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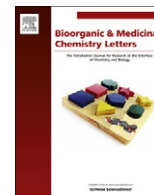

A specific molecular beacon probe for the detection of human prostate cancer cells

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ABSTRACT

The small-molecule, water-soluble molecular beacon probe **1** is hydrolyzed by the lysate and living cells of human prostate cancer cell lines (LNCaP), resulting in strong green fluorescence. In contrast, probe **1** does not undergo significant hydrolysis in either the lysate or living cells of human nontumorigenic prostate cells (RWPE-1). These results, corroborated by UV–Vis spectroscopy and fluorescent microscopy, reveal that probe **1** is a sensitive and specific fluorogenic and chromogenic sensor for the detection of human prostate cancer cells among nontumorigenic prostate cells and that carboxylesterase activity is a specific biomarker for human prostate cancer cells.

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Prostate cancer is the most common malignancy in American men and the second leading cause of cancer mortality.[1] There are no symptoms during this cancer's early stage; patients may die due to lack of early detection and treatment.[2] Therefore, there is an urgent need to develop effective chemical methods for the detection of prostate cancer in its early stages, potentially allowing us to control the cancer from spreading and, ultimately, cure the aggressive disease.[2]

At present, a chemical test measuring the level of prostate-specific antigen (PSA) in serum is the most effective method for the early detection of prostate cancer; its positive predictive value is, however, only 35%.[3] Therefore, the majority of patients will receive false-negative results, making them likely to lose the possibility of early detection, prevention, and treatment. Another method for the clinical diagnosis of human prostate cancer is through biopsy, which checks the patient's prostate tissues for cancerous cells.[4] The diagnosis and prognosis of prostate cancer might also be possible through analysis of the carboxylesterase activity in the cells; some reports have described increased carboxylesterase activity in the cells of other cancer types, such as human breast cancer,[5,6] relative to that in normal tissues. We are, however, unaware of any previous reports of increased carboxylesterase activity in human prostate cancer cells for the diagnosis and prognosis of prostate cancer cells.[7]

In a previous study, the carboxylesterase activity in breast cancer cells was determined through fluorescence monitoring, with fluorescein diacetate used as the substrate for the enzyme.[5] This activated aryl acetate is, however, labile (high automatic hydrolysis rate), making it unstable in water or buffer.[8] As a result, the fluorescence background was high and the signal to noise ratio was low.[5] Fluorescein diacetate also possesses limited water solubility.[8] One approach toward overcoming the limitations of that probe would be to develop a specific, water-soluble, ester-based fluorescent probe, such as an alkyl aliphatic acid ester, with a low rate of automatic hydrolysis.[8]

Recently, water-soluble 2-(2′-phosphoryloxyphenyl)-4-(3*H*)-quinazolinone ($Q_{2\text{-p}}$) derivatives have been applied to detect the activity of extra-cellular phosphatases around prostate cancer cells.[9] In addition, a couple of large molecules and fluorescent probes containing pyrenyl oligonucleotides have been employed in the detection of intra-cellular carboxylesterase activity in HeLa cells.[8] Notably, such pyrenyl probes have not been used for the detection of human

