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Development of dichloroacetamide pyrimidines as pyruvate dehydrogenases kinase inhibitors to reduce cancer cell growth: synthesis and biological evaluation†

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Pyruvate dehydrogenases kinases (PDKs) have recently emerged as an attractive target for anticancer treatment. Herein, we report the synthesis and biological evaluation of novel PDK1 inhibitors as anticancer agents. Of the newly synthesized compounds, *N*-(4,6-bis(4-(2-hydroxyacetyl)piperazin-1-yl)-2-methylpyrimidin-5-yl)-2,2-dichloroacetamide (40) is found to inhibit the growth of SF188 cancer cell with an IC_{50} value of 8.21 μM. Isothermal titration calorimetry (ITC) experiment reveals that compound 40 directly binds to PDK1 with a K_d value of 14.7 μM. Compound 40 inhibits PDK1 activity by 72.5 % at concentration of 40 μ, which could be a useful compound to explore the pharmacology of PDK1.

Targeting metabolic aberration has emerged as a promising strategy for cancer therapy over the last century.[1] Tumor cells exhibit a switch in metabolism from mitochondrial oxidative phosphorylation to cytoplasmic aerobic glycolysis.[2] This altered metabolism allows tumor cells to sustain higher proliferative rates and suppress apoptosis.[3] Moreover, the elevated lactate level in tumor environment contributes to the breakdown of the extracellular matrix, facilitates tumor cells mobility, and increases the metastatic potential.[4] Disruptions of these metabolic aberrations represent therapeutic opportunities in anticancer treatment.

In the process of glycolysis, glucose is converted to pyruvate, which is then further reduced to lactate in cancer cells. However, for normal cells, most of the pyruvate is oxidized and converted into acetyl-CoA in mitochondrion by pyruvate dehydrogenase complex (PDC), a gatekeeper enzyme that regulates the flux of the pyruvate into the mitochondria.[5, 6] However, the activity of PDC is tightly regulated by a variety of allosteric effectors and by reversible phosphorylation. The phosphorylation of PDC by PDKs results in its inactivation, while dephosphorylation of PDC by pyruvate dehydrogenase phosphatases (PDPs) restores its activity.[7] Inhibition of PDKs to increase the oxidative phosphorylation of glucose by activating PDC is an attractive therapeutic strategy to reverse the abnormal metabolic pathway and inhibit cancer cell proliferation.

Four PDK isoforms (PDK1, PDK2, PDK3, and PDK4) in mitochondria have been isolated and characterized.[8] PDK1 is mostly associated with cancer malignancy.[9] It had been reported that PDK1 was remarkably overexpressed in multiple human tumor such as lung cancer,[10] head squamous cancer,[9] myeloma,[11] and gastric cancer.[12] Over expression of PDK1 also correlated with poor prognosis and drug resistance.[13] Collectively, these suggest that PDK1 is a valid anticancer target.

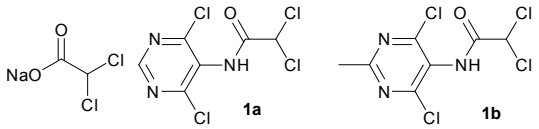

Fig. 1 Structures of DCA, compounds **1a** and **1b**

So far, a number of PDKs inhibitors have been reported, such as AZD7545,[14] Nov3r,[15] Pfz3,[16] PS10,[7] VER-246608,[17] and Radicicol,[18] but none of them are efficacious anticancer agents. In 2007, Michelakis *et al* reported that dichloroacetate (DCA, Fig. 1) was capable of inducing apoptosis in cancerous *in vitro*.[4] Since then, DCA has received considerable attention and rapidly entered Phase II clinical trials only three years after its anticancer activity was first reported. However, the high effective dosage (25-100 mg/kg body weight) appeared to limit its further clinical application.[19] Moreover, clinical data suggested that long-term DCA treatment of patients showed reversible limb motor weakness and demyelination of cerebral.

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† The authors declare no competing interests.

Electronic Supplementary Information (ESI) available: Supplementary information available for: Assay for lactate formation; Synthesis procedures for the target compounds; ^{1}H NMR, ^{13}C NMR, and HRMS data and spectra; Protocols for cell culture, cell viability assay, IncuCyte Zoom live cells image for the growth of the cancer cells, apoptosis detection by flow cytometry, PDK1 expression and purification, ITC Analysis, PDK kinase activity assay, molecule docking.

