Acetyl-CoA Carboxylase Inhibition by ND-630 Inhibits Fatty Acid Synthesis and Stimulates Fatty Acid Oxidation in Cultured Cells and in Experimental Animals

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ABSTRACT

Inhibition of acetyl-CoA carboxylase (ACC) reduces fatty acid synthesis and stimulates fatty acid oxidation and has the potential to favorably affect the morbidity and mortality associated with obesity, diabetes, and fatty liver diseases. ACC exists as two tissue-specific isozymes; ACC1 present in lipogenic tissues and ACC2 present in oxidative tissues. To achieve maximal effectiveness an ACC inhibitor should inhibit both isozymes.

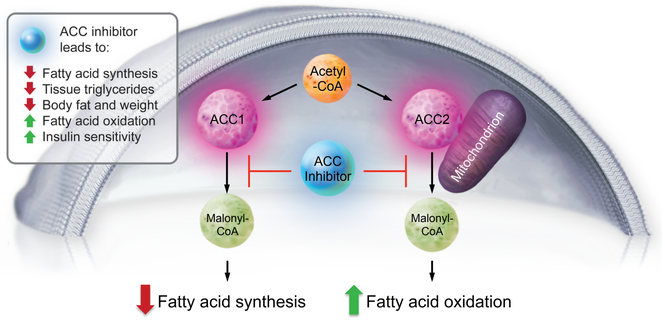
Most efforts to discover ACC inhibitors have focused on interactions within the carboxyltransferase (CT) domain of the enzyme active center. By contrast, our efforts have focused on the allosteric subunit dimerization site on the biotin carboxylase (BC) domain where Soraphen interacts. This is also the same site where the phospho-containing motif of phospho-ACC binds to prevent dimerization and subsequent activation of the enzyme.

Using state-of-the-art structure-based drug design techniques together with crystal structures of the BC domain of human ACC2, we identified a unique series of small molecule allosteric inhibitors that bind to the Soraphen binding site and inhibit enzymatic activity. Development of this series has yielded deep structure-activity relationships, sub-nanomolar enzyme inhibition, functional activity in HepG2 and C2C12 cells and favorable drug-like properties leading to *in vivo* proof of concept.

For example, the series representative ND-630 inhibits both human ACC1 and ACC2 (IC_{50} = 2.0 nM), inhibits HepG2 cell fatty acid synthesis (EC_{50} = 66 nM), stimulates C2C12 cell fatty acid oxidation (2-fold at 200 nM), inhibits rat hepatic fatty acid synthesis (ED_{50} = 0.14 mg/kg) and stimulates rat whole body fatty acid oxidation (minimum effective dose 3 mg/kg).

Together these observations suggest that allosteric ACC inhibition has the potential to favorably impact diabetes, obesity, fatty liver and lipid disorders.

FIGURE 1: Acetyl CoA Carboxylase (ACC): Master Regulator of Fatty Acid Synthesis & Oxidation



- Beneficial effects on lipids, blood glucose, weight, potentially diabetes and CV risk.
- Nimbus: **FIRST** small molecule allosteric inhibitor successfully targeting BC domain.

FIGURE 2: Nimbus Allosteric Inhibitors Show Promise for Both Metabolic Disease & Fatty Liver Disease

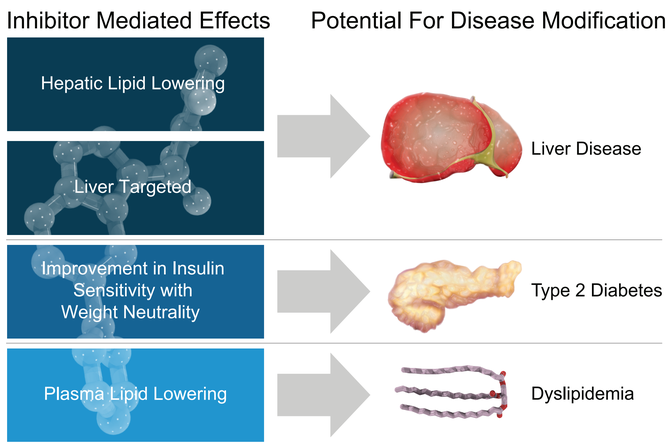
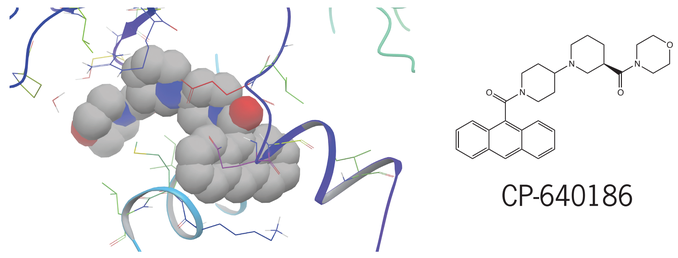
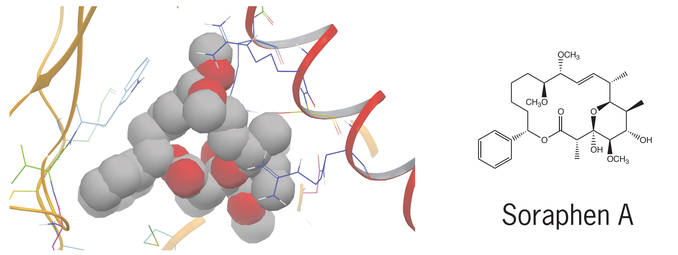


