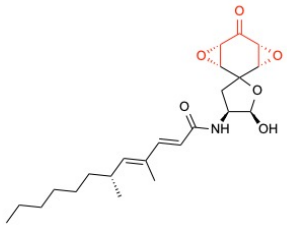
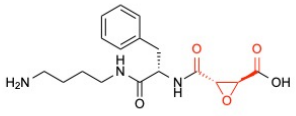
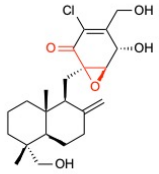
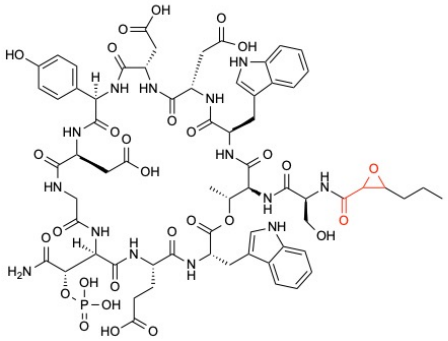
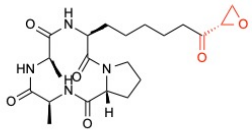
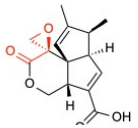
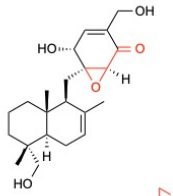
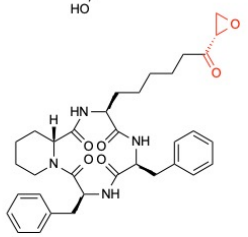
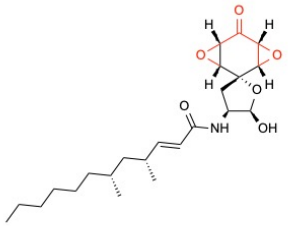
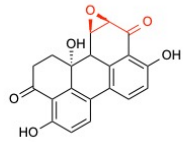
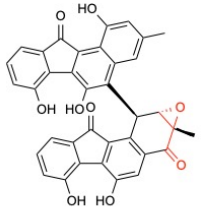
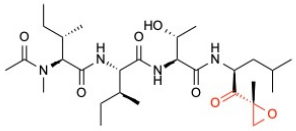
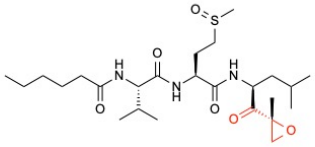
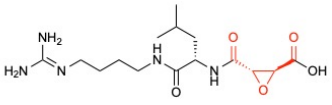
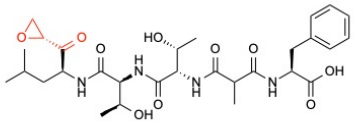
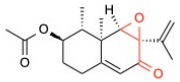
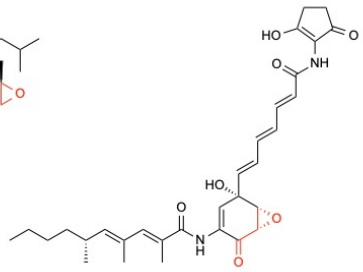










Remote Work Recap Redux: Epoxyketones

Theodore Berger

Gerwick Lab

Summer 2022

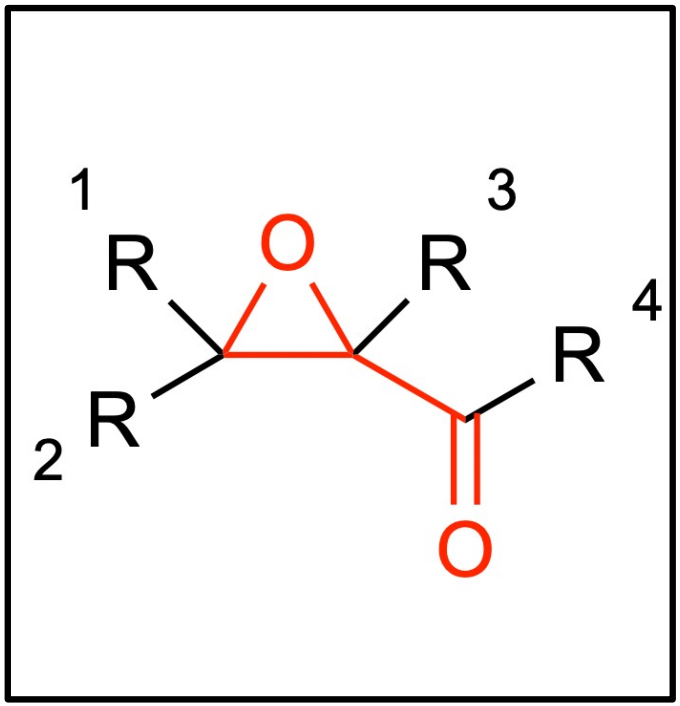









Agenda

- Objective
- Methodology
- Compound Example
- Notable Finds
- Mistaken Thoughts
- Takeaways and Summary
- In-Person Tasks
- Next Steps