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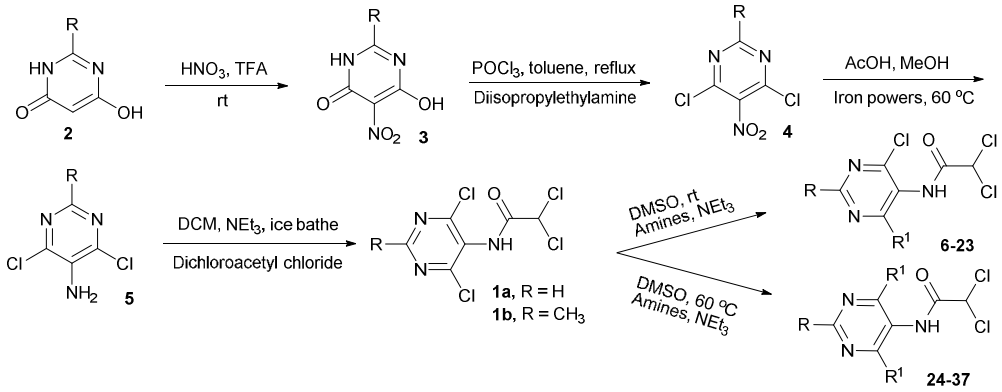
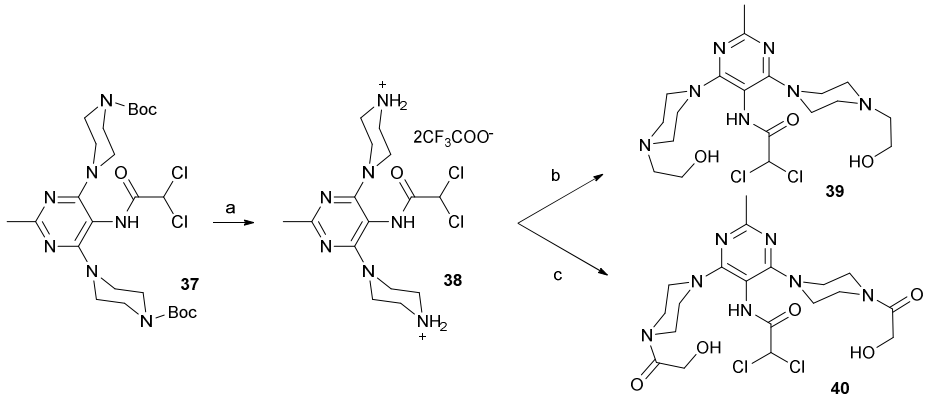
In our previous work,[2] we discovered the DCA derived compounds **1a** and **1b** (Fig. 1), which selectivity bound to the ATP pocket of PDK1 and exhibited moderate anti-proliferation activities against breast cancer cell line Michigan Cancer Foundation-7 (MCF-7) (ATCC). However, the binding of these two compounds to their target PDK1 is relatively weak. To further enhance the anticancer activities and binding affinity of this chemical series, herein we describe the optimization of compounds **1a** and **1b**, and their biological evaluation as PDK1 inhibitor.

The preparation of the target dichloroacetamide pyrimidines started from commercially available material **2**. The reaction of compound **2** with nitric acid (90% aqueous solution) yielded compound **3**, which was chloridized with phosphorus oxychloride to produce 2,6-dichloropyrimidine **4**,[20] and further reduced in the presence of by iron powers and acetic acid to afford 4,6-dichloro-5-aminopyrimidines **5**.[21] Finally, the dichloroacetamides **1a** and **1b** were prepared by the amidation of compounds **5** in dichloromethane with dichloroacetyl chloride.



Scheme 1 Synthetic routes for the mono-substituted dichloroacetamide pyrimidines **6**-**23** and bis-substituted dichloroacetamide pyrimidines **24**-**37**. For the compounds **14** and **23**, the reaction condition is DCM, **13**, and **22**, then was treated with TFA.

Then mono-substituted dichloroacetamide pyrimidines **6**–**23** were synthesized from the intermediates **1a** and **1b** by *N*-alkylation with various aliphatic amines at room temperature (Scheme 1). The bis-substituted pyrimidines **24**–**37** were successfully synthesized by increasing the reaction temperature to 60 °C (Scheme 1). At last, the *N*-Boc protection of bispiperazine pyrimidine **37** was subjected to the deprotection to produce compound **38**, which was then treated with bromoethanol or glycolic acid to afford target compounds **39** and **40** with yields of 64.7 % and 24.7%, respectively (Scheme 2). All prepared compounds were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS spectra.