FIGURE 3: Previous Approaches Identified Inhibitors of the ACC CT Domain (ex. CP-640186)



- Most CT-domain inhibitor series identified exhibited poor drug-like properties.

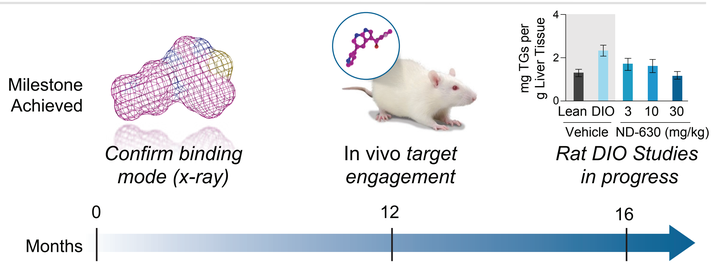
FIGURE 4: Nimbus' Unique Approach Identified Potent ACC BC Domain Inhibitors Targeting Soraphen Binding Site



- Nimbus took an orthogonal approach and designed inhibitors that bound to the Soraphen binding site in the BC domain of the ACC complex.

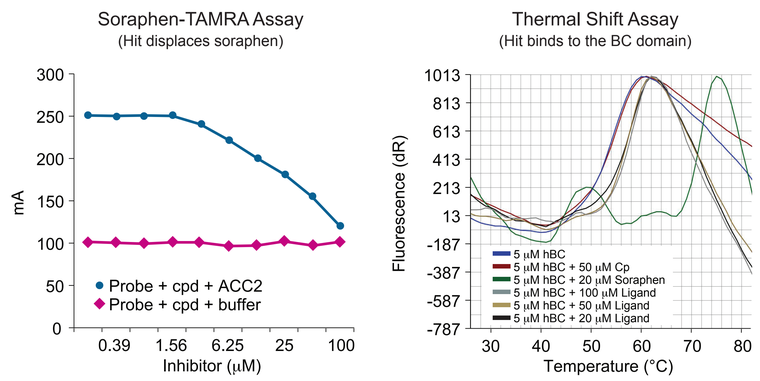
FIGURE 5: Nimbus Approach has Delivered ACC Inhibitors with Demonstrated *in vivo* PoC in 12 Months

Ideas	1.3MM	5,000	10,000
Total synthesized compound		175	225
Potency ACC1, ACC2	**8,000 nM**	**<10 nM**	**<1 nM**
Milestone Achieved	*Confirm binding mode (x-ray)*	In vivo *target engagement*	*Rat DIO Studies in progress*



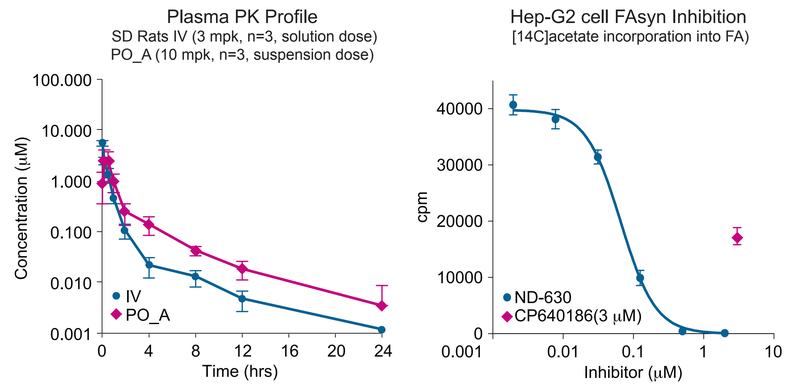
- A structure-based virtual screen of 1.3M lead-like molecules was performed utilizing Schrodinger's computational tools including WaterMap and Glide. This led to the identification of a high quality family of hit molecules with micromolar inhibitory effects against the enzyme. Co-crystal structures of members of this series in the human BC domain confirmed the model and our approach.
- In an iterative design fashion over the next 12 month period, the potency of this family of hits were improved 1000x utilizing the computational model focusing on the replacement or stabilization of high energy hydration sites within the Soraphen binding site.
- Simultaneous to the potency improvements, drug-like properties were optimized to deliver Development Candidate quality molecules that are currently being profiled in chronic models of metabolic syndrome and diabetes.

