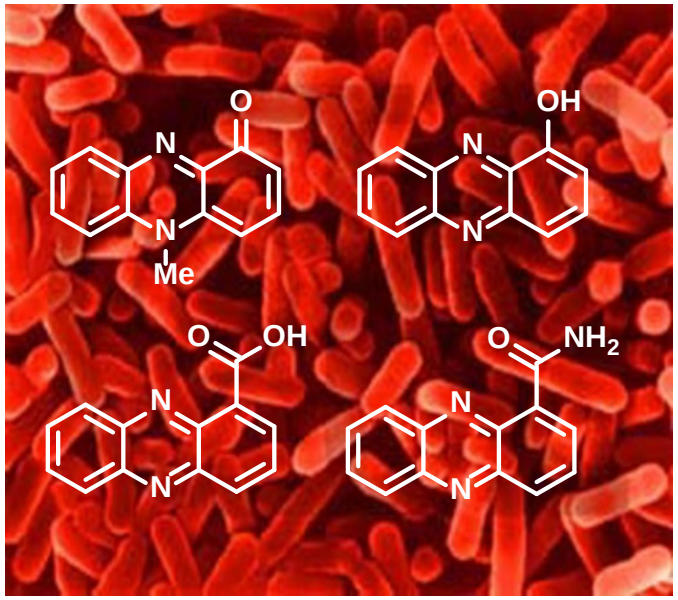
Huigens Lab: Natural Products Inspired Drug Discovery

Phenazine Antibiotics



Pseudomonas aeruginosa

Inspired Library Synthesis

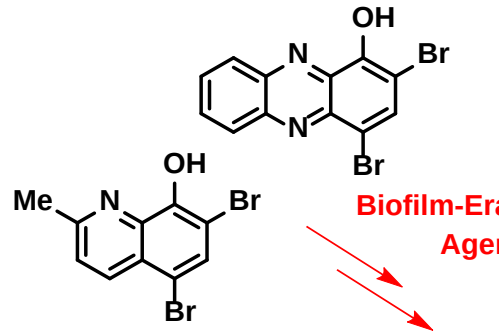
Phenotypic Screening

Planktonic Bacterial Cells

Staphylococcal Pathogens

Surface Attachment

Biofilm Dispersion



Biofilm-Eradicating Agents

Colonization → **Growth/Development** → **Mature Biofilm**

Bacterial Biofilms
resistant to antibiotics and immune responses

= bacterial cell

= EPS

Huigens Lab Wrecking Crew (Summer 2015)

Where do I even start?!?!

Yasmeen, Thandie and Dr. Huigens' Mission Impossible?

Melanie and Minh trying to get out of this picture?

Sam yelling, "we kill biofilms"?

Hongfen thinking, "I'm flying"?

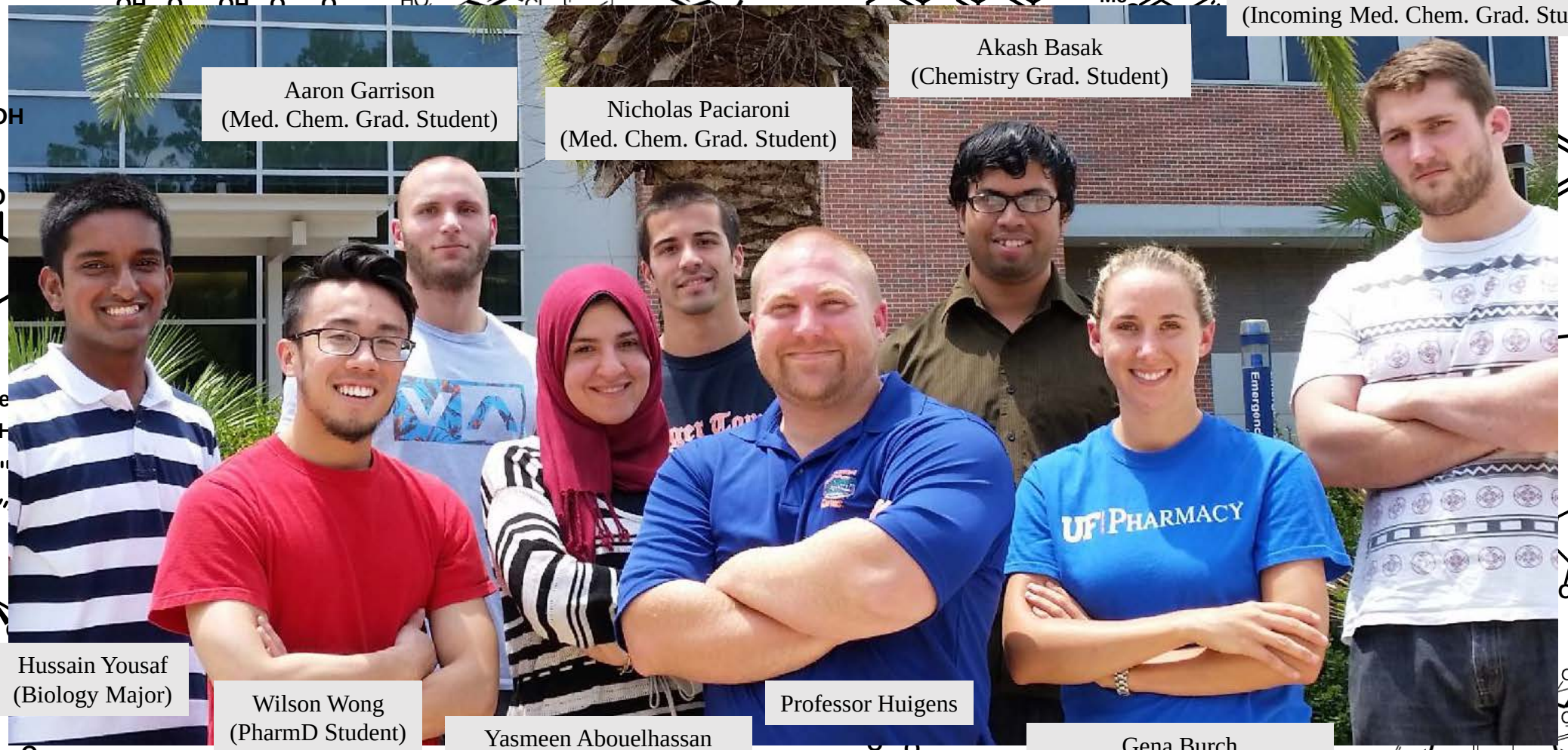
Aaron's double biceps pose?

Chip and Nick thinking "is this really happening"?

Hussain and Tho thinking, "we have experiments to do, stop playing around"?

Akash giving (three) bunny ears to Tho?

Huigens Lab Wrecking Crew (Summer 2014)

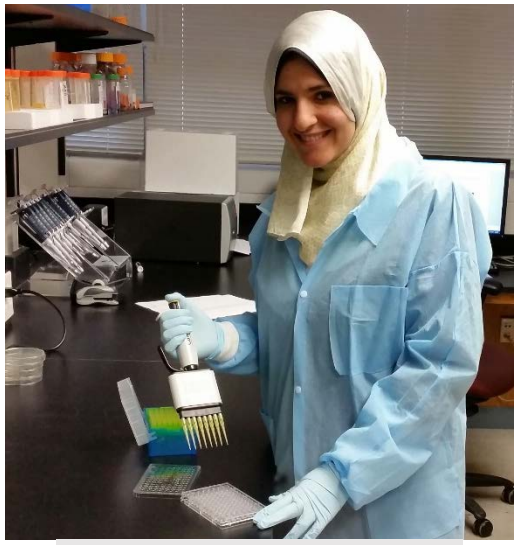


Huigens Lab – August 2013

Meet Our Graduate Students In Action!

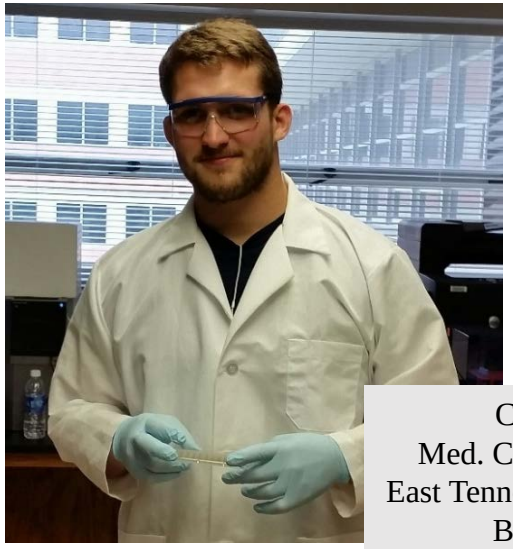
Nicholas Paciaroni
Med. Chem. Grad. Student
Clemson University
B.S. Chemistry

Aaron Garrison
Med. Chem. Grad. Student
University of South Florida
B.A. Biochemistry

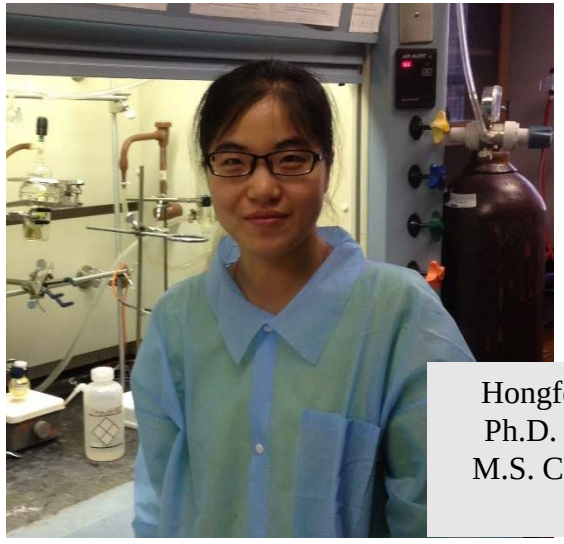


Yasmeen Abouelhassan
Med. Chem. Grad. Student
Cairo University
B.S. Pharmacy

Akash Basak
Chemistry Grad. Student
IIT Kanpur
M.S. Chemistry



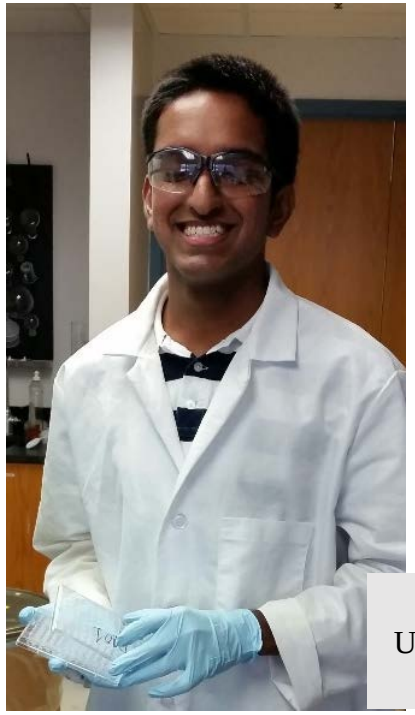
Chip Norwood
Med. Chem. Grad. Student
East Tennessee State University
B.S. Chemistry



