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Synthesis and biological evaluation of novel 2-alkoxycarbonylallylester phosphonium derivatives as potential anticancer agents

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ABSTRACT

Several phosphonium derivatives have been synthesized from Baylis-Hillman (BH) reaction derived allyl bromides and aryl phosphines as mitochondria targeting anticancer agents. *In vitro* cell proliferation inhibition studies on various solid tumor cell lines indicate that most of the compounds exhibit IC_{50} values in μM concentrations. Further studies reveal that β-substituted BH bromide derived phosphonium derivatives enhance the biological activity to low μM IC_{50} values. *In vitro* metabolic studies show that the lead candidate compound **16** inhibits the production of mitochondrial ATP, increases the proton leak within the mitochondrial membrane and abolishes the spare respiratory capacity in a concentration dependent manner.

Cancer cells alter several energetic and biosynthetic pathways to meet the demands of uncontrolled and rapid cell proliferation. In fact, reprogrammed metabolism has been recognized as one of the critical hallmarks of many cancers[1]. Glycolysis and mitochondrial oxidative phosphorylation (OxPhos) are processes that produce the majority of cellular energy. Tumors characteristically exhibit elevated glycolysis even in the presence of sufficient amounts of oxygen[2–6]. Numerous recent studies have revealed the critical role of mitochondrial OxPhos in generating a large portion of ATP in cancer cell[7–13]. The glycolytic end product pyruvate is shuttled into the mitochondria and is converted to acetyl CoA to initiate the citric acid cycle. Additionally, glutamine and fatty acids are oxidized in the mitochondria as fuel substrates, and many of the OxPhos intermediates are utilized in the Kreb's cycle for the synthesis of fatty acids, amino acids, and nucleotides; highlighting a crucial role of the mitochondria in proliferation. Recent studies indicated that inhibition of mitochondrial function would lead to severe ATP depletion and dysfunction of the TCA cycle, starving cancer cells of critical components for cell survival and proliferation[7–13]. Apart from energy generation, mitochondria are also involved in the generation of reactive oxygen species, metabolite production, maintenance of intracellular Ca^{2+} homeostasis, modulation of cell death pathways, and regulation of signaling pathways linked to cell proliferation and differentiation. The multiple functions of mitochondria play a critical role in cancer cell survival, drug resistance, relapse, and metastasis[14–18]. Hence selective targeting of the mitochondria is an important therapeutic target for cancer treatment.

The mitochondrial membrane potential is established on the outer and inner membranes that shuttle several small molecules through these membranes. In highly oxidative cancer cells, the mitochondrial TCA cycle and electron transport chain operate at elevated levels resulting in proton gradients that offer a biophysical means of drug delivery to the mitochondrial matrix[19–22]. Due to this, the possibility of cations with large surface area to cross mitochondrial membranes and accumulate in the matrix is increased. Triphenylphosphonium (TPP^+) based cations have hydrophobic surfaces and can diffuse readily through the inner membrane. Lipophilic TPP^+ based small molecules sufficiently

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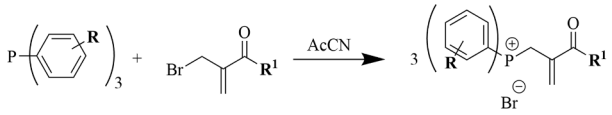
delocalize their positive charge across a large surface area and thus do not require any specific transporters for mitochondrial translocation[19–22]. In addition, TPP^+ cations have been shown to localize inside the mitochondria matrix compared to the cytoplasm, in some cases there is 1,000-fold concentration differences. These TPP^+ cations have also been extensively studied as mitochondrial targeting anticancer and other agents[19–22].

