Synthesis and Application of Novel Benzothiazole Fluorescent Probes

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Abstract: As a new detection method, fluorescent probe plays a very important role in many fields. The variety of probe is more and more, the specificity of detection is more and more strong, the range is more and more wide, the detection sensitivity is more and more good. Compared with the traditional detection method, the fluorescence probe has higher selectivity and no obvious toxic and negative effect, and has good sensitivity and simple and convenient operation, can be very good for living cells or living animals, plants and other advantages of tracking imaging, Therefore, the development of new fluorescent probes has now become the focus of scientific research. In many fluorescence probes, the small molecule fluorescence probe has definite element composition and molecular weight, and has many advantages in synthesis, analytical performance, reproducibility and biological imaging. This article using ethanol as solvent, 2-aminophenol and 5-methylsalicylaldehyde as starting materials. A fluorescence probe targeting 2-(benzo [D] thiazole-2-yl) -6-(hydrazone methyl) -4-methylphenol (BTH-MPH) was designed and synthesized. Its structure was characterized by hydrogen spectrum, carbon spectrum and mass spectrum analysis. The absorption spectrum of the probe was measured by fluorescence photometer, and the cytotoxicity of the probe was measured by CCK-8 method, and the imaging of the cells was detected by confocal laser microscopy. The results show the target probe has the correct structure, pH range 3-8, and strong absorption peak at 510 and 570 nm. The cell survival rate was above 92.34% after 24 hours incubation at 7 concentrations in the range of 0-50 μm, the target probe can be used to detect living cells.

Keywords: Benzothiazole; Fluorescent Probe; Cytotoxicity

1. Introduction

Fluorescence is a common luminescence phenomenon in nature. When a fluorescent molecule is excited by energy such as light, electricity and chemistry, the electrons will transition from the ground state to the excited state, emission of photons by radioactive decay back to the ground state to produce fluorescence [1] .Fluorescence probe is a kind of small molecule compound which can produce fluorescence signal response to ph, temperature, ion and so on.[2].Compared with instrumental techniques, organic small molecule fluorescence probes have the advantages of simple synthesis, better batch-to-batch reproducibility and easy purification, it is a small molecule fluorescence probe that can transform the interaction between molecules into fluorescence signal tool. Is an important tool widely used in modern scientific and medical fields, such as analytical and environmental chemistry, biochemistry, materials science, clinical diagnosis, molecular biology, biotechnology, etc.[3]. Fluorescence probe has many advantages in tumor marker detection Such as real-time imaging, no trauma, easy to operate, both economic and fast. The organic small molecule fluorescence probes have the advantages of easy modification, easy adjustment of spectra, good Biocompatibility and easy metabolism by organisms, it has been widely used in cell imaging, molecular marking and real-time imaging [4-6] .In addition to the advantages of conventional fluorescent probes, the small molecule fluorescent probe has the advantages of definite molecular weight and element composition, good repeatability and small molecular weight, it can be distributed evenly and reproducibly throughout the organism for easy cell imaging of biological samples [7-8] .Benzothiazole as an important fluorescence backbone, typical rigid planar structure and delocalization system, benzothiazole and its derivatives have excellent optical properties. Compared with inorganic

luminescent materials, benzothiazole derivatives have the characteristics of high luminescent efficiency and controllable, and their applications in organic fluorescent dyes and fluorescent probes have been rapidly developed [9-10] ,The structure of benzothiazole is easy to be modified by modifying different sites of benzothiazole to increase the degree of conjugation, structural rigidity, planarity, introduction of hetero-atom and push-pull electron system, to regulate the optical and electronic properties of benzothiazole molecules, the effects of different modification methods on the linear absorption, fluorescence emission and other photophysical properties of benzothiazole derivatives were investigated by spectroscopic methods [11-12]. The construction of new fluorescent dyes and their design principles can explain the relationship between the chemical structure and photophysical properties of fluorophores at the molecular level. After modifying the structure of benzothiazole, a series of benzothiazole fluorescent probes and dyes have been developed in recent years. The delocalized large π bonds and rigid planar structures in benzothiazole molecules give their derivatives the advantages of high fluorescence quantum yield, high Stokes shift and high molar absorptivity [13-14] ,In recent years, a series of functional fluorescent dyes based on benzothiazole framework have been reported, which have excellent optical properties, widely used in chemistry, biomedicine, materials science and the environment [15--16].In this paper, a fluorescence probe for 2-(benzo [D] thiazole-2-yl) -6-(hydramethyl) -4-methylphenol (BTH-MPH) was designed and synthesized from 2-aminophenothiophenol and 5-methylsalicylaldehyde, it is expected to be widely used in the field of biology.

