Primary Research Interests

1) Discovery of phenazine antibiotic inspired bacterial biofilm-eradicating agents!

2) Ring distortion of indole alkaloids for drug discovery!


Yasmeen hard at work to kill pathogenic bacteria!

Zach preparing for columns!

Chip super zoned in on his NMR spectra (left)!

Our new 600 MHz NMR makes Chip smile, for sure!
Fen is all smiles in the lab!

Fen coaching Zach up on purification!

Daniel smiles before work...

...now he’s all business!
Chip & Will Action Photos!

Will (left & below) was part of our SSTP program for high school students at UF. In summer 2016, Chip taught Will about organic chemistry – theory and practice in the lab – for 7 weeks!

Dr. Huigens gives a seminar to the SSTP students each year on organic chemistry (reaction mechanisms), drug discovery and the importance of young people finding a passion for science.
Away Action Photos!

Let’s get excited about medicinal chemistry and drug discovery!

Tenure tour seminar at UIC Department of Medicinal Chemistry & Pharmacognosy.

Dr. Huigens with Dr. Nile (UNCG) at an ACS Conference in SF (4/2017). Dr. Nile was the Department chair of UNCG Chemistry and an outstanding mentor to Dr. Huigens during his undergraduate days.
Recent Progress Synthetizing New Halogenated Phenazine (HP) Biofilm-Eradicating Agents

A) Halogenated Phenazine (HP)
Biofilm Eradicators & Anti-tuberculosis Agents

- Phenylenediamine Condensation
  - No regioselectivity
  - Limited substrate availability

- Wohl-Aue Reaction
  - Low reaction yields
  - Several side products
  - Harsh conditions

B) Methoxyphenazine Intermediates

- Reductive Cyclization
- Cross-Coupling

- "Standard" Diarylamines
- "Inverted" Diarylamines

Permits complete regioselectivity at 6-, 7-, and 8-positions
Convergent Synthesis of Precursors to HP Biofilm-Eradicating Agents

Continued next slide

<table>
<thead>
<tr>
<th>Aniline</th>
<th>Diarylamine</th>
<th>1-Methoxyphenazine</th>
<th>Aniline</th>
<th>Diarylamine</th>
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<td>( \text{R} - \text{NH}_2 )</td>
<td>( \text{R} - \text{O}_2\text{N} )</td>
<td>( \text{R} - \text{N} - \text{N} - \text{O}_2\text{N} )</td>
<td>( \text{R} - \text{NH}_2 )</td>
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<td>10 (94%)</td>
<td>11 (71%)</td>
<td>20 (83%)</td>
<td>21 (60%)</td>
<td>22 (83%)</td>
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<td>31 (80%)</td>
<td>32 (83%)</td>
<td>33 (97%)</td>
<td>42 (71%)</td>
<td>43 (15%)</td>
<td>31 (71%)</td>
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<td>( \text{F}_3\text{C} )</td>
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<td>23 (38%)</td>
<td>44 (84%)</td>
<td>45 (64%)</td>
<td>46 (71%)</td>
<td>47 (53%)</td>
<td>48 (64%)</td>
</tr>
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Average Buchwald-Hartwig (BH) Cross-Coupling Yield: 70%
Average Reductive Cyclization (RC) Yield: 68%

For full details regarding this route, see: J. Med. Chem. 2018, 61, 3962–3983.
### End Game to Diverse HP Analogues for Biological Evaluation

**A)**

![Chemical Structures](Image1)

1. **1-OMe Phenazine**
2. **Series A**
   - 1) BB\(_3\)
   - 2) NBS
3. **Series B**
   - 1) NBS
   - 2) BB\(_3\)

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<th>R(^3)</th>
<th>R(^4)</th>
<th>X</th>
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<th>NBS Yield</th>
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<tr>
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<td>47(^\text{b})</td>
<td>H</td>
<td>NE(_2)</td>
<td>H</td>
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<td>Br</td>
<td>94%</td>
<td>53%</td>
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<td>H</td>
<td>H</td>
<td>Br</td>
<td>87%</td>
<td>73%</td>
<td>A</td>
<td>65</td>
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</tbody>
</table>

| 32              | H     | Me    | H     | H     | H  | 96%           | 88%       | B      | 66 |
| 33              | H     | Et    | H     | H     | H  | 92%           | 83%       | B      | 67 |
| 36              | Me    | H     | Me    | H     | H  | 82%           | 55%       | B      | 68 |
| 42              | H     | Cl    | H     | H     | H  | 90%           | 96%       | B      | 69 |

