[01583] The amount of phospho-ERK was determined using an antibody specific for the phosphorylated form of ERK and compared to the amount of GAPDH. Primary antibodies used for the detection were added as follows: Phospho-ERK(Cell Signaling cs-9101) diluted 1:500 and GAPDH(Millipore MAB374) diluted 1:5000 in Licor block + 0.05%Tween 20. The plates were incubated for 2 hours at room temperature. The plates were washed with PBS + 0.05% Tween 20.

[01584] Secondary antibodies used to visualize primary antibodies were added as follows: Anti-rabbit-680 diluted 1:1000 and Anti-mouse-800 diluted 1:1000 both in Licor block +0.05% TweeN_20, and were incubated for 1 hour at room temperature. The plates were washed with PBS +0.05% Tween 20. A 100 µL aliquot of PBS was added to each well and the plates were read on a Li-Cor Odyssey CLX plate reader.

[01585] The phospho-ERK(Thr202/Tyr204 signal was normalized to the GAPDH signal for each well and percent of DMSO control values were calculated. IC50 values were generated using a 4-parameter fit of the dose response curve

[01586] The results for exemplary compounds of Formula (I) are shown in Table 3. ND is not determined.

Table 3

Inhibition of KRas G12D-mediated Phosphorylation of ERK by Exemplary Compounds of Formula (I)

Example No.	IC_{50} (nM)	Example No.	IC_{50} (nM)
1	3159.1	234	409.0
2	721.4	235	338.6
3	10283.1	236	2626.3
4	8530.0	238	4656.9
5	8193.8	239	2951.1
6	11518.2	240	>16666.7
10	8115.5	241	>16666.7
11	1078.2	242	>16666.7
12	4905.2	243	0.8
14	4843.5	244	763.0
15	>16666.7	245	23.4
16	3834.5	246	1.0
18	3742.5	247	4310.5
19	>16666.7	248	1082.8

22	1888.7	249	7705.6
24	2890.0	250	7477.7
26	4282.7	251	10.3
27	1275.8	252	0.8
28	11736.0	253	2.0
29	13630.9	254	2428.8
30	9600.4	255	673.4
31	2425.6	256	>16666.7
32	>50000	257	530.7
33	2052.1	258	1460.4
34	4207.8	259	3.9
35	11332.1	260	>16666.7
36	107.8	263	7641.0
40	4241.8	274	4706.4
41	6647.2	275	7058.7
42	637.6	276	3789.2
43	6204.1	277	3675.0
44	2290.1	278	104.4
45	840.0	279	7401.8
47	4445.6	280	6115.7
48	4875.6	281	97.8
49	2123.1	282	4.9
50	15404.5	283	7.0
51	12066.6	284	17.4
52	8380.0	286	1733.1
54	>16666.7	287	8067.4
60	>16666.7	290	110.5
63	186.7	291	46.5
64	3971.0	292	1696.0
65	1332.6	296	8472.0
68	7530.9	297	6562.0
69	>16666.7	298	1228.7
71	>16666.7	299	>5555.6
72	7260.8	301	3173.2
73	>5555.6	302	1993.3
74	5465.1	304	1415.1
75	>16666.7	308	>5555.6
76	7412.9	312	1877.6
78	>16666.7	314	3.7
81	8288.3	321	>16666.7
82	>16666.7	322	664.8
84	5722.3	324	863.9
85	>16666.7	325	>16666.7
87	10553.8	326	121.6
91	5514.1	327	31.4

93	>16666.7	328	896.9
98	388.6	330	11.8
102	7955.9	331	10826.0
103	>16666.7	332	>16666.7
104	>16666.7	334	2909.3
106	3096.3	335	10440.5
109	330.5	336	1057.2
110	>16666.7	337	1620.7
114	10259.6	338	>16666.7
115	9514.0	339	8659.5
118	>16666.7	340	51.5
119	3700.2	341	837.3
120	2092.9	342	1001.2
121	4077.1	343	1616.5
122	>16666.7	344	8069.6
124	11087.4	345	1468.2
126	3470.6	346	1351.5
127	>16666.7	347	458.9
131	>16666.7	348	778.6
132	2558.7	351	>16666.7
133	2048.8	356	11920.1
134	895.4	360	8353.4
135	4820.9	362	10457.0
139	5245.1	364	322.0
141	>16666.7	365	3949.0
152	>50000	366	7.0
153	2369.2	367	111.8
156	1786.9	370	10730.6
157	7998.7	372	10.5
158	4222.3	373	>16666.7
159	>16666.7	375	1310.3
160	6930.7	376	582.3
161	>16666.7	380	>16666.7
162	>16666.7	381	708.4
163	>16666.7	382	>16666.7
164	>16666.7	384	398.2
165	11068.5	385	>16666.7
167	16142.4	386	320.3
168	>16666.7	388	40.2
173	10504.3	389	780.7
174	12816.8	390	6108.7
175	>5555.6	393	7579.3
178	>16666.7	395	1089.1
179	71.2	396	8173.3
180	>16666.7	397	>16666.7

181	12839.1	399	509.9
182	13549.2	400	260.6
183	5252.5	401	603.5
185	7.1	403	2881.0
186	3383.1	404	8331.6
187	450.1	405	297.0
189	24.1	406	440.3
190	16314.3	408	238.0
191	5047.0	410	8.0
193	8517.9	411	745.3
195	2461.5	412	4779.3
197	>16666.7	413	2156.3
198	2425.5	418	2741.5
199	464.8	419	2776.1
201	13915.2	420	7816.4
202	10186.8	422	2357.8
203	2183.2	424	680.9
204	306.7	425	5414.1
205	242.1	426	228.0
206	899.6	427	1981.7
208	>16666.7	428	6964.4
209	>16666.7	429	1696.0
210	>16666.7	430	141.0
211	13506.7	431	74.3
214	>16666.7	432	106.3
215	>16666.7	433	43.2
216	51.0	434	6.7
219	2032.0	435	1822.4
220	1344.9	436	3989.5
221	15840.8	438	1630.8
223	4272.7	439	12.8
224	6375.8	440	8.4
225	3058.3	441	4.1
226	6745.4	442	1162.0
227	2976.0	443	160.0
228	13718.5	444	365.0
229	11330.3	445	130.0
231	2806.1	451	78.3
232	15.2	452	13.7
233	15568.1		