Scheme 2 Synthetic routes for the bispiperazine pyrimidines. Reagents and conditions: (a) DCM, TFA, rt; (b) MeOH, NEt_3, 2-bromoethan-1-ol; (c) DMF, oxalyl chloride, glycolic acid, NEt_3, DCM, rt.

Next we used Alamar Blue assay to test the antiproliferative activity of the prepared compounds against the glioblastoma cell line SF188, colon cancer cell line RKO, and MCF-7 (all purchased from ATCC). As shown in Table 1, all of the mono-substituted, and some of the bis-substituted pyrimidines displayed relative low inhibition rates at 40 μM comparing with compounds **1a** and **1b**. Interestingly, the bis-substituted *N*-Boc pyrimidines **30** and **37** showed improved antiproliferative activity against the three cancer cell lines at 40 μM. In particular, the inhibition rates of **30** and **37** against SF188 are 45.20 % and 74.63 %, respectively. After the removal of the *N*-Boc group from **37** and further optimization, the resultant compounds **39** and **40** exhibited superior anticancer activity. It can be seen that both mono-substituted (**6**–**23**) and bis-substituted pyrimidines (**24**–**29**, **31**–**36** and **38**) with substituents of small/moderate size do not offer potency enhancement, which could be rationalized by our molecular docking studies (see below). On the other hand, introduction

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of bulky Boc group promotes anticancer activity (**37**), which is further improved in cases of compounds **39** and **40**.

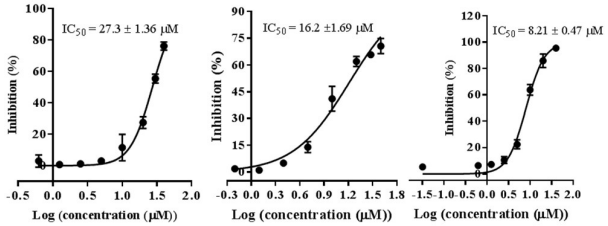
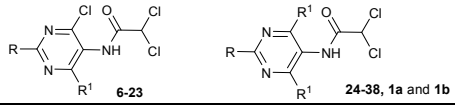


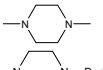
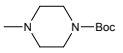
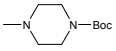
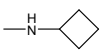
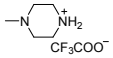
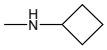
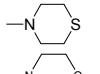
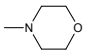
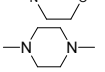
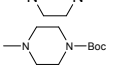
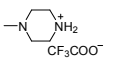
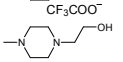
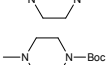
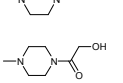
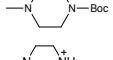
Fig. 2 Dose-response curves of compound **37** (left)**, 40** (right) and **1b** (middle) against the SF188 cancer cells.

We have observed that PDKs are highly expressed in SF188 and RKO cancer cell lines but moderately expressed in MCF-7 (unpublished results). Hence, SF188 cancer cell line was selected for dose response studies on compounds **37**, **40** and **1b** and further evaluations. Notably, the most potent compound **40** exhibited an improved IC_{50} value of 8.21 μM against the SF188 cancer cells (see Fig 2). Then, we used IncuCyte® Zoom Live-Cell Analysis System (Essen BioScience Company) to monitor the growth of SF188 cancer cell after being treated with compound **40** at 40 μM for 24, 48, and 72 h. As shown in Fig. 3, compound **40** inhibits the growth of SF188 cancer cell in a time dependent manner.

To explore the mechanism of cancer cells death, compounds **1b** and **40** were used to induce SF188 apoptosis, which was examined by using Annexin V-FITC/PI FACS assay. As shown in Fig. 4, the percentages of apoptosis for SF188 cells treated with compound **40** in 10, 20, and 40 μM for 24 h were 6.3, 9.3, and 13.4 %, respectively. This indicates that compound **40** induces the apoptosis of SF188 cells in a dose dependent manner. Clearly, the apoptotic rates in the presence of compound **40** at 20 μM outperformed compound **1b** at 40 μM.

