Supplemental Figures and Legends

The Natural Product Butylcycloheptyl Prodiginine Binds Pre-miR-21, Inhibits Dicer-Mediated Processing of Pre-miR-21, and Blocks Cellular Proliferation

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Figure S1: Results of 3682 pure natural products screened *via* DSF to identify modulators of pre-miR-21 thermal stability *in vitro*. Related to Figure 1.

Figure S2: Cytotoxicity data on select pre-miR-21 modulating compounds. Related to Table 1.

Figure S3: Chemical structures of control compounds 5-fluorouracil (5-FU) and navitoclax. Related to Figure 1 and Table 1.

Figure S4: Differential scanning calorimetry of pre-miR-21 (30 μM) with or without bPGN (300 μM). Related to Figure 1.

Figure S5: Hexachlorophene and regorafenib were previously identified as Dicer inhibitors. Related to Figure 3.

Table S1: List of 32 active natural products identified from the 3682 natural product compounds screened *via* DSF for modulators of pre-miR-21 thermal stability *in vitro*. Related to Table 1.

TableS2: 41 predicted miR-21 target genes annotated from NanoString analysis. Related to Figure 5.

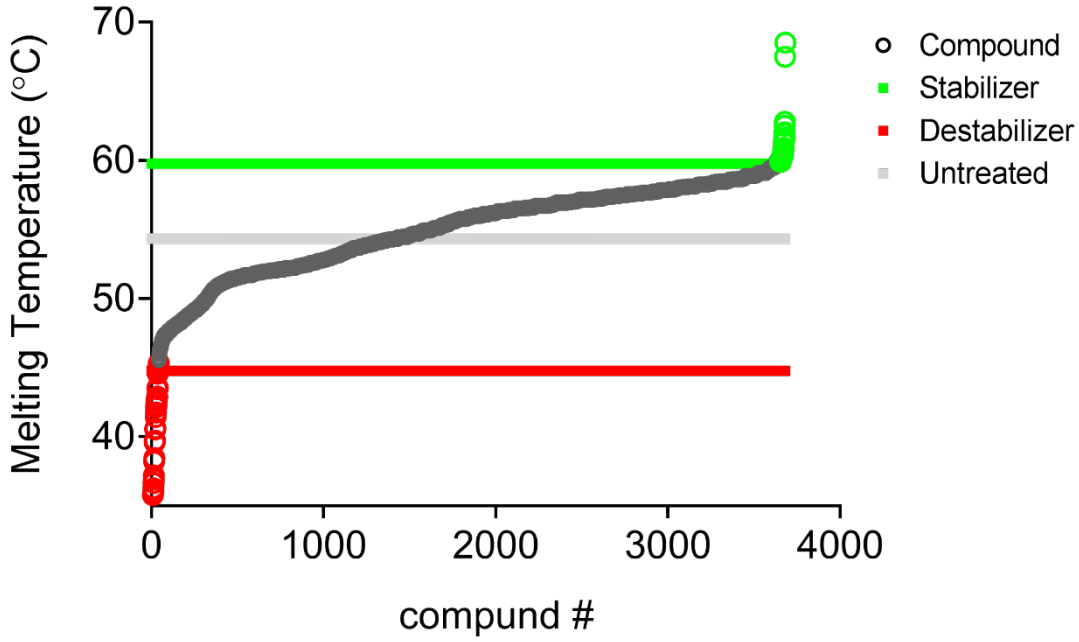


Figure S1: Results of 3682 pure natural products screened *via* DSF to identify modulators of pre-miR-21 thermal stability *in vitro*, related to Figure 1. A stabilizing control (spermidine, green) and destabilizing control (methoctramine, red) were used to calculate statistical significance to define hits. Compounds in green are stabilizing hits (increased melting temperature) while compounds in red had a destabilizing effect (decreased thermal temperature). A total of 32 compounds were found to significantly modulate pre-miR-21 thermal stability *in vitro*.

