ligand *bbp*

2,6-Pyridinedicarboxylic acid (0.50 g) and o-phenylenediamine (0.70 g) were dispersed in orthophosphoric acid (10 cm^3), and heated for 4 h at 220 °C. Whilst still hot, the resulting solution was poured into cold distilled water (1 dm^3) with vigorous stirring. The resulting blue precipitate was collected by filtration, then dispersed in 500 cm^3 of hot 10% (w/v) Na_2CO_3 (aq) and stirred for 30 min. The insoluble material was collected by filtration, dispersed in cold distilled water (800 cm^3), and adjusted to pH 4 using 10% (v/v) HCl (aq) then recovered by filtration and recrystallized from the minimum amount of methanol. The final product obtained was a white powder (96%, 0.90 g) with a melting point in excess of 250 °C.

2.2. Synthesis of solid *bbp*:urea and *bbp*:thiourea

bbp:urea complex: A solution of urea (0.020 g, 33.3 mmol) in methanol was added dropwise to a methanolic solution of **bbp** (0.104 g, 33.3 mmol) and allowed to stir for 25 min. The clear solution was evaporated to get the solid **bbp**:urea complex.

bbp:thiourea complex: A solution of thiourea (0.020 g, 26.3 mmol) in methanol was added dropwise to a methanolic solution of **bbp** (0.082 g, 26.3 mmol) and allowed to stir for 25 min. The clear solution was evaporated to get the solid **bbp**:thiourea complex.

3. Results and discussion

Supramolecular complex formation between **bbp** and thiourea was studied by optical techniques such as UV/visible and fluorescence spectroscopic titration in dry acetonitrile. The solution state properties of receptor **bbp** was evaluated by careful addition of 0.1 equivalents of thiourea aliquots at regular intervals to a cuvette containing **bbp** and the spectral changes were recorded by means of UV/visible spectroscopy. Our observations suggest that on increasing the concentration of thiourea, progressive decrease of absorbance in the initial absorption band (Fig. 2a) having λ_{max} at 327 nm occurred and a new peak at 249 nm with higher absorbance develops which is due to the formation of stable complex between **bbp** and thiourea. This group of spectra shows formation of an isosbestic point at 263 nm indicating the presence of at least one species at equilibrium.

Thus the gradual decrease in the band absorbance at 327 nm with the formation of a new higher absorbance blue-shifted band at 249 nm with a sharp isosbestic point confirms the formation of hydrogen-bonded complex between **bbp** and thiourea. The low concentrations at which these spectroscopic changes were observed clearly reveal that **bbp** can be used for the recognition of thiourea. The inset plot inserted in Fig. 2a shows that a limiting value is reached on forming a 1:1 adduct between **bbp** and thiourea.

Similar titration was performed by careful addition of 0.1 equivalents of urea aliquots at regular intervals to **bbp**. On increasing the concentration of urea, the initial absorption band (Fig. 2b) having a λ_{max} at 327 nm showed a marginal but progressive decrease in absorbance with broadening and formation of a clear isosbestic point at 277 nm, indicating the presence of at least one stable species at equilibrium. The inset of Fig. 2b shows that a limiting value is reached on forming a 1:1 adduct between **bbp** and urea.

The formation of supramolecular complex between **bbp** and thiourea was also studied by the use of fluorescence titration experiments. The changes observed in the fluorescence spectra of a solution of **bbp** in acetonitrile on addition of up to 1.0 equivalents thiourea is represented in (Fig. 3a). A large quenching (>54%) in intensity of the 375 nm band was observed on the addition of 1.0 equivalents of thiourea indicating that on formation of the hydrogen bonded complex between thiourea and **bbp**, the excited state was modified considerably leading to the quenching of fluorescence. Therefore, formation of supramolecular complex alters the optical properties of **bbp** in solution and could be employed for sensing essential guests such as thiourea having large atoms such as sulfur.

Fluorescence titration was also carried out by the addition of 0.1 equivalents of urea to **bbp** where the 375 nm band in the spectrum was diminished by over 70% of the initial intensity (Fig. 3b). A large quenching in intensity was observed up to the addition of 0.5 equivalents of urea after which the changes were minor.

