VANDERBILT UNIVERSITY

Molecular Design and Synthesis Center (MDSC) in Support of Chemical Biology

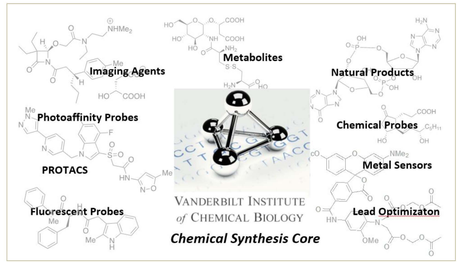
VANDERBILT INSTITUTE *of* CHEMICAL BIOLOGY

ABSTRACT

The Molecular Design and Synthesis Center (MDSC) at Vanderbilt Institute of Chemical Biology plays a crucial role in supporting organic and medicinal chemistry needs. Its mission is to provide high-quality chemical synthesis and compound analysis for biomedical researchers, including lead development, functional probe design, large-scale synthesis, and quality control. The MDSC collaborates with investigators from Vanderbilt, VUMC, and external institutions, contributing to research, consulting, and educational activities.

MDSC CAPABILITIES

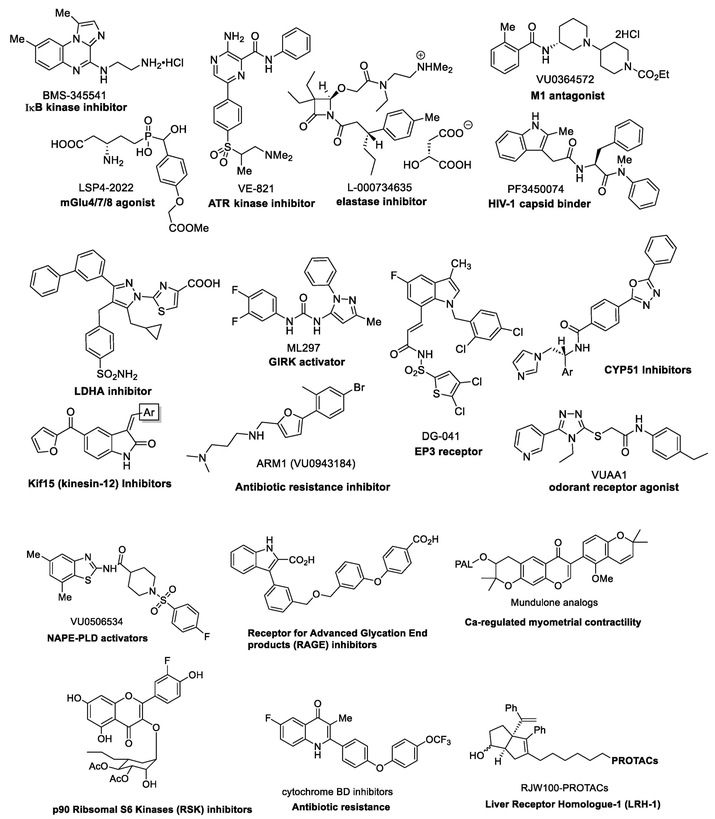
- ❑ Custom synthesis of compounds
- ❑ Synthesis and design of small molecular probes
- ❑ Medicinal chemistry (improve potency, PK)
- ❑ Synthesis of biotinylated/fluorescently labeled molecules
- ❑ Large scale synthesis (up to 100g)
- ❑ Dye Synthesis
- ❑ Nucleotide/Peptide synthesis
- ❑ Compound libraries
- ❑ GMP synthesis
- ❑ DMPK and Formulation study
- ❑ HPLC purification
- ❑ Analytical support (NMR, MS)



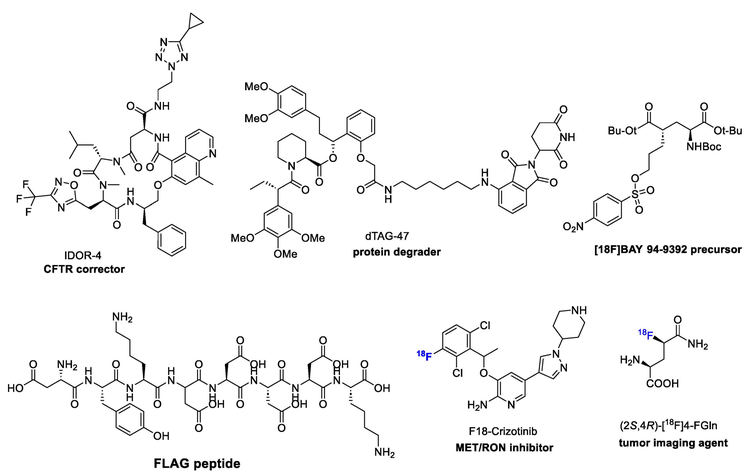
EQUIPMENT

- ❑ A technology-driven approach significantly accelerates compound synthesis using advanced equipment such as the **Biotage microwave reactor, benchtop large-scale reactor**, and **large-scale rotary evaporator**
- ❑ LC/MS equipment provides detailed reaction and purity monitoring (**Agilent 1260 &1200 system**)
- ❑ **ISCO** automated normal/reverse phase column purification systems yield high purity products and intermediates.
- ❑ **GILSON HPLC** equipment enables reverse phase purification and chiral separations on a range of scales, from milligrams to grams.
- ❑ Small Molecule **NMR** (Bruker 400 MHz, MRB4 12th floor)
- ❑ Compound Registration and Storage (**Chemcart** registration, DeltaSoft)

