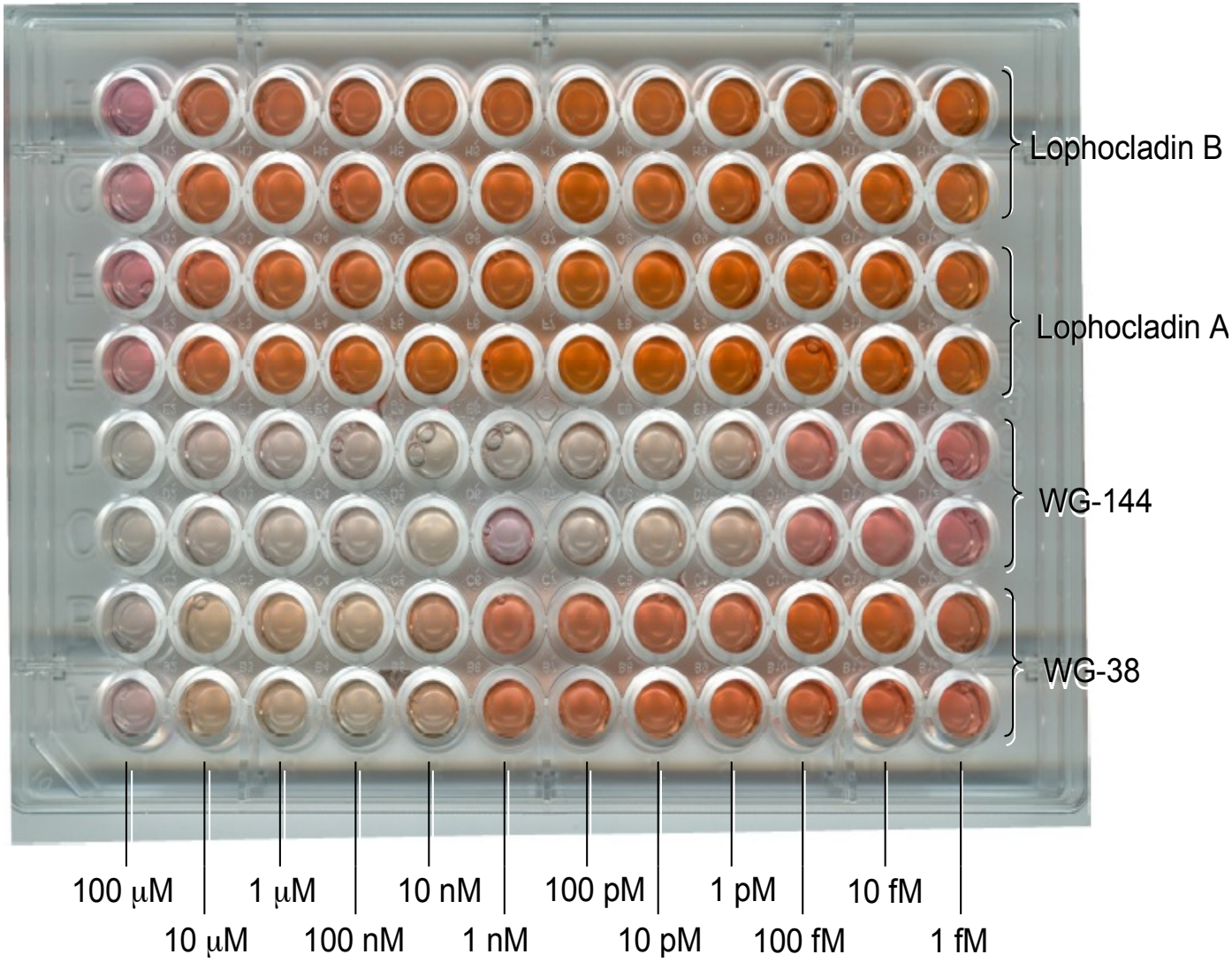
Research Collaboration with Moores Cancer Center
Discovery of Antiangiogenic Natural Product Leads