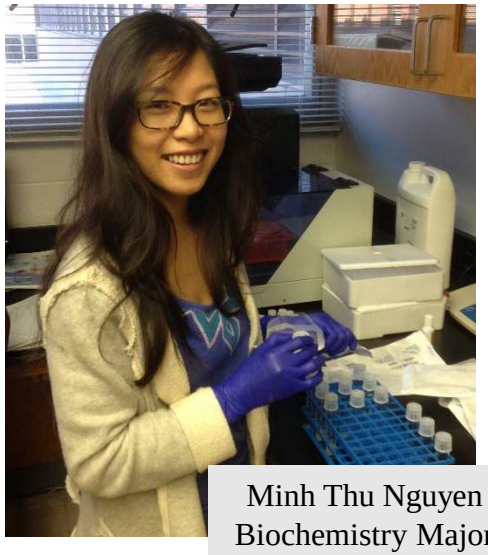
Hongfen Yang
Ph.D. Student
M.S. Chemistry

Gena Burch
PharmD/Ph.D. Student
B.S. Chemistry
Hillsdale College

Pharmacy, Undergraduate, High School Group Members



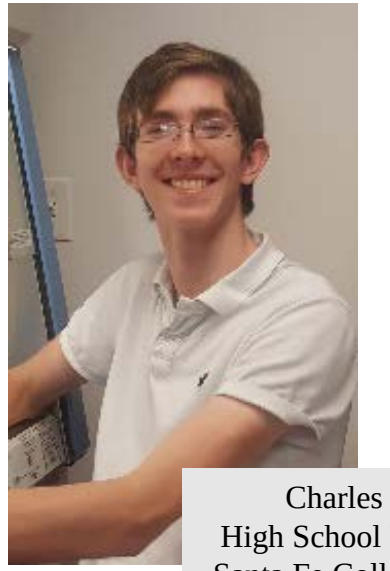
Hussain Yousaf
University of Florida
B.S. Biology



Minh Thu Nguyen
Biochemistry Major
University of Florida
University Scholar

Austin Arnold
Chemistry Major
Santa Fe/UF

Melanie Rolfe
PharmD Student
Marymount University
B.S. Biology



Charles Mock
High School Researcher
Santa Fe College Student

Former Group Members

Dr. Nicholas Borrero
Ph.D., University of Florida
(Grad. Advisor: Prof. Aaron Aponick)
Current Position: Acme Bioscience, Inc.

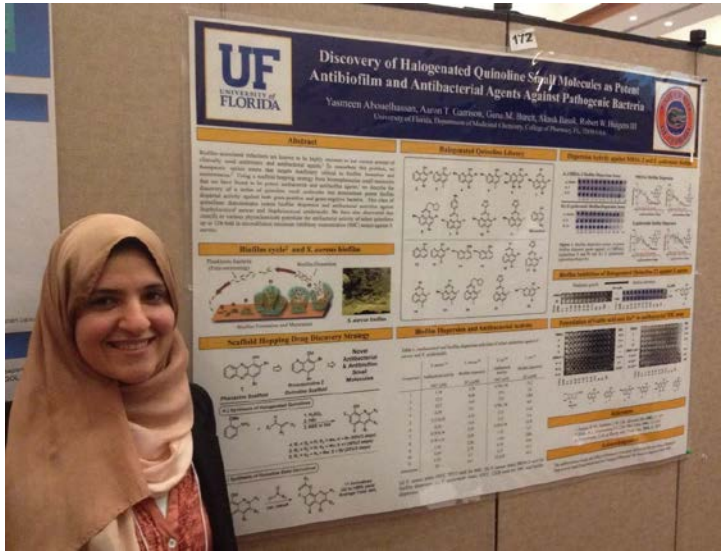
Benjamin Duong
PharmD Student
University of Florida

Wilson Wong
PharmD Student
University of Florida

Tho Nguyen
University of Florida
B.S. Chemistry

Thandiwe Jolly
University of Florida
B.S. Chemistry

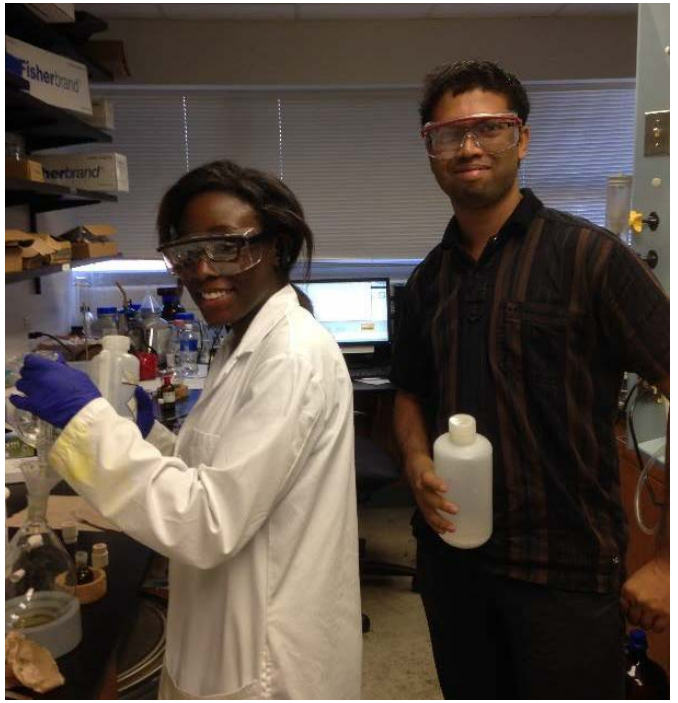
Sam Goldstein
High School Researcher



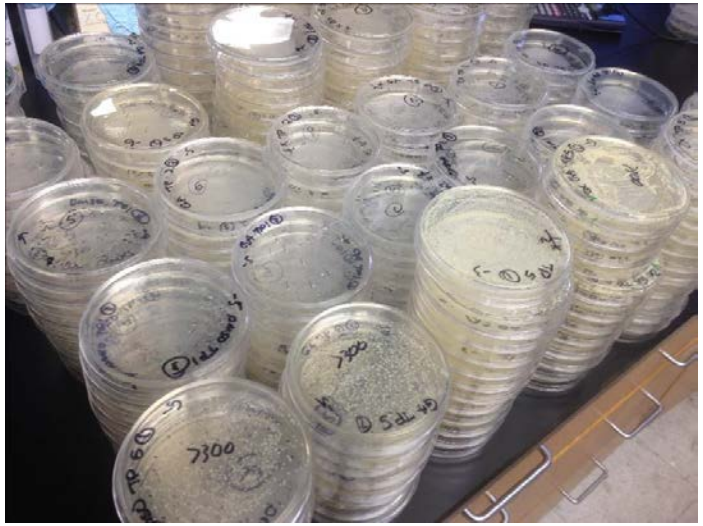
Yasmeen presents her work at UF's graduate student research day.

Gena wins poster award at COP Annual Research Showcase!

"I swear, the handle just fell off."
(a true story by Nick P.)



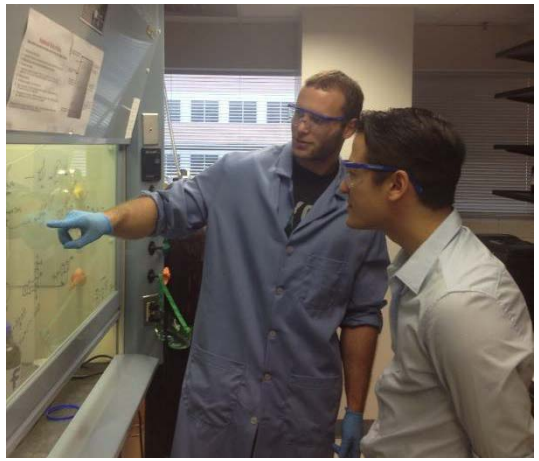
Akash…always mentoring in style.



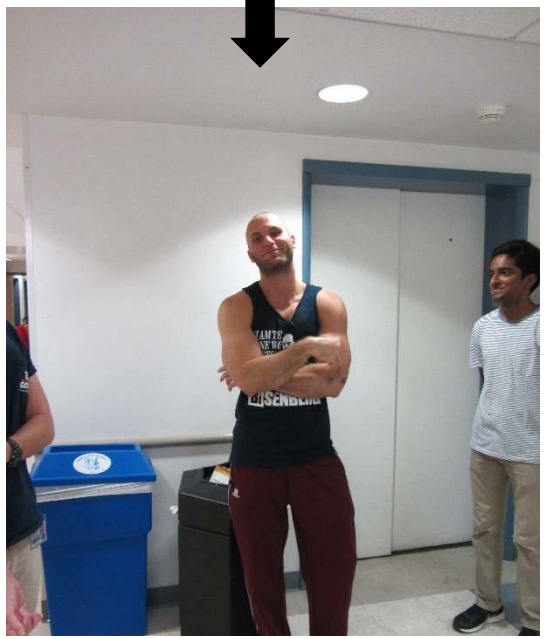
Colony counts DONE!

Instant Classic!!

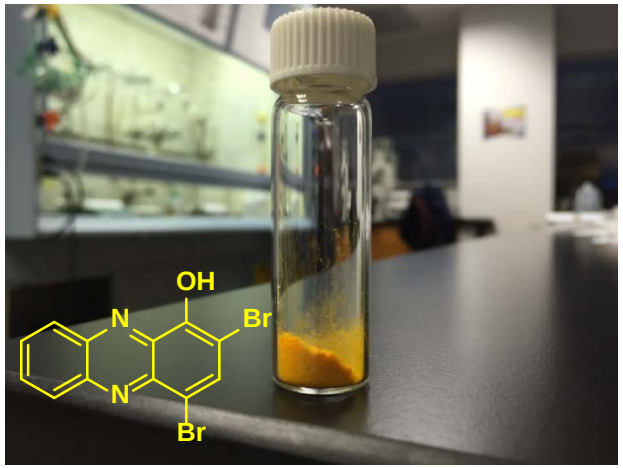
Heading back to the lab…a lot of bacterial biofilms are about to be in trouble.



