Bioorganic & Medicinal Chemistry Letters 19 (2009) 2804–2807

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

Brian Raymer [a,*], Michael Kavana [a], Allen Price [b], Bing Wang [a], Lisa Corcoran [a], Raviraj Kulathila [c], James Groarke [c], Theirry Mann [a]

[a] Novartis Institutes for BioMedical Research, 100 Technology Square, Cambridge, MA 02139, United States
[b] Emmanuel College, 400 The Fenway, Boston, MA 02115, United States
[c] Novartis Institutes for BioMedical Research, 250 Massachusetts Avenue, Cambridge, MA 02139, United States

ARTICLE INFO

Article history:
Received 16 January 2009
Revised 20 March 2009
Accepted 23 March 2009
Available online 26 March 2009

Keywords:
BODIPY
Soraphen
Acetyl-CoA carboxylase
ACC
Biotin carboxylase

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.

© 2009 Elsevier Ltd. All rights reserved.

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.

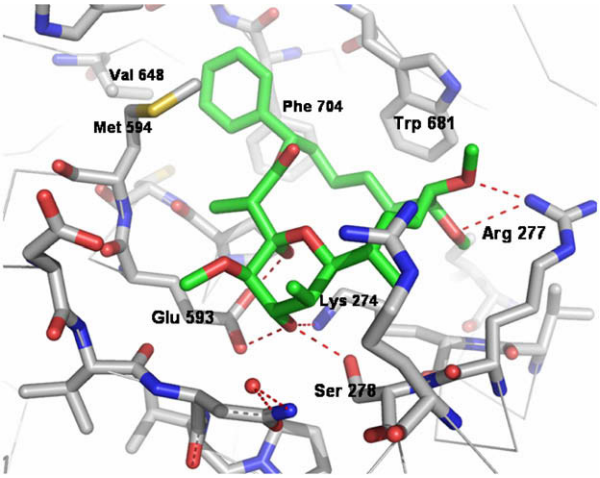
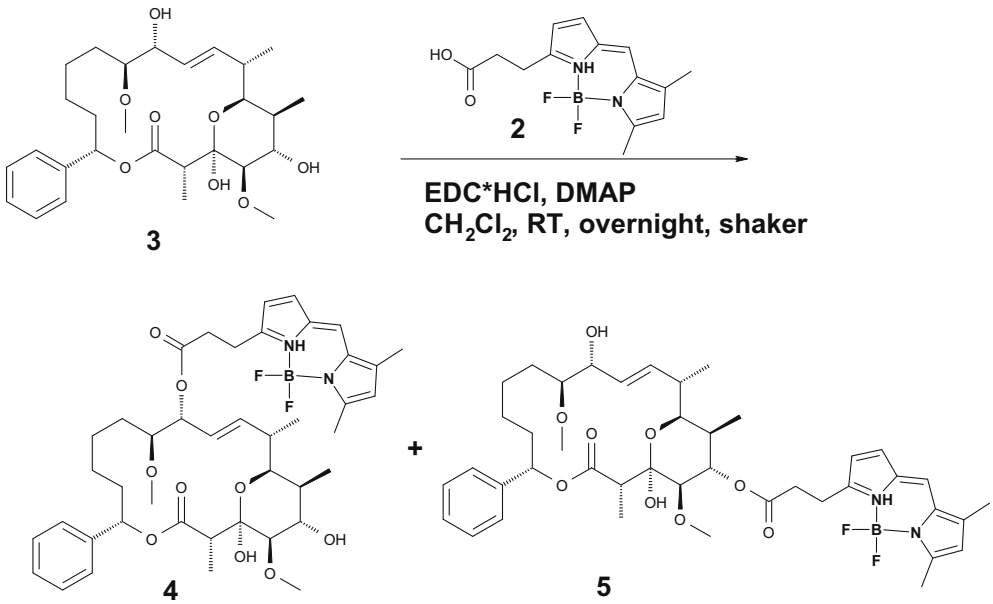


Figure 1. Crystal structure of Soraphen A (**1**, green) bound in the biotin carboxylase domain of ACC (gray). Water molecules are red. C-16 (shown in foreground interacting with Arg277) was identified as an ideal point of substitution due to the orientation of the methoxy substituent towards solvent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

* Corresponding author. Tel.: +1 617 871 7694; fax: +1 617 871 7042.
E-mail address: brian.raymer@novartis.com (B. Raymer).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.03.107



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]

