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Synthesis, characterization, and evaluation of rhodamine based sensors for nerve gas mimics

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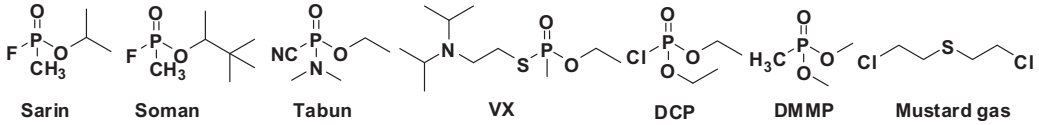
ABSTRACT

Six new rhodamine-B based compounds were synthesized (**1**–**6**) and used as fluorescent turn-ON sensors for diethyl chlorophosphate (DCP) in aqueous media at pH 7.0. Compound **1** and **3** gave high fluorescent enhancement with DCP compared to the other compounds. Very high selectivity and sensitivity were observed as these compounds did not show significant fluorescent enhancement with dimethyl methylphosphonate (DMMP), HCl, and metal ions, such as Na^{+}, K^{+}, Ca^{2+}, Cr^{3+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}. Depending on the way the sensor is presented, results are instantaneous or observed over some minutes.

1. Introduction

Nerve gas agents are highly toxic organophosphonates, which can inhibit acetylcholinesterase, a highly critical enzyme in nerve function.[1] Nerve gas agents irreversibly inhibit the serine esterase activity of acetylcholinesterase via the phosphorylation of the serine residue at the active site. This triggers rapid and fatal consequences, such as paralysis of the central nervous system.[2] The nerve agents of most concern include Sarin (GB), Tabun (GA), and Soman (GD), which can be fatal in minutes when inhaled[3] or absorbed through the skin.[4] Different types of detection methods have been developed so far based on fluorescence,[5] enzymes,[6] interferometry,[7] surface acoustic wave sensing,[8] and electrochemistry.[9] However, most methods suffer certain drawbacks due to lack of selectivity, operational complexity or limited portability. We report herein a series of rhodamine B based sensors with very high selectivity toward nerve gas stimulants, with the potential of easy operation and portability.

Rhodamine-based compounds have properties, such as very high molar extinction coefficients and high fluorescent quantum yields, ideal for use as chemosensors.[10] It is well known that the equilibrium between the non-fluorescent colorless ring-closed form and the highly fluorescent pink-colored ring-open form, provides a better model for the development of turn-on sensors. The equilibrium between the two forms is highly sensitive to the pH of the medium, the ring-open form being predominant in acidic conditions. Cations can trigger the change in structure between the spirocyclic and open-cycle form[11] and therefore rhodamine-based compounds have been well established as sensors for metal ions, such as Cu^{2+},[12] Fe^{3+},[13] Pb^{2+},[14] Hg^{2+},[15] and Cr^{3+}.[16] Recently, Kang et al. have reported some rhodamine hydrazides as sensors for nerve agent mimics, such as diethyl chlorophosphate (DCP) in the solid phase. They show that rhodamine hydrazides are much better sensors for DCP than rhodamine amides due to a proposed involvement of hydrazide nitrogen in binding with DCP.[17] Here we report a series of rhodamine-based compounds as sensors for nerve gas agents for the first time in solution.



In order to avoid the interference from inorganic acids, such as HCl or HBr, we decided to use a buffer system to study the binding of nerve gas mimics. Nerve gas agents, such as Sarin, VX, Soman, and Mustard Gas hydrolyze in aqueous systems with half-lives varying

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from minutes to days. For example, the half-life of Sarin is 2340 min at pH 7.5 while Soman has an even longer half-life.[18] Therefore, a water based-system can be used to detect nerve gas agents even though DCP, the chlorine containing analog we used, has a shorter half-life in water[19] compared to fluorine-containing nerve agents. In addition, Sarin and Soman generate HF upon hydrolysis.[20] Therefore all the compounds were tested with HCl to show that sensors should interact with the nerve gas and not the nerve gas degradation products. The compounds were also tested with DMMP to study the importance of leaving group (Cl) of DCP in binding with sensors.

