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Tetrahedron Letters
Tetrahedron Letters 48 (2007) 47–50

Utilization of 2,6-bis(2-benzimidazolyl)pyridine to detect toxic benzene metabolites

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Received 27 September 2006; revised 24 October 2006; accepted 2 November 2006
Available online 21 November 2006

Abstract—The tridentate ligand 2,6-bis(2-benzimidazolyl)pyridine has the ability to detect toxic benzene metabolites such as phenol, hydroquinone, resorcinol, catechol and *p*-benzoquinone by simple techniques like UV/vis and fluorescence spectroscopy. The formation of a stable supramolecular complex between 2,6-bis(2-benzimidazolyl)pyridine and hydroquinone was confirmed by X-ray analysis.

Benzene and its metabolites which include phenols and quinones are carcinogens, known to cause the breakage and rearrangement of chromosomes in human, animal and yeast cells. Hydroquinone (benzene-1,4-diol) and catechol present in cigarette smoke are metabolites of benzene which result from the pyrolysis of naturally occurring flavonoids found in tobacco.[1] It has been demonstrated by several physiological studies that 'hydroquinone' and its oxidation product '*p*-benzoquinone' are responsible for immunosuppression as well as myelotoxicity and are known to inhibit mitogen-stimulated activation of both T and B lymphocytes.[2] Besides their carcinogenic properties, these metabolites of benzene are present in several naturally occurring biomolecules such as Coenzyme Q (CoQ), in the form of hydroquinone or quinone fragments in their structures, due to which they are able to undergo redox interconversions in living cells.[3] Among the several biochemical roles of CoQ, the most important one is in electron transport chain process, passing reduced species to the acceptors and in the direct electron transfer towards oxygen. CoQ also has structural resemblance with the vitamin K group of compounds that possess quinine rings. Thus, metabolites of benzene also play an important role in biological cycles.

In spite of their important relevance to biology, there are very few synthetic receptors that form supramolecular adducts with these small phenolic compounds.[4] In a recent communication, we showed that 2,6-bis(2-benzimidazolyl)pyridine, **1** (Fig. 1), is an efficient receptor for binding urea with high affinity.[5] It was observed that the host–guest complex which formed between **1** and urea was very stable, where **1** utilized not only its cavity but also the imine nitrogen located on the outer core to form stable donor–acceptor complexes with urea. Encouraged by these results, we explored the ability of **1** as a host to detect metabolites of benzene. Herein, we demonstrate that **1** forms hydrogen-bonded complexes with various metabolites of benzene even in the presence of a competitive solvent environment, which

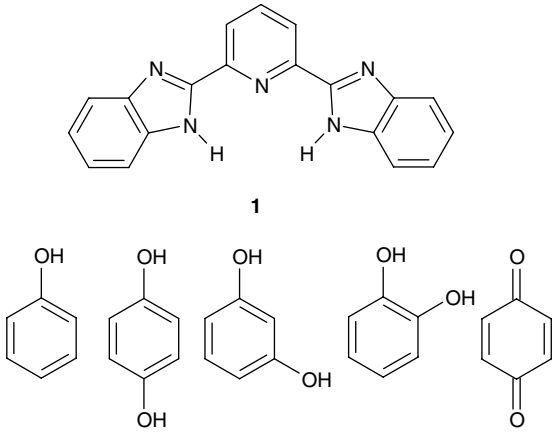

Figure 1. 2,6-Bis(2-benzimidazolyl)pyridine **1**, phenol, hydroquinone, resorcinol, catechol and *p*-benzoquinone.

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doi:10.1016/j.tetlet.2006.11.022

is proof of their potential application in chemical and biological sensing.