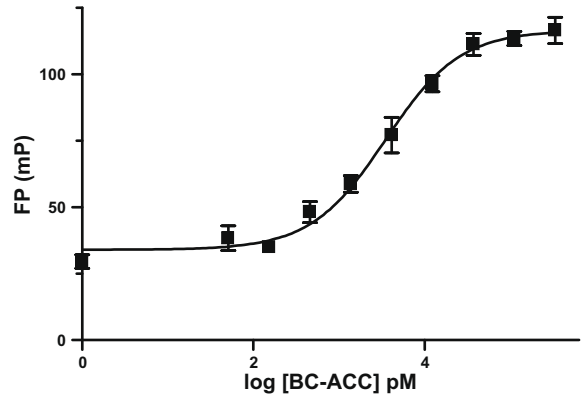


Figure 3. Titration of ACC BC domain versus Bodipy-labeled Soraphen A analog **4**.[16] The K_{obs} of **4** is 1/2 the concentration used in the assay indicating tight binding (<5 nM).

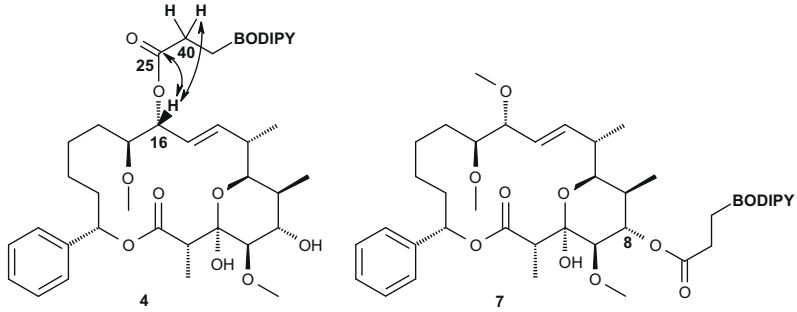


Figure 2. NMR correlations for BODIPY-labeled Soraphen derivative **4** and structure of reference compound **7**.

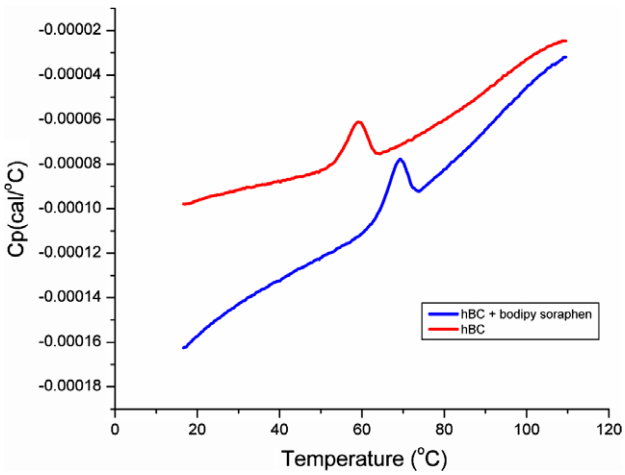


Figure 4. Comparison of temperature shifts by differential scanning calorimetry. The lower curve (blue) represents the temperature shift caused by the addition of **4**.[17]

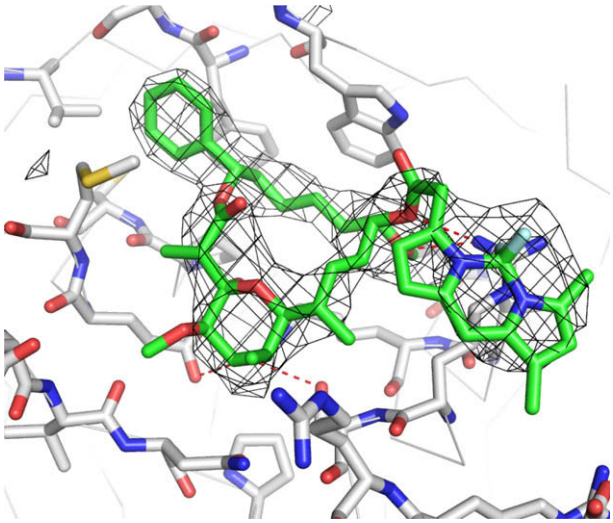


Figure 5. Crystal structure of Bodipy-labeled Soraphen A (**4**, green) bound in the biotin carboxylase domain of ACC (gray). The BODIPY label is oriented towards solvent space and away from the binding pocket.

