J. Biol. Chem. Chron. **2018, 4***(2),* 48-53
ISSN (Print): 2454 – 7468
ISSN (Online): 2454 - 7476
www.eresearchco/jbcc/

Synthesis of Resorufin Based Chromogenic and Fluorescent Probe for Detection of Mercury

Milind Shamrao Thakare[1*] and Pravin Madhavrao Yeole[2]

[1 &*] *Synthetic Organic Research lab, Department of chemistry, Pratap college Amalner, Maharashtra, INDIA*
[2] *P. G. Research Lab, R. L. College Parola, Maharashtra, INDIA*
[*] *Correspondence: E-mail: milindthakare75@gmail.com*

(Received 18 Aug, 2018; Accepted 09 Sept, 2018; Published 19 Sept, 2018)

ABSTRACT: Mercury in its lipophilic and inorganic form is a major threat to the environment and to human health. It is therefore highly necessary to develop a chemosensor for mercury detection. A novel fluorescent probe for mercury, 7-(vinyloxy)-3H-phenoxazin-3-one has been designed and synthesized by anchoring resorufin (helping as a fluorophore) with vinylic ether moiety. In present work we have designed a fluorescence strategy based on the π-electrophilicity. The Probe is essentially nonfluorescent which upon reaction with Hg(II) undergoes oxymercuration of vinylic doublebond followed by cleavage, complemented by the generation of strong fluorescence to detect traces of mercury in biological samples. The resorufin based probe responds selectively to mercury species, while reluctant or little responsive toward other analyte metal ions. Significant colorimetric and fluorescence change from colourless to pink through the anchoring and unanchoring process is being observed.

Keywords: Mercury; Resorufin; Chemosensor; Chromogenic and Fluorescence.

INTRODUCTION: Mercury contamination is still a universal problem due to geological events and the extensive use of mercury species in modern human activities.[1-4] Mercury exists in numerous forms metallic, organic, and inorganic to which people may be exposed through their occupation.[5-10] Elemental and lipophilic organo-mercury are toxic to the central and peripheral nervous systems. The inhalation of mercury vapours can produce damaging effects on the nervous, digestive and immune systems, lungs and kidneys,[11-14] and may be fatal. The inorganic salts of mercury are corrosive to the skin, eyes and gastrointestinal tract, and may induce kidney toxicity if consumed .Ingestion of methylmercury-contaminated fish and grain triggered the epidemics in Japan and in Iraq specifically known as Minamata disease.[15-16] The toxicity of Hg^{2+} in human caused by the coordination with biological ligands such as proteins, DNA and enzymes[17-18] due to its affinity towards thiol group. Its significant hazards to public health because of its presence in drinking water.

Considering the facts associated with mercury poisoning, efficient detection and quantification tools for mercury species which have been developed for evaluating their contamination in the environment and living species. For instance, mercury is generally quantified by Cold vapor atomic absorption spectroscopy[19-20] or Inductively coupled plasma mass spectroscopy[21-22] or Gas chromatography[23]. Although these methods are quantitative and powerful, the analyses require large and expensive instruments, highly trained personnel, and tedious maintenance. Other methods which have been reported were based on various strategies like reaction based strategy and complexation based strategies, with different fluorophores like Fluorescein, Coumarin , Rhodamine, Pyrene.[24-33] The development of rapid, cost-effective colorimetric sensors for the easy and fast detection of toxic metal ions by the naked eye without resorting to any expensive instruments is still an active ongoing research area.

A chemodosimetric approach based on mercury-triggered reaction has emerged as a powerful tool in this area. These reaction-based probes can provide superior selectivity towards Hg^{2+} with large spectroscopic changes. In the present work, we devised a Hg-responsive fluorogenic probe whose molecular composition is elaborately designed to make the inner chemical environment optimal for the sensing mercury.

In continuation of our work on metal sensing using fluorescent probes.[34] We herewith described a colorimetric and fluorometric mercury assay system which

is visible to the naked-eye, and this method can be used as Hg-monitoring system. Probe was designed based on resorufin as the chromophore, vinylic ether group as the cleavable group through mercury initiated hydrolysis reaction. Resorufin is selected as the chromophore based on its excellent chromogenic and fluorogenic signal behaviors. Resorufin is a strong pink fluorescent dye with maximal emission, is visible to the naked-eye as a pink color, has good water-solubility, and can be applied in biological analysis with easy signal readout and nontoxic properties, and is used to analyze intracellular processes without cell damage. The alkylation of the hydroxy of resorufin effectively quenches its fluorescence emission.

