European Journal of Pharmaceutical Sciences 123 (2018) 43–55

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Anticancer effects of some novel dichloroacetophenones through the inhibition of pyruvate dehydrogenase kinase 1

Shao-Lin Zhang[a,1], Zheng Yang[a,1], Xiaohui Hu[b], Harapriya Chakravarty[a], Kin Yip Tam[a,*]

[a] Faculty of Health Sciences, University of Macau, Taipa, Macau, China
[b] Drug Development Core, Faculty of Health Sciences, University of Macau, Taipa, Macau, China

ARTICLE INFO

Keywords:
Dichloroacetophenone
Pyruvate dehydrogenase kinase
Pyruvate dehydrogenase complex
Anticancer activity
Metabolic reprogramming

ABSTRACT

Targeting pyruvate dehydrogenase kinase 1 (PDK1) has been suggested as a potential anticancer strategy. 2,2-dichloroacetophenone (DAP) is a PDK1 inhibitor exhibiting weak anticancer potency and poor selectivity. The current study describes the characterization of three potent compounds **54**, **55** and **64**, which tightly bound to PDK1 with K_d values of 1.29, 0.97, and 0.58 μM, respectively, and activated pyruvate dehydrogenase complex with EC_{50} values of 0.68, 0.49, and 0.33 μM, respectively. In contrast with DAP, these analogues were more potent and selective against PDK1, reduced proliferation and survival of NCI-H1975 cells, and suppressed tumor growth in a NCI-H1975 xenograft mouse model. Moreover, compounds **54**, **55** and **64** depolarized mitochondrial membrane potential, induced cell apoptosis, decreased extracellular lactate formation, and increased reactive oxygen species production in NCI-H1975 cells. They may serve as potential modulators to regulate mitochondrial function and reprogram metabolism in cancer cells which could represent promising compounds for further development of potent PDK1 inhibitors.

1. Introduction

Cancer cells exhibit an altered cell metabolism, which represents by decreased oxidative phosphorylation (OXPHOS) and increased aerobic glycolysis, relative to normal cells (Warburg, 1956). This is likely due to an increased demand on rapid ATP generation for proliferation and survival, as well as an increased rate of glycolysis (Vander Heiden et al., 2009), known as the Warburg Effect, which is one of the hallmarks of caner (Tekade and Sun, 2017). Therefore, targeting cell metabolism to specifically disrupt these processes could be a promising strategy in cancer therapy (Vander Heiden, 2011).

It is reported that pyruvate dehydrogenase complex (PDC) is a gatekeeper enzyme, enabling irreversible oxidative decarboxylation of pyruvate to generate acetyl-CoA, which facilitates the tricarboxylic acid (TCA) cycle to produce ATP for cell energy consumption (Patel and Korotchkina, 2006). The structure of PDC consists of four major catalytic parts, including pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2), dihydrolipoamide dehydrogenase (E3), and E3-binding protein (E3BP) (Yu et al., 2008). Pyruvate dehydrogenase kinases (PDKs) are the integral part of PDC, which facilitate binding to the L2 domain located on the E2 protein of the complex (Patel et al., 2009). Attachment of PDKs to E2 domain of the complex allows the kinase to reach the core of E1 subunit, which can phosphorylate specific serine residues, such as Ser232, Ser293, or Ser300, to suppress the activity of the complex (Hiromasa et al., 2006). On the contrary, dephosphorylation of PDC by two pyruvate dehydrogenase phosphatases (PDPs) can restore its activity, which, together with PDKs, regulates the process of catalyzing pyruvate into acetyl-CoA as the source of ATP generation (Zhang et al., 2015). In most cancer cells, however, energy is produced *via* an increased rate of aerobic glycolysis, rather than mitochondrial OXPHOS. The resulting intra-cytosol lactate fermentation could be partially due to the dysfunction of mitochondrial respiration, which could attribute to the inhibition of PDC activity by PDKs overexpression (Saunier et al., 2016). Among the PDK isoforms, PDK1 is most frequently associated with cancer development, and has been shown to be significantly up-regulated in different tumor types (Hur

Abbreviations: PDK, pyruvate dehydrogenase kinase; PDC, pyruvate dehydrogenase complex; MMP, mitochondrial membrane potential; ROS, reactive oxygen species; OXPHOS, oxidative phosphorylation; TCA, tricarboxylic acid; PDPs, pyruvate dehydrogenase phosphatases; DCA, dichloroacetate; DAP, 2,2-dichloroacetophenone; ITC, isothermal titration calorimetry; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide; TMRM, tetramethyl rhodamine methyl ester; DCDMH, 1,3-dichloro-5,5-dimethylhydantoin

* Corresponding author.
E-mail address: kintam@umac.mo (K.Y. Tam).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ejps.2018.07.026
Received 7 June 2018; Received in revised form 5 July 2018; Accepted 11 July 2018

et al., 2013; Koukourakis et al., 2007; Wigfield et al., 2008). Moreover, it is reported that the activity of PDK1 is increased by a number of oncogenic tyrosine kinases. This occurs during post-transcriptional modification, resulting in tumor growth when PDC activity is blocked by PDK1 activation (Hitosugi et al., 2011). These results suggest that PDK1 could be a potential target for cancer therapy.

