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Recognition of Adenosine Monophosphate and $H_2PO_4^-$ using Zinc Ensemble of New Hexaphenylbenzene Derivative: Potential Bioprobe and Multichannel Keypad System

Vandana Bhalla,*,† Varun Vij,† Manoj Kumar,*,† Parduman Raj Sharma,‡ and Tandeep Kaur‡

Department of Chemistry, UGC-Center for Advanced Studies-1, Guru Nanak Dev University, Amritsar, Punjab, India, and Department of Cancer Pharmacology, Indian Institute of Integrative Medicine, Canal Road, Jammu-180001, India

vanmanan@yahoo.co.in; mksharmaa@yahoo.co.in

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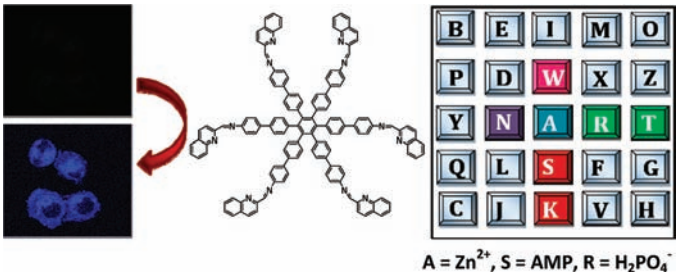
ABSTRACT



Zinc ensemble of hexaphenylbenzene derivative 3 exhibits sensitive response toward adenosine monophosphate (AMP) and $H_2PO_4^-$ ions. Further, the application of derivative 3 as a multichannel molecular keypad could be realized in the presence of inputs of Zn^{2+} ions, $H_2PO_4^-$ ions, and AMP.

Among various anions, phosphates are of particular interest as they are key substrates for many biochemical reactions and are main components of biomolecules.[1,2] Recently, metal-based receptors, particularly Zn(II) complexes, have been reported for fluorescent sensing of biphosphylated, triphosphylated, and polyphosphylated species. However, a few reports are available on metal-based receptors for fluorescent sensing of monophosphylated species.[3]

Our research work involves the development of artificial chemosensors based on different scaffolds such as calix[4]arenes, thiacalix[4]arene, and terphenyl for soft metal ions of clinical and environmental interest and construction of molecular switches and logic-based molecular devices.[4] In continuation of this work, in the present manuscript, we have chosen hexaphenylbenzene (HPB) as the core group for preparing different types of chemosensors selective for soft metal ions. The hexaphenylbenzene (HPB) motif has gained considerable attention due to its unique propeller-shaped structure and its usage in the construction of organic materials suitable for organic light emitting diodes (OLED). However, numerous possibilities

† Guru Nanak Dev University.
‡ Indian Institute of Integrative Medicine.

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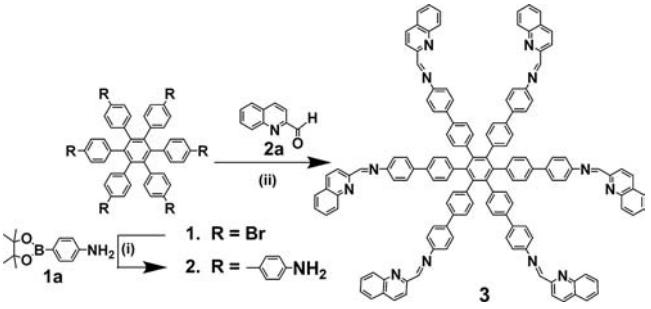
exist for the use of novel self-assembled architectures based on haxaphenylbenzene derivatives in host–guest chemistry in general. In light of this, we have designed and synthesized a symmetrically substituted star-shaped hexaphenylbenzene derivative **3** appended with quinoline moieties, which shows remarkable fluorescence enhancement in the presence of Zn^{2+} ions and upholds exceptional selectivity over other physiologically relevant divalent cations.[5] Derivative **3** responds to Zn^{2+} ions even in blood serum milieu, is cell permeable and exhibits dramatic fluorescence enhancement on binding with zinc ions present in the PC-3 cells without the need for additional extracellular zinc, unlike many reported sensors,[6] and thus is a promising candidate for biological use. Zinc is the second most abundant transition metal ion present in the human body and is required as a key component of numerous enzymes and transcription factors.