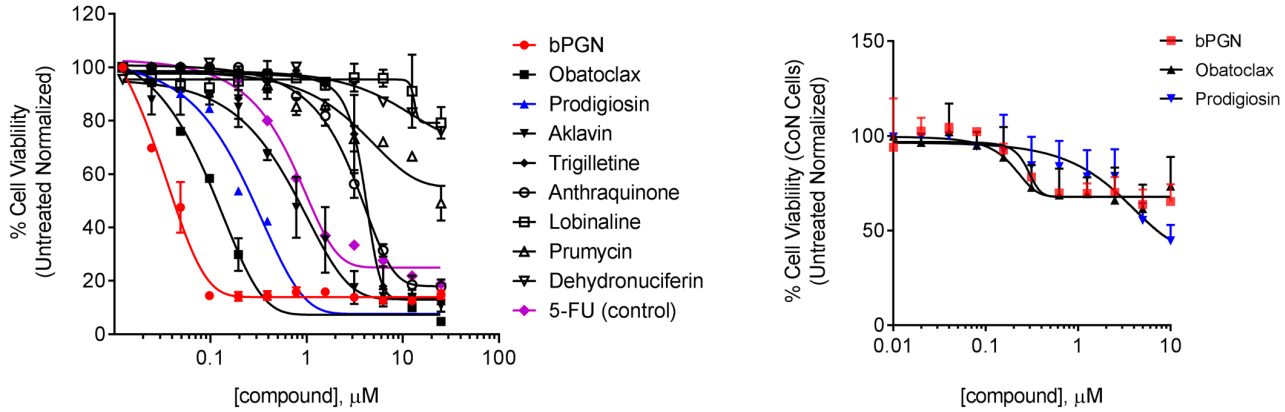
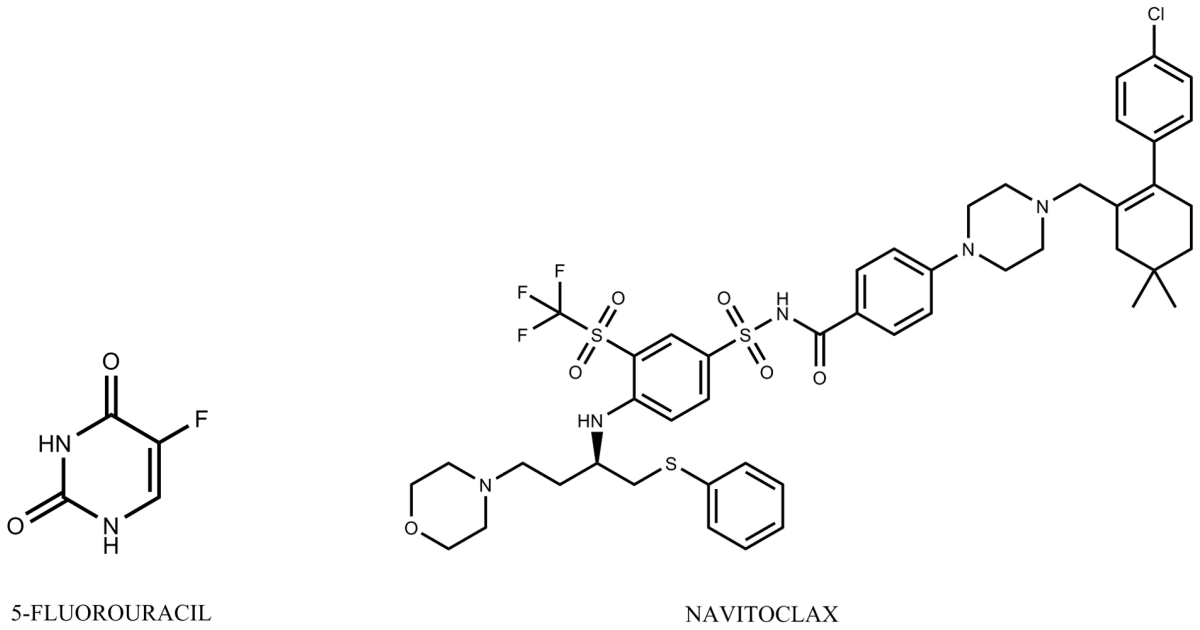
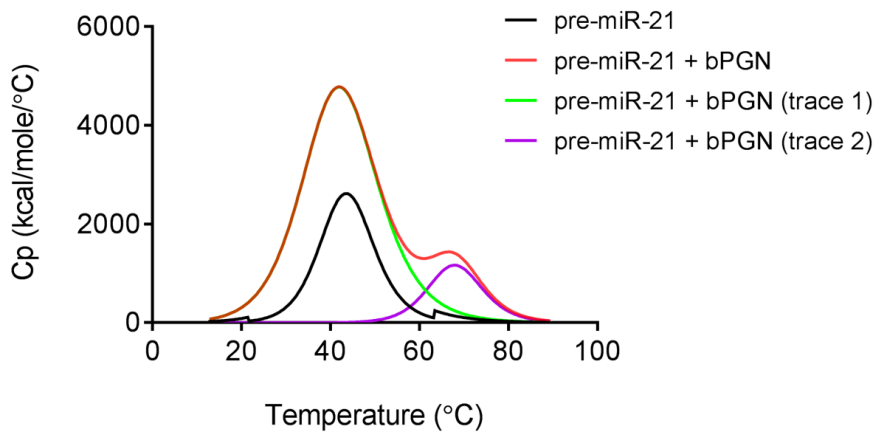