Aaron Garrison…happy about being at his hood.



…not too happy about being away from his hood.



The grind!

Paciaroni got it! (in the NMR lab)

The Quiet Before The Storm

Empty labs in April 2013.
Ready to get to work!

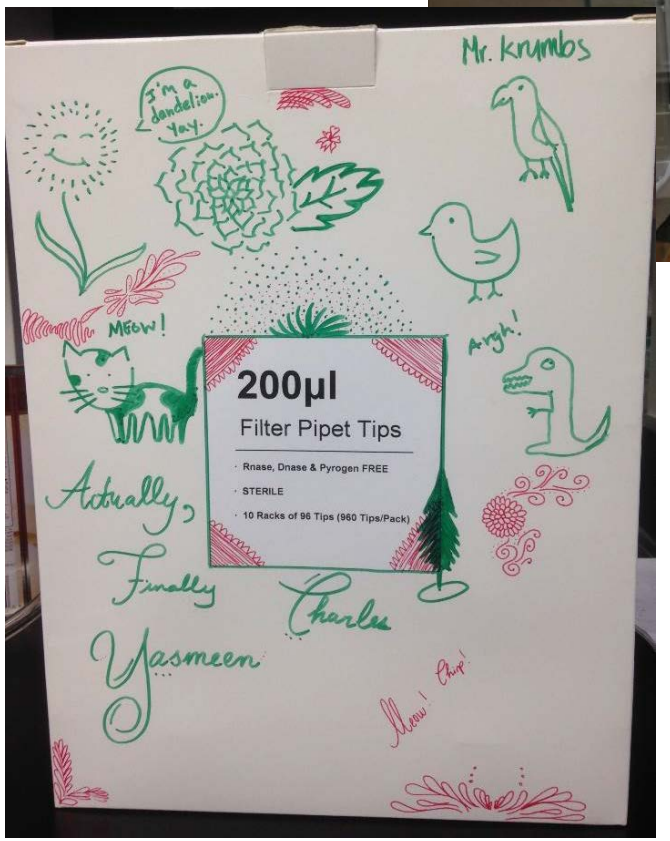
Working to change the world!
June 2015.

More Quiet Before The Storm

April 2013.

Working to change the world!
June 2015.

Microbiology!

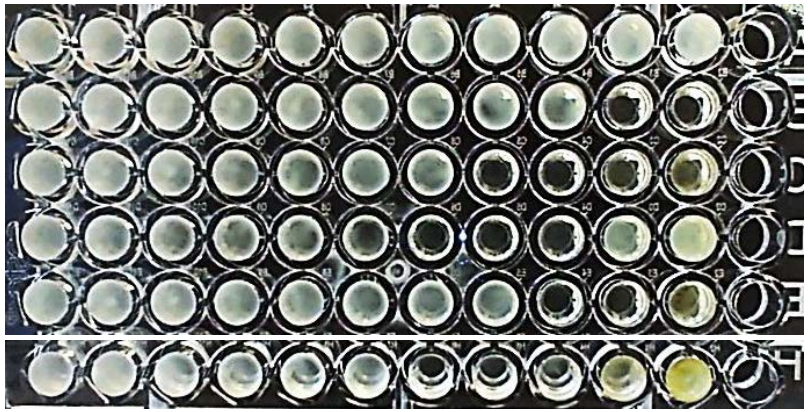


S. aureus Biofilm Clearance

0.2 0.4 0.8 1.6 3.13 6.25 12.5 25 50 100 200 -- µM

MRSA-2 Biofilm Eradication with Bromophenazines

MRSA-2 Biofilm Eradication Assay



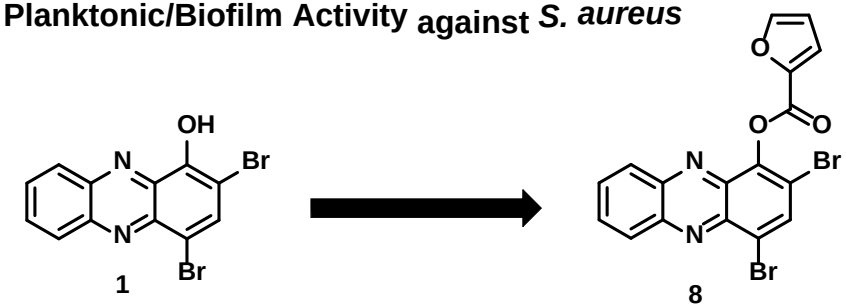
	MBEC (μM)
A: DMSO	--
B: 8**	100**
C: 9	250
D: 10	125
E: 1*	200**
F: 2	125

2 3.9 7.8 15.6 31.3 62.5 125 250 500 1000 2000 -- μM
* 0.78 1.56 3.13 6.25 12.5 25 50 100 200 400 800 -- μM
** 0.2 0.39 0.78 1.56 3.13 6.25 12.5 25 50 100 200 -- μM

	MBEC (μM)
A: DMSO	--
B: 1*	100*
C: Vancomycin	>2,000

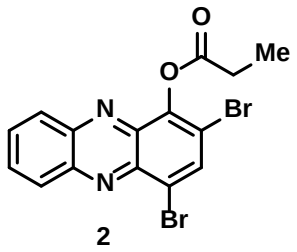
2 3.9 7.8 15.6 31.3 62.5 125 250 500 1000 2000 -- μM
* 0.78 1.56 3.13 6.25 12.5 25 50 100 200 400 800 -- μM

Planktonic/Biofilm Activity against *S. aureus*

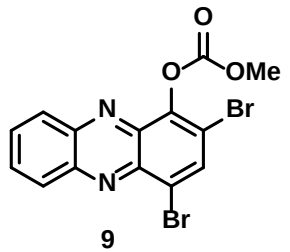


MIC: 1.56 μM (planktonic)
EC50: 29.3 μM (biofilm clearance)
MBEC: 100-200 μM (biofilm eradication)

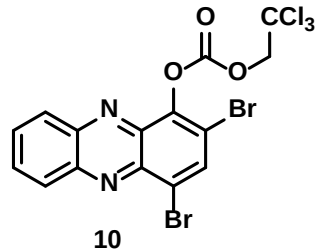
MIC: 0.78 μM
EC50: 1.4 μM
MBEC: 62.5-100 μM



MIC: 1.56 μM
MBEC: 125 μM



MIC: 1.56 μM
MBEC: 250 μM



MIC: 0.78 μM
MBEC: 125 μM

Vancomycin

MIC: 0.78 μM
MBEC: >2,000 μM

MIC values against *S. aureus* ATCC 29213
MBEC values against MRSA-2 (clin. isolate)

Yasmeen Abouelhassan, Aaron Garrison

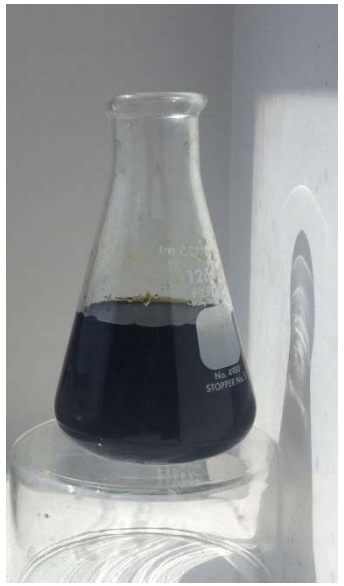
RSC Advances **2015**, *5*, 1120-1124.

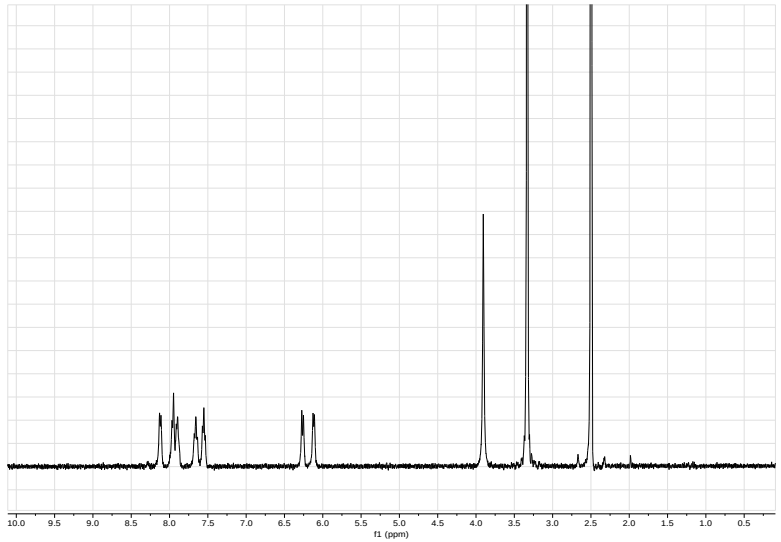
Getting After It!!

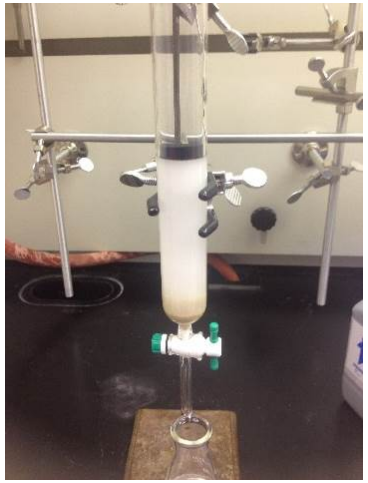
Dr. Nick helps to unpack our first large glassware order.

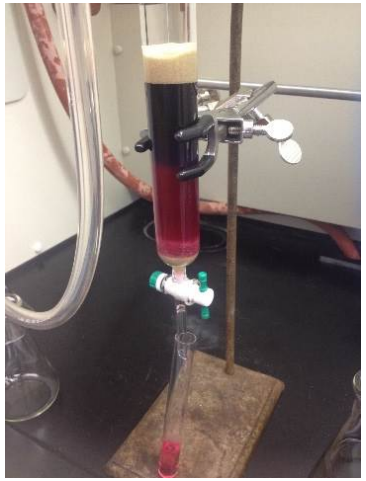
Fen cranks out some new compounds!