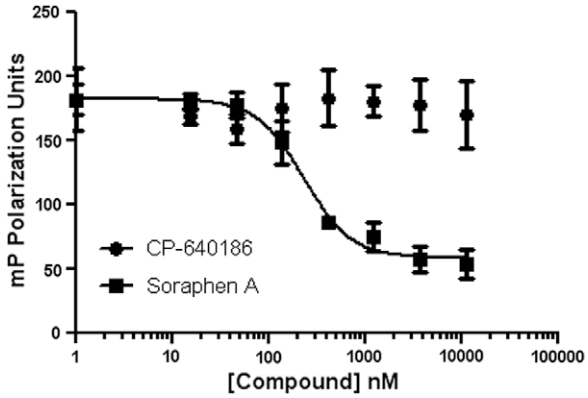


Figure 6. Selected ACC inhibitors titrated in dose response with **4**.[19]

With the binding mode of the BODIPY-labeled Soraphen A derivative **4** established, we investigated its utility to identify ACC inhibitors which bind specifically to the biotin carboxylase domain. A known carboxytransferase binder, CP-640186, and a known Soraphen site biotin carboxylase domain binder, Soraphen A, were run in dose titration with **4** (Fig. 6).[18] As expected, Soraphen A, but not CP-640186 showed a dose response with **4**.

In conclusion, we have synthesized BODIPY-labeled Soraphen A derivative **4**, which has been shown to bind at the Soraphen A binding site of the biotin carboxylase domain by X-ray crystallography and biophysical methods. The compound binds with high affinity to the enzyme and can be used to identify ACC inhibitors that bind to the Soraphen site of the biotin carboxylase domain.

Acknowledgements

The Novartis Natural Products Unit is gratefully acknowledged for supply of Soraphen analogs. Special thanks to James Wareing, Robert Damon and Gary Copolla for helpful discussion and Michael Sachs for protein purification.

References and notes

1. Tong, L.; Harwood, H. J., Jr. *J. Cell. Biochem.* **2006**, *99*, 1476. and references therein.
2. (a) Savage, D. B.; Choi, C. S.; Samuel, V. T.; Liu, Z.-X.; Zhang, D.; Wang, A.; Zhang, X.-M.; Cline, G. W.; Yu, X. X.; Geisler, J. G.; Bhanot, S.; Monia, B. P.; Shulman, G. I. *J. Clin. Invest.* **2006**, *116*, 817; (b) Mao, J.; DeMayo, F. J.; Li, H.; Abu-Elheiga, L.; Gu, Z.; Shaikenov, T. E.; Kordari, P.; Chirala, S. S.; Heird, W. C.; Wakil, S. J. *PNAS* **2006**, *103*, 8552. and references therein.
3. Vahlensieck, H. F.; Pridzun, L.; Reichenbach, H.; Hinnen, A. *Curr. Genet.* **1994**, *25*, 95.
4. Total synthesis of Soraphen A: (a) Abel, S.; Faber, D.; Hueter, O.; Giese, B. *Synthesis* **1999**, 188; (b) Abel, S.; Faber, D.; Huter, O.; Giese, B. *Angew. Chem.* **1994**, *106*, 2522.
5. For recent synthetic studies towards Soraphen A see: (a) Park, S. H.; Lee, H. W. *Bull. Korean Chem. Soc.* **2008**, *29*, 1445; (b) Eweas, A. F. *Synth. Commun.* **2008**, *38*, 1541; (c) Vincent, G.; Mansfield, D. J.; Vors, J.-P.; Ciufolini, M. A. *Org. Lett.* **2006**, *8*, 2791; (d) Diaz-Oltra, S.; Murga, j.; Falomir, E.; Carda, M.; Peris, G.; Marco, J. A. *J. Org. Chem.* **2005**, *70*, 8130.
6. a http://probes.invitrogen.com.; b Molecular Probes; Invitrogen Corp.: Carlsbad, CA, **2006**.; (c) Burgess, K.; Loudet, A. *Chem. Rev.* **2007**, *107*, 4891.
7. For recent synthesis and use of BODIPY-labeled bioactive compounds see: (a) Arnold, L. A.; Ranaivo, P.; Guy, R. K. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5867; (b) Jones, L. H.; Randall, A.; Napier, C.; Trevethick, M.; Sreckovic, S.; Watson, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 825.