Figure S2: **Cytotoxicity data on select pre-miR-21 modulating compounds, related to Table 1.** HCT-116 cells were treated with 9 DSF-active compounds (0-25 μM) to determine the 50% minimum growth inhibitory concentration (GI_{50}) using a XTT cell viability assay. 5-FU was used as a control. Normal colon cells (ATCC CRL-1790) were also treated with **1**, **2** and **3** ranging from 0 - 10 μM to determine the GI_{50} using the XTT cell viability assay. All experiments were performed in triplicate.



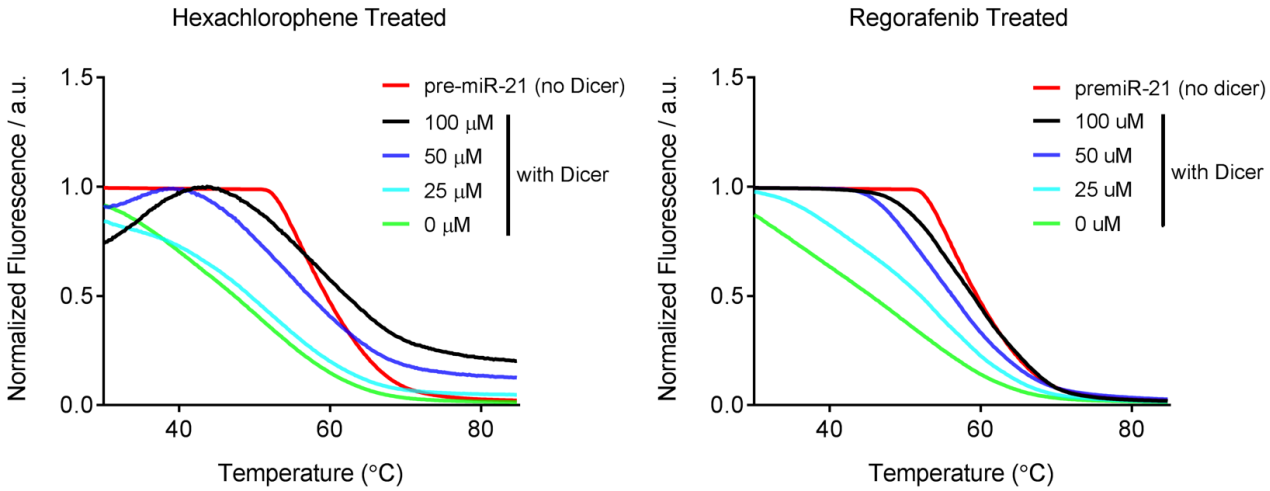
5-FLUOROURACIL NAVITOCLAX

Figure S3: **Chemical structures of control compounds 5-fluorouracil (5-FU) and navitoclax, related to Figure 1 and Table 1.**



	T_m (°C)	ΔHcal (kcal/mol)	ΔHv (kcal/mol)
pre-miR-21	43.77 ± 0.1	42.6 ± 0.5	49.0 ± 0.8
pre-miR-21 (trace 1)	42.3 ± 0.07	108.1 ± 0.8	34.9 ± 0.3
pre-miR-21 (trace 2)	68.0 ± 0.22	19.1 ± 0.7	56.5 ± 2.3

Figure S4: **Differential scanning calorimetry of pre-miR-21 (30 µM) with or without bPGN (300 µM)**, r**elated to Figure 1.** Two distinct thermal curves (trace 1 and 2) are observed after treatment with **1**, indicating the presence of two major species of pre-miR-21.



	SAMPLE	T_m (°C)		
10U DICER			Hexachlorophene	Regorafenib
NO	miR-21	52.4 ± 0.4		
	pre-miR-21	59.7 ± 0.7	59.7 ± 0.7	59.6 ± 0.4
YES	0 μM compound		46.7 ± 0.8	43.9 ± 0.6
	25 μM compound		49.2 ± 0.1	51.3 ± 0.3
	50 μM compound		56.3 ± 0.5	56.1 ± 0.3
	100 μM compound		61.5 ± 0.7	58.9 ± 0.5

Figure S5: **Hexachlorophene and regorafenib were previously identified as Dicer inhibitors, related to Figure 3.** Here, we performed a DSF based assay to determine if Dicer (10U) processing of pre-miR-21 (10 μM) is inhibited in the presence of hexachlorophene and regorafenib. The reaction was incubated in 37 ^{o}C up to 36 hr, after which RNA was purified and assayed using DSF at 1 μM final RNA concentration.