**Average Demethylation Yield:** 89%  **Average Bromination Yield:** 71%

**B)**

**Active HPs**
- **52**: MRSA MIC: 0.4 μM, MtB MIC: 6.25 μM
- **66**: MRSA MIC: 12.5 μM, MtB MIC: 25 μM
- **53**: MRSA MIC: 0.1 μM, MtB MIC: 6.25 μM
- **67**: MRSA MIC: 37.5 μM, MtB MIC: 25 μM
- **56**: MRSA MIC: 0.08 μM, MtB MIC: 50 μM
- **68**: MRSA MIC: 4.69 μM, MtB MIC: 50 μM
- **69**: MRSA MIC: 4.69 μM, MtB MIC: 6.25 μM

**MtB Analogues**
- **52**: MRSA MIC: 0.4 μM, MtB MIC: 6.25 μM
- **66**: MRSA MIC: 12.5 μM, MtB MIC: 25 μM
- **53**: MRSA MIC: 0.1 μM, MtB MIC: 6.25 μM
- **67**: MRSA MIC: 37.5 μM, MtB MIC: 25 μM
- **56**: MRSA MIC: 0.08 μM, MtB MIC: 50 μM
- **68**: MRSA MIC: 4.69 μM, MtB MIC: 50 μM
- **69**: MRSA MIC: 4.69 μM, MtB MIC: 6.25 μM

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**J. Med. Chem. 2018, 61, 3962–3983.**
A.) Calgary Biofilm Device (CBD) assay for MBC/MBEC determination of select HPs against MRSA BAA-1707. B.) Live/dead fluorescence imaging of MRSE biofilms following treatment with HP 61. C.) UV-Vis evaluation of QuAOCOM 86 stability in LB media. D.) MRSA BAA-1707 agar diffusion assay with HP 61 and HP QuAOCOM 87: a) DMSO, b) HP 61, c). HP QuAOCOM 87 (prodrug, structure not shown here; see publication for full details.)
Exciting New Findings With This Work! Stay Tuned!
Cancer Screening Results from Yohimbine Ring Distortion Library

Figure 2. A) Heat map matrix summarizing primary biological screening results of the yohimbine ring distortion library (biological activity scale key: red = inhibition; yellow = no change; green = activation), including the structures of six validated hit compounds. Rows correspond to compounds tested. Columns correspond to primary biological activity screens (1-10) and concentrations tested (200, 100, 10 and 1 µM). 1) Parental HCT116 and 2) HCT116\(^{\text{HIF-1\alpha-/-HIF-2\alpha-/-}}\) cell viability; 3) RAW264.7 cell viability; 4) RAW264.7 NO production; 5) LNCaP cell viability; 6) LNCaP cell ARE activity; 7) MDA-MB-231-ARE-luc cell viability; 8) MDA-MB-231-ARE-luc cell ARE activity; 9) S. aureus and 10) A. baumannii growth. B)−E) Validation experiments of hit compounds. B) HIF-dependent anticancer activity. Cell viability of parental HCT116 and HCT116\(^{\text{HIF-1\alpha-/-HIF-2\alpha-/-}}\) cells was determined after 48 h exposure using MTT assay (p-values: ≤ 0.01 **, ≤ 0.05 *; pairwise student t-test comparing cell viability between parental HCT116 and HCT116\(^{\text{HIF-1\alpha-/-HIF-2\alpha-/-}}\) cells at a given concentration). C) NO production and cell viability. Production of NO and cell viability of RAW264.7 cells were determined after 24 h exposure using Griess reagent and MTT assay, respectively (p-values: ≤ 0.01 **, ≤ 0.05 *; pairwise student t-test comparing relative NO production and viability of RAW264.7 cells at a given concentration). D) MDA-MB-231-ARE-luc cell ARE activity and E) LNCaP cell ARE activity. ARE inhibition in MDA-MB-231-ARE-luc and ARE activation in LNCaP cells after 24 h exposure were validated by effects on endogenous NQO1 transcript levels measured by RT-qPCR. ACTB expression was used as an internal control for normalization. All validation data are presented relative to vehicle control (0.5% DMSO). Brusatol (125 nM) and sulforaphane (10 µM) served as positive controls for MDA-MB-231-ARE-luc ARE inhibition and LNCaP ARE activation assays, respectively.

