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Identification of pyruvate dehydrogenase kinase 1 inhibitors with anti-osteosarcoma activity

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ABSTRACT

Overexpression of pyruvate dehydrogenase kinases (PDKs), especially PDK1 has been observed in a variety of cancers. Thus, targeting PDK1 offers an attractive opportunity for the development of cancer therapies. In this letter, we reported the identification of two novel PDK1 inhibitors as anti-osteosarcoma agents. We found that **TM-1** and **TM-2** inhibited PDK1 with the IC_{50} values of 2.97 and 3.41 μM, respectively. Furthermore, **TM-1** and **TM-2** dose-dependently reduced phosphorylation of pyruvate dehydrogenase complex in MG-63 osteosarcoma cells. Finally, **TM-1** and **TM-2** were found to inhibit the proliferation of MG-63 cells with the EC_{50} values of 14.5, and 11.0 μM, respectively, meaning **TM-1** and **TM-2** could be promising leads for the discovery of potent PDK1 inhibitors.

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Cancer cells feature a switch in metabolic profile from mitochondria-based glucose oxidative phosphorylation to cytoplasm-based glycolysis, even under normoxia. This altered cellular metabolic pathway, first recognized by Otto Warburg in 1924,[1] meets the survival and proliferation needs during the process of tumor progression. Thus, targeting this cancer-specific metabolic remodeling may offer therapeutic opportunities in cancer treatment.[2]

Pyruvate dehydrogenase kinases (PDKs) are mitochondrial enzymes that inhibit the activity of pyruvate dehydrogenase complex (PDC), an important gatekeeper enzyme which links glycolysis to the Krebs cycle.[3] PDKs negatively regulate the activity of PDC by reversible phosphorylation, which occurs independently on three different serines (Ser232, Ser293, and Ser300).[4] To date, four isoforms namely PDK1, PDK2, PDK3, and PDK4 in mitochondria have been isolated and characterized in terms of their differences in activity, tissue distribution, and regulations.[5] PDK1 is the only isoform reported to phosphorylate all the three serines and is closely associated with cancer malignancy.[6] It was reported that PDK1 was overexpressed in various cancers such as osteosarcoma cancer, lung cancer, head squamous cancer, and gastric cancer.[7] In addition, PDK1 activity was up-regulated at the post-transcriptional level by diverse oncogenic tyrosine kinases in cancer cell.[6b,8] This enhancement of PDK1 activity could lead to a promotion of the Warburg effect and tumor growth.[9] Furthermore, overexpression of PDK1 protected cancer cells from anoikis, while depletion of PDK1 restored the susceptibility to anoikis.[10] Collectively, these evidences suggested that PDK1 was a viable anticancer target.[11] Currently, a large amount of PDKs inhibitors have been reported in scientific literature.[12] However, none of which has successfully entered into the clinical use. Herein, we reported the use of several biological assays and molecular docking for the discovery of novel PDK1 inhibitors with good anti-proliferative activity on MG-63 cancer cells.

As the starting point of our efforts to discover new potent PDK1 inhibitor, we carried out an enzyme-linked immunosorbent assay (ELISA) to screen an in-house library of ~1000 small molecules. All the compounds were evaluated at an initial concentration of 10 μM against PDK1, and dichloroacetate (DCA) was used at 10 mM as a positive control. In the process, **TM-1** and **TM-2** (Fig. 1) were found to be capable of remarkably inhibiting PDK1 activity, with the inhibition rates were 80.5%, and 83.3% at 10 μM, respectively. Then DCA and the two inhibitors were further evaluated to measure the IC_{50} values against PDK1 activity. As shown in Fig. 2, the IC_{50} value for DCA was 5.1 mM. In contrast, **TM-1** and **TM-2** dramatically inhibited PDK1 activity, with the IC_{50} values of 2.97, and 3.14 μM, over 1000-fold more potent than DCA. Furthermore, it was observed that DCA, **TM-1** and **TM-2** also inhibited PDK2 kinase activity. The IC_{50} value for DCA against PDK2 was 9.0 mM, while for **TM-1** and **TM-2**, the IC_{50} values were 5.2 and 10.9 μM, respectively.

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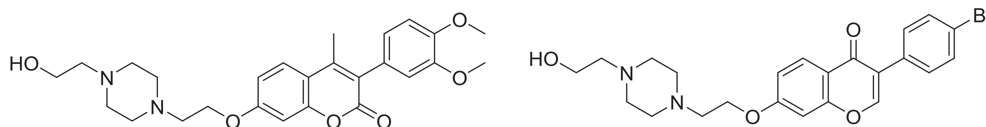

Fig. 1. Chemical structures of the identified **TM-1** (left) and **TM-2** (right).

