

Supporting Information

Gli Pathway-Targeted Co(III) Schiff Base Complexes Inhibit Migration of Basal Cell Carcinoma Cells

Caroline E. Bond^a, Keaton D. Olson^{a,‡}, Metehan Punar^{a,‡}, Lillian B. Friedman^a, Jian-Hong Tang^{a,c}, Minrui Luo^a, Matthew D. Bailey^a, Robert A. Holmgren^b, and Thomas J. Meade^{a,†}

^a. Departments of Chemistry, Molecular Biosciences, Neurobiology, and Radiology, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208-3113

^b. Department of Molecular Biosciences, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208-3113

^c. Author now affiliated with School of Future Technology, University of Chinese Academy of Sciences, Beijing 101408, P. R. China

[‡] These authors contributed equally

[†]Correspondence author: tmeade@northwestern.edu

Table of Contents

Section 1: Spectroscopic Data: MALDI and Circular Dichroism (CD)	2
Section 2: ASZ Experiment Data	4
Section 2.1: ASZ Transwell Migration Experiment: Day 1	4
Section 2.2: ASZ Transwell Migration Experiment: Day 2	4
Section 2.3: ASZ Transwell Migration Experiment: Day 3	6
Section 3: ASZ Stock Solution Concentrations	9

Section 1: Spectroscopic Data: MALDI and Circular Dichroism (CD)

