Screening a Kinase Inhibitor Library for Mitochondrial Dysfunction

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KEY FINDING

Mitochondrial toxicity from compound exposure can be pinpointed with coordinated OCR/ECAR analysis and mechanism of action assays.

Identifying Mitochondrial Toxicity

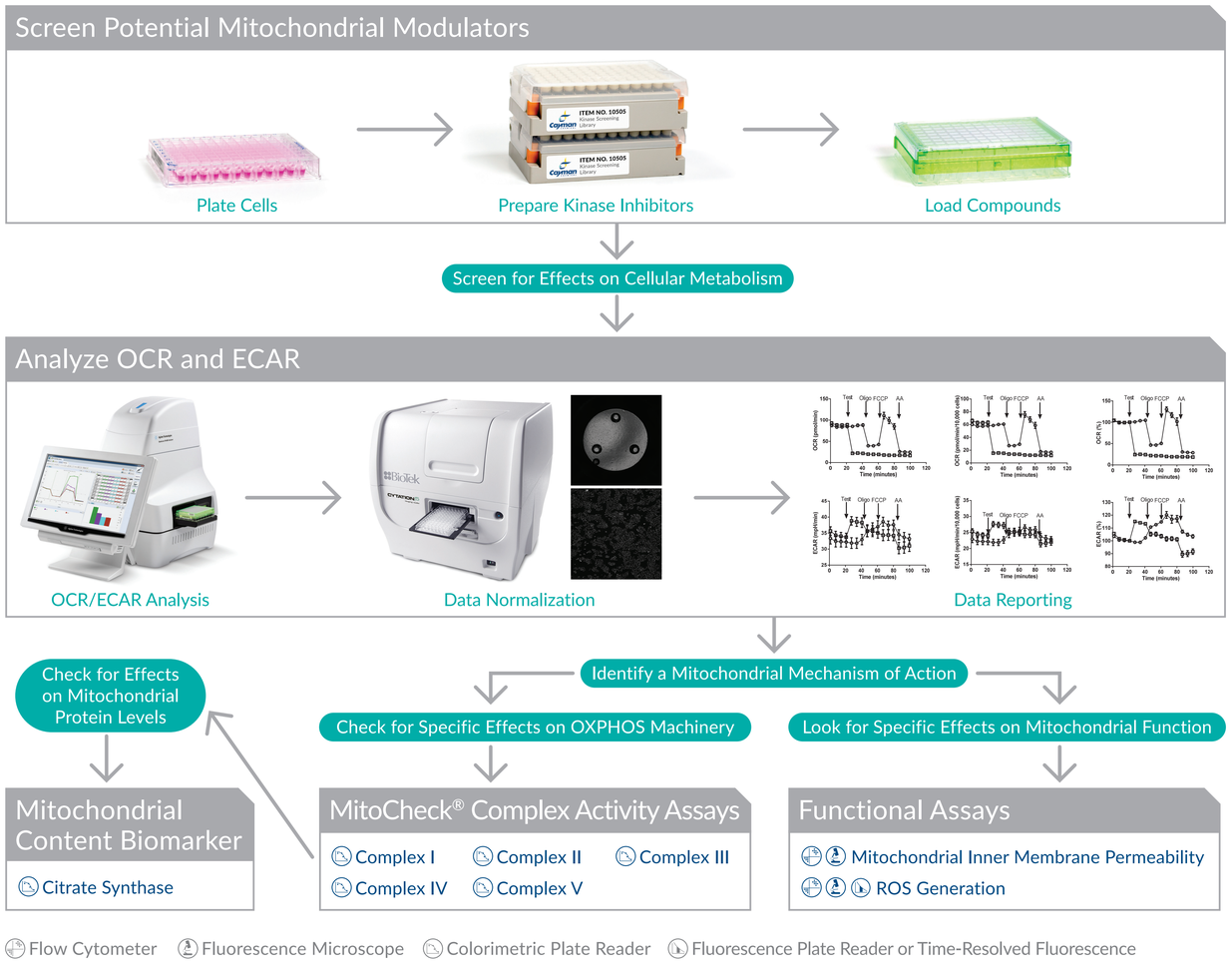
Mitochondria, in addition to their role as primary producers of ATP, play several important roles in maintaining cellular homeostasis. For the mitochondrion to maintain this role, proper function is essential. The bulk of the biochemical processes occurring in the mitochondrion are dependent on a chemiosmotic proton gradient, which is generated and maintained by the oxygen-consuming electron transport chain (ETC) in combination with a proton impermeable inner mitochondrial membrane (IMM). It is because of this dependency that any perturbations to the ETC, IMM, or the machinery that utilizes the proton gradient can have severe consequences on the cell as a whole.