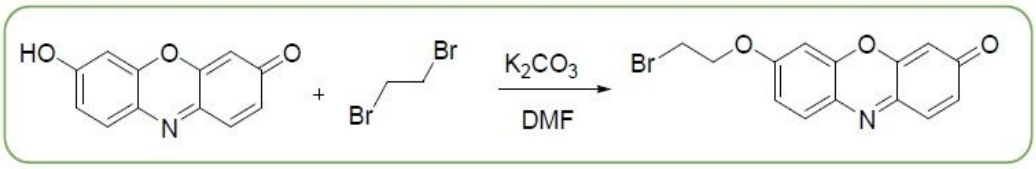
MATERIAL AND METHODS: All reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified and dried by standard methods. Redistilled water was used throughout all experiments. All reactions were carried out in oven or flame dried vials with magnetic stirring under nitrogen atmosphere. Dried solvents and liquid reagents were transferred by oven-dried syringes or hypodermic syringe cooled to ambient temperature in a desiccators. All experiments were monitored by analytical thin layer chromatography (TLC). TLC was performed on pre-coated silica gel plates. After elution, plate was visualized under UV chamber. Further visualization was achieved by staining $KMnO_4$ and charring on a hot plate.

^1H NMR spectra and ^{13}C NMR spectra were recorded with Bruker AV, 200/400/500 MHz spectrometers in appropriate solvents using TMS as internal standard or the solvent signals as secondary standards and the chemical shifts are shown in δ scales. Multiplicities of ^1H NMR signals are designated as s (singlet), br. s (broad singlet), d (doublet), dd (doublet of doublet), t (triplet) and m (multiplet) All measurements were carried out at room temperature. Fluorescence spectra measurements were performed with a Perkin–Elmer UV/Vis Spectrophotometer and a Photon Technology International, Quanta Master 400 Spectrofluorometer, respectively, in degassed spectral grade solvents.

Design and Synthesis of Probe: Alkylation of resorufin (Scheme: 1) followed by base catalyzed dehydrohalogenation reaction (Scheme: 2) offers desired probe which on mercury promoted hydrolysis (Scheme 3) selectively converts nonfluorescent molecule to fluorescent molecule.

General procedure for synthesis of Probe:

Step 1: Synthesis of 7-(2-bromoethoxy)-3H-phenoxazin-3-one (Compound-A) - To a solution of resorufin (1 mmol) and K_2CO_3 (2 mmol) in DMF was added 1,2-dibromopropane (1 mmol) in one portion under an atmosphere of N_2. The reaction mixture was stirred at 60°C overnight. After evaporation of the solvent, the resulting light brown material was dissolved in DCM (100 mL). After removal of insoluble materials by Celite filtration, the filtrate was concentrated to give the product from which desired product was isolated as a solid by silica-gel column chromatography (eluent: hexane:EA = 1:1) in 60 % yield.



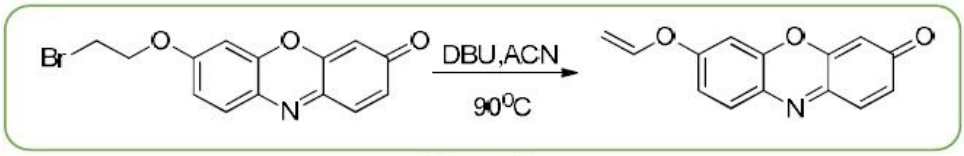
Scheme: 1
(Compound A)

NMR ($CDCl_3$, 500 MHz): δ (ppm) = 3.63 (2 H, t, J = 6.3 Hz,), 3.72 (2 H, t, J = 5.7 Hz), 6.33 (1 H, d, J = 1.8 Hz), 6.82-6.86 (2 H, m), 6.95 (1 H, dd, J = 9.1, 1.8 Hz), 7.43 (1 H, d, J = 9.7 Hz), 7.72 (1 H, d, J = 9.1 Hz) ,13C NMR (CDCl3, 125 MHz): δ (ppm) = 29.6 , 66.5, 100.9, 107.0, 114.1, 128.7, 131.8, 134.5, 134.9, 145.8, 145.9, 150.0, 162.8, 186.5; FT-IR 1606.7 cm–1 (C=O). M.S.:[M + Na]+: 353.0. Found: 353.1.

