BIOGRAPHICAL SKETCH

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

NAME: **Isaac Thomas Schiefer**

eRA COMMONS USER NAME (credential, e.g., agency login): **ISCHIE2**

POSITION TITLE: **Assistant Professor,** Department of Medicinal and Biological Chemistry, College of Pharmacy and Pharmaceutical Sciences, University of Toledo

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Toledo (Toledo, Ohio)	B.Sc.	2007	Medicinal and Biological Chemistry
University of Illinois at Chicago (Chicago, Illinois)	Ph.D.	2012	Medicinal Chemistry
University of Illinois at Chicago (Chicago, Illinois)	Res. Assoc.	2012	Drug Discovery
Northwestern University (Evanston, Illinois)	Res. Fellow	2012-2013	Chemical Biology

A. PERSONAL STATEMENT

The research in the Schiefer lab is focused on CNS drug discovery. We are a genuine multi-disciplinary research group. Specific activities carried out in the Schiefer lab include: organic synthesis; bioanalytical characterization of efficacy (in collaboration); *in vitro* metabolic stability and reactivity analysis; *in vivo* analysis of brain:plasma ratio and pharmacokinetics (using HPLC and LC-MS-MS); analysis of pharmacodynamic markers of efficacy (via ELISA, western blot, or microscopy); and *in vivo* behavioral efficacy studies using murine models of learning, memory, and motor function. Our projects can be broadly classified into two categories: 1) target identification; and 2) hit-to-lead optimization. Target identification projects focus on using techniques in chemical biology to understand the molecular targets and mechanism of action of small molecules with interesting CNS activity. Ideally the knowledge gained during target ID studies will be used to produce novel proprietary small molecules, followed by hit-to-lead optimization.

B. POSITIONS AND HONORS

2006-2007 Honors Student Lab Researcher, University of Toledo, Toledo, OH, with William S. Messer Jr.
2007-2009 Teaching Assistant, University of Illinois College of Pharmacy
2007-2012 Graduate Research, Dept. of Medicinal Chemistry, UIC, with Gregory R. J. Thatcher
2009-2012 NMR Facility Technician, Department of Medicinal Chemistry, UIC
2012 Postdoctoral Res. Assoc., Collaborative Engagement for Novel Therapeutic Research and Enterprise (UICentre- drug discovery at UIC), University of Illinois College of Pharmacy
2012-2013 Postdoctoral Res. Fellow, Dept. of Chemistry, Northwestern University, with Richard B. Silverman
2013-2015 Res. Assist. Prof., Dept. of Medicinal and Biological Chemistry, University of Toledo, Toledo, OH
2015-pres Assist. Prof., Dept. of Medicinal and Biological Chemistry, University of Toledo, Toledo, OH
2016-pres Associate Director, Shimadzu Laboratory for Pharmaceutical Research Excellence, U of Toledo

Honors and Achievements

Departmental Honors in Medicinal and Biological Chemistry, University of Toledo, 2007
Oscar Robert Olberg Prize for Excellence in Medicinal Chemistry, University of Illinois at Chicago (UIC), 2011
Young Investigator Scholarship, Alzheimer's Drug Discovery Foundation (ADDF), UIC, 2012 (spring)
Young Investigator Award, Alzheimer's Drug Discovery Foundation (ADDF), UIC, 2012 (fall)
Takeda Pharmaceuticals Award for Excellence in Research, UIC, 2012

Howard Hughes Medical Institute (HHMI) Mentoring Fellow, Northwestern University, 2012-2013
Kellogg's Business for Scientists Executive Training Certificate, Northwestern University, 2013
New Investigator Award, American Association of Colleges of Pharmacy (AACP), 2014-2015
New Investigator Research Grant, Alzheimer's Association (AA), 2015-2017
License to Distribute Dangerous Drugs, Laboratory/Research, Ohio State Board of Pharmacy, 2018-present

Memberships and other experience

2004- Member, Kappa Psi Pharmaceutical Fraternity
2007- Member, American Chemical Society (ACS)
2008- Member, American Chemical Society: Medicinal Chemistry Division
2011- Member, Society for Neuroscience (SfN)
2013- Member, American Stroke Association/American Heart Association (ASA/AHA)
2013- Member, American Association of Colleges of Pharmacy (AACP)
2014- Peer review (ad-hoc): ACS Medicinal Chemistry Letters; Scientific Reports (Nature); ChemMedChem; Medicinal Research Reviews Organic & Biomolecular Chemistry; Chemistry Central
2018 Guest Editor, Journal: *Nitric Oxide*, Special Issue: 'Pharmacology & Medicinal Chemistry of Nitric Oxide'
2018 Study Section, Alzheimer's Disease and Related Dementias (ZRG1 MDCN-R 54), *ad hoc*