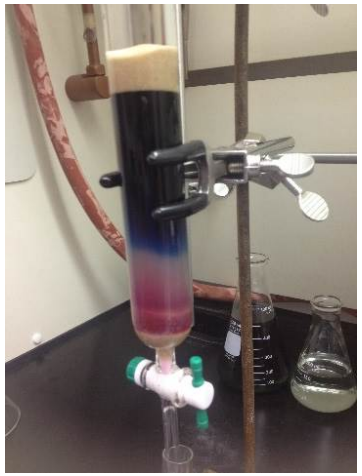
Synthesis of Pyocyanin (Nature’s Inspiration)



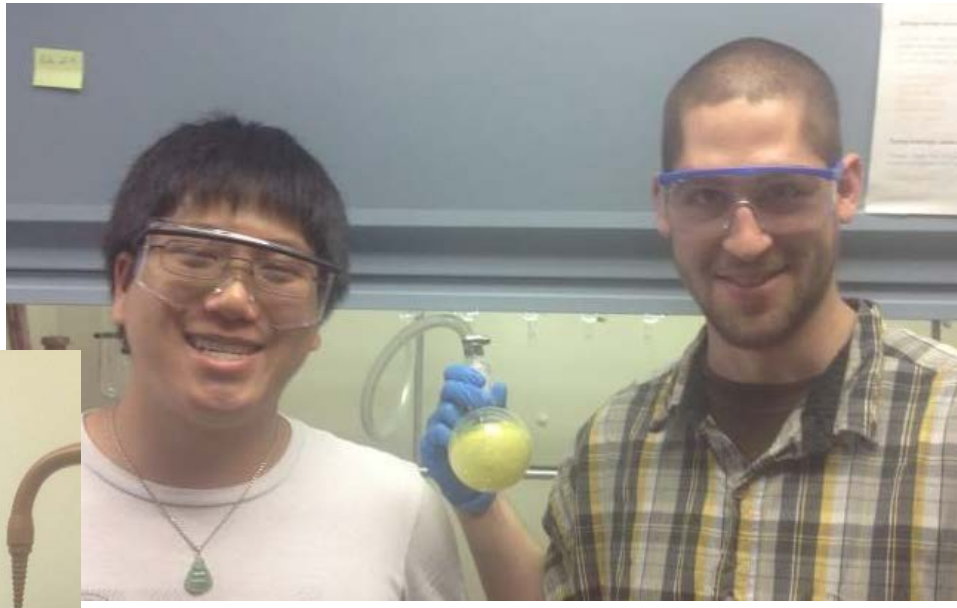


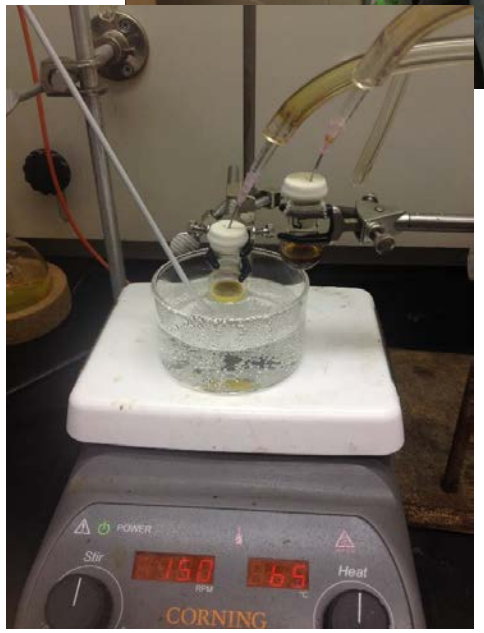


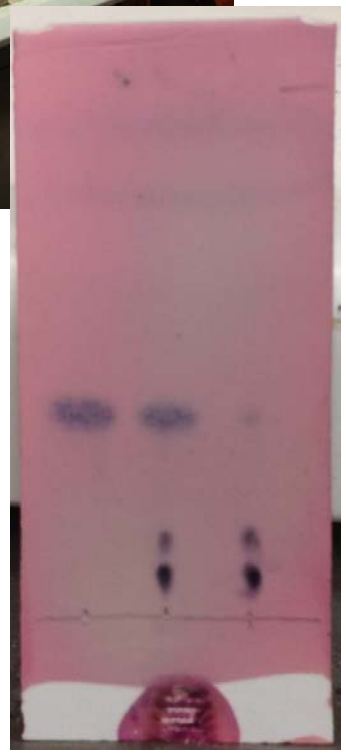


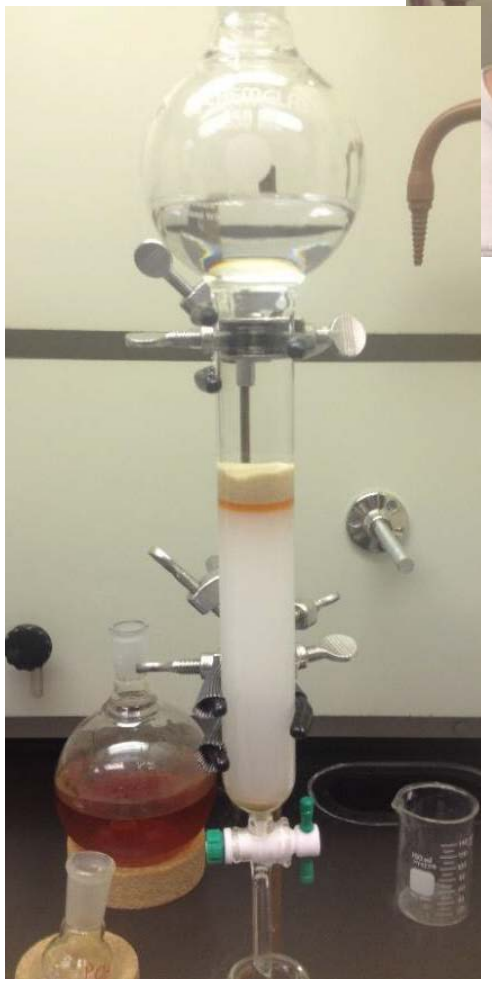


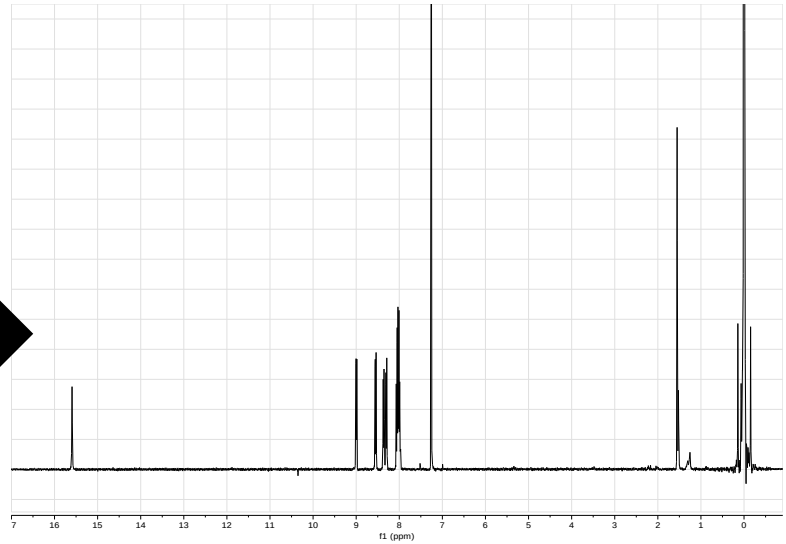
Developing Innovative Synthesis Strategies in Drug Discovery





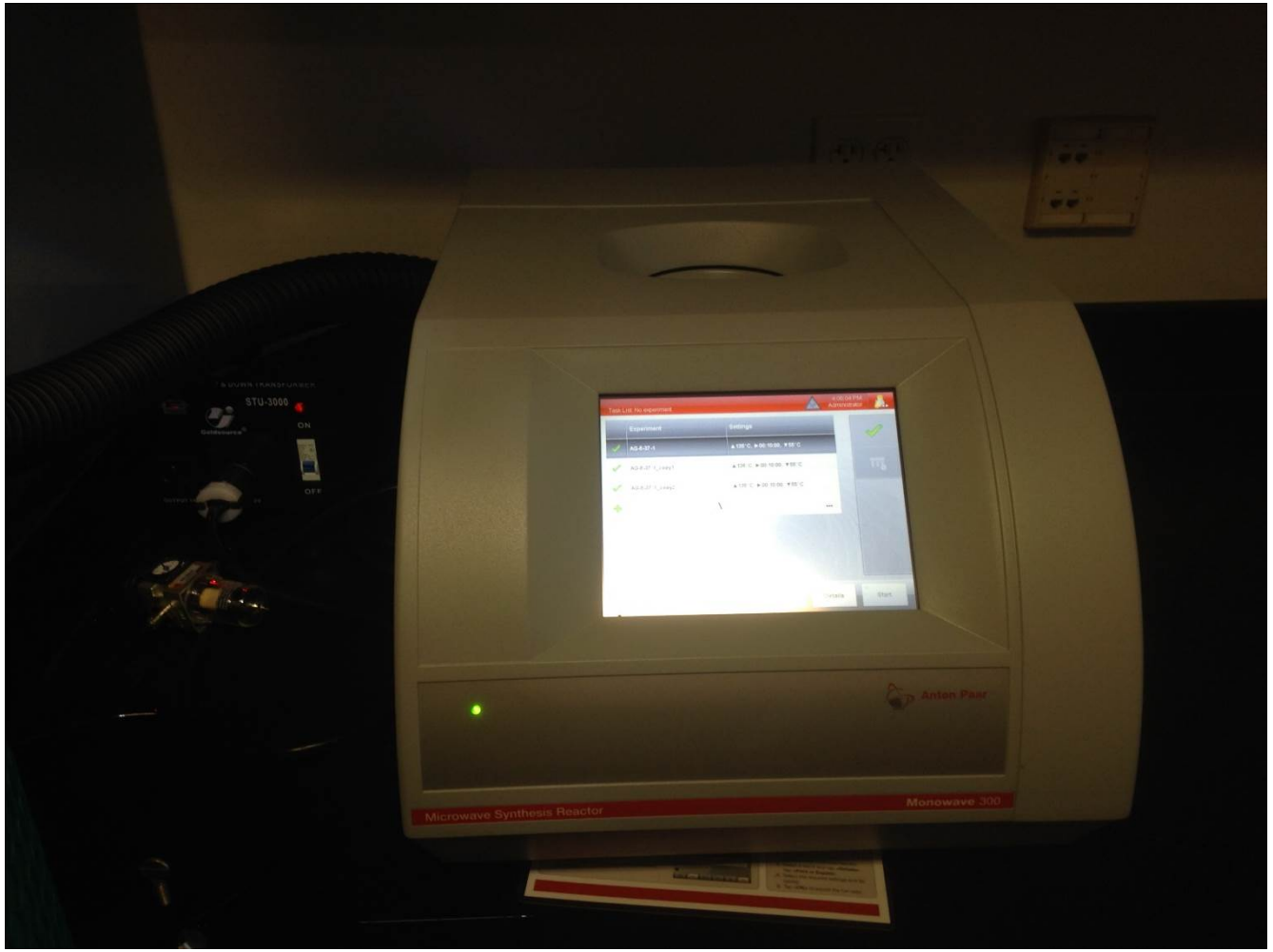






New Organic Synthesis Equipment

Anton Parr Microwave Reactor



Benchtop Ozone Generator (used in a hood)

Novel Small Molecules for Drug Discovery and Chemical Biology

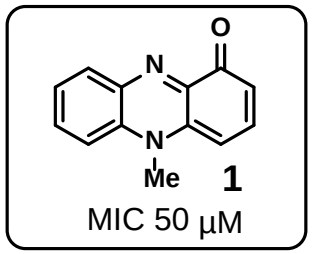
Dr. Borrero running an NMR of a new compound.