Step 2: Synthesis of 7-(vinyloxy)-3H-phenoxazin-3-one (probe) - To a stirred solution of compound A (1mmol) in CH_3CN (10 ml) was added DBU(1,8 diazabicyclo[5.4.0]undec-7-ene) (2 mmol). The mixture was stirred at 90˚C for 12 h under N_2 where the reaction was completed by TLC monitored. Then the solvent was evaporated by vacuum. Water (20 ml) was added and the solution was extracted with DCM (20 ml*3). The combined organic layers was washed with brine, dried over Na_2SO_4 and evaporated under vacuum. The residue was purified by silica gel column chromatography to give dsired probe as paly solid.

1H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 8.7 Hz, 1H), 7.36 (d, J = 9.8 Hz, 1H), 7.18 (s, 1H), 7.15 (dd, J = 8.7, 2.5 Hz, 1H), 6.80 (dd, J = 9.8, 2.0 Hz, 1H), 6.27 (d, J = 2.0 Hz, 1H), 6.02-5.88 (m, 1H), 5.44-5.36 (m,

1H), 5.31 (dd, J = 10.4, 1.1 Hz, 1H), 13C NMR (101 MHz, $CDCl_3$) δ 186.24, 153.57, 152.40, 149.26, 144.37, 135.24, 134.80, 131.31, 131.23, 130.65, 120.14, 118.54, 109.09, 107.33 HRMS (ESI): calcd for (M+Na)+ 262.23.



Scheme: 2

Spectral Characterization of Compound-A and Probe:

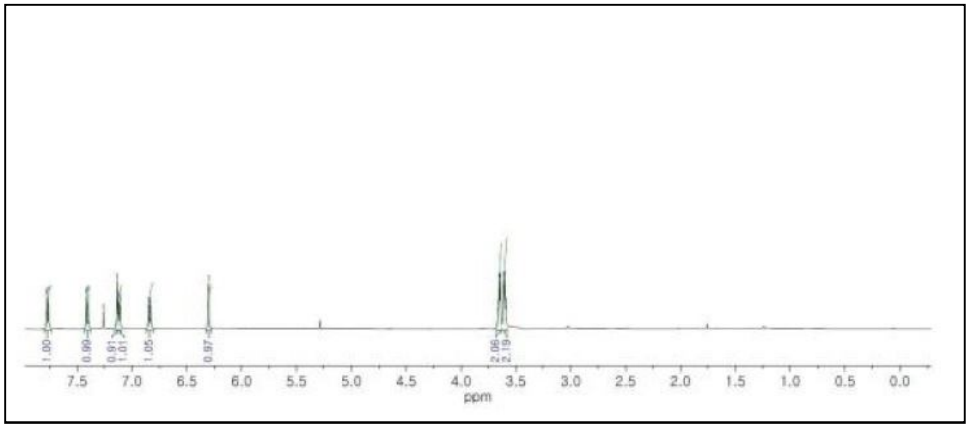


Figure 1: 1H NMR of compound A.

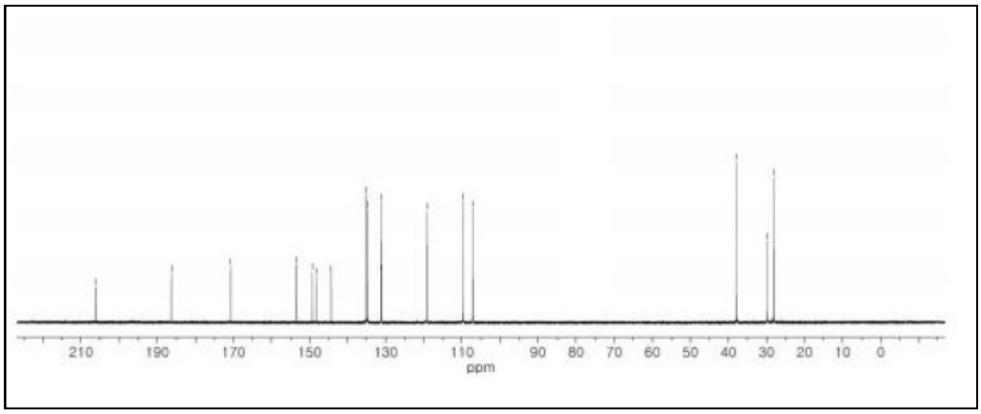


Figure 2: 13C NMR of compound A.

