1. General method of UV-Vis and fluorescence titration

Path length of the cells used for absorption and emission studies is 1 cm. For UV-Vis. and fluorescence titrations, stock solution of **RHENTU** is prepared (50 μM) in CH_3CN/water (4/1, v/v) HEPES (0.1M) buffer. Working solutions of **RHENTU** and ClO_4^- are prepared from their respective stock solutions. Fluorescence measurements are performed using 5 nm x 5 nm slit width. All the fluorescence and absorbance spectra are taken after 5 minutes of mixing of ClO_4^- to **RHENTU**.

2. Calculation of Quantum Yield

Fluorescence quantum yields (Φ) are estimated by integrating the area under the fluorescence curves using the equation,

where A is the area under the fluorescence spectral curve and OD is the optical density of the compound at the excitation wavelength[1]. Rhodamine B is used as quantum yield standard (quantum yield is 0.65 in basic ethanol)[2] for measuring the quantum yields of the probe and its ClO_4^- adduct.

3. Job's plot (using fluorescence technique)

A series of solutions containing **RHENTU** and ClO_4^- are prepared such that the total concentration of ClO_4^- and **RHENTU** remain constant (50 μM) in all the sets. The mole fraction

(X) of **RHENTU** is varied from 0.1 to 0.9. The fluorescence intensity at 569 nm is plotted against the mole fraction of **RHENTU** in solution.

4. Calculation of detection limit

To determine the detection limit, fluorescence titration of **RHENTU** with ClO_4^- is carried out by adding aliquots of micromolar concentration of ClO_4^-. From the concentration at which there occurs a sharp change in the fluorescence intensity multiplied with the concentration of **RHENTU** give the detection limit.[3]

Equation used for calculation of detection limit (DL)

$DL = C_L \times C_T$

C_L = Concentration of probe; C_T = Concentration of ClO_4^- at which fluorescence enhanced.

Thus;

$DL = 1 \times 10^{-6} \times 0.1 \times 10^{-6} = 0.1 \times 10^{-6} = 1 \times 10^{-7}$

Experimental

General procedures. High-purity HEPES, rhodamine B, and ethylenediamine are purchased from Sigma Aldrich (India). KSCN and $NaClO_4$ are purchased from Merck (India). Solvents used are of spectroscopic grade. Other chemicals are of analytical reagent grade and used without further purification except when specified. The Na^+ or K^+ salts of anions have been used. Mili-Q Milipore® 18.2 MΩ cm^{-1} water is used throughout all the experiments. A JASCO (model V-570) UV–Vis spectrophotometer is used for recording absorption spectra. FTIR spectra are recorded on a JASCO FTIR spectrophotometer (model: FTIR-H20). Mass spectra are performed on a QTOF Micro YA 263 mass spectrometer in ES positive mode. ^1H NMR spectra are

recorded using Bruker Avance 600 (600MHz) in DMSO-d_6. Melting point measurement is done by VEEGO digital melting point apparatus. Elemental analysis is performed using Perkin Elmer CHN-Analyzer with first 2000-Analysis kit. The steady-state fluorescence emission and excitation spectra are recorded with a Hitachi F-4500 spectrofluorometer. All pH measurements are performed with Systronics digital pH meter (model 335).

Imaging system. The imaging system is composed of an inverted fluorescence microscope (Leica DM 1000 LED), digital compact camera (Leica DFC 420C), and an image processor (Leica Application Suite v3.3.0). The microscope is equipped with a 50 W mercury arc lamp.

Preparation of cells. Pollen grains are collected from fresh mature buds of *Allamanda puberula* (Aapocynaceae), a common ornamental plant with bell shaped bright yellow flower by crashing stamens on a sterile petriplate and suspending them in normal saline. After crashing the stamina debrishes are removed by filtering through a thin layer of non absorbant cotton and the suspended pollens are collected by centrifugation at 5000 rpm for five minutes. The pollen pellet is then washed twice in normal saline and incubated in a solution of $NaClO_4$ (0.1 mg mL^{-1}) for one hour at ambient temperature. After incubation they are again washed in normal saline and observed under fluorescence microscope in presence and absence of the probe. Both perchlorate treated and untreated cells are stained with the probe to observe under fluorescence microscope.

***Synthesis of N*-(rhodamine- B)lactam-ethylenediamine**

N-(rhodamine- B)lactam-ethylenediamine is prepared according to the literature mothod.[4]

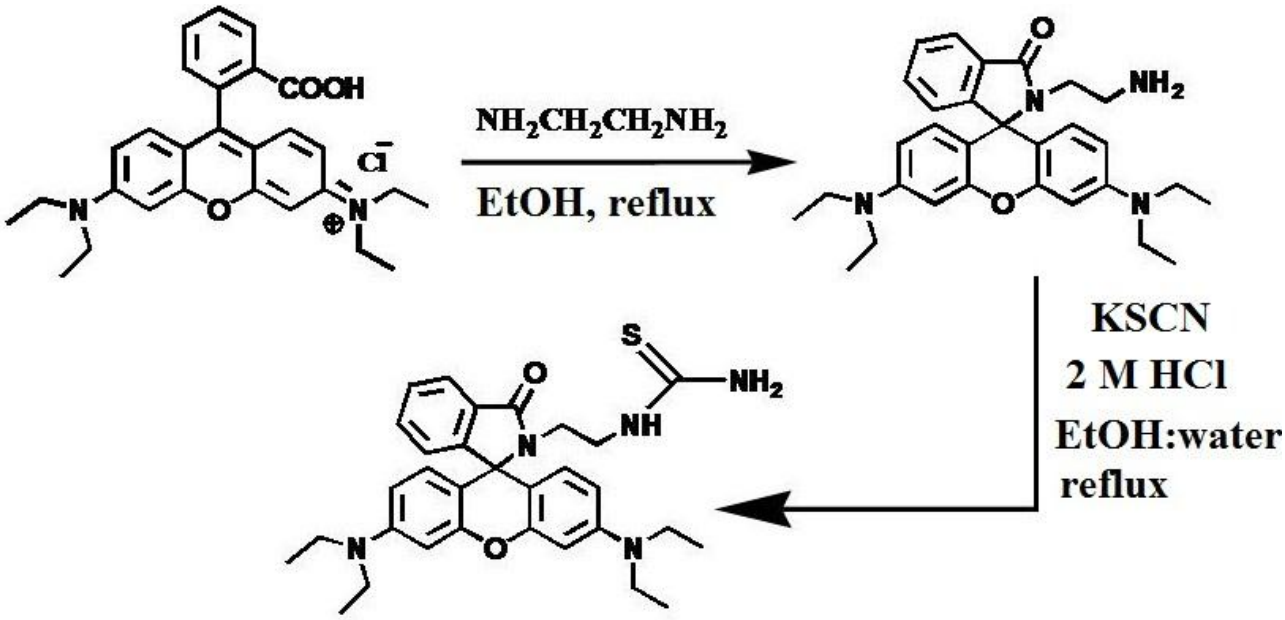
Synthesis of RHENTU

A simple one-step reaction of N-(rhodamine- B)lactam-ethylenediamine with KSCN in acidic media (Scheme S1) have yielded **RHENTU**. Briefly, a mixture of N-(rhodamine- B)lactam-ethylenediamine (1.0 g, 2.06 mmol) and KSCN (0.99 g, 10.30 mmol) is refluxed in ethanol/2 M aqueous HCl (4:1) for 10 h. The reaction mixture is allowed to cool to room temperature, neutralized with $NaHCO_3$ when a pink solid is obtained. The crude solid is subjected to silica gel (200–300 mesh) chromatography using ethyl acetate–petroleum ether (2:1, v/v) to afford the desired product (0.678 g, 55% yield) as a faint purple solid.[5] The product is further recrystallized from acetonitrile to obtain pure **RHENTU**. M. P., 229^{o}C (± 2^{o}C); ^{1}H NMR (600MHz, DMSO-d_6) (ESI, Fig. S16): 1.1 (12H, m, J = 6.0 Hz); 1.64 (1H, s); 2.19 (2H, m, 12.0 Hz); 3.01 (2H, m, J = 6.0 Hz); 3.04 (2H, m, J = 6.0 Hz); 3.33 (8H, d, J = 6.0 Hz); 6.38~6.29 (4H, m, J = 6.0 Hz); 6.43 (1H, m, J = 6.0 Hz); 7.00 (1H, m, J = 6.0 Hz); 7.50 (3H, m, 6.0 Hz); 7.77 (1H, m, J = 6.0 Hz). QTOF – MS ES^{+} (ESI, Fig. S17): $[M + H]^{+}$ = 544.39. Elemental analysis data as calculated for $C_{31}H_{37}N_5O_2S$ (%): C, 68.48; H, 6.86 and N, 12.88. Found (%): C, 68.42; H, 6.91 and N, 12.74. FTIR (cm^{-1}) (ESI, Fig. S18): υ(NH) 3414.59, υ(C=O) 1678.41 and υ(COC) 1118.42

Synthesis of [RHENTU-ClO_4^-] adduct

To the CH_3CN solution of **RHENTU** (0.025 g, 0.045 mmol, 8 mL), aqueous solution of $NaClO_4$ (0.003 g, 0.023 mmol, 2 mL) water is added drop-wise under stirring condition. The stirring is continued for 5 minutes and the solvent is allowed to evaporate at room temperature.