Table 1 Antiproliferative assay on the prepared compounds against SF188, RKO and MCF-7 cancer cell lines.



Cp. ID	R	R^1	Yield (%)	Growth inhibition (%) for cancer cell lines at 40 μM			Cp. ID	R	R^1	Yield (%)	Growth inhibition (%) for cancer cell lines at 40 μM		
				SF188	RKO	MCF-7					SF188	RKO	MCF-7
6	H		75.1	8.91 ± 0.9	8.70 ± 0.72	22.54 ± 1.8	24	H		87.9	12.08 ± 1.1	13.14 ± 0.6	12.07 ± 1.2
7	H		77.9	16.99 ± 1.5	7.48 ± 0.81	20.77 ± 1.7	25	H		73.5	6.30 ± 0.54	5.32 ± 0.87	10.53 ± 1.2
8	H		83.9	9.79 ± 1.2	12.21 ± 1.0	7.43 ± 0.7	26	H		74.9	14.20 ± 1.3	9.43 ± 0.4	12.74 ± 1.5
9	H		76.8	11.54 ± 0.4	8.87 ± 0.7	9.28 ± 0.9	27	H		69.2	14.36 ± 1.2	13.68 ± 1.7	12.51 ± 0.3
10	H		81.2	11.70 ± 1.1	12.22 ± 1.4	7.69 ± 1.0	28	H		84.3	9.81 ± 0.4	12.67 ± 0.7	13.31 ± 1.3
11	H		78.5	11.06 ± 0.9	14.49 ± 1.7	7.04 ± 1.0	29	H		82.7	12.51 ± 1.2	14.92 ± 1.9	24.88 ± 2.0
12	H		79.4	16.99 ± 0.7	12.38 ± 1.5	12.88 ± 1.3	30	H		61.5	45.20 ± 3.8	42.88 ± 1.1	53.80 ± 6.5
13	H		84.1	17.20 ± 2.0	17.85 ± 1.2	16.39 ± 0.8	31	CH_3		60.7	17.03 ± 0.7	12.41 ± 1.0	15.42 ± 1.2
14	H		96.2	2.79 ± 0.9	10.75 ± 1.0	11.05 ± 0.5	32	CH_3		72.4	20.73 ± 1.5	15.13 ± 1.2	15.20 ± 1.7
15	CH_3		86.4	17.32 ± 1.3	6.08 ± 0.5	20.77 ± 1.7	33	CH_3		79.1	17.29 ± 1.1	13.78 ± 0.8	14.38 ± 1.2
16	CH_3		83.1	35.13 ± 2.7	13.89 ± 1.0	15.59 ± 0.8	34	CH_3		76.2	8.41 ± 0.4	5.32 ± 1.0	16.35 ± 1.2
17	CH_3		74.4	6.30 ± 0.7	5.29 ± 0.4	14.89 ± 1.1	35	CH_3		80.6	12.66 ± 1.3	13.07 ± 0.8	17.04 ± 1.1
18	CH_3		69.2	16.06 ± 0.4	5.37 ± 0.1	39.05 ± 2.7	36	CH_3		73.8	11.06 ± 1.0	14.49 ± 0.9	21.05 ± 1.8
19	CH_3		72.1	14.45 ± 1.1	12.85 ± 0.8	18.36 ± 0.7	37	CH_3		69.5	74.63 ± 5.4	57.01 ± 3.1	85.72 ± 6.3
20	CH_3		73.5	9.25 ± 0.6	9.64 ± 1.2	8.57 ± 0.4	38	CH_3		89.4	10.32 ± 0.7	13.95 ± 1.5	11.05 ± 1.2
21	CH_3		77.4	13.21 ± 1.5	13.64 ± 1.1	13.66 ± 0.7	39	CH_3		64.7	49.07 ± 5.0	51.73 ± 4.2	57.61 ± 2.7
22	CH_3		64.1	9.32 ± 1.0	6.89 ± 0.2	12.37 ± 1.4	40	CH_3		24.7	95.4 ± 5.0	86.34 ± 6.5	89.05 ± 7.4
23	CH_3		93.2	1.97 ± 0.4	5.66 ± 0.7	6.38 ± 0.8	1a	H	Cl	78.5	85.46 ± 5.2	48.49 ± 3.4	70.13 ± 4.5
							1b	CH_3	Cl	59.8	71.1 ± 6.5	43.31 ± 2.1	69.56 ± 2.5