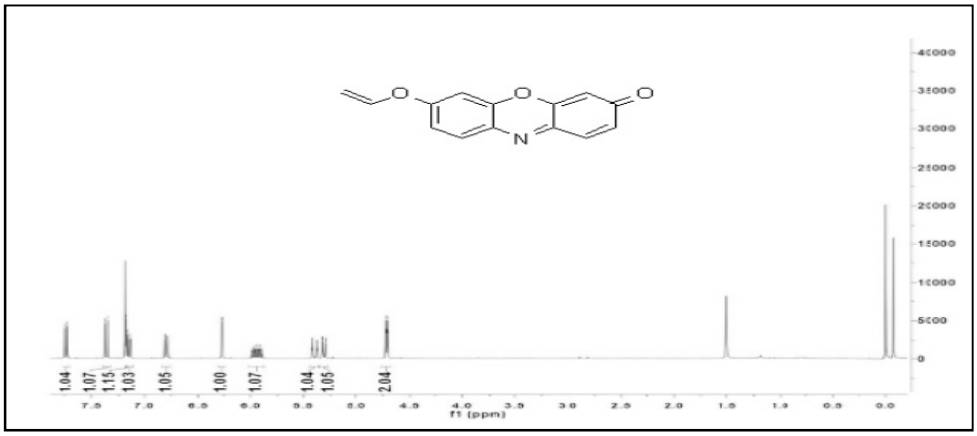


Figure 3: 1H NMR of Probe.

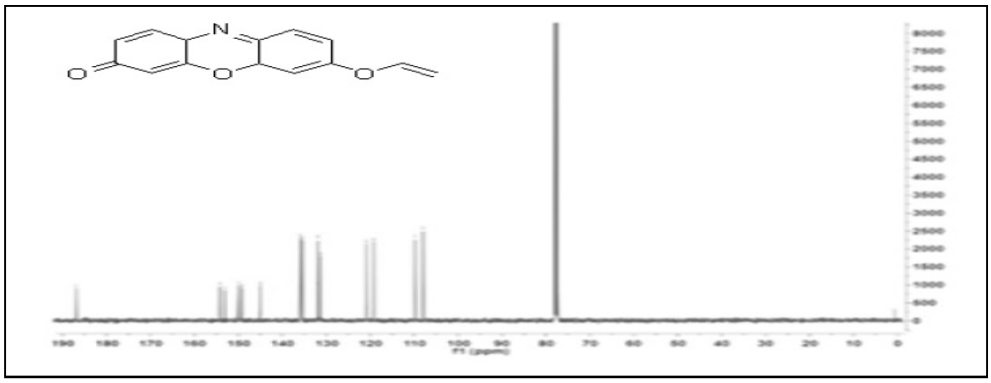
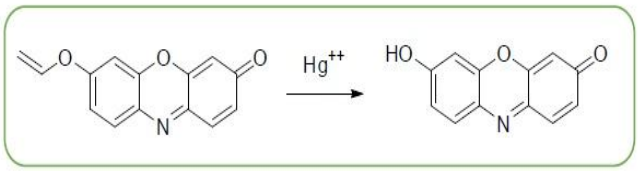


Figure 4: 13 C NMR of probe.

RESULTS AND DISCUSSION:

Response of probe 1 to mercury sensing experiment: Probe 1 was prepared by the reaction of the 7-hydroxy group of resorufin 1,2 dibromoethane. This on later treatment with DBU undergoes 1-2 elimination to produce desired probe in good to moderate yield. The absorption and fluorescence spectra of probe before and after reaction with Mercury are shown in Figure 5 .Probe 1 exhibited a very weak absorption in the long wavelength region. However, upon reaction with Hg^{++} (Scheme 3), its solution not only displayed a distinctive color change from nearly colorless to pink (Figure 5), but also produced strong signal at 575 nm. Moreover, probe 1 itself was weakly fluorescent, this low background signal was due to the strong quenching effect of the vinylic ether unit. However, its reaction with mercury in (Figure 6) fluorescence enhancement. The fluorescence enhancement of probe was attributed to the mercury-triggered cleavage reaction causing the release of free resorufin λ emission 595nm.