A number of PDK inhibitors with different mechanisms have been reported. Dichloroacetate (DCA), an analogue of pyruvate, was reported as a PDK inhibitor for the treatment of diabetes, lactic acidosis, and myocardial ischemia (Abdelmalak et al., 2013; Bersin and Stacpoole, 1997; Stacpoole et al., 1978). Although it has been used for cancer therapy recently (Bonnet et al., 2007; Michelakis et al., 2010), the high effective dosage hampered its routine application in clinical practice (Stacpoole et al., 2008). AZD7545 binds to the lipoamide-binding site of PDK2 with its trifluoromethylpropanamide warhead, which represents a PDK2 inhibitor (Mayers et al., 2003). On the contrary, dihydroxyphenyl sulfonylisoindoline derivatives (Tso et al., 2017), 4,5-diarylisoxazoles (Meng et al., 2014) and VER-246608 (Moore et al., 2014) are reported as ATP-competitive inhibitors, which bind to the ATP pocket. Dichloroacetophenone was previously identified as PDK inhibitor using a high throughput microtiter plate assay (Espinal et al., 1995), whereas one of its analogues, 2,2-dichloroacetophenone (DAP) was reported to be able to target PDK1 for suppression of cancer development in acute myeloid leukemia (AML) both *in vitro* and *in vivo* (Qin et al., 2016). In addition to PDK1 inhibition, DAP also showed off-target effects on various signaling pathways, such as PI3K (Qin et al., 2016) and EGFR (Yang et al., 2017), which might lead to potential side effects. Herein, we report some novel DAP analogues, which are identified as PDK1 inhibitors by using kinase-, enzyme- and cell-based assays. We have shown that these novel inhibitors re-activated the OXPHOS in cancer cells leading to apoptosis. The observed anticancer effects in cells were found to translate well in a NCI-H1975 xenograft model. Our results suggested that selective inhibition of PDK1 could be a promising approach to inhibit cancer cell proliferation.

2. Materials and methods

2.1. Chemical synthesis

All building blocks were bought from Sigma-Aldrich Corporation (USA), JK chemical (India), Wako (Japan) and Acros Organics (USA), and were used as received. Solvents were purchased from Anaqua Chemicals Supply Inc. Limited (Hong Kong). The chemical structures of the compounds were confirmed by ^{1}H NMR and ^{13}C NMR spectroscopy using a Bruker AV-400 instrument. High resolution mass spectra (HRMS) were obtained on a Xevo G2-XS QTof spectrometer (Waters, USA). Melting points (M.p.) were determined using a SMP10 instrument (Stuart Company, UK). Reactions were monitored by thin-layer chromatography (TLC, France) and carried out on commercial silica gel plates (GF254, Germany), which could be visualized under UV light. Flash column chromatography was performed with silica gel (200–300 mesh) purchased from Hong Kong Labware Co. Ltd. The purity of synthesized compounds was better than 95% as determined by HPLC equipped with UV detector (Agilent 1260 Infinity HPLC, USA). The HPLC separation was accomplished by a C-18 column (150 × 4.6 mm Zorbax SB-C18, 5 μm) using an 8-min water-acetonitrile gradient with a mobile phase flow rate of 1 mL/mL and injection volume of 50 μL.

To preparing compounds **54**, **55** and **64**, a mixture of NH_4Cl (0.14 g, 2.5 mmol), 1-(4-ethyl-3-nitrophenyl)ethan-1-one (0.96 g, 5.0 mmol) or 1-(4-isopropyl-3-nitrophenyl)ethan-1-one (1.04 g, 5.0 mmol) or 1-(4-isopropoxy-3-nitrophenyl)ethan-1-one (1.12 g, 5.0 mmol), and 20.0 mL of acetonitrile was stirred for 5 min, respectively. Then DCDMH (1.95 g, 10 mmol) was added, and the mixture was stirred for 16 h at 40 °C. After completion of the reaction, solvent was removed and ethyl acetate (20 mL) was added. The ethyl acetate was then washed twice with water (20 mL). The organic phase was collected and dried over anhydrous sodium sulfate. Finally the solvent was evaporated under reduced pressure. The residues were purified by silica gel column chromatography.