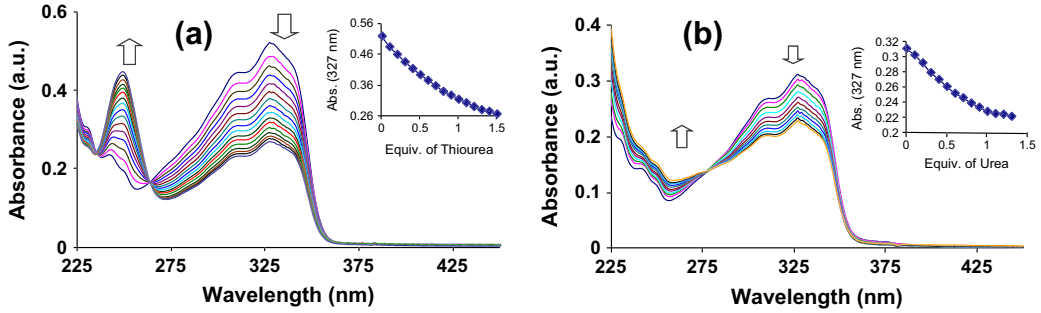

Fig. 2. (a) UV/visible spectra of **bbp** (9.5×10^{-6} M in dry CH_3CN) during titration with thiourea from 0 to 1.5 equivalents (v/v). Inset-titration profile of the band at 327 nm corresponding to the **bbp**:thiourea. (b) UV/visible spectrum of receptor **bbp** (6.35×10^{-6} M in dry CH_3CN) during titration with urea from 0 to 1 equivalents (v/v). Inset-titration profile of the band at 327 nm corresponding to the **bbp**: urea H-bonded complex.

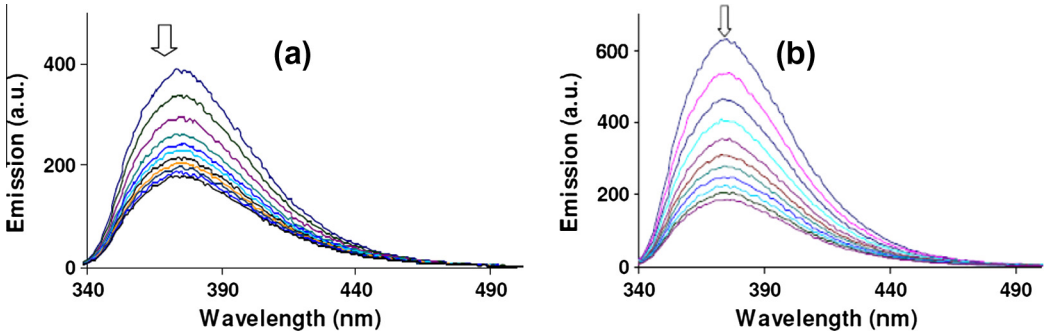

Fig. 3. (a) Emission spectra of receptor **bbp** (2.27×10^{-7} M in dry CH_3CN) during the titration with thiourea from 0 to 1 equivalents (v/v). (b) Emission spectrum of receptor **bbp** (3.54×10^{-7} M in dry CH_3CN) during the titration with urea from 0 to 1 equivalents (v/v).

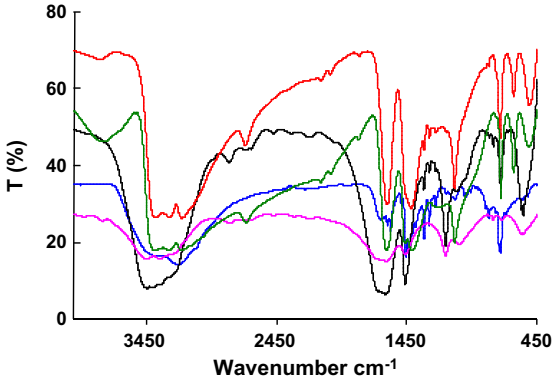

Fig. 4. FT-IR spectra of **bbp** (_____), **urea** (_____), **thiourea** (_____), **bbp**:urea (_____) and **bbp**:thiourea (_____) complexes.

