Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Temple University – Of The Commonwealth System of Higher Education

2. **Reporting Period (start and end date of grant award period):** 01/01/2010 - 12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Germaine A Calicat, MA

4. **Grant Contact Person's Telephone Number:** 215-204-7655

5. **Grant SAP Number:** 4100050909

6. **Project Number and Title of Research Project:** Project 19 – Temple PET/SPECT Imaging Tracer Initiative

7. **Start and End Date of Research Project:** 01/01/2011 – 11/30/2013

8. **Name of Principal Investigator for the Research Project:** Magid Abou-Gharbia, Ph.D.

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 590,731.42

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Abou-Gharbia, Magid	Principal Investigator	5%	$33,011
Childers, Wayne	Co-Investigator	20%	$55,218
Annadurai, Siva	Post-doctoral Fellow	100%	$98,046
Canney, Daniel	Co-Investigator	5%	$19,806
Korzekwa, Kenneth	Co-Investigator	5%	$22,561
Pratico, Dominico	Co-Investigator	5%	$22,006
Tucker, Jalia	Research Assistant (Departed 08/2012)	15%	$5,357
Tyree, Keyana	Research Assistant (Replaced J. Tucker 08/2012)	15%	$5,455
Ye, Min	Research Assistant	15%	$19,084
Chu, Jin	Research Assistant (11/2011 – 6/1012)	5%	$2,211

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
Fan, Rong	Graduate Student	50%

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes___X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
PET Imaging Agent for Brain 5-Lipoxygenase as a Diagnostic Tool for Alzheimer's Disease	☐NIH ☐ Other federal (specify:_____ _____) ☒ Nonfederal source (specify: Alzheimer's Drug Discovery Foundation)	08/2012	$139,340	$0

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes__X_____ No_____

If yes, please describe your plans:

Currently, in vivo autoradiography studies are under way to determine the suitability of our lead molecule as an imaging agent in rodent and human brain tissue (normal and Alzheimer's brain tissue) and in rodent brains in vivo. These data will be used to solicit funding to label the molecule with carbon-11 and perform PET imaging studies. If the PET labeled ligand proves to be useful, additional funding would then be sought to support IND-enabling studies to advance the molecule to clinical trials. At that stage, potential development partners would also be approached for collaborative funding or licensing.

12. Future of Research Project. What are the future plans for this research project?

Future plans include labeling the lead molecule with carbon-11, running PET imaging studies in rodent and human brain tissue (normal and Alzheimer's brains) and running imaging studies in normal and rodent brains in vivo (normal and genetically generated animal models of AD, e.g., Tg2576 and 3xTg transgenic mice). A successful outcome would support the advancement of the molecule to IND-enabling studies to determine if the imaging ligand could be used in humans to diagnose or monitor Alzheimer's disease. We are also continuing to synthesize and test 5-LO ligands to identify second generation compounds in the event that the autoradiography studies do not support the use of MC-200022 as an imaging ligand.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				1
Female				
Unknown				
Total				**1**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				1
Unknown				
Total				**1**

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				1
Unknown				
Total				**1**

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____X____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes____X____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

The project established the collaborative capacity and expertise at Temple University to design, synthesize and evaluate and imaging ligands. As this capacity grows it will provide Temple with an internal resource to support its translational medicine and basic research needs.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes___X_____ No_____

If yes, please describe the collaborations:

As a result of the project we have established the following collaborations:

- Dr. Graham Jones (Northeastern University, Boston, MA) in the area of radioligand synthesis methodology
- Dr. Jinbin Xu (Washington University, St Louis, MO) in the areas of autoradiography and tissue /small animal imaging (normal and Alzheimer's brains, normal animals and animal models of Alzheimer's disease)
- Dr. Robert Mack (University of Pennsylvania, Philadelphia, PA) in the area of radioligand design and synthesis methodology
- Dr. Surendra Gupta (American Radiolabeled Chemicals, Inc.), in the area of radiosynthesis

16(B) Did the research project result in commercial development of any research products?

Yes_____ No___X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No___X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

The ultimate goal of the project is to identify a suitable ligand for 5-lipoxygenase (5-LO) that can be used as a PET/SPECT tracer ligand for imaging and quantifying 5-LO in the CNS. Data suggest that 5-LO is elevated in the brains of Alzheimer's disease (AD) patients compared to age-matched controls and that CNS-derived 5-LO may play a role in the occurrence of two major hallmarks of AD, the formation of neurotoxic beta-amyloid proteins and the over-phosphorylation of tau protein. Thus, a non-invasive CNS 5-LO imaging agent could provide a valuable tool for the study of the role of 5-LO in AD as well as a potential diagnostic agent. Three aims were identified to guide the project:

- Aim 1: Identify appropriate 5-LO inhibitors or inhibitor scaffolds as starting points
- Aim 2: Identify Prodrugs that will adequately deliver 5-LO PET tracers into the brain
- Aim 3: Identify synthetic routes to 5-LO prodrug imaging candidates that are amenable to radiolabeling with PET and/or SPECT radioisotopes

The progress achieved at fulfilling the three specific aims will be discussed separately below.

<u>Aim 1: Identify appropriate 5-LO inhibitors or inhibitor scaffolds as starting points</u>.

Aim 1 was fully achieved. The quinolone-pyran scaffold possesses the appropriate balance of 5-LO inhibitory potency and physicochemical properties to serve as a good starting point for designing a PET/SPECT imaging ligand.

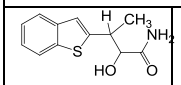
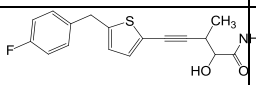
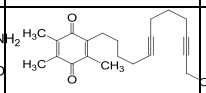
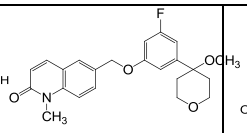
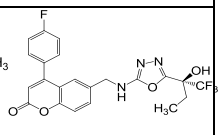
CNS imaging agents, especially those that use short-lived positron emission tomography (PET) radioisotopes, must possess a specific combination of biochemical, pharmacological and physicochemical properties to provide good signal-to-noise ratios and sensitive, quantifiable results (Table 1).[1] In addition, compounds that have already advanced to clinical trials or are within the same chemical scaffold as compounds that have qualified for advancement to the clinic provide advanced starting points that can be quickly advanced to IND enabling studies because their ADME and toxicity profiles are known or can be predicted. Therefore, we began by analyzing the published literature on known classes of 5-LO inhibitors to identify starting points for the project.

Table 1. Optimal Properties for a CNS PET Imaging Ligand.