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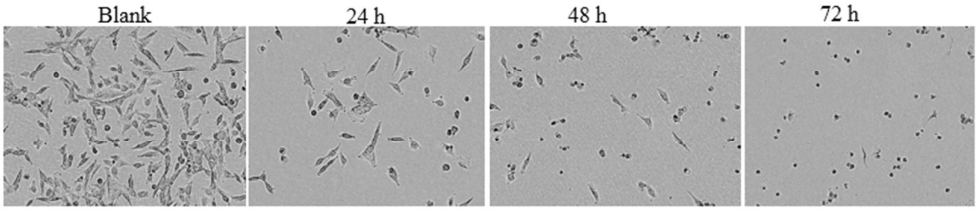

Fig. 3 Growth of SF188 cancer cells after treated with compound **40** at 40 μM for 24 h, 48 h, and 72 h. The images were taken by using IncuCyte Zoom live cells images (Essen BioScience Company).

With improved anticancer activity of compound **40**, we sought to address the question whether this compound interacted with its supposed target PDKs. Specifically, PDK1 solution was titrated with compound **40** in an isothermal titration calorimetry (ITC) experiment. A moderate K_d value (14.7 μM) was observed, suggesting that compound **40** interacted directly with PDK1 (see Fig. 5A). In contrast, the interaction of the starting compound **1b** with PDK1 was undetectable under the same conditions.

Next, PDK1 kinase activity was evaluated in the presence of compound **1b** and **40** using Kinase-Glo® Luminescent Kinase Assays (Promega). As shown in Fig. 5B, compound **40** reduced the consumption of ATP in the presence of a substrate peptide fragment (amino acid sequences: RYHGHSMSDP, which is a fragment around S293 of PDC),[2] indicating that compound **40** inhibited PDK1 activity. In Fig. 5C, compound **40** at 10, 20, and 40 μM reduced PDK1 activities by 24.3, 53.7, and 72.5 %, respectively. Inhibition of PDK1 activates PDC, leading to a switch of pyruvate consumption from lactate production to oxidative phosphorylation in the mitochondrion. Consequently, the lactate formation in cancer cell should be reduced after treatment with PDKs inhibitor. With this notion in mind, the lactate production was evaluated by Nova Bioprofile Flex analyzer (Nova Biomedical). As shown in Fig. S1, compound **40** decreases the lactate formation in SF188 cancer cells. The extent of lactate formation in the presence of compound **40** at 10 μM was similar to that of compound **1b** at 40 μM.

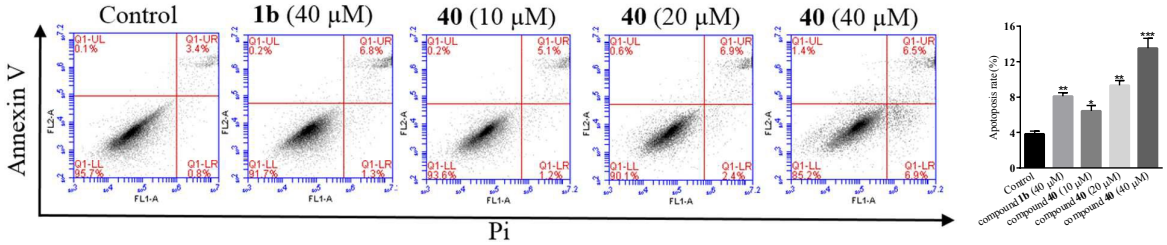

Fig. 4 Flow cytometer analysis of cell apoptosis. The cells were treated with compound **40** and **1b** for 24 h and then stained with FITC Annexin V / PI. Cells in the lower right quadrant indicate PI positive/ Annexin V negative, late apoptotic, or necrotic cells. The cells in the upper right quadrant indicate Annexin V-positive/PI negative, early apoptotic cells. * $P < 0.05$, versus control group.

