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Synthesis of a dansyl-labeled inhibitor of 17β-hydroxysteroid dehydrogenase type 3 for optical imaging

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ABSTRACT

The steroidogenic enzyme 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) is a therapeutic target in the management of androgen-sensitive diseases such as prostate cancer and benign prostate hyperplasia. In this Letter, we designed and synthesized the first fluorescent inhibitor of this enzyme by combining a fluorogenic dansyl moiety to the chemical structure of a known inhibitor of 17β-HSD3. The synthesized compound **3** is a potent fluorogenic compound (λ_{ex} = 348 nm and λ_{em} = 498 nm). It crosses the cell membrane, keeps its fluorescent properties and is distributed inside the LNCaP cells overexpressing 17β-HSD3, where it inhibits the transformation of 4-androstene-3,17-dione into the androgen testosterone (IC_{50} = 262 nM).

The enzyme 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) plays a critical role in androgen metabolism. In fact, an impairment in the expression of this enzyme can lead to hermaphroditism in men.[1] In the presence of cofactor NADPH, 17β-HSD3 catalyzes the transformation of 4-androstene-3,17-dione (4-dione) into the androgen testosterone (T).[1–3] Mainly located in testicular leydig cells, this enzyme is responsible for approximately 60% of the production of circulating androgens in men.[4,5] 17β-HSD3 is also found in blood platelets, megakaryocytes as well as in some prostate cancer tumors.[6–8] Since it plays a central role in testosterone production, 17β-HSD3 has been recognized as a promising therapeutic target to reduce the circulating level of androgens and, thus, to inhibit androgen-sensitive tumor proliferation.[9]

In the last decades, improvements have been made in the development of 17β-HSD3 inhibitors. Some steroidal and non-steroidal inhibitors of 17β-HSD3 have been synthesized,[9–12] though none of them is enrolled in a clinical trial to date,[13] including notably the non-steroidal inhibitor STX2171 that showed antitumor activity against xenografts of LNCaP cells overexpressing 17β-HSD3.[14] In our laboratory, many generations of inhibitors have been synthesized as androsterone (ADT) derivatives.[15–22] A hydrophobic side chain at position C-3 was first designated as the best pattern for an 17β-HSD3 inhibitor design. Moreover, a subsequent series of ADT derivatives bearing a substituted hydrophobic piperazine moiety at position C-3β converged to the identification of inhibitor RM-532-105 (Scheme 1).[20] This steroidal inhibitor of 17β-HSD3 (IC_{50} = 6 nM in 17β-HSD3-HEK-293 transfected cells) showed its capacity to reduce the plasma levels of T and dihydrotestosterone, two potent androgens, in rats.[21]

In the drug development process, imaging is an inescapable tool in studying fundamental biological processes as well as clinical investigations.[23–25] For instance, fluorescent steroid conjugates maintaining high affinities for steroid receptors were designed for mechanistic and diagnostic purposes.[26–28] Among available fluorophores, the dansyl group possesses interesting fluorescent properties and has been largely used to generate fluorescent tools exploitable in optical in vitro and ex vivo imaging. As examples, we can mention fluorescent labeled glycosides to probe the enzyme sialyltransferase, fluorescent derivatives of cyclooxygenase-2 selective inhibitor celecoxib, fluorescent probe of the antitumor agent thiophene-3-carboxamide analog for visualizing cell distribution, dansylated molecules to study cholesterol behavior in cell membranes and fluorogenic probes for imaging of the enzyme 17β-HSD10 in cells.[23,29–32]

Tracking an inhibitor within the living cells by direct imaging could be a strategic approach in understanding its cell distribution. Since no 17β-HSD3 fluorescent inhibitor had been synthesized to date, and given the fact that the dansyl group is one of the smallest known fluorophores, we were prompted to take advantage of this opportunity to design and synthesize the first fluorescent 17β-HSD3 inhibitor. Interestingly, the C3-side chain of RM-532-105

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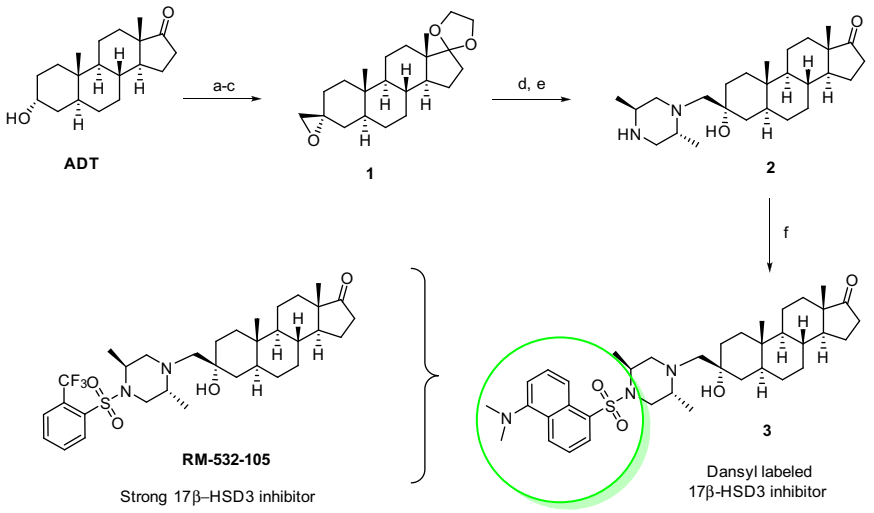

