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TITLE: Furanyl Fatty Acid Inhibition of FABP5 as a Mechanism for Treatment and Prevention of Cancer

PRINCIPAL INVESTIGATOR: Gregory Tochtrop

CONTRACTING ORGANIZATION: Case Western Reserve University
Cleveland, OH 44106

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Gregory Tochtrop

E-Mail: gpt6@case.edu

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Case Western Reserve University
10900 EUCLID AVE
CLEVELAND OH 44106-1712

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14. ABSTRACT

: We propose that inhibition of FABP5 represents a novel approach to diverting endogenous RA from pro-proliferative (PPAR δ) to anti-proliferative (RAR) receptors, and further propose the use of furan-containing fatty acids as agents to target RA to RAR. We hypothesize that this pharmacologic inhibition will prevent the oncogenic effects of FABP5 overexpression in highly relevant breast cancer models that display a high ratio of FABP5:CRABP-II expression.

15. SUBJECT TERMS

None provided

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INTRODUCTION: Retinoic acid (RA) is a potent anticarcinogenic agent that functions by regulating the expression of multiple genes through its ability to activate two nuclear receptors: RA receptors (RAR) and the peroxisome proliferator–activated receptor δ (PPARδ). However, RA's utility as a therapeutic agent is limited by RA-resistance that is acquired in some tumors, and the paradoxical observation that in some tumors RA actually potentiates tumor growth. The key to regulating the partitioning of RA between these two opposing pathways lies in the two proteins that deliver RA to their respective transcription factors: cellular RA-binding protein II (CRABP-II), which targets the hormone to RAR, and fatty acid binding protein 5 (FABP5), which transports it to PPARδ. Based on our RA signaling model we predict that by blocking FABP5, furanyl-FAs will specifically divert RA to RAR and consequently will overcome RA-resistance and suppress the growth of FABP5-overexpressing tumors. The goal of this work is to further investigate this partitioning between RAR and PPARδ, investigate the metabolic fate of furanyl-FAs, and determine if these molecules can serve as effective chemopreventive agents.

KEYWORDS: Furan fatty acid (FFA); retinoic acid (RA); retinoic acid receptor (RAR); peroxisome proliferator–activated receptor δ (PPARδ); fatty acid binding protein (FABP); cellular RA-binding protein II (CRABP-II); and fatty acid binding protein 5 (FABP5).

ACCOMPLISHMENTS: The initial year of this award was marked by the sudden and unexpected death of Noa Noy, PI of the accompanying award: 16-1-0700 (BC151494P1), and subsequent transfer to its current PI, Liraz Levi that coincided with her Assuming a faculty position at CWRU School of Medicine. This has hampered our ability to make progress on the key areas of the proposal that were earmarked for Dr. Noy's Laboratory.

Major Goals:

Specific Aim 1. Define the ability of furanyl-FAs to perturb the CRABP-II/FABP5 signaling balance.

1.1. Define a structure-activity relationship for naturally occurring furanyl-FAs.
1.2. Examine the ability of high affinity FABP5-binding furanyl-FAs to target RA to the CRABP-II/RAR path.
1.3. Determine the ability of furanyl-FAs to inhibit the growth of cultured carcinoma cells.

Specific Aim 2. Define the metabolic fate(s) of furanyl-fatty acids.

2.1. Develop a comprehensive understanding of the metabolic and catabolic fate(s) of furanyl-FAs using a mass isotopomer approach in perfused organ systems.
2.2. Examine the rate(s) of metabolism/catabolism in normal tissues, and cancer cell lines.

Specific Aim 3. Assess the effects of furanyl-FAs on mammary tumor development *in vivo*.

3.1. Assess the efficacy of furanyl-FAs in inhibiting tumor development in xenograft mouse models of breast cancer.
3.2. Test the ability of furanyl-FAs to prevent tumor formation in the transgenic MMTV-Neu/Erb-B2 model of mammary carcinogenesis.

Given the above, our primary areas of emphasis has been on activities that primarily resided in the Tochtrop Laboratory.

Specific Aim 1. Define the ability of furanyl-FAs to perturb the CRABP-II/FABP5 signaling balance.