Scheme: 3

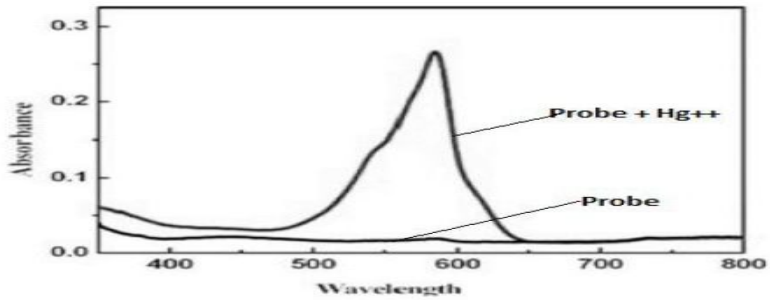


Figure 5: Absorption spectra at 25^0 C in PBS buffer (10 mmol /L, pH 7.4, 30%CH3CN, v/v).clearly shows that the probe alone with PBS buffer merely shows negligible absorbance but on addition of Hg^{++} there is tremendous increase in absorption at 575 nm.

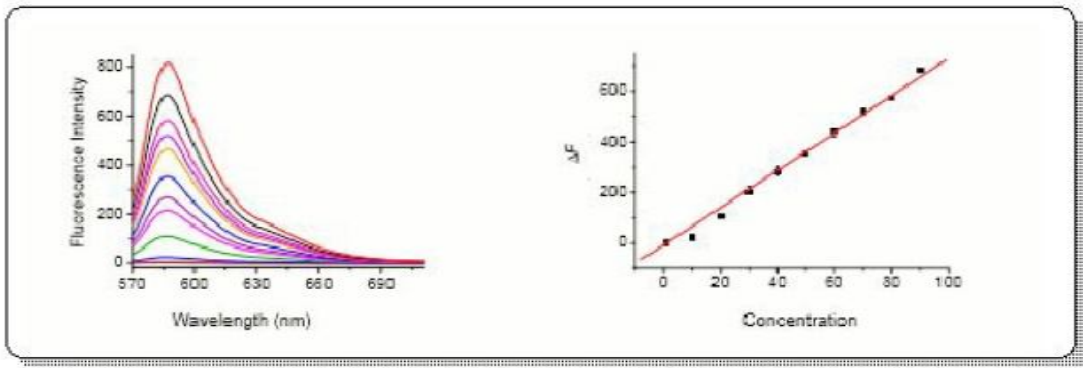
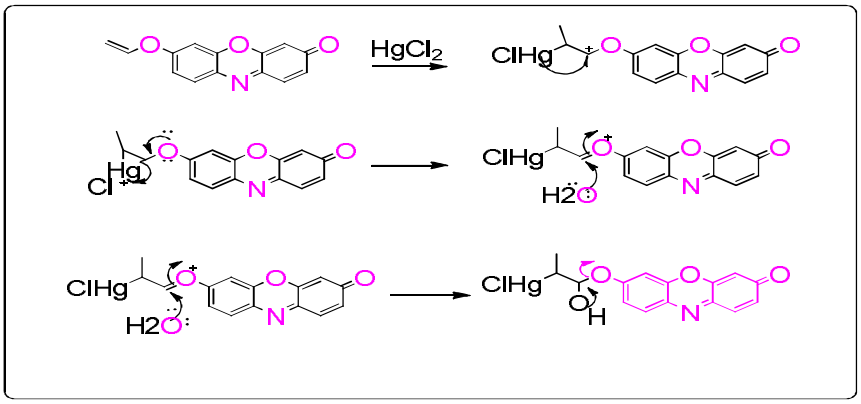


Figure 6: Fluorescence changes of probe 1 in presence of Hg (0-100 µM) in buffer λemission 595nm,linearity plot of fluorescence emission after gradual addition of Hg^{++}.