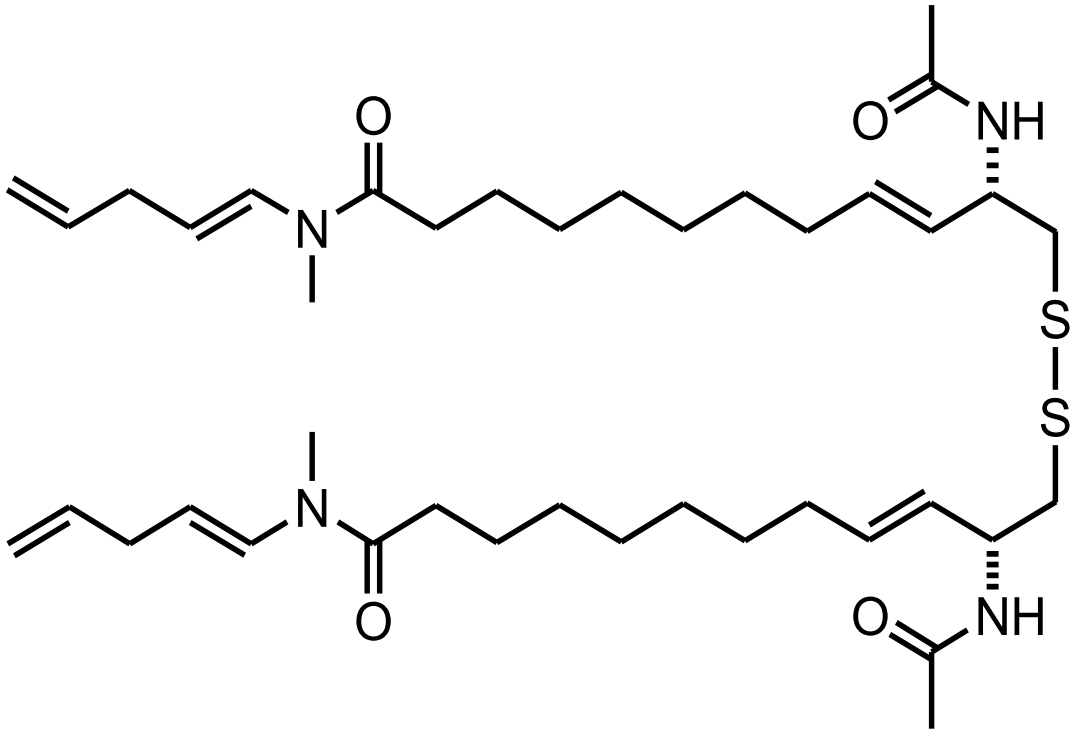
- Dennis Carson and Wolf Wrasidlo
- Screening of Marine Cyanobacterial Natural Products to HUVEC cells

Extended Range Anti-Proliferation Screen



W. Wrasidlo - MCC

Structure of Somocystinamide A (WG-144) from a Fijian Strain of *Lyngbya majuscula*



- Original isolation Nogle & Gerwick, *Org. Lett*. **2002**, *4*, 1095-1098
- Somocystinamide A modestly toxic to Neuro-2a cells @ 1 μM
-Testing at Moore's CC, 99% Cell Kill in HUVEC cells at 10 pM

T-lymphoblastic leukemia apoptosis DAPI Stain of Molt4 cells before and after treatment with 1 µM of WG-144

Initial

after 6 hr. exposure

W. Wrasidlo - MCC

Summary of Cell Proliferation Assays with WG-144 (Somocystinamide)

Cell Line	IC50	
U266 myeloma	5.83 uM	
M21 Melanoma	1.28 uM	
PC3 prostate cancer	0.97 uM	
TJK304	0.83 uM	
NB7 neuroblastoma	0.81 uM	
NB7 (caspase 8 positive)	0.012 uM	Caspase 8 Expressing
A-549 Lung cancer	0.046 uM	
Pancreatic metastatic mouse carcin.	0.008 uM	
HUVEC primary endothelial cells	0.000004 uM	