Affinity	Ki < 10 nM; $B_{max}/K_D \geq 10$
Specific Binding	Total/nonspecific ≥ 2:1
Lipophilicity	logP or logD ≈ 1-3.5
TPSA	< 90
Aqueous Solubility	> 100 ug/mL
CNS Penetration	*In vitro*: Pe > 10^{-5} cm/sec *In vivo*: Brain/Plasma > 1
Protein Binding (Brain & Plasma)	< 50%
Metabolism	Defined; Radioisotope not cleaved from parent
Safety and Toxicology	No critical findings, 14 day; No adverse effects at doses 5000x anticipated dose
Plasma Clearance	$t_{1/2}$ = 30-90 minutes

Inhibitors of 5-LO are characterized into three classes (Table 2).[2] Hydroxyureas such as zileuton use coordination to the iron ion in the 5-LO active site as a major contributor for binding. The most potent member of this class described to date is ABT-761. AA-861 is a representative of the second class of 5-LO inhibitors. These lipophilic compounds bind to the arachidonic acid binding site and lock the iron in the ferrous state. The third class of 5-LO inhibitor binds to the arachidonic acid binding site but has no redox activity. ZD-2138 and setileuton (MK-0633) are recently described examples of this class of 5-LO inhibitor, but several chemical scaffolds possessing this activity have been disclosed.

Table 2. Representatives of the Three Classes of 5-LO Inhibitors.

	Iron Chelators		**Redox Inhibitors**	**Arachidonic Acid Ligands**	
	 Zileuton	 ABT-761	 AA-861	 ZD-2138	 Setileuton
5-LO, IC_{50} (human white blood cells)	600 nM	23 nM	800 nM	12 nM	4 nM
logD	1.86	3.33	3.38	2.6	4.44
TPSA*	63.3	65.6	54.4	48.0	92.5

*TPSA = Topological Polar Surface Area

No binding assays for 5-lipoxygenase have been described in the literature. Therefore, we used inhibition of arachidonic acid turnover in human white blood cells, the most commonly reported data in the 5-LO inhibitor literature, as a potency surrogate for affinity. ABT-761, the result of extensive SAR studies around zileuton, is the most potent iron chelating 5-LO inhibitor reported to date. However, the reported slow metabolism and long in vivo half-live of ABT-761 (15 – 16 hours) makes it unsuitable for use as an imaging ligand.[3] As a class, the redox inhibitors are not potent enough to be considered as imaging ligand candidates. Two chemical series of arachidonic acid ligands, represented by ZD-2138 and setileuton, displayed suitable 5-LO inhibitory potency to be investigated further. These two scaffolds were further recommended by the fact that both compounds were previously advanced to clinical trials.

Table 3. Inhibitory Data, N2A Neuroblastoma Cells

Compound	**5-LO IC_{50} N2A Cells**	**IC_{50} range WBC***
zileuton	2.2 uM	1 - 2.6 uM
AA-861	1.05 uM	0.3 - 1.5 uM
ZD-2138	0.012 uM	0.010 - 0.023 uM
setileuton	0.008 uM	0.004 uM

* WBC = white blood cells

To confirm the 5-LO inhibitory potency reported in the literature and to measure the 5-LO inhibitory effect of the compounds in a system derived from CNS tissue, we developed a 5-LO arachidonic acid turnover assay using human N2A neuroblastoma cells and a commercially available 5-LO kit (Cayman Chemicals, Ann Arbor, MI).

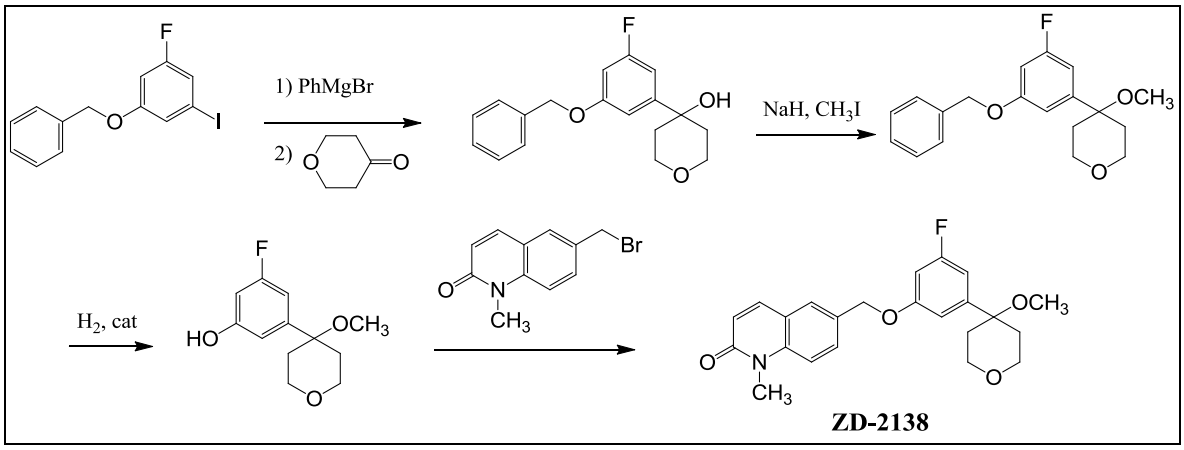


Figure 1. Synthesis of ZD-2138.