2. Results and discussion

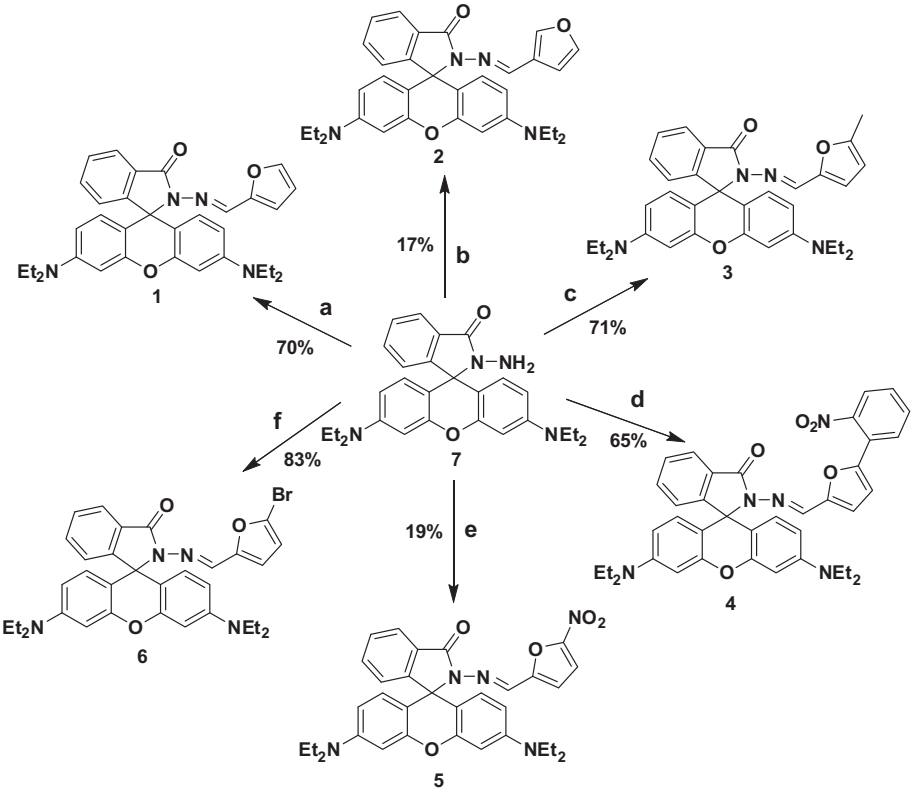
2.1. Synthesis and characterization

We have synthesized six new rhodamine B derivatives with an electron rich furan moiety. We use different substituent groups in the furan ring to study the effect of electronics and sterics on the sensitivity of the sensor toward DCP. We expect DCP to bind with sensors via the carbonyl O, and imine N similar to what Kang et al.[17] suggested. The preparation of chemosensors **1**–**6** is shown in Scheme 1. Chemosensors **1**–**6** were synthesized using Schiff-base condensation between the amine-containing compound **7**[12a] and the corresponding aldehyde in ethanol. The yields were very high except for compound **2** and **5**, which were obtained in only 17% and 19% yield, respectively. Single crystals of **1**, **2**, **3**, **4**, and **6** were grown in acetonitrile at room temperature. The structures were well characterized using ^{1}H NMR, ^{13}C NMR, mass spectrometry, elemental analysis, and X-ray crystallography[22] (Supplementary data).

formed colorless solutions that were stable for over a week. Generally rhodamine-based compounds are protonated at acidic conditions and emit strong fluorescence. The colorless solutions were very weakly fluorescent and showed no absorption above 450 nm, properties, which are characteristic of the predominant ring-closed spirolactam. The predominant spirolactam form was further confirmed by observation of the characteristic carbon resonance near 66 ppm for each of the compounds.

Compound **1** registered the highest emission intensity with DCP among all the compounds tested during the study (Fig. 1). The fluorescence spectrum of **1** peaked at 583 nm after the addition of 34 equiv of DCP and corresponding to delocalization in the xanthene moiety of rhodamine. There was a significant fluorescent intensity enhancement (>165-fold) as the solution turned pink, a color change clearly visible to the naked eye. The linearity of the $F_0/(F-F_0)$ versus 1/[DCP] plot (Fig. S17, Supplementary data) confirms the formation of 1:1 complex between **1** and DCP. The binding constant was calculated using the Benesi–Hildebrand method[21] and the values for all the compounds are summarized in Table 1. Initially, compound **1** showed no absorption band above 400 nm, but a new absorption band around 550 nm with a shoulder at 520 nm appeared upon the addition of DCP (Fig. 2). Interestingly, the addition of 34 equiv of Cu^{2+} also yielded a color change but no fluorescence enhancement was observed. Thus the compound senses copper, but copper is not an interferant because of the different behavior of **1** with Cu^{2+} and DCP.