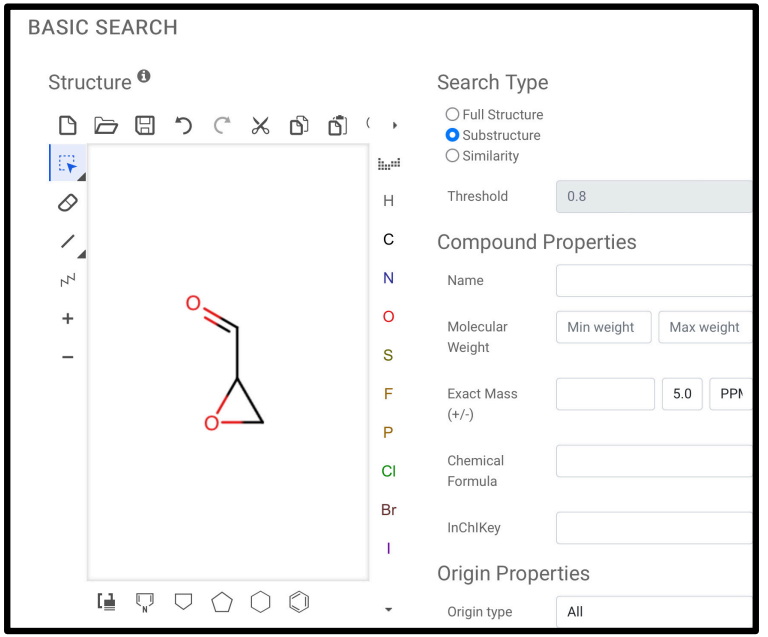
Objective

- Establish a comprehensive resource on epoxyketone-containing compounds and their biological activities
- Data Collected:
  - Compound Name
  - SMILES String, InChI and PubChem ID
  - Natural Product? (Y/N) and Source
  - Publication DOI(s)
  - Biological Activity? (Y/N)
    - If Y:
      - Type of Activity (Cytotoxic, antibacterial, etc.)
      - Mechanism of Action
      - Protein Target

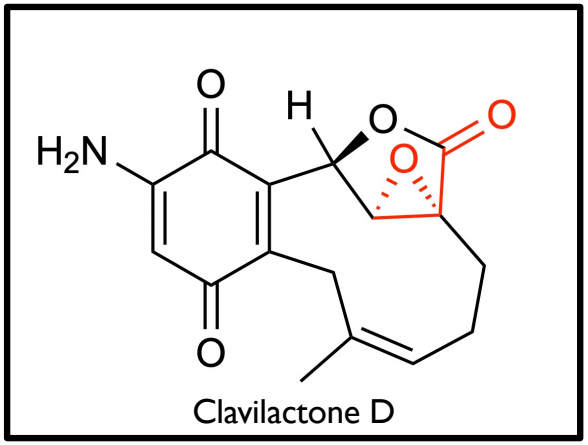


Methodology

- Initial search using keywords "epoxyketone" and "natural product" in Google Scholar
- Conducted basic search on NP Atlas using epoxyketone SMILES and "substructure" option:
  - Search returned 544 compounds
  - Read primary DOI looking for biological activity
    - If there is:
      - Use NP Atlas SMILES to search Sci-Finder for compound profile
      - Populate spreadsheet with SMILES, InChI, and name from Sci-Finder + Primary DOI from NP Atlas
      - Use references linked to Sci-Finder compound profile to populate other activities, mechanism(s) of action, and protein target(s) if information is available
    - If not mentioned:
      - See if references linked to Sci-Finder compound profile mention activity
    - If compound has been tested and there isn't:
      - It is placed in separate sheet with name and primary DOI
    - If compound has not been tested:
      - It is placed in the same spreadsheet with a yellow highlighted box to denote its untested state
  - Review articles linked for compounds with known activity, but paper does not mention biological application



