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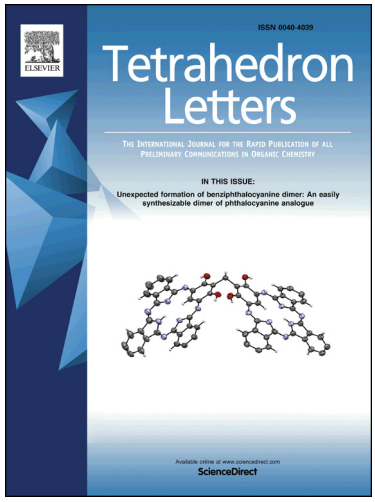

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A Novel HPQ-based Turn-on Fluorescent Probe for Detection of Fluoride Ions in Living Cells

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ABSTRACT

2-(2'-hydroxyphenyl)-4(3*H*)-quinazolinone (HPQ) has been reported as a precipitating fluorescent molecule with excellent optical properties, such as large Stokes shift and strong fluorescence intensity. HPQF, a novel HPQ-based turn-on probe for localizable detection of fluoride ions, was designed, synthesized and fully characterized by ^{1}H NMR, ^{13}C NMR and HRMS. As a chemogenic fluoride probe, the *tert*-butyldiphenylsilane moiety of HPQF can be easily cleaved by fluoride. After spontaneous 1, 6-elimination, HPQ molecule was generated to emit fluorescence under the excitation light. Further study shows that HPQF exhibited high selectivity and sensitivity for detection of fluoride. In addition, HPQF was utilized for the detection of fluoride in living cells.

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Fluoride ions detection attracts many chemists' interest owing to its association with plenty of environmental, biological and chemical processes.[1] For example, excessive intake of fluoride could lead to many diseases such as fluorosis, urolithiasis, acute gastric, kidney injuries and even cancer.[2] However, fluoride ions also play an important role in dental health and has potential use for osteoporosis treatment.[3-5] In addition, fluoride could even be used in military such as nerve gas monitor[6] and nuclear weapon manufacture.[7] Therein many literatures have been reported for fluoride ions detection based on different mechanisms and technology over the past decades. Among them colorimetric and fluorescence sensors have attracted the greatest interest as they have high sensitivities and low limit of detection and are easy to performed even in live cell and in vivo.[8, 9] Enormous amounts of fluoride ions probes have been developed and the sensing strategies included reaction-based chemosensors,[10-12] fluoride-boron interaction-based chemosensors[13], hydrogen bond or π-π interaction based chemosensors[14], metal ions binding based chemosensors and nanoparticles based sensors.[15-18] For chemogenic fluoride probes, desilylation of Si-O bond was applied frequently due to its high sensitivity and selectivity.

Herein we developed a novel fluorescent probe based on the structure 2-(2'-hydroxyphenyl)-4(3*H*)-quinazolinone (HPQ), which is a solid-state fluorophore and has two-photon properties. HPQ was completely insoluble in aqueous media but could emit strong fluorescence in solid state.[19, 20] HPQ possessed many optical properties, such as large Stokes shift, intense luminescence and excellent photostability.[19] As known, poor solubility of HPQ leads to the precipitating fluorescent feature through excited state intramolecular proton transfer (ESIPT).[21] Blockade of phenolic group of HPQ could dismiss the ESIPT mechanism to result in turn-on probes. Given these advantages, HPQ was employed for further modifications to imaging certain enzymes, such as phosphatases, esterases, lipases, glucuronidase, lactamases and peptidases through corresponding trigger reactions.[22-25] Most recently, Tan and his co-workers designed and synthesized a HPQ-based probe for localizable and photoactivatable bioimaging with spatiotemporal and two-photon properties.[21] Blocked by the 2-nitrobenzyl group, a photolabile group which can be removed by ultra-violet light, HPQ cannot emit fluorescence any more. When the phenolic group was released by the light, precipitating fluorescence occurred. With a certain quaternary ammonium moiety which could target mitochondria, localizable imaging was realized in living cells. In addition, two tautomers of HPQ have different fluorescence. This feature is similar with some other fluorescent probes, especially acid-responsive probes.[26-29]