The binding ability of receptor **1** was evaluated by titrating 0.1 equiv of hydroquinone aliquots into a solution of **1** in acetonitrile at regular intervals and recording the changes in UV/vis and fluorescence spectra. It was observed that on increasing the concentration of hydroquinone, a progressive decrease of the intensity in the initial absorption band (Fig. 2) having λ_{max} at 327 nm associated with the π–π^* transition of **1** occurred. Concurrently, a new peak at 294 nm with higher intensity developed which is due to the formation of a stable complex between **1** and hydroquinone. This study showed the formation of an isosbestic point at 307 nm, indicating the presence of at least one species at equilibrium. The inset in Figure 2 shows the changes in titration profile of the band at 327 nm corresponding to the **1**:hydroquinone H-bonded complex. It was observed that the spectral features reached a limiting value only after the addition of 2.0 equiv of hydroquinone (though the changes observed in the spectra on addition of 1.1–2.0 equiv of hydroquinone were very minor compared to the initial ten additions). Thus the gradual decrease in the band intensity at 327 nm and formation of a new higher intensity blue-shifted band at 294 nm with a clear isosbestic point is proof of the formation of a hydrogen-bonded complex between **1** and hydroquinone. The association constant (K_a) for the 1:1 complex was calculated[6] to be 441 M^{-1}, which is the highest value for a supramolecular adduct of any host with hydroquinone. The 33 nm blue shift in the absorption spectra would not have occurred unless there were interactions between the hydroquinone and the imidazole ring of receptor **1**. The low concentrations at which these spectroscopic changes were observed clearly reveal that receptor **1** possesses excellent properties as a host material for recognizing phenolic guests in high affinity.

The changes observed in the fluorescence spectra of a solution of **1** in acetonitrile on addition of up to 2.0 equiv of hydroquinone are shown in Figure 3. A large quenching in intensity of the 375 nm band was

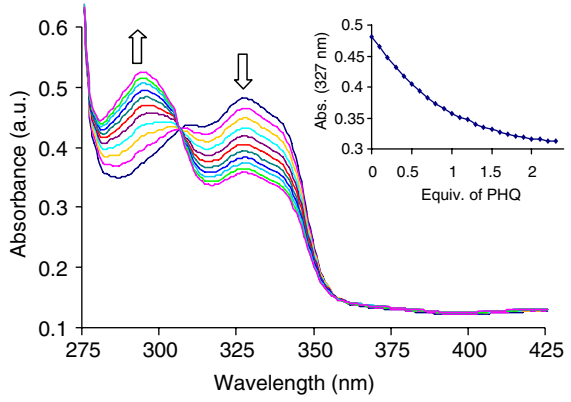

Figure 2. UV/vis spectra of **1** (8.46×10^{-6} M in dry CH_3CN) during titration with hydroquinone from 0 to 2 equiv (v/v). Inset—titration profile of the band at 327 nm corresponding to **1**:hydroquinone H-bonded complex.

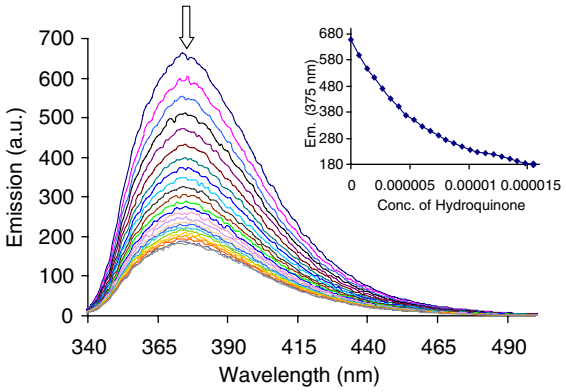

Figure 3. Emission spectra of **1** (7.05×10^{-7} M in dry CH_3CN) during the titration with hydroquinone from 0 to 2 equiv (v/v). Inset—plot of the emission intensity (375 nm) of **1** as a function of hydroquinone concentration.

observed up to the addition of 1.0 equiv of hydroquinone indicating that on formation of the donor–acceptor complex between hydroquinone and **1**, the excited state is modified leading to the quenching of fluorescence. The changes in the fluorescence spectra on adding further aliquots of hydroquinone to **1** were very minor, which is in agreement with the results of UV/vis titration. Therefore, formation of a donor–acceptor supramolecular complex modifies the optical properties of **1** in solution, which could be employed for sensing neutral guests. Similar UV/vis (Fig. 4) and fluorescence (Fig. 5) titration experiments of **1** were also performed with phenol, resorcinol, catechol and *p*-benzoquinone. The spectral variations observed for **1** on titrating these metabolites in a 1:1 ratio were unique for each guest, thereby demonstrating the selective nature of **1** as a sensor for recognizing different guest molecules.[7]