Compound Example

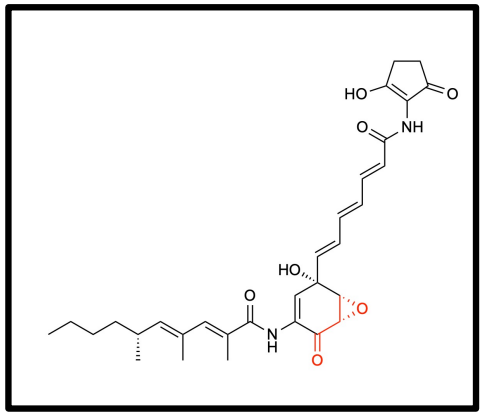


Clavilactone D

SMILES String	O=C1[C@@]23[C@H](O2)[C@](O1)(C4=C(C(=O)C=C(N)C4=O)C/C(/C)=C\CC3)[H]
InChI	InChI=1S/C16H15NO5/c1-7-3-2-4-16-14(22-16)13(21-15(16)20)11-8(5-7)10(18)6-9(17)12(11)19/h3,6,13-14H,2,4-5,17H2,1H3/b7-3-/t13-,14-,16-/m1/s1
PubChem ID	138977652
Natural Product or Synthetic?	Natural
Source of NP (if applicable)	Clitocybe clavipes
DOI for PRIMARY literature	10.1016/S0031-9422(99)00506-3
Additional relevant citations (1)	10.1016/S0006-2952(00)00278-1
Biological Activity (Y/N)?	Y
Type of Biological Activity 1	Antiproliferative (Antitumor)
Known MoA? (1)	Inhibition of cell growth
Known Protein Target? (1)	Receptor Tyrosine Kinases (Ret/ptc1, EGF-R, v-Abl) & Receptor Serine/Threonine Kinase p34cdc2

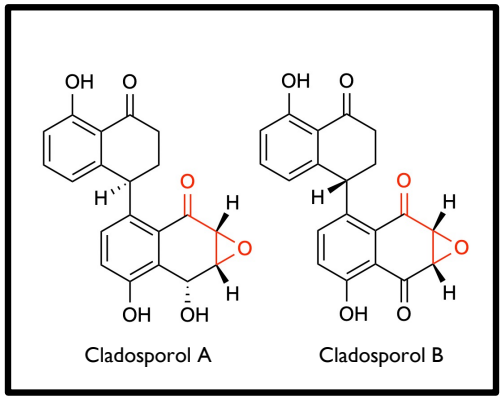
Notable Finds

- Manumycin A was effective against gram-negative bacteria (*Anaplasma phagoctyophilum)*
  - Prevention of ERK pathway activation upon infection – a pathway also involved in biogenesis and excretion of exosomes in some cancers (prostate)



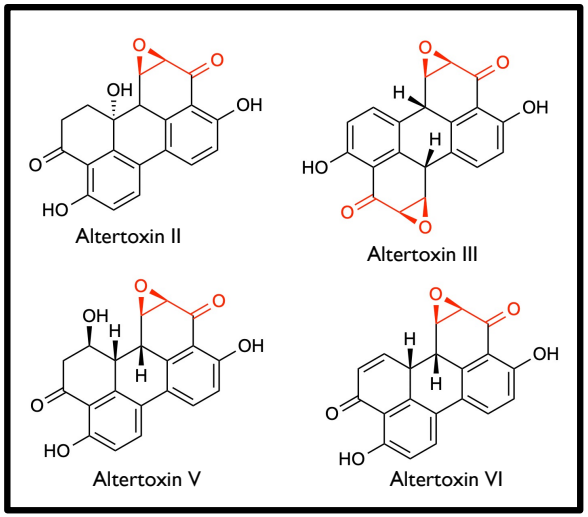
1. 10.1099/jmm.0.029231-0
2. 10.1016/j.canlet.2017.08.020

- Cladosporol A and B may have potential weight loss and antineoplastic applications
  - Agonists of PPARγ that inhibited adipogenesis and had antiproliferative effects against HT-29 cells through modulation of adiponectin and leptin



3. 10.1016/j.bbagen.2021.129973

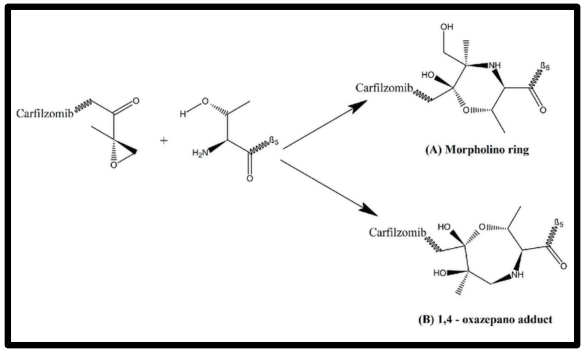
- Altertoxin II, III, V, and VI exhibited antiviral activity against HIV by inhibiting reverse transcriptase