The Baylis-Hillman (BH) reaction is a well-established C—C bond forming reaction in organic chemistry to synthesize functionalized allyl alcohols and amines under simple reaction conditions[23–26]. The allyl alcohols can be readily converted into corresponding allyl bromides under standard bromination conditions. These bromides are known to undergo reactions with a wide variety of nucleophiles in S_N2 or S_N2' fashion to provide further functionalized synthetic intermediates[23–26]. We recently reported a protocol by converting BH bromides to their corresponding aryl acetates by reacting them with arylcarboxylic acids. Several of these aryl acetates showed significant *in vitro* cell proliferation inhibition properties against various cancer cells. We envisioned that the aryl acetates acted as a leaving group for cellular nucleophiles to provide the cytotoxicity[27]. Our long-standing interest in the development of novel small molecule anticancer agents[27–32] has prompted us to synthesize and evaluate novel functionalized phosphonium salts derived from BH bromides and aryl phosphines. We hypothesized that these novel phosphonium salts could be targeted to mitochondria to develop them as anticancer agents. Based on the reactivity of the alkoxycarbonylallyl ester group to cellular nucleophiles, we also envisioned that these agents would interact with mitochondrial nucleophiles to potentially disrupt mitochondrial integrity and energetics leading to inhibition of cancer cell proliferation.

To synthesize the aryl phosphonium salts, initially, we utilized BH reaction derived methyl 2-(bromomethyl)acrylate as a model substrate. The BH product was synthesized in two steps by the reaction of paraformaldehyde with methyl acrylate in the presence of DABCO followed by the bromination of resulting alcohol. The bromide was reacted with various mono-substituted triaryl phosphines including triphenyl, 4-methyl, 4-methoxy and 4-fluoro triaryl, tris(2-furyl) and cyclohexyl diphenyl phosphines. In all the cases, 2-(alkoxycarbonyl)-allyl-TPP derivatives **1**–**5** were obtained in good yields after purification by recrystallization (Scheme 1).

The synthesized phosphonium derivatives **1**–**5** were evaluated for their *in vitro* cell proliferation inhibition properties against several solid tumor cell lines by utilizing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay. Human breast cancer MCF7, human triple-negative breast cancer MDA-MB-231, human pancreatic cancer MiaPaCa-2, human colorectal adenocarcinoma WiDr, murine metastatic breast cancer 4T1, and murine breast cancer 67NR cell lines were utilized in this study. From these studies, we found that the tris(4-methylphenyl) phosphonium **2** and tris(4-methoxyphenyl) phosphonium **4** were more biologically potent on all the tested cells lines with IC_{50} values ranging from 1 to 10 μM (Table 1). The electron donating character of the tolyl and methoxy groups may stabilize the cation and enhance the ability of compounds **2** and **4** to diffuse down into the mitochondrial matrix, leading to higher mitochondrial accumulation and potency. A cyclohexyldiphenyl phosphonium synthesized from the corresponding phosphine and BH bromide showed relatively lower activity (9–67 μM) compared its triaryl derivatives. A tris(2-furyl) phosphonium derivative synthesized from tris(2-furyl) phosphine and BH bromide didn't show any significant activity even at 100 μM concentration indicating that this compound is not sufficiently lipophilic to diffuse into mitochondrial matrix.



R=H, R^1=OMe, **1** (76%), R=Me, R^1=OMe, **2** (79%), R=F, R^1=OMe, **3** (84%)
R=OMe, R^1=OMe, **4** (77%), R=OMe, R^1=O*t*Bu, **5** (82%), R=H, R^1=O*t*Bu, **6** (79%)
R=H, R^1=$N(CH_3)_2$, **7** (71%)

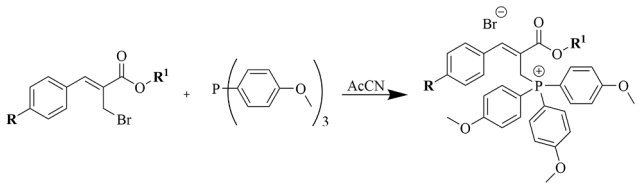
Scheme 1. Synthesis of 2-carbonyl-allyl phosphonium bromides.

Table 1
Cell proliferation inhibition (IC_{50}) values of Baylis-Hillman aryl phosphonium bromide salts (MTT assay). IC_{50} values reported as average values ± SEM from three separate experiments.