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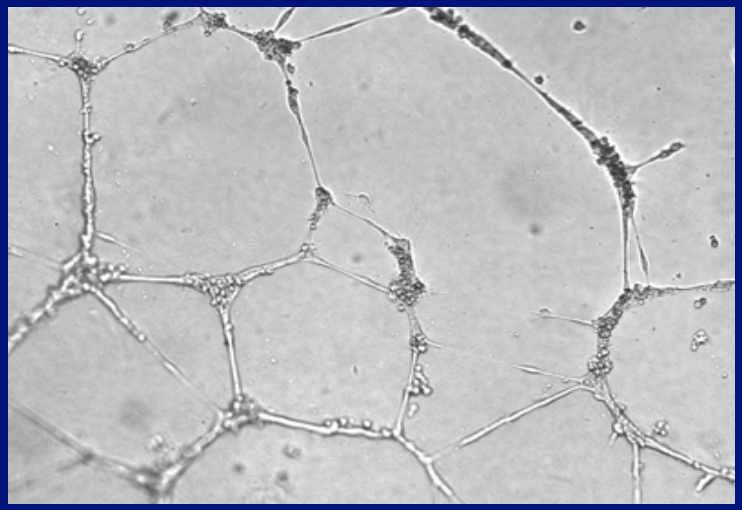
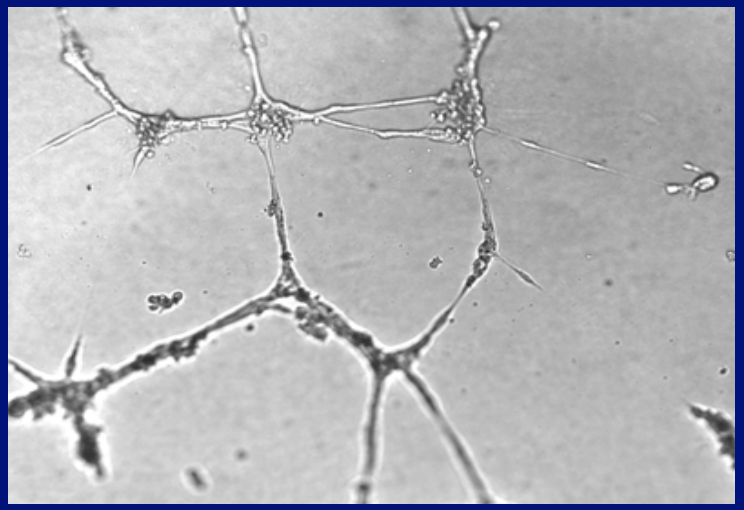
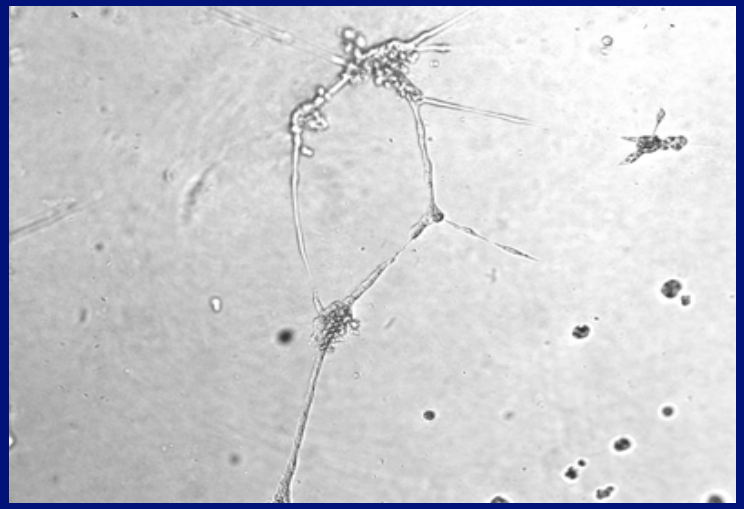
Somocystinamide inhibits tube formation on HUVEC /matrigel



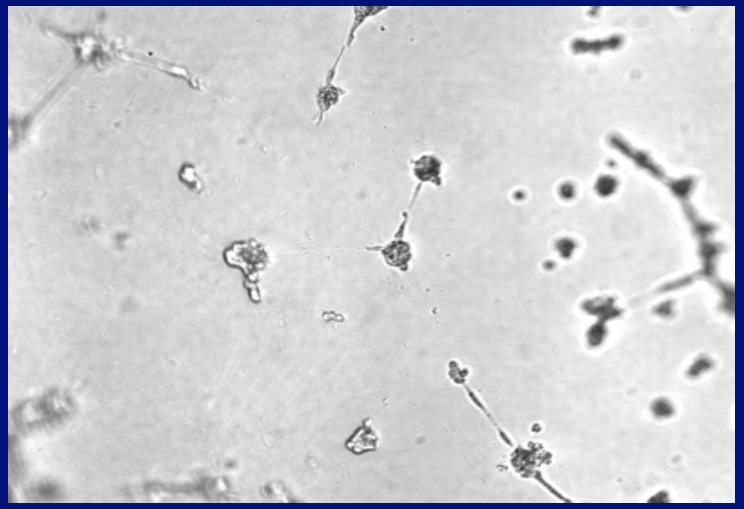
Control (culture medium)



WG-144 (1 pM)