Current Members

Ongoing Project Highlights
Yasmeen Abouelhassan (5th year grad student)

Senior Division Winner for Her Oral Presentation (UF COP Research Showcase)

Poster Presentation Award (UF Drug Discovery Symposium)

Yasmeen has been critical to establishing our antibacterial discovery program.

Currently, Yasmeen is using RNA-seq technology to elucidate the mode of action for our biofilm-eradicating agents.

Chemical Biology Approach to Study Novel Antibacterials & Bacterial Biofilms
Chip Norwood (4th year grad student)

Chip sharing his incredible work at our most recent COP Research Showcase.

Chip has been a warrior in the lab and we cannot wait to share his exciting work with the world!

Keep crankin', Chip!

Drug Discovery Pipeline

Optimization of New Lead
Hongfen Yang (3rd year grad student)

Fen is working to develop modular synthetic approaches to explore new halogenated phenazine molecules, including new prodrugs.

Fen recently published some of her exciting work in *Scientific Reports!*

**Synthetically Tunable Biofilm-Killing Scaffold**

![Chemical structure](image)

Modular Synthetic Approaches $\leftrightarrow$ Novel Prodrug Development
Zach Johnson just completed his first year of pharmacy school here at UF. This summer, Zach worked with Fen to synthesize novel anticancer agents for an NIH funded project we are part of. Keep crankin’ Zach!

Daniel Garcia is an undergraduate student from Florida Atlantic University. Daniel joined our UF SURF summer program to gain research experience in organic synthesis. Daniel has worked with Chip this summer to advance our ring distortion chemistry. Keep crankin’ Daniel!
Huigens Lab
PhD Graduates
Hall of Fame!
Dr. Aaron Garrison – Congrats on your PhD!!

Dr. Garrison is an aggressive experimentalist in the lab and continuously put new ideas into play during his graduate work!


Dr. Akash Basak – Congrats on your PhD!!

Dr. Basak worked extremely hard on multiple projects to finish great publications and cover art selections!


Both covers developed by Dr. Basak
Dr. Nicholas Paciaroni – Congrats on your PhD!!

Dr. Paciaroni crushing his research talk at the second annual UF Drug Discovery Symposium.

Best student research talk award.

Dr. Paciaroni unleashed a Herculean effort during his graduate studies and I am extremely proud of his work!

Hot Articles from Our Lab (Selected By Publishing Journals)

Progress towards novel biofilm-eradicating agents!

UF College of Pharmacy News About Our Work!

College of Pharmacy researchers develop novel ring distortion strategy to fight diseases

Published: February 1st, 2017

ChemBioChem journal features UF medicinal chemistry researchers as cover art

Published: February 23rd, 2017

Medicinal chemist Dr. Rob Huigens named 2016–17 College of Pharmacy Teacher of the Year

Published: January 25th, 2017
Researchers find novel compounds kill biofilms, may eliminate persistent bacterial infections

Published: Nov 3rd, 2015

Fine-tuning chemistry to destroy bacterial biofilms

Published: June 10th, 2016

Watch the YouTube Video here: https://www.youtube.com/watch?v=rHQq84OTbqs

Yasmeen Abouelhassan receives award from Association for Academic Women

Yasmeen Abouelhassan, a Ph.D. student in the department of medicinal chemistry, is the recipient of the Emerging STEM Scholar Award from UF’s Association for Academic Women.
Biofilm-killing pumpkins have been seen in the Gainesville area.

*In silico* hit compounds from our ring distortion library ready to be validated in biochemical and functional assays with new collaborators!

*Keep it coming!*
Lab outing to see *Rogue One* (Dec, 2016)

Group outing before Drs. Garrison and Basak leave to begin postdocs! (Dec, 2017)