The beginning of the Huigens Lab small molecule library for drug discovery.

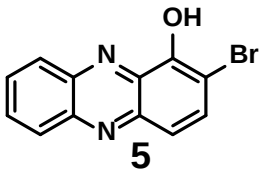
Focused on the prize of making a great lab!

June 2015

First MIC experiment against *Staphylococcus aureus* (by Professor Jin!)

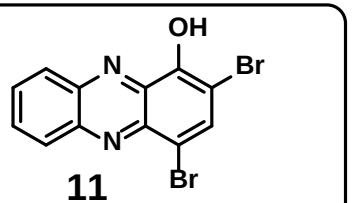


MIC 50 μM



MIC 6.25 μM

Marine Phenazine Antibiotic (*Streptomyces* sp.)

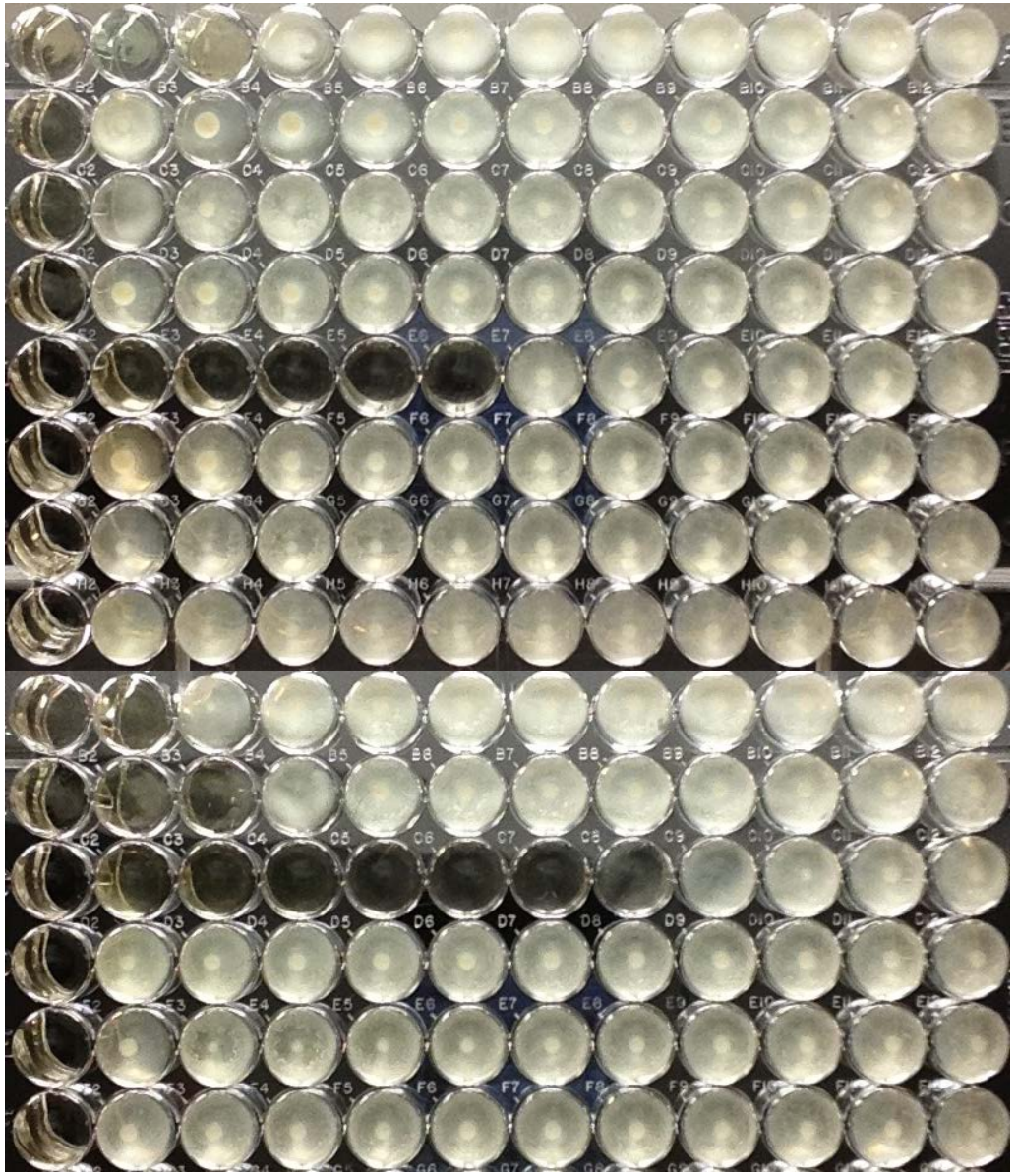


MIC 1.56 μM (0.55 μg/mL)

32-fold more potent than pyocyanin

A B C D E F G H I J K L

1
2
3
4
5
6
7
8
9
10
11
12
13
DMSO



MIC = minimum inhibitory concentration

A.	**No bacteria**
B.	**100 μM**
C.	**50 μM**
D.	**25 μM**
E.	**12.5 μM**
F.	**6.25 μM**
G.	**3.13 μM**
H.	**1.56 μM**
I.	**0.78 μM**
J.	**0.39 μM**
K.	**0.20 μM**
L.	**0.10 μM**

Identical MIC results against *S. epidermidis*

Prof. Shouguang Jin

Org. Biomol. Chem. **2014**, *12*, 881-886.

Paper #1 (Great Job Team)

Organic & Biomolecular Chemistry

COMMUNICATION

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Cite this: *Org. Biomol. Chem.*, 2014, **12**, 881

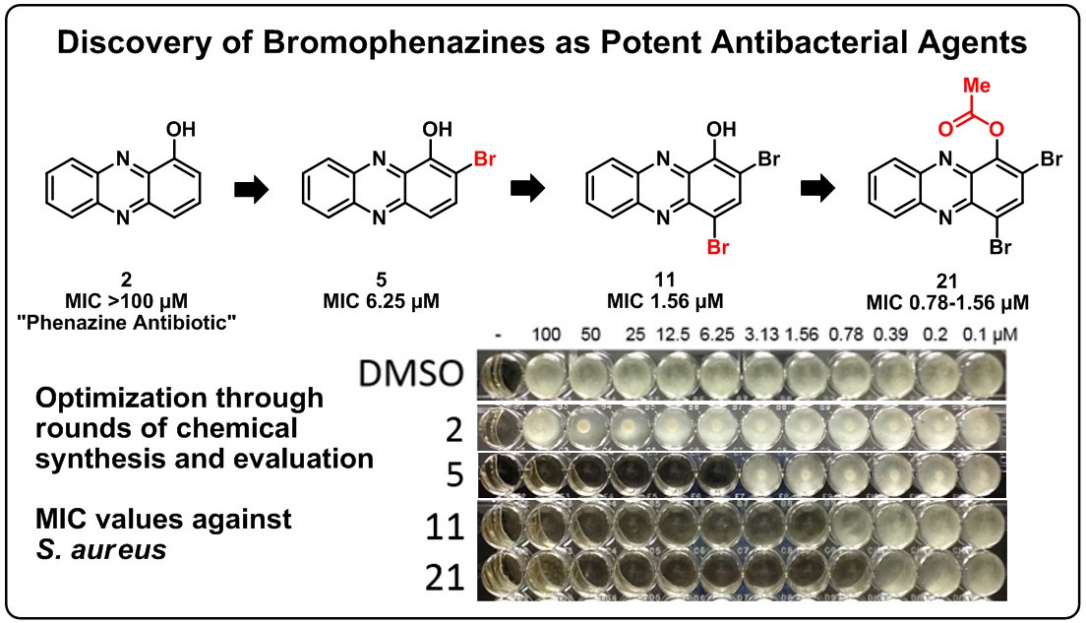
Received 3rd December 2013,
Accepted 17th December 2013

DOI: 10.1039/c3ob42416b

www.rsc.org/obc

Phenazine antibiotic inspired discovery of potent bromophenazine antibacterial agents against *Staphylococcus aureus* and *Staphylococcus epidermidis*†

Nicholas V. Borrero, Fang Bai, Cristian Perez, Benjamin Q. Duong, James R. Rocca, Shouguang Jin and Robert W. Huigens III*