The formation of a supramolecular complex between **1** and hydroquinone in the solid state was confirmed by means of single crystal X-ray analysis.[8] The ORTEP view (Fig. 6) shows that **1** forms a stable supramolecular adduct with hydroquinone through the imine nitrogen located on its outer core via hydrogen-bonding. The O1–H···N2 (imine) distance was found to be 2.67 Å, which indicates a fairly good association between **1** and hydroquinone. The hydroquinone molecule lies

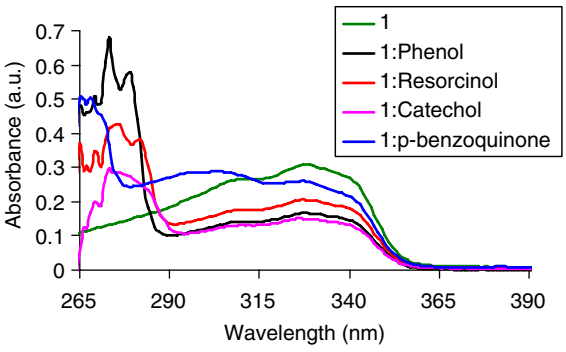

Figure 4. UV/vis spectra of **1** with phenol, resorcinol, catechol and *p*-benzoquinone in 1:1 ratio.

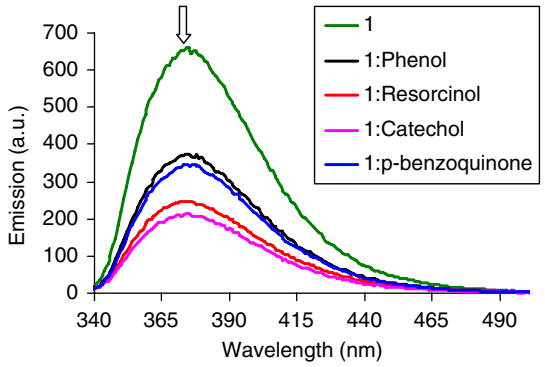

Figure 5. Emission spectra of **1** with phenol, resorcinol, catechol and *p*-benzoquinone in 1:1 ratio.

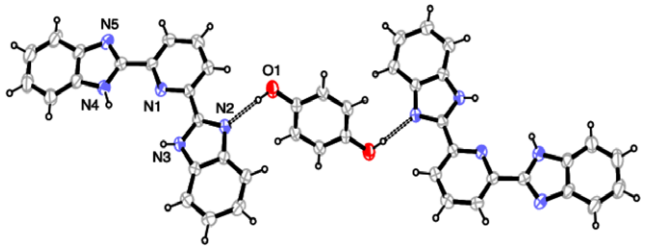

Figure 6. ORTEP diagram of the hydrogen-bonded **1**:hydroquinone complex in the ratio 1:1. Solvent molecules are omitted for clarity.

outside the cavity of **1** and seemed to deaggregate two receptor molecules by acting like a spacer between them. The inner cavity of **1** forms bonds with a molecule of water having bond distances of 3.00 Å for (N3–H···O2) and 2.94 Å for (N4–H···O2). To our knowledge, this is the first crystallographic report showing a novel supramolecular complex between the imine nitrogen of receptor **1** with any phenolic guest. We were also successful in obtaining crystals of this complex in several other solvents such as ethanol,[9] acetonitrile and mixtures of these solvents with water indicating that even in competitive environments, **1** prefers to form a supramolecular complex with hydroquinone. However, the bond distance between hydroquinone and **1** was shortest in the crystals that were grown in methanol. By acting as a spacer, hydroquinone seemed to also deaggregate molecules of receptor **1** in the solution state as observed by the increased intensity of the blue shifted peak until the formation of a stable supramolecular complex. These results are apparent indications that **1** is a smart receptor capable of recognizing different guest molecules depending on their size and nature by utilizing the NH groups on the inner cavity[5] and/or the nitrogen lone pairs on the outer core to form self-assembled supramolecular complexes with high affinity. It is important to note here that even in the presence of solvents like methanol and ethanol that would compete strongly with hydroquinone to form a supramolecular adduct, **1** is selective for hydroquinone and forms stable crystals that could be isolated and analyzed by X-ray diffraction.

