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14. ABSTRACT

This project aims to develop synthetic multifunctional compounds as therapeutics for polycystic kidney disease (ADPKD). We have shown that two parent compounds, 11β-dichloro and 11β-dipropyl, are effective in preventing and delaying cystic growth in orthologous gene mouse models of human ADPKD. To guide the development of new compounds, the mechanism by which 11β compounds achieve their efficacy and selectivity against cystic kidney cells is being investigated. During the last funding period, we continued the work on probing the mechanism of therapeutic efficacy of 11β compounds in animals through induction of mitochondrial reactive oxygen species (ROS). We validated the efficacy of 11β-dipropyl compound in the adult mouse model of ADPKD extending the efficacy noted in perinatal models of the disease. In parallel, through our collaboration with the Essigmann group at MIT, we have continued the synthesis of new molecules from the 11β family, which will inform through a structure-activity relationship studies, the key molecular features required for activity and provide additional hints about the mechanism of action. These aims will permit design and testing of compounds with improved pharmacological properties.

15. SUBJECT TERMS
Polycystic kidney disease, ADPKD, *PKD1*, polycystin-1, therapeutic, apoptosis, mitochondria, reactive oxygen species, unfolded protein response

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Table of Contents

1. INTRODUCTION

This project aims to develop synthetic multifunctional compounds as therapeutics for autosomal dominant polycystic kidney disease (ADPKD). In collaboration with the Essigmann group at MIT, we have shown that two parent compounds, 11β-dichloro and 11β-dipropyl, are effective at preventing and delaying cystic growth in mouse models of orthologous genes for human ADPKD. To guide the development of new compounds, we are exploring the mechanism by which 11β compounds achieve their efficacy and selectivity for cystic cells in vitro and in vivo. One arm of the project focuses on the synthesis of new molecules from the 11β family, which will inform, through a structure-activity relationship studies, the key molecular features required for activity against kidney cyst cells and in so doing provide additional hints about the mechanism of action. A second arm of the project focuses on the development of an in vitro cell culture model that can be used to screen the new molecules for improved efficacy and selectivity; such molecules will be validated in the established perinatal and adult-onset ADPKD mouse models and pave the way towards their preclinical and clinical development.

During the last funding period, we made progress on validating cell culture models that recapitulate the biological consequences of 11β compounds. The LLC-PK1 *Pkd1* null kidney epithelial cell lines we developed last year show good promise for toxicity assays when coupled with the total cellular ATP measurements (Cell-Titer Glo Assay). We also investigated aspects of the mechanism by which these compounds affect Pkd knockout cell cell culture models. Additionally, through our partnering efforts with our collaborators at MIT, we completed the synthesis of 5 new 11β analogs, and a 6th analog will be completed soon. The new compounds were tested for efficacy and selectivity in cell culture models, providing new structure-activity information. Moreover, we have continued probing the mechanism of toxicity of 11β compounds in animals specifically focusing on induction of mitochondrial reactive oxygen species (ROS) and testing the 11β-dipropyl compound in the adult mouse model of ADPKD.

2. KEYWORDS

Polycystic kidney disease, polycystic disease, ADPKD, *PKD1*, *PKD2*, therapeutic, polycystin-1, apoptosis, mitochondria, reactive oxygen species, unfolded protein response, mouse model.

3. ACCOMPLISHMENTS

What were the major goals of the project?

Our major goals were:

- Synthesize and characterize 11β analogs with different linkers and/or alkyl substituents (90% completed)
- Using the cell culture model, evaluate the efficacy and selectivity of the 11β analogs in a structure-activity study (90% completed)
- Using the cell culture model, investigate the role of apoptosis, mitochondrial metabolism and unfolded protein response (UPR) in the toxicity and anti-PKD effects of 11β compounds (50% completed).
- Test 11β-dipropyl in the adult onset PKD mouse model (75% completed).

What was accomplished under these goals?

a. Synthesis and characterization of 11β analogs (Essigmann, MIT)

One central goal of this project was to synthesize a series of 11β analogs and perform structure-activity studies that will inform which chemical and structural features of the molecule are essential for activity in kidney cyst cells. Additional goals of this effort were to obtain a better lead compound with perhaps a simpler chemical structure. To this end, we varied the structure of the linker (carbamate, secondary amine or both) and the structure of the aniline moiety. The 11β analogs synthesized are shown in Figure 1.

Although not originally proposed, we synthesized an additional analog (compound **5**), in which the dipropyl arms of the 11β-dipropyl are replaced with methyl groups. This analog constitutes the smallest variation from the 11β-dipropyl structure, and its synthesis was facilitated by readily-available starting materials.

The synthetic schemes used for the synthesis of compounds **1-6** are shown in Figures 2 and 3. Except for compound **6**, which is anticipated to be completed within 1-2 months, all compounds were synthesized, isolated and purified with yields >100 mg each (Table 1). All compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, MS and UV-Vis.