2.Experimental Method and Materials Used

2.1 Principal Instruments and Reagents

Fluorescent photometer (F-7000, Hithchi, Japan) ,Inverted microscope (CKX31SF, Olympus Corporation) ,Micro-melting point apparatus (X-4, made by Shanghai Instrument Electric Physical Optical Instrument Co. , Ltd.) , cell culture box (BPN-80CRH, Shanghai Yiheng Science Instrument Co. , Ltd.) , cell culture box (C21, Biospherix, USA) , laser confocal microscopy (FV1200 + IX83, Olympus, Japan) ,1H NMR and 13C NMR were performed using DPX400 or DPX600 nuclear magnetic resonance spectrometer .Electronic Precision Balance (RS232C, Shanghai Yueping Scientific Instrument Co. , Ltd.) ,Automatic cell counter (IC1000, Shanghai Ruiyu Biotechnology Co. , Ltd.),Rotary evaporator (Zhengzhou Great Wall Technology Industry & Trade Co. , Ltd.),DF-101S Magnetic stirrer (Zhengzhou Great Wall Science, Industry and Trade Co. , Ltd.) , three-use ultraviolet analyzer (Shanghai Qingpu Luxi Instrument Factory) .

Ethanol, 5-methylsalicylaldehyde, 2-aminophenol were purchased from West Asia Reagent Co., Ltd.The trifluoroacetic acid, hydrazine hydrate (analytically pure) and hexamethylenetetramine were purchased from Bellway Technologies Ltd., paclitaxel from Märklin, other solvents and inorganic salts were analytically pure, shanghai Guoyao Group Chemical Reagent Co., Ltd.

Cell line: non-small-cell lung carcinoma cell A549 was purchased from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences.

2.2 Experimental Operation Process

2.2.1 The synthesis of compounds

Accurately weigh 2-Amino-Benzenethiol 15 mmol and 5-methylsalicylaldehyde 15 mmol, Place in a clean 50ml reaction bottle, Add anhydrous ethanol 30ML, stir on Magnetic stirrer, add 1 mmol hydrochloric acid 600 μL and 1 mmol hydrogen peroxide 600 μL while stirring, reaction temperature 0 ° C, TLC to track the reaction, At the end of the reaction, the mixture was filtered and washed with ether for 3 times, then dried naturally and recrystallized with anhydrous ethanol to obtain compound 1(gray solid) .The compound was dissolved in the appropriate trifluoroacetic acid in 10 mmol and 20 mmol hexamethylene tetramine. Refluxing at 80° C, stirring overnight, TLC tracing until the reaction is complete, the reaction is completed, then the reaction is cooled to room temperature, and pH is adjusted with a 2 mmol L potassium hydroxide solution until the yellow solid is completely precipitated. After the reaction was completed, the solvent was removed by vacuum evaporation and washed with water to obtain a fluorescence probe of the target compound 2-(benzo [D] thiazole-2-yl) -6-(hydramethyl) -4-methylphenol (BTH-MPH) .

2.2.2 Spectral properties of BTH-MPH

Preparation of pH solution,0.1 mol L citric acid

and 0.2 mol L Disodium phosphate,pH ranges from 3.0 to 8.0,The probe was mixed with different pH solutions, and the concentration of the probe in the final system was 10 μm, and the fluorescence of BTH-MPH was determined using an excitation wavelength of 377 nm.