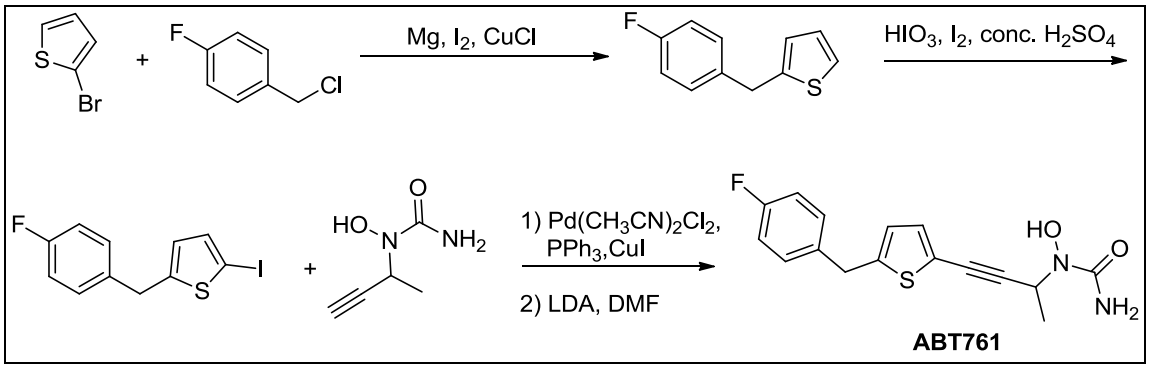


Figure 2. Synthesis of ABT-761

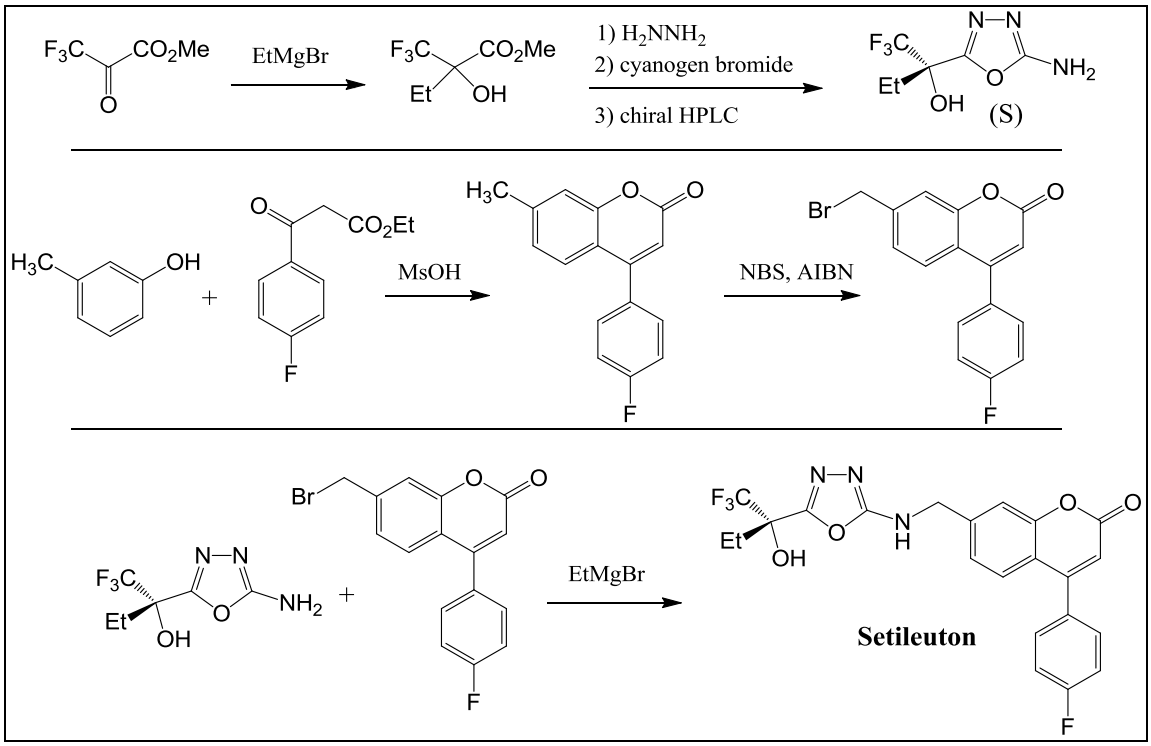


Figure 3. Synthesis of Setileuton.

The representative compounds shown in Table 1 were tested in the N2A-derived assay and their 5-LO inhibitory activity was found to be in agreement with the ranges reported in the literature using human white blood cells (Table 3). For this assay, zileuton, ABT-761 and AA-861 were obtained from commercial sources. ZD-2138, ABT-761 and setileuton were synthesized using variations on the synthetic procedures described in the literature.[4-6] The synthetic schemes for ZD-2138, ABT-761 and setileuton are shown in Figures 1, 2 and 3, respectively.

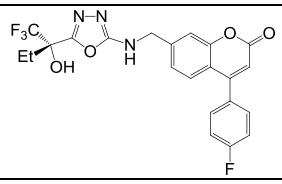
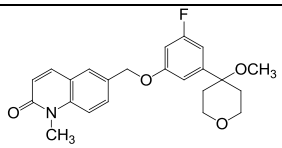
These results confirmed ZD-2138 and setileuton as the best starting points from the point of view of potency. The logD of ZD-2138 was within the desired range for a potential imaging ligand and the topological polar surface area (TPSA) was within the range predicted for

compounds that display good CNS penetration.[7] The logD and TPSA of setileuton were not as optimal as that seen for ZD-2138, but the greater 5-LO inhibitory potency of setileuton encouraged us to include it in the next level of assessment.

Setileuton and ZD-2138 were examined for their in vitro and in vivo pharmacokinetic parameters (Table 3 and Figures 3 & 4). Setileuton was relatively stable in mouse and human liver microsomes. This in vitro stability was mirrored in the compound's in vivo half-life of 7 hours in mice. Brain concentrations measured at 4 hours post-administration revealed that only about 20% of the drug penetrated into the CNS. This poor CNS penetration was likely due to the compounds high lipophilicity and tight binding to plasma proteins (> 99% bound). Furthermore, the high partitioning into microsomes (> 99%) raised concerns about non-specific binding for setileuton. While still not optimal, the in vitro and in vivo PK parameters for ZD-2138 were more encouraging. The compound was metabolized (and cleared) more quickly. Plasma protein binding and microsomal partitioning were lower and, most significantly, the compound distributed well into the CNS, with a brain/plasma ratio = 2.

These data supported prioritization of ZD-2138's quinolone-pyran scaffold for further optimization as a 5-LO PET imaging ligand.

Table 4. In Vitro and In Vivo PK Parameters for ZD-2138 and Setileuton.

		
	Setileuton	**ZD 2138**
Potency vs 5-LO (N2A cells)	IC_{50} = 8 nM	IC_{50} = 12 nM
Microsomal Stability* $T_{1/2}$, minutes	> 60 (MLM) > 60 (HLM)	8 (MLM) 10 (HLM)
Protein Binding Free, Unbound Fraction	< 1%	3%
Microsomal Partitioning Free, Unbound Fraction	< 1%	29%
In Vivo PK – $T_{1/2}$ (hrs)	7	7
In Vivo PK – V_D (L/kg)	6	5
Brain/Plasma Ratio - 4 hr post	0.2	2

* MLM = Mouse Liver Microsomes; HLM = Human Liver Microsomes.

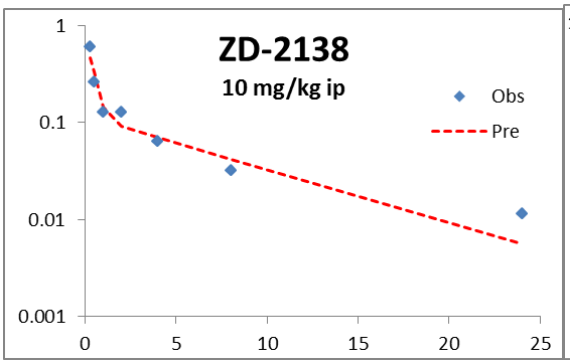


Figure 3. PK of ZD-2138 following a dose of 10 mg/kg ip.