CHEMICAL PROBES IN BIOMEDICAL RESEARCH

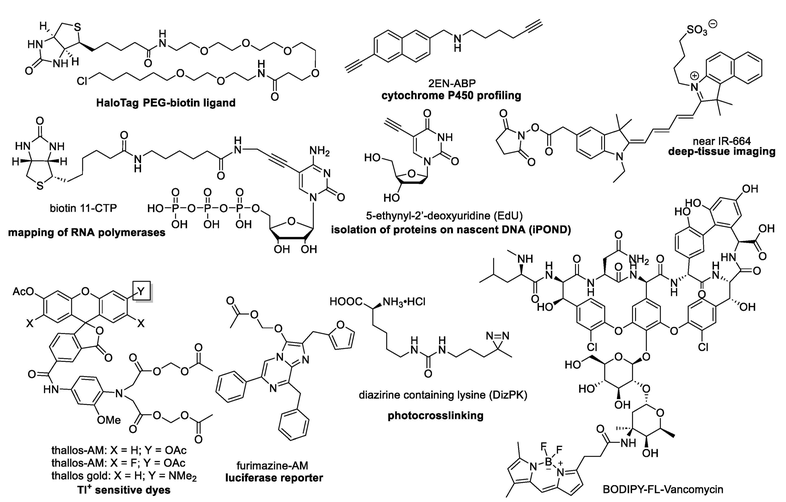


PEPTIDES and PROTACs and PET IMAGING AGENTS

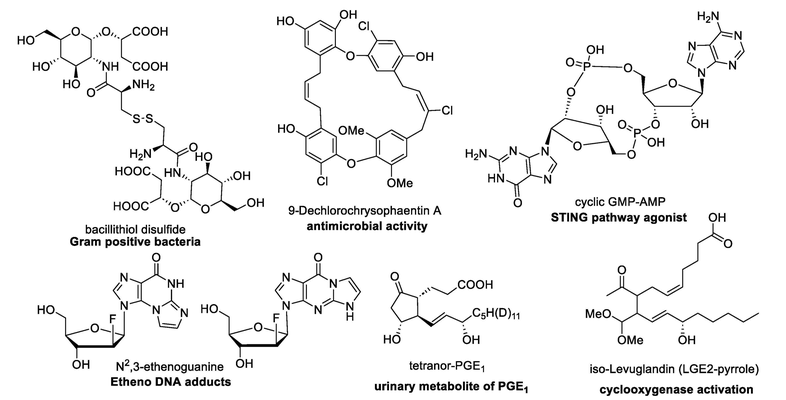


ACS Chemical Biology **2021**, *16*, 787-793

FLUORESCENT AND AFFINITY PROBES



RARE NATURAL PRODUCTS AND METABOLITES



SERVICE RATES

DESCRIPTION	BASE PRICE	EXTERNAL NON-PROFIT	EXTERNAL FOR-PROFIT
NMR	$116.31/hour	$168.07	$244.47
HPLC	$116.31/hour	$168.07	$244.47
LCMS	$116.31/hour	$168.07	$244.47
ISCO	$116.31/hour	$168.07	$244.47
Synthesis Service	$133.55/hour	$188.97	$274.87

Visit Molecular Design and Synthesis Center (MDSC) website:

https://medschool.vanderbilt.edu/syncore/

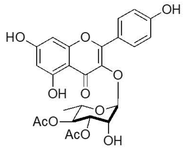
Optimization Studies of a Kaempferol Glycoside Chemotype as Selective RSK2 Inhibitor

Zach Austin[1], Eric B. Wright[4], Somnath Jana[5], Ian Romaine[5], Alex Allweil[1], Kwangho Kim[5], Gary A. Sulikowski[1-3], Alex Waterson[1-3], Deborah Lannigan[3-4]

[1]Department of Chemistry, Vanderbilt University, Nashville, TN, USA. [2]Department of Pharmacology, Vanderbilt University, Nashville, TN, USA. [3]Vanderbilt Institute of Chemical Biology, Vanderbilt University, Nashville, TN, USA.
[4]Department of Pathology, Microbiology, and Immunology, Vanderbilt University, Nashville, TN, USA. [5]Molecular Design & Synthesis Center, Vanderbilt University, Nashville, TN, USA. [5]

ABSTRACT

p90 Ribsomal S6 Kinases (RSK), a family of Ser/Thr kinases, have been linked to various cancers and are thought to be a suitable target for therapeutic discovery. In a high-throughput screening effort, Lannigan and co-workers[1] discovered a selective RSK2 inhibitor identified from extracts of the South American plant *Forsteronia refracta*. The RSK2 selective inhibitor was identified as a kaempferol glycoside and given the name SL0101. Since the discovery of SL0101's activity, a number of analogues have been synthesized in efforts to optimize activity. Following the initial synthetic efforts of the O'Doherty group, attention has turned toward addressing the unfavorable DMPK properties to generate an improved compound for *in vivo* studies.



SLO101

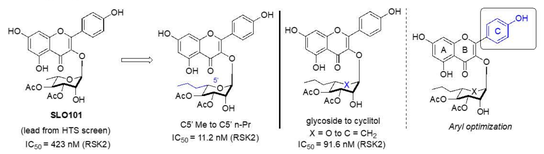
(lead from HTS screen)

IC_{50} = 89 nM (RSK2)

1. Smith, J.A., Poteet-Smith, C.E., Xu, Y., Errington, T.M., Hecht, S.M. and Lannigan, D.A. (2005) Identification of the first specific inhibitor of p90 ribosomal S6 kinase (RSK) reveals an unexpected role for RSK in cancer cell proliferation. Cancer Res.65: 1027-1034

BACKGROUND

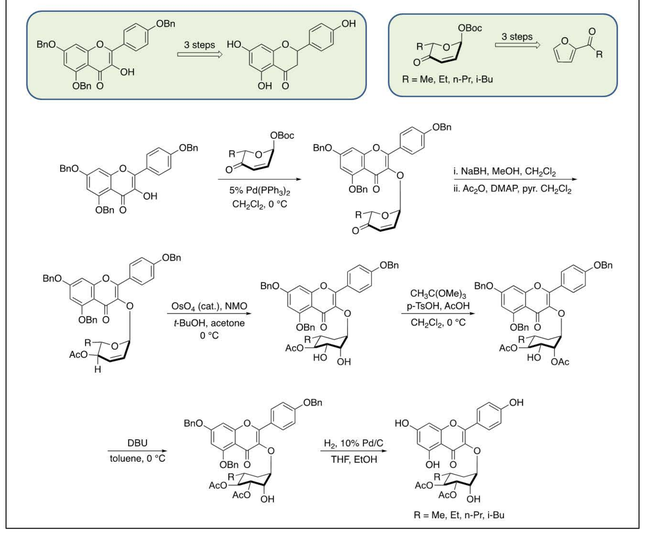
- p90 Ribsomal S6 Kinases (RSK) represents a family of Ser/Thr kinases implicated in various cancers, indicating they could serve as viable targets for drug discovery[1].
- SL0101, extracted from *Forsteronia refracta,* demonstrated potent, selective inhibition of RSK2 in a dual high-throughput screen.
- O'Doherty and co-workers synthesized numerous analogues of the lead compound (SL0101). Early SAR studies determined several functionalities integral to potency: (1) Replacing the C5' methyl group with an n-propyl group increased potency[2]; (2) Replacing the O-glycoside (X = O bond with a cyclitol (X = CH_2) maintained potency and increased metabolic stability; (3) Preserving the C-4'-hydroxy group proved integral to RSK inhibition.