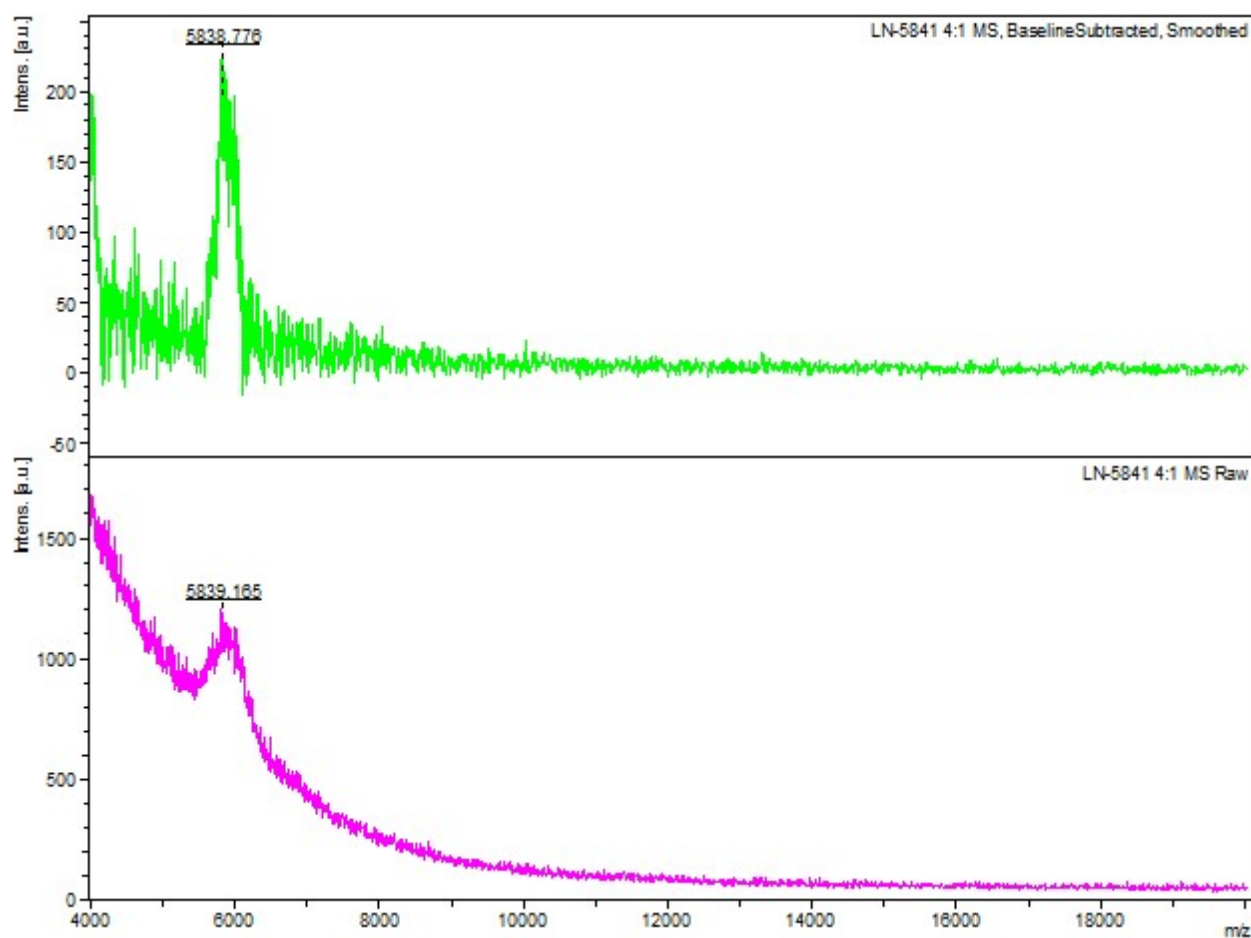


Figure S1: MALDI performed on Co-Gli ssDNA to confirm identity. Bottom) raw data for isolated ssDNA from HPLC purification. Top) Smoothened MALDI data for the Co-Gli ssDNA after applying a baseline subtraction. MS (MALDI-TOF) m/z: $[M - H]^-$ Calcd for $C_{188}H_{255}CoN_{62}O_{105}P_{17}S_6$: 5838.972; Found: 5838.776.