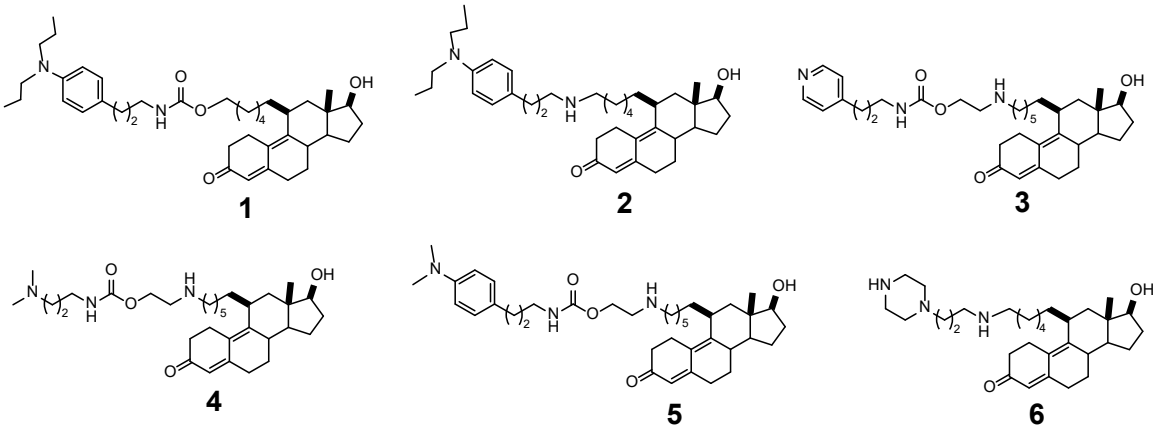

Figure 1. Chemical structures of the 11β-dipropyl analogs synthesized for structure-activity studies.

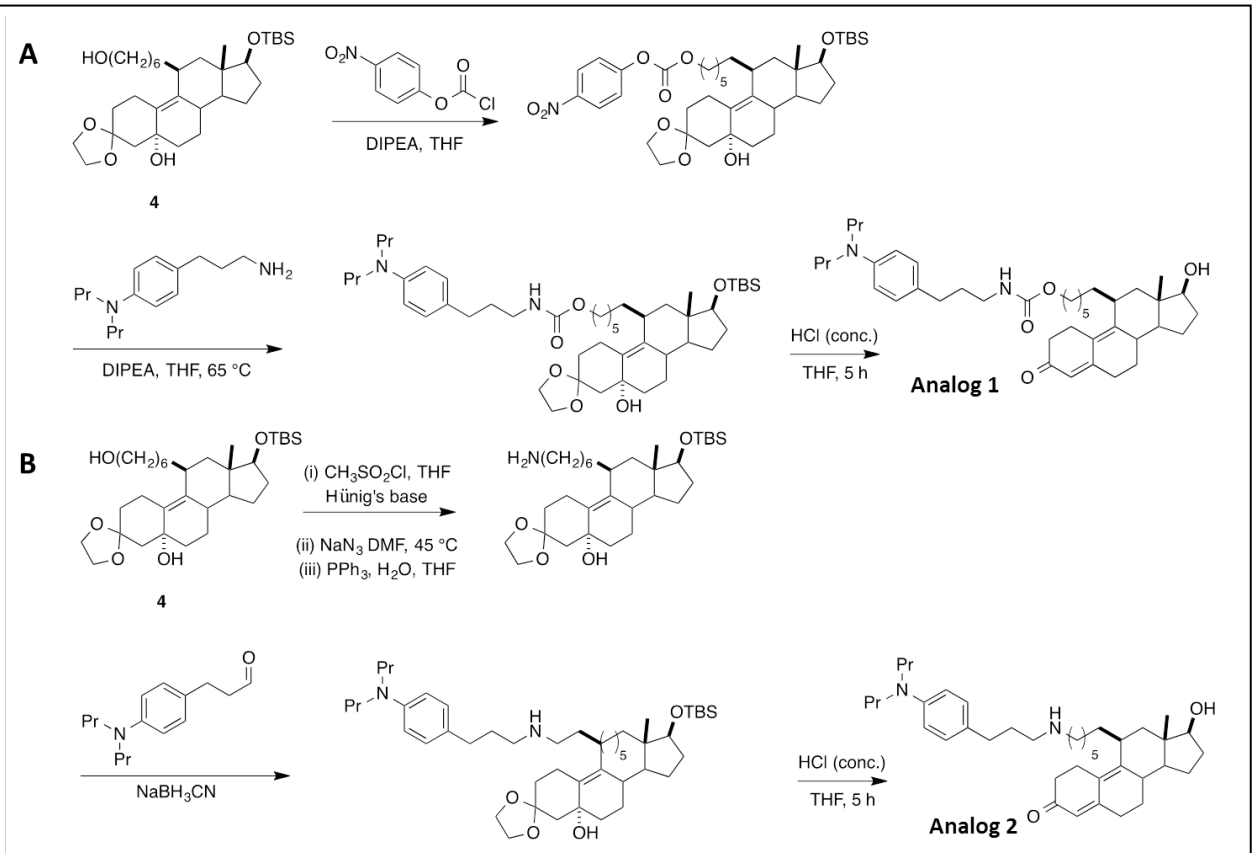

Figure 2. Chemical synthesis schemes for 11B-dipropyl analogs. A) The synthesis scheme for analog **1**, which features a linker with only the carbamate moiety. B) The synthesis scheme for analog **2**, which features a linker with only the secondary amine moiety.

