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Synthesis and characterization of a BODIPY-labeled derivative of Soraphen A that binds to acetyl-CoA carboxylase

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ABSTRACT

BODIPY-labeled Soraphen A derivative **4** was synthesized and characterized as an acetyl-CoA carboxylase (ACC) binder. Biophysical measurements indicate that the molecule binds in the biotin carboxylase domain where Soraphen A has been shown to bind. The fluorescent label of the BODIPY can be used to biophysically identify a compound that binds to the Soraphen A site of the biotin carboxylase domain versus the carboxytransferase domain of ACC.

Acetyl-CoA carboxylase (ACC) catalyzes the transformation of acetyl-CoA to malonyl-CoA. The enzyme contains two major domains; the biotin carboxylase domain (BC) and the carboxytransferase domain (CT) connected by the biotin carboxyl carrier protein (BCCP) and is catalytically active as a dimer[1] The enzyme has two isoforms: ACC1 is located in intracellular space and ACC2 is located on the surface of mitochondria. Inhibition of one or both ACC isoforms leads to lower intracellular malonyl-CoA levels which has been shown to increase the rate of fatty-acid oxidation and lower the rate of fatty-acid synthesis in cells.[2] The resulting shift in fatty-acid metabolism causes a decrease in muscle, liver and adipose tissue triglycerides in animal models. Long term inhibition may lead to a reduction in body fat, hyperinsulemia and an increase in insulin sensitivity.

Based on our desire to differentiate small molecule ACC inhibitors based on their binding site and mode of inhibition at the enzyme, we designed a tool compound for biophysical studies by attaching a fluorescent moiety to a known ACC binder, Soraphen A (**1**).[3–5] A useful dye for this purpose is 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene, (BODIPY), because of its efficient absorption of ultra-violet radiation and fluorescence emission peaks at 520–650 nM as well as an appropriate profile for fluorescence polarization measurements.[6,7] Many BODIPY derivatives are available and suitable functional groups on the target molecule enable straightforward derivitization.

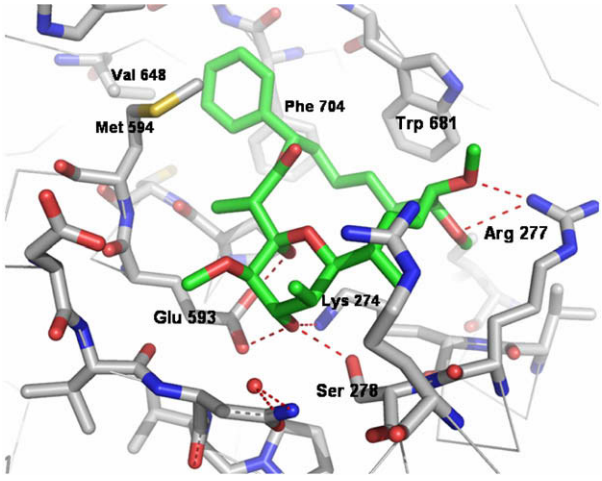

Figure 1. Crystal structure of Soraphen A (**1**, green) bound in the biotin carboxylase domain of ACC (gray). Water molecules are red. C-16 (shown in foreground interacting with Arg277) was identified as an ideal point of substitution due to the orientation of the methoxy substituent towards solvent space and away from the binding pocket.

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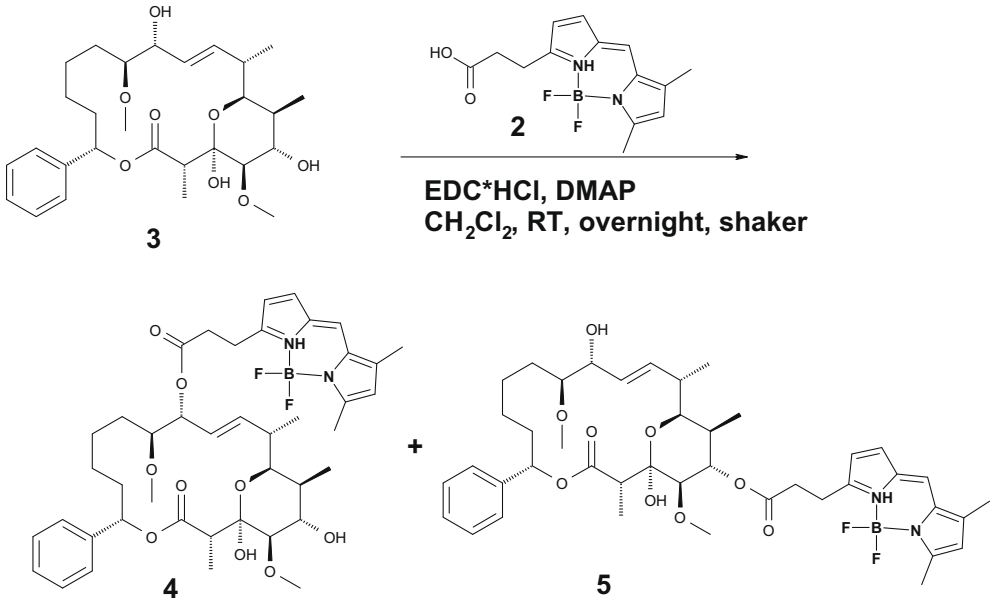