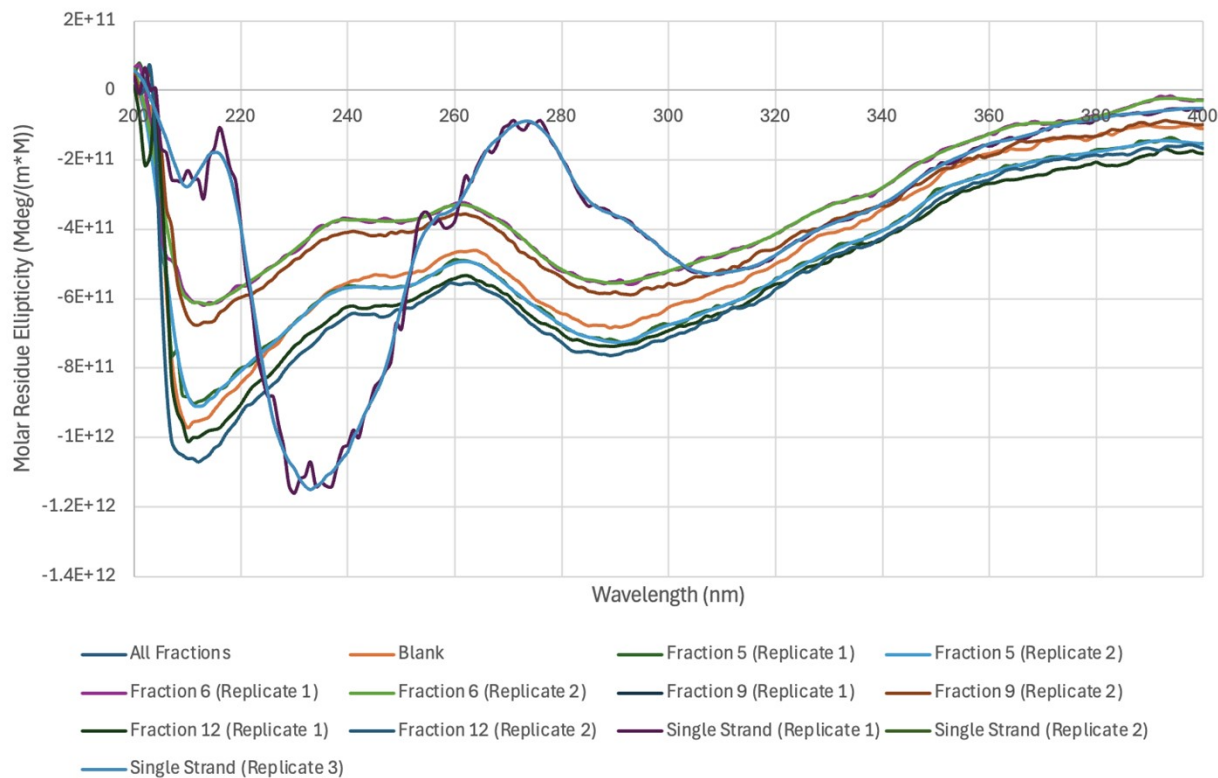


Figure S2: Circular Dichroism of Single Stranded and Double Stranded CoSB DNA. Post-DNA annealing fractions were collected from a G25 desalting column. CD was then run on each fraction for determination of annealing completion.

Section 2: ASZ Experiment Data

Section 2.1: ASZ Transwell Migration Experiments: Day 1

Drug	Volume of Cell Solution (uL)	Volume of Media (uL)
CoGli-GOPEI	94	906
GOPEI	420	1080
Cobalt	420	1080
CoGli-GOPEI	220	780
Vismodegib	168	832
GANT-61	201	799
GANT-61	214	786

Table S1: Transwell Migration Experiments of ASZ cells on Day 1. Each Experiment utilized a 6-well plate (Corning) with approximately 300,000 cells per well via hemocytometry. With the exception of Experiment 4, each Experiment's well was diluted to a final volume of 1mL at the end of day 1: Experiment 4 was the first Experiment that was conducted and was carried out at 1.5mL. However, it was realized that resources would only allow for all of the remaining Experiments to be carried out if their final volumes were diluted to 1mL.

Section 2.2: ASZ Transwell Migration Experiments: Day 2

Drug	Dose (uM)	Volume of Media (uL)	Volume of Drug (uL)
CoGli-GOPEI	0	900	0
	4	529	371
	8.5	112	788
GOPEI	0	900	0
	4	899.5	0.53
	8.5	898.8	1.13

Cobalt	0	900	0
	4	887.4	12.6
	8.5	873.3	26.75
CoGli-GOPEI	0	900	0
	1	807.3	92.7
	2.5	668	231.8
Vismodegib	0	900	0
	0.1	898.2	1.8
	0.25	895.5	4.5
	0.5	891	9
	1	882	18
GANT-61	0	900	0
	0.5	899.8	0.195
	1	899.6	0.381
	2.5	899.1	0.95
	5	898.1	1.91
GANT-61	0	900	0
	10	896.2	3.81

Table S2: Transwell Migration Experiments of ASZ Cells on Day 2. On this day, the appropriate drug and its concentration were added into each well for each Experiment during media swapping (with the drug for each

Experiment specified in the leftmost column of the table and the dose for a given drug in the next column over). After appropriate drug addition, each well was diluted to a final volume of 900uL.