C. CONTRIBUTION TO SCIENCE

As An Independent Investigator

1. The over-arching goal of my lab is best described as 'small molecule drug discovery for the CNS'. At present, the central thrust of my lab has been toward the development of nitric oxide (NO) mimetics as novel therapies for neurodegeneration. Specifically, we have worked with two classes of molecules; 1) furoxans, attenuated thiol-dependent NO mimetics; and 2) reactive oxygen species (ROS)-sensitive nitrones which are converted into NOS inhibitors upon reaction with superoxide. In the case of furoxans, we have recently provided the first description of *in vivo* effects of a furoxan target at the CNS, including the ability of a furoxan to reverse scopolamine induced deficits in step through passive avoidance and observations of a furoxan in the brain 12 hr after oral administration- a remarkable long half-life for an NO mimetic. Our furoxans are unique as NO mimetics because they provide slow NO release (termed 'attenuated NO mimetics'). We provided a preliminary pharmacokinetic curve using LC-MS/MS and demonstrated that our furoxans have a brain:plasma ratio ~1 and a long systemic half-life.

    i. Horton, A.; Nash, K.; Tackie-Yarboi, E.; Kostrevski, A.; Novak, A.; Raghavan, A.; Tulsulkar, J.; Alhadidi, Q.; Wamer, N.; Langenderfer, B.; Royster, K.; Ducharme, M.; Hagood, K.; Post, M.; Shah, Z. A.; **Schiefer, I. T.** Furoxans (Oxadiazole-4N-oxides) with Attenuated Reactivity are Neuroprotective, Cross the Blood Brain Barrier, and Improve Passive Avoidance Memory. *Journal of Medicinal Chemistry,* **2018**; 61(10), 4593-4607.
    ii. Schiefer, I.; Shah, Z.; inventors; The University of Toledo, USA. assignee. Furoxans as therapies for neurodegenerative disorders; **2018**, WO2018093762A1.
    iii. Nash, K. M.; **Schiefer, I. T.*;** Shah, Z.A.* Development of a reactive oxygen species-sensitive nitric oxide synthase inhibitor for the treatment of ischemic stroke. *Free Radicals in Biology and Medicine*, **2018**; 115: 395-404. *denotes co-corresponding authors

As a Collaborator

2. To this point, collaborative work carried out in the Schiefer lab centralizes around Dr. Schiefer's appointment as Associate Director of the Shimadzu Laboratory for Pharmaceutical Research Excellence. In this capacity we have aided with the isolation/identification of small molecules from complex mixtures using semi-preparative HPLC and 2D-NMR. We have also quantitated small molecules in biological matrices using LC-MS/MS. Representative publications for these collaborations are given below. Additionally, the Dr. Schiefer is a Co-I on two NIH funded projects in which the Schiefer lab is currently carrying out synthetic work.

i. Obrenovich, M. E.; **Schiefer, I. T.**; Bongiovanni, R.; Li, L.; Donskey, C. J.; Jaskiw, G. E. Quantification of Phenolic Acid Metabolites in Humans by LC-MS – A Structural and Targeted Metabolomics Approach. *Bioanalysis*, **2018**, 10(19):1591-1608.
ii. Kurogi, K.; Yoshihama, M.; Horton, A.; **Schiefer, I. T.**; Krasowski, M. D.; Hagey, L. R.; Williams, F. E.; Sakakibara, Y.; Kenmochi, N.; Suiko, M.; Liu, M. Identification and characterization of 5α-cyprinol-sulfating cytosolic sulfotransferases (Sults) in the zebrafish (Danio rerio). *Journal of Steroid Biochemistry and Molecular Biology*, **2017**, 174, 120-127.
iii. Alasmari, F.; Crotty Alexander, L. E.; Nelson, J. A.; **Schiefer, I. T.**; Breen, E.; Drummond, C. A.; Sari, Y. Effects of chronic inhalation of electronic cigarettes containing nicotine on glial glutamate transporters and α-7 nicotinic acetylcholine receptor in female CD-1 mice. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. **2017**, 77, 1-8.
iv. Yamamoto, A.; Kurogi, K.; **Schiefer, I. T.**; Liu, M.; Sakakibara Y.; Suiko M., et al. Human Cytosolic Sulfotransferase SULT1A3 Mediates the Sulfation of Dextrorphan. *Biological & Pharmaceutical Bulletin* **2016**; 39(9):1432-6.