Rhodamine derivatives are well known to give a color change in the presence of acids and metals.[13–17] We tested our sensor systems with a range of potential interferents including HCl and metals, such



Scheme 1. Synthesis of **1**–**6**. Compound **7** was refluxed with the corresponding aldehyde in ethanol: a. 2-furaldehyde, b. 3-furaldehyde, c. 5-methyl-2-furaldehyde, d. 5-(2-nitrophenyl)-2-furaldehyde, e. 5-nitro-2-furaldehyde, f. 5-bromo-2-furaldehyde.

2.2. Sensing studies

All the spectroscopic studies were performed in 50% CH_3CN, 50% 0.01 M Tris–HCl buffer (pH=7.0) medium in which compounds as Na^{+}, K^{+}, Ca^{2+}, Cr^{3+}, Fe^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, and Pb^{2+}. Furthermore we tested our compounds with dimethyl methylphosphonate (DMMP), another organophosphate interferent. Only Cr^{3+} showed a slight emission enhancement among the metals and

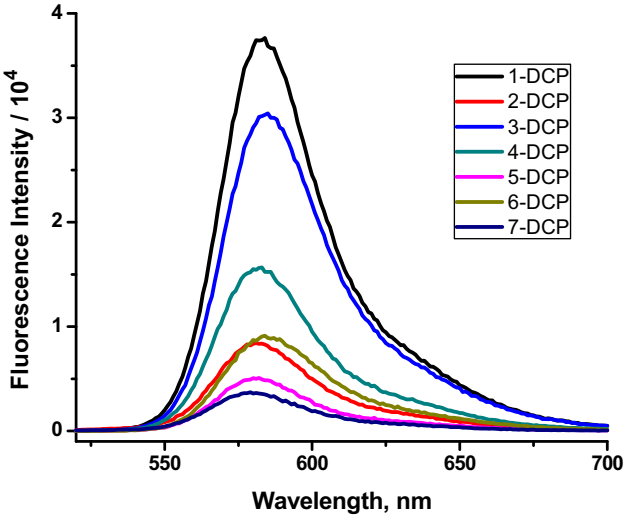


Fig. 1. Fluorescence spectra of compounds **1**–**7** (10 μM) with DCP (340 μM) in 50% CH_3CN, 50% 0.01 M Tris–HCl buffer (pH=7.0) (λ_{ex}=510 nm).

Table 1
Association constants of compounds **1**–**6** with DCP

Compound	Association constant, K/M^{-1}
1	3.0×10^3
2	1.69×10^3
3	3.4×10^3
4	2.1×10^3
5	17
6	1.75×10^3

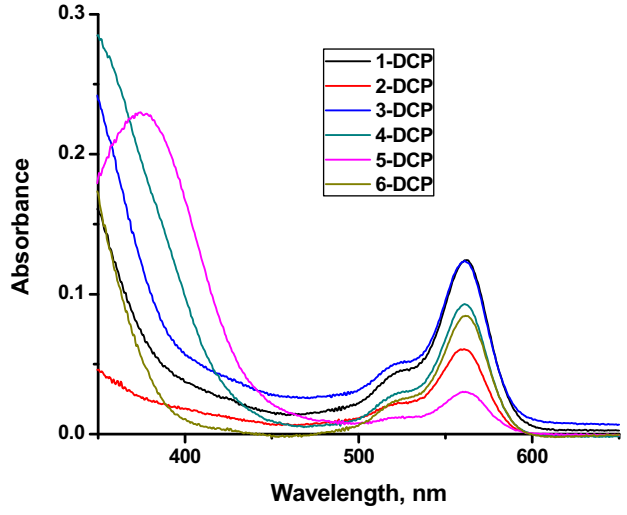


Fig. 2. UV–vis spectra of compounds **1**–**6** (10 μM) with DCP (340 μM) in 50% CH_3CN, 50% 0.01 M Tris–HCl buffer (pH=7.0).

other analytes used in the experiment (Fig. 3). This indicates that compound **1** is highly selective toward DCP. As **1** did not give any response to DMMP, it is clear that the leaving group (chlorine) of DCP plays a key role in binding with sensor **1** to trigger the color change. In order to prove the importance of furan moiety in sensing DCP, we tested compound **7**, which does not possess a furan moiety. It showed slight emission enhancement with DCP (Fig. 1) indicating that the furan moiety is important for DCP sensing.