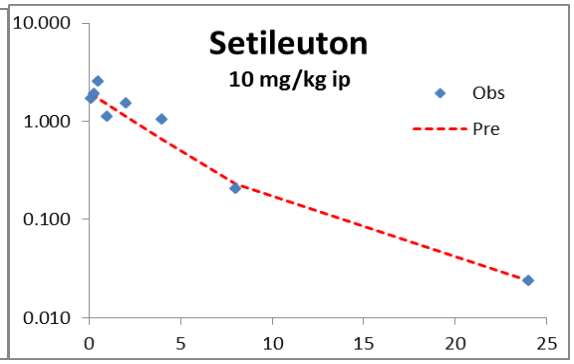


Figure 4. PK of Setileuton Following a dose of 10 mg/kg ip.

Aim 2: Identify Prodrugs that Will Adequately Deliver 5-LO PET Tracers Into the Brain

Aim 2 has been partially achieved. A series of (R)-zileuton derived prodrugs was designed and synthesized. Several of these prodrugs were shown to have suitable plasma stability. Development of a robust in vitro brain stability assay has been problematic and has prevented this aim from being fully achieved but is on-going. Data from the in vitro brain stability assay, when generated, will be used to prioritize 2-3 prodrugs for the in vivo PK studies that will determine whether or not full success is achieved in this aim.

As a backup strategy to Aim 1, a prodrug approach was examined. While not common, prodrug approaches have been successfully used to arrive at PET ligands for imaging in the CNS.[8-10] Zileuton was chosen as the prototype parent drug for this aim since it possesses an in vivo half-life that is suitable for PET imaging (2 hours).[11] The potency of zileuton is not adequate for PET imaging, but a study detailing the work that led to the more metabolically stable and more potent analog ABT-761 provides possible insight into how to increase 5-LO inhibitory potency and even further reduce in vivo half-life.[12] Therefore, the strategy was to use zileuton as a prototype of the hydroxyurea scaffold and determine if a suitable prodrug could be designed. Encouraging results would then trigger an SAR study to identify more potent, rapidly clearing analogs that could be combined with the prodrug approach to deliver high, rapidly cleared CNS concentrations of the parent compound.

Since (R)-zileuton is considered to be the active enantiomer, the first step was to obtain suitable quantities of the stereoisomer. This was accomplished on a 10 g scale using supercritical fluid chromatography on a chiral HPLC column. (R)-zileuton was then converted to a variety of carbamate and carbonate analogs using the synthetic methodology outlined in Figure 5. Since lipophilicity plays a major role in CNS penetration, a range of lipophilicities was designed into the prodrug candidates.

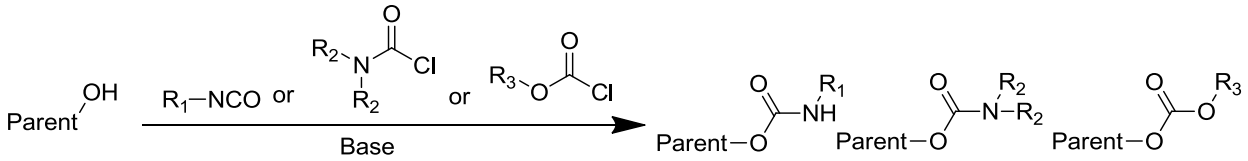
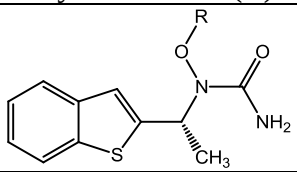
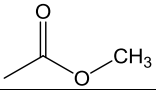
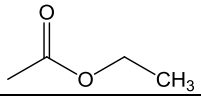
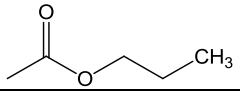
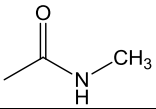
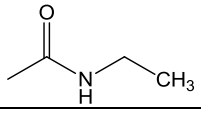
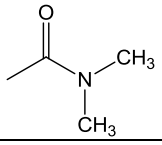
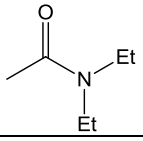
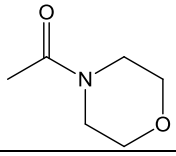


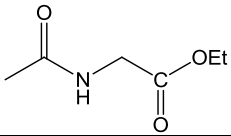
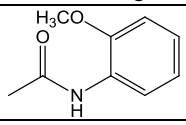
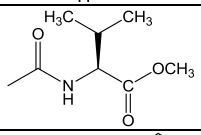
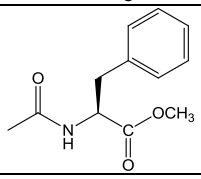
Figure 5. General Synthetic Scheme for Zileuton Prodrug Synthesis

Compounds were then screened for stability in C57bl/6 mouse plasma (the background strain for the murine Alzheimer's disease transgenic models like Tg2576 and 3xTg) on a 96-well platform at 37°C using procaine as a positive control. Data for a representative set of the prodrug candidates prepared to date are shown in Table 5.

Table 5. Mouse Plasma Stability of Selected (R)-Zileuton Prodrugs.



Entry	R	logP	MW	Mouse Plasma Stability % Remaining @ 1 hr (37C)
1		2.84	294	0
2		3.22	308	1
3		3.67	322	4
4		2.25	293	76
5		2.66	307	86
6		2.81	307	71
7		3.64	335	100
8		2.29	349	27

9		2.38	365	100
10		3.82	385	100
11		3.19	393	90
12		3.89	441	6

As can be seen, stabilities ranged from 0% remaining to 100% remaining after 1 hour @ 37C. Carbamates were more stable than corresponding carbonates and, with the exception of the morpholine derivative (Entry 8), steric bulk around the carbamate group enhanced stability. The prodrug derived from valine methyl ester was relatively stable (90% remaining after 1 hour), while a similar prodrug candidate derived from phenylalanine methyl ester was rapidly hydrolyzed by plasma. All compounds were concomitantly screened for aqueous solubility to insure that the plasma stability results were not influenced by solubility issues (data not shown).