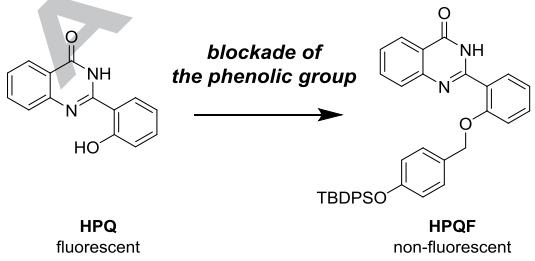
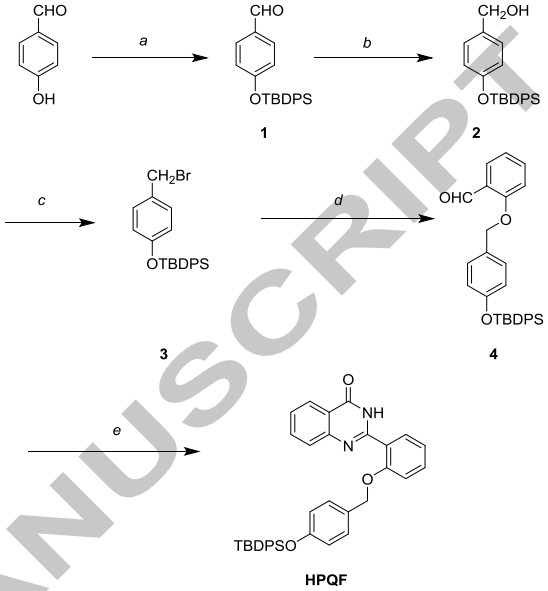


Fig. 1 Schematic illustration of the design of HPQF.

Inspired by this work, we attempted to introduce the *tert*-butyldiphenylsilane (TBDPS) group into HPQ (Fig. 1). As shown in Scheme 1. HPQF can be synthesized through five steps with satisfied yield.

All the chemicals were purchased from Adamas-Beta (Shanghai, China). $CDCl_3$ was purchased from Cambridge Isotope Laboratory. Milli-Q water was supplied by a Milli-Q Plus System (Millipore Corp., Breford, USA). All fluorescence measurements were carried out on a Shimadzu RF-5301 fluorescence spectrometer with both excitation and emission slits set at 5.0 nm.



Scheme 1 Synthetic route to HPQF. (a) TBDPSCl, imidazole, DMF, r.t; (b) $NaBH_4$, MeOH; (c) PBr_3, dichloromethane; (d) K_2CO_3, 2-hydroxybenzaldehyde, r.t; (e) 2-aminobenzamide, *p*-TSA, DDQ.

4-hydroxybenzaldehyde(2.45 g, 20 mmol) was dissolved in dichloromethane (30 mL) followed by imidazole (1.31 g, 19.2 mmol) added at room temperature for 15 min. tert-butylchlorodiphenylsilane (TBDPSCl, 5.2 mL) was added and stirred overnight. Washed by water (100 mL×3) then dried over anhydrous sodium sulfate before being filtered and the volatiles removed under vacuum. The residue was purified by a silica gel column chromatography to give compound 1 as a white solid (3.97 g, 54.6%).[30] ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) : 9.80(s, 1H), 7.75 ~ 7.44 (m, 12H), 6.91 (d, J = 8.4 Hz, 2H), 1.06 (s, 9H). ESI-MS $C_{23}H_{24}O_2Si$ $[M+Na]^+$ calcd 383.1, found 383.1.