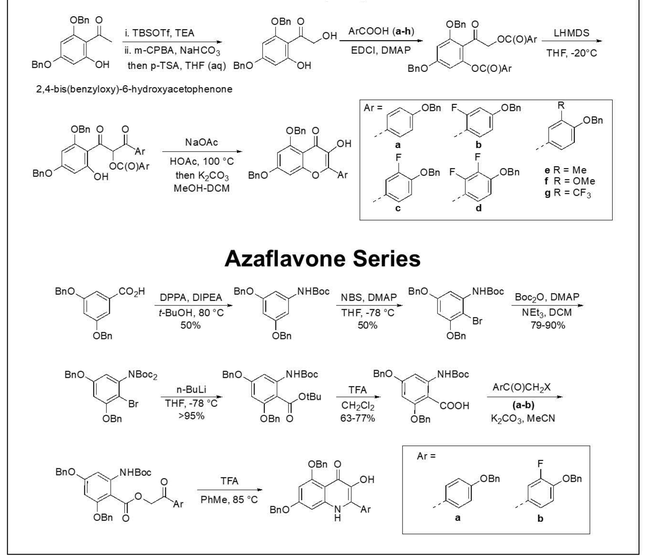
1. Ludwik, K. A.; Campbell, J. P.; Li, M.; Li, L.; Sandusky, Z. M.; Pasic, L.; Sowder, M. E.; Brenin, D. R.; Pietenpol, J. A.; O'Doherty, G. A.; Lannigan, D. A. Development of a RSK Inhibitor as a Novel Therapy for Triple Negative Breast Cancer. Mol. Cancer Ther. 2016, 15, 2598–2608.
2. Li, M.; Li, Y.; Ludwik, K. A.; Sandusky, Z. M.; Lannigan, D. A.; O'Doherty, G. A. Stereoselective Synthesis and Evaluation of C6''-Substituted 5a-Carbasugar Analogues of SL0101 as Inhibitors of RSK1/2. Org. Lett. 2017, 19, 2410–2413

O'DOHERTY & 2nd GENERATION (VU) CHEMICAL SYNTHESIS

SL0101 & Analogues (O'DOHERTY SYNTHESIS)



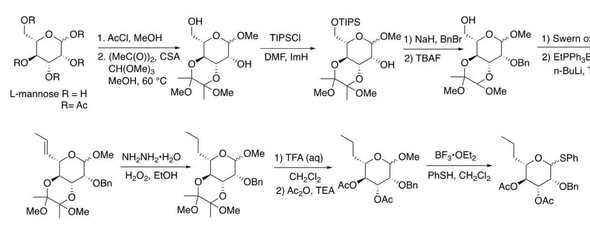
2nd GENERATION (VU) SYNTHESIS



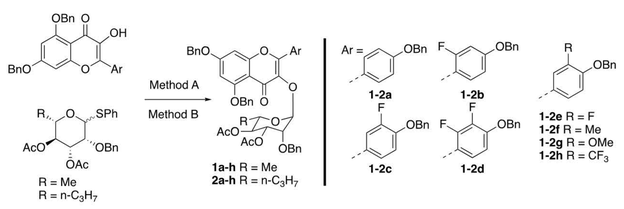
Azaflavone Series

GLYCOSYLATION METHOD (VU) & ACTIVITY / DMPK DATA

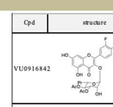
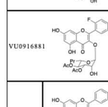
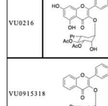
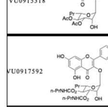
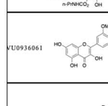
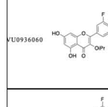
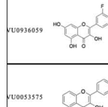
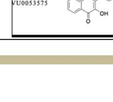
2nd GENERATION (VU) SYNTHESIS



General Glycosylation Methods



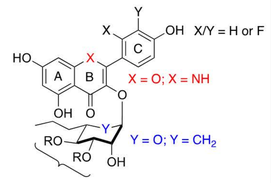
Method A: Conversion of thioether to bromide with Br_2 then glycosylation with Ag_2O
Method B: Glycosylation with NIS and TfOH

Cpd	structure	RSK2 IC50 (nM)	Mouse, HBF = 90 CL_{in}	Mouse, HBF = 90 Pred CL_{hep}	Rat, HBF = 55 CL_{in}	Rat, HBF = 55 Pred CL_{hep}	Human, HBF = 21 CL_{in}	Human, HBF = 21 Pred CL_{hep}
VU0916842		66	>5376.72	>88.52	634.23	63.04	219.99	19.17
VU0916881		11	>5376.72	>88.52	1199.59	66.14	201.44	19.02
VU0216		92	>5376.72	>88.52	1086.91	65.76	1046.92	20.59
VU0915318			>5376.72	>88.52	2845.49	68.32	283.72	19.55
VU0917592		26	NA	NA	NA	NA	NA	NA
VU0936061			1387.1	84.52	716.33	63.77	221.6	19.18
VU0936060			284.99	68.4	541.96	61.99	50.86	14.86
VU0936059			NA	NA	NA	NA	NA	NA
VU0053575			1296.77	84.16	654.12	63.23	670.6	20.36

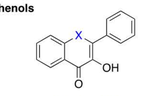
METABOLISM SAR

Functional Group Liability to address

1. phenols (A ring)
2. acetates (vs carbmates)
3. flavone (vs azaflavone)
4. glycoside versus cyclitol

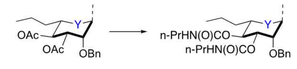


1. phenols



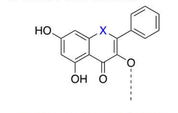
X = O or NH
purchased from supplier

2. acetates vs carbamates



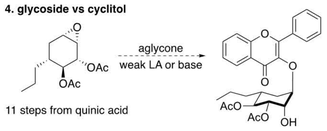
Y = O; Y = CH_2
conversion of acetates to carbamates (after glycosylation)

