DOCTORAL THESIS

Development of fluorescent chemosensors based on different signal transduction mechanisms

You, Qihua

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Abstract

A series of fluorescent probes based on different signal transduction mechanisms for the detection of Fe^{3+}, Zn^{2+}, histidine and pH was designed and synthesized. Their photophysical properties, binding abilities and the further application in cell imaging were fully evaluated.

Building on the groundwork of our previous study, molecular scaffold **19** has been appended to spirobenzopyran fluorophore to furnish a highly selective and sensitive Zn^{2+} sensor. To broaden the application scope of this trifunctional receptive molecule, **19** was incorporated onto rhodamine, antipyrine and coumarin moieties to give **20**, **21** and **23**, respectively. Probe **20** operative on a chelation-enhanced fluorescence mechanism exhibited highly selective response to Fe^{3+} with 2:1 stoichiometry of **20**-Fe^{3+} complex. However, a possible tendency of probe **20** to hydrolyze induced by Fe^{3+} and the unsuccessful attempt of cell imaging would limit its application scope.

Probe **21** with *O*-*N*-*N*-*N*-*N*-ligand showed a highly selective and sensitive detection of Zn^{2+}. The probe displayed suppressed response to Cd^{2+} which is the most common interference ion in zinc metal detection. The binding of Zn^{2+} to probe **21** inhibited the photoinduced electron transfer process originating from the lone pair of the nitrogen atom in the antipyrine moiety to quinoline fluorophore. Therefore, a turn-on fluorescent probe was developed. A moderate binding constant with 1:1 stoichiometry of **21**-Zn^{2+} complex was established by fluorescence titration. The binding mechanism was fully explained by ^{1}H NMR

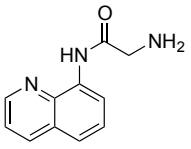
titration. To our delight, probe **21** was successfully applied for recognizing Zn^{2+} in living cells.

The preparation of probe **23** was achieved by appendage of **19** to coumarin derived fluorophore and the probe exhibited a good selectivity and fluorescent turn-off property to Cu^{2+}. The 1:1 stoichiometry of **23**-Cu^{2+} ensemble can serve as an efficient probe for the detection of histidine and biothiols. In the presence of NEM, the influence of biothiols could be eliminated. Furthermore, this sensing ensemble was also used in the detection of histidine in hard-to-transfect U87MG cells with very low cytotoxicity.

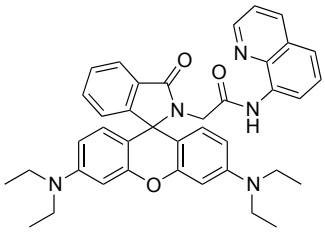
Based on our group's previous work on the spiropyran platform, a novel ratiometric near-infrared pH probe **27** operating on an excited-state intramolecular electron transfer mechanism was developed. The pK_a was calculated to be 5.9 and the ring-opening/ring-closing mechanism triggered by protons was reasonably explained by ^1H NMR titration. However, this spiropyran-based probe was found to be unsuitable for cell imaging.

To continue the innovation of pH sensing and extend its application in bioimaging, a series of ratiometric pH probes **32** and **38** characterized by their high quantum yield working in the NIR range was developed. The appendage of *N*,*O*-disubstituted hemiaminal ether moiety onto coumarin fluorophore with C=C double bond conferred the sensory material with the ability to display a pH-dependent ratiometric output operating on the ring-opening/ring-closing mechanism. The pK_a of **32** and **38** were 6.9 and 5.8 – 6.0, respectively, which

rendered them suitable for pH measurement in near-neutral and acidic media. A preliminary work of intracellular pH measurement was also conducted and promising results were obtained.



19



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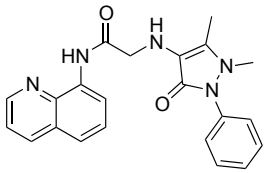

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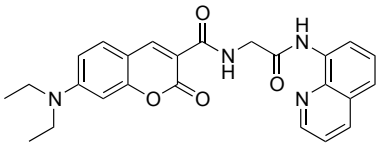

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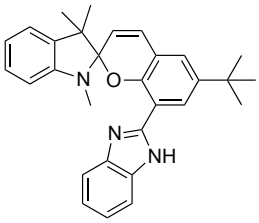
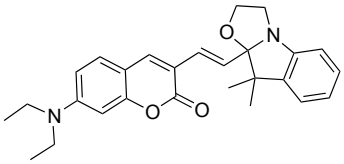
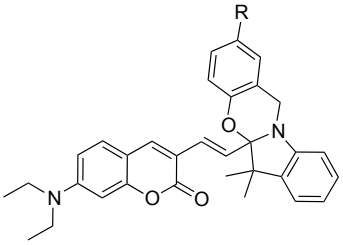


27



32



38-H: R = H
38-CF_3: R = CF_3
38-OMe: R = OMe

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