Supplemental Tables and Legends

	NSC ID#	Assigned Name	ΔT_m	Effect
1	38270	Olivomycin	-15.8	Destabilizer
2	3590	Calcium leucovorin	-14.0	Destabilizer
3	18355	2-Pyridin-3-ylacetohydrazide chloride	10.4	Stabilizer
4	70664	Heptopyranosylamine	-11.4	Destabilizer
5	76022	Taspine acetate	-11.1	Destabilizer
6	86005	Nogalarol	-15.7	Destabilizer
7	100290	Aklavin	-17.1	Destabilizer
8	106995	Lomofungin	8.62	Stabilizer
9	107067	Tirandamycin	3.69	Stabilizer
10	247562	Butylcycloheptyl prodiginine hydrochloride	3.38	Stabilizer
11	278619	Prumycin hydrochloride	8.41	Stabilizer
12	356217	Harmine derivative	-10.8	Destabilizer
13	692259	tetradecahydropicene-4a-carboxylic acid	5.54	Stabilizer
14	698298	Schweinfurthin C	4.16	Stabilizer
15	785154	dehydronuciferine	-15.7	Destabilizer
16	78571	Tris(3-methyl-1H-indole-2-yl)methane	6.94	Stabilizer
17	99A051A	Eilatin	7.78	Stabilizer
18	99B014A	Isobatzelline C	-18.1	Destabilizer
19	99B072A	3,4,5-tri-O-galloylquinic acid	4.45	Stabilizer
20	99B102A	Morin	-17.4	Destabilizer
21	99B137A	Aclacinomycin A	-16.9	Destabilizer
22	99B140A	Spectinabilin	-15.4	Destabilizer
23	49842	Vinblastine sulfate	-10.2	Destabilizer
24	65104	Septacidin	6.53	Stabilizer
25	85700	5-(2-(1,6-Dimethyl-1lambda(5)-quinolin-2-yl)vinyl)-8-quinolinol	-9.58	Destabilizer
26	102815	7-O-Methyl-epi-nogalarol	-17.6	Destabilizer
27	109444	Lobinaline hydrochloride	-14.0	Destabilizer
28	174280	Sodium butyrate	-32.6	Destabilizer
29	181486	trigilletine	5.48	Stabilizer
30	196524	eta-Rhodomycinone	-17.0	Destabilizer
31	324368	ALP	-12.1	Destabilizer
32	331757	anthraquinone hydrochloride	8.71	Stabilizer

Table S1: **List of 32 active natural products identified from the 3682 natural product compounds screened *via* DSF for modulators of pre-miR-21 thermal stability *in vitro*. Related to Table 1.**

Genes	log2 Fold Change	p-values
ACVR1C	2.09	0.0439
ACVR2A	0.846	0.000714
BCL2	0	
CDC25A	-2.16	1.29E-06
CDK6	0.878	0.00239
CDKN2C	-1.79	0.000103
DUSP8	2.18	3.30E-06
EFNA2	-0.339	0.0333
FGF18	0	
GNG12	0.34	0.00837
GPC4	0	
HGF	1.45	0.00968
IL12A	0.876	0.000189
IL6R	0.425	0.0389
JAG1	0.204	0.0419
LIFR	2.26	0.000639
MAP3K1	0.659	0.0149
MAP3K8	2.07	0.00542
MAPK1	0.158	0.0118
MAPK10	1.26	0.0367
MSH2	-0.665	0.0234
MYC	-0.298	0.00686
PBRM1	0	
PIK3R1	1.1	0.0102
PLD1	1.1	0.00368
PPP3CA	0.429	0.0256
PTEN	1.15	0.000537
RAD21	-0.556	0.0415
RASGRP1	1.32	0.00164
SKP2	-1.77	2.53E-06
SPRY1	0	
SPRY2	0.292	0.0465
SPRY4	0.669	6.06E-05
STAG2	0.875	0.00504
STAT3	0.823	2.14E-05
TGFB1	0.171	0.0208
TGFB2	0	
TGFBR2	0.255	0.0203
VHL	0.572	0.000286
WNT2B	2.35	0.000213
SOS2	1.87	2.43E-05

Table S2: **41 predicted miR-21 target genes annotated from NanoString analysis. Related to Figure 5.** Most notably, miR-21 target genes that are reported to play a role in suppressing cellular proliferation such as *PTEN*, *STAT3*, and *SPRY2* were all significantly upregulated by bPGN.