3. flavone vs azaflavone



X = O (synthesized in 6 steps)
X = NH (synthesized in 7 steps)

4. glycoside vs cyclitol



11 steps from quinic acid

FUTURE PLANS/DIRECTIONS

Develop a functional group relationship to high metabolic clearance in microsomal assay

1. Replace flavone aglycone with azaflavone employing newly established azaflavone synthesis
2. Compare acetate series to urethane series
3. Compare glycoside series to cyclitol series

Funding/Acknowledgements

1 R01 CA213201 (Lannigan, PI)
2P30CA068485 (VICC, Cancer Center Support Grant)

Studies on the Antimicrobial Marine Natural Product Chrysophaentin A and Congeners

Christopher R. Fullenkamp[1], Somnath Jana[2], Gabriel Pongdee[1], Yen-Pang Hsu,[3,4] Ellen M. Quardokus,[4] Gengxiang Zhao,[5] Carole A. Bewley[6], Michael VanNieuwenhze[3,4]* & Gary A. Sulikowski[1,2]

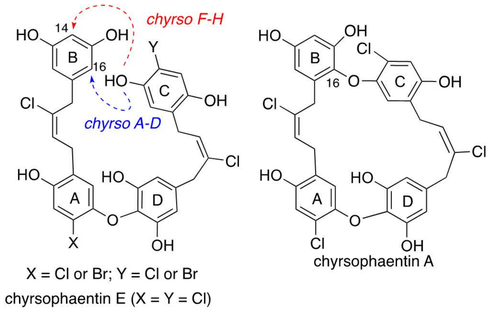
[1]Department of Chemistry, Vanderbilt University, Nashville, TN, USA. [2]Vanderbilt Institute of Chemical Biology, Vanderbilt University, Nashville, TN, USA. [3]Department of Chemistry, Indiana University, Bloomington, IN, USA. [4]Department of Molecular and Cellular Biochemistry, IN, USA. [5]Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Maryland, USA

ABSTRACT

Chrysophaentin A is an antimicrobial natural product isolated from the marine alga *C. taylori* in milligram quantity. Structurally, chrysophaentin A features a macrocyclic biaryl ether core incorporating two trisubstituted chloroalkenes at its periphery. A concise synthesis of iso- and 9-dechlorochrysophaentin A enabled by a Z-selective RCM cyclization followed by an oxygen to carbon ring contraction is described. Fluorescent microscopy studies revealed 9-dechlorochrysophaentins inhibited bacterial cell wall biosynthesis by disassembly of key divisome proteins, the conerstone cornerstone to bacterial cell wall biosynthesis and division. In unpublished work, chyrsophaentin A was shown to circular dichroism (CD) active indicating optical activity (enantiomerically enriched). In agreement, proton and carbon NMR spectra of 9-dechlorochrysophaentin A indicate temperature dependent atropisomer properties, further validated by preliminary computational studies. Current studies aim to access single enantiomer chrysophaentins and associate conformational properties to antimicrobial activity.

BACKGROUND

- Microbial secondary metabolites (natural products) have a rich history in the discovery and development of antibiotics for the clinical treatment of infections[1]
- Antimicrobial natural products serve not only as new antibiotic leads, but they have also identified new antimicrobial targets and provided insight into fundamental microbiology by study of their mechanism of action. For example, our understanding of bacterial cell wall biosynthesis has benefited from the discovery and study of beta-lactam and glycopeptide natural products
- In 2010 Bewley and co-workers[2] reported on the isolation, structure elucidation, and antimicrobial activity of chrysophaentins A-H. Chrysophaentins A-D and F-H are presumably derived from chrysophaentin E by oxidative cyclization of the C ring phenol at B ring carbons C16 and C14, respectively

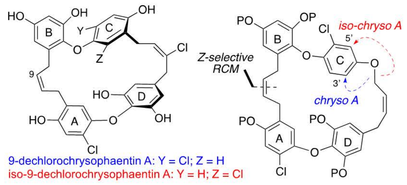


- The most prevalent metabolite, chrysophaentin A, displayed antimicrobial activity against *S. aureus*, methicillin-resistant *S. aureus* (MRSA), *E. faecium,* and vancomycin-resistant *E. faecium* (VREF)
- ***Study of chrysophaentin A was hindered due to unreliable supply from the producing marine alga (C. taylori) as well as failed attempts to produce chrysophaentins by in lab culturing of C. taylori*** [3]

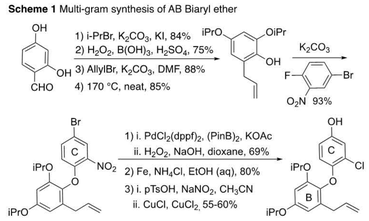
1. von Nussbaum F, Brands M, Hinzen B, Weigand S, Habich D. Antibacterial natural products in medicinal chemistry - Exodus or revival? *Angewandte Chemie-International Edition* **2006**; *45*: 5072-129
2. Plaza A, Keffer JL, Bifulco G, Lloyd JR, Bewley CA. Chrysophaentins A-H, antibacterial bisdiarylbutene macrocycles that inhibit the bacterial cell division protein FtsZ. *Journal of the American Chemical Society* **2010**;*132*: 9069-77.
3. Davison JR, Bewley CA. Antimicrobial chrysophaentin analogs identified from laboratory cultures of the marine microalga *Chrysophaeum taylorii*. *Journal of Natural Products* **2019**; *82*: 148-53

CHEMICAL SYNTHESIS OF 9-DECHLOROCHRYSOPHAENTIN A, ISO-9-DECHLOROCHRYSOPHAENTIN A AND CONGENERS

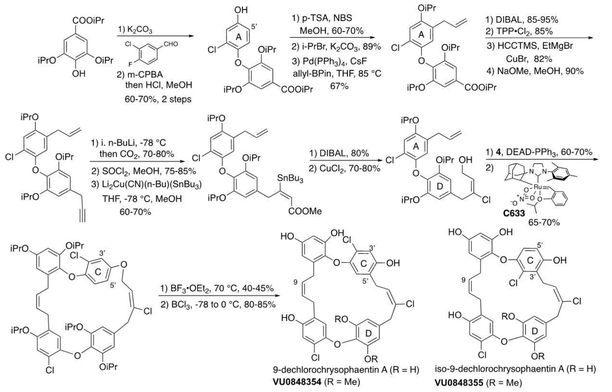
Our synthetic strategy featured two key steps: i. closure of the macrocyclic biaryl ether by way of a Z-selective ring-closing metathesis (RCM) reaction; and ii. Lewis acid mediated O to C alkyl migration which proved to bifurcate between C5' and C3' leading to iso- and 9-dechlorochrysophaentin A, respectively