The formation of supramolecular complexes of urea and thiourea molecules with **bbp** was also confirmed by FT-IR (KBr pellets) studies (Fig. 4). In case of urea, the —C═O stretching frequency should be observed at 1680 cm^{-1} [17]. The two hydrogen bonds formed by the urea oxygen with the two —NH hydrogen results in a red shift of the peak value to 1652 cm^{-1}. On the other hand —C═S stretching of thiourea appears at 729 cm^{-1} as expected and does not show any shift in the complex with **bbp**. However, the —NH_2 stretching frequency of thiourea, which should appear at 3360 cm^{-1}, shows a red shift to 3346 cm^{-1}. This suggests that the major contribution of bonding between thiourea and **bbp** is by the —NH_2 group of thiourea and not through the sulphur of —C═S. This is further evident from the fact that, the —NH stretching frequencies in urea appeared at 3423 cm^{-1} and in the complex it appeared at 3420 cm^{-1} indicating very minor shift. Moreover, the N—H peak of **bbp** at 3200 cm^{-1} is shifted to 3447 cm^{-1} in the 1:1 complex of urea:**bbp** and to 3390 cm^{-1} for 1:1 complex of thiourea:**bbp**. Calculated vibrational frequencies also follow a similar trend as we observed experimentally. The —C═O stretching frequency of urea (1792 cm^{-1}) shifts significantly to a lower wavelength (1741 cm^{-1}) in the **bbp** urea complex. But the —C═S stretching of thiourea (764 cm^{-1}) did not change much (740 cm^{-1}) (calculated frequencies are listed in Table S1 and some of the vibrational modes are mentioned in Table S2 in supporting information).

The effective binding between **bbp** and urea as well as thiourea (Fig. 5) were established by molecular geometry optimization in spin restricted shell wave function manner with hybrid functional B3LYP of Becke's exchange functional [18] and the correlation functional of Lee et al. [19] using the basis set 6-31+G(d,p). The calculations were carried with a developed version of Gaussian 03 [20]. The two hydrogen bonding interactions, X27-H25 and X27-H26 (both 2.511 Å) contribute to the binding stability of the thiourea complex. The bigger size of X27 in case of the thiourea restricts the closer approach of the guest molecule to the binding site. Much longer X27-N11 distance in the thiourea complex (3.948 Å) in comparison to the urea complex (3.459 Å) is evidence of it. The near perpendicular orientation of the thiourea molecule to the plane of the host molecule brings H31 atom nearer to N11 (2.074 Å) and maximizes the stability by another stronger H-bonding interaction. Importantly it was also observed that the binding constant value (calculated from UV/visible data) of the **bbp**: thiourea complex (40.88 M^{-1}) is lower in comparison to the urea complex (44.38 M^{-1}) which is attributed to the larger size of sulphur that prevents it to come closer into the cavity of **bbp**. The calculated energies of reaction for the formation of urea and thiourea co-crystals with **bbp** are −10.381 and −11.329 kcal/mol respectively. This is in agreement with the IR spectra where the data suggests that one of the —NH_2 of thiourea takes part in

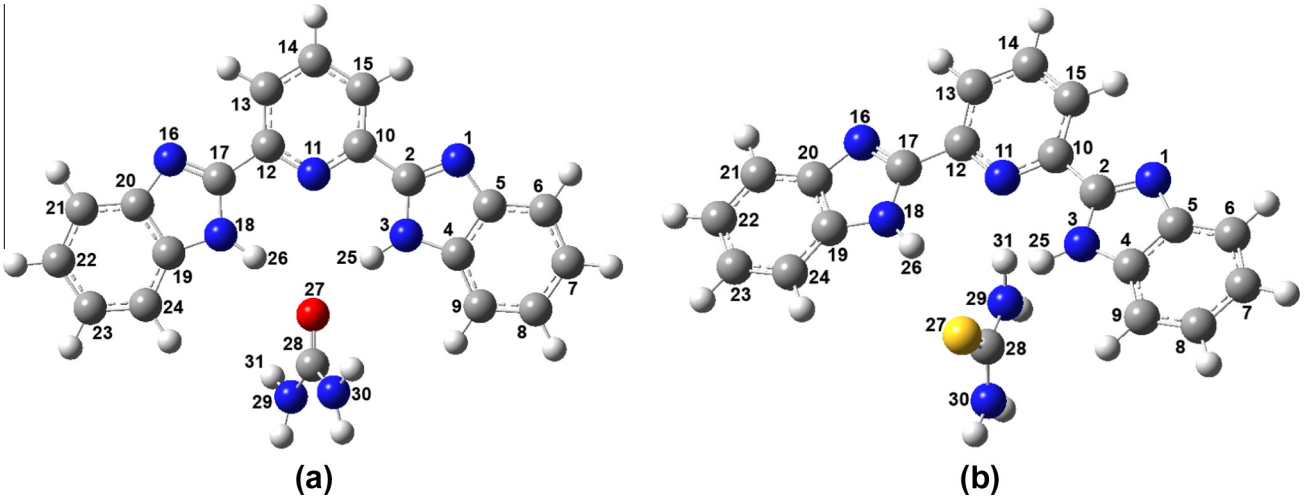

Fig. 5. Optimized structures of **bbp** complex with (a) urea and (b) thiourea.