Currently, efforts are underway to develop a mouse brain homogenate assay that will be used to examine the stability of the prodrugs. To date, attempts to develop this assay have met with limited success, as standard prodrugs known to hydrolyze in brain tissue have not hydrolyzed as expected in our hands. Studies are currently underway to optimize this assay. Once the assay has been validated, it will be used to study the (R)-zileuton derived prodrugs. Compounds that show rapid hydrolysis in brain tissue (2-3 prioritized prodrugs) will be examined for their in vivo pharmacokinetic profiles in plasma and brain. Encouraging results in these studies would trigger the SAR campaign described at the beginning of this section.

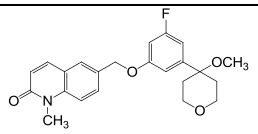
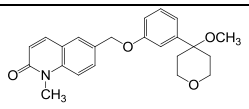
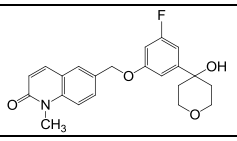
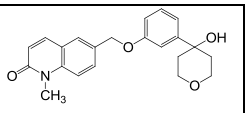
<u>Aim 3: Identify synthetic routes to 5-LO prodrug imaging candidates that are amenable to radiolabeling with PET and/or SPECT radioisotopes</u>

Aim 3 was fully achieved. Structure-activity and structure-property studies identified MC-200022 as having the best combination of 5-LO inhibitory potency and in vitro and in vivo physicochemical and ADME properties to qualify it as a potential PET/SPECT imaging ligand. A synthesis of MC-200022 that can be used to rapidly install the required radioisotope in the last step has been developed and implemented. This synthesis was used to prepared tritium-labeled MC-200022 which will be used in the next phase of the project.

A relatively thorough SAR study on the ZD-2138 pyran scaffold has been published.[5] From that work, the only other analog possessing enough 5-LO inhibitory potency to be considered

as a PET ligand was the des-fluoro analog MC-200022 (Table 6), with an IC_{50} value of 30 nM in human white blood cells, which translated to an IC_{50} value of 18 nM in our 5-LO turnover assay in N2A neuroblastoma cells. Interestingly, removal of the aromatic fluorine improved the ADME characteristics of the scaffold. The free, unbound fraction in plasma was estimated to be 14% for MC-200022 (compared to 3% for ZD-2138). A lower percentage of MC-200022 partitioned into microsomes (29%) compared to ZD-2138 (86%). These data suggest that much less non-specific binding would be expected with MC-200022 (3-5 fold less compared to ZD-2138). The in vivo half-life for MC-200022 (2.7 hours), while not optimal, was more in line with that needed for a PET ligand, and the compound partitioned into the CNS well (brain/plasma ratio = 1.8). It was hypothesized that removal of the methyl group on the methoxy moiety pendant to the pyran might give a compound with even better ADME properties. This analog had not been described in the prior art. We therefore prepared the hydroxyl analog MC-200023. While the protein binding and microsomal partitioning characteristics of MC-200023 were better than those seen with MC-200022 and the 5-LO inhibitory potency in our N2A neuroblastoma cell system was suitable, the compound was surprisingly stable to metabolism, resulting in an unacceptable in vivo half-life of 3.6 hours. Removal of the fluorine atom from MC-200023 gave MC-200024, which also had suitable 5-LO inhibitory potency. However, the stability of the compound to oxidative metabolism in mouse and human liver microsomes suggested that, like MC-200023, its in vivo half-life would be too long for use as an imaging agent. Thus we chose to pursue additional studies on MC-200022.

Table 6. 5-LO Data and ADME Parameters for ZD-2138, MC-200022, MC-200023 and MC-200024.

				
	ZD 2138	**MC-200022**	**MC-200023**	**M-200024**
logD	2.6	2.45	2.2	2.1
TPSA	48	48	59	59
5-LO (N2A cells)	IC_{50} = 0.012 uM	IC_{50} = 0.018 uM	IC_{50} = 0.011 uM	IC_{50} = 0.020 uM
Microsomal Stability* $T_{1/2}$, minutes	8 (MLM) 10 (HLM)	12 (MLM) 11 (HLM)	48 (MLM) > 60 (HLM)	> 60 (MLM) > 60 (HLM)
Protein Binding Free, Unbound Fraction	3%	14%	11%	ND
Microsomal Partitioning Free, Unbound Fraction	29%	71%	93%	ND

In Vivo PK $T_{1/2}$ (hrs)	7	2.7	3.6	ND
In Vivo PK V_D (L/kg)	5	3.2	2.3	ND
Brain/Plasma Ratio 4 hr post	2	1.8	1.3	ND

* MLM = Mouse Liver Microsomes; HLM = Human Liver Microsomes.

Since there is no information on the amount of 5-LO in the brain, other than qualitative statements based on mRNA levels, and since there is no actual affinity data on the compounds being considered as potential PET imaging ligands, there is no way to determine the K_D/Bmax ratio. Therefore, the next logical step was to radiolabel MC-200022 with a traditional radioligand (^3H or ^{14}C) and perform autoradiography studies in isolated tissue and, if appropriate, whole animals to ascertain the sensitivity and resolution that the ligand would give. Encouraging results in these studies would support the initiation of PET labeling and PET imaging studies.

The first step was to develop a synthesis of MC-200022 that would install a radioisotope in the last step in a short and efficient manner. The procedure shown in Figure 6 was devised and used to synthesize MC-200022 in 100 mg quantities. The methoxy group is installed in the last step of the synthesis, which allows for the insertion of either [^3H] (for autoradiography studies) or [^{11}C] for positron emission imaging studies. The entire reaction time for the final step was 22 minutes, including HPLC purification. This synthetic method should be suitable for preparing [^{11}C]-labeled MC-200022 from [^{11}C]H_3I.