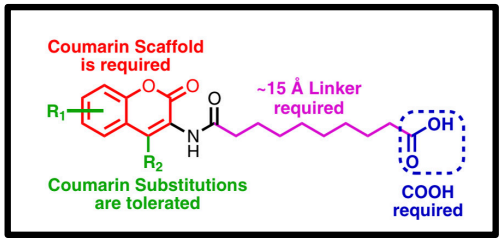
4. 10.1016/j.bmc.2014.08.039

Mistaken Thoughts (Part 1)

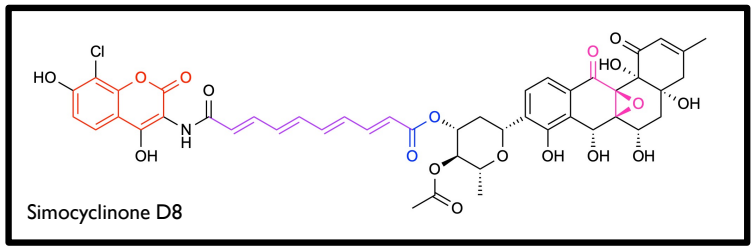
- For compounds populating the spreadsheet, epoxyketone was almost always part of the pharmacophore in compounds with known structure-activity relationships
  - Irreversible inhibitor due alkylation of target via epoxide ring opening (e.g. carfilzomib binding to catalytic threonine in beta-5 subunit of 20S proteasome)
- Exception mentioned in paper defining minimum pharmacophore for simocyclinone D8 inhibition of DNA gyrase does not include epoxyketone



7. 10.3389/fonc.2021.740796

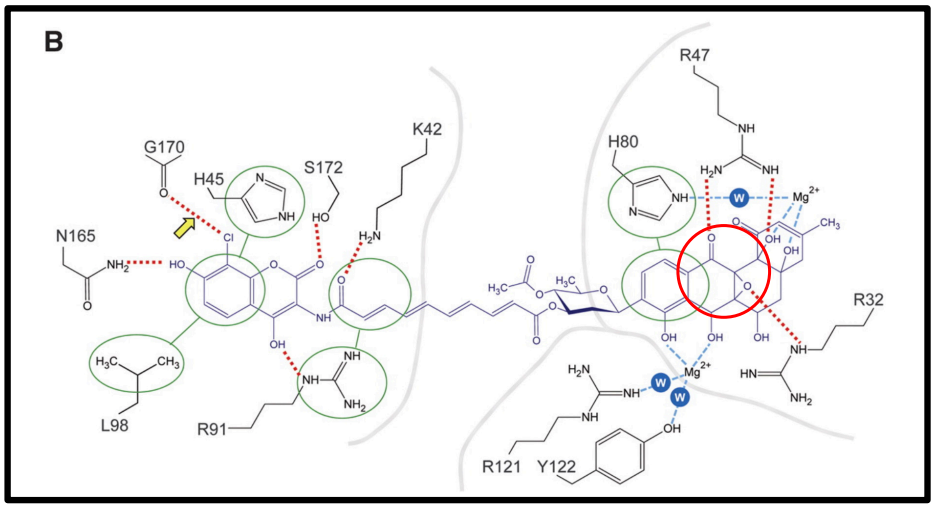


6. 10.1007/s00044-014-0942-z



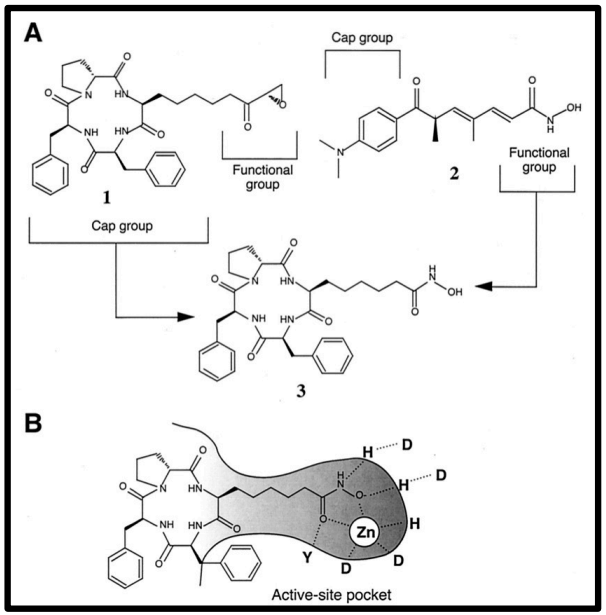
Mistaken Thoughts (Part II)