During Graduate Work

Other relevant contributions were made during my graduate work. I was extremely fortunate to begin my graduate work at the same time that a collaborative U01 grant was funded for my advisor (Gregory Thatcher, UIC) with Ottavio Arancio (Columbia) and Pavel Petukhov (UIC). This project had three “hit compounds” as potential novel calpain inhibitors. Calpain is a calcium dependent cysteine protease over-activated in AD patients. We evaluated three ‘hits’ which each had a generic peptidomimetic portion and a unique chemically reactive warhead (epoxide, furoxan, or cyclopropenone). At the end of year one, we selected epoxides for comprehensive development.

3. The majority of my graduate school effort was spent developing novel selective epoxide incorporating calpain inhibitors. I led the hands-on medicinal chemistry effort to advance the project throughout. I personally designed and synthesized over 50 analogs of the selected epoxide lead compound during the duration of the project (all compounds fully characterized by ^{1}H NMR, ^{13}C NMR, HRMS, and > 95 % pure by HPLC). I examined stability and reactivity of selected compounds by HPLC-UV. I characterized *in vitro* metabolites and measured *in vivo* bioavailability using LC-MS/MS. I acted as the key intermediary between the neurobiologist, bioanalytical chemist, computational chemist, and the PI/synthetic chemistry team. I actively corresponded with collaborators to define deadlines and ensure the timely delivery of compounds to colleagues and collaborators in order to advance the project. The highest endeavor culminated in the identification of two novel, synthetically accessible molecules that show promising efficacy and negligible toxicity *in vivo* (Therapeutic Index > 100, in murine model). A Notice of Allowance for all claims associated with the patent for this work was announced (04/19/2016) by the USPTO.

    i. **Schiefer, I. T.**; Tapadar, S.; Litosh, V.; Siklos, M.; Scism, R.; Wijewickrama, G. T.; Chandrasena, E. P.; Sinha, V.; Tavassoli, E.; Brunsteiner, M.; Fa’, M.; Arancio, O.; Petukhov, P.; Thatcher, G. R. J. Design, Synthesis, and Optimization of Novel Epoxide Incorporating Peptidomimetics as Selective Calpain Inhibitors. *Journal of Medicinal Chemistry* **2013**, 56, 6054–68.
    ii. Novel cysteine protease inhibitors and uses. Arancio, Ottavio; Fa, Mauro; **Schiefer, Isaac T.**; Thatcher, Gregory; US 20150045393.
    iii. Fà, M.; Zhang, H.; Staniszewski , A.; Saeed, F.; Shen, L. W.; **Schiefer, I. T.**, Siklos, M.; Tapadar, S.; Litosh, V.; Libien, J.; Petukhov, P.; Teich, A. F.; Thatcher, G. R. J.; Arancio, O. Novel Selective Calpain 1 Inhibitors as Potential Therapeutics in Alzheimer’s Disease. *Journal of Alzheimer’s Disease* **2016**, 49, 707-721.

4. Furoxan development was discontinued as the primary development emphasis during the U01 because furoxans could not be designed as effective calpain inhibitors. With permission from my advisor, I continued intermittent furoxan development in my spare time (i.e., Saturday morning project) while still meeting deadlines of epoxide development. I designed and synthesized a couple dozen novel neuroprotective NO mimetic furoxan (oxadiazole-N-oxides) containing peptidomimetics. I monitored reactivity of selected furoxans with cysteine using HPLC and identified reaction products by LC-MS/MS. I examined NO release and cysteine reaction rate using colorimetric assays, and correlated reactivity/NO release to observed neuroprotection. I spear-headed the effort to provide the first report of neuroprotective cognition enhancing

effects for furoxans. My hard work was rewarded by Dr. Thatcher, who has given me his blessing to continue furoxan development. I was also involved with profiling of protein S-nitrosation by different NO donors. In this study, we showed that nitrates, such as nitroglycerin and furoxans produce negligible S-nitrosation.

i. **Schiefer, I. T.**; VandeVrede, L.; Fa`, M.; Arancio, O.; Thatcher, G. R. J. Furoxans (1,2,5-Oxadiazole-N-Oxides) as Novel NO Mimetic Neuroprotective and Procognitive Agents. *Journal of Medicinal Chemistry* **2012**, 55 (7), 3076-3087.
ii. Sinha, V.; Wijewickrama, G. T.; Chandrasena, R. E.; Xu, H.; Edirisinghe, P. D.; **Schiefer, I. T.**; Thatcher, G. R. Proteomic and Mass Spectroscopic Quantitation of Protein S-nitrosation Differentiates NO-donors. *ACS Chemical Biology* **2010**, 5, 667-680.