2.2.3 BTH-MPH is toxic to living cells

Lung cancer cell A549 was cultured to logarithmic phase, digested with trypsin, centrifuged at 1200r for 4 min, cells were collected, after removing supernatant, cell suspension was swabbed with fresh cell culture medium and counted by cell counter, adjusted cell density 5× 10^4 cells/mL, Add the beaten, diluted cell fluid to the cell culture plate (96-well) , adding 100 μL per well, and place in the incubator at a temperature of thirty-seven ° C with a 5% CO_2 concentration. After the cells adhered to the wall, the probe was added at the concentrations of 0,1,5,10,15,20,40,50 μm/L for 6 times. After 24 hours of incubation, The cells were washed three times with PBS, 100 μL of new culture medium was added to each well and 10 μL of CCK-8 solution was added to each well.

2.2.4 BTH-MPH imaging in a living cell model

A549 cells at logarithmic growth stage were seeded in small dishes (confocal only) with a cell density of about 1 × 10^5 cells/mL, placed in a 37 ° c 5% CO_2 incubator and attached to the wall, and the probe was added at a final concentration of 20 μm/L, After incubation for 1 hour, the probe solution was removed, PBS was washed 3 times, and paclitaxel solution was added. The concentration of paclitaxel was 5,10 and 20 μm/L, respectively, except for the control, pBS was washed 3 times, fixed with 4% formaldehyde for 15 min, PBS washed 3 times, 1.5 ml PBS was added to each dish, observed under confocal, and photographed.

3 Results and Discussion

3.1Characterization of Target Probe

The target probe is a bright yellow solid, m.p.162.4-167.6 ℃,1H NMR (600 MHz, DMSO-d6)δ: 8.14(s,1H),8.12(T,J=3Hz,2H),8.05(s,1H),7.55(t, J=12Hz,1H),7.45(S,1H),7.28(m,J=9.6Hz,1H),2.34(s,3H).13C NMR (151 MHz, CDCl3) δ:163.46,153.80,151.92,141.99,135.61,132.08,128.44,127.94,126.74,125.29,122.68,122.33,121.05,119.52,20.51. TOF MS m/z:C15H13N3OS, Theoretical value 283.35, Measured values 284.08[M+1]+.

3.2 PH Spectral Properties of BTH-MPH

To evaluate the effect of pH on the stability of the probe, the probe BTH-MPH was added to different pH values, As can be seen from Figure 1-3 in the range of acidity, the fluorescence intensity increases with the increase of acidity, When the pH is 3.0, the fluorescence intensity is the strongest and the absorption peak at 510nm is the largest, In the range of alkalinity, the fluorescence intensity increased with the increase of alkalinity. When pH was 8.0, the absorption peak at 570nm was the largest.

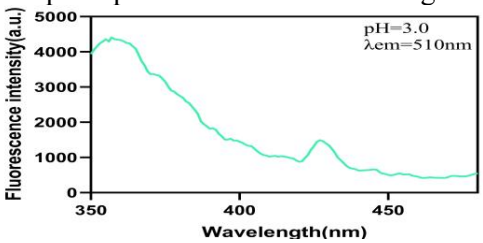

Figure 1. Fluorescence Characteristics of BTH-MPH at pH 3

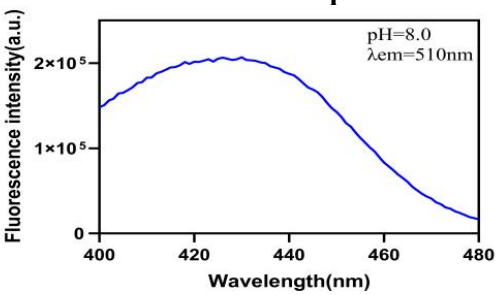

Figure 2. Fluorescence Characteristics of BTH-MPH at pH 8

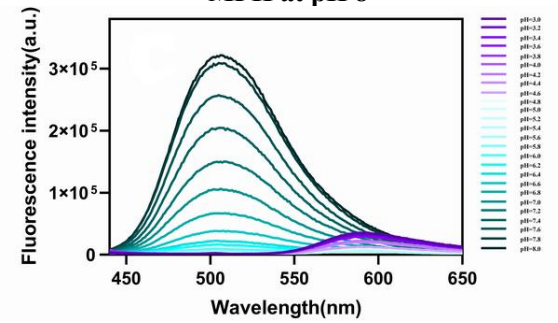

Figure 3. pH Fluorescence Properties of BTH-MPH

3.3 Cytotoxicity

The fluorescence probe was used to test the cytotoxicity of A549,The results are shown in Table 1, A549 cells were incubated for 24 hours in the range of probe concentration 0-20 μm, the cell survival rate was above 95% and basically non-toxic, and the cell survival rate was above 92.34% in the range of probe concentration 20-50 μm, the toxicity is minimal, Six repetitions,