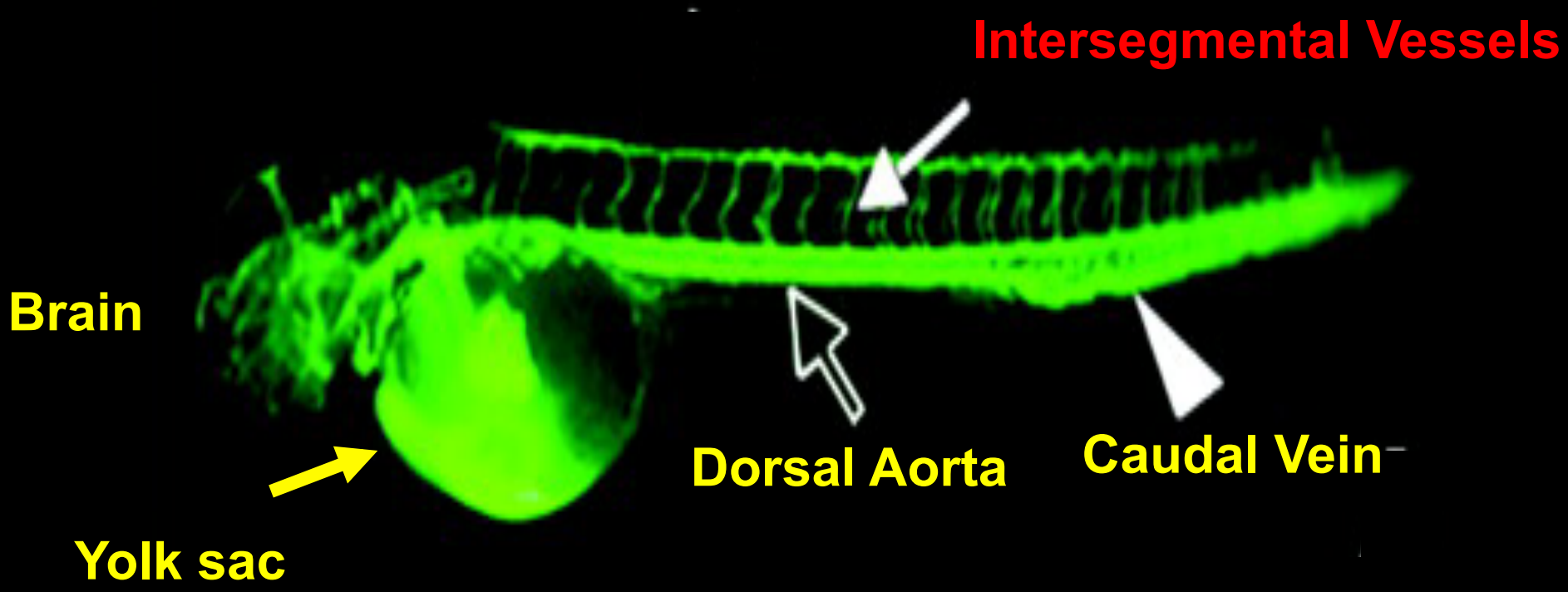


WG-144 (100 pM)

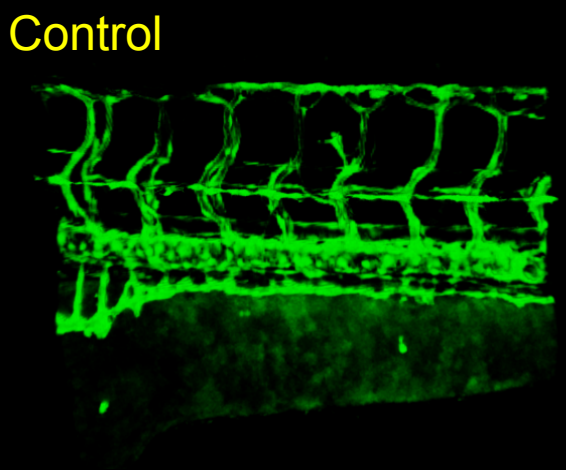


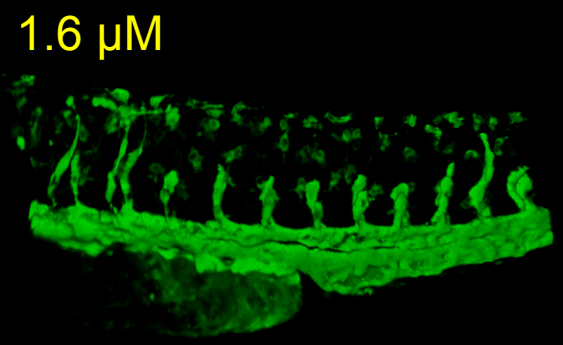
WG-144 (10 nM)