The fluorescence studies of sensor **1** were also performed in acetonitrile and the results were completely different from those of

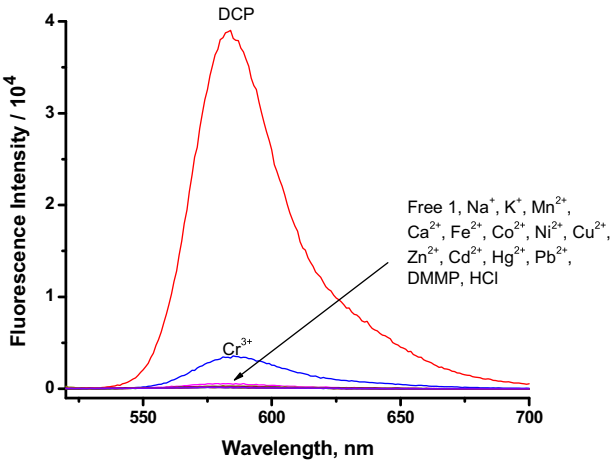


Fig. 3. Fluorescence spectra of compounds **1** (10 μM) with DCP, DMMP, HCl, and metals (340 μM) in 50% CH_3CN, 50% 0.01 M Tris–HCl buffer (pH=7.0) (λ_{ex}=510 nm).

the buffer system. There was a significant emission intensity enhancement (>1400-fold) with 1.0 equiv of Cr^{3+} (Fig. S1, Supplementary data). In addition, a significant fluorescence enhancement (ca. 900-fold) was observed with Hg^{2+} for Sensor **1**, while Pb^{2+}, Zn^{2+}, Cd^{2+}, and Fe^{2+} showed very slight interference at the same concentration. Compound **1** gave an instantaneous and far higher response with DCP in acetonitrile (Fig. S2, Supplementary data) but the sensitivity was low compared to the metal ions in acetonitrile. That motivated us to use an acetonitrile/buffer system, which gave an excellent sensitivity toward DCP.

Compound **2** was designed to understand the importance of the position of the furan oxygen in sensing DCP. Interestingly, the emission enhancement for **2** (13-fold, Fig. 1) with DCP is significantly lower than for compound **1**. This example proves that 2-substitution of the furan produces a better DCP sensor than 3-substitution possibly due to an electronic interaction during binding. However this was not directly confirmed. Compounds **4**–**6** bear electron-withdrawing groups, which can lower the electron density of the furan ring. We selected a strong electron-withdrawing group ($-NO_2$) and a weak electron-withdrawing group (−Br) to compare the effectiveness. Compound **4** showed a fluorescence enhancement (>23-fold) with DCP (34 equiv), but the observed fluorescence intensity was much lower than that of compound **1** and **3** with DCP. This behavior can be attributed to the effect of the electron-withdrawing group at the fifth position of the furan ring. In addition, the bulky 2-(2-nitrophenyl)furan moiety of compound **5** may also sterically hinder the binding of DCP. Interestingly, compound **4** gave a reasonable emission enhancement (10-fold) with Cu^{2+} while none of the other compounds gave any significant fluorescence enhancement with Cu^{2+} (Fig. S4, Supplementary data). Furthermore, the fluorescence enhancement observed with Cr^{3+} was insignificant when it compared with the **4**-DCP and **4**-Cu^{2+} adducts. Compounds **5** and **6** produced extremely low emissive solutions compared to the other compounds, and as expected, both **5** and **6** yielded extremely low emission intensities with DCP (Fig. 1) due to the presence of electron-withdrawing group at the fifth position of the furan ring. In fact the binding constant for **5** with DCP was found to be very low (K=13 M^{-1}). These observations indicate that a strong electron-withdrawing group ($-NO_2$) significantly lowers the sensitivity of the compound toward DCP. Bromine, a weak electron-withdrawing group, produces a similar yet smaller effect compared to the compound bearing nitro group.

Conversely, compound **3** was designed to study the effect of an electron-donating group at the fifth position of the furan ring. There was significant fluorescence intensity increase (>150-fold)

upon the addition of DCP. Furthermore, the calculated binding constant is 3.4×10^3 M^{-1}, which is the highest of the compounds studied. This clearly indicates that an electron-donating group can improve the sensitivity of the sensor. In addition, **3** showed a slight emission enhancement with Cr^{3+}, which however is insignificant compared to DCP.

2.3. Kinetic study

The fluorescence spectrum of **1** was recorded as a function of time, as the color development was not instantaneous in the buffer environment. As shown in Fig. 4, there is no fluorescence intensity enhancement during the first 4 min with 340 μM of DCP. The emission intensity enhanced dramatically after 4 min and was static after 9 min. The color development accelerated with higher concentrations of DCP. The kinetics of the reaction was studied with respect to temperature and concentration of DCP (Fig. 5). As expected, reaction rates were increased with elevated temperature and concentration. The color development was observed in 40 s at 70 °C with 340 μM of DCP.