Mechanistic study:



CONCLUSION: In conclusion, we have proposed a new approach consisting of protection and deprotection of fluorophore for the recognition of Hg(II), which now a days a widely spread contamitant in ecosystem. The present probe is highly sensitive and selective enough in determination of mercury .We strongly believe that this approach of detection of notorious Hg (II) will definitely catch the attention scientific community and henceforth many probes based on this strategy may appear in near future The system described herein is highly efficient and highly sensitive in recognition of Hg(II) which represents a simple vinylic ether anchored Resorufin based fluorescent probe for Hg(II) ions, it combines the selectivity of Hg(II) efficient cleavage of vinylic ether bonds with the high sensitivity of fluorescence detection. In summary, the novel chromogenic and fluorescent probe based on the release of resorufin upon addition of Hg(II),the probe displayed drastic changes in UV-vis absorption and fluorescence emission intensities selectively for Hg(II).We expect that this probe shall be further useful in identification of Hg(II) at cellular level.

ACKNOWLEDGEMENT: The author sincerely thanks Principal Pratap College, Amalner and Principal, R. L. College, Parola for providing necessary facilities.

REFERENCES:

1. Renzoni A, Zino F, Franchi E (1998), Mercury levels along the food chain and risk for exposed populations, *Environ Res*, 77, 68.
2. Von Burg R., Greenwood M.R. (1991) Handbook on Metals in Clinical and Analytical Chemistry *VCH, Weinheim*, 1045.
3. Boening D. W. (2000) Ecological effects, transport, and fate of mercury: a general Review, *Chemosphere*, 40 ,1335,
4. Richardson S. D., Temes T. A. (2005) Water analysis: emerging contaminants and current issues - NCBI, *Anal Chem.*, 77, 3807.
5. Renzoni A., Zino E. Franchi (1998) Mercury levels along the food chain and risk for exposed populations, *Environ. Res.*, 77, 68.
6. Gustin M. S., Coolbaugh M. F., Engle M. A., Fitzgerald B. C. (2003) Environmental Forensics: Contaminant Specific Guide, *Environ. Geol.*, 43, 339.
7. Clarkson T. W., Magos L., Myers G. J. (2003) The toxicology of mercury-current exposures and clinical manifestations, *New Engl J Med*, 30(349), 1731.
8. Harris H. H., Pickering I. J. and George G. N. (2003) The chemical form of mercury in fish, *Science*, 301 (5637), 1203.
9. Nolan E. M. and Lippard S. J. (2008) Tools and Tactics for the Optical Detection of Mercuric Ion*Chem Rev*., 108(9), 3443.
10. Wang Q, Kim D, Dionysiou D. D., Sorial G. A. and Timberlake D.(2004) Sources and remediation for mercury contamination in aquatic systems--a literature review, *Environ Pollut.*, 131(2), 323.
11. M. F. Wolfe, S. Schwarzbach, R. A. Sulaiman (1998) Effects of mercury on wildlife: a comprehensive review, *Environ. Toxicol and Chem.*, 17, 146.
12. Boening D. W. (2000) Ecological effects, transport, and fate of mercury: a general review, *Chemosphere*, 40, 1335.
13. Tchounwou P. B., Ayensu W. K., Ninashvili N., Sutton D., (2003) Environmental exposure to mercury and its toxicopathologic implications for public health, *Environ. Toxicol.*, 18 ,149,
14. Nolan E. M., Lippard S. J., (2008) Tools and tactics for the optical detection of mercuric ion, 108, 3443

15. Takeuchi T., Morikawa N., Matsumoto H., Y. Shiraishi (1962)Grating test of contrast sensitivity in patients with Minamata disease, *Acta Neuropathol*, 2,40

16. Harada M. (1995) Minamata disease: methylmercury poisoning in Japan caused by environmental pollution, *CritRev Toxicol*, 25, 1.

17. Carvalho C. M. L., Chew E. H., Hashemy S. I., Lu J, A. Holmgren (2008) Inhibition of the human thioredoxin system. A molecular mechanism of mercury toxicity, *J Biol Chem*, 283, 11923.

18. Clarkson T. W., Magos L., Myers G. J. (2003) The toxicology of mercury-current exposures and clinical manifestations, *New Eng J Med*, 349, 1731.

19. Vil'pan Y. A., Grinshtein I. L., Akatov A. A., Gucer S. (2005) Direct atomic absorption determination of mercury in drinking water and urine using a two-step electrothermal atomizer, *J. Anal. Chem.*, 60, 38.