Scheme 1. Synthesis of Quinoline-based Hexaphenylbenzene Derivative, (i) THF/Toluene (1:1), NaOH(aq), $PdCl_2(PPh_3)_2$; (ii) DMF, rt



In this context, design of new zinc imaging tools suitable for studies in living cells is of great interest. Besides, the Zn^{2+} ensemble of compound **3** acts as potential fluorescent probe for $H_2PO_4^-$ and AMP. Moreover, the application of derivative **3** with the inputs of Zn^{2+} ions, $H_2PO_4^-$ ions, and AMP could be realized as a multichannel molecular keypad. To the best of our knowledge, this is the first report where a hexaphenylbenzene derivative **3** has been used for selective sensing of Zn^{2+} ions and **3-Zn^{2+}** ensemble shows selective sensing of phosphate and AMP. In addition, derivative **3** works as a multichannel molecular keypad system with the inputs of Zn^{2+} ions, $H_2PO_4^-$ ions, and AMP, which could be of interest in molecular computing.[7]

The 6-fold Suzuki–Miyuara cross coupling of hexakis-(4-bromophenyl)benzene **1**[8] with 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolane-2-yl)aniline **1a**[4b] furnished hexakis-(4-amino(1,1′-biphenyl)-4′-yl)benzene **2** in 70% yield (Scheme 1), which on condensation with 2-quinolinecarboxaldehyde **2a** in *N*,*N*-dimethylformamide at room temperature for 3 h furnished compound **3** in 71% yield (Scheme 1). The structures of compound **2** and **3** were confirmed from their spectroscopic and analytical data (Supporting Information, S4–S10).

To evaluate binding ability of compound **3** toward different metal ions, we carried out UV–vis and fluorescence experiments in ethanol/THF (3:1) by adding aliquots of different metal ions (Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, Co^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+}, Ag^+, Ba^{2+}, Mg^{2+}, K^+, Na^+, and Li^+) as their perchlorate salts. The absorption spectrum of compound **3** (10 μM) in ethanol/THF (3:1) solution exhibits two absorption bands at λ_{abs} 287 and 355 nm corresponding to hexaphenylbenzene and quinoline, respectively. Upon addition of Zn^{2+} ions (0–20.0 equiv), the band at 355 nm disappeared and the band at 287 is

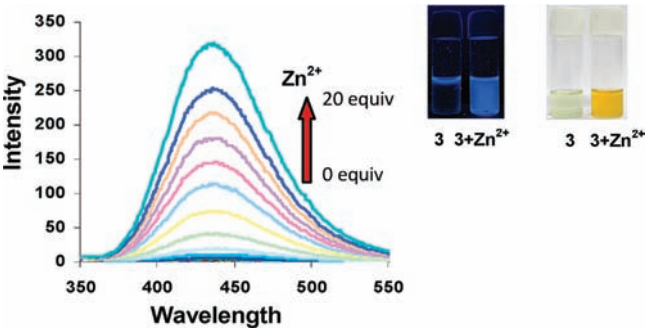


Figure 1. Fluorescence spectrum of **3** (**5** μM) in the presence of Zn^{2+} ions in ethanol/THF (3:1, v/v) at λ_{ex} = 287 nm. (Inset) Change in (left) fluorescence and (right) color on addition of 20 equiv of Zn^{2+} ions in 5 μM solution of compound **3** in ethanol/THF (3:1, v/v).

red-shifted to 305 nm (Supporting Information, Figure S1). Two isosbestic points are observed at 296 and 325 nm, indicating formation of **3-Zn^{2+}** complex. In the fluorescence spectrum, compound **3** (5 μM) does not exhibit any fluorescence emission in ethanol/THF (3:1) (Figure 1) when excited at λ_{ex} = 287 or 355 nm. This is due to photoinduced electron transfer (PET)[9] from imino nitrogen to a photoexcited hexaphenylbenzene moiety. Upon addition of increasing amounts of Zn^{2+} ions (0–20 equiv) to the solution of **3** in ethanol/THF (3:1), a fluorescence emission band appeared at 438 nm (Figure 1). This fluorescence emission band is attributed to the formation of the **3-Zn^{2+}** complex due to interaction between Zn^{2+} ions and imino nitrogens and the nitrogen atoms of the quinoline moieties as a result of which the PET from the imino nitrogens to the

(5) The receptor **3** is better in comparison to the Zn^{2+} sensors reported in the literature (Supporting Information, S21).

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hexaphenylbenzene moiety is suppressed resulting into fluorescence enhancement. Under the same conditions as used above for zinc ions, we also tested the fluorescence response of compound **3** to other metal ions such as Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, Co^{2+}, Pb^{2+}, Ni^{2+}, Cd^{2+}, Ag^{+}, Ba^{2+}, Mg^{2+}, K^{+}, Na^{+}, and Li^{+} but no change in emission was observed in the presence of these metal ions (Supporting Information, Figure S2 and S3). Further, to check the practical applicability of compound **3** as Zn^{2+} sensor, we carried out competitive experiments in the presence of 20 equiv of Zn^{2+} mixed with Pb^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, Ni^{2+}, Cd^{2+}, Co^{2+}, Mg^{2+}, Ba^{2+}, Ag^{+}, K^{+}, Na^{+}, and Li^{+} of at 2000 equiv (Supporting Information, Figure S4), and no significant variation in the fluorescence intensity change was found by comparison with or without the other metal ions. The detection limit of compound **3** as a fluorescent sensor for the analysis of Zn^{2+} ions was found to be 4.5×10^{-6} M.

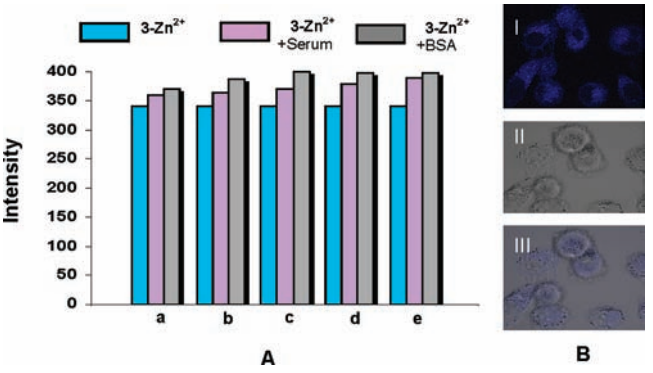

Figure 2. (A) Histograms showing the fluorescence response of **3** (5 μM) with Zn^{2+} (100 μM) in the presence of BSA or blood serum in ethanol/THF, 3:1 at pH = 7.0. The **3-Zn^{2+}** was titrated with varying amounts (μL) of either BSA or serum. (a)10 μL; (b) 20 μL; (c) 30 μL; (d) 40 μL; (e) 50 μL. (B) Fluorescence and brightfield images of PC3 cells lines (on right). (I) Blue fluorescence images of cells treated with probe **3** (1.0 μM) only for 20 min at 37 °C. (II) Brightfield image of (I). (III) Overlay of (I) and (II).

Fitting the changes in the fluorescence spectra of compound **3** with Zn^{2+} ions using the nonlinear regression analysis program SPECFIT gave a good fit and demonstrated that 3:1 stiochiometry (metal:receptor) was the most stable species in the solution with binding constant (log β) = 11.0215 (Supporting Information, S15 and S18). The 3:1 (metal:receptor) stiochometry was further confirmed by MALDI-TOF spectrum of **3-Zn^{2+}** complex where a moleucular ion peak at 2136.6941 was observed, which corresponds to [(M + **3-Zn^{2+}**) + H^{+} + Na^{+}] complex (Supporting Information, S12). To elucidate the binding mode of receptor **3** with zinc perchlorate, the IR spectrum of **3-Zn^{2+}** complex was recorded (Supporting Information, S11). The important change was in absorption band corresponding to imino group, which shifts from 1625.51 to 1595.02 cm^{-1}.

Biological applicability of **3** to sense Zn^{2+} ions was checked by carrying out fluorescence titrations of *in situ* prepared Zn^{2+} complex and titrating it by varying the concentrations of blood serum[10] as well as bovine serum albumin (BSA), capable of capturing Zn^{2+} ions. As is evident from the histogram (Figure 2A), no significant change in the fluorescence intensity of the emission band of ensemble **3-Zn^{2+}** at 438 nm was observed either in presence of blood serum milieu or in the presence of BSA (Supporting Information, Figure S8 and Figure S9).

We also evaluated the potential biological application of the receptor **3** for detection of Zn^{2+} ions present in prostate

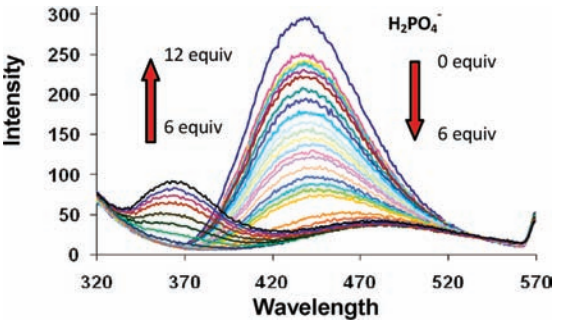

Figure 3. Fluorescence spectrum of **3-Zn^{2+}** (5 μM in ethanol/THF, 3:1) on addition of phosphate ions.

cancer (PC-3) cells since the malignant prostate cells possess the ability to accumulate high zinc levels. The prostate cancer (PC3) cell lines were incubated with receptor **3** (5.0 μM in ethanol/THF, 3:1), in RPMI-1640 medium for 20 min at 37 °C and washed with phosphate buffered saline (PBS) buffer (pH 7.4) to remove excess of receptor **3**. Microscopic images showed a strong blue fluorescence which indicates that the compound **3** complexes with labile Zn^{2+} ions present in cells (Figure 2B).

Further, the binding ability of the **3-Zn^{2+}** ensemble[11] in ethanol/THF (3:1) was studied toward different anions and biomolecules (F^{-}, Cl^{-}, Br^{-}, I^{-}, NO_3^{-}, OH^{-}, ClO_4^{-}, OAc^{-}, CN^{-}, $H_2PO_4^{-}$, AMP, ADP, and ATP). The binding studies exhibited the fast, sensitive, and distinct response toward monophosphate ions and AMP in comparison to the other anions, ADP and ATP (Supporting Information, Figure S11). In the fluorescence spectrum, upon addition of $H_2PO_4^{-}$ ions, the emission band at 438 nm was quenched and a new blue-shifted band at 366 nm was observed (Figure 3). This result indicates the weakening of the existing 3-Zn^{2+} bond due to interaction of phosphate ions with Zn^{2+} ions. On the other hand, addition of AMP leads to enhancement of emission intensity along with slight blue shifting of signal from 438 to 431 nm

(10) The blood serum was isolated by centrifugation of the fresh blood sample of a healthy volunteer after fasting at 4000 rpm for 20 min at 4 °C. The stock solution of blood serum was prepared by dissolving 100 μL of serum in 1 mL solution of HEPES buffer at pH = 7.0

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(Supporting Information, Figure S10). This result suggests that AMP is bound to Zn^{2+} ions without breaking the existing 3-Zn^{2+} bond. The fluorescence enhancement on addition of AMP to 3-Zn^{2+} can be attributed to the additional hydrogen bonding between hydrogen of phosphate group of AMP and nitrogen of the quinoline moiety. Such type of emission behavior in the presence of AMP has been previously reported.[12] In the case of other anions such as F^-, Cl^-, Br^-, I^-, NO_3^-, CH_3COO^-, CN^-, ClO_4^-, and OH^- and ADP and ATP, no significant change in emission intensity is observed. The stronger binding affinity of **3-Zn^{2+}** ensemble toward phosphate ions and AMP over other anions including acetate and halide ions may be attributed to strong coordination of Zn^{2+} to the monophosphate unit.[13] It was found that the **3-Zn^{2+}** ensemble has detection limit of 10^{-8} and 9×10^{-7} for $H_2PO_4^-$ ions and AMP, respectively.