FTIR (cm^{-1}) (ESI, Fig. S19): υ(NH) 3416.49, υ(C=O) 1637.08, υ(ClO_4^-) 1086.84, υ(ClO_4^-) 627.39.



Scheme S1

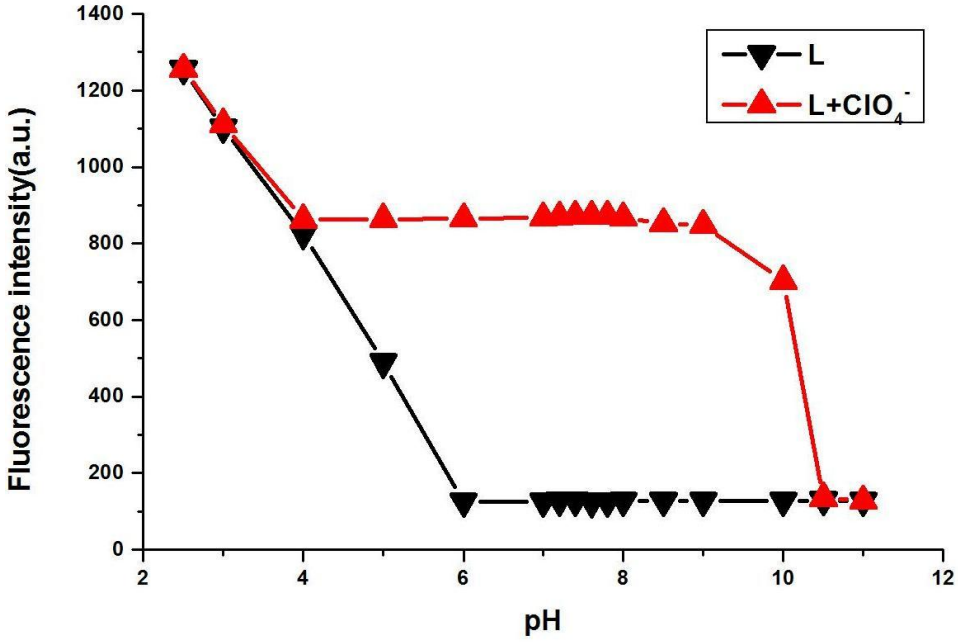


Fig. S1 Influence of pH on the emission intensities of **RHENTU** (50 µM) and **RHENTU** (50 µM) - ClO_4^- (175 µM) adduct in CH_3CN: water (4:1, v/v), (λ_{ex} = 520 nm).

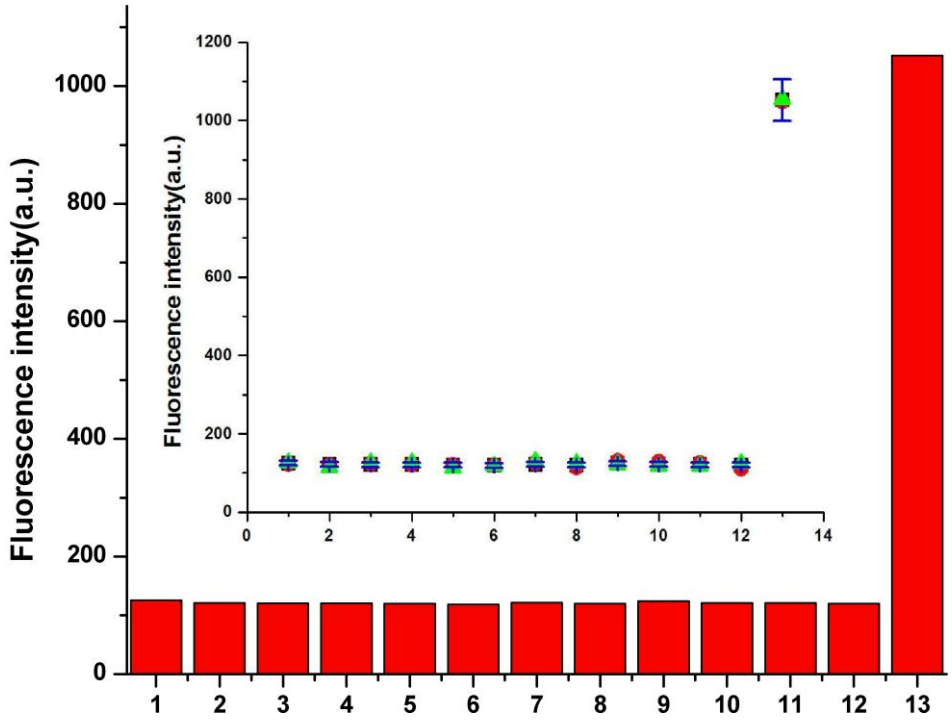


Fig. S2 Emission intensity changes of **RHENTU** (50 μM) in presence with F^-, Cl^-, Br^-, I^-, N_3^-, NCO^-, $H_2PO_4^-$, SO_4^{2-}, OAc^-, NO_3^-, NO_2^-, CN^-, ClO_4^- (200 μM) in HEPES buffered (0.1 M) CH_3CN: water (4:1, v/v, pH, 7.4). Inset: error bars related to the fluorescence intensity changes of **RHENTU** (50 μM) in presence of above ions (200 μM), N = 3 (from left to right).

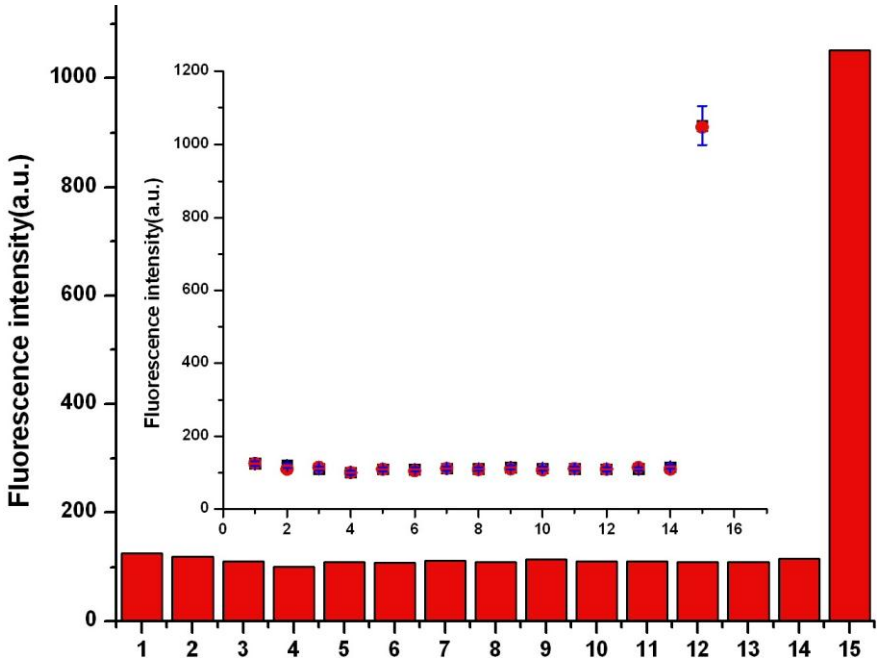


Fig. S3 Fluorescence intensity changes of **RHENTU** (50 µM) in presence of Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Cu^{2+}, Ni^{2+}, Fe^{3+}, Zn^{2+}, cysteine, methionine, glutathione, H_2O_2, OCl^{-} and ClO_4^{-} (200 µM) in HEPES buffered (0.1 M) CH_3CN: water (4:1, v/v, pH, 7.4). Inset: error bars related to the emission intensity changes of **RHENTU** (50 µM) in presence of above species (200 µM), N = 3, (from left to right). .