To a solution of compound 1 (2.67 g, 7.4 mmol) in methanol (100 mL) in ice bath, sodium borohydride (627 mg, 16.5 mmol) was added slowly. After being stirred for 3 h, the reaction mixture was poured into water to quench the rest sodium borohydride. The volatiles were removed to minimum volume and extracted with ethyl ether (100 mL×3) and dried over anhydrous sodium sulfate. The solvent was removed in vacuum to afford the compound 2 as colorless oil (2.67 g, 99.4%).[30] ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) : 7.68 ~ 7.44 (m, 12H), 7.07 (d, J = 8.4 Hz, 2H), 6.68 (d, J = 8.5 Hz, 2H), 1.03 (s, 9H). ESI-MS $C_{23}H_{26}O_2Si$ $[M+Na]^+$ calcd 385.2, found 385.2.

To a solution of compound 2 in dichloromethane (1.83 g), phosphorus tribromide (0.5 mL) was added slowly at 0 ℃ and stirred overnight. The mixture was poured into aqueous sodium bicarbonate, extracted with dichloromethane (50 mL × 3) and dried over anhydrous sodium sulfate. The solvent was removed to give title compound as colorless oil (1.07 g, 49.8 %).[30] ^{1}H NMR (300 MHz, DMSO-d6) δ (ppm) : 7.66 (d, J = 7.0 Hz, 4H), 7.49 ~ 7.42 (m, 6H), 7.23 (d, J = 8.0 Hz, 2H), 6.69 (d, J = 8.1 Hz,

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2H), 4.60 (s, 2H), 1.03 (s, 9H). ESI-MS $C_{23}H_{25}BrOSi$ [M-^{81}Br]$^+$, [M-^{79}Br]$^+$, calcd 347.2, 345.2 found 347.2, 345.2.

2-hydroxybenzaldehyde (273.0 mg, 2.2 mmol)was dissolved in acetonitrile (4 mL), then potassium carbonate (655.2 mg, 4.7 mmol) was added and stirred at room temperature for 10 min. Compound 3 (850.6 mg, 2.0 mmol) was added and stirred overnight. The solvent was removed, washed with water and extracted with ethyl acetate. After filtering, the volatiles removed and the residue was purified on a silica chromatography to give the title compound as yellowish solid (625.2 mg, 67.1 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) : 10.48 (s, 1H), 7.84 ~ 7.82 (m, 1H), 7.71 (d, J = 6.4 Hz, 4H), 7.53 ~ 7.35 (m, 7H), 7.16 (d, J = 8.3 Hz, 2H), 7.05 ~ 7.00 (m, 2H), 6.78 (d, J = 8.5 Hz, 2H), 5.03 (s, 2H), 1.10 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) : 189.75, 135.73, 135.47, 129.91, 128.70, 128.31, 127.75, 120.85, 119.89, 113.12, 70.40, 26.47ESI-MS $C_{30}H_{30}O_3Si$ [M+Na]$^+$ calcd 489.2 found 489.2.

Compound **4** (550.6 mg, 1.18 mmol) and 2-aminobenzamide (177.3 mg, 1.3 mmol) were dissolved in ethanol (40 mL) and stirred at 0 ℃ for 10 min to form precipitation. The resulting mixture was refluxed for 30 min. p-TSA (20 mg) was added and the mixture was refluxed for 1 h. After cooled to room temperature, 2, 3-dichloro-5,6-dicyano-1,4-benzoquinone (270.8 mg, 1.2 mmol) was added and stirred overnight with exposed to the air. The precipitation was filtered out, washed with cold ethanol and dried to give the HPQF (300.2 mg, 43.7 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) : 10.96 (s, 1H), 8.53 (d, J = 6.99 Hz, 1H), 8.29 (d, J = 7.86 Hz, 1H), 7.77 ~ 7.70 (m, 6H), 7.48 ~ 7.35 (m, 8H), 7.20 ~ 7.13 (m, 3H), 7.07 (d, J = 8.31 Hz, 1H), 6.81 (d, J = 8.37 Hz, 2H), 5.19 (s, 2H), 1.11 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) : 161.69, 157.05, 156.05, 150.88, 149.43, 135.60, 134.45, 133.08, 132.73, 131.58, 130.03, 128.92, 127.90, 127.65, 126.48, 126.47, 122.14, 121.36, 120.54, 120.34, 113.91, 71.55, 26.57. ESI-HRMS $C_{37}H_{34}N_2O_3Si$ [M+Na]$^+$ calcd 605.22309 found 605.22552.