	Substituents		Cell line IC_{50} values (μM)					
No.	R	R^1	MCF-7	MDA-MB-231	MiaPaCa-2	WiDr	4T1	67NR
1	H	OMe	19.9±3.3	28.9±12.7	27.4±5.0	7.74±2.1	14.8±4.8	58.4±11.6
2	Me	OMe	2.43±0.4	3.79±0.2	2.66±0.2	1.48±0.3	1.78±0.4	5.34±1.2
3	F	OMe	47.0±5.6	91.1±2.8	>100	19.0±3.3	61.9±6.5	73.9±13.0
4	OMe	OMe	2.78±0.6	4.87±0.8	3.60±0.5	1.62±0.2	1.85±0.2	10.6±1.5
5	H	O*t* Bu	1.99±0.2	2.88±0.3	2.87±0.4	2.08±0.3	2.76±0.3	7.57±1.1
6	OMe	O*t* Bu	0.98±0.4	2.69±0.3	1.39±0.6	0.81±0.1	1.08±0.2	2.17±0.8
7	H	$N(CH_3)_2$	>100	>100	>100	81.2±7.0	>100	>100

To further understand the SAR of these compounds, the alkoxy ester group was modified by replacing the methyl group with metabolically more stable *t*Bu and *N,N*-dimethyl groups. The required BH bromides were synthesized via literature known protocols[28,33,34]. The reaction of these bromides with triphenyl phosphine in acetonitrile, followed by recrystallization in ether gave the corresponding phosphonium bromides **5**–**7** in good yields (Scheme 1). Cell proliferation inhibition studies of **5**–**7** showed an increase in potency with *t*Bu substitution compared to methyl group. The enhanced carbon content and lipophilic nature of the *t*Bu group may allow for better tissue diffusion and biological potency. Moreover, the *t*Bu ester is metabolically more stable to enzymatic hydrolysis than the methyl ester and is a preferred option over the methyl ester for *in vivo* studies. Surprisingly, the metabolically stable *N,N*-dimethyl amide derivative **7** did not show any significant activity even at high concentrations of 100 μM in all the tested cell lines (Table 1). This could be explained due to the lesser nucleophile accepting capability of alkyl amides compared to corresponding esters resulting in the reduced biological potency; further illustrating that the ester moiety is necessary for reactivity with intracellular nucleophiles and subsequent biological activity. Encouraged by the higher activity of the *t*Bu ester in **5**, the *t*Bu bromide was reacted with tris(4-methoxyphenyl)phosphine and recrystallized in ether to obtain the corresponding phosphonium bromide **6**. This compound showed even further enhancement in cell proliferation inhibition from the combination of enhanced lipophilicity and better cationic stabilization due to methoxy groups (Table 1).

We further carried out SAR studies on the β-substitution of BH bromides to explore the effect of β-substitution on biological activity. To synthesize the required β-substituted BH bromides, a variety of electron withdrawing and donating aromatic aldehydes were used. These bromides were obtained under standard BH reaction and subsequent bromination conditions. Since tris(4-methylphenyl) phosphine provided higher cell proliferation properties, phosphonium derivatives **8**–**16** were synthesized using β-substituted BH bromides with this phosphine



R=H, R^1=Me, **8** (86%), R=Me, R^1=Me, **9** (75%), R=CN, R^1=Me, **10** (86%)
R=NO_2, R^1=Me, **11** (83%), R=OMe, R^1=Me, **12** (83%), R=F, R^1=Me, **13** (89%)
R=Cl, R^1=Me, **14** (89%), R=Br, R^1=Me, **15** (87%), R=Br, R^1=*t*Bu, **16** (78%)

Scheme 2. Synthesis of phosphonium salts with tris(4-methoxyphenyl)phosphine.