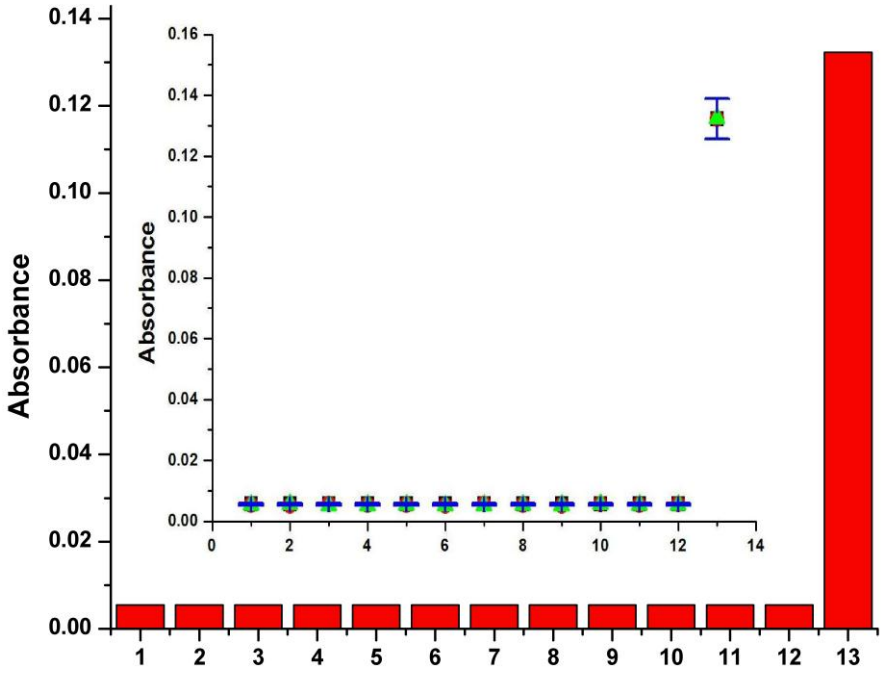


Fig. S4 Absorbance changes of **RHENTU** (50 µM) in presence with F^-, Cl^-, Br^-, I^-, N_3^-, NCO^-, $H_2PO_4^-$, SO_4^{2-}, OAc^-, NO_3^-, NO_2^-, CN^-, ClO_4^- (200 µM) in HEPES buffered (0.1 M) CH_3CN: water (4:1, v/v, pH7.4). Inset: error bars related to the absorbance changes of **RHENTU** (50 µM) in presence of above ions (200 µM), N = 3, (from left to right).

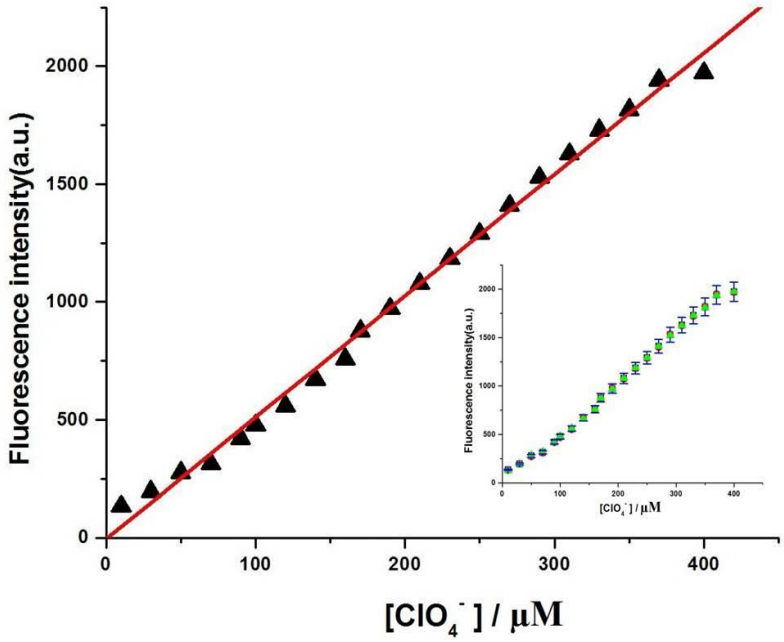


Fig. S5 Plot of emission intensity (at 569 nm) of **RHENTU** (50 μM) as a function of externally added ClO_4^- (10 - 400 μM), (R^2 = 0.99). Inset: error bars related to the emission intensity changes of **RHENTU** (50 μM) as a function of externally added ClO_4^- (10-400 μM), N = 3.

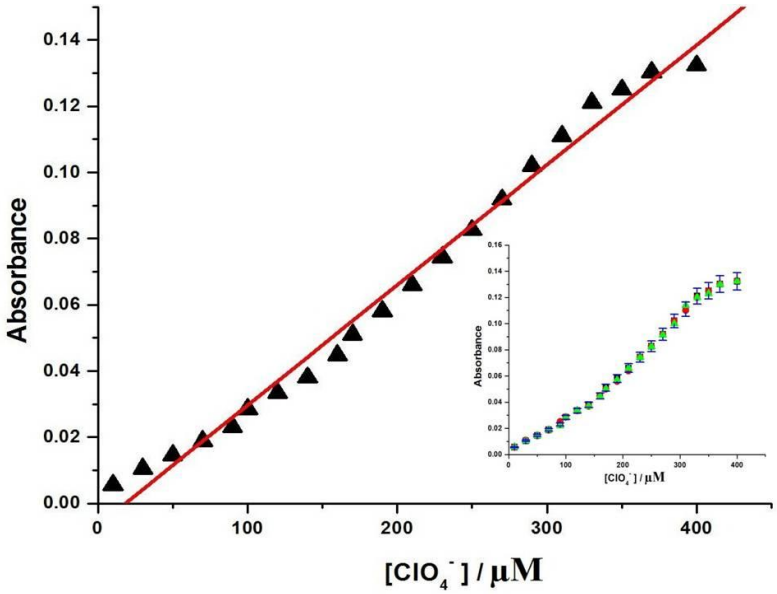


Fig. S6 Plot of absorbance (at 541 nm) of **RHENTU** (50 µM) as a function of externally added ClO_4^- (10 - 400 µM), (R^2 = 0.99), Inset: error bars related to the absorbance of **RHENTU** (50 µM) as a function of externally added ClO_4^- (10-400 µM), N = 3.

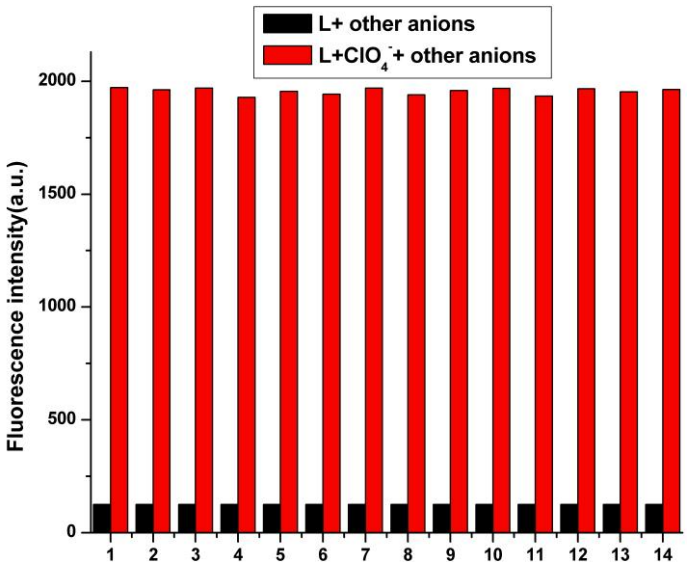


Fig. S7 Plot of emission intensity of [**RHENTU**-ClO_4^-] system in presence of different anions : **RHENTU** (50 μM) + ClO_4^- (400 μM) + other anions (400 μM), where other anions = (**1**- F^-, **2**- Cl^-, **3**- Br^-, **4**- I^-, **5**- N_3^-,**6**- NCO^-, **7**- $H_2PO_4^-$, **8**- SO_4^{2-}, **9**- OAc^-, **10**- NO_3^-, **11**- NO_2^-, **12**- CN^-, **13**- **Mix**. (solutions of F^-, Cl^-, Br^-, I^-), **14**- **Mix**. (solutions of NCO^-, $H_2PO_4^-$, SO_4^{2-}, OAc^-, NO_3^-) in HEPES buffer (0.1 M, CH_3CN: water, 4:1,v/v), (λ_{ex} = 520 nm).