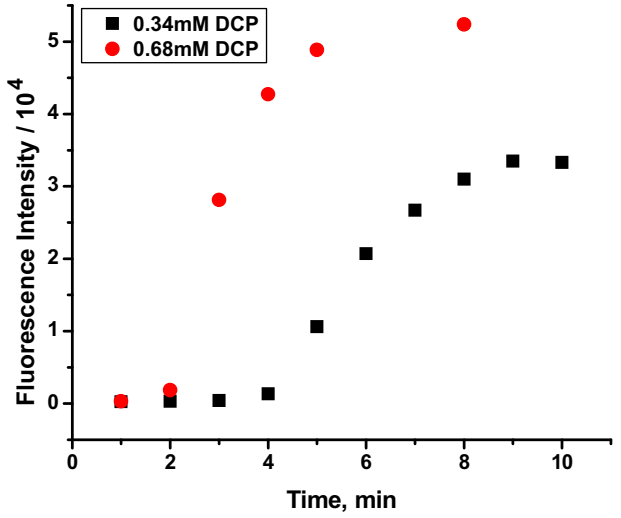


Fig. 4. Change in fluorescence intensity of **1** (10 μM) in the presence of DCP (0.34 mM and 0.68 mM) with time in 50% CH_3CN, 50% 0.01 M Tris–HCl buffer (pH=7.0) (λ_{ex}=510 nm).

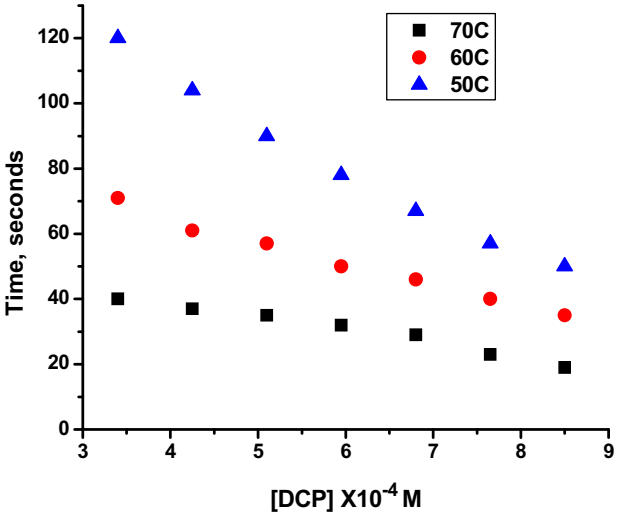


Fig. 5. Time required by **1** (10 μM) for the color change with DCP in 50% CH_3CN, 50% 0.01 M Tris–HCl buffer (pH=7.0).

2.4. Sensing mechanism

A ^1H NMR titration was performed to shed light on the binding of DCP with compound **1** (Fig. 6). Continuous addition of DCP resulted in a shortening and broadening of the imine hydrogen peak at δ 8.95. Interestingly, the peaks corresponding to the furan hydrogens did not show any significant change with the addition of DCP, while the intensities of some of the xanthene ring hydrogens decreased and broadened. DCP is expected to trigger the formation of the highly fluorescent ring-open form involving carbonyl oxygen and imine nitrogen as Kang et al. postulated for the protonation of rhodamine hydrazides.[17] As **1** did not interact with DMMP, it is clear that the chlorine leaving group of DCP plays a key role in binding with sensor **1** to trigger the color change (Fig. 7). It is well known that ethylenediaminetetraacetic acid (EDTA) can remove metals from a rhodamine–metal complex, thereby—causing the formation of the ring-closed non-fluorescent form. Based on an interaction between the phosphorus of DCP and the carbonyl of rhodamine, it is expected that a similar competitive interaction is occurring between the polarizable P=O of DCP and carbolylates of EDTA causing the removal of DCP from the sensor, which results in the formation of ring-closed spirolactam (Fig. S10, Supplementary data).

2.5. Sensor performance

Detection limits were calculated for compound **1** with DCP in 50% CH_3CN, 50% 0.01 M Tris–HCl buffer (pH=7.0) as it registered the highest fluorescence enhancement with DCP. The detection limit was found to be 170 μM at room temperature and improved at 70 °C (147 μM). In addition, a low detection limit of 17 μM was found in CH_3CN. The visible color changes from colorless to pink also permit the identification of nerve gas agents with the naked eye. The new design clearly improves the detection of nerve gas agents, as the unmodified **7** showed a very weak signal compared to other compounds. Swager[5a] and Rebek[5b] have used intramolecular cyclization mechanism to detect DCP, which is irreversible and the sensor system can be used only once. In contrast, our system is reversible and can be reusable. In addition, our sensors were very selective as they did not give any fluorescence enhancement with DMMP, metal ions or especially HCl, with which the reported sensors had problems.[5a] In terms of operational complexity and limited portability, our sensors are very easy to handle and even could be used in the field.