1.1: *Synthesis of all naturally occurring furanyl-FAs in quantities that will allow further analysis in vitro and in vitro. Synthesis of a thiophene and pyrrole-containing furanyl-FA.* These activities have been completed as detailed below. The synthetic targets are shown in Figure 1

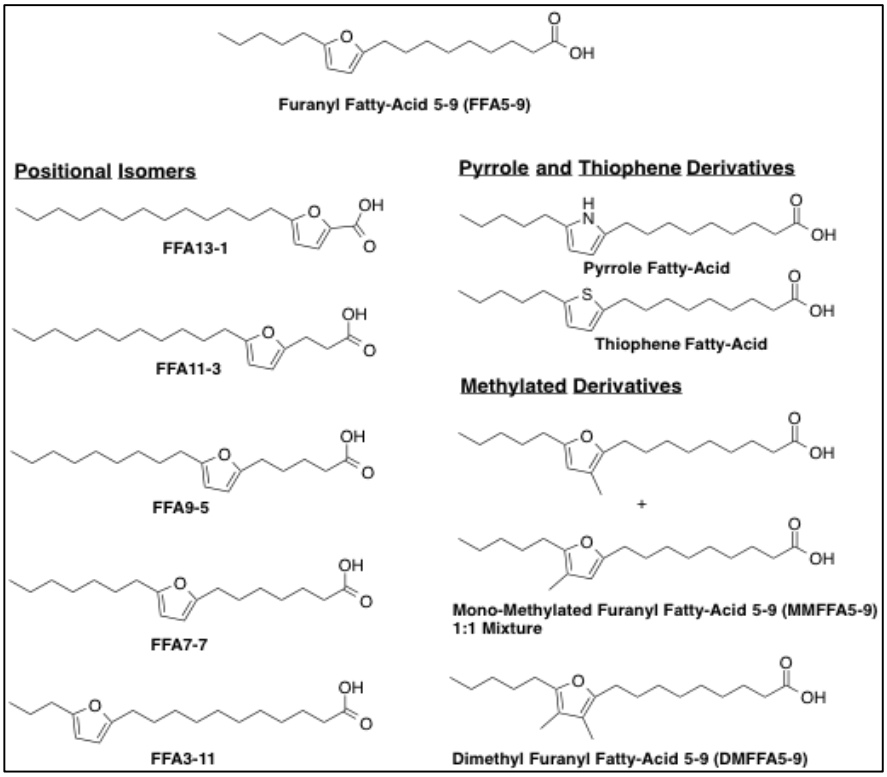
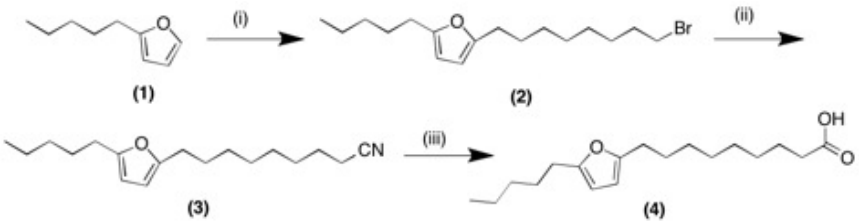


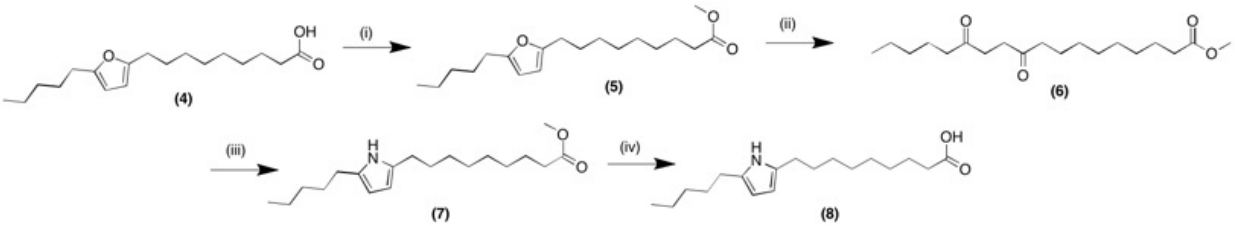
Figure 1. Positional, pyrrole and thiophene derivatives of FFA 5-9. Positional variants are distinguished by chain length on either side of the furan or an added methyl group. Pyrrole and thiophene derivatives replace the furan oxygen with a nitrogen and sulfur, respectively.