Several kinase inhibitors released by the FDA have been given black box warnings for cardio- and hepatotoxicity due to mechanisms resulting from mitochondrial dysfunction.[1] Therefore, mechanisms used to screen for mitochondrial toxicity in a high-throughput manner are rapidly becoming more and more valuable.

1. Zhang, J., Salminen, A., Yang, X., *et al.* Effects of 31 FDA approved small-molecule kinase inhibitors on isolated rat liver mitochondria. *Arch. Toxicol.* **91(8)**, 2921-2938 (2017).

Screening Workflow

A library of kinase inhibitors (Cayman Item No. 10505) was analyzed for mitochondrial toxicity effects within human hepatocarcinoma cells (Huh7) utilizing the Agilent Seahorse XF96 and the BioTek Cytation™ 5 Cell Imaging Multi-Mode Reader. Compounds were screened at a concentration of 1 µM with the goal of identifying kinase inhibitors that cause mitochondrial dysfunction by affecting oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Once identified, the mechanism of toxicity can be further elucidated by measuring the effects of these compounds on ETC function in isolated mitochondria using Cayman's MitoCheck® mitochondrial function assays.

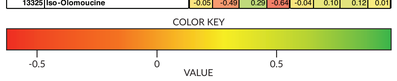


Compound Heat Map

Item No.	Product Name	OCR Test	OCR Oligo	OCR FCCP	OCR AA	ECAR Test	ECAR Oligo	ECAR FCCP	ECAR AA
10398	TG003	0.04	-0.47	0.03	-0.79	-0.03	0.14	0.13	0.04
10459	PKC 412	0.00	-0.51	0.15	-0.79	-0.03	0.12	0.10	0.03
10460	Doramapimod	0.08	-0.49	0.35	-0.70	-0.02	0.13	0.15	0.01
10461	Paclitaxel	0.02	-0.52	-0.24	-0.75	0.00	0.14	0.13	0.04
10483	Erlotinib	0.05	-0.50	-0.27	-0.73	-0.04	0.15	0.11	0.03
10565	NVP-BEZ235	-0.01	-0.54	0.14	-0.75	-0.01	0.13	0.12	0.02
10735	Phthalazinone pyrazole	0.04	-0.50	0.35	-0.71	-0.01	0.17	0.17	0.04
10793	AG-879	0.72	0.57	0.10	-0.72	0.41	0.13	0.06	-0.08
10954	1-NA-PP1	0.06	-0.49	0.37	-0.65	-0.01	0.15	0.16	0.03
10997	Torin 1	0.01	-0.57	0.20	-0.74	-0.05	0.08	0.09	-0.01
11022	BIBF 1120	0.09	-0.46	0.29	-0.67	-0.04	0.13	0.15	0.02
11029	SMI-4a	0.15	-0.41	-0.33	-0.72	0.03	0.16	0.16	0.03
11140	CAY10657	0.01	-0.53	0.20	-0.74	0.00	0.17	0.18	0.10
		0.09	-0.47	0.36	-0.70	-0.02	0.14	0.14	0.04
11314	Chelerythrine (chloride)	0.16	-0.44	0.58	-0.69	-0.03	0.14	0.17	0.02
