**Electronic Supplementary Informations**

**A radical approach for fluorescent turn ‘on’ detection, differentiation and bioimaging of methanol**

Virendra Kumar,² Ajit Kumar,² Uzra Diwan,² Manish Kumar Singh,² and K. K. Upadhyay²*  

*Department of Chemistry (Centre of Advanced Study), Faculty of Science, Banaras Hindu University, Varanasi, Uttar Pradesh-221005, India.  
²Department of Zoology (Centre of Advanced Study), Faculty of Science, Banaras Hindu University, Varanasi, Uttar Pradesh-221005, India.  
E-mail: drkaushalbhu@ yahoo.co.in; kku@bhu.ac.in, Tel No.: +91-542-6702488

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**Experimental section**
1.1. **Synthesis of RC**: RC was synthesized by adding 2.0 mM acetonitrile solution of 7-(diethylamino)-2-oxo-2H-chromene-3-carbaldehyde to the equimolar acetonitrile solution of 3-aminorhodanine followed by constant stirring for three hours at room temperature (**Scheme 1**). A brick red solid was precipitated which was filtered and washed with diethyl ether and finally dried under vacuum over anhydrous CaCl₂. **RC** was characterized through various spectroscopic techniques like IR, ¹H & ¹³C NMR spectral studies along with mass determination through ESI-MS (**ESI; Fig. S1-S4**).

**Scheme 1**: Synthesis of **RC**

**Spectroscopic characterization data**: **Yield**: 86%. **IR/cm⁻¹**: 2971, 2929, 1741, 1709, 1620, 1598, 1563, 1513, 1483, 1379, 1350, 1311, 1295, 1259, 1233, 1188, 1133, 1078, 1029, 955, 904, 875, 796, 761, 688, 637; **¹H NMR (300 MHz, CDCl₃, 298K, TMS)**: δ = 1.233-1.279 (t, 6H, CH₃), 3.433-3.504 (q, 4H, CH₂), 4.077 (s, 2H, CH₂), 6.490 (s, 1H, Ar-H), 6.613-6.635, (d, 1H, Ar-H), 7.369-7.399 (d, 1H, Ar-H), 8.587, (s, 1H, Ar-H), 8.740 (s, 1H, –CH=N) δ ppm; **¹³C NMR (75
1.2. **Apparatus:** IR Spectra were recorded with a Perkin-Elmer spectrometer using KBr pellets. The corresponding $^1$H NMR and $^{13}$C NMR spectra were recorded in CDCl$_3$ with a JEOL AL 300 FT NMR Spectrometer instrument using tetramethylsilane (Si(CH$_3$)$_4$) as an internal standard. $^1$H and $^{13}$C chemical shifts are reported in parts per million (ppm) relative to the residual proton signal of the deuterated solvents. Mass spectrometric analysis was carried out on a MDS Sciex API 2000 LCMS spectrometer while HRMS of RO was recorded at Water-Q-Tof Premier-HAB213. The electronic spectra and UV-visible titrations were carried out room temperature (298 K) on a UV-1700/1800 Pharmaspec spectrophotometer with quartz cuvette (path length=1 cm). The emission spectra were recorded at JY HORIBA Fluorescence spectrophotometer.

1.3. **Materials:** All the reagents and solvents for synthesis were purchased from Sigma-Aldrich and were used without further purification. All reactions were carried out using commercial-grade solvents.

1.4. **Theoretical Calculations:** The geometric and energy optimizations were performed with the Gaussian 03 program based on the density functional theory (DFT) method. Becke’s three parameter hybrid functional with the Lee-Yang-Parr correlation functional (B3LYP) was employed for all the calculations. The 3-21G** basis set was used to treat all atoms.

1.5. **X-ray diffraction studies:** Single crystal X-ray diffraction measurements were carried out on an Oxford Diffraction Xcalibur system with a Ruby CCD detector using graphite-monochromated MoKα radiation ($\lambda = 0.71073 \, \text{Å}$). All the determinations of unit cell and intensity data were performed with graphite-mono-chromated Mo-Kα radiation ($\lambda = 0.71073 \, \text{Å}$). Data for the ligand and metal complexes were collected at room temperature/liquid nitrogen temperature. The structures were solved by direct methods, using Fourier techniques, and refined by full-matrix least-squares on $F^2$ using the SHELXTL-97 program package.