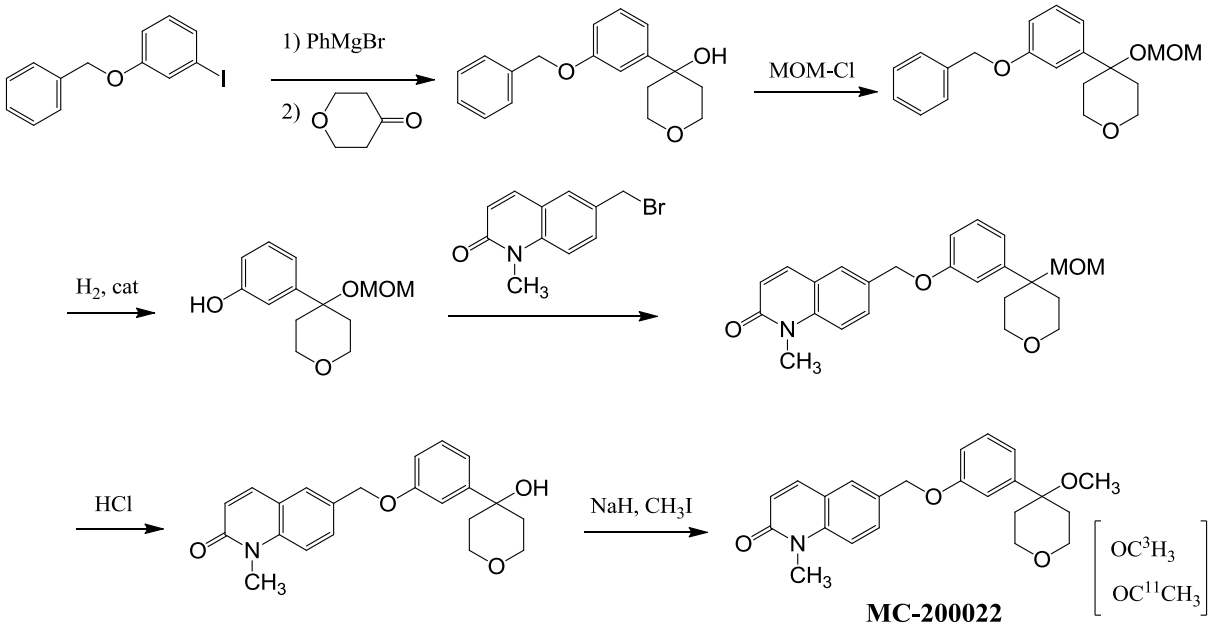


Figure 6. Synthesis of MC-200022 for Radioisotope Labeling Activities.

To obtain tritiated MC-200022, we established a collaboration with Dr. Surendra Gupta, American Radiolabeled Chemicals, Inc. (St. Louis, MO). Dr. Gupta was able to prepare 5 mCi of [^{3}H]-labeled MC-200022 at a specific activity of 80 Ci/mmol using the last step of the synthesis shown in Figure 6.

We have established a collaboration with Dr. Jinbin Xu of Washington University, St. Louis to perform the autoradiography studies needed to ascertain the suitability of MC-200022 as a potential imaging ligand. Dr. Xu is an expert in the field of CNS- and oncology-based functional radioimaging. Dr. Xu has access to both transgenic and normal rodent brains and normal and AD human brain tissue. In collaboration with Dr. Xu, we will:

1) Determine the affinity of [^{3}H]-MC-200022 for 5-LO using saturation autoradiography binding techniques and then use that data to measure 5-LO levels in different brain regions. Differences in normal and AD-related 5-LO expression will also be measured in rodent (transgenic) and human (AD) brain tissue and compared to normal age-matched controls.
2) Determine non-specific binding, signal-to-noise ratios and other parameters needed to assess the suitability of MC-200022 as a potential imaging ligand
3) Establish a high throughput competitive 5-LO binding assay for screening second generation 5-LO imaging radiotracers (the first assay of its kind).

This work is currently in progress using the [^{3}H]-MC-200022 prepared by American Radiolabeled Chemicals, Inc. and is currently being funded through sources outside of the health research funds.

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12) Bouska, J. J.; Bell, R. L.; Goodfellow, C. L.; Stewart, A. O.; Brooks, C. D. W.; Carter, G. W. *Drug. Metab. Disp*. **1997**, *25*, 1032-1038.
13)

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?

_____Yes

_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1.				☐Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published

3.				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X____ No_____

If yes, please describe your plans:

The results of the ongoing autoradiography studies will be available within 6 months. At that time we expect to present them at a suitable scientific conference and submit them to a suitable high impact journal. Following those studies, we plan to advance the project to the next stage, which would involve labeling the molecule with the PET radioligand and carrying out imaging studies in normal and Alzheimer's brain tissue (rodent and human) as well as in vivo imaging in rodent animals of Alzheimer's disease. The results of those studies would also be presented at scientific conferences and submitted to a high impact journal for publication.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No____x_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items

a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information*

for only those key investigators whose biosketches were not included in the original grant application.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Magid Abou-Gharbia	POSITION TITLE Associate Dean for Research, Professor of Medicinal Chemistry and Director of Moulder Center for Drug Discovery Research
eRA COMMONS USER NAME (credential, e.g., agency login) aboumag	

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Cairo University, Cairo, Egypt	B.Sc.	1966-1971	Pharmacy
Cairo University, Cairo, Egypt	M.Sc.	1971-1974	Medicinal Chemistry
University of Pennsylvania, Philadelphia, PA	Ph.D.	1974-1979	Organic Chemistry
Fels Research Institute, Temple University, Philadelphia, PA Philadelphia, PA	NIH Postdoc	1979-2002	Cancer Research Medicinal Chemistry

A. Personal Statement

I am currently Associate Dean for Research, Professor of Medicinal Chemistry and Director of Moulder Center for Drug Discovery Research. I have over twenty-six years of pharmaceutical research experience in Drug Discovery and Development directing research projects in multiple therapeutic areas in Neuroscience, Cardiovascular Metabolic diseases, Oncology, Infectious diseases and immunology which resulted in the identification and development of eight marketed drugs such as Effexor, Pristiq, Torisel, Mylotarg, Tygacil and several New Chemical Entities (NCEs) currently at various stages of development, including the recent FDA approval of the tyrosine kinase inhibitor Bosutinib for treatment of chronic myelogenous leukemia (CML). I will utilize my expertise in Drug Discovery to effectively coordinate the medicinal chemistry and molecular modeling efforts on the research project in collaboration with Dr. Childers.

B. Positions and Honors

<u>Positions and Employment</u>

1982 - 1987 *Research Scientist*, Wyeth Pharmaceuticals, Radnor, PA
1987 - 1991 *Associate Director*, CNS Medicinal Chemistry, Wyeth-Ayerst Research, Princeton, NJ
1991 - 1994 *Director*, Medicinal Chemistry, CNS Research, Wyeth-Ayerst Research, Princeton, NJ
1994 - 1997 *Assistant Vice President*, Global Chemical Sciences, Wyeth-Ayerst Research, Princeton, NJ
1997 - 2004 Vice President & Department Head, Chemical & Screening Sciences, Wyeth Research, Collegeville, PA. (Department of 350 scientists)
2005 - 2008 *Senior Vice President & Department Head*, Chemical & Screening Sciences, Wyeth Research, Collegeville, PA. Directing research efforts of 500 chemists and biologists in the area of Drug discovery which led to discovery of 6 marketed drugs.
2008 - Present Associate Dean for Research, Professor of Medicinal Chemistry and Director of the Moulder Center for Drug Discovery Research (MCDDR), School of Pharmacy, Temple University. Overseeing of research efforts focusing on the design and synthesis of small molecules therapeutics in multiple disease areas. Center's objective include; Discovery of biologically

active drug candidates and synthesis of chemical leads to probe and test biological hypotheses, facilitating collaborative research within Temple University researchers and with external collaborators in the US and abroad and training undergraduate and graduate students, junior faculty, Postdoctoral Fellows and visiting scientists on state-of-the art enabling technologies and modern Drug discovery and Development.