(Scheme 2). We then carried out MTT based cell proliferation inhibition studies of the phosphonium derivatives **8**–**16** on designated cancer cell lines. These studies indicated that β-aryl substituted phosphonium salts **8**–**16** exhibited increased potency when compared to the β-unsubstituted derivatives **1**–**7** (Table 2, Table 1). Specifically, the derivative **15** exhibited activity at low μM concentrations against all the cell lines (Table 2). The increase in activity could be due to additional lipophilicity of β-aryl substituted derivatives and increased shuttling of these derivatives into mitochondrial matrix. We then carried out further synthetic modifications by substituting the methyl ester with the *t*Bu ester as it provided higher activity. Similar to our earlier observed results with *t*Bu derivatives **5** and **6**, it was found that *t*Bu derived tris(4-methoxyphenyl)phosphine **16**, exhibited enhanced biological potency in low μM IC_{50} values against all of the cell lines tested (Table 2).

Since compounds **12, 15**, and **16** exhibited potent cell proliferation at low μM and nM concentrations in the MTT assay, these compounds were also evaluated using another cell proliferation inhibition method called sulforhodamine-B (SRB) assay. The MTT assay is dependent on intact mitochondrial function to reduce the MTT to formazan where the absorbance represents the compound's cell viability potential. Since the candidate compounds are targeted to mitochondria, the MTT results may be a result of a false positive showing mitochondrial function as a surrogate of cell proliferation. In this regard, the SRB assay was included to confirm the cell proliferation inhibition properties of these molecules. The SRB assay allows for the evaluation of cell proliferation based on cellular protein content which is a more direct measure of protein content than mitochondrial viability. These results indicated that compounds **12, 15**, and **16** had retained comparable cell proliferation inhibition properties in low μM to nM potent IC_{50} values (Table 3) validating the mitochondrial targeting ability of these compounds.

We further carried out metabolic assays with the lead candidate compound **16** using widely employed Seahorse XFe96® MitoStress test on highly oxidative, metabolically plastic 4T1 cells and more glycolytic triple negative breast cancer cells MDA-MB-231 (**S1, S2, S3**). Here, cells treated with test compound, followed by the successive addition of known electron transport chain inhibitors oligomycin, carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP), and rotenone + antimycin A respectively. The changes in oxygen consumption rates (OCR) were recorded in real time to obtain numerous parameters of mitochondrial respiratory function including ATP production, proton leak, maximal respiration, and spare respiratory capacity (Figs. 1 and 2). In a mitochondrial stress test, successive injections of test compound and known inhibitors alter different electron transport chain processes allowing for the evaluation of the acute effects of test compound on mitochondrial function.

Lead compound **16** was screened at different concentrations to observe any concentration dependent changes in oxygen consumption rate (OCR). Compound **16** exhibited a dose-dependent decrease in maximal respiration (Fig. 1**A, S2**). This decrease in maximal respiration

Table 2
Cell proliferation inhibition (IC_{50}) values comparing trimethoxy phenyl phosphonium bromide salts (MTT assay). IC_{50} values reported as average values ± SEM from three separate experiments.

	Substituents		Cell line IC_{50} values (μM)					
No.	R	R^1	MCF-7	MDA-MB-231	MiaPaCa-2	WiDr	4T1	67NR
8	H	Me	2.67±0.7	9.27±1.4	4.59±3.1	2.74±4.9	7.20±4.9	4.87±1.8
9	Me	Me	7.47±3.3	9.79±2.0	0.34±0.2	2.58±1.2	5.23±2.5	2.78±1.1
10	CN	Me	4.49±0.4	12.5±1.3	7.86±1.7	4.87±1.3	20.4±1.3	>100
11	NO_2	Me	8.83±3.0	8.29±0.5	5.59±1.3	5.95±1.1	4.73±0.5	28.7±17.7
12	OMe	Me	1.40±0.4	3.70±0.7	1.37±0.3	1.14±0.2	3.36±2.3	4.59±0.2
13	F	Me	1.42±0.3	7.41±1.6	1.29±0.1	2.14±0.4	4.57±2.2	5.14±1.1
14	Cl	Me	1.37±0.2	3.01±0.5	0.59±0.2	1.32±0.4	0.98±0.1	ND
15	Br	Me	1.24±0.5	1.91±0.4	0.73±0.1	0.90±0.4	1.21±0.2	2.9±0.4
16	Br	OtButyl	0.64±0.4	2.1±0.3	0.36±0.1	0.95±0.1	0.86±0.2	0.62±0.1

Table 3
Cell proliferation inhibition (IC_{50}) values of the phosphonium bromide salts (SRB assay). IC_{50} values reported as average values ± SEM from three separate experiments.