8. A structure of human ACC2 complexed with Soraphen A has recently appeared in the literature: Cho, Y. S.; Lee, J. I.; Shin, D.; Kim, H. T.; Cheon, Y. H.; Seo, C. I.; Kim, Y. E.; Hyun, Y. L.; Lee, Y. S.; Sugiyama, K.; Park, S. Y.; Ro, S.; Cho, J. M.; Lee, T. G.; Heo, Y. S. *Proteins* **2007**, *70*, 268.
9. (a) Protein was concentrated to 10 mg/ml in 25 mM HEPES pH 8.0, 150 mM NaCl, 1 mM TCEP, 5% glycerol, and then mixed with Soraphen A powder and left overnight at 4 °C. The complex was centrifuged and placed the 1% DMSO on top of the Soraphen A precipitate, and mixed well. The complex was incubated for an hour and the centrifuged to remove the precipitate (un dissolved Soraphen A powder). A sparse-matrix method was employed using Hampton research screens. Crystal screen, Crystal screen2, Peg/ion screen and Cryo screen to identify initial screen at 19 °C. Well diffracting crystals were obtained by conventional hanging drop where 1.5 μl of the protein was equilibrated with 1.5 μl of well buffer, in 0.1 M Tris pH 7.5–8.5, 2.6 M–3.2 M sodium formate. The crystals were flash frozen in liquid nitrogen. The crystals diffracted to 2.3 Å. Data was collected at in house generator, Raxis IV.; (b) The structures of ACC2-BC·Soraphen A complex was refined to 2.3 Å. The protein crystallized in the $C222_1$ spacegroup, with two molecules per ASU. This structure showed disorder in the same regions as the unliganded structure. The final R factors (R_{work} = 0.200, R_{free} = 0.242) show excellent agreement between the model and the data. Stereochemical analyses of the structure with PROCHECK (Laskowski, R. A., MacArthur, M. W., Moss, D. S., Thornton, J. M. *J. App. Crystallogt.* **1993**, *26*, 283.) and WHATCHECK (Hooft R.W.W., Vriend G., Sander C., Abola E.E. *Nature* **1996**, **381**, 272.) show good stereochemistry. PDB codes: 3GID (Soraphen bound), 3GLK (enzyme only).
10. Amino acid residues L238-I760 of the human ACC2 protein was purified from an E.coli strain expressing the protein as an N-terminal His tagged/TEV cleavable fusion. The cell pellet containing the expressed protein was lysed in 50 mM Hepes buffer, 500 mM NaCl, 5% glycerol, 1 mM TCEP and 1 mM PMSF at pH 8.0. The soluble fraction was batch bound to a Nickel sepharose resin, washed and eluted with 250 mM imidazole. The fractions containing the BC domain were pooled, digested with TEV protease to remove the His tag and then loaded onto an anion exchange column in the following buffer: 25 mM HEPES, 30 mM NaCl, 1 mM TCEP and 5% glycerol. The anion exchange column was developed with an NaCl gradient from 30 mM to 500 mM NaCl. The fractions containing the BC domain were pooled, concentrated and loaded onto a preparative sizing column in the following buffer: 25 mM HEPES, 150 mM