3. Conclusion

In conclusion, we have synthesized six new rhodamine-based compounds in high yields and determined the effect of the furan ring and its substitution upon DCP binding. Introducing a furan ring increased the sensitivity toward DCP as compound **7** with no furan ring showed less fluorescence enhancement compared to all the other compounds. The study determined that compounds **1** and **3** are the best sensor for of those studied. In addition, they show very high selectivity toward DCP over the common interferences used in the study. These results indicate that the presence of an electron-withdrawing group hampers the binding of DCP as it lowers the electron density of the furan ring and the imine nitrogen. The reaction rate increases dramatically with heating, so that **1** showed the color change with DCP in 40 s at 70 °C. That none of the compound showed any interaction with DMMP indicates the importance of the leaving group for the binding mechanism. Furthermore, the binding was found to be reversible, as the pink color disappears upon the addition of EDTA.

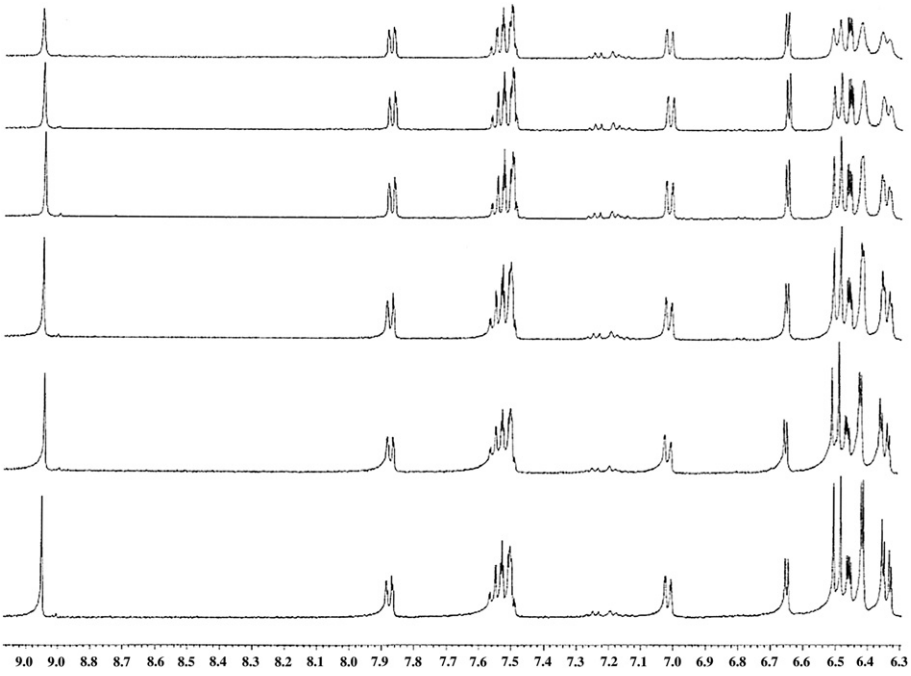


Fig. 6. ^{1}H NMR (CD_3CN) spectra of **1** with DCP (0, 0.75, 1.5, 3, 4, 6 equiv from bottom to top).

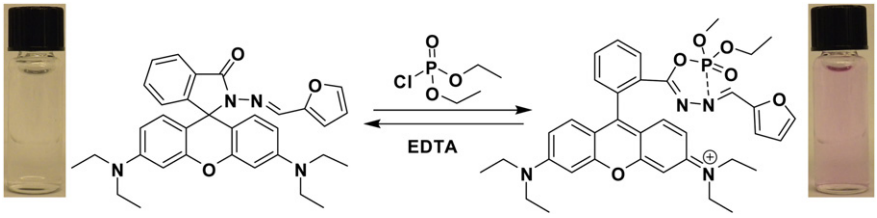


Fig. 7. The possible DCP binding mechanism that triggers the formation of colored ring-open form.