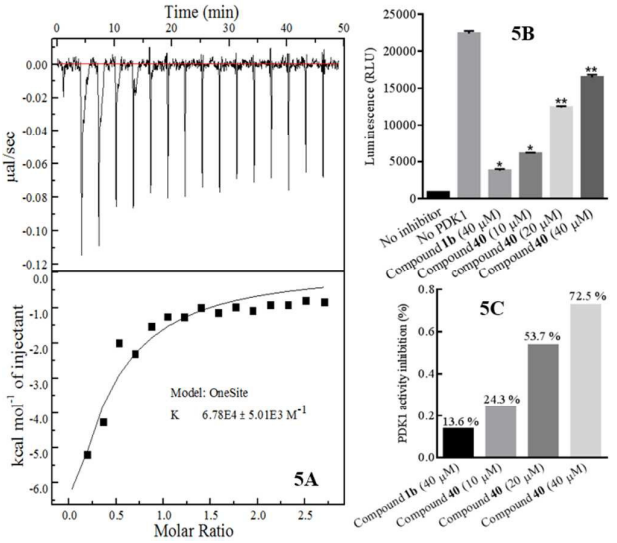

Fig. 5A ITC analysis of compound **40** binding to PDK1. Upper showed the raw data of the titration, in which the power output in microcalories per second is measured as a function of time in minutes; 5B: PDK1 kinase activity assay as indicated by ATP consumption in the presence of a substrate peptide fragment. * $P < 0.05$, versus no inhibitor control group; 5C: Reduced PDK1 activity after treatment of compounds **1b** and **40**.

To rationalize the interactions between the PDK binding site and compound **40**, molecule docking study was undertaken. All crystal structure data were obtained from the protein data bank. Since the structure of PDK1-ATP complex is not available, we selected PDK2-ATP complex (PDB code: 2BU2) as the template for docking. PDK1 (PDB code: 2Q8F) and PDK2 share high similarity in their structures, with an alignment Root Mean Square (RSM) less than 1.5 angstrom. More importantly, all critical residues involved in ATP binding are conserved between PDK1 and PDK2. These justify the use of the PDK2-ATP structure as template in our docking studies. As shown in the Fig. 6, compounds **1b** and **38** (as well as other analogues with small/moderate sized substituents; data not shown) were not within reach of the two conserved amino acids Asp 282 and Thr 346, which formed hydrogen bonds with ATP. When compound **39** was docked to the ATP pocket, we observed hydrogen bond interaction between the compound and Asp282. Docking of compound **40** to the ATP pocket showed hydrogen bond interactions of the compound with Asp 282 and Thr 346. These could contribute to the enhanced potency

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of compound **40** as compared with compounds **1b** and **39**. Apparently, compounds 39 and 40 are useful hits for the molecular design of inhibitors that specifically bind to the ATP pocket of PDKs. Further work on hit expansion based on compound 40 is being carried out in our laboratory. Results will be reported in due course.

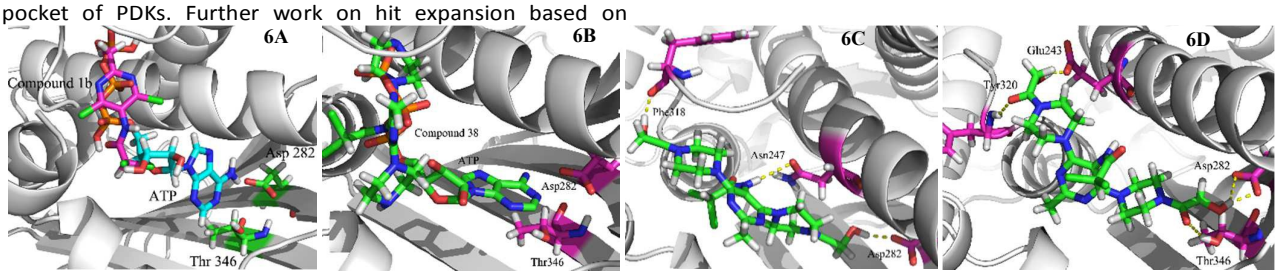

Fig. 6 Molecular modelling of compounds in the ATP binding pocket of PDK2. 6A: compound **1b** and ATP in the ATP binding pocket of PDK2; 6B: compound **38** and ATP in the ATP binding pocket of PDK2; 6C: compound **39** in the ATP binding pocket of PDK2; 6D: compound **40** in the ATP binding pocket of PDK2 (PDB code: 2BU2). All the amino acid residues and compounds are shown as stick models, H-bonds are shown as yellow dashed lines. The 3D graphical presentations were drawn by PyMol.