These results suggest that the probe BTH-MPH is relatively safe and has good Biocompatibility and low cytotoxicity, suggesting that the probe molecule BTH-MPH can be used for further cellular imaging experiments in A549 cells.

Table 1. Effect of Probe on Cytotoxicity

BTH-MPH μM	0	5	10	
Cell survival rate%	99.12±0.78	98.78±0.56	97.34±0.23	
BTH-MPH μM	15	20	40	50
Cell survival rate%	96.87±0.45	95.12±0.64	93.21±0.67	92.34±0.78

3.4 Cell Imaging

Tumor cells are different from normal cells in their morphology, metabolism and growth pattern. Fluorescent probes can target tumors, The combination of fluorescent dye probe and tumor marker can achieve the goal of tumor labeling and research through fluorescence imaging observation, or the change of fluorescence signal. The fluorescence probe has high sensitivity, high specificity and multifunction, and the interference to the studied cells is very small.

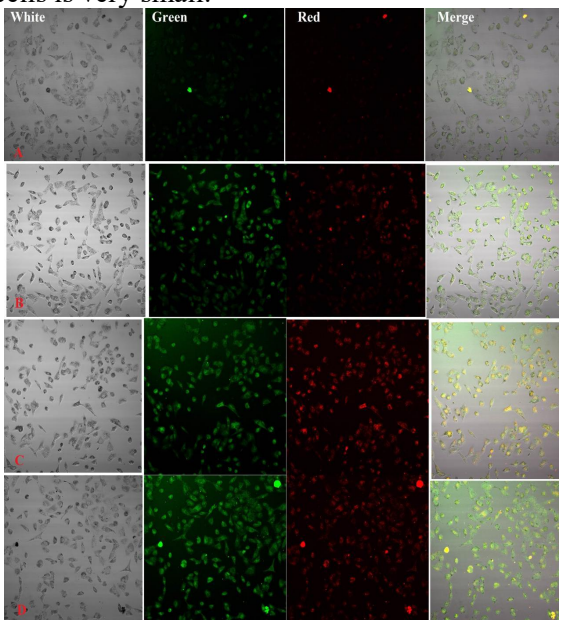

Figure 4. Confocal Fluorescence Imaging of A549 Cells Incubated with BTH-MPH (20 μm/L)

Note: In control a, the concentration of paclitaxel was 5 μm/L in B, 10 μm/L in C and 20 μm/L in D

s very small. Thiazole-based fluorescent probes for cell imaging have the following advantages: It has good light stability and brightness in biological environment, minimizes background noise and maximizes spatial resolution; The cytotoxicity is very small and is able to maintain a balance between hydrophilicity and hydrophobicity in terms of membrane permeability and cell retention [17].As can be seen from Figure 4, The red fluorescence and the green fluorescence can be observed obviously. With the increase of the concentration of paclitaxel, the fluorescence intensity increases gradually. The probe BTH-MPH could image live A549 cells with good cell permeability.

4 Conclusion

The fluorescence probe has unique advantages, such as easy operation, fast analysis speed, strong selectivity, high sensitivity and no damage to the detection sample, Therefore, this method is widely used in biology, medicine, chemistry, microbiology, environmental science and other fields, especially suitable for living cells, sub-cellular level of small molecule recognition and visual imaging. With the development of molecular biology, synthetic chemistry and molecular pharmacology, the search for organic small molecule fluorescence probes with high sensitivity, high specificity and low toxicity has become a new method for detecting diseases, new Directions for biological development.

The structure of the synthesized probe was determined by hydrogen spectrum, carbon spectrum and mass spectrum. The probe has a wide applicable range of pH (3.0-8.0) , can be used for the detection of living cells.

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