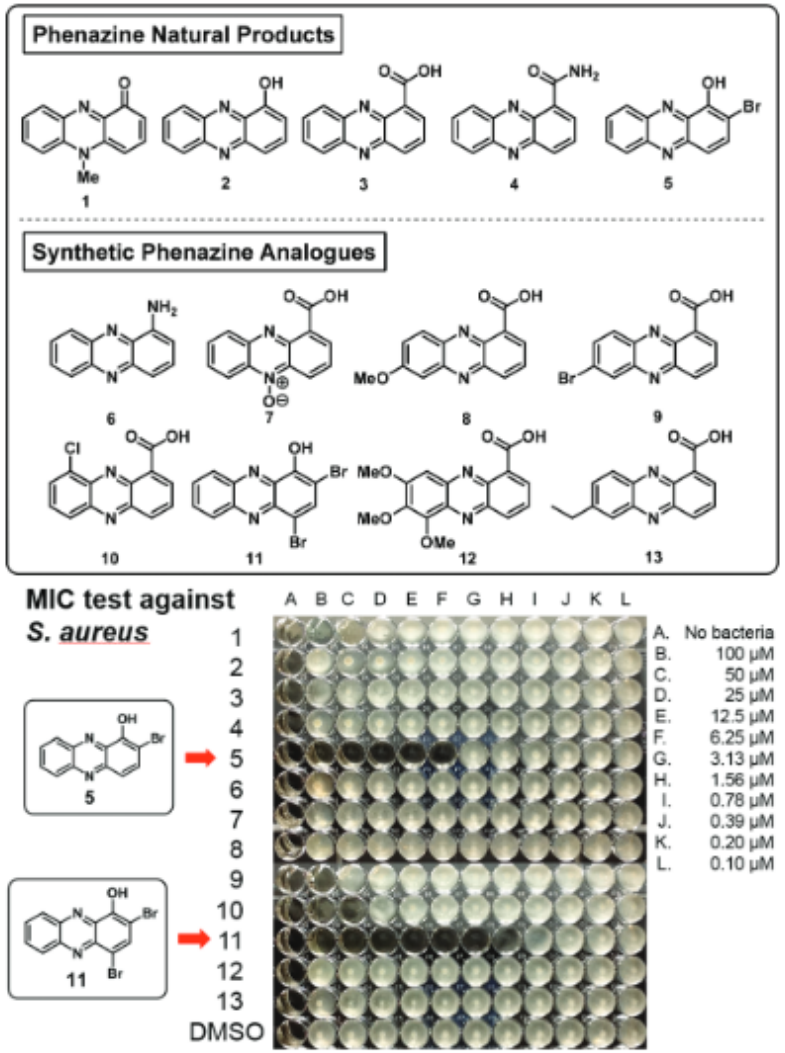


Fig. 1 Initial library of 5 phenazine natural products and 8 synthetic phenazines in head-to-head MIC experiments against *Staphylococcus aureus*.

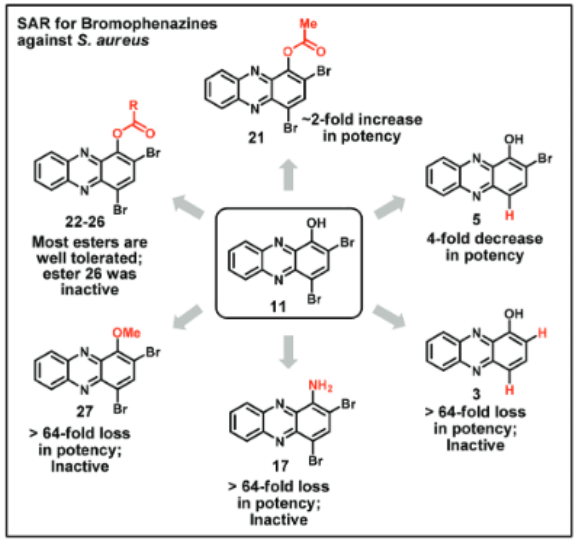


Fig. 3 Detailed structure–activity relationship (SAR) for this novel class of antibacterial agents against *S. aureus*.

Bioorganic & Medicinal Chemistry Letters 24 (2014) 5076–5080

Contents lists available at ScienceDirect

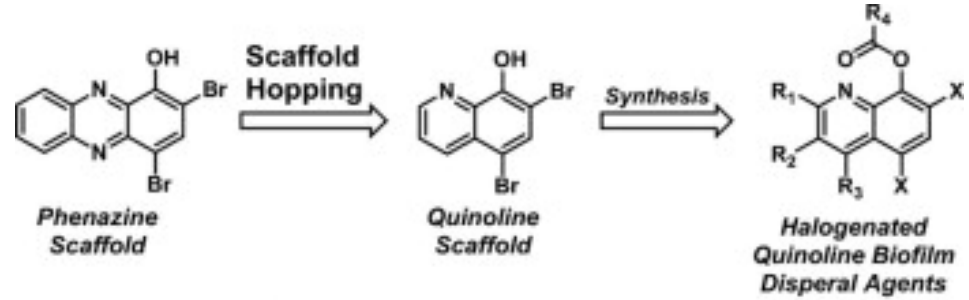
Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of quinoline small molecules with potent dispersal activity against methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms using a scaffold hopping strategy

Yasmeen Abouelhassan, Aaron T. Garrison, Gena M. Burch, Wilson Wong, Verrill M. Norwood IV, Robert W. Huigens III*

University of Florida, Department of Medicinal Chemistry, College of Pharmacy, 1600 SW Archer Rd., Gainesville, FL 32610, USA



RSC Advances

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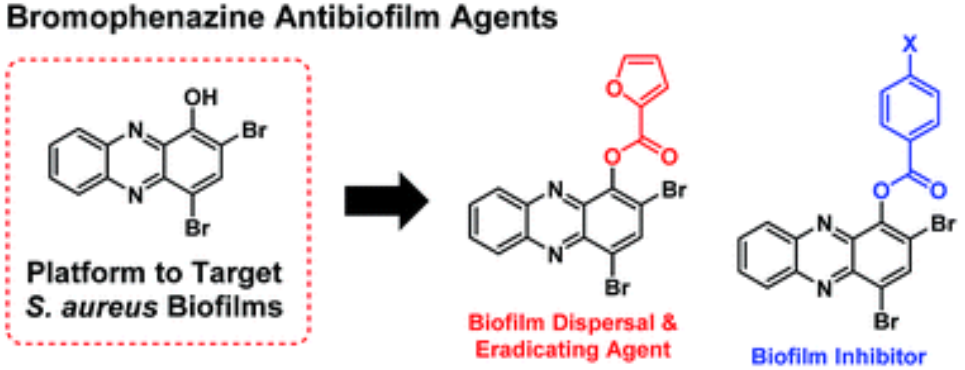
Cite this: *RSC Adv.*, 2015, 5, 1120

Received 15th August 2014
Accepted 25th November 2014

DOI: 10.1039/c4ra08728c

Bromophenazine derivatives with potent inhibition, dispersion and eradication activities against *Staphylococcus aureus* biofilms†

Aaron T. Garrison,‡[a] Fang Bai,‡[bc] Yasmeen Abouelhassan,[a] Nicholas G. Paciaroni,[a] Shouguang Jin[b] and Robert W. Huigens III*[a]



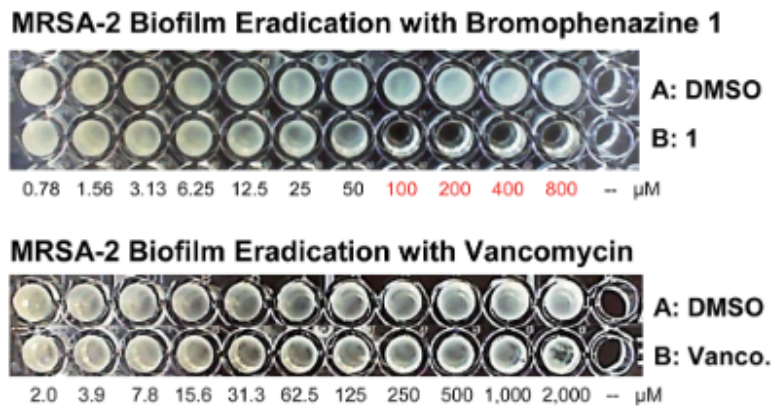


Fig. 3 Biofilm eradication of MRSA-2 with bromophenazine **1** (MBEC = 100–200 μM) and vancomycin (MBEC > 2000 μM).

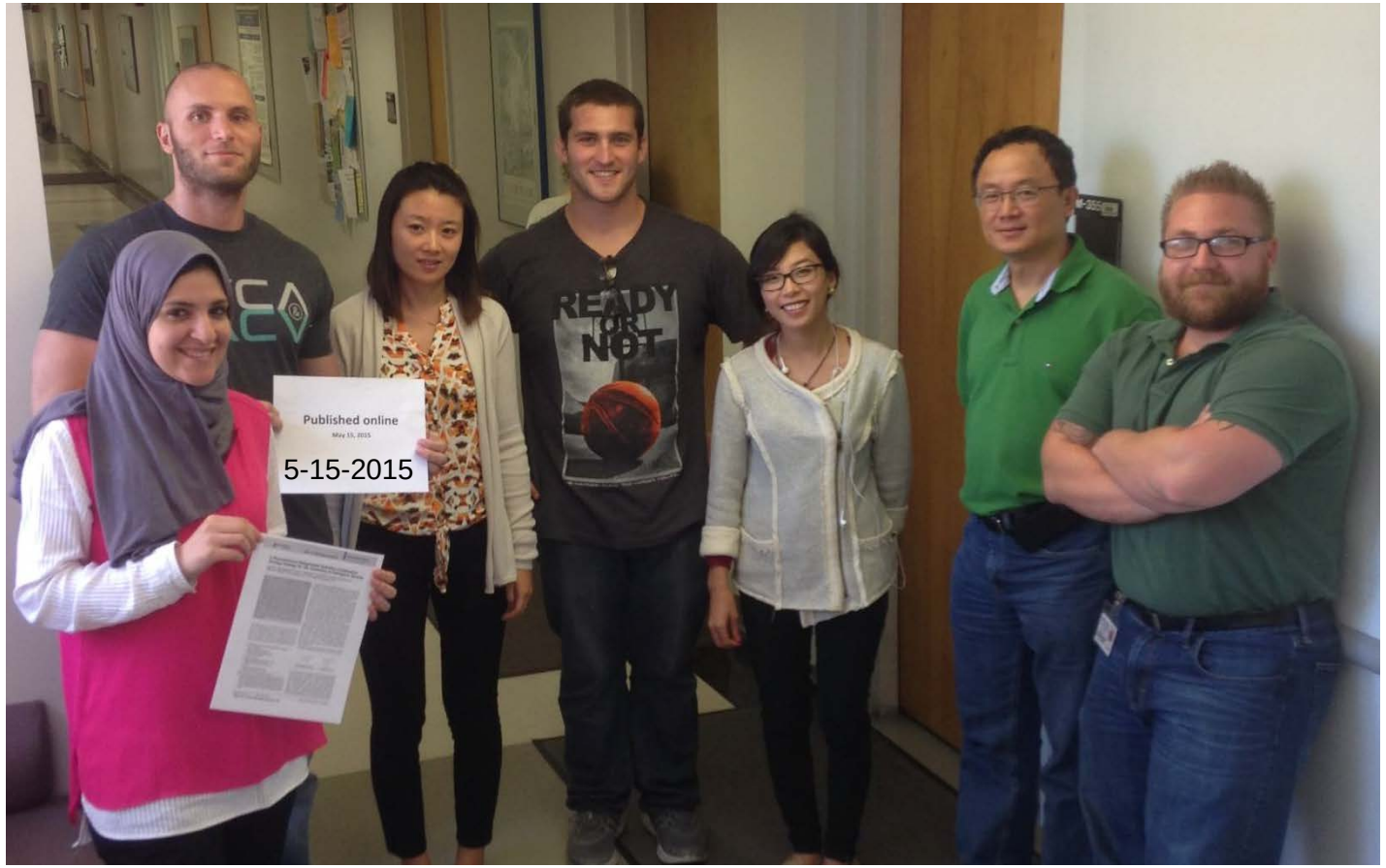
ChemPubSoc Europe

DOI: 10.1002/cmdc.201500179

CHEMMEDCHEM Communications

A Phytochemical–Halogenated Quinoline Combination Therapy Strategy for the Treatment of Pathogenic Bacteria