4. Experimental

4.1. Compound 1

A mixture of compound **7** (0.5 g, 1.09 mmol) and 2-furaldehyde (0.14 g, 1.45 mmol) in 10 ml of ethanol was refluxed for 12 h. A precipitate formed during the reaction was filtered after cooling down to room temperature. The precipitate was washed with ethanol and dried to obtain **1** as a red solid (0.41 g, 70%). Mp=204–206 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.14 (12H, t, J=6.9 Hz), 3.31 (8H, q, J=6.9 Hz), 6.23 (2H, dd, J=8.8, 2.6 Hz), 6.33 (1H, dd, J=3.3, 1.4 Hz), 6.41 (2H, d, J=2.6 Hz), 6.53 (2H, d, J=8.8 Hz), 6.57 (1H, d, J=3.4 Hz), 7.05 (1H, d, J=6.2 Hz), 7.37 (1H, d, J=1.1 Hz), 7.43 (2H, m,), 7.97 (1H, d, J=8.1 Hz), 8.19 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.7, 44.3, 65.6, 98.0, 105.5, 108.2, 111.6, 112.1, 123.5, 123.6, 127.9, 128.2, 133.5, 136.0, 143.9, 149.0, 150.7, 152.6, 152.8, 165.1. ESI-MS: 535.42 $[M+1]^+$. Anal. Calcd for $C_{33}H_{34}N_4O_3$: C, 74.13; H, 6.41; N, 10.48. Found: C, 73.79; H, 6.80; N, 10.59.

4.2. Compound 2

A mixture of **7** (1.0 g, 2.2 mmol) and 3-furaldehyde (0.24 g, 2.5 mmol) in 20 ml of ethanol was refluxed overnight. The reaction mixture was allowed to cool down to room temperature and the precipitate was filtered off. The precipitate was washed with ample amount of ethanol, ether, and dried to obtain **2** as a purple solid (0.2 g, 17%). Mp=182–184 °C; ^{1}H NMR (400 MHz, acetone-d_6): δ 1.13 (12H, t, J=6.9 Hz), 3.37 (8H. q, J=6.9 Hz), 6.35 (2H, m), 6.47 (5H, m), 7.11 (1H, d, J=7.6 Hz), 7.47 (1H, s), 7.58 (2H, m), 7.80 (1H, s), 7.87 (1H, d, J=7.3 Hz), 9.23 (1H, s). ^{13}C NMR (100 MHz, acetone-d_6): δ 12.0, 44.0, 66.1, 97.7, 106.8, 106.8, 107.9, 122.8, 124.0, 124.3, 127.8, 128.5, 130.4, 133.2, 142.0, 144.2, 144.8, 148.9, 151.2, 153.6, 163.9. ESI-MS: 535 $[M+1]^+$. Anal. Calcd for $C_{33}H_{34}N_4O_3$: C, 74.13; H, 6.41; N, 10.48. Found: C, 73.74; H, 6.80; N, 10.50.

4.3. Compound 3

A mixture of **7** (1.0 g, 2.2 mmol) and 5-methyl-2-furaldehyde (0.29 g, 2.6 mmol) in 20 ml of ethanol was refluxed overnight. The reaction mixture was allowed to cool down to room temperature and the precipitate was filtered off. Precipitate was washed with ample amount of ethanol and dried to obtain **3** as a pink solid (0.85 g, 71%). Mp=193–194 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.14 (12H, t, J=6.9 Hz), 2.26 (3H, s), 3.31 (8H, q, J=6.9 Hz), 5.92 (1H, s), 6.23 (2H, d, J=8.4 Hz), 6.41 (3H, s), 6.55 (2H, d, J=8.8 Hz), 7.03 (1H, d, J=6.9 Hz), 7.41 (2H, m), 7.93 (1H, s), 7.97 (1H, d, J=6.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.7, 13.9, 44.3, 65.4, 98.0, 105.5, 108.0, 108.2, 114.3, 123.5, 127.9, 128.0, 128.1, 133.4, 135.7, 148.9, 149.0,

152.6, 152.8, 154.7, 165.1. ESIMS: 549 $[M+1]^+$. Anal. Calcd for $C_{34}H_{36}N_4O_3$: C, 74.43; H, 6.61; N, 10.21. Found: C, 74.06; H, 7.00; N, 10.43.