11445	Tunicamycin	0.04	-0.44	0.32	-0.64	-0.02	0.12	0.14	0.01
11491	AZD 7762	0.05	-0.55	0.50	-0.73	-0.06	0.02	0.08	-0.07
11569	GSK1059615	-0.04	-0.57	0.13	-0.74	-0.03	0.07	0.15	-0.01
11609	Ruxolitinib	0.04	-0.49	0.35	-0.72	-0.04	0.14	0.13	0.01
11658	Necrostatin-1	0.03	-0.42	0.10	-0.72	-0.03	0.10	0.10	-0.04
11793	SB-505124	-0.02	-0.50	-0.26	-0.70	-0.02	0.12	0.13	0.03
11811	INK128	0.16	-0.52	-0.27	-0.72	-0.03	0.05	0.05	-0.03
12076	Canertinib (hydrochloride)	0.09	-0.49	0.26	-0.70	-0.04	0.11	0.11	0.03
13031	SB-431542	-0.01	-0.14	-0.21	-0.27	-0.05	-0.07	-0.06	-0.11
13032	PD 173074	0.06	-0.51	0.04	-0.69	-0.05	0.02	0.05	-0.02
13033	Valproic Acid (sodium salt)	0.01	-0.53	0.18	-0.74	0.02	0.16	0.17	0.09
13034	PD 0325901	0.04	-0.48	0.07	-0.68	0.01	0.14	0.14	0.00
13067	SB 203580	0.00	-0.51	0.13	-0.71	-0.02	0.15	0.14	0.06
13108	VX-702	0.05	-0.50	0.22	-0.70	0.00	0.14	0.13	0.04
13109	Emodin	0.11	-0.51	-0.62	-0.60	0.00	0.09	0.07	-0.05
13122	CHIR99021	0.08	-0.49	-0.25	-0.71	-0.02	0.12	0.11	-0.01
13123	BIO	0.03	-0.51	0.24	-0.71	0.00	0.16	0.16	0.01
13139	Imatinib (mesylate)	0.02	-0.53	0.18	-0.71	-0.06	0.09	0.11	-0.04
13159	Sunitinib (malate)	0.06	-0.48	0.11	-0.67	-0.02	0.10	0.12	0.00
13166	Gefitinib	0.03	-0.52	0.18	-0.72	0.00	0.14	0.15	0.03
13198	PP2	0.01	-0.50	0.06	-0.74	-0.01	0.11	0.11	-0.01
13242	3-Methyladenine	0.07	-0.50	0.15	-0.72	0.01	0.16	0.15	0.02
13298	Bisindolylmaleimide I	0.11	-0.40	0.17	-0.58	0.00	0.09	0.10	-0.05
13299	Bisindolylmaleimide IV	0.10	-0.37	0.09	-0.53	-0.04	0.07	0.06	-0.04
13300	Bisindolylmaleimide V	0.05	-0.32	-0.05	-0.58	-0.01	0.12	0.09	0.01
13303	NSC 663284	-0.02	-0.46	0.36	-0.60	0.00	0.13	0.14	0.05
13305	D 4476	-0.01	-0.25	0.07	-0.32	-0.03	0.09	0.10	0.03
13308	NU 7026	-0.02	-0.48	0.28	-0.68	-0.01	0.12	0.13	0.03
13311	Gö 6983								
13312	H-9 (hydrochloride)	-0.02	-0.44	0.42	-0.57	-0.01	0.14	0.15	0.07
13314	Indirubin-3'-monoxime	-0.01	-0.47	0.34	-0.61	-0.01	0.10	0.11	0.01
13317	NU 6102	-0.03	-0.47	0.35	-0.59	-0.02	0.12	0.13	0.03
13318	KN-62	-0.01	-0.33	0.20	-0.61	-0.01	0.11	0.11	0.00
13319	KN-93	-0.07	-0.49	0.31	-0.63	0.01	0.14	0.15	0.04
13322	CGP 57380	-0.07	-0.46	0.42	-0.56	-0.01	0.14	0.15	0.05
13325	Iso-Olomoucine	-0.05	-0.49	0.29	-0.64	-0.04	0.10	0.12	0.01