- Study of simocyclinone D8 bound to DNA gyrase A supports epoxyketone being necessary for binding and inhibition



8. 10.1126/science.1179123

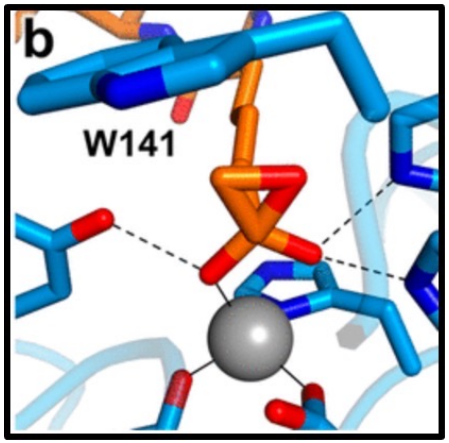
- Synthesis of trapoxin A analog that binds reversibly to histone deacetylase provides possible reasoning for this discrepancy



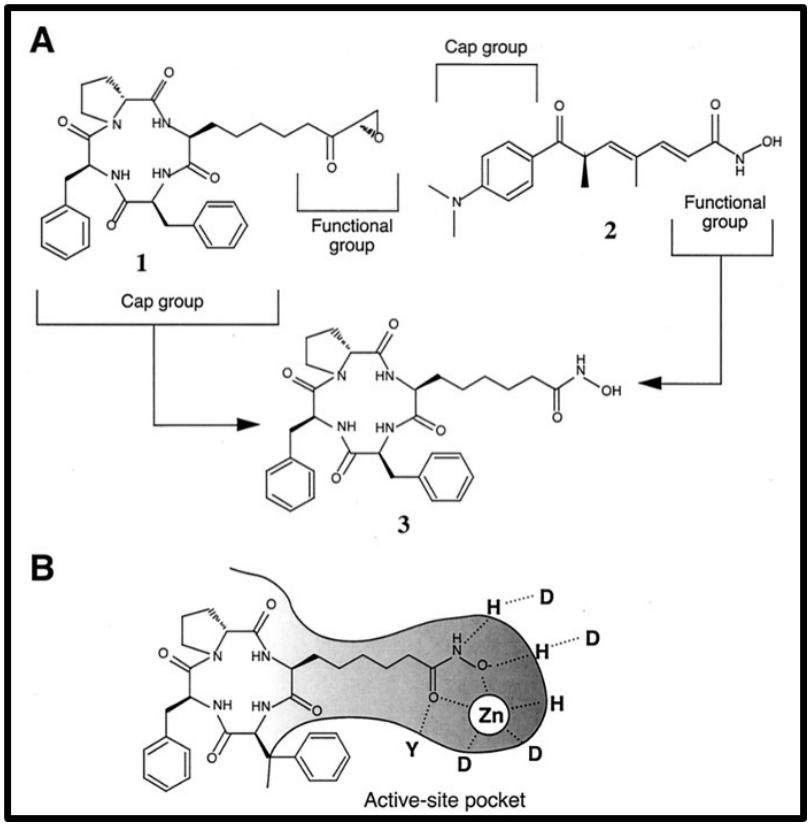
9. 10.1073/pnas.98.1.87

Mistaken Thoughts (Part III)

- Ketone moiety of trapoxin A's epoxyketone undergoes nucleophilic attack by water to form a geminal diol(ate)



10. 10.1021/acschembio.7b00330



Takeaways and Summary

- Assessing compounds for biological activity is completely different from researching their mechanisms and protein targets
- Structure-activity relationship studies are essential for pinpointing mechanisms of action and protein targets
  - Molecular docking could provide starting points to begin these efforts to elucidate these mechanisms and targets
- Medicinal chemistry and optimizing natural products is integral to applying these compounds for human use
- Chemical structures may be infatuating but take a logical step back before forming any conclusions!
- As of August 29:
  - 314 biologically active compounds
  - 102 biologically inactive compounds/activity not noted in the literature

In-Person Tasks and Next Steps

- This summer has allowed me to become more accustomed to my in-person lab work
- I have refined skills I learned during the spring and learned new tasks essential for our lab work:
  - Making media
  - Using the lyophilizer and autoclave

Next Steps:

- Continue to populate the spreadsheet
- Work with Jehad to build up my organic chemistry skillset
- Learn as much as I can

Thank You

- Nicole and Jehad
- Dr. Gerwicks
- The Gerwick Lab

Citations

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