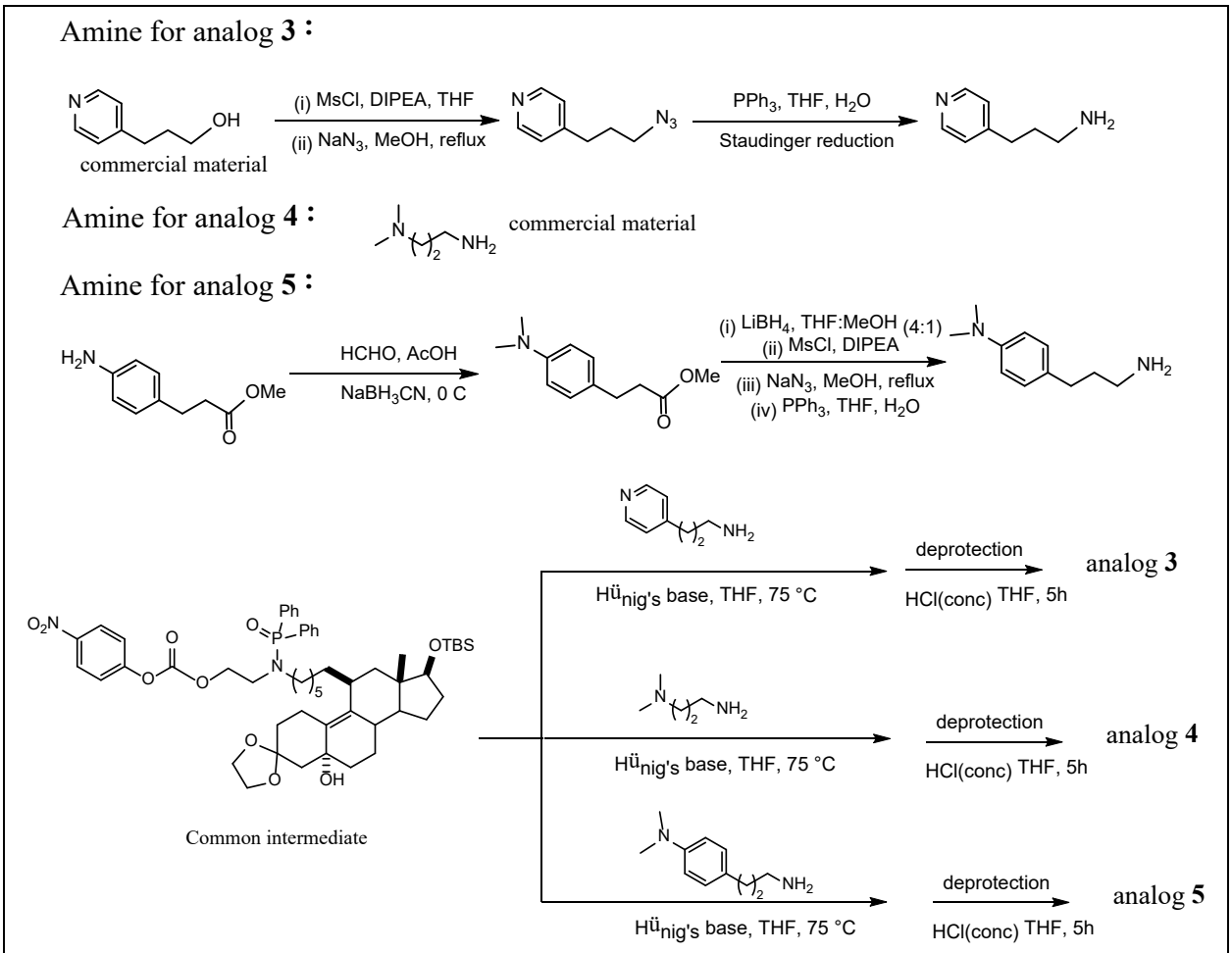

Figure 3. Chemical synthesis schemes for 11β-dipropyl analogs **3-5**.

b. Physico-chemical characterization of the 11β-dipropyl analogs (Essigmann, MIT)

All the logP values have been estimated using the tools available at www.molinspiration.com, which estimate logP based on the equation of Horvath et al. The pKa values for all compounds were estimated using the Evans pKa tables available at www.evans.rc.fas.harvard.edu. The compounds were dissolved in pure ethanol and their UV-Vis spectra were recorded. All compounds show the absorption peak at 305 nm, characteristic of the steroid moiety; however, the experimentally-determined extinction coefficient at 305 nm varies considerably across compounds (Table 1).

Table 1. Physico-chemical properties of 11β-dipropyl analogs. Structures are shown in Figure 1.

Compound	Amount synthesized (mg)	MW (g/mol)	logP	pKa	Extinction Coefficient at 305 nm (M^{-1} cm^{-1})
1	568	632.92	8.62	11	5752
2	107	588.91	8.36	10.5	4415
3	120	577.80	5.14	11	24390
4	127	543.78	4.41	11	32100
5	116	619.88	6.53	11	33030
6	*N/A*	497.76	3.84	10.5	*N/A*
11β-dipropyl	800	675.88	8.25	11	22500

c. Structure-activity study of 11β-analogs in the LLC-PK1 cell line model (Somlo/Essigmann, Yale/MIT)

The commercially available kidney cell line LLC-PK1 (ATCC CL-101) was evaluated over the previous funding periods as a possible cell culture model for testing 11β compounds activity. LLC-PK1 is a porcine kidney proximal tubule cell line from the Hampshire pig that is routinely used to study epithelial cell function. The advantages of this line over other cell lines are as follows: i) it is a well characterized kidney epithelium cell line; ii) the cells exhibit lateral growth inhibition, which allows formation of stable monolayers, without the need to inactivate a growth-driving oncogene (e.g. SV40); iii) easy to culture under standard conditions; iv) it readily forms cilia which are a structure central to the pathogenesis of ADPKD. The team at Yale employed CRISPR-Cas9 genome editing technology to generate two isogenic cell lines derived from LLC-PK1 in which either *Pkd11* or *Pkd2* genes were deleted. The cell biologic properties of these cells were evaluated by the Yale group. The isogenic wild-type and the two mutant cell lines were then evaluated in cell culture for response to 11β by the Essigmann team at MIT.

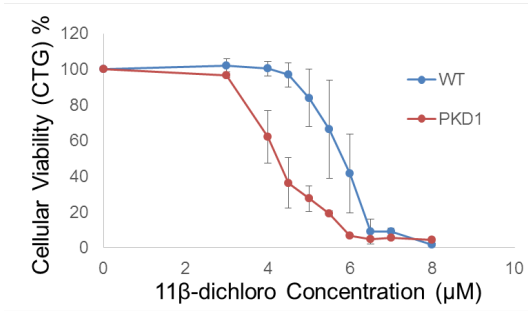

Figure 4. Dose responses to 11B-dichloro of LLC-PK1 cell lines (WT and *Pkd1*$^{-/-}$).