Immediately prior to the imaging experiments, the cells were washed with phosphate-buffered solution (PBS), incubated with HPQF stock solution (final concentration 20 μM) for 60 min at 37 °C, then washed with PBS for three times. After incubated with 100 μM TBAF for another 0.5 h at 37 °C, the HepG2 cells were washed with PBS three times and imaging. Confocal fluorescence imaging were observed under an LSM700 confocal microscope (Zeiss, Oberkochen, Germany). Excitation wavelength of the laser was 405 nm. Emissions were set at 470–530 nm.

Over the past decades, the strategy that desilylation of Si-O bond was utilized frequently due to the high sensitivity and selectivity. Si-O bond can be attacked by nucleophilic fluoride ions easily, then the HPQ can be released through 1, 6-elimination. As shown in Fig. 1 and Scheme 1, HPQF was designed, synthesized and fully characterized by ^{1}H, ^{13}C NMR and HRMS (Detailed spectra can be found in Supplementary Materials).

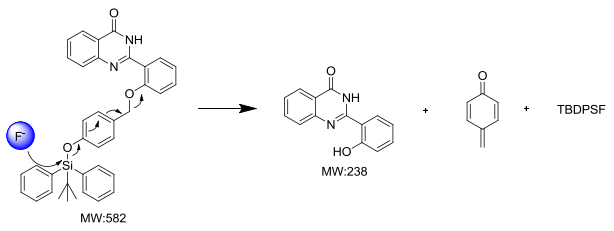

Fig. 2 Proposed mechanism of the turn-on detection of fluoride by HPQF.

The response of HPQF to fluoride ions was investigated. Since HPQ is insoluble in aqueous media, HPQF exhibited quite poor solubility due to its hydrophobic TBDPS group. Therefore, we attempted to use organic solvents to make HPQF dissolved. Enlightened by Tan and his co-workers[21], tetrahydrofuran (THF) was chosen to be the solvent to conduct the consequent reaction. The Si-O bond of the HPQF can be easily cleaved by fluoride ions to give the fluorescent product HPQ via spontaneously 1, 6-elimination (Fig. 2). Once the phenolic hydroxyl group was uncaged, the fluorescence can be excited by UV light. To confirm the desilylation mechanism, LC-MS method was used to give convinced evidence. HPQ was also synthesized to be a reference compound. As shown in the liquid choromatography (Fig. S4), the retention time of HPQ and HPQF are 3.36 min and 5.76 min, respectively. After the reaction conducted, the peak of HPQF decreased significantly and the peak of HPQ arose consequently, even though the baselines of the chromatograms were not smooth enough. Obviously, the mass spectra results of the corresponding time are consistent with the molecular weight of HPQ and HPQF (Fig. S5 & S6).

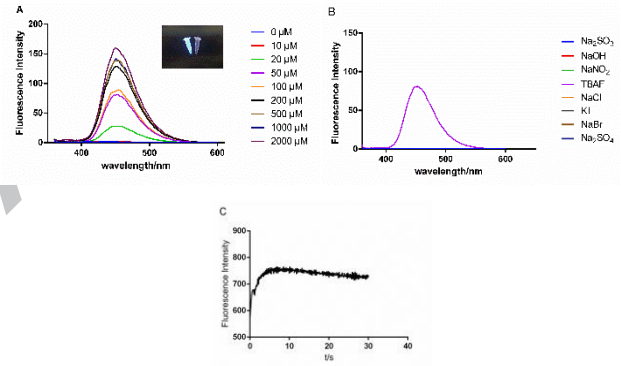

Fig. 3 (A) Fluorescence spectra of 200 μM HPQF response to different concentration (0 – 2000 μM) TBAF, inset was the reaction mixture (left) and HPQF solution (right); (B) Selectivity experiments of HPQF against a cast of anions. TBAF was dissolved in THF and the reaction was conducted in THF after adding HPQF. Other reactions were all carried on in aqueous media; (C) Kinetic curve of HPQF with TBAF in THF solution. Excitation wavelength 350 nm.