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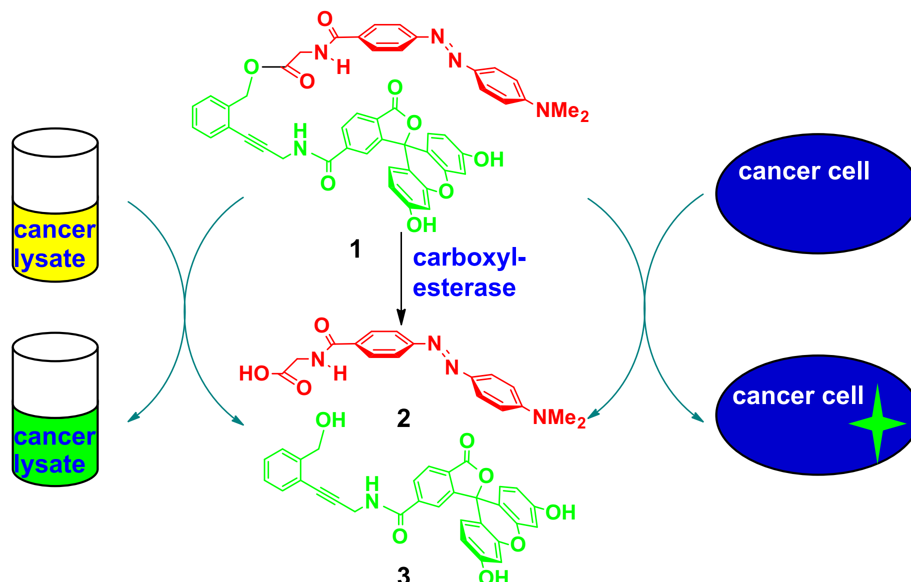
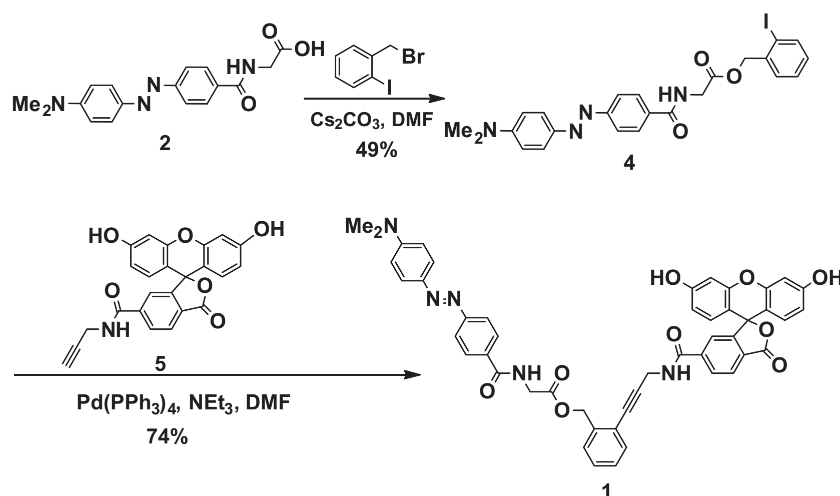


Scheme 1. Schematic representation of the operation of probe **1** for detection of human prostate cancer cells.

prostate cancer cells. Therefore, a small-molecule with better cell permeability and a probe with specific fluorescence appear necessary for monitoring the carboxylesterase activity in human prostate cancer cells.

We have designed the molecular beacon probe **1** as a substrate for monitoring carboxylesterase activity (Scheme 1). This molecule features one aliphatic ester group and two aromatic acid amide groups; only the ester group will be hydrolyzed readily by carboxylesterase.[10] Aryl acid amide groups are typically less-reactive substrates for most carboxylesterases, amidases and proteases.[11,12] In addition, we expected this probe to be resistant to hydrolyzes catalyzed by intra- and extra-cellular phosphatases, nucleases, and phosphodiesterases in prostate cancer cells due to probe **1** is non phosphate salt/ester. Furthermore, probe **1** incorporates a fluorophore—fluorescein, a water-soluble unit[13] at physiological pH (7.4)—and a typical fluorescence quencher (dabcyl group).[14] In addition, the structure features an ortho-substituted phenyl group that acts as a bridge to bring the fluorescein and dabcyl units together. This design makes it possible to ensure effective fluorescence quenching of the fluorescein moiety by the dabcyl group. Taken together, we expected these features to make probe **1** a specific substrate for carboxylesterases. After hydrolysis of **1** by a carboxylesterase through acyl substitution, *N*-dabcylglycine (**2**) will be expelled, causing the fluorescein unit to become fluorescent (i.e., no longer quenched). We synthesized the molecular beacon probe **1** from the dabcyl derivative **2** through Williamson ether synthesis[15] and palladium-catalyzed Sonogashira coupling[16,17] with compound **5** (Schemes 2 and S1–S3). We also prepared compounds **3** and **6** for NMR spectroscopic studies (Fig. 1, Schemes S2 and S4).

To demonstrate whether the designed and synthesized **1** has a hairpin like structure and whether it is a new molecular beacon probe, and to determine whether fluorescence quenching was possible between the fluorescein and dabcyl units in **1**, we investigated their intramolecular hydrogen bonding and π–π stacking interactions using ^{1}H NMR spectroscopy. We monitored the chemical shifts of the protons of the probe **1**, its synthetic precursor **6**, and its related compound **3** under the same conditions (in acetone-d_6, at a concentration of 38.0 mM, and at a temperature



Scheme 2. Synthesis of **1**.

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