	Substituents		Cell line IC_{50} values (μM)					
No.	R	R^1	MCF-7	MDA-MB-231	MiaPaCa-2	WiDr	4T1	67NR
12	OMe	Me	0.17±0.1	2.19±0.4	0.31±0.1	0.76±0.2	1.83±0.2	2.56±0.4
15	Br	Me	0.17±0.1	0.94±0.4	0.29±0.1	0.81±0.2	1.25±0.2	1.78±0.3
16	Br	OtButyl	0.063±0.1	0.58±0.1	0.20±0.1	0.14±0.1	0.5±0.1	0.47±0.1

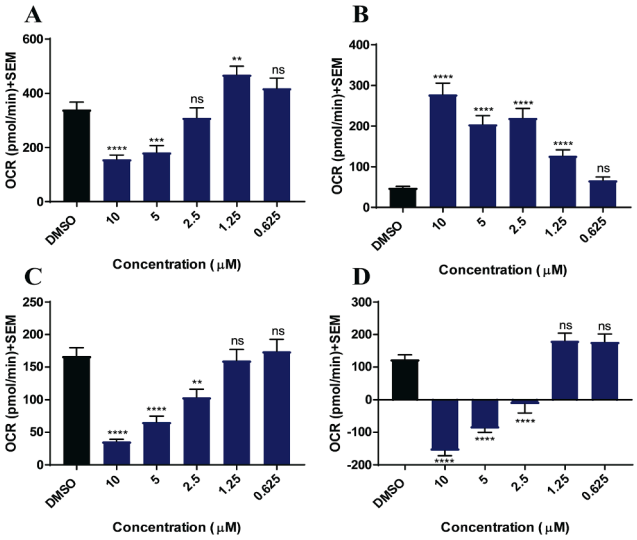


Fig. 1. Seahorse XFe96® mitochondrial stress test parameters of serial diluted **16** on 4T1 cell line. (A) Maximal respiration (B) Proton leak (C) ATP production (D) Spare respiratory capacity. OCR values of were calculated using wave software. The average + SEM values of four independent experimental values were calculated. Repeated measures one-way ANOVA was used to calculate statistical significance ($P < 0.05$) between treatment concentrations vs. DMSO control. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

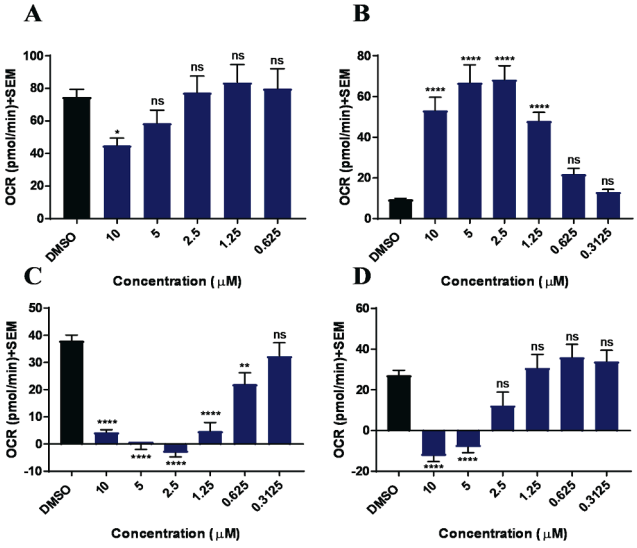


Fig. 2. Seahorse XFe96® mitochondrial stress test parameters of serial diluted **16** on MDA-MB-231 cell line. (A) Maximal respiration (B) Proton leak (C) ATP production (D) Spare respiratory capacity. OCR values of were calculated using wave software. The average + SEM values of three independent experimental values were calculated. Repeated measures one-way ANOVA was used to calculate statistical significance ($P < 0.05$) between treatment concentrations vs DMSO control. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