1.6. **Cell Imaging Studies:** *E. coli* strains (DH5-α) were grown in LB media at 37° C overnight in shaker incubator. The cells were collected in sterile water and vortexed to make the suspension homogeneous. These cell cultures were incubated with RC
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(10µM) from 1.0 mM stock in 50mM phosphate buffer (pH 7.54) for 1 hour. The treated cells were examined by the excitation range from 450-490 nm and emission range from 500-560 nm on a fluorescence microscope (Nikon-E800, Japan).

References:


S2. (a) G. M. Sheldrick, SHELXL-97, Program for X-ray Crystal Structure Refinement, Göttingen University, Göttingen, Germany, 1997; (b) G. M. Sheldrick, SHELXS-97, Program for X-ray Crystal Structure Solution, Göttingen University, Göttingen, Germany, 1997.
Figure S1: $^1$H NMR spectrum of RC in CDCl$_3$: 
Figure S2: $^{13}$C NMR spectrum of RC:
Figure S3: IR spectrum of RC:
Figure S4: Mass spectrum of RC:

[M+H]$^+$

Chemical Formula: $C_{12}H_{11}N_3O_3S_2$

Molecular Weight: 375.4652
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**Figure S5:** UV-visible absorbance spectrum of RC in different solvent:

![UV-visible absorbance spectrum of RC in different solvent](image)
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**Figure S6a:** Selective visible color changes of **RC** in various solvents; from left to right: CHCl$_3$, DCM, Toluene, THF, Ethylacetate, ACN, Acetone, MeOH, EtOH, Propanol, Butanol, DMF, and DMSO

![Selective visible color changes of RC in various solvents](image)

**Figure S6b:** Selective fluorescence color changes (Under UV light) of **RC** in various solvents; from left to right: **RC**, MeOH, EtOH, Propanol, Butanol, Acetone, Ethyl acetate, Toluene, DCM, CHCl$_3$, THF, ACN, DMF, DMSO and Water.

![Selective fluorescence color changes (Under UV light)](image)
Figure S7: $^1$H NMR spectrum of RO in CDCl$_3$: 

![NMR Spectrum](image)
Figure S8: Proposed mechanism of nucleophile attack of methanol over RC:

Where, \( R = \text{Coumarin} \)
Figure S9: IR spectrum of RO:
Figure S10: Overlay IR spectrum of RC and RO:
Figure S11: Mass spectrum of RO:

Chemical Formula: C_{16}H_{27}N_{2}NaO_{3}S_{2}
Exact Mass: 430.0871
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**Figure S12:** HOMO-LUMO orbitals of RC and RO their calculated energy and energy gaps are shown:
**Figure S13**: Fluorescence reaction time profile of receptor **RC** at 0.5μM in MeOH:
**Figure S14**: Calibration curve of RC in water with increasing MeOH%:

\[
y = 79599x + 113827 \\
R^2 = 0.9954
\]
**Table S1**: Important bond length and bond angle of RO:

<table>
<thead>
<tr>
<th>Atom</th>
<th>Bond Length(Å)</th>
<th>Atom</th>
<th>Bond angle</th>
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<tr>
<td>S(2)-C(16)</td>
<td>1.786(3)</td>
<td>C(16)-S(2)-C(15)</td>
<td>100.1(1)</td>
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<tr>
<td>S(2)-C(15)</td>
<td>1.752(2)</td>
<td>C(9)-O(1)-C(13)</td>
<td>122.7(2)</td>
</tr>
<tr>
<td>S(1)-C(15)</td>
<td>1.643(2)</td>
<td>N(3)-N(2)-C(14)</td>
<td>116.1(2)</td>
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<tr>
<td>O(1)-C(9)</td>
<td>1.377(3)</td>
<td>C(17)-O(4)-C(18)</td>
<td>115.1(2)</td>
</tr>
<tr>
<td>O(1)-C(13)</td>
<td>1.381(3)</td>
<td>N(2)-N(3)-C(15)</td>
<td>119.2(2)</td>
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<tr>
<td>N(2)-N(3)</td>
<td>1.383(2)</td>
<td></td>
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<td>N(2)-C(14)</td>
<td>1.271(2)</td>
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<tr>
<td>O(4)-C(17)</td>
<td>1.343(4)</td>
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<tr>
<td>O(4)-C(18)</td>
<td>1.445(3)</td>
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<td>O(2)-C(13)</td>
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<td>N(3)-C(15)</td>
<td>1.339(4)</td>
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Showing intermolecular hydrogen bonding in **RO**
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**Table S2:** Crystal data and structure refinement for **RO:**