Other Experience and Professional Memberships

1998 - 2008	Member, Board of Visitors, Temple University School of Pharmacy, Philadelphia, PA.
2000 - Present	Member of Scientific Advisory Board, Widener University, Chester, PA
2001 - 2004	Chair, Scientific Advisory Board, Rider University, Lawrenceville, NJ
2003 - Present	Adjunct Professor, School of Pharmacy, University of Ferrara, Ferrara, Italy
2004 - 2008	Chemical Biology Discussion Group Program Committee Member, New York Academy of Sciences.
2004 - Present	Member of Scientific Advisory Board for Biotechnology & Pharmaceutical Industry, Ministry of Health, Cairo, Egypt.
2007 - Present	Adjunct Professor, School of Pharmacy, Center for Drug Discovery (CDD), Northeastern University, Boston, MA
2008 - Present	Visiting Professor, Wolfson Center for Age Related Diseases, School of Biomedical and Health Sciences, King's College, London.
2008 - Present	Member, Board of Visitors, College of Science and Technology (CST), Temple University, Philadelphia, PA

Honors

ACS Fellow (2010),Induction to Medicinal Chemistry Hall of Fame by ACS Division of Medicinal Chemistry (2008), Science & Technology Medal, R&D Council of New Jersey (2008), Alfred Burger Award in Medicinal Chemistry (2008), Heroes of Chemistry Award: For the Discovery of Torisel for treatment of renal carcinoma (2008), Pioneer Award, American Institute of Chemists (AIC) Chemical Heritage Foundation (2007), Fellow of the Royal Society of Chemistry (FRSC), London, UK (2006), New Jersey Researcher of the Year (Hall of Fame inductee (2006),"Trailblazer Award" Minority in Research Science Award from Science Spectrum Magazine (2006), "Script Award" Best R&D Team –Wyeth Research & Development Executive Team (RADEX)(2006),"Script Award" Best Discovery Management Team – Wyeth Discovery Executive Committee (2005), New Jersey Inventors Hall of Fame Award (2004), Procter Medal: Awarded by the Philadelphia Drug Exchange (PDE), American Pharmaceutical Association (2003), Guest Commencement Speaker: Graduating class of College of Science & Technology (CST), Temple University, Egyptian Pharmaceutical Society (2002), Earle B. Barnes Award – ACS: Awarded by American Chemical Society (ACS) for outstanding achievements in Chemical Research Management (2001), POCC Award: Awarded by Philadelphia Organic Chemists Club (POCC) for significant contributions to organic chemistry and service to the scientific community (2001), Prolific Inventor of the Decade, US Patent & Trademark: In their Technology Assessment & Forecast Report citing inventors who hold > 70 issued US Patents during 1987-1997 (1998), Wyeth-Ayerst Patent Award: For issued US patents in 1996 (most issued patents in one year 25 US patents) (1997), Wyeth-Ayerst Teamwork Award: For outstanding teamwork and contributions in Global CNS strategic planning (1994), Wyeth-Ayerst Exceptional Achievement Award: In recognition of outstanding personal achievement and contributions to discovery and development at Wyeth (1992),Wyeth-Ayerst Patent Award: Three-time recipient (consecutively) for issued patents for compounds in clinical development (Zalospirone, Carvotroline, & Adatanserin) (1990 – 1992)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.

Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Wayne E. Childers	POSITION TITLE Associate Professor and Associate Director, Moulder Center for Drug Discovery Research
eRA COMMONS USER NAME (credential, e.g., agency login) WCHILDERS01	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Vanderibilt University, Nashville, TN	BA	1975-1979	Chemistry
University of Georgia, Athens, GA	PhD	1979-1984	Organic Chemistry
Johns Hopkins Univ. School of Medicine, Baltimore, MD	Postdoc	1984-1987	Bioorganic Chemistry; Drug Design

A. Personal Statement

I am currently an Associate Professor in the Department of Pharmaceutical Sciences, and I am actively conducting research in the area of medicinal chemistry and I actively consult in this area. I have over 23 years of pharmaceutical research experience in drug discovery and development in numerous therapeutic areas. Projects under my supervision have identified several New Chemical Entities currently at various stages of development. I am well versed in all phases of drug identification, from early hit identification to lead optimization and scale-up. In addition to my experience in drug design, my bioorganic chemistry background gives me a good working knowledge of pharmacological principals and how chemicals interact with biological targets at the molecular level. I will use my expertise to coordinate the medicinal chemistry, molecular modeling and in vitro physicochemical/ADME analysis in collaboration with Dr. Abou-Gharbia.

B. Positions and Honors

Professional Experience

1984-1987, *Assistant Adjunct Professor*, Dept. of Chemistry, Bucknell University, Lewisburg, PA
1987-1990, *Senior Scientist*, CNS Medicinal Chemistry, Wyeth-Ayerst Research, Princeton, NJ
1990-1992, *Senior Research Scientist I*, CNS Medicinal Chemistry, Wyeth Research, Princeton, NJ
1992-2000, *Senior Research Scientist II*, Chemical & Screening Sciences, Wyeth Research, Princeton, NJ
2000-2006, *Principal Research Scientist II*, Chemical & Screening Sciences, Wyeth Research, Princeton, NJ
2006-2009, *Principal Research Scientist III and Group Leader*, Chemical & Screening Sciences, Wyeth Research, Princeton, NJ

2009-2010, *Principal Research Scientist IV & Group Leader*, Chemical Sciences, Pfizer Global Research, Princeton, NJ
2010-Present, *Associate Professor of Pharmaceutical Sciences and Associate Director of the Moulder Center for Drug Discovery Research,*Temple University School of Pharmacy, Philadelphia, PA

Awards and Honors

DuPont Research Scholarship, 1981
N.I.H Training Grant Fellowship in cancer drug development, 1984-1987
Wyeth-Ayerst Teamwork Award for CNS Global Strategy Taskforce, 1994
Wyeth-Ayerst Teamwork Award for Neurodegeneration Discovery Team, 1997
Wyeth Teamwork Award for 5-HT_{1A} Antagonist Discovery Team, 1998
Wyeth Exceptional Achievement Award, 2000
Wyeth CNS Pipeline Award, 2006
Inducted into Rho Chi Honor Society, 2012
TUSP Golden Apple Award for Innovative Teaching, 2013
Member, American Chemical Society
Member, Society for Neuroscience
Member, New York Academy of Science