provides insight that this derivative is indeed targeting the mitochondria and perturbing function. Due to the reactivity of the allyl carbonyl ester moiety in **16** to cellular nucleophiles, the decrease in maximal respiration could be related to covalent modification of intramitochondrial nucleophilic species. Localization of **16** into the mitochondria is further supported by the significant and dose-dependent proton leak across the mitochondrial inner membrane (Fig. 1**B**). The mitochondria require a proton gradient across the inner membrane for ATP synthase to function where maintenance of membrane integrity allows for compartmentalization of protons that are ultimately used as the motive for oxidative phosphorylation of ADP. If this proton gradient is not present within the cell, the cells undergo a severe energy crisis and redirect metabolites towards other metabolic pathways. Mitochondrial damage and loss of membrane integrity can result in leaking of protons across the mitochondrial membranes, in line with the hypothesized mechanism of action of compound **16**. Due to the reliance on the proton gradient to generate ATP, a significant proton leak and loss of membrane potential by the addition of **16**, not surprisingly, results in the dose dependent decrease in ATP production (Fig. 1**C**).

Spare respiratory capacity is the ability of the cell to respond to changes in energy demand. Addition of **16** resulted in a negative spare respiratory capacity at higher concentrations followed by a return to normal capacity with lower concentrations (Fig. 1**D**). The candidate **16** could be damaging mitochondrial membrane to such a large extent that the basal respiration rate after compound injection is greater than the obtained maximal respiration after FCCP injection. The increase in spare respiratory capacity seen at lower concentrations can be attributed to less membrane damage which is supported by the lack of significant proton leak at lower concentrations. A similar trend was observed on MDA-MB-231 where an initial decrease in maximal respiration but rates quickly return to baseline by the addition of **16** (Fig. 2**A**). Comparable to 4T1, there was a significant dose-dependent proton leak in MDA-MB-231 (Fig. 2**B**). This proton leak subsequently reduced ATP production (Fig. 1**C**). This result could be due to the fact that MDA-MB-231 is more energetically rigid when compared to the energetically plastic 4T1[35]. MDA-MB-231 is highly reliant on glycolysis for ATP production with a small contribution from OxPhos (**S1**). Disrupting mitochondrial membrane potential and substantial proton leak would effectively halt all ATP produced in the mitochondrial derived from glycolytic end products. Interestingly, even at concentrations of **16** where there is a significant proton leak, spare respiratory capacity was shown to recover. At high concentrations of **16** there could be abolishment of mitochondrial integrity rendering the mitochondria unable to carry out oxidation of metabolic substrates and therefore consume oxygen to recover energy needs. At lower concentrations there could be enough impact on mitochondrial integrity to cause a proton leak but the mitochondria are still functioning to try to return the proton gradient to generate energy. Overall the Seahorse XFe96® MitoStress test indicates that the lead candidate compound perturbs the mitochondrial membrane potential as supported by the proton leak and inhibition of ATP production.

The candidate compounds **12** and **16** were screened against normal human dermal fibroblast cell line (HDFa) to evaluate their effect on normal cells. Candidate **12** had an IC_{50} value of 23.9 ± 1.6 μM and candidate **16** had an IC_{50} of 5.5 ± 0.5 μM which is 5–20 times and 2–5 times more selective towards cancer cells respectively. Additionally, to explore the general tolerability and translational potential of these class of compounds, we have conducted a preliminary systemic toxicity study of the compound **12**. In this regard, CD-1 mice (n = 6) were treated with **12** at 10 mg/kg 6 days a week for 14 days. Based on this study, it was found that **12** is generally well tolerated as evidenced by normal grooming pattern, zero mortality, and no significant weight loss of the treated mice (**S4**).