In the previously proposed synthesis of furanyl fatty acid positional variants, the structural backbones were constructed via basic substitution reactions (S_N1) utilizing nBuLi, a furan base **(1)**, and one or more dibromoalkanes (Scheme 1,). The resulting brominated species **(2)** were then converted to nitirile derivatives **(3)** via a second substitution reaction and then hydrolyzed into the resulting fatty acid **(4)** following treatment with KOH[9]. Mono and dimethylated furans were synthesized using the same synthesis.

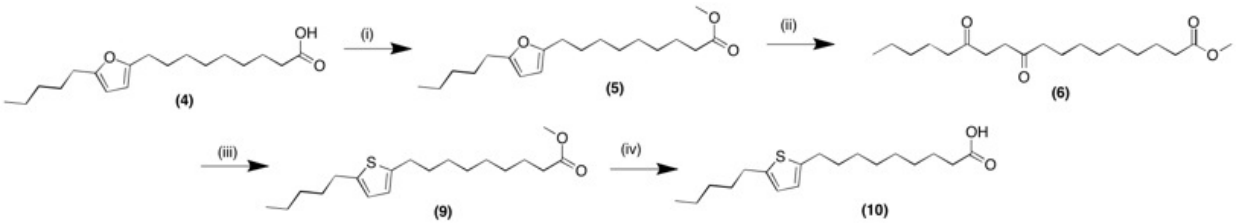
The synthesis of the pyrrole and thiophene derivatives is designed to utilize readily available FFA5-9 **(4)** in a simple Paal-Knorr heterocyclization. Once esterified **(5)**, the furanyl species will then undergo hydrolysis to a diketone intermediate **(6)**[11]. This intermediate will then undergo a Paal-Knorr cyclization to either a pyrrole **(7)**[12] (Scheme 2) or thiophene ester **(9)**[13] (Scheme 3) followed by hydrolysis to the pertinent fatty acid **(8,10)**[14].



Scheme 1. Example synthesis of FFA5-9. (i) 1. n-BuLi (1.1 equiv). 2. 1,8-dibromooctane (3 equiv.), 69.4% (ii) KCN (10 equiv.), TBAB (0.2 equiv.), $CHCl_3$:H_2O 1:1, reflux 16 hr, 75% (iii) KOH (10 equiv.), EtOH:H_20 1:1, reflux 17hr, 97.4%.



Scheme 2. Synthesis of pyrrole derivative. (i) CH_2N_2 (4 equiv.), diethyl ether, 42% (ii) 10% aq. H_2SO_4**,** MeOH, reflux, 12hr, 98.9% (iii) NH_4OAc (10 equiv.), EtOH, stir rt 24 hr, 96% (iv) 6M aq. KOH (1.85 equiv.), EtOH, reflux 10 min, 48.5%



Scheme 3. Synthesis of thiophene derivative. (i) CH_2N_2 (4 equiv.), diethyl ether, 42% (ii) 10% aq. H_2SO_4**,** MeOH, reflux, 12hr, 98.9% (iii) Lawesson's reagent (1.2 equiv.), toluene, 84.3% (iv) 6M aq. KOH (1.85 equiv.), EtOH, reflux 10 min, %

1.2: *Examine the ability of high affinity FABP5-binding furanyl-FAs to target RA to the CRABP-II/RAR path;* ***1.3:*** *Determine the ability of furanyl-FAs to inhibit the growth of cultured carcinoma cells.*

These studies are ongoing in the Levi Lab.

Specific Aim 2. Define the metabolic fate(s) of furanyl-fatty acids.

2.1. Develop a comprehensive understanding of the metabolic and catabolic fate(s) of furanyl-FAs using a mass isotopomer approach in perfused organ systems.