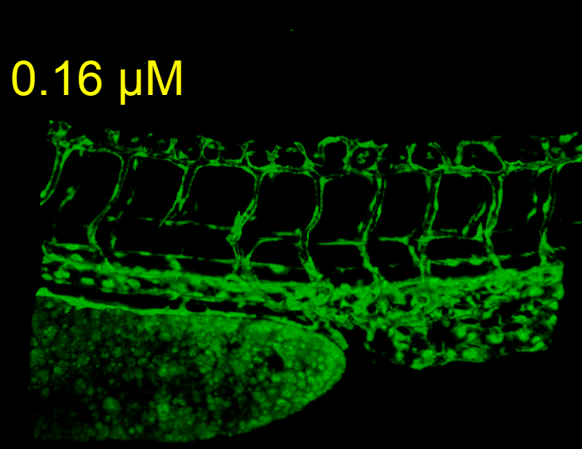
Zebrafish Angiogenesis is Mediated by VEGF (fli-gfp transgenic fish)

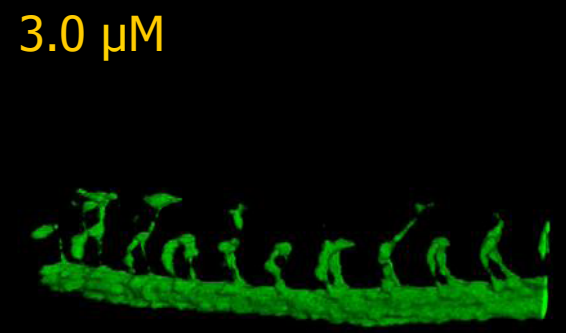


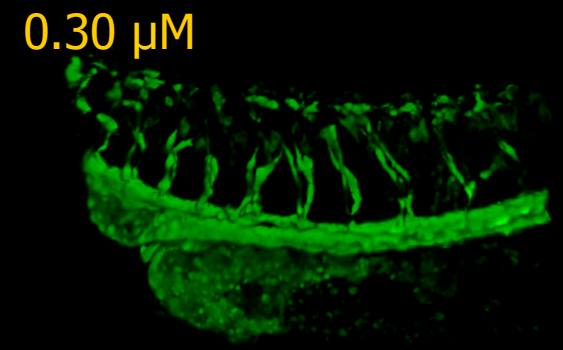
Dose Response for Somocystinamide A