Yasmeen Abouelhassan,[a] Aaron T. Garrison,[a] Fang Bai,[b, c] Verrill M. Norwood IV,[a] Minh Thu Nguyen,[a] Shouguang Jin,[b] and Robert W. Huigens, III*[a]



Communications

A Phytochemical–Halogenated Quinoline Combination Therapy Strategy for the Treatment of Pathogenic Bacteria

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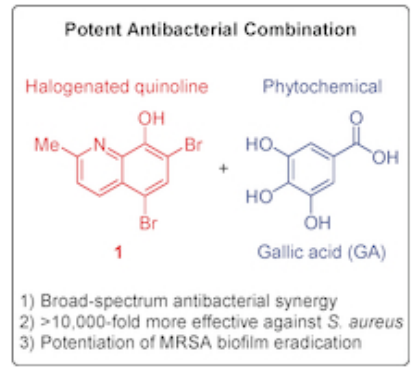
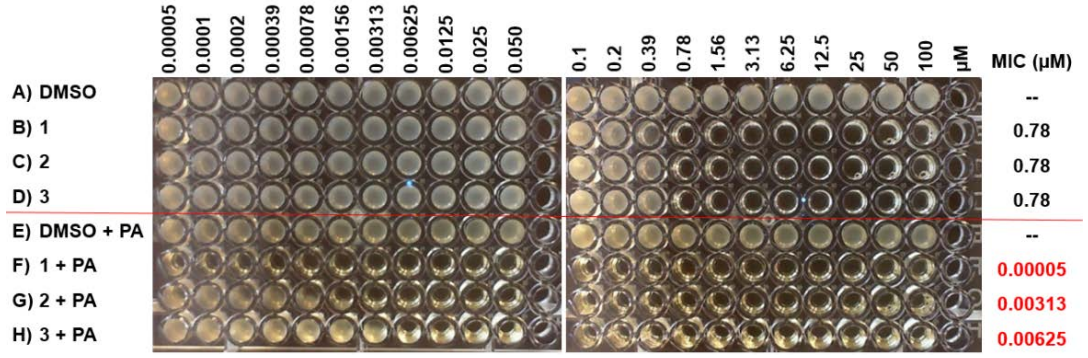
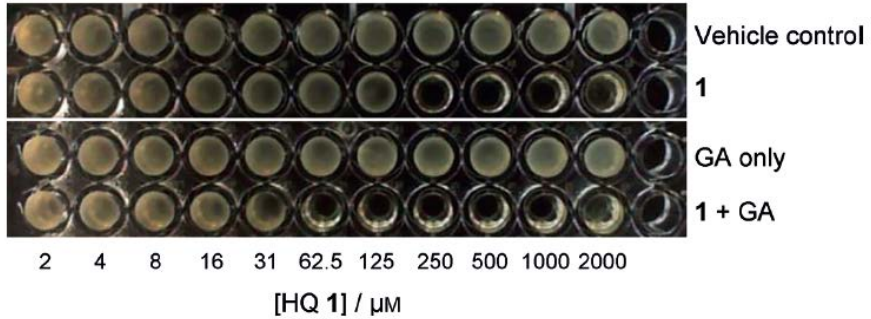


Quite the combo! Halogenated quinolines and select phytochemicals, in particular gallic acid (GA), possess unique antibacterial synergy against several pathogenic bacteria, including: *Staphylococcus aureus*, *S. epidermidis*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*. Here, GA is shown to potentiate the growth inhibitory properties of **1** (>10 000-fold) against *S. aureus* while also potentiating biofilm eradication activities against a methicillin-resistant *S. aureus* (MRSA) clinical isolate (fourfold).

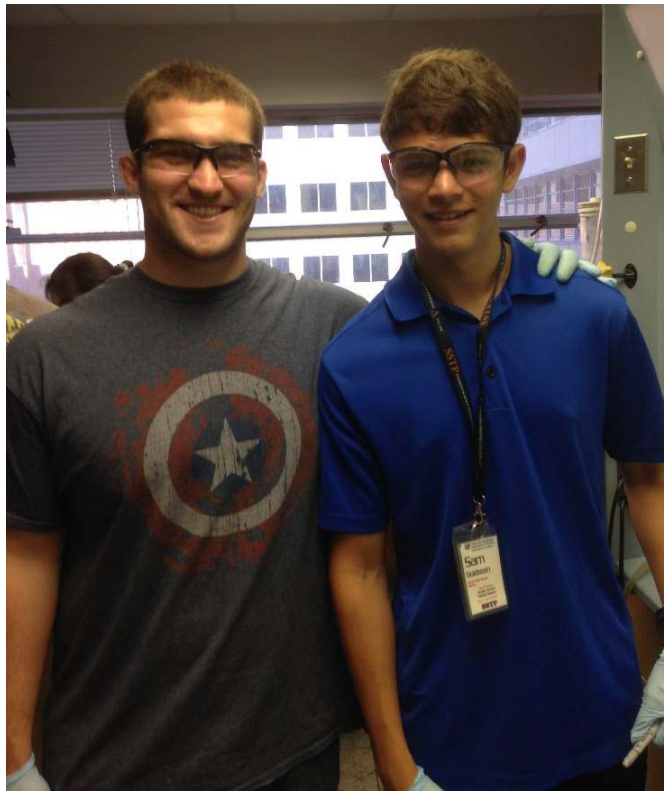
S. aureus (ATCC 29213) Antibacterial Potentiation Assay



Biofilm eradication assay with MRSA-2



Outreach: UF SSTP and Homeschool Students

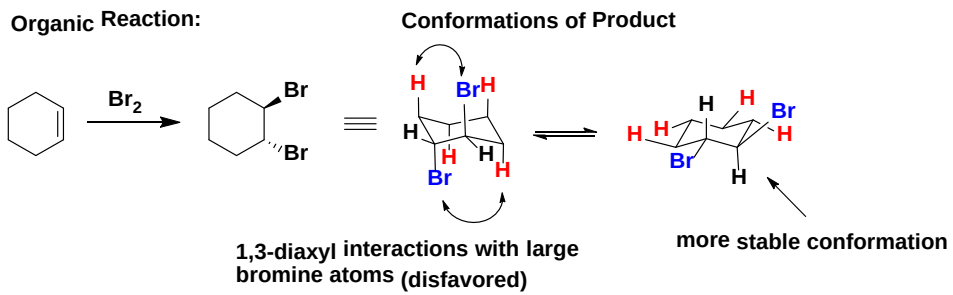


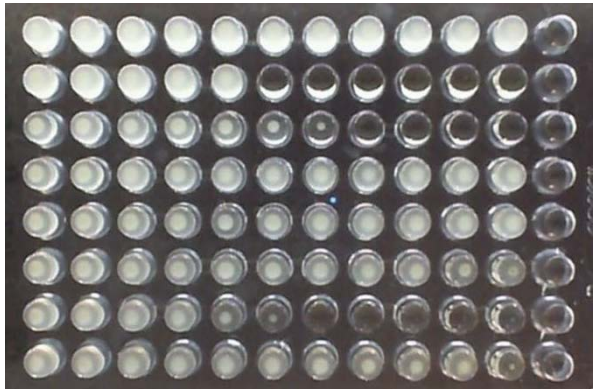
Graduate Student Mentor Chip Norwood (left) with Sam Goldstein (right) High School Researcher in the UF SSTP summer 2015

http://www.cpet.ufl.edu/students/sstp/

Charles Mock (left) former Homeschool High School Researcher and current Santa Fe College Student with Gradate Student Mentor Yasmeen Abouelhassan (right)