BIOGRAPHICAL SKETCH

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Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Kenneth Korzekwa	POSITION TITLE Associate Professor Department of Pharmaceutical Sciences Temple University School of Pharmacy
eRA COMMONS USER NAME (credential, e.g., agency login) kkorzekwa	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
New Mexico State University, Las Cruces, NM	BS	12/80	Chemical Engineering
University of Washington	PhD	2/86	Medicinal Chemsitry

C. Personal Statement

My research involves developing models to predict intracellular concentrations in the presence of transporters. These models will be most applicable for use within the pharmaceutical industry for drug discovery and development. When at the NIH and the University of Pittsburgh, my research focused on the

enzymology and kinetics of the cytochrome P450s. Models to predict metabolism developed at NIH and University of Pittsburgh were used to form the startup company Camitro Corporation, which I joined as VP of Research. When at Camitro, I led the research efforts for a company that built predictive ADME (Absorption, Distribution, Metabolism and Elimination) models for the industry. Camitro was sold after three years and I joined Merck in the Department of Drug Metabolism two years later. At Merck, I was in charge of the In Vitro Groups and focused my efforts on human pharmacokinetics prediction. I also worked with the computational chemistry groups to improve Merck's internal ADME models. After spending time at another startup company (Alchemie/Ethos), I joined Temple Universwity as an Associate Professor in the School of Pharmacy. When at AllChemie/Ethos I was responsible for preclinical development, and ran the chemistry efforts for the last year. This involved in vitro and in vivo preclinical studies, including outsourcing, pricing and budgeting for all preclinical and IND enabling studies, including pharmacokinetics, toxicology and safet pharmacology. These experiences left me with the belief that careful and coordinated preclinical studies would result in more rapid lead optimization and more successful clinical trials. This provides the background and the motivation to produce better tools for human PK prediction.

D. Positions and Employment

1980-1981, Chemical Engineer, Union Carbide Corp., Charlestown, WV
1986-1990, PRAT Fellow/Senior Staff Fellow NIH, NHLBI, Laboratory of Chemical Pharmacology
1990-1995, Senior Staff Fellow, NIH, NCI, DCE, Laboratory of Molecular Carcinogenesis
1995-1999, Associate Professor of Medicine, University of Pittsburgh, Center for Clinical Pharmacology
1999-2002, Vice President of Research, Camitro Corporation, Menlo Park, CA
2002-2003, Vice President of Research, ArQule Corporation, Woburn, MA
2004-2017, Director/Distinguished Senior Investigator, Merck Research Laboratories, West Point, PA
2007-2009, Vice President, Preclinical Research and Development, Ethos Pharmaceuticals, Wayne, PA
2009-2010, President, Reach Therapeutics Inc., Wayne, PA
2010-, Associate Professor, Department of Pharmaceutical Sci., Temple Univ. School of Pharmacy

Honors and Awards
Recipient of NIH Training Grant
Recipient of NIH Pharmacology Research Associate Training Fellowship

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Domenico Pratico	POSITION TITLE Associate Professor of Pharmacology
eRA COMMONS USER NAME PRATICO	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Univ. of Rome "La Sapienza", Rome, Italy	M.D.	1986	Medicine
Univ. of Rome "La Sapienza", Rome, Italy	Board Certification	1991	Internal Medicine

A. Personal statement: My contribution to this project will draw on my 15 years of work in the field of neurodegeneration. My main research area is clinical pharmacology with a special focus on the cellular and molecular aspects of oxidative neurobiology, and their role in the pathogenesis of chronic neurodegenerative diseases such as Alzheimer's disease by using both cellular and animal models. To this end, we use neuronal and non-neuronal cells stably expressing wild type human APP as well as the Swedish mutant. These are excellent in vitro models to investigate Amyloid beta metabolism and APP processing and their modulation by old and newly developed chemical probes. For the animal models, we have transgenic mice which express human APP with the Swedish mutant, also known as Tg2576, or APP and PS1 human mutants, and triple transgenic mice (3xTg), which by expressing at the same time APP/PS1 and a mutant human tau develops both the amyloid plaques and the tau deposits (i.e., neurofibrillary tangles) in their brains. My lab is well equipped for biochemistry, molecular biology and immunohistochemistry studies looking at the main AD-like neuropathologies in these animals. Importantly, these mice develop also behavioral deficits (i.e. memory and learning), which we can easily detect by using the contextual and cued fear conditioning paradigms all well established in our lab. My role in this proposal is inspired by our previous work in AD and transgenic model of AD (ref. # 1-7;11-14) the Lipoxygenases (ref. #8-10), and more recent work on the 5-Lipoxyegnase and the development of the AD-like phenotype, which has just been published in Annals of Neurology (ref.#15). Because my lab has pioneered the research area of 5LO and AD, and generated a unique mouse model (i.e., Tg2576/5LO$^{-/-}$), we are best qualified to carry out the studies proposed in this research project. In summary, I have a demonstrated record of successful and productive research in an area of high relevance to neurodegenerative diseases, and my expertise and experience have prepared me well to lead this research program.

B. Positions and Honors

Positions

1986-1988 Internship, Department of Clinical Medicine, University Hospital of Rome, Italy.

1989-1992 Residency in Internal Medicine, Hospital of the University of Rome "La Sapienza", Italy.

1988-1992 Res. Fellow, Haemocoagulation Unit, University of Rome "La Sapienza", Italy.

1992-1994 Res. Fellow, Centre for Cardiovasc. Science, University College Dublin, Mater Hospital, Dublin, Ireland.

1994-1995 Research Fellow, Univ. Pennsylvania, School of Medicine.

1996-1997 Research Associate, Univ. Pennsylvania, School of Medicine.

1998-2003 Assistant Professor, Dept. Pharmacology, Univ. Pennsylvania, School of Medicine.

2004-2007 Associate Professor, Dept. Pharmacology, Univ.Pennsylvania, School of Medicine.

2008- Associate Professor of Pharmacology, Dept. Pharmacology, Temple University

Honors

European Bristol-Myer-Squibb Post Doctoral Fellowship for Cardiovascular Research. Italian Research Council Fellowship for Hemocoagulation Research. Biomedicine Investigator Award for Excellence in Scientific Research. American Federation for Aging Research. Irvine H. Page Arteriosclerosis Research Award. American Federation for Aging Research Award. Italian Society for Hemostasis and Thrombosis Award. Neurosciences Education and Research Foundation Award. The CART Fund. Dorothy Dillon Eweson Lectureship.