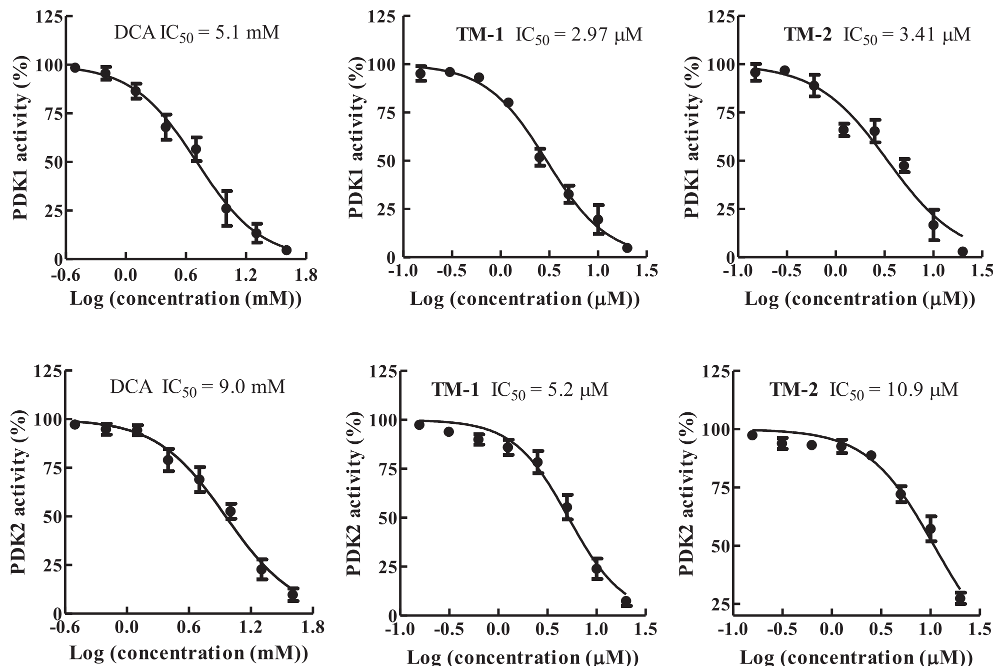

Fig. 2. IC_{50} values of DCA, **TM-1** and **TM-2** against PDK1 and PDK2 kinases activity. The inhibitory activities of the compounds on PDK1 and PDK2 were determined by ELISA. The values were reported as the mean ± SD.

We then tested the cellular activity of **TM-1** and **TM-2** on PDKs inhibition by using a high content analysis, which examined the impact on the downstream PDC phosphorylation. As shown in Fig. 3, DCA, **TM-1** and **TM-2** decreased the phosphorylation of both Ser293 and Ser232. Moreover, the percentages of the phosphorylation reduction of Ser293 by **TM-1** at 3, 6, and 12 μM were 18.7, 39.7, and 78.3%, respectively, while for **TM-2**, the percentages were 17.6, 49.3, and 75.3%, suggesting the reduction of phosphorylation of both Ser293 and Ser232 sites followed dose-dependent manners.

The cellular activity of **TM-1** and **TM-2** were further verified by using the immunoblotting analysis. MG-63 cancer cells were treated with DCA, **TM-1** and **TM-2** at desire concentrations for 24 h, then PDC phosphorylation of Ser293 and Ser232 was analyzed by western blotting experiment. As shown in Fig. 4, DCA at 10 mM, **TM-1** and **TM-2** at 6 or 12 μM dramatically reduced the PDC phosphorylation of both Ser293 and Ser232. Moreover, the PDC phosphorylation of both sites was significantly decreased in a concentration-dependent manner.

Molecular docking then was undertaken to verify the interactions between PDK1 and the two inhibitors. Since the crystal structure of PDK1-ATP complex is not available in protein data bank, we chose PDK2-ATP complex as the template for docking. PDK1 and PDK2 share a high similarity in their structures, with an alignment

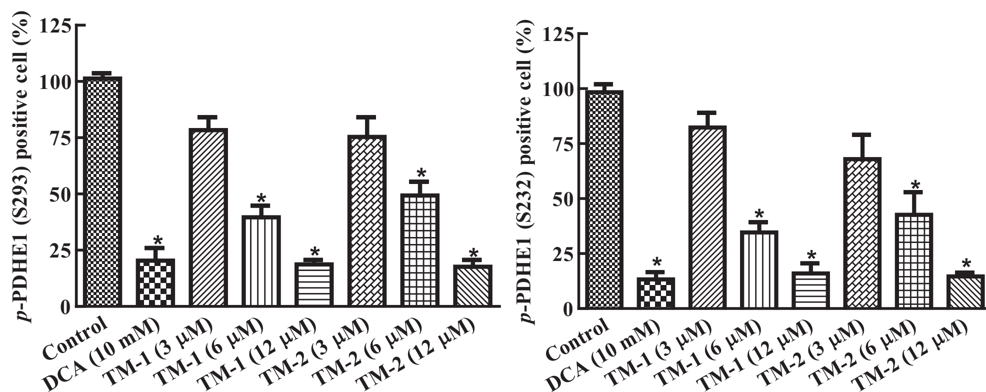

Fig. 3. Cellular activity of DCA, **TM-1** and **TM-2** on PDKs inhibition. MG-63 cancer cells were treated with DCA, **TM-1** and **TM-2** at desired concentrations for 12 h. The decrease of PDC phosphorylation of Ser293 and Ser232 was a reflection of PDKs inhibition.

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