Scheme 1. Synthesis of BODIPY-labeled Soraphen derivatives **4** and **5**.[13]

Selection of an appropriate point to attach a BODIPY label to Soraphen A was facilitated by crystal structures of Soraphen A bound to the biotin carboxylase domain of ACC (Fig. 1).[8–10] The C-16 methoxy group was oriented towards solvent space and away from the surface of the binding pocket; presumably allowing for substitution of a large moiety such as an alkyl chain linked BODIPY and said derivative would bind with adequate efficiency for biophysical studies.

The propionic acid BODIPY derivative **2** was selected as the coupling partner for **3**, a demethylated analog of Soraphen A.[11] The coupling reaction was facilitated by EDC and DMAP and afforded a 53% yield of **4**, a BODIPY-labeled Soraphen A analog with 85% purity by NMR (Scheme 1). The major byproduct was regioisomeric acylation product **5,** which could be removed by careful chromatography.[12] Further purification using supercritical CO_2 HPLC with methanol afforded 98% pure material by LC/UV (210 nM–550 nM).

The regiochemistry of derivative **4** was confirmed by long range proton–carbon (H-16 to C-25) and proton–proton (H-16 to H-40) NMR correlations (Fig. 2). The regiochemistry of derivative **5** was assigned by comparison of the C-8 proton shift of reference compound **7** (5.05 ppm), synthesized in a similar manner to **5**.

The BODIPY-Soraphen analog **4** was found to bind to the ACC2 BC domain with an apparent K_d of 3 nM, (Fig. 3). Since the concentration of **4** was kept low (5 nM) the affinity was not measured accurately but is presumed to be less than 5 nM. Moreover, **4** was confirmed to directly bind to the BC domain of the ACC enzyme by causing a 10 °C increase in thermal stability of the protein using a differential scanning calorimetry assay (Fig. 4). An X-ray crystal structure of BODIPY-labeled Soraphen A derivative **4** complexed to human ACC2 BC domain was solved, establishing the similar binding modes of both **4** and Soraphen **A** (Fig. 5).[14,15]

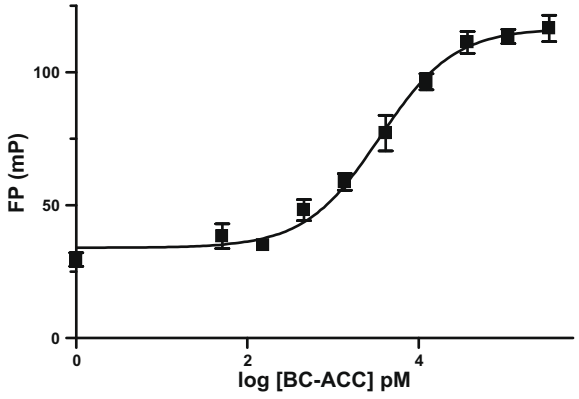

Figure 3. Titration of ACC BC domain versus Bodipy-labeled Soraphen A analog **4**.[16] The K_{obs} of **4** is 1/2 the concentration used in the assay indicating tight binding (<5 nM).

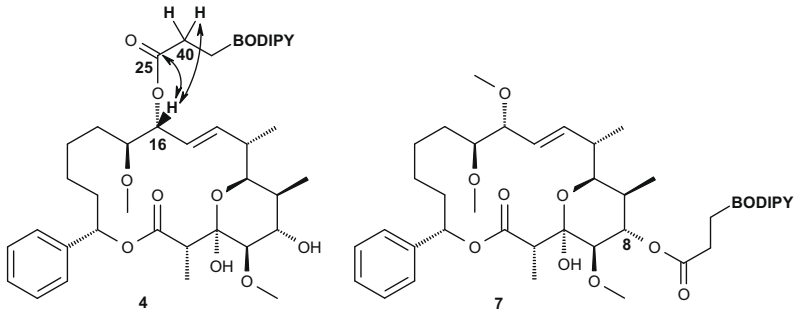

Figure 2. NMR correlations for BODIPY-labeled Soraphen derivative **4** and structure of reference compound **7**.