Scheme 1 Multi-gram synthesis of AB Biaryl ether



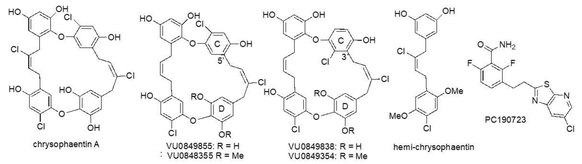
Synthesis of iso- and 9-Dechlorochrysophaentin A and Congeners VU0848354 and VU0848355 and their Antimicrobial Activity[4]



4. Fullenkamp, C. R.; Hus, Y-P.; Quardokus, E. M.; Zhao, G.; Bewley, C. A.; VanNieuwenhze, M.; Sulikowski, G. A. Synthesis of 9-dechlororchrysophaentin A enables studies revealing bacterial cell wall biosynthesis inhibition phenotype in *B. subilis*. *J. Am. Chem. Soc.* **2020**, 142, 16161-16166

CHRYSOPHAENTINS INHIBIT CELL WALL BIOSYNTHESIS BY A UNIQUE MOA

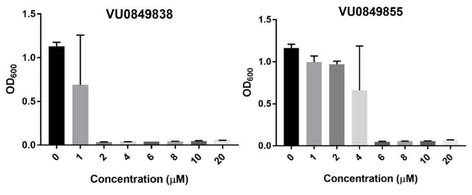
Antimicrobial activity of chrysophaentins and comparison to known FtsZ inhbitior PC190723[4]



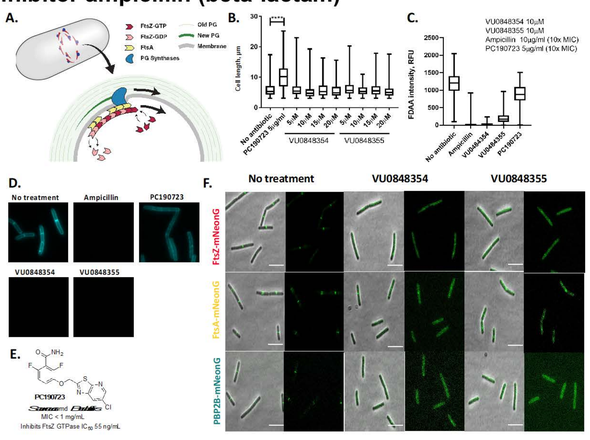
	MIC$_{50}$ (μg/mL)				
	S. aureus	MRSA	*E. faecalis*	VRE	*E. coli*
Chrysophaentin A	2	2	2	2	n/a
VU0849855	2.5	2.5	2.5	5	n/a
VU0848355	4.2	4.2	4.2	8.4	n/a
VU0849838	1.1	1.1	0.3	2.2	n/a
VU0848354	2.1	2.1	4.2	4.2	n/a
Hemichrysophaentin	10	10	5.0	10	n/a
PC190723	0.4	0.4	n/a	n/a	n/a

	Inhibition GTPase IC$_{50}$ (μM)	
	S. aureus FtsZ	*E. coli* FtsZ
Chrysophaentin A	9-67	9-67
VU0849855	10	9.5
VU0848355	30	50
VU0849838	10	6.5
VU0848354	25	45
Hemichrysophaentin	40	18
PC190723	n/a	n/a

In *B. subtilis*



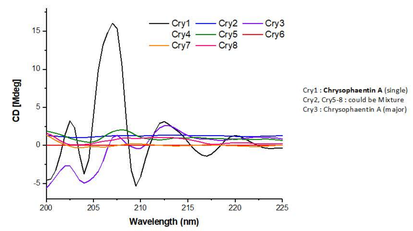
Chrysophaentins inhibit bacterial cell wall biosynthesis by a novel mechanism. Comparison to known FtsZ inhbitior PC190723 and known cell wall biosynthesis inhibitor ampicillin (beta-lactam) [4]



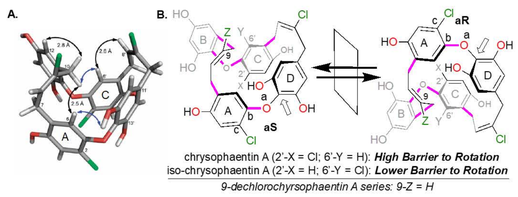
Chrysophaentins have a unique mode of inhibiting cell wall biosynthesis in *B. subtilis*. **A**. Fluorescently labeled proteins (FtsZ/A) and PBP2B allow visualization of peptidoglycan (PG) synthesis. **B**. The FtsZ inhibitor PC190723 produces the expected cell lengthening phenotype, but not '354 or '355. **C** and **D**. Ampicillin, '354 and '355 inhibit peptidoglycan synthesis, FtsZ inhibitor PC190723 does not. **E**. Structure of PC190723, MIC and GTPase-FtsZ inhibition. **F**. '354 and '355 displace FtsZ/A and penicillin binding protein PBP2B, a novel phenotype.

ATROPISOMERS?