In conclusion, several phosphonium salts **1**–**16** were synthesized from different BH bromides as mitochondria targeting anticancer agents. *In vitro* cell proliferation studies indicated that the tris-(4-methoxyphenyl)phosphine was the optimal phosphine compared to the other aryl phosphines in providing more potent IC_{50} values in the all tested cancer cell lines. Further SAR studies indicated that β-substituted BH bromide derived phosphonium derivatives enhanced the biological activity to low μM or nM IC_{50} values. The MTT based cell proliferation inhibition values were validated by observing similar IC_{50} values with an SRB assay. Preliminary *in vitro* metabolic studies indicated that the lead candidate compound **16** inhibited the production of ATP, increased the proton leak within the mitochondrial membrane and abolished the spare respiratory capacity in a concentration dependent manner. A limitation of developing mitochondrial directed molecules include toxicity resulting from the nonselective targeting of both healthy and diseased tissues by the derivatives. Overall, these compounds have excellent potential to be developed as potential anticancer agents based on the ease of preparation and observed low μM to nM IC_{50} cell proliferation inhibition values.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128136.

References

1 Hanahan D, Weinberg R. Leading edge review hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646–674.
2 Wu Z, Wu J, Zhao Q, Fu S, Jin J. Emerging roles of aerobic glycolysis in breast cancer. *Clin Transl Oncol*. 2020;22(5):631–646.
3 Abdel-Wahab AF, Mahmoud W, Al-Harizy RM. Targeting glucose metabolism to suppress cancer progression: prospective of anti-glycolytic cancer therapy. *Pharmacol Res*. 2019;150:104511. https://doi.org/10.1016/j.phrs.2019.104511.
4 Lau AN, Vander Heiden MG. Metabolism in the Tumor Microenvironment. *Annu Rev Cancer Biol Annu Rev Cancer Biol*. 2019;2020:17–40.
5 Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. *Nat Rev Cancer*. 2011;11(2):85–95.
6 Roma-Rodrigues C, Mendes R, Baptista PV, Fernandes AR. Targeting tumor microenvironment for cancer therapy. *Int J Mol Sci*. 2019;20(4):840.
7 Cassim S, Vučetić M, Ždralević M, Pouyssegur J. Warburg and beyond: The power of mitochondrial metabolism to collaborate or replace fermentative glycolysis in cancer. *Cancers (Basel)*. 2020;12(5):1119.
8 Roth KG, Mambetsariev I, Kulkarni P, Salgia R. The mitochondrion as an emerging therapeutic target in cancer. *Trends Mol Med*. 2020;26(1):119–134.
9 Vyas S, Zaganjor E, Haigis MC. Mitochondria and cancer. *Cell*. 2016;166(3):555–566.
10 Porporato PE, Filigheddu N, Pedro J-S, Kroemer G, Galluzzi L. Mitochondrial metabolism and cancer. *Cell Res*. 2018;28(3):265–280.
11 Spinelli JB, Haigis MC. The multifaceted contributions of mitochondria to cellular metabolism. *Nat Cell Biol*. 2018;20(7):745–754.
12 Fulda S, Galluzzi L, Kroemer G. Targeting mitochondria for cancer therapy. *Nat Rev Drug Discov*. 2010;9(6):447–464.
13 Burke PJ. Mitochondria, bioenergetics and apoptosis in cancer. *Trends in Cancer*. 2017;3(12):857–870.
14 Pfanner N, Warscheid B, Wiedemann N. Mitochondrial proteins: from biogenesis to functional networks. *Nat Rev Mol Cell Biol*. 2019;20(5):267–284.
15 Martínez-Reyes I, Chandel NS. Mitochondrial TCA cycle metabolites control physiology and disease. *Nat Commun*. 2020;11(1):1–11.
16 Giacomello M, Pyakurel A, Glytsou C, Scorrano L. The cell biology of mitochondrial membrane dynamics. *Nat Rev Mol Cell Biol*. 2020;21(4):204–224.
17 Guerra F, Arbini AA, Moro L. Mitochondria and cancer chemoresistance. *Biochim Biophys Acta – Bioenerg*. 2017;1858(8):686–699.
18 Jia D, Park J, Jung K, Levine H, Kaipparettu B. Elucidating the metabolic plasticity of cancer: mitochondrial reprogramming and hybrid metabolic states. *Cells*. 2018;7(3):21. https://doi.org/10.3390/cells7030021.