Preliminary work with the LLC-PK1 cell lines showed that they could be a good cell culture model for testing sensitivity and specificity to 11β compounds. A dose response with 11β-dichloro showed that the *Pkd1*$^{-/-}$ knockout cells are more sensitive to 11β than the wild-type parental line (Figure 4). The cellular viability in this case was measured using the CellTiter-Glo reagent from Promega, which gives a luminescent signal proportional to the amount of ATP present in each well. For reasons that are currently under investigation, the CellTiter-Blue reagent we used for measuring the viability in other cell lines in previous studies did not work with the LLC-PK1 cell lines.

Dose-response experiments were set up using the LLC-PK1 cell lines and the 11β analogs. The initial experiment used doses between 0-10 μM. Under these conditions, only compounds **5** and 11β-dipropyl showed substantial toxicity. Subsequently, the doses tested for compounds **1**-**4** were increased (up to 300 μM) to determine their toxic range. Of these, compound **1** did not show any toxicity at concentrations as high as 300 μM. Based on the dose response curves, IC50 values (concentration at which cell viability is decreased by 50%) were estimated (Table 2).

Table 2. The toxicity (IC50 concentrations) of 11β analogs in the LLC-PK1 cell line model.

Compound	$IC50_{WT}$ (μM)	$IC50_{PKD1}$ (μM)
1	>300	>300
2	90	110
3	48	52
4	42	60
5	3.1	3.4
11β-dipropyl	2.6	2.9

The structure-activity study provided novel insights into the structural requirements for activity against PKD cells. Compounds **1** and **2**, which feature simpler linkers [carbamate-only (**1**), or secondary amine-only (**2**)], were significantly less active than compounds having both functional groups in the linker. This result suggested that the bifunctional linker is important for activity, with the amine functionality ostensibly helping with solubility and charge, and the carbamate providing conformational rigidity. Compounds **3** and **4** provided insight into the requirements for the left-hand side of the molecule. The aromatic ring alone (compound **3**) or the aliphatic dimethyl amine (**4**) were not as effective as the dimethyl-aniline compound **5**, which features both structural features. Moreover, compound **5** showed a very similar activity to that of 11β-dipropyl, suggesting that the length of the alkyl groups on the tertiary aniline is not a significant contributor to the toxicity of 11β-dipropyl.

One puzzling observation that emerged from the structure-activity study was that none of the compounds (including 11β-dipropyl) showed a consistent selectivity towards the *Pkd1*$^{-/-}$ LLC-PK1 knockout cells. The IC50 values are relatively close for the less active compounds (**2**,**3**,**4**) and statistically indistinguishable for the most active compounds (**5**, 11β-dipropyl). Moreover, the trend suggests the WT strain is more sensitive than the mutant. This is in contradiction with prior data that showed reproducible selectivity for both 11β-dichloro and 11β-dipropyl towards the mutant cell line. A number of possibilities may have contributed to this result, including a clonal effect perhaps from off target CRISPR/Cas9 activity leading to a rescue mutation in the Pkd1 mutant cell line. We will further investigate this by examining two independent *Pkd1* null clonal LLC-PK1 cells lines as well as two independent *Pkd2* null clonal LLC-PK1cell lines to determine whether this is a general feature of LLC-PK1 cells carrying *Pkd1* and *Pkd2* mutations or an aberration of this particular cell line. The Yale group has already produced all 4 requisite knockout cells along with the isogenic parental line from which they were made.

d. Investigation of the mechanism of toxicity of 11β-analogs: ROS generation (Essigmann, MIT)

Our previous work has shown that 11β compounds (specifically 11β-dichloro) kill cells by deregulating mitochondrial respiration, which leads to increased oxidative stress, and by increasing the level of ER stress which manifests as the unfolded protein response (UPR). Either of these perturbations may be influencing and promoting the other, with the end result being cell death by apoptosis.

A preliminary study was setup to determine the ability of the 11β-dipropyl analogs to induce ROS generation. Cells were exposed to compounds (**2-5**) at concentrations near their IC50 for 4 h. The compounds were washed off and then the cells treated with the ROS dye CM-H2DCFDA, a compound that upon cellular uptake and oxidation becomes fluorescent. The median fluorescence for each treatment condition was then evaluated by flow cytometry and compared with the median fluorescence of vehicle (DMSO) treated cells. Preliminary results indicated that compounds **2, 5** and 11β-dipropyl at doses of 100 μM, 3 μM and 3 μM, respectively seemed to be able to generate an increase in fluorescence (ROS), while compounds **3** and **4** at doses of 50 μM each had no effect. However, given the considerations about clonal cell line effects discussed in part above (c), these observations are preliminary and will be further investigated in a panel of cell lines.

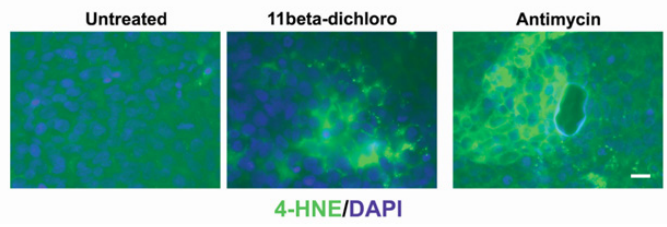

Figure 5. Detection of 4-HNE induced by 11β-dichloro treatment in cells. The green fluorescence denotes 4-HNE staining. The nuclei are stained with DAPI.

e. Examining the mechanism of toxicity of 11β compounds in tissues. (Somlo, Yale).