In summary, we have shown that **1** is an efficient receptor that can recognize several toxic metabolites of benzene with high sensitivity and selectivity. The binding constant (441 M^{-1}) calculated from the spectroscopic data showed that **1** is the highest affinity receptor which binds hydroquinone solely through hydrogen bonds. The formation of a stable hydrogen-bonded supramolecular complex between **1** and hydroquinone was also confirmed by X-ray structure analysis. In terms of simplicity, selectivity, binding strength and ease of molecular recognition, **1** is an extremely efficient receptor that recognizes different metabolites of benzene with high efficiency, thereby extending its utility as a neutral receptor.

Acknowledgements

The authors are grateful to the Council of Scientific and Industrial Research (CSIR) and Department of Science and Technology (DST), India, for financial support.

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7. The UV/vis (Varian Cary 50 Bio UV–vis Spectrophotometer) titration graphs of **1** with phenol, catechol, resorcinol and *p*-benzoquinone showed progressive decreases in intensity, development of new blue shifted peaks and formation of one isosbestic point which were specific for each guest. Fluorescence (Varian Cary-Eclipse Fluorescence Spectrophotometer) titrations of **1** with the above four guests showed quenching of fluorescence in different proportions indicating that the excited state of **1** was modified with these different guests.
8. Crystal data of **1** and hydroquinone in 1:1 ratio: A solution of hydroquinone (0.030 g, 0.272 mmol) in methanol was added dropwise to a methanolic solution of **1** (0.085 g, 0.272 mmol) and allowed to stir for 5 min. The vial containing the clear solution of the above mixture was allowed to stand (rt) for 24 h leading to the formation of crystals that were suitable for X-ray diffraction analysis. The data collection of single crystals was performed on a Bruker Nonius Smart Apex II X-ray single crystal diffractometer (CCD). Cell constants and orientation matrices for data collection were obtained from least-square refinement with a set of 45 narrow-frame (0.5° in ω) scans. The structure was solved by direct methods and refined by full-matrix least-squares calculations with SHELX97 software. All hydrogen atoms attached to heteroatoms were located in the difference Fourier map and refined with isotropic displacement coefficients. $C_{23}H_{22}N_5O_3$, $M = 416.46$, monoclinic, $P2(1)/n$, $a = 11.185(3)$ Å, $b = 14.395(5)$ Å,

$c = 13.548(5)$ Å, $\beta = 103.03(2)°$, $V = 2125.1(12)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.302$ g cm^{-3}, $\mu = 0.089$ mm^{-1}, $R_1 = 0.0825$, $wR_2 = 0.3102$. CCDC—604676 contains the supplementary crystallographic data for this letter. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

9. Crystal data of **1** and hydroquinone in ethanol in 1:1 ratio: A solution of hydroquinone (0.030 g, 0.272 mmol) in ethanol was added dropwise to an ethanolic solution of **1** (0.085 g, 0.272 mmol) and allowed to stir for 5 min. The vial containing the clear solution of the above mixture was allowed to stand (rt) for 48 h leading to the formation of crystals that were suitable for X-ray diffraction analysis. The data collection of single crystals was performed on a Bruker Nonius Smart Apex II X-ray single crystal diffractometer (CCD). Cell constants and orientation matrices for data collection were obtained from least-square refinement with a set of 45 narrow-frame (0.5° in ω) scans. The structure was solved by direct methods and refined by full-matrix least-squares calculations with SHELX97 software. All hydrogen atoms attached to heteroatoms were located in the difference Fourier map and refined with isotropic displacement coefficients. $C_{24}H_{24}N_5O_3$, $M = 430.48$, monoclinic, $P2(1)/n$, $a = 11.3692(3)$ Å, $b = 14.3746(5)$ Å, $c = 13.6532(3)$ Å, $\beta = 102.425(2)°$, $V = 2179.05(11)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.312$ g cm^{-3}, $\mu = 0.089$ mm^{-1}, $R_1 = 0.0676$, $wR_2 = 0.2284$. CCDC—621837 contains the supplementary crystallographic data for this letter. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.