5. My earliest work focused on the evaluation of nitric oxide chimeras (hybrid molecules containing drug pharmacophores and nitrate moieties) for the CNS. One publication added to the structure activity relationship surrounding gamma-secretase modulation by non-steroidal anti-inflammatory drugs and their hybrid nitrates. At the time, flurbiprofen was being investigated clinically for AD. Ultimately, flurbiprofen failed in the clinic. Additionally, I carried out synthetic and bioanalytical work for series of NO-SSRI's. Interestingly, these molecules reduced anxiety in a similar fashion to the SSRI's of interest (fluoxetine and paroxetine) without requiring a long-duration of treatment to see an effect. SSRI's are notorious for requiring administration for weeks prior to seeing significant anxiolytic effects. Enhancement of BDNF is implicated as a mechanism of SSRI activity. The NO-SSRI hybrids were shown to increase BDNF after acute administration, while the SSRI's required multiple doses.

i. **Schiefer, I. T.**; Abdul-Hay, S.; Wang, H.; Vanni, M.; Qin, Z.; Thatcher, G. R. Inhibition of Amyloidogenesis by Nonsteroidal Anti-inflammatory Drugs and Their Hybrid Nitrates. *Journal of Medicinal Chemistry* **2011**, 54 (7), 2293-2306.
ii. Abdul-Hay, S.; **Schiefer, I. T.**; Chandrasena, R. E. P.; Li, M.; Abdelhamid, R.; Wang, Y.-T.; Tavassoli, E.; Michalsen, B.; Asghodom, R. T.; Luo, J.; Thatcher, G. R. J. NO-SSRIs: Nitric Oxide Chimera Drugs Incorporating a Selective Serotonin Reuptake Inhibitor. *ACS Medicinal Chemistry Letters* **2011**, 2 (9), 656-661.

Complete List of Published Work in MyBibliography

https://www.ncbi.nlm.nih.gov/sites/myncbi/1fIg7lwJP2yA-/bibliography/47585629/public/?sort=date&direction=descending

D. RESEARCH SUPPORT

ACTIVE

R01AG057598-01 (Schiefer [PI]) 04/15/2018 - 01/31/2023 25 % calendar
NIH-NIA
"Development of Attenuated Furoxans as Novel Therapies for Alzheimer's Disease"

The major goals of this project is to carry out hit-to-lead optimization to develop a novel class of nitric oxide mimetics, known as furoxan, for the treatment of Alzheimer's disease.

R03DA045833-01A1 (Schiefer [PI]) 04/01/2018 - 03/30/2020 10 % calendar
NIH-NIDA
"In Vivo Photoaffinity Labeling using Casper Zebrafish for Target Identification"

The major goal of this work is to develop a novel platform for target identification for psychoactive drugs. This includes the synthesis of photoaffinity probes related to methamphetamine and methinone and performing in vivo photoaffinity in pigment free zebrafish known as casper fish.

R56AG005214-23A1 (Ellis [PI]) 09/30/2017 - 8/31/2018 11 % calendar
NIH-NIA
“Signal shaping via multiple allosteric sites on oligomeric muscarinic receptors”

The major goal of this work is to profile muscarinic receptors using bivalent allosteric modulators. My lab is synthesizing novel probes to characterize muscarinic receptors.

1R03DA045350-01A1 (Hall [PI]) 07/15/2018 - 06/30/2020 10 % calendar
NIH-NIDA
“High Throughput Approaches To Determining The Lethality Of Synthetic Psychoactive Cathinones Using Danio Rerio Larvae ”

The major goal of this work is to develop a novel platform for target identification for psychoactive drugs. This includes the synthesis of photoaffinity probes related to methamphetamine and methinone and performing in vivo photoaffinity in pigment free zebrafish known as casper fish.

COMPLETED

NIRG-15-363739 (Schiefer [PI]) 10/01/2015 - 09/31/2017
Alzheimer’s Association
“PK/PD study on a Prototype Attenuated NO Mimetic Furoxan”

New Investigator Award (Schiefer [PI]) 01/01/2014 - 12/31/2014
American Association of Colleges of Pharmacy
“Development of NO Mimetic Furoxans as Novel Agents for Alzheimer's Disease Therapy”