The previous mechanistic study of 11β toxicity demonstrated the ability of the compound to localize to the mitochondrion, disrupt the flow of electrons through the electron transport chain and induce formation of ROS. Therefore, we investigated whether these findings extend to the in vivo situation and 11β induces ROS in cystic kidneys. We evaluated ROS by staining for the lipid peroxidation biomarker 4-hydroxynonenal (4-HNE), a common oxidative stress-induced cellular byproduct. Recent evidence suggests that the bulk of 4HNE in a cell is formed from the oxidation of mitochondria-specific phospholipid cardiolipin, and thus, it primarily reflects mitochondrial oxidative stress. First we established the specificity of the anti-4-HNE antibody by treating IMCD3 cells (an established kidney cell line) with antimycin A (a known ROS stressor) and 11β-dichloro (Figure 5); both drugs elicited a positive 4-HNE signal compared with DMSO treated cells.

Next we investigated the status of 4-HNE in the 11β-treated mice. Cystic kidneys the early onset *Pkhd1-Cre*; *Pkd1*$^{fl/fl}$ model were harvested and stained with 4-HNE (Figure 6). 4-HNE staining was substantially increased in the cystic kidneys treated with 11β, but only in the DBA positive cells (i.e., *Pkd1*$^{-/-}$ cyst forming cells), suggesting a specific induction of oxidative stress in the cells lining the cysts (Figure 6, bottom row). By contrast, no 4-HNE signal was detected in wild-type non-cystic proximal tubule epithelia in the 11β-treated kidneys, or in any of the vehicle-treated kidneys. These observations are consistent with and bolster previous observations (described in the proposal for this project) that the transcriptional levels of oxidative stress

inducible genes catalase (CAT) and Cu-Zn superoxide dismutase (SOD1) were significantly increased in the cystic kidneys treated with 11β.

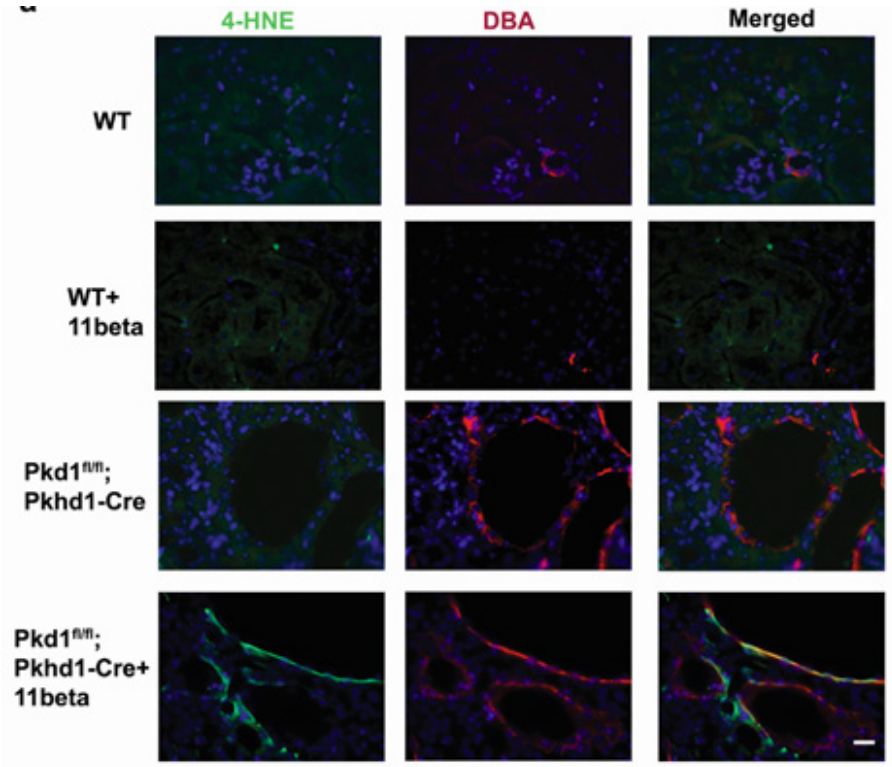

Figure 6. 11β-dichloro induces specific accumulation of the oxidative stress biomarker 4-HNE in cystic cells of the neonate mouse model of ADPKD (*Pkd1$^{fl/fl}$; Pkhd1-Cre*). DBA staining is characteristic for the collecting ducts that harbor the mutant cells and form cysts in this model.

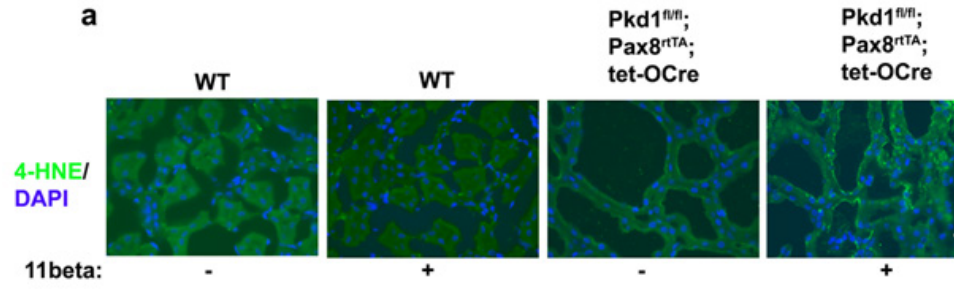

Figure 7. 11β-dichloro induces specific accumulation of the oxidative stress biomarker 4-HNE in cystic cells of the adult mouse model of PKD (*Pkd1$^{fl/fl}$; Pax8^{rtTA};TetO-Cre*); right panel. Cell nuclei are stained with DAPI.