Item No.	Product Name	OCR Test	OCR Oligo	OCR FCCP	OCR AA	ECAR Test	ECAR Oligo	ECAR FCCP	ECAR AA
13332	(S)-Glycyl-H-1152 (hydrochloride)	-0.04	-0.47	0.16	-0.61	0.01	0.11	0.12	0.03
13333	Bisindolylmaleimide VII (acetate)	0.01	-0.45	0.12	-0.58	0.00	0.06	0.04	-0.06
13334	Bisindolylmaleimide IX (mesylate)	0.01	-0.41	0.21	-0.50	-0.02	0.05	0.04	-0.06
13337	ST638	-0.05	-0.50	0.30	-0.66	0.02	0.13	0.18	0.08
13338	SU6656	-0.03	-0.47	0.21	-0.60	-0.12	0.02	0.03	-0.07
13341	LY364947	-0.05	-0.45	0.31	-0.58	-0.05	0.07	0.09	0.00
13344	SB 203580 (hydrochloride)	-0.06	-0.48	0.23	-0.60	0.01	0.11	0.12	0.04
13371	CAY10621	-0.02	-0.48	0.26	-0.62	-0.03	0.11	0.11	0.01
13576	YM-201636	0.03	-0.44	0.31	-0.53	-0.16	-0.02	0.03	-0.11
13601	ZM 447439	-0.03	-0.47	0.08	-0.62	-0.04	0.11	0.13	0.02
13622	AS-041164	-0.04	-0.51	0.14	-0.69	-0.04	0.10	0.12	0.00
13641	NVP-AEW541 (hydrochloride)	-0.03	-0.48	-0.17	-0.62	-0.01	0.07	0.08	0.00
13643	PP242	-0.03	-0.51	0.10	-0.61	0.02	0.09	0.09	0.03
13653	ABT-869	-0.02	-0.47	0.14	-0.60	0.02	0.13	0.11	0.04
13687	CAY10622	-0.04	-0.47	0.18	-0.60	-0.02	0.10	0.11	0.02
13812	17β-hydroxy Wortmannin	-0.04	-0.49	0.14	-0.63	0.01	0.13	0.13	0.04
13838	CAY10626	-0.05	-0.50	0.07	-0.62	-0.01	0.09	0.08	0.02
13873	SU 6668	-0.04	-0.48	0.20	-0.60	-0.05	0.03	0.01	-0.08
18218	PHA-767491	-0.02	-0.46	0.14	-0.59	0.00	0.12	0.12	0.02
62575	N,N-Dimethylsphingosine (d18:1)	-0.01	-0.43	0.17	-0.56	-0.01	0.13	0.14	-0.01
70920	LY294002	-0.02	-0.48	0.14	-0.64	-0.02	0.14	0.12	0.00
70970	U-0126	-0.02	-0.44	0.14	-0.57	0.02	0.13	0.12	0.01
81590	Staurosporine	-0.01	-0.44	0.32	-0.56	0.01	0.19	0.27	-0.02
		-0.01	-0.46	0.23	-0.60	-0.01	0.14	0.14	0.02
9000980	AS-605240 (potassium salt)	-0.01	-0.45	0.15	-0.59	-0.02	0.09	0.13	-0.01
10004914	O-1918	-0.03	-0.46	0.17	-0.59	0.00	0.16	0.15	0.04
10005583	Y-27632 (hydrochloride)	-0.06	-0.44	0.17	-0.57	-0.02	0.12	0.12	0.00
10006148	Leelamine	-0.03	-0.44	0.28	-0.57	-0.03	0.12	0.13	-0.01
10006726	PD 98059	0.09	-0.46	-0.04	-0.70	-0.08	0.10	0.12	-0.05
10006727	PD 169316	-0.03	-0.50	0.08	-0.71	-0.01	0.21	0.28	0.11
10007349	TGX-221	0.01	-0.50	-0.02	-0.71	-0.06	0.13	0.12	-0.04
10007653	(S)-H-1152 (hydrochloride)	0.00	-0.56	0.06	-0.80	-0.04	0.14	0.17	-0.04
10007707	AS-605240	0.00	-0.53	0.05	-0.73	-0.05	0.12	0.18	-0.05
10007907	Sphingosine (d18:1)	0.01	-0.57	-0.02	-0.78	-0.03	0.12	0.11	-0.09
10008131	JNJ-10198409	0.00	-0.50	0.19	-0.71	-0.03	0.13	0.15	-0.05
10008614	Leelamine (hydrochloride)	-0.02	-0.51	0.06	-0.72	-0.03	0.14	0.14	-0.05
		-0.03	-0.51	0.08	-0.70	-0.03	0.15	0.16	-0.05
10008618	Lauric Acid Leelamide	-0.01	-0.50	0.11	-0.71	-0.02	0.18	0.14	-0.02
10009052	AS-252424	-0.02	-0.53	-0.08	-0.73	-0.17	-0.02	0.04	-0.15
10009078	CAY10505	0.03	-0.47	0.08	-0.72	0.01	0.11	0.17	0.00
10009209	PI-103	-0.01	-0.58	-0.01	-0.81	-0.03	0.10	0.14	0.04
10009210	PIK-75 (hydrochloride)	-0.02	-0.52	0.00	-0.71	0.00	0.13	0.13	-0.01
10009222	Sphingosine Kinase Inhibitor 2	-0.01	-0.51	-0.04	-0.72	0.01	0.18	0.18	0.01
10009366	Piceatannol	-0.01	-0.51	0.07	-0.72	-0.07	0.11	0.11	-0.05
10009557	SC-1	-0.09	-0.56	-0.13	-0.73	0.00	0.12	0.14	-0.06
10009569	(R)-Roscovitine	-0.01	-0.51	0.04	-0.72	-0.03	0.15	0.15	-0.04
10009644	Sorafenib	0.00	-0.51	0.02	-0.74	0.00	0.14	0.13	-0.04
10010043	CAY10561	0.00	-0.45	0.19	-0.64	-0.04	0.18	0.18	-0.02
10010175	AS-604850	0.08	-0.45	0.00	-0.71	-0.01	0.13	0.17	0.01
10010177	PI3-Kinase α Inhibitor 2	0.00	-0.53	-0.01	-0.72	-0.03	0.12	0.14	0.02
10010233	CAY10567	0.00	-0.51	-0.08	-0.72	0.00	0.16	0.16	0.03
10010236	ML-9	-0.03	-0.51	0.05	-0.71	-0.02	0.14	0.16	0.00