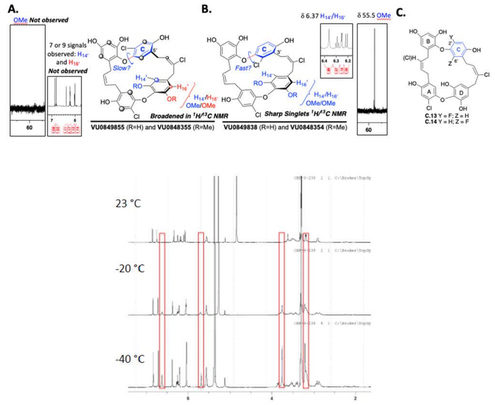
A. Chrysophaentin A shows a CD spectrum



B. Hindered rotation around the highlighted bonds impart atropisomerism and consequently chrysophaentin A may exist as one of the two enantiomers. Also shown below is an estimated lowest energy structure of chrysopophaentin A based NMR data



C. Temperature dependent behavior of 9-dechlorochrysophaentin (compared to iso-series) support atropisomer behavior



FUTURE PLANS/DIRECTIONS

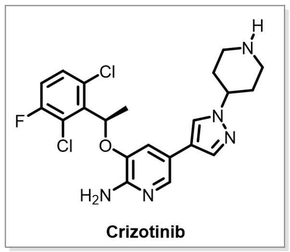
- Resolution of racemic mixture employing Mosher esters derived from phenol released phenol (following O to C migration (cf. above, Synthesis of iso- and 9-dechlorochrysophaentin….)
- Synthesis of C ring analogs (chloro to fluoro and chloro to methyl, to examine dependence of atropisomer properties on C ring substitution pattern.

Progress toward the Radiosynthesis of the [^{18}F]Crizotinib

KyuOk Jeon[1], Adam J. Rosenberg[2], Yiu-Yin Cheung[2], Kwangho Kim[1], Bhuminder Singh[3]

[1]Molecular Design and Synthesis Center, Vanderbilt Institute of Chemical Biology, Vanderbilt University, [2]Vanderbilt University Institute of Imaging Science, Vanderbilt University Medical Center, [3]School of Medicine, Vanderbilt University Medical Center

Background



EGF receptor (EGFR) is a receptor tyrosine kinase (RTK) that is overexpressed in over 50% of colorectal cancer (CRC) cases where it is linked to metastasis and poor prognosis.

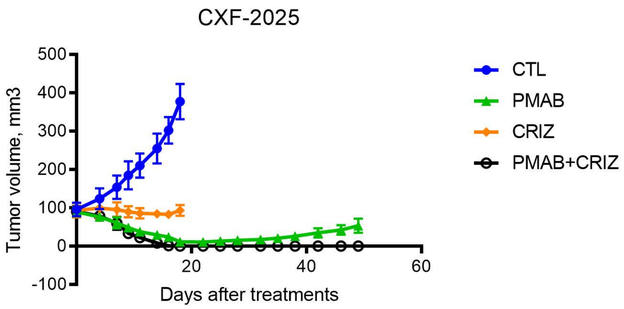
Cetuximab, an EGFR-targeting monoclonal antibody is approved by US-FDA to treat advanced wild-type KRAS CRC.

Cetuximab, as monotherapy, is effective in only about 10% of CRCs and resistance frequently emerges.

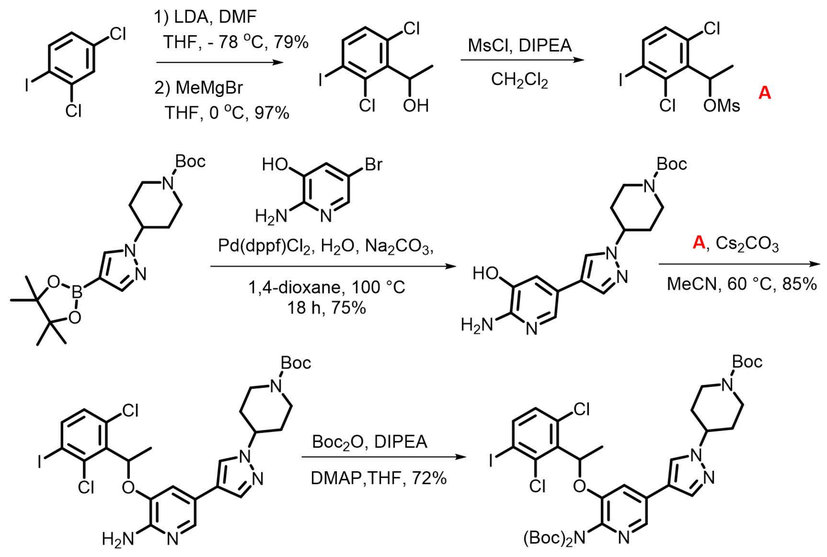
There is a pressing need 1) to identify those patients most likely to respond (or not respond) to cetuximab and 2) to devise treatment strategies that would prevent resistance and/or enhance cetuximab response.

We propose to address these needs by aggressively pursuing our observations that enhanced activity of multiple RTKs (e.g. MET and RON) confers de novo and acquired cetuximab resistance, which may be overcome by addition of the dual MET/RON inhibitor, crizotinib.

Delayed Panitumumb Resistance in CRC PDXs by Crizotinib

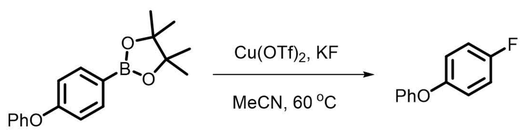


Synthesis of Common Intermediate



Cold Fluorination

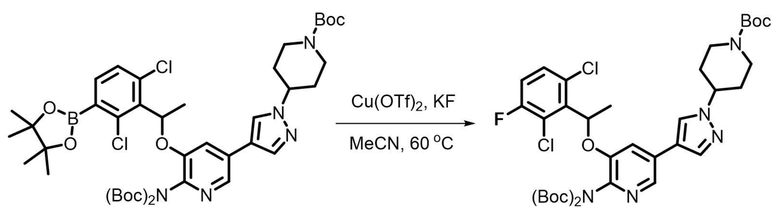
Model Study



In a grovebox
MeCN (distilled w/ CaH_2)

Conformed by ^{1}H, ^{19}F NMR

Reference: *J. Am Chem. Soc.* **2013**, *135*, 16292-16295.