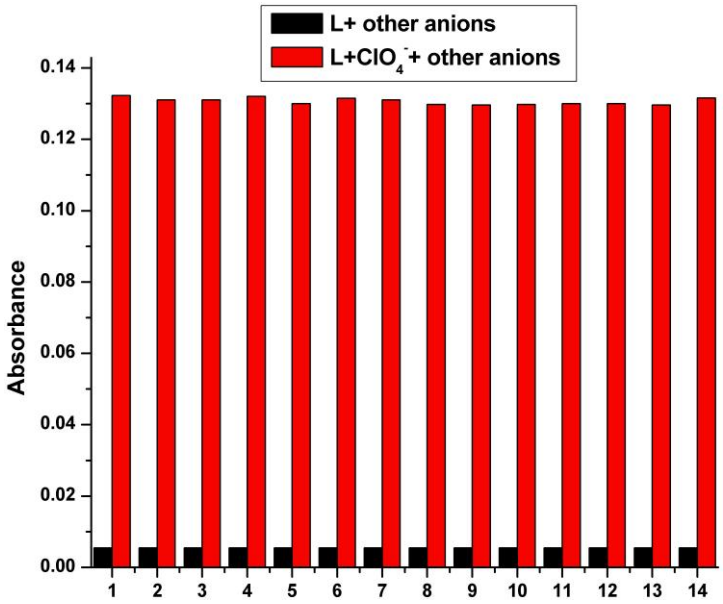


Fig. S8 Plot of absorbance of [**RHENTU**-ClO_4^-] system in presence of different anions : **RHENTU** (50 μM) + ClO_4^- (400 μM) + other anions (400 μM), where other anions = (**1**- F^-, **2**- Cl^-, **3**- Br^-, **4**- I^-, **5**- N_3^-,**6**- NCO^-, **7**- $H_2PO_4^-$, **8**- SO_4^{2-}, **9**- OAc^-, **10**- NO_3^-, **11**- NO_2^-, **12**- CN^-, **13**- **Mix**. (solutions of F^-, Cl^-, Br^-, I^-), **14**- **Mix**. (solutions of NCO^-, $H_2PO_4^-$, SO_4^{2-}, OAc^-, NO_3^-) in HEPES buffer (0.1 M, CH_3CN: water, 4:1,v/v).

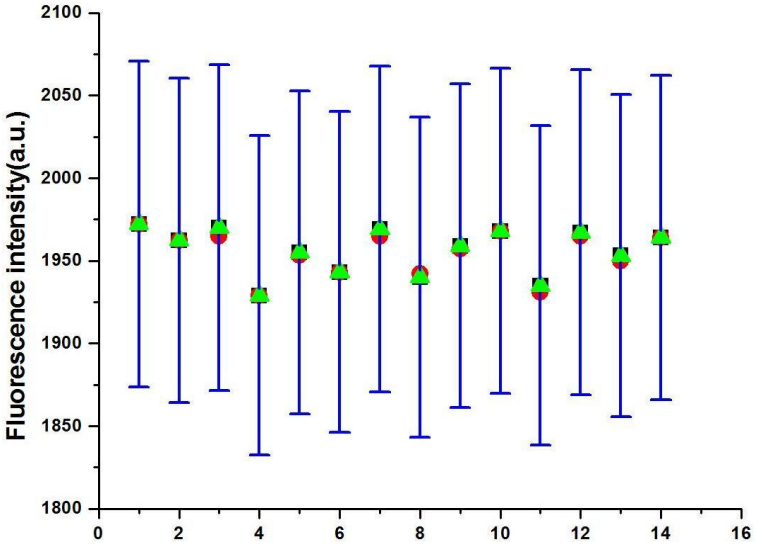


Fig. S9 Error bars related to the fluorescence intensity of [**RHENTU**-ClO_4^-] system in presence of different anions : **RHENTU** (50 μM) + ClO_4^- (400 μM) + other anions (400 μM), where other anions = (**1**- F^-, **2**- Cl^-, **3**- Br^-, **4**- I^-, **5**- N_3^-,**6**- NCO^-, **7**- $H_2PO_4^-$, **8**- SO_4^{2-}, **9**- OAc^-, **10**- NO_3^-, **11**- NO_2^-, **12**- CN^-, **13**- **Mix**. (solutions of F^-, Cl^-, Br^-, I^-), **14**- **Mix**. (solutions of NCO^-, $H_2PO_4^-$, SO_4^{2-}, OAc^-, NO_3^-), N = 3.

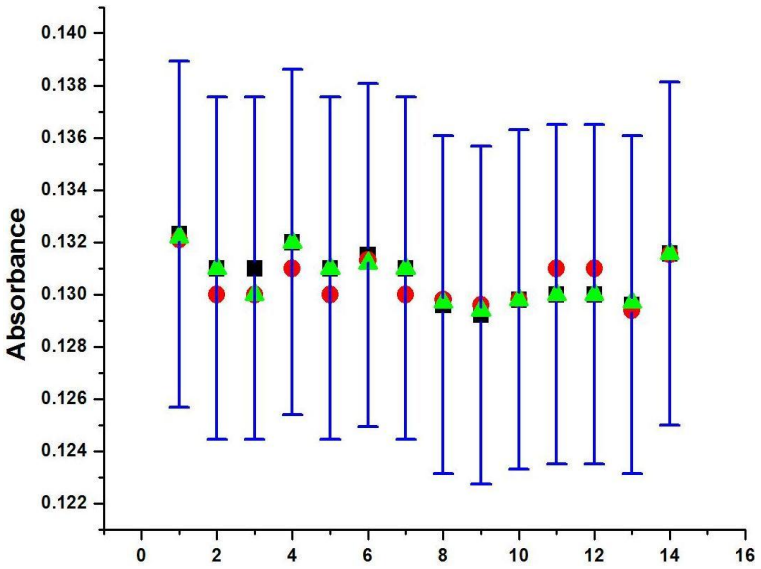


Fig. S10 Error bars related to the absorbance of [**RHENTU**-ClO_4^-] system in presence of different anions : **RHENTU** (50 μM) + ClO_4^- (400 μM) + other anions (400 μM), where other anions = (**1**- F^-, **2**- Cl^-, **3**- Br^-, **4**- I^-, **5**- N_3^-,**6**- NCO^-, **7**- $H_2PO_4^-$, **8**- SO_4^{2-}, **9**- OAc^-, **10**- NO_3^-, **11**- NO_2^-, **12**- CN^-, **13**- **Mix**. (solutions of F^-, Cl^-, Br^-, I^-), **14**- **Mix**. (solutions of NCO^-, $H_2PO_4^-$, SO_4^{2-}, OAc^-, NO_3^-), N = 3.

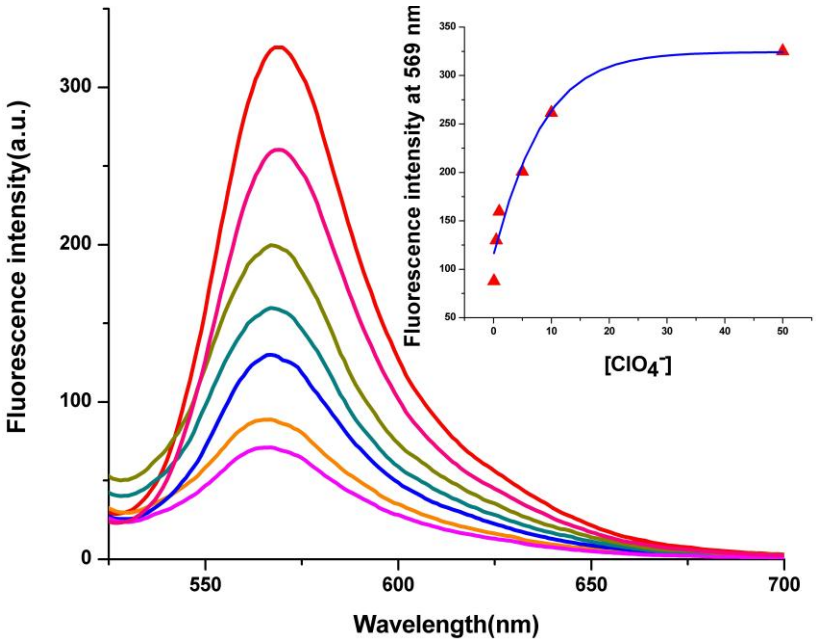


Fig. S11 Changes in the fluorescence spectra of **RHENTU** (0.1 µM) in HEPES buffer (0.1 M, CH_3CN: water, 4:1, v/v, pH 7.4) upon gradual addition of ClO_4^- (0, 0.1, 0.5, 1, 5, 10, 50 µM), (λ_{ex} = 520 nm, λ_{em} = 569 nm). Inset: plot of emission intensities of **RHENTU** (0.1 µM) as a function of ClO_4^- (0.5-50 µM, R^2 = 0.99).