The Pax8 adult model also recapitulated these mechanistic insights; cystic cells displayed higher level of 4-HNE staining (Figure 7), and catalase mRNA levels were significantly increased in treated whole kidney extracts (data not shown). The occurrence of a common mechanism in both the early onset and late adult models is strongly suggested that this is a fundamental and common aspect of the mechanism of action of 11β compounds.

f. Investigating the efficacy of 11β-dipropyl in the adult onset PKD mouse mode (Somlo, Yale).

Testing 11β-dipropyl activity in the adult onset ADPKD mouse model (*Pkd1$^{fl/fl}$; Pax8^{rtTA};TetO-Cre*) was begun in the last funding period. The complete study is still ongoing due to the long duration of the treatment period (to age >24 weeks), the low efficiency in getting all 4 alleles combined and the normal variation in this model necessitating higher number of mice per group (8-9 each for vehicle vs 11β-dipropyl-treated). Although the study is still ongoing, we present preliminary observations that suggest that 11β-dipropyl is effective in slowing down cyst growth in the adult animal model as it is in the early onset model. We observed decreased kidney/body weight ratio, decreased average cystic index and statistically significant improvement in kidney function (i.e., lower levels of blood urea nitrogen) in the treated animals (Figure 8). Analyses of more animals in both groups (vehicle vs 11β-dipropyl treated) will continue in the current funding period to achieve the sample size and statistical power to draw robust conclusions.

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

An undergraduate research student, Sienna Li (Yale University) conducted research for this project during an NIH funded summer student fellowship. Parisa Westergengling, a medical student from Germany also

contributed to the project. Tobias Staudner, a medical student from Erlangen University in Germany is currently fully engaged in scholarly enrichment research activities of the Yale University Section of Nephrology.

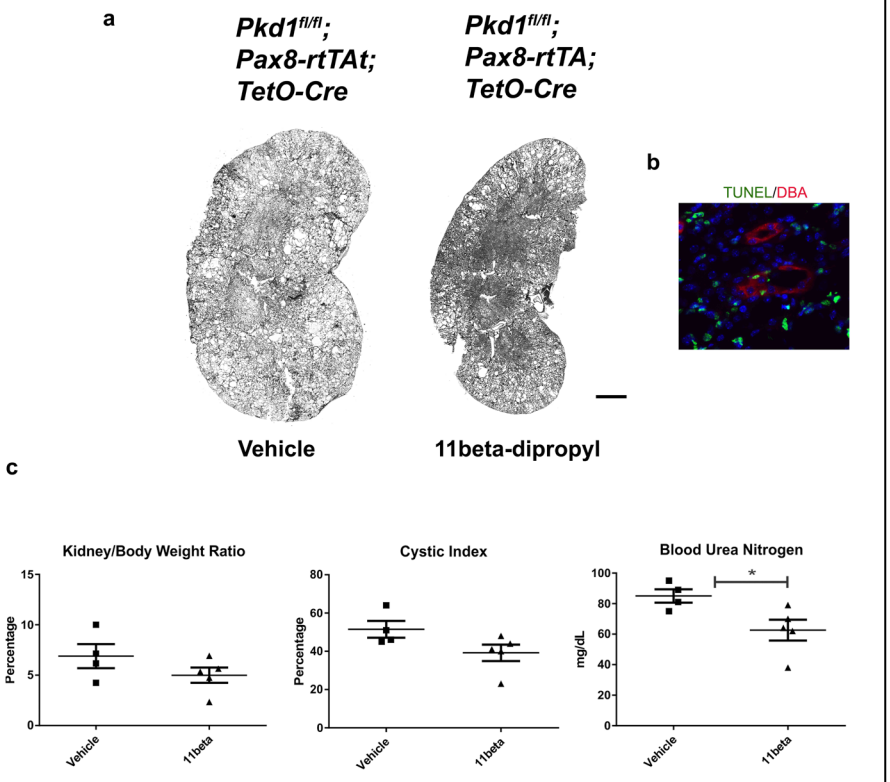

Figure 8. 11β-dipropyl ameliorates cystic disease in the adult mouse model of ADPKD (*Pkd1*$^{fl/fl}$*; Pax8*^{rtTA}*;TetO-Cre)*. a, H&E cross-sections of typical kidney specimens from vehicle and 11β-dipropyl treated animals; b, 11β-dipropyl induces extensive apoptosis in DBA-positive tubules; c, Quantification of kidney weight/body weight ratio, cystic index and BUN (n=4 controls, n=5 treated). The dots denote individual animals; large bar indicates the mean, and error bars are +/- 1 SD. The drug induces a statistically significant decrease in BUN (*p=0.038) with both the kidney to body weight ratio and cystic index trending towards an improvement.

How were the results disseminated to communities of interest?

Results from this project were disseminated in the form of oral and poster presentations at the 2016 and 2017 (upcoming) annual meetings of the American Society of Nephrology. The titles of the published abstracts are included in Section 6. Data were also presented in research conferences in Nephrology and Genetics at Yale.

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

During the next reporting period, we shall continue our work to define the mechanism of action of 11β compounds against PKD cells in cell culture, and their efficacy in animal models of ADPKD. First, we shall complete the synthesis of the last 11β-dipropyl analog proposed (compound **6**), and repeat the structure activity-study. Concomitantly, we shall thoroughly characterize the genotype of multiple clonal LLC-PK1 cell lines and establish whether our preliminary findings are an inherent feature of this class of cells or specific to particular clonal line. In addition, we have *Pkd1* and *Pkd2* gene knockout cell lines of IMCD3 cells which are another well accepted kidney epithelial cell model. We will investigate this cell type if the LLC-PK1 findings are not the result of clonal effects. We shall also introduce a re-expression cell line, in which an exogenous *Pkd1* gene is expressed in the *Pkd1* knockout background; this cell line is another control for any off-target genetic in the CRISPR/Cas9 editing procedure. This will establish whether this is a broader property of multiple renal epithelia or just LLC-PK1 cells. The outcome of this investigation will dictate the next steps regarding the role of cell models (Somlo, Yale; Essigmann, MIT).