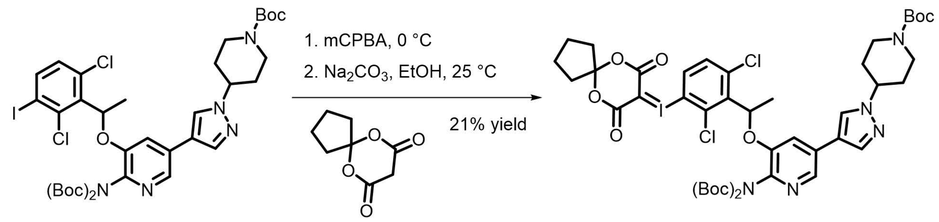


In a glovebox
MeCN (distilled w/ CaH_2)

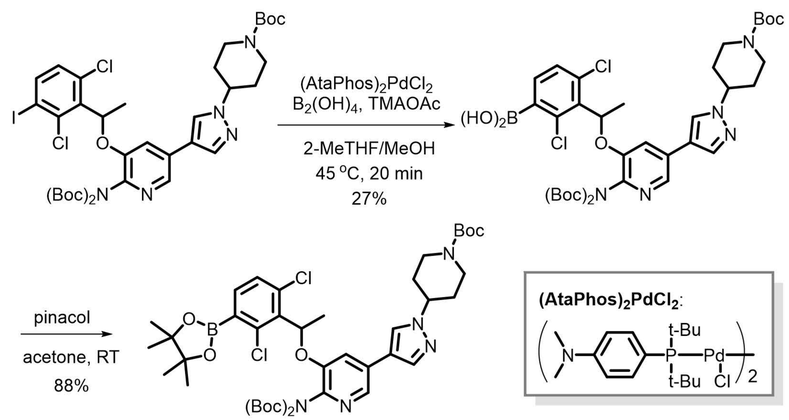
$Cu(OTf)_2$: 8 eq
KF: 8 eq
Reaction time: 24 h

^{1}H NMR: messy
^{19}F NMR: very weak signal

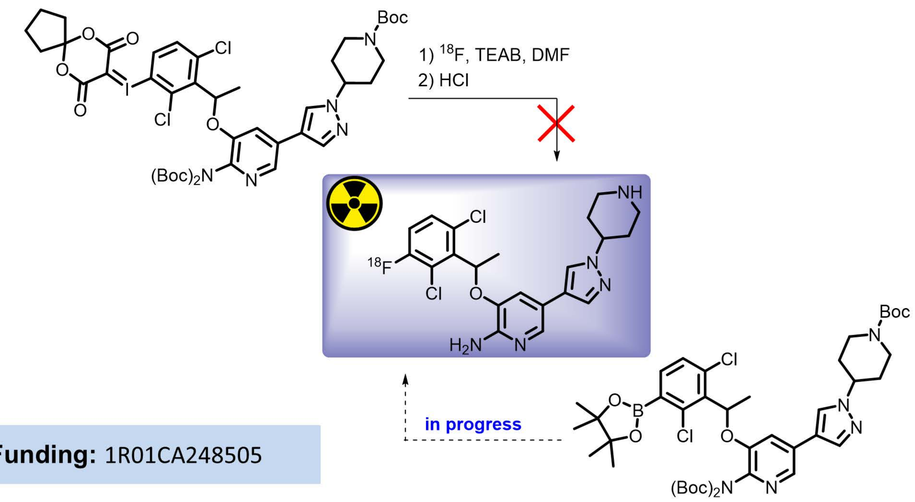
Synthesis of Precursor: Spirocyclic Hypervalent Iodine(III) complex



Synthesis of Precursor: Boronic acid and Boronic acid pinacol ester



Automated Radiofluorination



Funding: 1R01CA248505

Synthesis of Thio-cyclic-GMP-AMP and Conjugates as Novel STING Agonists

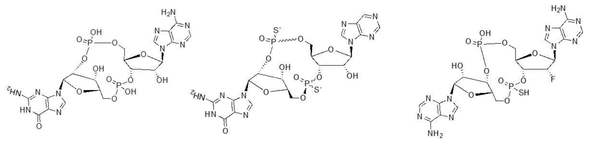
Plamen Christov[1], Blaise R. Kimmel[2], Karan Arora[2], Taylor L. Sheehy[3], Jessalyn J. Baljon[3], Gary Sulikowski[1], John T. Wilson[1-4]

[1]Vanderbilt Institute of Chemical Biology, Vanderbilt University, Nashville, TN, USA. [2]Department of Chemical and Biomolecular Engineering, Vanderbilt University, Nashville, TN, USA, [3]Department of Biomedical Engineering, Vanderbilt University, Nashville, TN, USA, [4]Vanderbilt-Ingram Cancer Center, Nashville, TN, USA

ABSTRACT

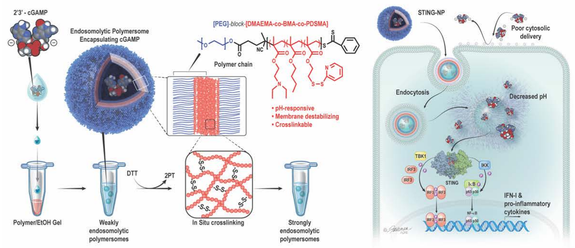
- There is a significant unmet need for new strategies to enhance response rates to immune checkpoint blockade (ICB) through the development and optimization of immunostimulatory drugs.
- Cyclic dinucleotide (CDN) agonists of stimulator of interferon genes (STING) are a promising class of immunotherapeutics that activate innate immunity to increase tumor immunogenicity.
- The development of unique CDN variants that can be conjugated to drug carriers via cleavable linkers offers potential to increase their activity and specificity to widen their therapeutic window.

2'3'cGAMP **ADU-S100** **Thio-F-CDA**



BACKGROUND

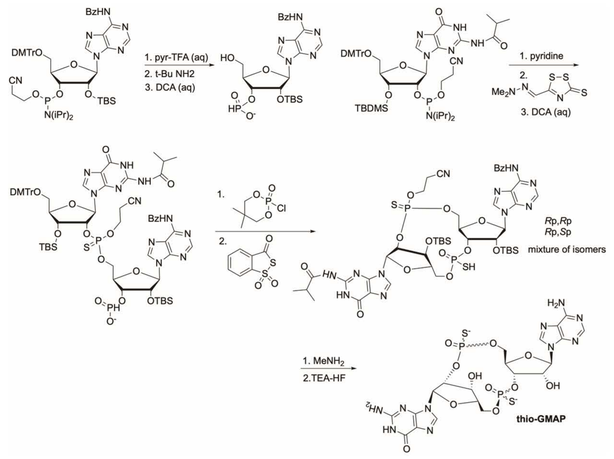
- The stimulator of interferon of genes (STING) pathway plays an important role in immune recognition of cancer. STING activation triggers a multifaceted innate immune response that mobilizes endogenous antitumor T cell immunity.
- STING recognizes several cyclic dinucleotides (CDNs) and various CDNs are entering clinical trials.
- CDNs have poor drug like properties and their efficacy and utility is limited by rapid clearance, low cellular uptake, and inefficient cytosolic delivery.
- We have previously developed STING-activating nanoparticles (STING-NPs) that improve their pharmacological properties, opening a therapeutic window for enhancing responses to immunotherapy.
- However, STING-NPs accumulate significantly in the liver and CDNs leak from the particle during circulation.
- Towards addressing these barriers, we are developing CDNs with reactive handles and cleavable linkers for integration into diverse drug carriers.