Item No.	Product Name	OCR Test	OCR Oligo	OCR FCCP	OCR AA	ECAR Test	ECAR Oligo	ECAR FCCP	ECAR AA
10010237	Tricirribine	0.00	-0.51	-0.05	-0.71	0.02	0.17	0.14	0.01
10010238	Erbstatin analog	-0.02	-0.50	-0.09	-0.71	0.00	0.16	0.19	0.06
10010239	Kenpaullone	0.00	-0.49	-0.03	-0.70	-0.02	0.15	0.16	0.01
10010240	Olomoucine	-0.05	-0.51	-0.17	-0.69	-0.03	0.13	0.15	0.01
10010242	AG-494	0.00	-0.53	-0.02	-0.72	-0.02	0.09	0.06	-0.08
10010243	AG-825	-0.05	-0.52	-0.06	-0.74	-0.05	0.08	0.10	-0.05
10010244	AG-1478	-0.01	-0.50	-0.07	-0.70	-0.03	0.11	0.15	-0.05
10010246	SB-216763	-0.03	-0.53	-0.07	-0.74	-0.05	0.12	0.14	-0.04
10010247	SB-415286	-0.03	-0.51	0.01	-0.70	-0.01	0.12	0.16	-0.02
10010248	AG-17	0.10	-0.27	-0.39	-0.73	0.18	0.10	0.08	-0.10
10010249	H-8 (hydrochloride)	0.06	-0.39	-0.12	-0.55	0.09	0.21	0.26	0.04
10010265	LFM-A13	-0.01	-0.48	-0.01	-0.67	-0.01	0.16	0.16	0.00
10010267	SC-514	0.04	-0.49	-0.03	-0.67	-0.02	0.16	0.15	-0.02
10010275	Apigenin	0.00	-0.50	-0.04	-0.67	-0.03	0.16	0.15	-0.02
10010300	AG-18	-0.01	-0.49	-0.02	-0.68	-0.03	0.03	0.04	-0.13
10010302	DRB	-0.02	-0.52	-0.03	-0.75	-0.02	0.15	0.15	0.00
10010309	RG-13022	0.05	-0.48	0.08	-0.67	-0.03	0.08	0.06	-0.06
10010310	RG-14620	0.04	-0.46	0.03	-0.66	-0.01	0.10	0.12	-0.01
10010311	AG-490	-0.01	-0.50	0.07	-0.69	-0.01	0.10	0.09	-0.05
10010312	AG-82	-0.02	-0.48	0.14	-0.69	-0.06	0.01	0.00	-0.16
10010313	AG-99	-0.01	-0.51	0.07	-0.72	0.01	0.10	0.09	-0.05
10010314	AG-213	-0.03	-0.53	0.14	-0.73	-0.08	-0.01	-0.02	-0.16
10010315	AG-183	-0.05	-0.56	0.01	-0.77	-0.07	-0.10	-0.17	-0.34
10010329	Lavendustin C	-0.01	-0.51	0.11	-0.70	0.01	0.12	0.14	0.00
		-0.01	-0.52	0.12	-0.72	0.01	0.14	0.15	-0.01
10010375	5-Iodotubercidin	-0.07	-0.55	0.09	-0.76	0.01	0.14	0.13	-0.04
10010399	SB 202190	-0.02	-0.52	0.13	-0.71	0.01	0.13	0.14	-0.01
10010400	CAY10571	-0.04	-0.51	0.00	-0.72	0.01	0.12	0.12	-0.02
10010422	Nilotinib	0.03	-0.49	0.08	-0.70	-0.08	0.05	0.07	-0.07
10010466	SP 600125	-0.03	-0.52	0.00	-0.72	-0.01	0.10	0.11	-0.02
10010541	L-threo-Sphingosine (d18:1)	-0.02	-0.52	0.14	-0.73	0.01	0.12	0.10	0.00
10010556	H-89 (hydrochloride)	-0.05	-0.53	0.05	-0.71	0.01	0.17	0.18	0.05
10010559	HA-1077 (hydrochloride)	-0.02	-0.48	0.09	-0.68	0.01	0.13	0.15	0.02
10010568	AG-370	0.00	-0.51	0.02	-0.72	-0.01	0.13	0.13	0.00
10010591	Wortmannin	-0.01	-0.51	0.08	-0.72	0.02	0.15	0.15	-0.01
10010592	AG-1296	-0.03	-0.52	0.02	-0.73	0.00	0.11	0.11	-0.04
10011246	WHI-P131	-0.03	-0.51	0.02	-0.71	-0.01	0.09	0.10	-0.02
10011247	CAY10574	-0.02	-0.51	0.02	-0.72	-0.01	0.10	0.12	-0.01
		-0.04	-0.53	-0.04	-0.74	-0.03	0.09	0.10	-0.01
10011249	CAY10576	-0.01	-0.48	0.04	-0.69	0.00	0.11	0.12	-0.02
		-0.03	-0.52	-0.02	-0.74	0.00	0.14	0.14	0.03
10011251	TWS119	0.00	-0.50	-0.06	-0.69	-0.02	0.09	0.09	-0.03
10011255	NSC 210902	-0.02	-0.51	-0.05	-0.72	0.00	0.14	0.14	0.01
10011256	CAY10577	-0.02	-0.49	-0.04	-0.69	0.01	0.14	0.15	0.00
10011264	CAY10578	-0.03	-0.51	0.04	-0.71	0.03	0.15	0.19	0.00
10012431	PD 184161	-0.02	-0.50	0.00	-0.70	0.00	0.10	0.10	-0.03
10012591	CCT018159	-0.01	-0.50	-0.03	-0.68	0.00	0.09	0.11	-0.06
10012600	Myricetin	-0.03	-0.52	-0.06	-0.74	0.01	0.12	0.12	-0.02
10527	Necrostatin-5	-0.03	-0.53	-0.06	-0.73	0.00	0.10	0.11	-0.06
10008005	OSU03012	-0.02	-0.50	0.01	-0.70	0.01	0.12	0.14	-0.03
10010301	CAY10554	-0.01	-0.52	-0.04	-0.73	0.02	0.13	0.14	0.00