2.1.1. Chemical characteristics of 2,2-dichloro-1-(4-ethyl-3-nitrophenyl) ethan-1-one (54)

White oil with yield of 72.8%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.61 (d, J = 2 Hz, 1H), 8.28 (dd, J = 2, 8 Hz, 1H), 7.56 (d, J = 8 Hz, 1H), 6.58 (s, 1H), 3.04 − 2.99 (m, 2H), 1.35 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 183.93, 149.43, 145.48, 133.47, 131.92, 129.88, 126.04, 67.67, 26.44, 14.52; HRMS (ESI) calcd for $C_{10}H_{10}Cl_2NO_3$ $[M + H]^+$, 262.0038; found, 262.0035; HPLC t_R = 5.20 min, purity 98.96%.

2.1.2. Chemical characteristics of 2,2-dichloro-1-(4-isopropyl-3-nitrophenyl) ethan-1-one (55)

White solid with yield of 88.5%. M.p. 149 − 150 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.44 (d, J = 2 Hz, 1H), 8.29 (dd, J = 2, 8 Hz, 1H), 7.66 (d, J = 8 Hz, 1H), 6.57 (s, 1H), 3.52 − 3.45 (m, 1H), 1.36 (s, 3H), 1.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 183.89, 149.83, 149.09, 133.13, 129.56, 128.48, 125.35, 67.67, 29.09, 23.28; HRMS (ESI) calcd for $C_{11}H_{12}Cl_2NO_3$ $[M + H]^+$, 276.0194; found, 276.0199; HPLC t_R = 5.26 min, purity 97.30%.

2.1.3. Chemical characteristics of 2,2-dichloro-1-(4-isopropyl-3-nitrophenyl) ethan-1-one (64)

White solid with yield of 67.7%. M.p. 136 − 137 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.54 (d, J = 2.4 Hz, 1H), 8.29 (dd, J = 2.4, 8.8 Hz, 1H), 7.18 (d, J = 8.8 Hz, 1H), 6.56 (s, 1H), 4.87 − 4.81 (m, 1H), 1.46 (s, 1H), 1.45 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 183.28, 155.69, 140.32, 135.27, 127.69, 122.54, 114.96, 73.52, 67.66, 21.66; HRMS (ESI) calcd for $C_{11}H_{12}Cl_2NO_4$ $[M + H]^+$, 292.0143; found, 292.0148; HPLC t_R = 5.15 min, purity 97.22%.

2.2. PDK1 kinase activity assay

PDK1 kinase activity was measured with the Kinase-Glo® Luminescent kit (Promega). Firstly, 25 μL of double-distilled water and 5 μL assay buffer (10×, 250 mM Tris-HCl, 10 mM EDTA, 5 mM EGTA, 10 mM DTT, 50 mM $MgCl_2$) were added to 96-well plates. Then 5 μL of compounds (50 μM) were added to each wells, except the two control wells containing 5 μL of 1% DMSO PBS buffer. Subsequently, 5 μL ATP (10 μM) and 5 μL peptide (50 μM, amino acid sequences: RYHGHSMSDP, which is a fragment flanking Ser293 of PDC E1 subunit) were added to all the wells. Then 5 μL PDK1 (20 μM) in protein buffer (50 mM K_3PO_4, 250 mM KCl, 2 mM $MgCl_2$, pH = 7.4) was added to all the wells to initialize peptide phosphorylation except the control well, where 5 μL protein buffer was added instead. At last, the plate was mixed and incubated at 37 °C for 30 min. Then 50 μL of the appropriate Kinase-Glo® reagent was added to each well. The plate was shake gently, and incubated for another 10 min at room temperature. The luminescence was recorded on a SpectraMax M5 Microplate Reader. Based on this assay, PDK1 kinase activities were determined in the presence of **54**, **55** or **64** at 10, 5, 2.5 μM.

2.3. PDC primary enzymatic assay

PDC primary enzymatic assay was performed as previously described (Espinal et al., 1995). Initially, PDC was acetylated to enhance the intrinsic PDKs activity. Pig PDC (Sigma, CAS 9014-20-4) containing intrinsic kinase activity PDKs was incubated for 40 min at 37 °C in buffer A (40 mM Mops (pH = 7.20), 0.5 mM EDTA, 30 mM KCl, 1.5 mM $MgCl_2$, 0.25 mM acetyl-CoA, 0.05 mM NADH, 2 mM dithiothreitol, 10 mM NaF). Then, ATP-containing buffer A was added to the enzyme to trigger kinase reaction, in which PDKs catalyzed the phosphorylation of dehydrogenase in the presence or absence of the compounds. The