Conclusions

In this paper, we descried the synthesis and identification of novel PDK1 inhibitors. Their structures were confirmed by ^{1}H NMR, ^{13}C NMR, and HRMS. Biological assays revealed that compound **40** inhibited the cancer cell proliferation with an IC_{50} value of 8.21 μM against the SF188 cancer cells. ITC experiment suggested that compound **40** bound to PDK1 with a K_d value of 14.7 μM. Kinase activity assay showed that compound **40** in 10, 20, and 40 μM reduced PDK1 activity by 24.3, 53.7, and 72.5 %, respectively. Molecule modeling study indicated that compound **40** bound to ATP pocket of PDKs, potentially forming four hydrogen bonds with amino acid residues Glu 243, Asp 282, Thr 320, and Thr 346. Compound 40 could be a probe molecule to explore the pharmacology of PDK1.

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Notes and references

1 D. A. Tennant, R. V. Durán and E. Gottlieb, *Nat. Rev. Cancer,* 2010, **10**, 267.
2 S.-L. Zhang, X. H. Hu, W. Zhang and K. Y. Tam, *J. Med. Chem.*, 2016, **59**, 3562.
3 R. J. DeBerardinis, J. J. Lum, G. Hatzivassiliou and C. B. Thompson, *Cell Metab.*, 2008, **7**, 11.
4 S. Bonnet, S. L. Archer, J. Allalunis-Turner, A. Haromy, C. Beaulieu, R. Thompson, C. T. Lee, G. D. Lopaschuk, L. Puttagunta, S. Bonnet, G. Harry, K. Hashimoto, C. J. Porter, M. A. Andrade, B. Thebaud and E. D. Michelakis, *Cancer Cell*, 2007, **11**, 37.
5 E. D. Michelakis, G. Sutendra, P. Dromparis, L. Webster, A. Haromy, E. Niven, C. Maguire, T.-L. Gammer, J. R. Mackey, D. Fulton, B. Abdulkarim, M. S. McMurtry and K. C. Petruk, *Sci. Transl. Med.*, 2010, **2**, 31ra34.
6 S.-L. Zhang, X. H. Hu, W. Zhang, H. K. Yao and K. Y. Tam, *Drug Discov. Today*, 2015, **20**, 1112.
7 S. C. Tso, X. Qi, W. J. Gui, C. Y. Wu, J. L. Chuang, I. Wernstedt-Asterholm, L. K. Morlock, K. R. Owens, P. E. Scherer, N. S. Williams, U. K. Tambar, R. M. Wynn and D. T. Chuang, *J. Biol. Chem.*, 2014, **289**, 4432.
8 W. Zhang, S.-L. Zhang, X. H. Hu and K. Y. Tam, *Int. J. Biol. Sci.*, 2015, **11**, 1390.
9 S. M. Wigfield, S. C. Winter, A. Giatromanolaki, J. Taylor, M. L. Koukourakis and A. L. Harris, *Br. J. Cancer*, 2008, **98**, 1975.
10 M. I. Koukourakis, A. Giatromanolaki, G. Bougioukas and E. Sivridis, *Cancer Biol. Ther.*, 2007, **6**, 1476.
11 S. Fujiwara, Y. Kawano, H. Yuki, Y. Okuno, K. Nosaka, H. Mitsuya and H. Hata, *Br. J. Cancer*, 2013, **108**, 170.
12 H. Hur, Y. Xuan, Y. B. Kim, G. Lee, W. Shim, J. Yun, I. H. Ham and S. U. Han, *Int. J. Oncol.*, 2013, **42**, 44.
13 B. Daniel, R. Uta, H. Jannis, K. Hans, E. Jan, C. Hannes, M. Kurt, S. Martin and W. Steffen, *World J. Urol.*, 2013, 31, 1191.
14 J. A. Morrell, J. Orme, R. J. Butlin, T. E. Roche, R. M. Mayers and E. Kilgour, *Biochem. Soc. Trans.*, 2003, 31, 1168.
15 T. D. Aicher, R. C. Anderson, G. R. Bebernitz, G. M. Coppola, C. F. Jewell, D. C. Knorr, C. Liu, D. M. Sperbeck, L. J. Brand, R. J. Strohschein, J. Gao, C. C. Vinluan, S. S. Shetty, C. Dragland, E. L. Kaplan, D. DelGrande, A. Islam, X. Liu, R. J. Lozito, W. M. Maniara, R. E. Walter and W. R. Mann, *J. Med. Chem.*, 1999, **42**, 2741.
16 P. Gahlot and R. