Rationally Designed Circularly Arranged Sextuple Molecule with Dimethoxyphenolic Tentacles for Ample Hunting of Cyanide

Palwinder Singh,* Harpreet Kaur, and Harpreet Singh

UGC Sponsored Centre for Advanced Studies, Department of Chemistry, Guru Nanak Dev University, Amritsar 143005, India

ABSTRACT: Herein, we report the design, synthesis, and cyanide-scavenging behavior of circularly arranged sextuple molecule 4. The six syringaldehyde units carrying equal number of dimethoxyphenolic moieties projecting at the periphery make the molecule highly efficient for cleaning up cyanide from the aqueous solution. The stoichiometric data 1:6 showed that six units of cyanide interact with one unit of compound 4. The association constant of the compound for cyanide was $2.5 \times 10^4 \text{M}^{-1}$, and its detection limit for cyanide was 10 nM. The compound was also found to remove cyanide bound to cytochrome $c$ oxidase.

1. INTRODUCTION
The affinity of $\text{CN}^-$ for $\text{Fe}^{2+}$ of cytochrome $c$ (Cyt $c$) makes it one of the highly toxic anions $^{1-8}$ because Cyt $c$–CeOX (cytochrome $c$ oxidase) complex is the last enzyme in the respiratory electron transport chain and the blockage of this pathway limits the oxygen supply that ultimately proves lethal. $^{7,8}$ In addition to the available reports on cyanide sensing $^{9-18}$ and sequestering compounds such as hydroxocobalamin, $^{19-24}$ dicyanocobalt(III) porphyrins, $^{25-28}$ vitamin B12 analogues, $^{29,30}$ and hexahydrated dichlorides of cobalt(II), $^{31-34}$ it was found recently that compounds $^{1-3}$ (Chart 1) were capable of removing cyanide from the aqueous medium and human blood serum $^{35,36}$ through the more prevalent keto-form of their phenolic moiety and disposing it off in the form of COOH. Hence, it was logically hypothesized that the presence of more such phenolic groups in the molecule may increase its cyanide-sensing capacity and that the capturing of cyanide per molecule of the receptor becomes more effective. Therefore, it was planned to introduce six 3-(4-hydroxy-3,5-dimethoxybenzylidene)indolin-2-one units on the benzene core, and consequently, compound 4 (Chart 2) was designed.

2. RESULTS AND DISCUSSION
2.1. Chemistry. Compound 5 was obtained by the methylation of syringaldehyde, and it was made to react with oxindole by heating at 145 °C to obtain compound 6 (Scheme 1). Compound 6 was procured as inseparable E- and Z-isomers in the ratio 4:1. For the synthesis of compound 8, first mesitylene was treated with formaldehyde and $\text{HBr}$–$\text{HAc}$ to get compound 7 and then compound 7 was refluxed with $\text{Br}_2$ in 1,2-dibromoethane wherein compound 8 was obtained. Further reaction of compounds 6 and 8 in acetonitrile (ACN) in the presence of $\text{K}_2\text{CO}_3$ resulted into the replacement of all the six $\text{Br}$ of compound 8 with compound 6, and consequently, compound 9 was procured. Selective demethylation of compound 9 was achieved by using $\text{AlCl}_3$ in dichloromethane (DCM) providing desired compound 4 (Scheme 1). The energy-minimized geometry of compound 4 indicates circular shape of the molecule with the six 3-(4-hydroxy-3,5-dimethoxybenzylidene)-indolin-2-one units projecting alternatively upward and downward of the benzene plane. All the six phenolic units are directed outward from the central hydrophobic core (Chart 2).

2.2. Cyanide-Scavenging Studies. Addition of compound 4 [1 $\mu$M, dimethyl sulfoxide (DMSO)–$\text{H}_2\text{O}$ (1:9) v/v] to the aqueous solutions of different anions changed the color of the solution containing cyanide (Figure 1). The UV–vis spectrum of compound 4 [1 $\mu$M, DMSO–$\text{H}_2\text{O}$ (1:9), pH 7.0] showed an absorption maximum at 373 nm. Incremental addition (1 $\mu$L at each step) of cyanide (1 $\mu$M in H$_2$O) to the solution of
compound 4 resulted in the decrease in intensity of the absorption band at 373 nm with concomitant emergence of a new band at 535 nm (Figure 2). The spectral changes were observed until the addition of 16 equiv of cyanide (very small changes after the addition of 10 equiv CN\(^-\)). The band at 373 nm was diminished, and the other one at 535 nm was highly intense. At this stage, the compound–cyanide solution was dark orange in color. Apparently, the compound interacted with CN\(^-\) and consequently resulted in the change of the UV–vis spectrum as well as the color of the solution of compound 4.

The selective and competitive binding of the compound with cyanide was ascertained with the help of appropriate experiments, and the results are depicted in Figure 3. The stoichiometry of compound–cyanide, found through Job’s plot of continuous variation, was 1:6 (Figure 4), and the association constant \((K_a)\) was 2.5 \(\times\) 10\(^4\) M\(^{-1}\) (Supporting Information). The detection limit of compound 4 for cyanide was 10 nM (Supporting Information). It was observed that in
comparison to compounds 1−3, compound 4 was more effective in the detection of cyanide even at lower concentration than that of compounds 1−3 (Table S1).

Assisted removal of compound 4 of cyanide from an aqueous solution was demonstrated with a physical experiment. Addition of 10 μL of 1 μM cyanide to 1 mL of 10 nM solution of compound 4 in acetone−water (1:9) turned the color of the solution to orange, indicating that 10 nM CN$^-$ is detected by the compound (Figure 5A). The aqueous part of this solution left after repeated (4−5 times) extraction with ethyl acetate did not respond to the addition of compound 4 (Figure 5A), indicating that all the cyanide was bound to the compound and extracted with ethyl acetate. The observations were confirmed by performing a control experiment (Figure 5B) in which the aqueous solution of cyanide was extracted 4−5 times with ethyl acetate, but still the aqueous part responded to compound 4, indicating that cyanide was not removed with ethyl acetate. Similar experiments with compounds 1 and 3 required, respectively, 10 μM and 200 nM compound for the removal of 10 μM and 10 nM cyanide from the respective aqueous solution (Table S1).

Therefore, in consistent with the design of the molecule, compound 4 was capable of selective and competitive detection of CN$^-$. The mass spectrum of the solution of compound 4 with cyanide (solution obtained after the last addition of cyanide in the UV−vis experiment) showed peaks at m/z 2081 and 2230 supporting the addition of cyanide to the compound and subsequent hydrolysis of the CN group to COOH (Figure 6, Supporting Information).

The compatibility of compound 4 with the CcOX pathway as well as the removal of Cyt c−CcOX bound cyanide was screened through enzyme immunoassay. Complementing the enzymatic activity of CcOX, Cyt c−Fe$^{3+}$ was oxidized to Fe$^{2+}$ in the presence of CcOX, and hence, the absorbance intensity at 550 nm gets decreased (Figure 7A). Incremental addition of cyanide to CcOX was made, and the resulting solution was added to the solution of Cyt c. A stepwise increase in the absorbance intensity at 550 nm (Figure 7B) was observed, which indicated the blockage of CcOX with CN$^-$. Further addition of compound 4
Figure 6. Mass spectrum of the solution of compound 4 and cyanide showing the reaction of cyanide with compound (m/z 2081 [M + H]+) and subsequent hydrolysis of CN to COOH (m/z 2230 [M + Na]+).

Figure 7. Working of the compound with cytochrome c oxidase: (A) UV–visible absorbance of 5.5 μM cyt c in assay buffer was monitored at a wavelength of 550 nm on incremental (10 μL) addition of CeOx (diluted in enzyme buffer). Decrease in absorbance was due to the oxidation of Fe2+ to Fe3+ of Cyt c by CeOx. (B) Change in absorbance at 550 nm of Cyt c on addition of CeOx solution along with 0–3 μM CN−, showing decrease in the enzymatic activity of CeOx in the presence of cyanide. (C) Absorbance intensity of solution obtained at step “B” was decreased on addition of 2 μM compound 4, indicating removal of cyanide from CeOx by the compound. (D) (a) No absorbance at 550 nm in the presence of compound 4 (10 μM) and 160 μM CN− and (b) addition of more CN− increased the absorbance intensity at 550 nm.

3. CONCLUSIONS

In conclusion, the rational modification of compounds 1–3 to compound 4 has significantly improved its cyanide-scavenging capacity. Supporting the design of the molecule, in comparison to compounds 2 and 3, the present compound was capable of removing cyanide from the aqueous solution and CeOx at much lower concentration than that of compounds 2 and 3. Detailed studies with the compound using animal models will be reported in the near future.

4. EXPERIMENTAL SECTION

1H and 13C NMR spectra were recorded on JEOL 400 MHz and Bruker 500 MHz NMR spectrometers, respectively, using CDCl3 as the solvent. Chemical shifts are given in parts per million with tetramethylsilane as the internal reference. Both 1H and 13C NMR spectra of compounds 9 and 4 were recorded by keeping the relaxation time 5–7 s. It seems that the CH2 and central benzene signals are buried inside the molecule and hence difficult to pick up though the integration in 1H NMR spectrum and number of carbons are correct. Mass spectra were recorded on a Bruker microOTOF-Q II mass spectrometer. Reactions were monitored by thin-layer chromatography (TLC) on glass plates coated with silica gel GF-254. Column chromatography was performed with 60–120 mesh silica. Infrared (IR) and UV–vis spectral data were recorded on FTIR Agilent CARY 630 and BIOTEK Synergy H1 Hybrid Reader instruments, respectively.

4.1. Synthesis of 3,4,5-Trimethoxybenzaldehyde (5).37

To the stirred solution of syringaldehyde (4 g, 21.9 mmol) in dimethylformamide (50 mL), K2CO3 (4.54 g, 32.92 mmol), and KI (catalytic amount) were added. The reaction was allowed to stir overnight at room temperature. After the completion of reaction, it was quenched by adding water and extracted with ethyl acetate. The organic layer was separated, dried over Na2SO4, and concentrated under vacuum to procure pure product 5, creamish white solid (90%), mp 75–76 °C, δH (400 MHz, CDCl3): 7.94 (s, 9H, OCH3), 7.13 (s, 2H, ArH), 7.07 (d, 1H, ArH), 6.97 (d, 1H, ArH), 6.88–6.90 (d, J = 7.86 Hz, 2H, ArH), 6.81–6.83 (d, J = 7.95 Hz, 2H, ArH), 6.73–6.75 (t, J = 7.70 Hz, 1H, ArH), 6.58–6.60 (d, J = 7.70 Hz, 1H, ArH), 6.78–6.80 (m, 1H, ArH), and 3.90 (s, 9H, OCH3). HRMS (ESI) m/z: for C16H12O6 [M + H]+ calcld, 293.0729; found, 293.0725.

4.2. Synthesis of Compound 6. 3,4,5-Trimethoxybenzaldehyde (2 g, 10.20 mmol) and oxindole (1.35 g, 13.58 mmol) were heated at 145 °C for 1 h. The reaction mass was purified by column chromatography to obtain compound 6, yellow solid (80%), mp 146–150 °C, δH (500 MHz, CDCl3): 7.90 (s, 9H, OCH3), 7.95 (d, 1H, ArH), 7.50–7.55 (m, 1H, ArH), 7.26–7.31 (m, 1H, ArH), 7.05–7.08 (t, J = 7.80 Hz, 1H, ArH), 6.79–6.83 (d, J = 7.95 Hz, 1H, ArH), 6.73–6.75 (d, J = 7.95 Hz, 1H, ArH), 6.76–6.78 (d, J = 7.95 Hz, 1H, ArH), 6.49–6.51 (d, J = 7.80 Hz, 1H, ArH), 6.35–6.37 (d, J = 7.80 Hz, 1H, ArH), and 3.90 (s, 9H, OCH3). HRMS (ESI) m/z: for C21H19O8 [M + H]+ calcld, 369.1256; found, 370.1259.

4.3. Synthesis of 1,3,5-Tris(bromomethyl)-2,4,6-trimethybenzene (7).38

To the mixture of mesitylene (2.4 g, 20
mmol), paraformaldehyde (2 g, 70 mmol), and glacial acetic acid (10 mL), 14 mL of 31% HBr–acetic acid solution was added rapidly. The reaction mixture was kept for 12 h at 95–110 °C and then poured into 100 mL of water. The solid was filtered, washed with water, and dried in vacuum to obtain a white solid 7 (91%), mp 187 °C, δH (400 MHz; CDCl3): 4.57 (s, 6H, CH3Br), 2.46 (s, 9H, CH3); δC (normal/DEPT-135; CDCl3): 15.5 (CH3), 30.0 (CH3), 133.3 (C), 138.0 (C).

4.4. Synthesis of Compound 8. Br2 (1.24 mL) was added dropwise over a period of 2 h to a stirred and boiling solution of compound 7 (2 g) in 1,2-dibromoethane (14 mL). Stirring and boiling were continued for another 22 h, the mixture was cooled down, and the deposited crystals were filtered and washed with 1,2-dibromoethane. Yield: 3 g (95%), yellow colored shining crystals, mp 307–308 °C. δH (400 MHz; CDCl3): 4.68 (s, 12H).

4.5. Synthesis of Compound 9. Solution of compound 6 (587 mg, 1.88 mmol) and K2CO3 (433.96 mg, 3.149 mmol) in dry DCM was stirred for 12 h. The reaction mixture was kept for 12 h at 95 °C, then poured into 100 mL of water. The solid was filtered, washed with diethyl ether to get compound 9 in 67% yield, mp 308 °C.

4.6. Synthesis of Compound 4. The reaction mixture obtained by the slow addition of anhydrous AlCl3 (198.40 mg, 1.34 mmol) in dry DCM was stirred for 12 h. The reaction was quenched with water and extracted with DCM. The organic layer was washed with brine, dried over Na2SO4, and evaporated only in the case of cyanide solution. As a control experiment, cyanide (10 μL, 1 μM) was taken in acetonewater (1:9, 1 mL). This orange colored solution was extracted with ethyl acetate (4 × 25 mL). Addition of compound 4 (10 μL, 1 μM) to the aqueous part (obtained after extraction with ethyl acetate) did not change the color of the solution, indicating absence of cyanide in the aqueous part. As a control experiment, cyanide (10 μL, 10 μM) was taken in acetonewater (1:9, 1 mL), and the solution was extracted was ethyl acetate (4 × 25 mL). Treatment of the aqueous part (left after extraction with ethyl acetate) with compound 4 (10 μL, 10 μM) turned the color of the solution orange, indicating the presence of cyanide in the aqueous part.

5. Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b01155.

■ ASSOCIATED CONTENT

5 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b01155.

H and 13C NMR spectra, mass spectra, IR spectra, stoichiometry, and binding constant (PDF)

■ AUTHOR INFORMATION

Corresponding Author

E-mail: palwinder_singh_2000@yahoo.com. Phone: 91-183-2258802 ext. 3278. Fax: 91-183-2258819 (P.S.).

ORCID

Palwinder Singh: 0000-0003-2332-5257
Notes
The authors declare no competing financial interest.

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