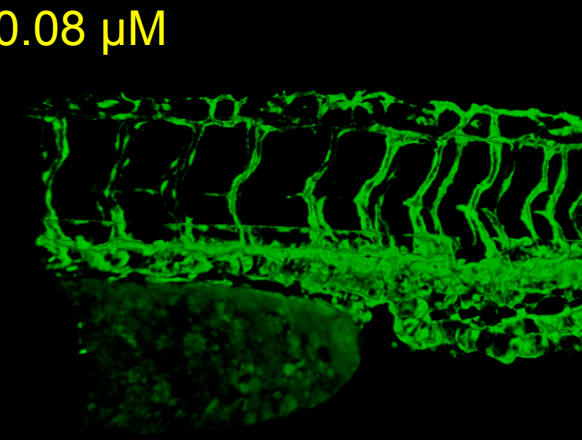




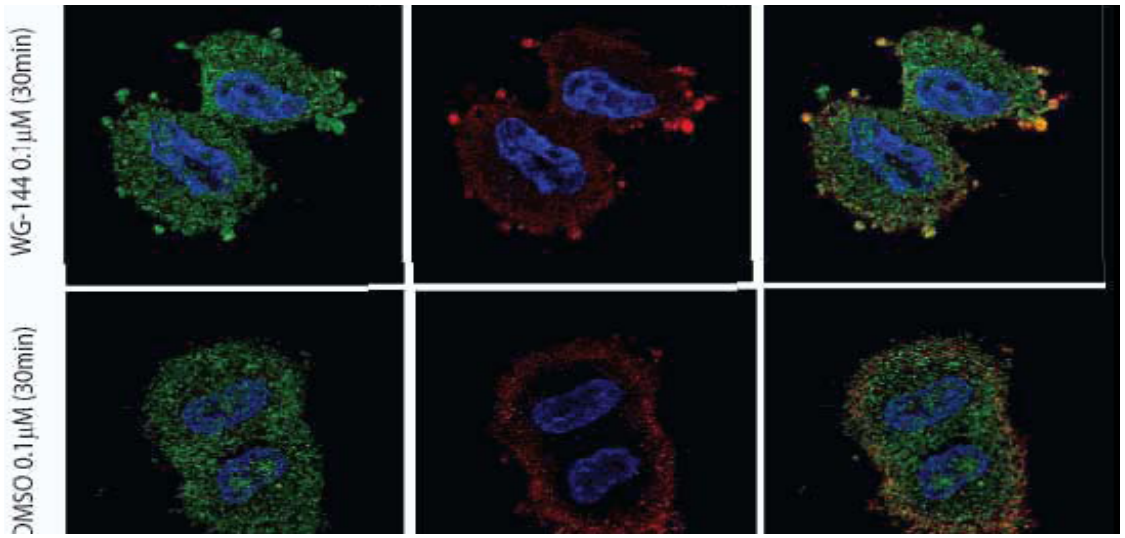




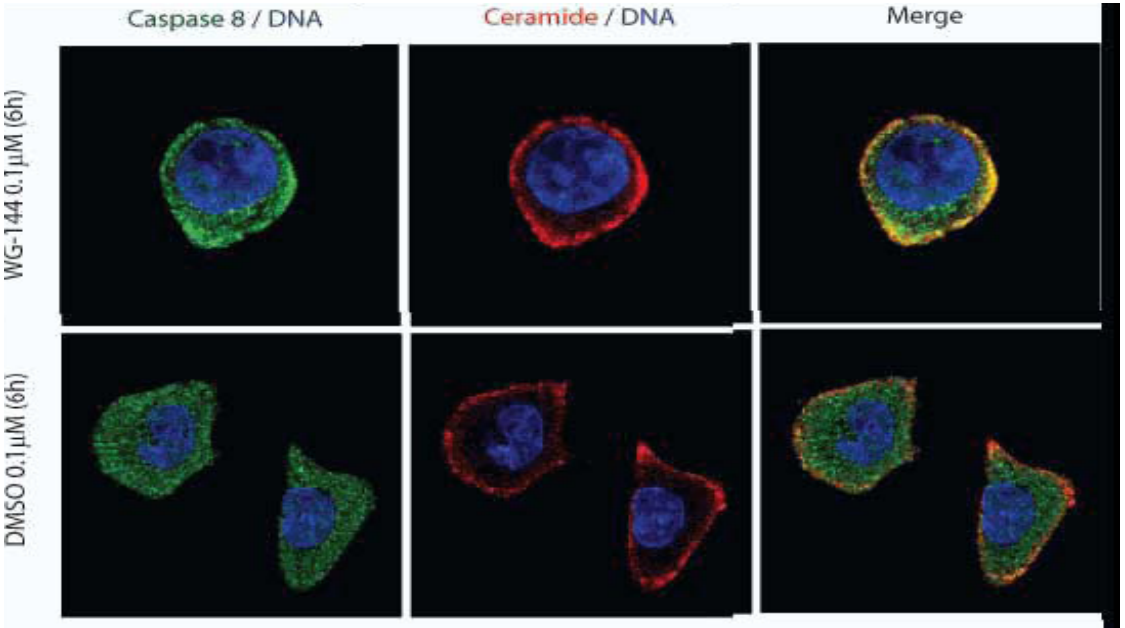




Co-localization of Ceramide and Caspase 8-GFP in Cells Treated with Somocystinamide A