Second, we shall characterize of the ability of the 11β-dipropyl analogs to induce oxidative stress and mitochondrial dysfunction. The ROS induction experiments will follow the outline described above (part d). The effect on the mitochondrial electron transport chain will be evaluated in isolated mitochondria using an oxygen sensor (Essigmann, MIT). Preliminary work on this assay is already underway.

Third, we shall complete the testing of the efficacy of 11β-dipropyl in the adult *Pkd1* inactivation model (*Pkd1*$^{flox/flox}$*;Pax8-rtta;TetO-Cre;* 12 weeks old) mouse model (Somlo, Yale).

Fourth, we shall perform an investigation of the transcriptional effects of 11β compounds, focusing on the 11β-dipropyl compound, both in cell culture (Essigmann, MIT) and in the animal models (Somlo, Yale). Preparation work to assemble a collection of probes specific for oxidative stress genes and other PKD specific genes (as part of a Qiagen RT^2 Profiler PCR Array) is currently underway. This probe set will be used to record and compare specific transcriptional responses between cell culture and animal models, in an effort to understand the molecular steps by which 11β compounds induce apoptosis in PKD cells.

4. IMPACT: *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

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

This project is making an impact in the area of therapeutics targeted at polycystic kidney disease (ADPKD). The 11β compounds show great efficacy in preventing cystic growth in mouse models, suggesting that they can be developed into clinical candidates. Furthermore, the 11β compounds work by inducing apoptosis in cystic cells, a mechanism of action relatively unique in the field of ADPKD. The concept of using drug-induced ROS generation to treat this disease is novel and, aside from our own laboratories, it could inspire others to develop ROS-generating drug candidates.

What was the impact on other disciplines?

The 11β compounds that will be developed in this project for treating PKD are likely to have an impact for the treatment of other diseases, including cystic diseases in other organs (i.e., liver) or proliferative diseases (i.e., cancer). It is noteworthy that 11β-dichloro has already shown efficacy against a number of tumor types in animal xenografts. Since other preclinical therapeutic efforts in ADPKD are directed at reducing cyst cell proliferation, the ability to concomitantly increased cyst cell apoptosis has the potential to markedly enhance these efforts.

What was the impact on technology transfer?

The new 11β compounds and derivatives synthesized in this project have the potential to qualify for composition of matter and use patents for treating ADPKD and related cystic diseases. In this regard, a recent communication from the USPTO conveyed optimism that intellectual property on the 11β-dipropyl compound, which has shown evidence of efficacy, will be issued. Issuance of intellectual property will be a step toward licensing to a company that can efficiently bring a drug candidate to clinical trials.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS: *The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change

We synthesized an additional 11β-dipropyl analog (compound **5**), in which the dipropyl groups of 11β-dipropyl were replaced with methyl groups. Among the analogs synthesized, this compound represents one of the smallest changes in the structure of the molecule; not surprisingly, its properties were very similar to the properties of 11β-dipropyl, suggesting that the length of the alkyl chains attached to the aniline nitrogen contribute very little to the activity of the molecule.

Actual or anticipated problems or delays and actions or plans to resolve them

As outlined in part d, the LLC-PK1 cell line used in the structure activity study gave inconsistent results, prompting consideration at this may be a clonal effect of a single cell line perhaps related to off target effects of the CRISPR/Cas9 system. As detailed in section C and the plans for the coming year, we will evaluate multiple independent clones of LLC-PK1 cells mutant for both *Pkd1* and *Pkd2*, evaluate mutant LLC-PK1 cells in which

the *Pkd1* cDNA has been re-expressed and, if the results continue to be confounding, evaluate mutant IMCD3 cells as well. These series of experiments are expected to resolve any gaps in understanding in our proposed studies.

Changes that had a significant impact on expenditures

While the change outlined above involved the synthesis of a new (additional) compound, the impact on expenditures was negligible, because the starting materials were readily available.

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.

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

Publications, conference papers, and presentations

Journal publications.

A draft manuscript that includes most of the preliminary data outlined in the proposal for this award, as well as some of the more recent data, is been completed and will be submitted this calendar year. The manuscript acknowledges the significant support from the Department of Defense for this project. The authors and title are as follows:

Fedeles BI, Fedeles SV, Ishikawa Y, Khumsubdee S, Campolo J, Rodrigues D, Gallagher AR, Westerling P, Krappitz M, Croy RG, Essigmann JM, Somlo S. “A synthetic anti-tumor agent ameliorates polycystic kidney disease by promoting apoptosis of cystic cells through increased oxidative stress” (in preparation).

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers, and presentations.

American Society of Nephrology published abstract:

Fedeles, S., Ishikawa Y., Gallagher R., Lee AH., Somlo S. Genetic interaction between *XBP1* and *Pkd1* modulates cyst progression in ADPKD. 2016, *J.Am.Soc.Nephrol.* (27); 211-12A

Website(s) or other Internet site(s)

Nothing to report

Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

The non-provisional patent application 14/515,441, entitled “Methods for treating polycystic kidney disease and polycystic liver disease” was filed jointly by MIT and Yale on October 15, 2014. While this application was

filed prior to the start of the funding for this project, it covers a broad range of compounds that could be used to treat polycystic kidney disease, including the lead compound 11β-dipropyl. Very recent communication from the attorney team handling this application informed us that the USPTO patent examiner has agreed to allow a number of the claims put forth in the patent application and that the patent will be issued in the near future.