19 Zielonka J, Joseph J, Sikora A, et al. Mitochondria-targeted triphenylphosphonium-based compounds: syntheses, mechanisms of action, and therapeutic and diagnostic applications. *Chem Rev*. 2017;117(15):10043–10120.
20 Armstrong JS. Mitochondrial Medicine: pharmacological targeting of mitochondria in disease. *Br J Pharmacol*. 2007;151:1154–1165.
21 Jonnalagadda SK, Wielenberg K, Ronayne CT, et al. Synthesis and biological evaluation of arylphosphonium-benzoxaborole conjugates as novel anticancer agents. *Bioorganic Med Chem Lett*. 2020;30(14):127259. https://doi.org/10.1016/j.bmcl.2020.127259.
22 Pathania D, Millard M, Neamati N. Opportunities in discovery and delivery of anticancer drugs targeting mitochondria and cancer cell metabolism. *Adv Drug Deliv Rev*. 2009;61(14):1250–1275.
23 Ma GN, Jiang JJ, Shi M, Wei Y. Recent extensions of the Morita-Baylis-Hillman reaction. *Chem Commun*. 2009;37:5496–5514.
24 Basavaiah D, Rao KV, Reddy RJ. The Baylis-Hillman reaction: a novel source of attraction, opportunities, and challenges in synthetic chemistry. *Chem Soc Rev*. 2007;36(10):1581–1588.
25 Basavaiah D, Naganaboina RT. The Baylis-Hillman reaction: a new continent in organic chemistry-our philosophy, vision and over three decades of research. *New J Chem*. 2018;42(17):14036–14066.
26 Kaye PT. Applications of the Morita–Baylis–Hillman Reaction in the Synthesis of Heterocyclic Systems. In: Advances in Heterocyclic Chemistry. Vol 127. Academic Press Inc.; 2019:101-152.
27 Ronayne CT, Solano LN, Nelson GL, et al. Synthesis and biological evaluation of 2-alkoxycarbonylallyl esters as potential anticancer agents. *Bioorg Med Chem Lett*. 2017;27(4):776–780.
28 Solano LN, Nelson GL, Ronayne CT, et al. Synthesis, in vitro, and in vivo evaluation of novel functionalized quaternary ammonium curcuminoids as potential anti-cancer agents. *Bioorg Med Chem Lett*. 2015;25(24):5777–5780.
29 Gurrapu S, Jonnalagadda SK, Alam MA, et al. Coumarin carboxylic acids as monocarboxylate transporter 1 inhibitors: In vitro and in vivo studies as potential anticancer agents. *Bioorg Med Chem Lett*. 2016;26(14):3282–3286.
30 Jonnalagadda S, Jonnalagadda SK, Ronayne CT, et al. Novel N, N-dialkyl cyanocinnamic acids as monocarboxylate transporter 1 and 4 inhibitors. *Oncotarget*. 2019;10(24):2355–2368.
31 Gurrapu S, Jonnalagadda SK, Alam MA, et al. Monocarboxylate transporter 1 inhibitors as potential anticancer agents. *ACS Med Chem Lett*. 2015;6(5):558–561.
32 Solano LN, Nelson GL, Ronayne CT, et al. Synthesis, in vitro, and in vivo evaluation of novel N-phenylindazolyl diarylureas as potential anti-cancer agents. Sci Rep. 2020;10(1). 33. Lardy SW, Schmidt VA. Intermolecular Aminoallylation of Alkenes Using Allyl-Oxyphthalimide Derivatives: A Case Study in Radical Polarity Effects. Eur J Org Chem. 2019;2019(40):6796-6799.
33 Grenning AJ, Van Allen CK, Maji T, Lang SB, Tunge JA. Development of asymmetric deacylative allylation. *J Org Chem*. 2013;78(14):7281–7287.
34 Simões RV, Serganova IS, Kruchevsky N, et al. Metabolic plasticity of metastatic breast cancer cells: adaptation to changes in the microenvironment. *Neoplasia (United States)*. 2015;17(8):671–684.