30 min after treatment With 100 nM Somocystinamide A



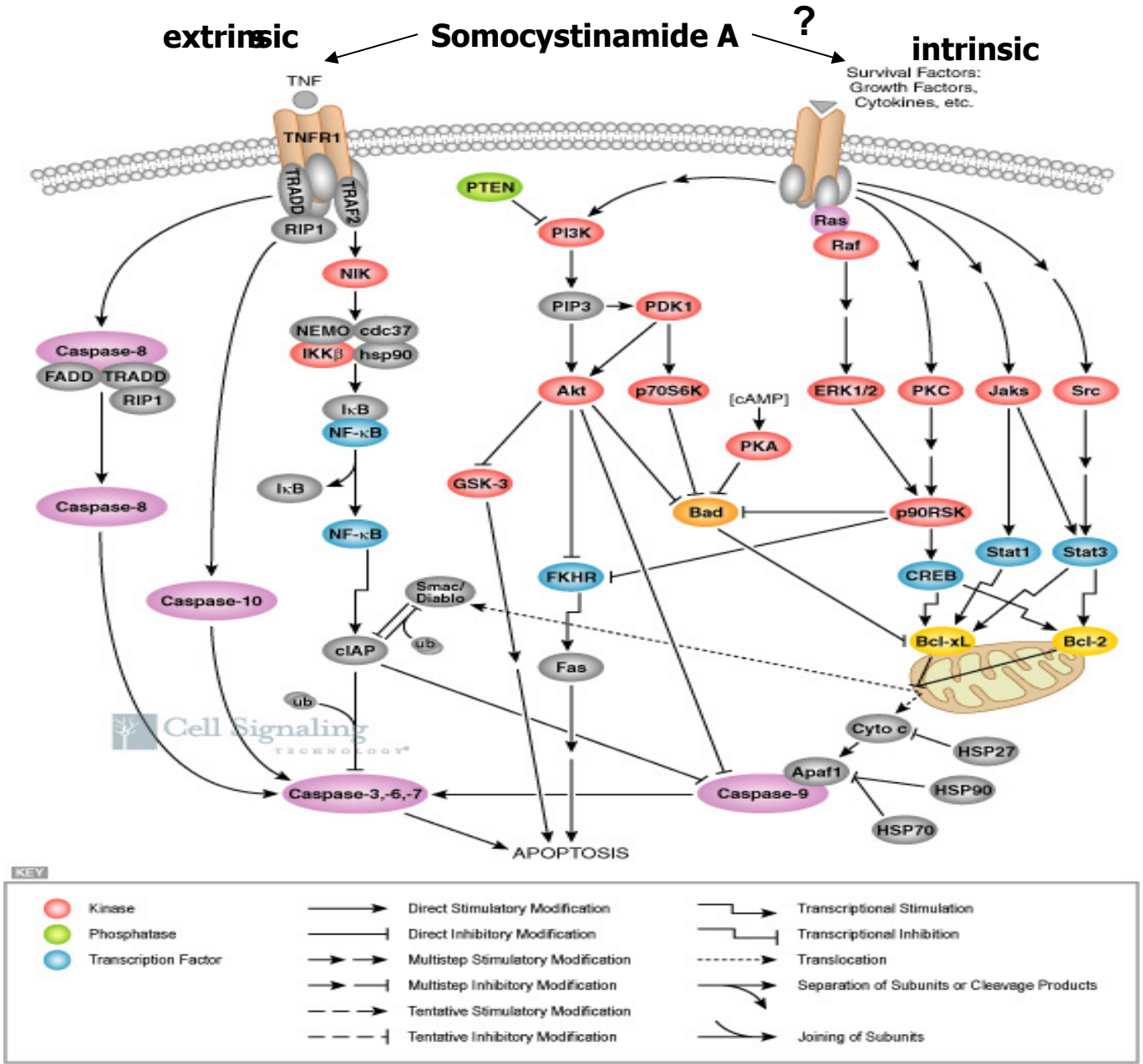
6 hours after treatment With 100 nM Somocystinamide A