Other Products

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	Somlo, Stefan
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	
Nearest Person Month Worked:	2.4
Contribution to Project:	Overall supervisory responsibility for the Yale site providing oversight of the project progress at Yale as well as ensuing coordination of the MIT site. Reviewing results, experimental design and quality control on a weekly basis and discussion of results particularly in the context of PKD.
Funding Support:	

Name:	Fedeles, Sorin
Project Role:	Associate Research Scientist
Researcher Identifier (e.g. ORCID ID):	
Nearest Person Month Worked:	6
Contribution to Project:	Performance of experiments including those related to mitochondrial biology in vivo and unfolded protein response studies.
Funding Support:	

Name:	Gallagher, Anna-Rachel
Project Role:	Associate Research Scientist
Researcher Identifier (e.g. ORCID ID):	
Nearest Person Month Worked:	6
Contribution to Project:	Performance of experiments including treatment of mice with 11B compounds, analyzing cell lines.
Funding Support:	

Name:	Tian, Xin
Project Role:	Associate Research Scientist
Researcher Identifier (e.g. ORCID ID):	
Nearest Person Month Worked:	3
Contribution to Project:	Mouse colony management for the PKD models used for the study.
Funding Support:	

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

Updated Other Support Pages are included in the Appendix 1.

What other organizations were involved as partners?

This is a COLLABORATIVE AWARD. Our collaboration partner is John Essigmann. Details are below:
Organization Name: Massachusetts Institute of Technology
Location of Organization: Cambridge, MA, USA.
Partner's contribution to the project
Financial support: None
In-kind support: Synthesis of compound used in the study.
Facilities: None
Collaboration: Compound synthesis and quality control; cell studies on mechanism of action of compounds; cell studies on mitochondrial function
Personnel exchanges: None
Other: None

8. SPECIAL REPORTING REQUIREMENTS

This is a COLLABORATIVE AWARD. An independent report from BOTH the initiating PI and Collaborating PI will be provided. The current report is from the Initiating PI (Stefan Somlo). Given the collaborative nature of the work, experiments that involve materials and expertise provided by both institutions are included in this report. The reports are therefore very similar. Throughout the report, the responsible PI and the site where the work was performed is included.

9. APPENDICES

Appendix 1: Stefan Somlo Updated Other Support Pages

Title:	Mechanisms of Polycystin and Cilia Function in ADPKD
Effort:	2.4 calendar months
Supporting Agency:	NIH/NIDDK
Grants Officer:	Krystle Nicholson, nicholsonk@niddk.nih.gov
Performance Period:	09/20/2013 – 5/31/2018
Level of Funding:	
Project Goals:	The major goal of this project is to determine the relationship between cilia and polycystin function in polycystic kidney disease.
Specific Aims:	The specific aims of this project are (1) to define the determinants of cyst progression whose activity following inactivation of polycystins is modulated by the presence or absence of intact cilia and (2) to determine the functional interplay between polycystins, cilia and α5β1 integrin in mechanisms of cyst progression
Overlap:	None

Title:	George M. O'Brien Kidney Center at Yale Core B: Mechanisms of Disease Models Core
Effort:	1.2 calendar months
Supporting Agency:	NIH/NIDDK
Grants Officer:	Amanada Linehan, linehana@mail.nih.gov
Performance Period:	09/01/2008 – 07/31/2018
Level of Funding:	
Project Goals:	This is a Core Center aimed at providing physiology, mouse and human genetics resource support to facilitate translational and clinical research projects that will advance the study of kidney diseases.
Specific Aims:	The specific aims of this project are (1) to provide services for modification of genes o interest in bacterial artificial chromosome (BAS) for use in transgenic mice (2) to provide services for isolation of primary tubule cells and cell lines from specific nephrc segments of mutant mouse strains (3) the generation of conditional knockout and knockin gene targeting strategies and constructs and (4) the education and general resources for investigators in mouse genetic applications.
Overlap:	None

Title:	Developing Therapies for Polycystic Kidney Disease
Effort:	1.2 calendar months
Supporting Agency:	Amy P. Goldman Foundation
Grants Officer:	R. Scott Johnston, scott.johnston@hklaw.com
Performance Period:	06/01/2017 – 05/31/2020
Level of Funding:	
Project Goals:	This project focuses on genetic re-expression of polycystin-2 in a mouse model to determine whether cyst formation is reversible.
Specific Aims:	The specific aims of this project are (1) to define the extent of reversibility of ADPKD (2) to discover the molecular changes that occur downstream of polycystin gene reactivation that are responsible for the improved in the course of ADPKD and (3) to explore whether chaperone therapy is effective in ADPKD resulting from partial loss c function of polycystin-1 due to missense mutations.
Overlap:	None

Title:	Cellular Energy Pathways as Novel Targets for the Therapy of Autosomal Dominant Polycystic Kidney Disease
Effort:	0.36
Supporting Agency:	Department of Defense
Grants Officer:	Susan Dellinger, susan.m.dellinger.civ@mail.mil
Performance Period:	09/01/2015 – 08/31/2018
Level of Funding:	
Project Goals:	The major goals of this project are to explore in cell culture and animal models the potential therapeutic utility of modulating cellular energy pathways in the setting of polycystic kidney disease.
Specific Aims:	The specific aims of this project are (1) to assess whether PDH kinase inhibition or AMPK activation, alone or in combination, corrects the perturbed energy metabolism ADPKD cells and slows cyst growth in vitro and in vivo and (2) to measure candidate metabolomic biomarkers and screen for additional biomarkers that correlate with disease severity and progression in ADPKD mice and cells and that are modulated by reduction of PDH kinase activity or AMPK activation. Dr. Somlo's role in the project is to oversee the animal models for the in vivo studies.
Overlap:	None