4.4. Compound 4

To a solution of **7** (1.0 g, 2.2 mmol) in 20 ml of ethanol was added 5-(2-nitrophenyl)-2-furaldehyde (0.57 g, 2.64 mmol). The resulting solution was refluxed overnight. The precipitate formed was filtered off after cooling down to room temperature. The precipitate was washed with ample amount of ethanol and air dried to get **4** as a brown solid (0.94 g, 65%). Mp=107–110 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.14 (12H, t, J=6.6 Hz), 3.31 (8H, q, J=6.6 Hz), 6.25 (2H, d, J=8.0 Hz), 6.43 (2H, s), 6.53 (3H, m), 6.65 (1H, s), 7.09 (1H, d, J=6.6 Hz), 7.36 (1H, t, J=7.5 Hz), 7.46 (2H, t, J=6.7 Hz), 7.54 (1H, t, J=7.5 Hz), 7.60 (1H, d, J=8.0 Hz), 7.82 (1H, d, J=7.3 Hz), 7.98 (1H, d, J=6.8 Hz), 8.36 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.7, 44.4, 66.0, 98.0, 105.5, 108.1, 112.1, 113.6, 123.4, 123.5, 123.7, 123.8, 127.9, 128.4, 128.7, 129.0, 131.8, 133.6, 135.8, 147.4, 148.5, 149.1, 151.7, 152.1, 153.0, 165.1. ESI-MS: 678 $[M+Na]^+$. Anal. Calcd for $C_{39}H_{37}N_5O_5 \cdot H_2O$: C, 69.52; H, 5.83; N, 10.39. Found: C, 70.25; H, 6.41; N, 10.70.

4.5. Compound 5

To a solution of **7** (1.0 g, 2.2 mmol) in 20 ml of ethanol was added 5-nitro-2-furaldehyde (0.37 g, 2.64 mmol). The resulting solution was refluxed overnight. The precipitate formed was filtered after cooling down to the room temperature. The precipitate was washed with ample amounts of ethanol and air dried to get **5** as a brown solid (0.24 g, 19%). Mp=120–123 °C; ^{1}H NMR (400 MHz, acetone-d_6): δ 1.14 (12H, t, J=6.9 Hz), 3.38 (8H, q, J=6.9 Hz), 6.38 (2H, d, J=8.8 Hz), 6.46 (2H, d, J=1.8 Hz), 6.50 (2H, d, J=9.1 Hz), 6.92 (1H, d, J=3.6 Hz), 7.11 (1H, d, J=7.6 Hz), 7.46 (1H, d, J=4.0 Hz), 7.58 (1H, t, J=7.3 Hz), 7.64 (1H, t, J=7.3 Hz), 7.93 (1H, d, J=7.3 Hz), 8.90 (1H, s). ^{13}C NMR (100 MHz, acetone-d_6): δ 12.0, 44.0, 66.3, 97.8, 105.5, 108.2, 113.0, 113.6, 116.8, 123.2, 124.0, 127.8, 128.6, 128.8, 134.2, 134.6, 149.1, 152.0, 152.6, 153.2, 164.8. ESI-MS: 580 $[M+1]^+$. Anal. Calcd for $C_{33}H_{33}N_5O_5 \cdot$ethanol: C, 67.18; H, 6.28; N, 11.19. Found: C, 67.18; H, 5.97; N, 12.31.

4.6. Compound 6

A mixture of **7** (1.0 g, 2.2 mmol) and 5-bromo-2-furaldehyde (0.45 g, 2.6 mmol) in 20 ml of ethanol was refluxed for 3 h. The reaction mixture was allowed to cool down to the room temperature and the precipitate was filtered. The precipitate was washed with ample amount of ethanol and dried to obtain **6** as a pink solid (1.12 g, 83%). Mp=203–204 °C; ^{1}H NMR (400 MHz, acetone-d_6): δ 1.13 (12H, t, J=6.9 Hz), 3.37 (8H, q, J=6.9 Hz), 6.36 (2H, m), 6.43 (2H, s), 6.51 (3H, m), 6.70 (1H, d, J=3.6 Hz), 7.06 (1H, d, J=7.6 Hz), 7.56 (2H, m), 7.87 (1H, d, J=6.9 Hz), 8.99 (1H, s). ^{13}C NMR (100 MHz, acetone-d_6): δ 12.0, 44.0, 66.0, 97.8, 106.1, 108.1, 114.1, 115.1, 116.8, 122.9, 123.8, 124.2, 127.8, 128.5, 129.1, 133.6, 137.2, 149.0, 152.1, 152.6, 153.2, 164.5. ESI-MS: 614$[M+1]^+$. Anal. Calcd for $C_{33}H_{33}BrN_4O_3$: C, 64.60; H, 5.42; N, 9.43. Found: C, 64.20; H, 5.62; N, 9.30.

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Supplementary data

General procedures, additional fluorescence, UV–vis, ^{1}H, ^{13}C NMR, and X-ray crystallographic data are included in the Supplementary data. Supplementary data related to this article can be found online at doi:10.1016/j.tet.2011.02.041.

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22. Crystallographic data has been deposited with the Cambridge Crystallographic Data Centre. CCDC numbers **1**–**4**, **6** are 730371, 759587, 759585, 759584, **6**: 759586.