Since the mechanism of sensing fluoride had been elucidated, we put our attention on investigating the response of HPQF to fluoride. TBAF was dissolved in THF and diluted into different concentration (0, 10, 20, 50, 100, 200, 500, 1000 and 2000 μM). HPQF was dissolved in DMSO to give 20 mM stock solution. All the reaction was conducted at room temperature for 30 min and measured using Shimadzu RF5301 spectrometer.

As shown in Fig. 3A & B, HPQF exhibited good concentration-dependent fluorescent properties and excellent selectivity towards fluoride ions. Fluoride can be detected very quickly by HPQF in a few seconds (Fig. 3C). Interestingly, the reaction conducted in THF resulted in blue fluorescence which was emitted by the keto form of HPQ molecule. We calculate the limit of detection out as 140 nM.

Since fluoride ions have multiple functions in physical and pathological conditions, image fluoride ions in living cells could provide clinicians more information for clinical diagnosis. HepG2 cells were incubated with HPQF stock solution (final concentration 20 μM) for 60 min and then exited under laser confocal microscopy to detect fluoride in living cells (Fig. 4). The cells was incubated with fluorides ions for 4 hours before the HPQF was added. And from the left picture below we could see

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that HPQF was converted to HPQ and emitted green fluorescence. Though we conducted the experiment in PBS, HPQF still showed good response to fluoride.

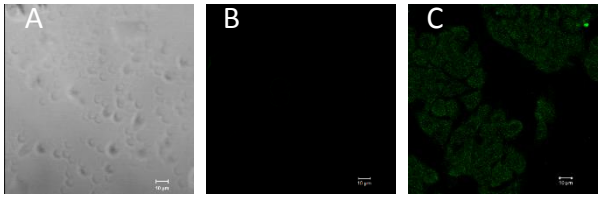

Fig. 4 Confocal imaging of fluoride (20μM) in HepG2 cells. (A) Bright field image of (B). (B) Image with no fluoride. (C) Image with fluoride ions incubation. λ_{ex}: 405nm. Scale bar: 10 μm.

In conclusion, we have designed and synthesized a novel HPQ-based probe for sensing fluoride in living cells. Given that HPQ has two photon properties, two photon imaging will be further investigated to realize in-depth imaging of fluoride in tissue pieces. Although detecting fluoride in living cells was realized, poor solubility was still a huge hindrance. To solve this problem, more derivatives containing water-soluble moieties would be designed and synthesized.

Acknowledgments

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2. HPQF exhibited high selectivity and sensitivity for detection of fluoride.

3. HPQF was utilized for the detection of fluoride in living cells.

Graphical Abstract

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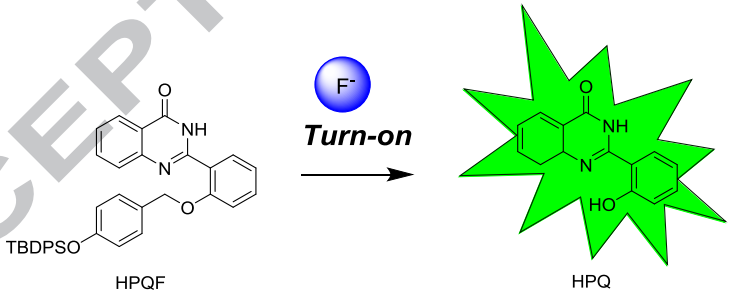

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