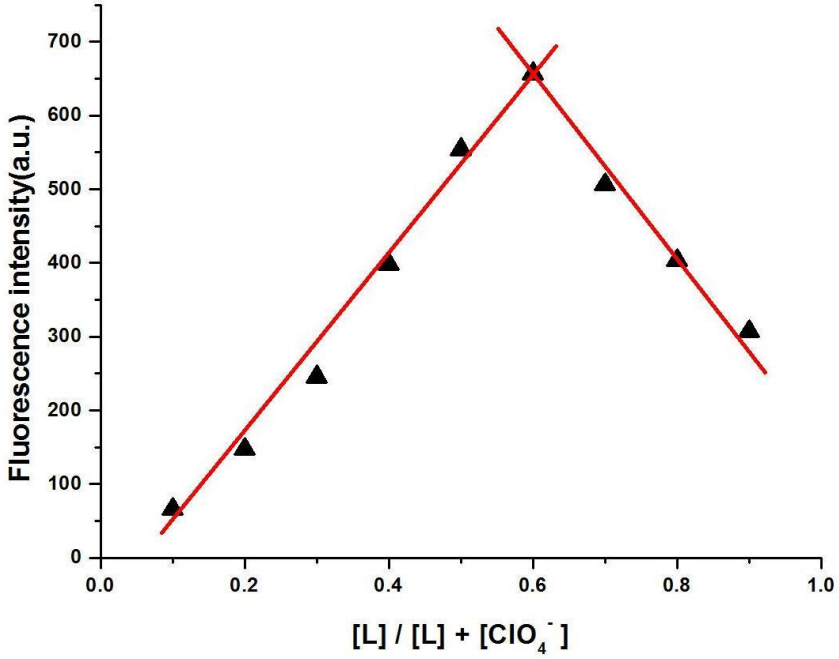


Fig. S12 Job's plot (stoichiometry determination of the [**RHENTU**-ClO_4^-] adduct) in HEPES buffer (0.1 M, CH_3CN /water = 4/1, v/v, pH 7.4). λ_{ex} = 520 nm, λ_{em} = 569 nm.

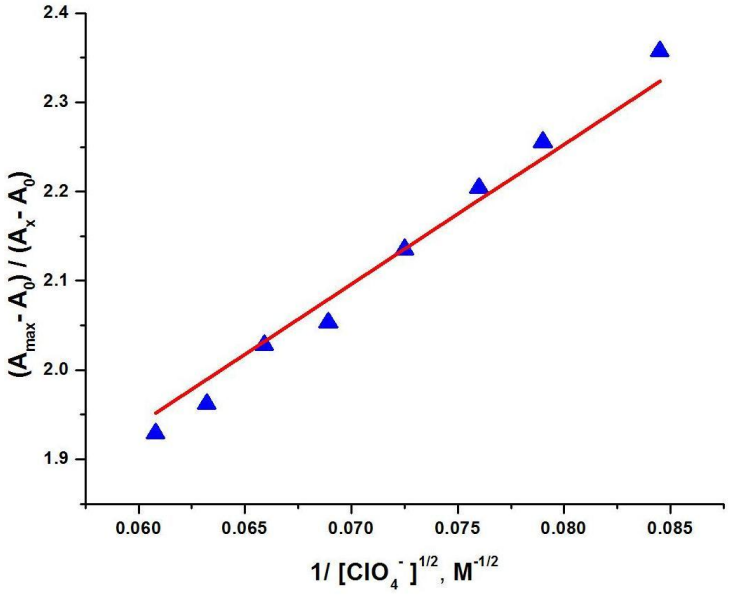


Fig. S13 Estimation of binding constant (K) of **RHENTU** for ClO_4^- (R^2 = 0.995 when n = 1/2).

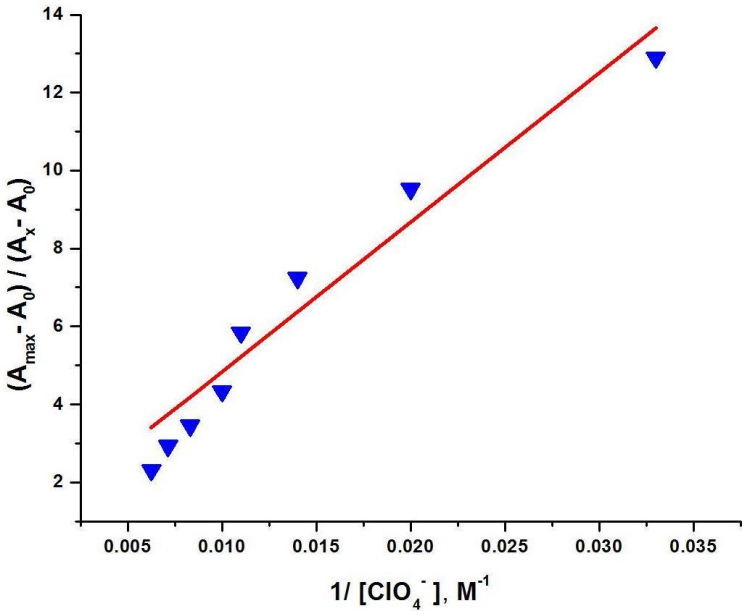


Fig. S14 Determination of binding constant (K) of **RHENTU** for ClO_4^- (R^2 = 0.883, n = 1).