Table 1
Important geometry parameters of single crystal and calculated structures.

Parameter	XRD data of **bbp**:urea crystal (X = O)	Urea-ligand (X = O)	Thiourea-ligand (X = S)
N11 X27	3.258	3.459	3.948
X27 N3	2.92	3.039	3.486
X27 N18	2.85	3.036	3.485
X27 H25	1.926	2.030	2.511
X27 H26	2.017	2.033	2.511
N3 H25	0.875	1.0187	1.020
N18 H26	0.905	1.019	1.020
N11 H31	5.209	5.319	2.075
N11 N29	5.281	5.556	3.096
N11 N30	5.348	5.531	5.223
H25 H26	2.974	2.932	3.219
N11 X27 C28	170.983	178.879	76.710
C10 N11 X27 N30	60.477	55.584	−92.000
N3 C2 C10 N11	−13.932	1.314	−9.551
N18 C17 C12 N11	2.433	1.187	9.548

Bond length and angle are in Angstrom (Å) and degree (°) respectively. For atom numbering see Fig. 5.

binding the **bbp** ligand as observed in the above optimized structure calculations (H31 interaction with N11) rather than the —C═S participation.

Table 1 summarizes the geometry parameters of calculated structures for both urea and thiourea complex with **bbp**. Single crystal X-ray parameters established earlier [8] for the urea complex are also placed for better comparison between the theoretical and experimental values. The minor deviation of the calculated values in this case is due to the solvent effects which are not taken into account in the calculated structures.

In our earlier report we had shown the formation of a supramolecular complex between the receptor **bbp** and urea [13]. The structure of **bbp**:urea complex (Fig. 5a) obtained via calculation is similar to that obtained from the single crystal X-ray structure except that the orientation of the urea molecule in the crystal structure is slightly more deviated from the aromatic plane than the calculated one. As observed from these figures, **bbp** forms 1:1 complex with urea molecule. The urea molecule fits into the cavity of **bbp** and is bound to the —NH protons of **bbp**. Here one receptor molecule donates two of its protons to the carbonyl oxygen of urea which is pointed inwards to the cavity. The molecular axis of urea molecule i.e. C28—O27 (as seen in the DFT structure, Fig. 5a) makes an angle of 171.0° with the plane of the pyridine ligand in the crystal structure while in the calculated structure, the angle is almost linear (178.9°). The angles between the two molecular planes obtained from single crystal and calculation are 60.5° and 55.6° respectively. The bond length between the carbonyl oxygen of urea and NH protons of **bbp** in crystal structure are of the order (N3—O27) 2.92 Å and (N18—O27) 2.85 Å while that obtained from calculation are both 3.04 Å.

In summary, we have established that a simple molecule such as **bbp** could be used as a receptor for binding guest molecules such as urea and thiourea. Larger size of thiourea sulfur atom compared to the oxygen in urea prevents it from entering the cavity of **bbp**, instead one of the thiourea —NH is pointed towards the cavity of **bbp** as seen from the molecular geometry optimization studies. This is also well supported by the IR data where a spectral shift of —NH peak occurs, whereas the position of —C═S peak remained unchanged. Additionally, the optimized structures obtained from DFT shows longer X27-N11 distance in the thiourea complex (3.948 Å) in comparison to the urea complex (3.459 Å). The near perpendicular orientation, for achieving better stability by another stronger H-bonding interaction between H31 atom and N11 (2.074 Å) of thiourea and **bbp** decreases the possibility of close packing which may be preventing the desired co-crystals to grow. The above results along with the UV/visible and fluorescence data indicate that **bbp** can be effectively used for binding important guest molecules. It can also be said that the cavity of **bbp** is not big enough to accommodate atoms with bigger radii such as sulphur resulting in a geometrical rearrangement of guest molecules. This study gives a new direction for the design of larger host molecules capable of effectively accommodating and binding important guest molecules such as urea and thiourea.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molstruc.2013.03.035.

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