FIGURE 6: *In vitro* Assays Confirm Binding of Early Hits to BC Domain Prior to Obtaining Co-crystal Structures



- Early in the program we utilized the Soraphen-TAMRA binding assay and BC domain thermal shift assays to provide us confidence that our inhibitors in fact were binding in the Soraphen binding site.
- These tools were utilized until the first Nimbus inhibitor was co-crystalized in the human BC domain. This co-crystal structure taught us our computational model was highly predictive and also provided additional information for WaterMap calculations.

FIGURE 7: ND-630 Displays Excellent Drug-like Properties



TARGET POTENCY
- ACC1 IC_{50} = 2 nM
- ACC2 IC_{50} = 2 nM
- HepG2 EC_{50} = 66 nM
- C2C12 FAOxn stim. 2x @ 200 nM

ADMET
- Low multispecies intrinsic clearance (human, mouse, rat, dog, monkey)
- High solubility (>300 μM)
- P450 inhib >50 μM, hERG >30 μM
- Protein binding (98%)
- DrugMatrixPanel 0/120 hits; 1000x window

RAT PK (10 mg/kg, PO)
- 37%F, Cmax 6 μM, Cl 33 mL/min/kg, AUC 3.4 μM.hr, T1/2 = 4.5h, Vss 2 L/kg
- Liver exposure: 81 μM
- Muscle exposure: 0.45 μM

PHARMACOLOGY
- Rat RQ MED = 3 mpk PO
- Rat FASyn ED50 = 0.14 mpk, PO

Confirmatory Co-crystal Structures

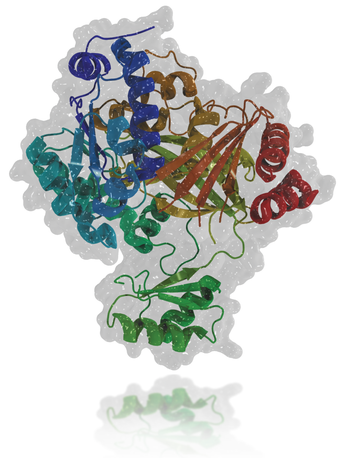
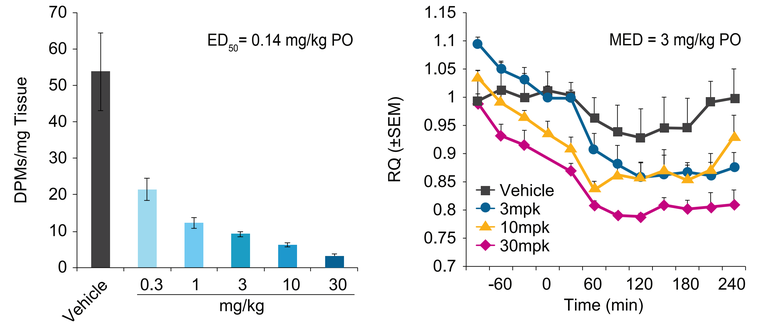


FIGURE 8: ND-630 *in vivo* Proof of Concept: Target Engagement in the Liver and Muscle



SUMMARY

- A structure-based virtual screen and drug design approach utilizing WaterMap was used to identify allosteric inhibitors of ACC that bind to the BC domain.
- Inhibitors were successfully optimized for excellent potency and drug-like properties in 12 months.
- ND-630 demonstrated *in vivo* proof of concept in pharmacologically relevant models of target engagement (rat FASyn ED_{50} = 0.14 mpk, PO & rat RQ MED = 3 mpk, PO).

NEXT STEPS

- Using this approach, a portfolio of nanomolar inhibitors with diverse functional-group driven bio-distribution patterns have been identified and are being utilized for a breadth of ACC-aligned indications.
- Nimbus will report on the detailed pharmacology of ND-630 and other molecules in metabolic disease models, diabetes models and oncology models (cancer metabolism) in the future.