Fig. 2 presents some of data from an orientation experiment with 2 mM Fa. Shown in Panel A is the time course of uptake of Fa from the perfusate.. By 1 hour, ~90% of total Fa (400 mmol) was taken up, a rate that is similar to uptake of regular long-chain saturated and unsaturated fatty acids. CG comparative analysis (with a control perfusion) showed that Fa proceeds through β-oxidation but that it does so at a greatly attenuated rate, resulting in effective accumulation of the parentalfuranyl-FA. This data further corroborates our previous cell culture data indicating that that furan-FAs are metabolically stable.

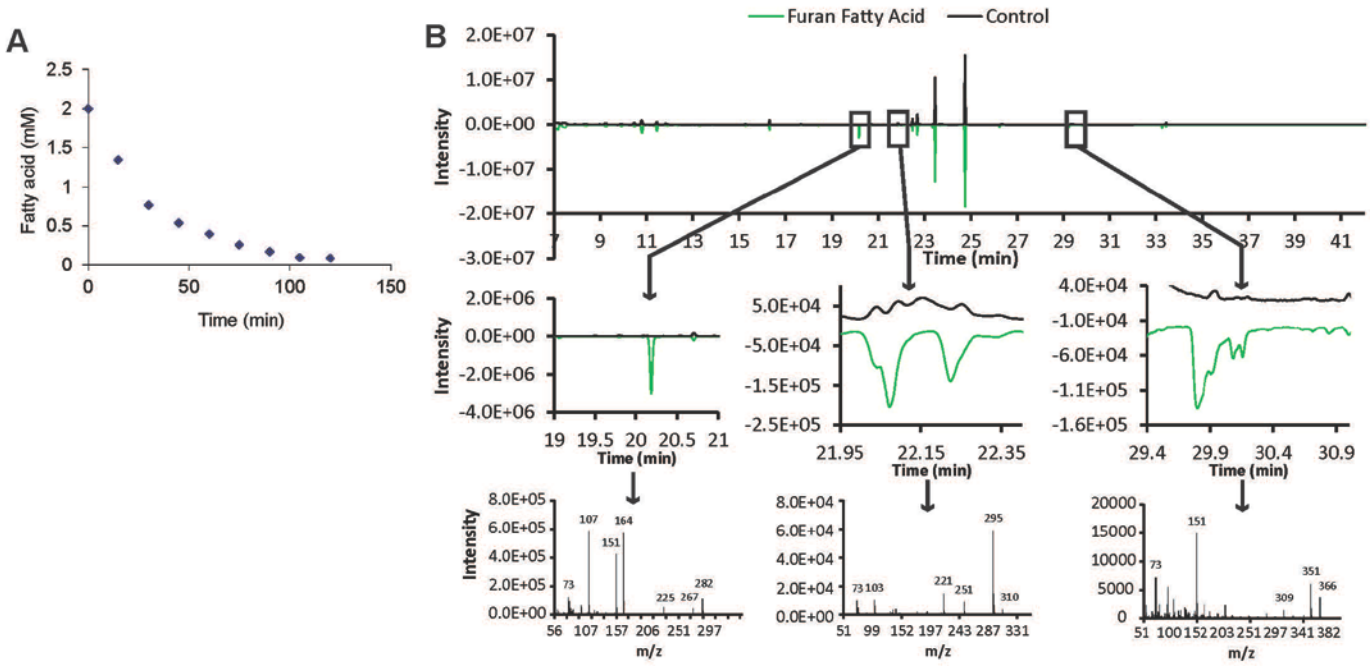


Figure 2. Metabolic analysis of FFAs A) The concentration of $\mathbf{F_a}$ was measured in the perfusate as a function of time. Quantitaion was performed via TMS derivitization and GC-MS utilizing an external standard. **B)** GC/MS traces of control and $\mathbf{F_a}$ perfusions. Difference analysis is used to identify novel metabolites, which are subsequently identified using mass spectra. The spectral expansion on the left shows an expansion of the GC-MS spectrum and the resulting mass spectra, which shows intact parent furanyl-FA, $\mathbf{F_a}$. The spectral expansions center and right indicate two predicted metabolites that we specifically searched the spectra for. Center shows the difference and mass spectra for a molecule we have tentatively assigned as the C_{16} furanyl-FA (indicating an $\mathbf{F_a}$ catabolism product via one cycle of β-oxidation). The expansion on the right shows the difference and mass spectra for a molecule we have tentatively assigned as the C_{14} furanyl-FA, which we ascribe to an $\mathbf{F_a}$ catabolism product derived from two cycles of β-oxidation.