Section 2.3: ASZ Transwell Migration Experiments: Day 3

Drug	Dose (uM)	Component:Volume in Well (uL)	Component:Volume in Insert (uL)
CoGli-GOPEI	0	Serum Media: 500	Serum Free Media: 500 *Cell Suspension:
	4	Serum Media: 294 CoGli-GOPEI: 206	Serum Free Media: 294 CoGli-GOPEI: 206 *Cell Suspension: 200
	8.5	Serum Media: 62 CoGli-GOPEI: 438	Serum Free Media: 62 CoGli-GOPEI: 438 *Cell Suspension: 200
GOPEI	0	Serum Media: 500	Serum Free Media: 300 Cell Suspension: 200
	4	Serum Media: 499.8 GOPEI: 0.3	Serum Free Media: 299.8 GOPEI: 0.3 Cell Suspension: 200
	8.5	Serum Media: 499.4 GOPEI: 0.63	Serum Free Media: 299.8 GOPEI: 0.63 Cell Suspension: 200
Cobalt	0	Serum Media: 500	Serum Free Media: 300 Cell Suspension: 200
	4	Serum Media: 493 Cobalt: 7	Serum Free Media: 293 Cobalt: 7 Cell Suspension: 200
	8.5	Serum Media: 485 Cobalt: 14.86	Serum Free Media: 285 Cobalt: 14.86 Cell Suspension: 200
CoGli-GOPEI	0	Serum Media: 500	Serum Free Media: 300 Cell Suspension: 200
	1	Serum Media: 449.5 CoGli-GOPEI: 50.51	Serum Free Media: 249.5 CoGli-GOPEI: 50.51

			Cell Suspension: 200
	2.5	Serum Media: 371.2 CoGli-GOPEI: 128.8	Serum Free Media: 371.2 CoGli-GOPEI: 128.8 Cell Suspension: 200
Vismodegib	0	Serum Media: 500	Serum Free Media: 300 Cell Suspension: 200
	0.1	Serum Media: 499 Vismodegib: 1	Serum Free Media: 299 Vismodegib: 1 Cell Suspension: 200
	0.25	Serum Media: 497.5 Vismodegib: 2.5	Serum Free Media: 297.5 Vismodegib: 2.5 Cell Suspension: 200
	0.5	Serum Media: 495 Vismodegib: 5	Serum Free Media: 295 Vismodegib: 5 Cell Suspension: 200
	1	Serum Media: 490 Vismodegib: 10	Serum Free Media: 290 Vismodegib: 10 Cell Suspension: 200
GANT-61	0	Serum Media: 500	Serum Free Media: 300 Cell Suspension: 200
	0.5	Serum Media: 499.9 GANT-61: 0.1058	Serum Free Media: 299.9 GANT-61: 0.1058 Cell Suspension: 200
	1	Serum Media: 499.8 GANT-61: 0.2116	Serum Free Media: 299.8 GANT-61: 0.2116 Cell Suspension: 200
	2.5	Serum Media: 499.5 GANT-61: 0.529	Serum Free Media: 299.5 GANT-61: 0.529 Cell Suspension: 200
	5	Serum Media: 498.9 GANT-61: 1.058	Serum Free Media: 298.9 GANT-61: 1.058 Cell Suspension: 200
GANT-61	0	Serum Media: 500	Serum Free Media: 300 Cell Suspension: 200
	10	Serum Media: 497.9 GANT-61: 2.116	Serum Free Media: 297.9 GANT-61: 2.116 Cell Suspension: 200

Table S3: Transwell Migration Assays of ASZ cells that were dosed with appropriate drug concentrations for a given Experiment. The assays were carried out in 24 well plates that contained inserts. After passaging the cells in Table **S2**, the inserts were filled with the appropriate drug concentration, and cell suspension, then diluted to a final volume of 500uL with serum free media. The wells for a given dose and Experiment were also filled with appropriate drug concentrations and then diluted to a final volume of 500uL with serum free media. Once the final volume of the well was acquired, their respective inserts (that were staged in empty wells) were transferred to their wells.

(* = Cell suspension was plated and adhered first with excess aspirated off after 2 hours. Then other components were added to the insert with already adhered cells)

Section 3: ASZ Stock Solution Concentrations

Compound	Stock Solution Concentration
Cobalt bis-amine	286.0 uM
GOPEI	1mL of 0.245 g/mL
CoGli-GOPEI	9.708 uM
Vismodegib	50 uM
GANT-61	2,362.7 uM

Table S4: Stock concentrations of Compounds used in the Transwell Migration Experiments.