20. Han F. X. X., Patterson W. D., Xia Y. J., Sridhar B. B., Y. Su, (2006) Rapid Determination of Mercury in Plant and Soil Samples Using Inductively Coupled Plasma Atomic Emission Spectroscopy, a Comparative Study, *Water Air Soil Poll.*, 170, 161.

21. Moreton J. A., Delves H. T. (1998) Simple direct method for the determination of total mercury levels in blood and urine and nitric acid digests of fish by inductively coupled plasma mass spectrometry, *J. Anal. Atom. Spectrom.*, 13, 659-665.

22. Chai Z. F., Zhang Z. Y., Feng W. Y., Chen C. Y., Xu D. D. and Hou X. L. (2004) Study of chemical speciation of trace elements by molecular activation analysis and other nuclear techniques J. Anal. At. Spectrom. 1926, *J. Anal. Atom. Spectrom.*, 19, 26.

23. Rupp E. C., Granite E. J., Stanko D. C. (2010) Method for detection of trace metal and metalloid contaminants in coal-generated fuel gas using gas chromatography/ion trap mass spectrometry, *Anal. Chem*, 82, 6315.

24. Song, F.; Watanabe, S.; Floreancig, P. E.; Koide, K. (2008) Oxidation –Resistant Fluoregenic Probefor Mercury Based on Alkynes Oxymercuration, *J. Am. Chem. Soc.*, 130, 16460.

25. Ando, S.; Koide, K. (2011) Development and application of Fluoregenic Probe for Mercury Based on Vinyl Ether Oxymercuration, *J. Am. Chem. Soc.*, 133, 2556

26. Santra, M.; Roy, B.; Ahn, K. H. (2011) A "Reactive" Ratiometric Fluorescent Probe for Mercury Species, *Org. Lett*., 13, 3422.

27. Ma, X.; Wang, J.; Shan, Q.; Tan, Z.; Wei, G.; Wei, D.; Du, Y.. (2012) A "Turn-on" Fluorescent Hg^{2+} Chemosensor Based on Ferrier Carbocyclization, *Org. Lett.*, 14, 820.

28. Ding Jun, Li Huiyang, Wang Can, Yang Jie, Xie Yujun, Peng Qian, Li Qianqian, and Li Zhen (2015) "Turn-On" Fluorescent Probe for Mercury(II): High Selectivity and Sensitivity and New Design Approach by the Adjustment of the π-Bridge ACS Appl. Mater. Interfaces, 7 (21), pp 11369–11376.

29. Nolan, E. M.; Lippard, S. J. A (2003) "Turn-On" Fluorescent Sensor for the Selective Detection of Mercuric Ion in Aqueous Media, *J. Am. Chem. Soc.*, 125, 14270.

30. Yoon, S.; Albers, A. E.; Wong, A. P.; Chang, C. J. (2005) Screening mercury levels in fish with a selective fluorescent chemosensor, *J. Am. Chem. Soc.*, 127, 16030.

31. Yuan, M.; Li, Y.; Li, J.; Li, C.; Liu, X.; Lv, J.; Xu, J.; Liu, H.; Wang, S.; Zhu, D. (2007) A Colorimetric and Fluorometric Dual-Modal Assay for Mercury Ion by a Molecule, *Org. Lett.*, 9, 2313

32. Yang, M. H.; Thirupathi, P.; Lee, K. H. (2011) Selective and Sensitive Ratiometric Detection of Hg(II) Ions Using a Simple Amino Acid Based Sensor, *Org. Lett.*, 13, 5028.

33. Zheng, H.; Zhang, X. J.; Cai, X.; Bian, Q. N.; Yan, M.; Wu, G.H.; Lai, X. W.; Jiang, Y. B. (2012) Ratiometric Fluorescent Chemosensor for Hg2+ Based on Heptamethine Cyanine Containing a Thymine Moiety, *Org. Lett.*, 14, 1986.

34. Patil Nitin T., Shinde Valmik S., Thakare Milind S., Kumar P. Hemant, Bangal Prakriti. R., Baruic Ayan Kumar and Patra Chitta Ranjan (2012) Exploiting the higher alkynophilicity of Au-species: development of a highly selective fluorescent probe for gold ions, *Chem. Commun.*, 48, 11229-11231.