NaCl, 1 mM TCEP, 5% glyerol, pH 8.0. The protein eluted as a single peak and its identity was confirmed by mass spectrometry.

11. (a) This compound was available in milligram quantities from biosynthetic methods see: Boehlendorf, B., Bedorf, N., Hoefle, G., Schummer, D., Sutter, M. Eur. Pat. EP358608.; (b) Boehlendorf, B., Bedorf, N., Hoefle, G., Schummer, D., Sutter, M. Eur. Pat. EP358607.
12. This material was of suitable purity to identify biotin carboxylase binders using biophysical methods. The regioisomeric byproduct was formed in approximately equal amounts to the desired product.
13. *Experimental procedure for the synthesis of* ***4***: To a glass vial with screw-cap septa was added desmethyl Soraphen A **3** (10 mg, 0.020 mmol), **2** (5 mg, 0.017 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (2 mg, 0.010 mmol), 4-di(methylamino)pyridine (2 mg, 0.016 mmol) and methylene chloride (0.20 mL). The septa was sealed, the vial wrapped in aluminum foil, and the resulting orange solution was shaken overnight. Analysis by TLC chromatography showed two product spots (RRT = 0.36 and 0.28 in 50% ethyl acetate/heptanes). Purification by preparative TLC (50% ethyl acetate/heptanes) and collection by methanol and methylene chloride washes of the excised product silica gel region (RRT = 0.36, vide supra) and subsequent rotary evaporation of the filtrate afforded desired product **4** (8 mg, 53% yield, 85% purity by NMR). This material was found to be suitable for use in biophysical assays and could be stored in a refrigerator wrapped in foil with minimal decomposition over one month. Samples of **4** isolated by preparative TLC were further purified by supercritical CO_2 chromatography (methanol co-solvent using an ethyl pyridine column) to afford 98% pure material. The regiochemistry of the analog was confirmed by long range proton–carbon (H-16 to C-25) and proton–proton (H-16 to H-40) NMR correlations. LC/HRMS: rt = 8.7 min, Intersil 3 μm C-18, 2.1 mm × 100 mm, 0.1% formic acid/acetonitrile, 5–95% over 10 min, M+NH_4 *m/z* 797.43497 $C_{42}H_{59}BF_2N_3O_9$. NMR: ^{1}H NMR (600 MHz, dichloromethane-d_2) δ ppm 0.92 (d, *J* = 6.79 Hz, 3H) 0.97 (d, *J* = 7.43 Hz, 3H) 1.04 (d, *J* = 7.06 Hz, 3H) 1.13–1.22 (m, 2H) 1.26–1.34 (m, 1H) 1.37–1.44 (m, 2H) 1.54–1.67 (m, 2H) 1.78–1.85 (m, 1H) 1.95–2.03 (m, 1H) 2.18 (s, 3H) 2.31–2.39 (m, 1H) 2.46 (s, 3H) 2.66–2.71 (m, 2H) 2.98 (dd, 2H) 3.09 (dd, *J* = 2.57, 1.01 Hz, 1H) 3.15–3.21 (m, 1H) 3.29–3.35 (m, 1H) 3.29–3.30 (m, 6H) 3.33 (d, 1H) 3.74 (dd, *J* = 10.55, 2.66 Hz, 1H) 3.86–3.98 (m, 1H) 4.26 (s, 1H) 5.30 (dd, *J* = 9.54, 2.02 Hz, 1H) 5.41–5.53 (m, 1H) 5.77 (dd, *J* = 11.00, 4.13 Hz, 1H) 6.08 (s, 1H) 6.13 (dd, *J* = 16.09, 3.71 Hz, 1H) 6.23 (d, *J* = 4.03 Hz, 1H) 6.85 (d, *J* = 3.94 Hz, 1H) 7.07 (s, 1H) 7.19–7.24 (m, 1H) 7.28 (d, *J* = 4.40 Hz, 4H).
14. (a) Otwinowski, Z.; Minor, W. *Methods Enzymol.* **1997**, *276*, 307; (b) Storoni, L. C.; McCoy, A. J.; Read, R. J. *Acta Crystallogr., Sect. D* **2004**, *60*, 432; (c) Emsley, P.; Cowtan, K. *Acta Crystallogr., Sect. D* **2004**, *60*, 2126; (d) Jones, T. A.; Zou, J. Y.; Cowan, S. W.; Kjeldgaard, M. *Acta Crystallogr., Sect. A* **1991**, *47*, 110; Kabsch, W. *J. Appl. Crystallogr.* **1993**, *26*, 795.
15. (a) The labeled Soraphen A compound binds in the same mode as the unlabeled ligand. The label lies outside the main binding pocket where it does not interfere with ligand binding. Although the density is rough for the label, the orientation and position strongly suggests a specific interaction between the fluourine atom, F54, and the guanidinium group of Arg 277.; (b) The protein inhibitor complex was prepared as mentioned in the above hACC2-BC complexed with Soraphen A. Initial screens were performed using Hampton research sparse-matrix as mentioned above. Crystals grew out of a 19 °C vapor diffusion tray with the well buffer, 0.1 M Tris pH 7.5, 0.86 M magnesium formate.
16. The assay was run in 20 mM Tris pH 7.4, 1 mM TCEP. ACC2 BC domain was titrated from 330 nM to 50 pM with a fixed concentration (5 nM) of **4** in 96 well 1/2 area non-binding black plates. The FP was read in an Envision plate reader with the FITC FP filter module.
17. ACC2 BC domain was dialyzed overnight into 20 mM Tris pH 7.4, 1 mM TCEP. Differential scanning calorimetry was run on either protein alone in dialysis buffer with 2% DMSO or 10 μM **4** added in DMSO to 2%. A Microcal capillary VP-DSC was used with a scan rate of 200 °C/h and a buffer trace was subtracted to give the shown traces.
18. (a) Harwood, H. J.; Petras, S. F.; Shelly, L. D., et al *J. Biol. Chem.* **2003**, *278*, 37099; (b) Zhang, H.; Tweel, B.; Li, J.; Tong, L. *Structure* **2004**, *12*, 1683.
19. The assay was run with 20 mM Tris pH 7.4, 1 mM TCEP, 150 nM ACC2 BC domain and 50 nM. Compound was added at 2% DMSO with concentrations ranging from 33 μM to 10 nM.