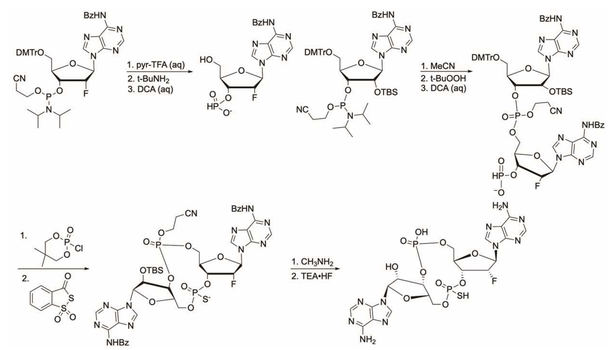
STING-activating nanoparticles (STING-NPs). *pH-responsive polymersomes encapsulate and mediate endosomal escape of CDNs in response to endolysosomal pH.*

SYNTHESIS OF CDN STING AGONISTS

Thio-cGAMP Synthesis

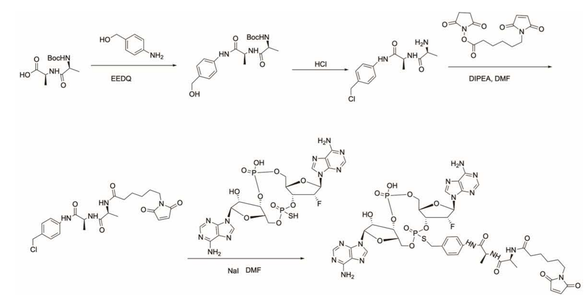


Mono-Thio-Mono-Fluoro-CDA

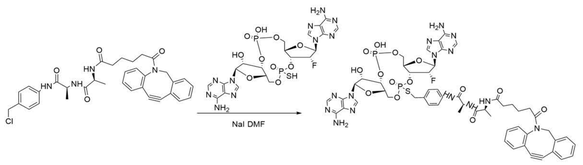


FUNCTIONALIZATION AND CHARACTERIZATION OF STING AGONISTS

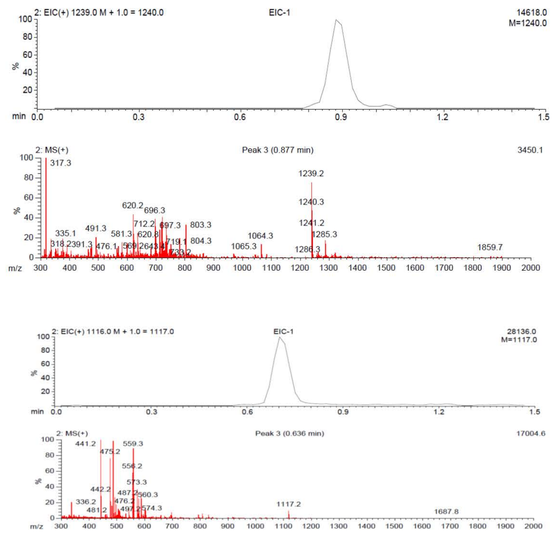
Maleimide-Dialanine-Mono-Thio-Mono-Fluoro-DA



DBCO-Dialanine-Mono-Thio-Mono-Fluoro-DA

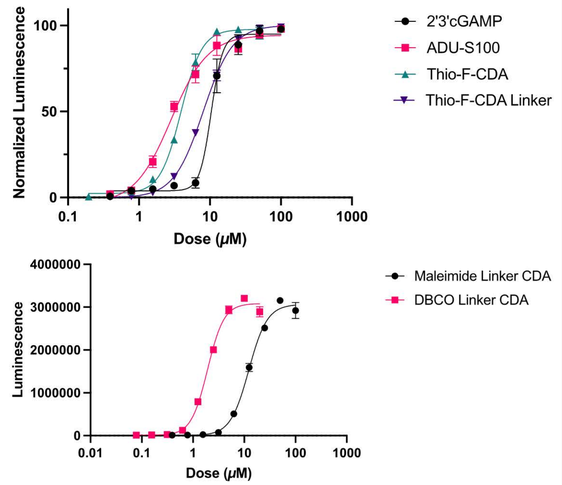


LC/MS

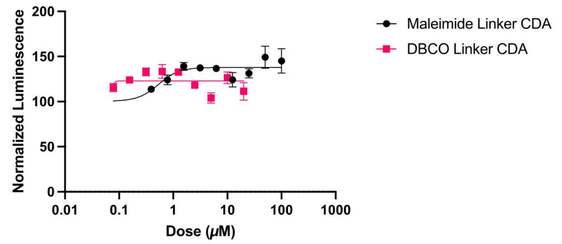


BIOLOGICAL ACTIVITY

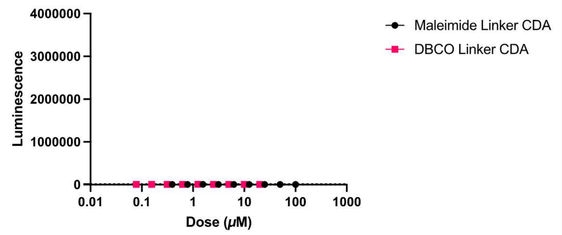
Activity of CDN Panel in THP1-Duals (Human Monocyte STING Reporter Cells)



Toxicity of CDN Panel in THP1-Duals



Activity in THP1-Duals with STING KO



SUMMARY AND FUTURE PLANS

- Thio modified CDNs were synthesized and enable functionalization with maleimide and DBCO reactive handles through an enzyme cleavable linker.
- Functionalized CDNs display STING-dependent immunostimulatory activity *in vitro*.
- Future studies are focused on ligating CDNs to diverse drug carrier platforms and evaluating efficacy in tumor models.

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CDMRP Congressionally Directed Medical Research Programs Department of Defense
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