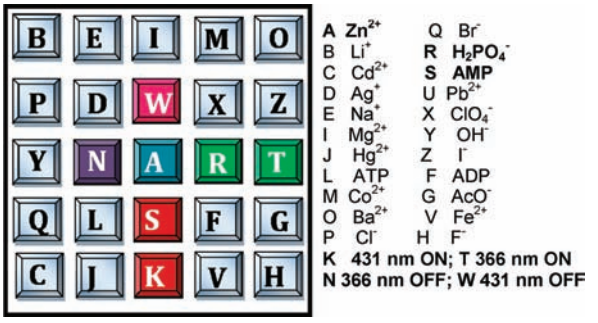

Figure 4. Multichannel keypad system.

Recently, there has been a lot of interest in development of molecular logic systems for construction of molecular devices[14] that have memory elements. Among these devices, the molecular keypad systems have drawn more attention as these systems are based on the sequence of the correct combination of chemical inputs that signifies a more secure and classified password. However, there are many reports on customary **ON**−**OFF** keypad locks that oscillate between **ON** state and **OFF** state, but there is no report of such a molecular device that generates two different ON states depending upon sequence of chemical inputs. Such circuits have shown a great interest in multiuser gadgets where the different personal user accounts can be operated by applying different passwords without the interference of other users. For instance, multichannel decoder, cellular phones, multichannel signaling, multiuser computers, ATM, and security alarms are generally based on these multichannel keypad locks.

Keeping this in view, we have constructed a multichannel keypad system based on chemical inputs that switches between two different fluorescent outputs. To construct this multichannel molecular keypad, we carried out fluorescence titrations of receptor **3** with Zn^{2+}, $H_2PO_4^-$ and AMP in different sequences in ethanol/THF, 3:1. The emission band was observed at 366 nm when Zn^{2+} ions were added to compound **3** followed by the addition of $H_2PO_4^-$.

In another sequence, the fluorescence emission band at 431 nm was observed when AMP was added after the addition of Zn^{2+} ions in chemosensor **3** (Supporting Information, Figure S11). Thus, depending upon the three different inputs (Zn^{2+}, $H_2PO_4^-$, and AMP), we have constructed a multichannel "**ON**−**ON**" keypad system in which the chemosensor **3** can switch between two different outputs. The three inputs Zn^{2+}, $H_2PO_4^-$, and AMP are designated as **A**, **R**, and **S**, respectively, and two outputs at 431 and 366 nm are designated by **K** and **T**, respectively. In the first sequence, **A** is added to **3** followed by the addition of **R** to generate the output **T** whereas in the reverse order, the output **N** is generated which represents the off state of emission at 366 nm. In the second sequence, **A** is added to **3**, followed by the addition of **S** to generate the output **K** whereas when the input sequence is reversed, it leads to the output **W**, which represents the off state of fluorescence emission at 431 nm. Thus, we can switch the chemosensor **3** in two different fluorescence emission wavelengths by changing the sequence of the three different inputs to generate a multichannel keypad (Figure 4) based on chemical inputs where every sequence characterizes a different password.

In conclusion, we designed and synthesized a novel hexaphenylbenzene-based derivative that showed fluorescence enhancement in the presence of Zn^{2+} ions. Derivative **3** can penetrate the cellular membrane of living PC-3 cells and exhibits high emission after binding the intracellular zinc. In addition, it works as a multichannel keypad system by using Zn^{2+}, phosphate, and AMP as chemical inputs.

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Supporting Information Available. Experimental procedure of **2** and **3** characterization data of **2** and **3**, ^1H, ^{13}C, mass spectra, IR spectrum. UV−vis and fluorescence spectra of different inputs. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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