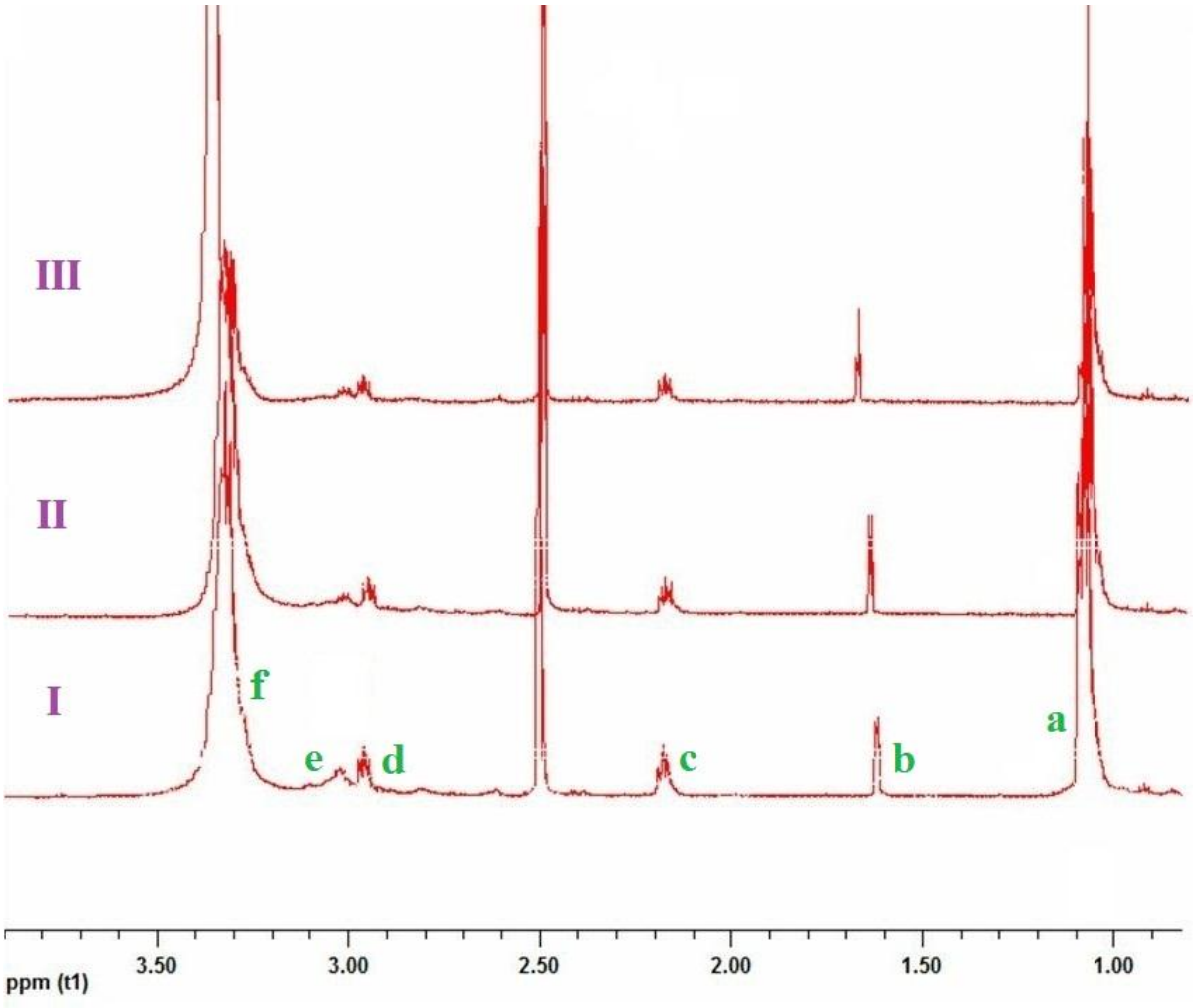


Fig. S15a Changes in the ^{1}H NMR spectra (aliphatic region) of **RHENTU** (1.8×10^{-2} M) in presence of $NaClO_4$ in DMSO-d_6: (I) **RHENTU**; (II) **RHENTU** with 2.5 equivalent of $NaClO_4$; (III) **RHENTU** with 5.0 equivalent of $NaClO_4$.

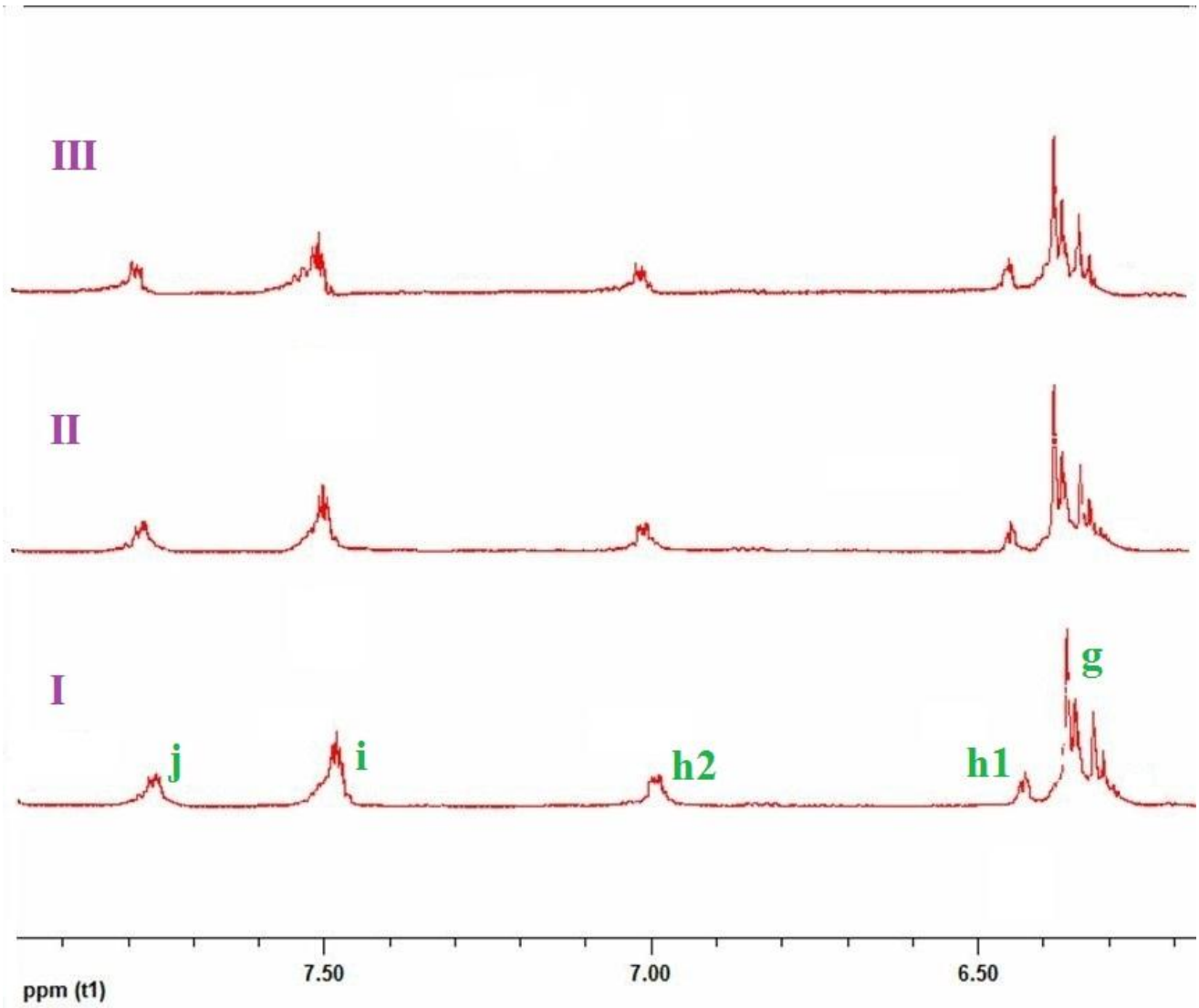


Fig. 15b Changes in the ^{1}H NMR spectra (aromatic region) of **RHENTU** (1.8×10^{-2} M) in presence of $NaClO_4$ in DMSO-d_6: (I) **RHENTU**; (II) **RHENTU** with 2.5 equivalent of $NaClO_4$; (III) **RHENTU** with 5.0 equivalent of $NaClO_4$.