2.2. *Examine the rate(s) of metabolism/catabolism in normal tissues, and cancer cell lines.*

Nothing to Report

Specific Aim 3. Assess the effects of furanyl-FAs on mammary tumor development in vivo.

3.1. *Assess the efficacy of furanyl-FAs in inhibiting tumor development in xenograft mouse models of breast cancer.*

3.2. *Test the ability of furanyl-FAs to prevent tumor formation in the transgenic MMTV-Neu/Erb-B2 model of mammary carcinogenesis.*

Nothing to Report

What opportunities for training and professional development has the project provided?

Tochtrop and Stewart attended the 2017 Lipids Gordon Conference. Stewart attended a professional development workshop at this conference.

How were the results disseminated to communities of interest?

Stewart gave a poster at the 2017 Lipids Gordon Conference.

What do you plan to do during the next reporting period to accomplish the goals?

Our main focus is to complete Aim 1, and specifically identify a small number of molecules that ww will be moving forward with to Aim 2 and Aim 3.

IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

We have developed methods to efficiently gain access to the complement of furan-containing fatty acids using chemical synthesis. This will allow scalability not possible through isolation from natural sources. Further, we have been able to show that these molecules will undergo beta oxidation, but that rate is greatly attenuated compared to standard fatty acids.

What was the impact on other disciplines?

We predict that the results we describe here will be important for the fields of organic synthesis and metabolism. In terms of synthesis, orthogonal alkylation of furan is important, and could be widely used. In terms of metabolism, it is generally not known what the fate of furan is in mammalian systems.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

CHANGES/PROBLEMS:

Changes in approach and reasons for change

1. The most significant change from the original proposal stems from the sudden and tragic death of the original PI, Noa Noy. This has led to significant delays in the activities detailed in the SOW to take place in Site 2. This has been mostly dealt with in the form of an extension of term of the grant in conjunction with the email from Mr. Darrell Beaver on 10/12/2017.
2. One change that we are requesting be allowed is to expand the scope of fatty acids to consider in the role of cancer chemopreventive agents. This proposal was based on our strong preliminary data on furan-containing fatty acids, but we would like to expand this to include common dietary fatty acids. For example, we have been able to show that palmitate displays similar effects as compared to F_a.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals.

Nothing to Report.

Significant changes in use of biohazards and/or select agents.

Nothing to Report.

PRODUCTS:

Publications, conference papers, and presentations

Nothing to Report.

Report only the major publication(s) resulting from the work under this award.

Nothing to Report.

Website(s) or other Internet site(s)

Nothing to Report.

Technologies or techniques

Nothing to Report.

Inventions, patent applications, and/or licenses

Nothing to Report.

Other Products

Nothing to Report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Gregory Tochtrop

Project Role: PI/Professor

Researcher Identifier (e.g. ORCID ID): https://orcid.org/0000-0003-2447-254X

Nearest person month worked: 3

Contribution to Project: Project oversight, and direct supervision of Ms. Stewart, Dr. Han, Ms. Shang, and completion of the metabolomics work reported here.

Name: Elizabeth Stewart

Project Role: Graduate Student

Researcher Identifier (e.g. ORCID ID): N/A

Nearest person month worked: 12

Contribution to Project: Contributions to small molecule synthesis, and biological contributions to Aim 1.

Name: Yong Han

Project Role: Research Associate

Researcher Identifier (e.g. ORCID ID): N/A

Nearest person month worked: 5

Contribution to Project: Ms. Small molecule synthesis.

Name: Grace Shang

Project Role: Research Assistant

Researcher Identifier (e.g. ORCID ID): N/A

Nearest person month worked: 5

Contribution to Project: Small molecule synthesis.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

Nothing to Report