D. Stupack - MCC

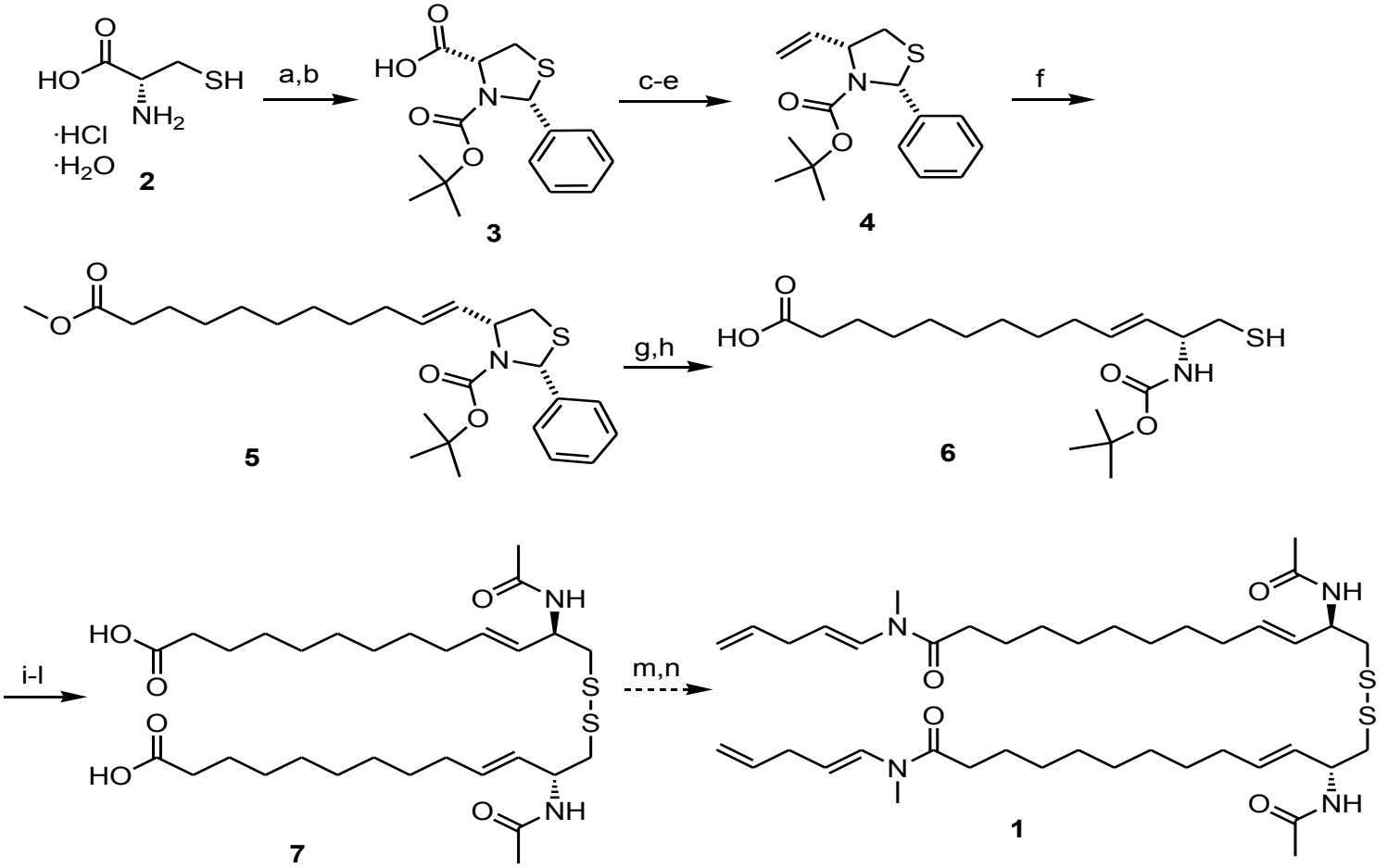
Proposed "Ceramide-Like" Fas-cap and Kill Mechansim of Somocystinamide A

- Partitioning of somocystinamide A to form sphingolipid-enriched membrane domains (lipid rafts)
- Translocation and super-aggregation of trimerized Fas/Trail
- Lateral segregation and co-localization into caps on one pole of cell
- Optimized Fas-signaling in caspase 8 expressing cells by
- a) Recruitment of cytoplasmic adapter protein FADD
- b) Binding of procaspase 8 (or 10) to FADD forming death inducing signaling complex (DISC)
- Direct activation of execution caspase 3
- Result: DNA fragmentation, chromatin condensation, membrane blebbing, apoptosis

Common Cell Signaling Path Leading to Apoptosis



Synthesis of Somocystinamide (Tak Suyama - SIO)



a) PhCHO, EtOH/H2O. b) Boc2O, NaOH, dioxane/H2O, 66% over 2 steps. c) BH3·Me2S, THF, 94%. d) (COCl)2, DMSO, Et3N, CH2Cl2, 92%. e) Ph3PCH3·Br, *n*-BuLi, THF, 62%. f) methyl 10-octenoate, 2nd generation Hoveyda catalyst, CH2Cl2, 81%. g) LiOH, H2O/THF. h) Na, NH3(l), 93% over 2 steps. i) CH2N2, Et2O/MeOH, 66%. j) TFA, CH2Cl2. k) Ac2O, Et3N, CH2Cl2, 85%. l) LiOH, H2O/THF, 100%. m) (COCl)2, DMF, CH2Cl2. n) MeNH2, 4-pentenal, pyridine, CH2Cl2.]