Toxicity Hits

Compounds having a greater than 20% impact on baseline, or FCCP addition, were tagged as potential mitotoxins. Of the 150 compounds screened, 10 compounds exhibited characteristics associated with mitochondrial toxicity.

Item No.	Product Name	Mechanism	Mitochondrial Effect	Previously Known?
10461	Paclitaxel	Microtubule disruption	Inhibition	Yes
10483	Erlotinib	RTK inhibitor	Inhibition	No for HepG2
10793	AG-879	RTK inhibitor	Uncoupling	Yes
11029	SMI-4a	Ser/Thr kinase inhibitor	Inhibition	Yes
11793	SB-505124	TGFβ Ser/Thr kinase inhibitor	Inhibition	Yes
11811	INK128	TORC1/2 inhibitor	Inhibition	Yes
13031	SB-431542	ALK5 inhibitor	Inhibition	No
13109	Emodin	Casein kinase inhibitor	Inhibition	Yes
13122	CHIR99021	GSK3 inhibitor	Inhibition	Yes
10010248	AG-17	Protein tyrosine kinase inhibitor	Inhibition	Yes

Exploring Potential Mechanisms for Toxicity

All compounds that caused inhibition did so following the addition of the uncoupler FCCP. As a protonophore, FCCP uncouples oxidative phosphorylation, maximizing OCR, when titrated properly.[2] At high concentrations, FCCP is known to inhibit OCR. Because of this, treatment of cells with compounds could potentially lower the cellular tolerance of FCCP. To determine this, FCCP titrations were performed in the presence of these compounds. Additionally, a better tolerated uncoupler, such as BAM15 (Cayman Item No. 17811) can be substituted. Since inhibition was not observed prior to the addition of the complex V inhibitor oligomycin, it is unlikely that these compounds inhibit complex V. Measuring the effects of these compounds on ETC function in isolated mitochondria can be further carried out using Cayman's MitoCheck® mitochondrial function assays in order to pinpoint the site of inhibition.

2. Kenwood, B.M., Weaver, J.L., Bajwa, A., *et al.* Identification of a novel mitochondrial uncoupler that does not depolarize the plasma membrane. *Mol. Metab.* **3(2)**, 114-123 (2013).

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