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<th><strong>Identification code</strong></th>
<th><strong>RO</strong></th>
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<tr>
<td><strong>CCDC No.</strong></td>
<td>980304</td>
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<tr>
<td>Empirical formula</td>
<td>C18 H21 N3 O4 S2</td>
</tr>
<tr>
<td>Formula weight</td>
<td>407</td>
</tr>
<tr>
<td>Temperature</td>
<td>293(2) K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073Å</td>
</tr>
<tr>
<td>Crystal system, space group</td>
<td>Triclinic, P -1</td>
</tr>
</tbody>
</table>
| Unit cell dimensions    | a = 6.9629(13) Å   alpha = 102.726(18) deg.  
                         | b = 10.231(3) Å    beta = 97.404(16) deg. 
                         | c = 14.781(2) Å    gamma = 94.658(18) deg. |
| Volume                  | 1012.0(3) Å³ |
| Z, Calculated density   | 2, 1.334 Mg/m3 |
| Absorption coefficient  | 0.291 mm-1 |
| F(000)                  | 428.0  |
| Crystal size            | 0.34 x 0.28 x 0.22 mm |
| Theta range for data collection | 2.97 to 28.99 deg. |
| Limiting indices        | -8<=h<=9, -9<=k<=13, -19<=l<=19 |
| Reflections collected / unique | 6963 / 4036 [R(int) = 0.0397] |
| Completeness to theta = 25.00 | 99.0 % |
| Max. and min. transmission | 1.00000 and 0.94362 |
| Refinement method       | Full-matrix least-squares on F2 |
| Data / restraints / parameters | 4036 / 0 / 248 |
| Goodness-of-fit on F2   | 0.960  |
| Final R indices [I>2sigma(I)] | R1 = 0.0574, wR2 = 0.0556 |
| R indices (all data)    | R1 = 0.1294, wR2 = 0.0712 |
| Largest diff. peak and hole | 0.213 and -0.161 e.Å-3 |
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**Table S3**: The selected experimental and calculated dihedral angles in **RC** and **RO**:

![Chemical structures of RC and RO](image)

**Dihedral angles of RC and RO**

- DA1 = C1-C2-C3=N1
- DA2 = C2-C3=N1-N2
- DA3 = C3-N1-N2-C4
- DA4 = N1-N2-C4-S1

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<tr>
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<tr>
<td></td>
<td><strong>RC</strong></td>
<td><strong>RO</strong></td>
</tr>
<tr>
<td>DA1</td>
<td>177.19</td>
<td>177.96</td>
</tr>
<tr>
<td>DA2</td>
<td>177.62</td>
<td>179.64</td>
</tr>
<tr>
<td>DA3</td>
<td>139.15</td>
<td>-179.97</td>
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<tr>
<td>DA4</td>
<td>-11.13</td>
<td>179.73</td>
</tr>
</tbody>
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**Table S4:** Theoretical calculation of absorption maxima of **RC** and **RO** in MeOH using TD-DFT study:

![Chemical structures of RC and RO](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Major transitions</th>
<th>Wavelength</th>
<th>Oscillator strength</th>
<th>Energy</th>
<th>Contributions of Excitation</th>
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| **RO** | 107 $\rightarrow$ 108  
0.63996 | 449.17 nm  
f=1.3047 | 2.7603 eV  
HOMO $\rightarrow$ LUMO $= 81.9$ |
| **RC** | 98 $\rightarrow$ 99  
0.63028 | 428.38 nm  
f=0.9064 | 2.8943 eV  
HOMO $\rightarrow$ LUMO $= 79.45$ |