Sam is learning organic chemistry theory and practice in our group

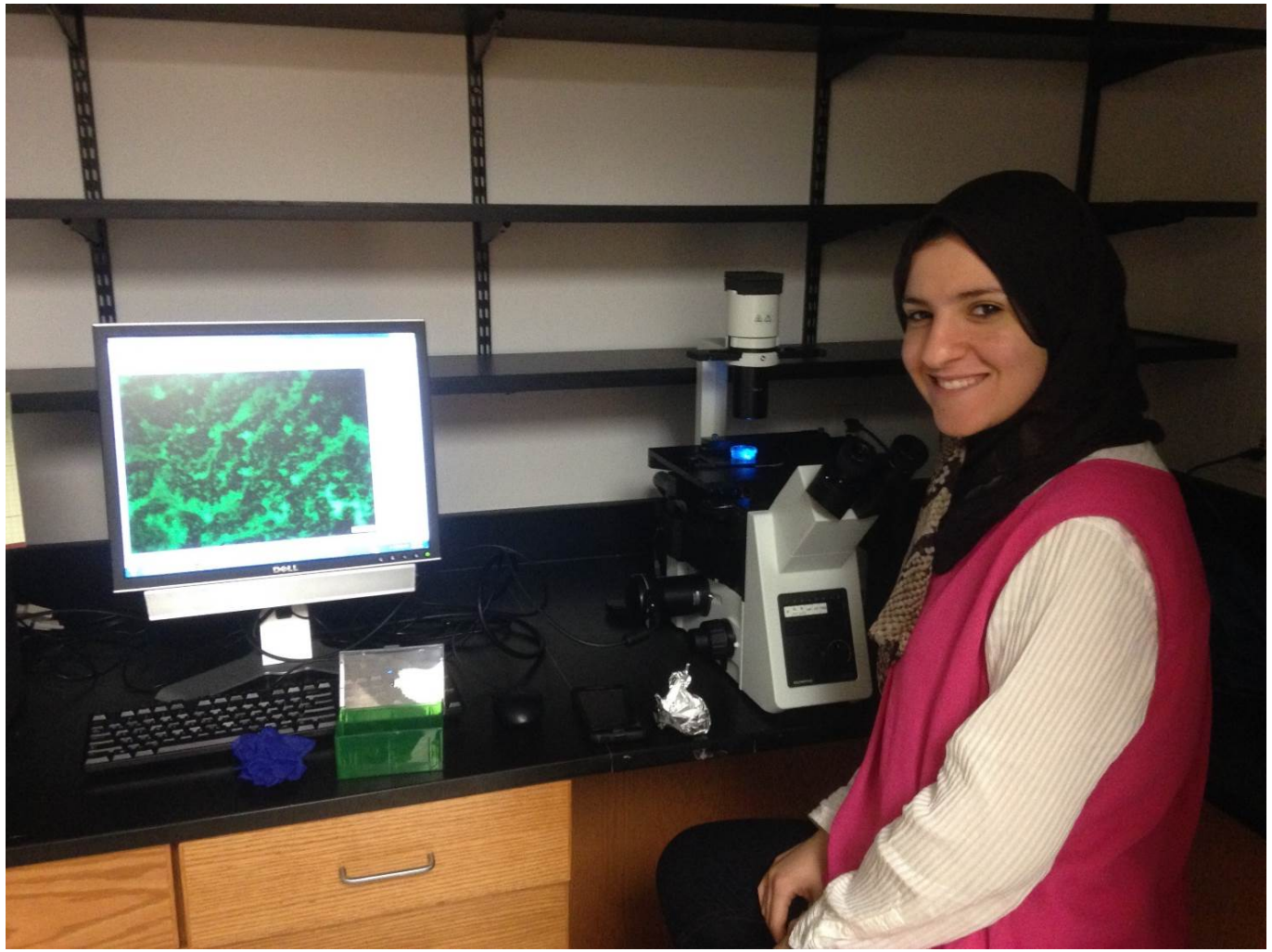


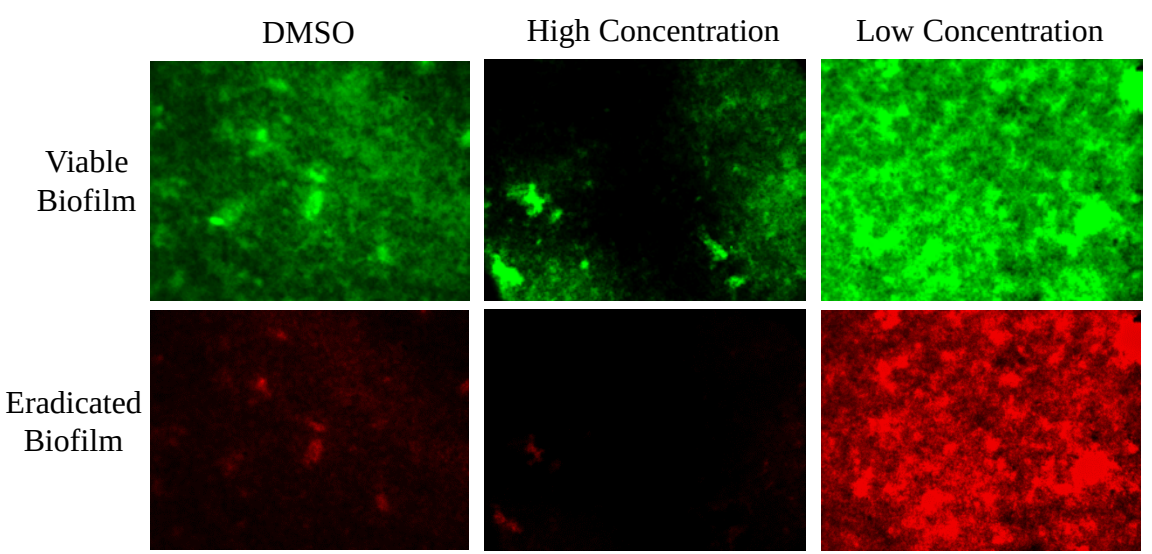


Charles is learning about the wonders of antibiotics and microbiology!

Fluorescence Microscope Images of Biofilms

(We thank the EPI and College of Pharmacy for providing additional funding for this microscope)





Yasmeen evaluating our new biofilm-eradicating small molecules.

Spreading the word! Huigens lab on the war path against bacteria!!

News & Updates

Dr. Huigens receives Young Investigator Award for Novel Biofilm-Eradicating Small Molecules

Robert W. Huigens III, Ph.D., an assistant professor of medicinal chemistry, was recently named one of two recipients of the Center for Biofilm Engineering Young Investigator Award. Dr. Huigens will be invited to present his research — titled Phenazine Antibiotic Inspired Discovery of Biofilm-Eradicating Small Molecules — at the semiannual Montana Biofilm Science and Technology Meeting in July. Awardees were chosen based on research quality and relevance of the material to the CBE's mission to advance fundamental interdisciplinary research and education related to the management of bacterial biofilms, and to transfer that knowledge to the realm of applied science and technology.

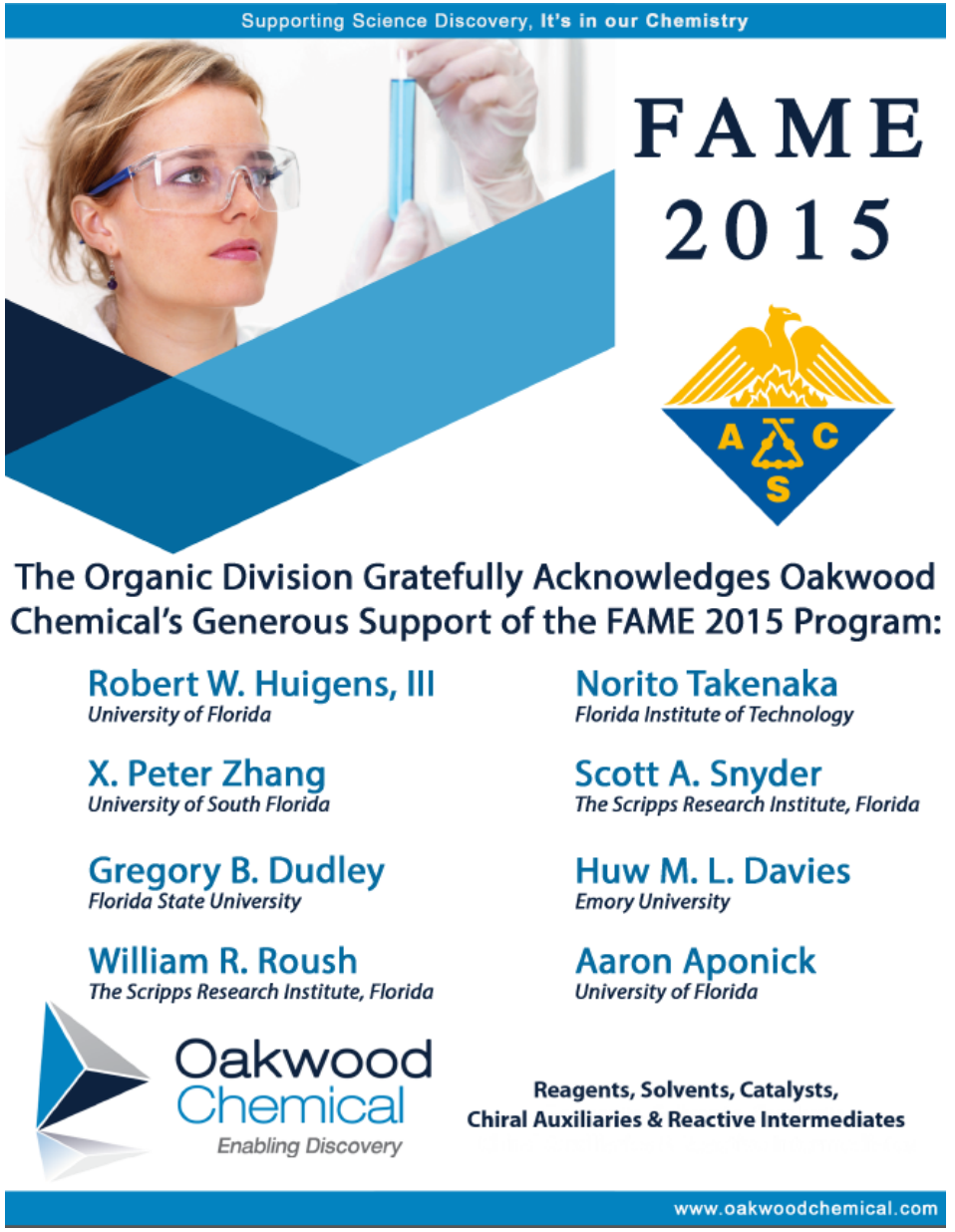
Dr. Huigens was also the recipient of a Young Investigator Award from the American Chemical Society (ACS; Division of Medicinal Chemistry), where he was invited to give a seminar on his group's novel biofilm-eradicating small molecules at the 249th national ACS meeting in Denver, Colorado, in March.

CBE Website: http://www.biofilm.montana.edu/resources/newsarchives/2015/18-03.html

Young Investigator Award Recipients

We are pleased to announce two awardees who will be presenting their biofilm research as Young Investigators at the July Montana Biofilm Meeting. **Robert Huigens**, assistant professor in medicinal chemistry at University of Florida, will present "Phenazine antibiotic inspired discovery of biofilm-eradicating small molecules." **Juan Pavissich**, postdoctoral researcher in biotechnology at Delft University of Technology in Delft, The Netherlands, will present "Coupling multi-scale in situ determination of biofilm mechanical properties to mathematical modeling of biofilm fluid-structure interaction."

The CBE launched the Young Investigator program in 2009 to encourage the participation of outstanding non-Montana State University biofilm investigators in our semi-annual Montana Biofilm Meetings. Targeting postdoctoral researchers and newly hired faculty, up to two investigators are invited to present research at each MBM and provided a $750 travel reimbursement and registration at the meeting.



Dr. Huigens gives invited seminar at FAME 2015 conference.

Professor Hergenrother Gives Seminar at UF!

January 2015

Rob Huigens, Paul Hergenrother, Nick Paciaroni, Aaron Garrison, Chip Norwood, Akash Basak (left to right)

Prof. Huigens Path to UF!

THE UNIVERSITY of NORTH CAROLINA GREENSBORO

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NC STATE UNIVERSITY

Ph.D. Chemistry
(with Christian Melander)

ILLINOIS

Postdoc Organic Chemistry
(with Paul Hergenrother)

UF | UNIVERSITY of FLORIDA

Assistant Professor
Medicinal Chemistry