Scheme 1. Synthesis of compound **3**, a dansyl-labeled 17β-HSD3 inhibitor mimic. Reagents and conditions: (a) $HOCH_2CH_2OH$, *p*-TSA, toluene, reflux (Dean Stark); (b) TPAP, NMO, molecular sieves, DCM, rt; (c) $(CH_3)_3SOI$, NaH, DMSO/THF, rt; (d) *trans*-dimethyl-piperazine, EtOH, 60 °C; (e) HCl (3 M)/acetone (1:1); (f) dansyl chloride; DIPEA, DCM, rt.

bears a phenyl sulfonamide functionality, which is structurally related to the dansyl group. We then replaced the phenyl sulfonamide of RM-532-105 by a dansyl group to generate compound **3** (Scheme 1). We were thus confident that this molecular mimic would be appropriate to generate a new and potent fluorescent inhibitor.

The chemical synthesis of compound **3** has been readily carried out in a similar way as previously reported for our 17β-HSD3 inhibitors.[15,20] Briefly, the C17-ketone of ADT was protected as a dioxolane, the C3-alcohol oxidized by TPAP and the corresponding C3-ketone reacted with dimethylsulfoxonium methylide to stereoselectively provide the oxirane **1**.[15] Aminolysis of **1** in presence of *trans*-dimethyl-piperazine generated the key piperazine intermediate **2**,[20] which was next reacted with dansyl chloride reagent to provide the corresponding sulfonamide **3** in good yield (71%) and high purity (98.8%). The characterization by IR, ^{1}H NMR, ^{13}C NMR and MS fully support the chemical structure of **3**.

The excitation and emission spectra of compound **3** are displayed in Figure 1. At a concentration of 50 μM, its maximum excitation was observed at 348 nM (major peak amplitude). When excited at this wavelength, the maximum emission peak was observed at 498 nm. Since compound **3** was synthesized from the reaction of dansyl chloride with intermediate **2**, and given that the dansyl moiety confers its fluorescent properties to the new labeled inhibitor, we also determined the excitation and emission spectra of dansyl chloride in the same conditions. As expected, the maximum excitation of dansyl chloride (340 nm) is close to the value of 348 nm obtained for **3**. Similarly, when excited at 340 nm, the maximum emission peak of dansyl chloride (520 nm) was also close to the value of **3** (498 nm). These data confirm that labeled inhibitor **3** kept the fluorescent properties of the dansyl group.

Once compound **3** was synthesized, characterized and its fluorescent properties confirmed, it was tested for its ability to inhibit 17β-HSD3 activity using the transfected LNCaP cells overexpressing 17β-HSD3 as model. This was done by measuring the transformation of [^{14}C]-labeled natural substrate 4-dione into [^{14}C]-T and by determining the concentration of **3** able to inhibit 50% of the enzyme activity (IC_{50}). As a result, the IC_{50} of the fluorescent compound **3** was found to be 262 nM, whereas the IC_{50} of the reference compound RM-532-105 was 85 nM (Fig. 2). Both tested compounds displayed 17β-HSD3 inhibitory activities in the nanomolar range, with a slight loss of activity (3 times) for compound **3** compared to our best 17β-HSD3 inhibitor (RM-532-105), which was optimized from previous structure–activity relationship (SAR) studies.[20]

Since the design and synthesis of the new 17β-HSD3 fluorescent inhibitor was done for in vitro and ex vivo purposes, it was fundamental to check whether this molecule crosses the cell membrane and still keeps its fluorescent properties. We chose two cell models

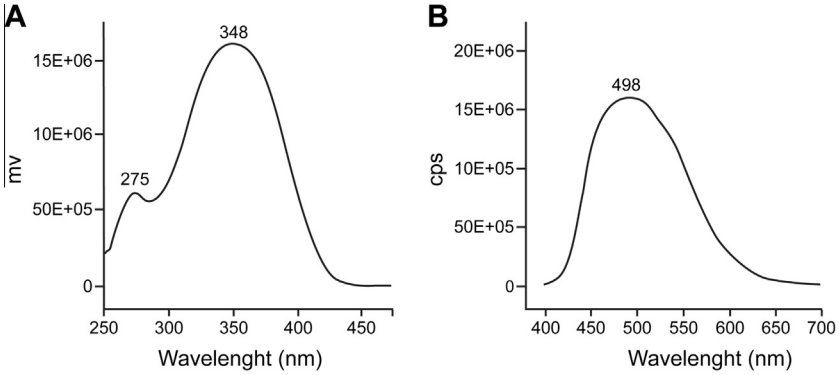

Figure 1. Excitation spectrum (A) and emission spectrum (B) of compound **3** (50 μM) in PBS.