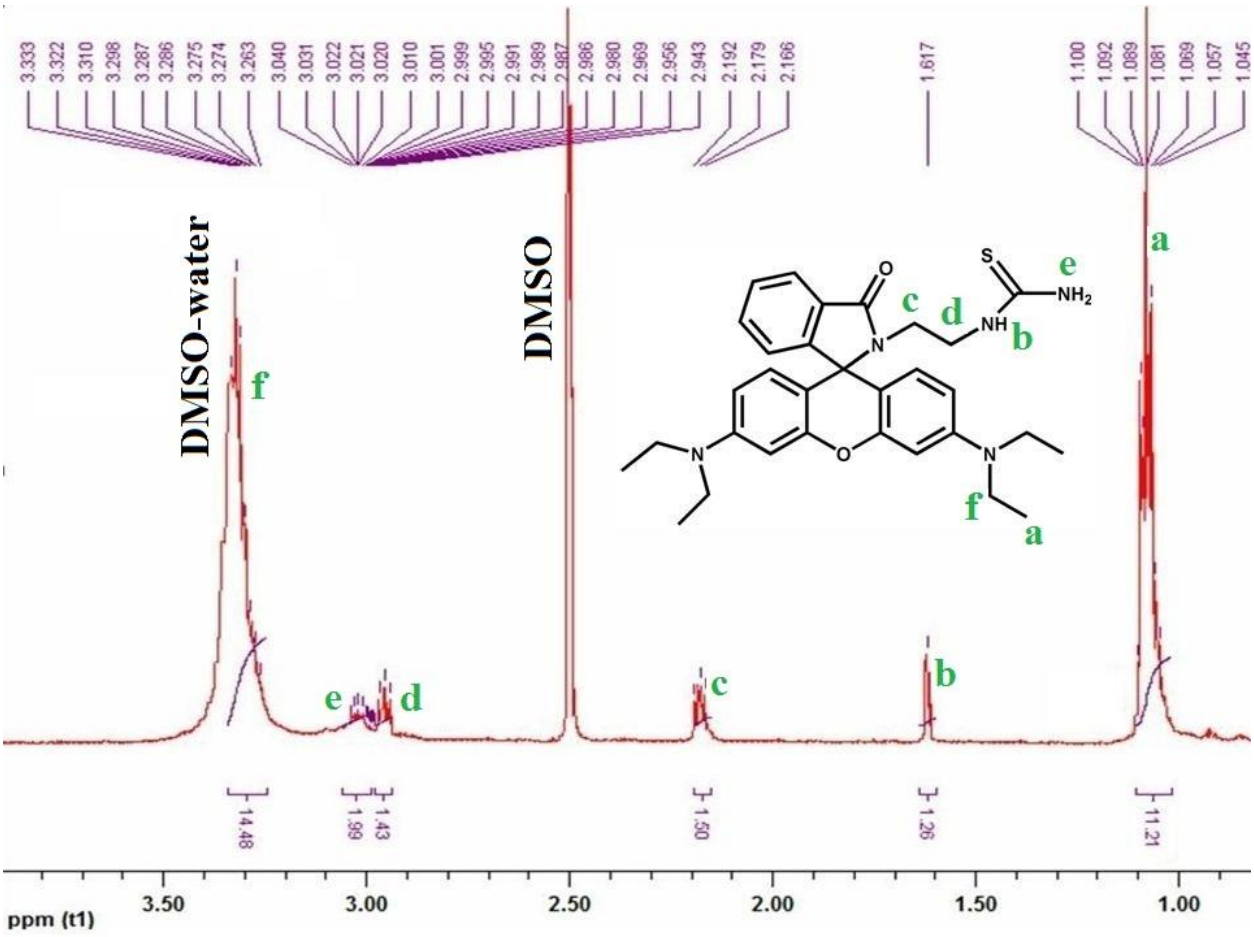


Fig. S16(a) ^{1}H NMR spectrum (aliphatic region) of **RHENTU** in DMSO-d_6

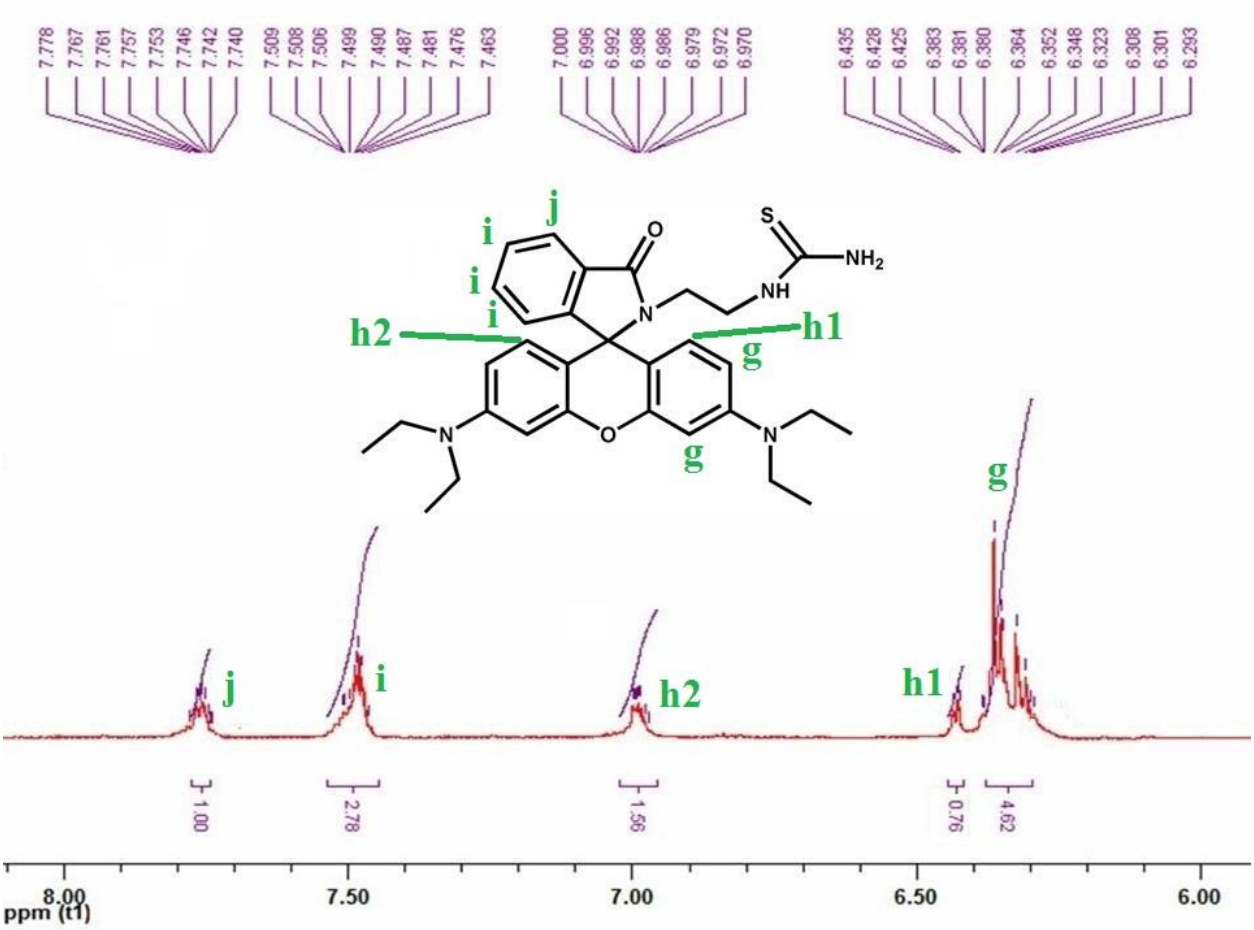


Fig. S16(b) ^{1}H NMR spectrum (aromatic region) of **RHENTU** in DMSO-d_6

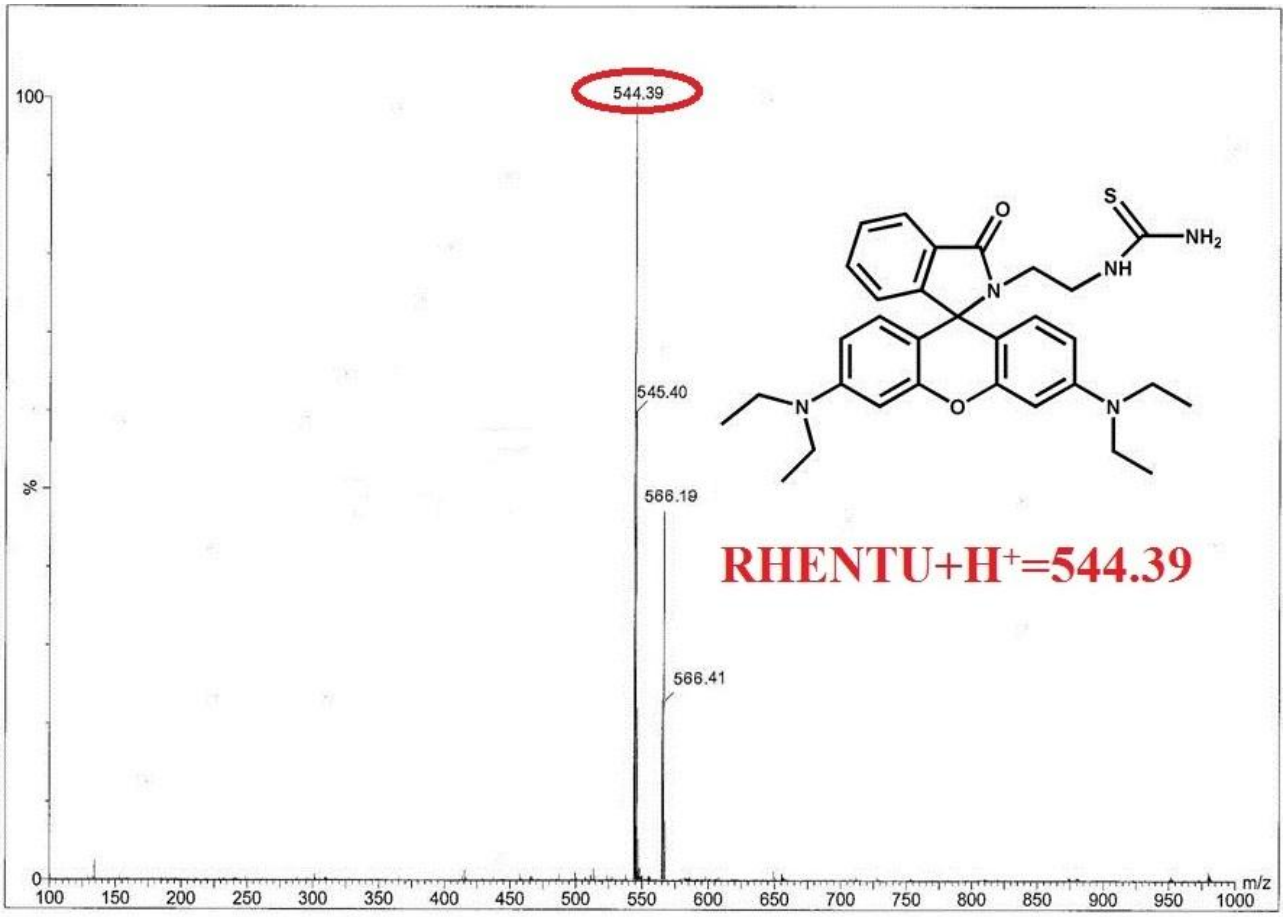


Fig. S17 QTOF-MS spectrum of **RHENTU**

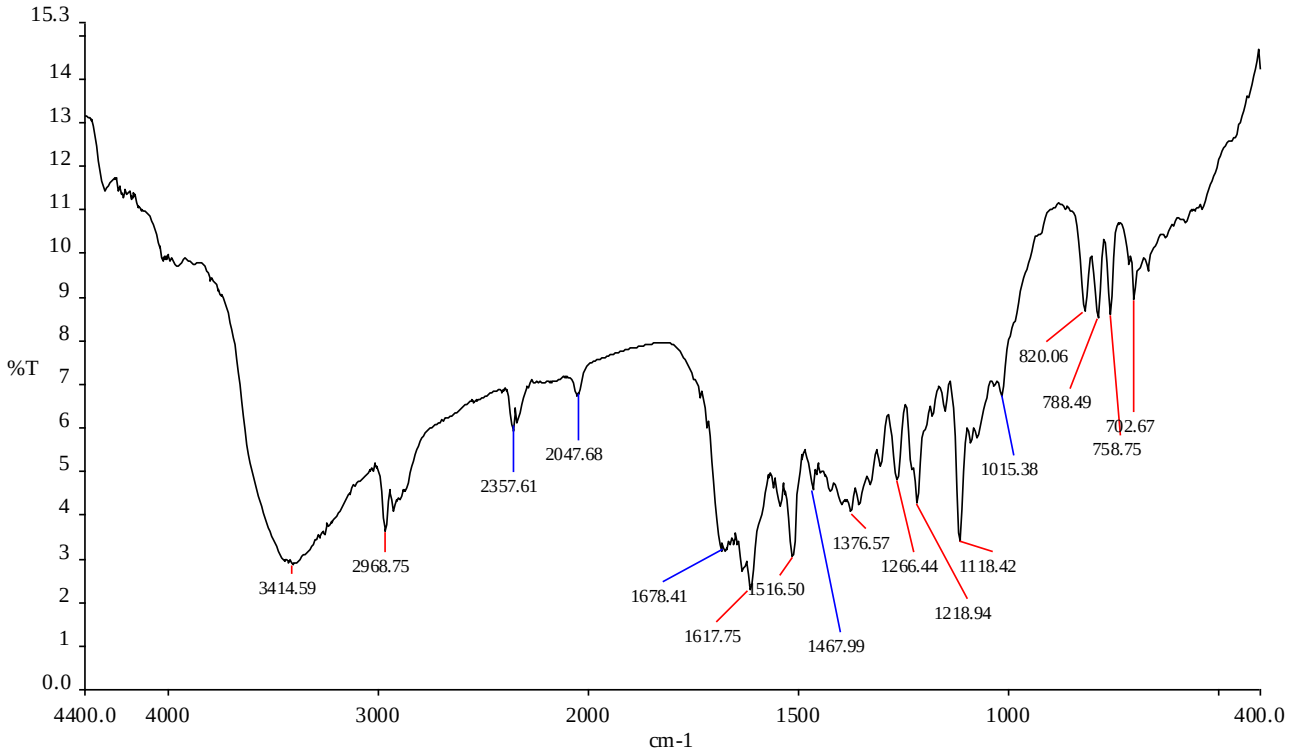


Fig. S18 FTIR spectrum of **RHENTU**.

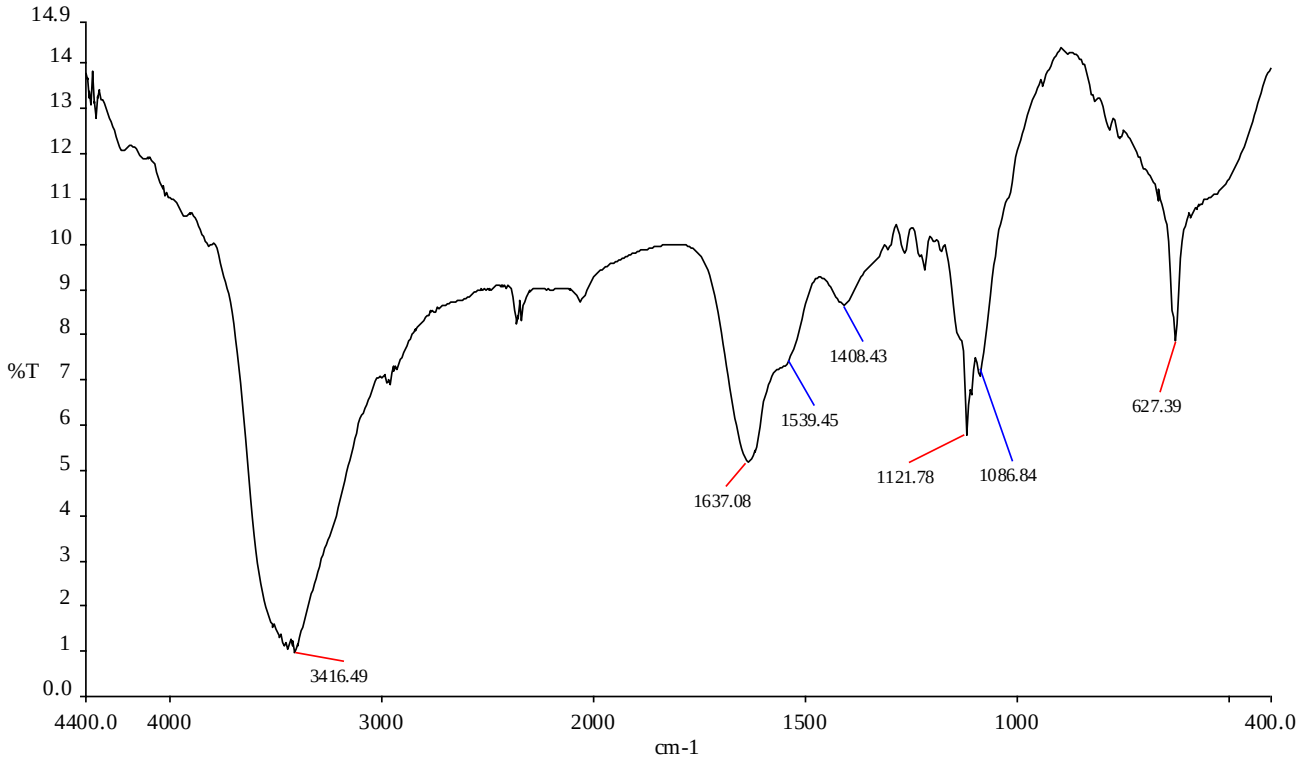


Fig. S19 FTIR spectrum of [**RHENTU**-ClO_4^-] adduct

Table S1 Changes in chemical shifts (δ ppm) of **RHENTU** during its ^{1}HNMR titration with ClO_4^-.

Protons	δ (ppm) for **RHENTU**		
	Free	In presence of 2.5 equivalent ClO_4^-	In presence of 5.0 equivalent ClO_4^-
a	1.10	1.10	1.10
b	1.61	1.64	1.67
c	2.19	2.19	2.19
d	3.01	3.01	3.01
e	3.04	3.05	3.06
f	3.33	3.33	3.33
g	6.38	6.39	6.40
h1	6.43	6.43	6.43
h2	7.00	7.00	7.00
i	7.50	7.51	7.52
j	7.77	7.78	7.79

References

(1) Austin, E.; Gouterman, M. *Bioinorg. Chem.*, **1978**, 9, 281.

(2) R. Kubin, *J. Lumin.*, 1983, **27**, 455.

(3) Long, G. L.; Winefordner, J. D. *Anal. Chem*., **1983**, 55, 712A.

(4) Wu, J.S.; Hwang, I.-C.; Kim, K. S.; Kim, J. S. *Org Lett*., **2007**, 9**,** 907.

(5) Zhao, M.; Yang, X.-F.; He, S.; Wang, L. *Sensors and Actuators B*., **2009**, *135*, 625.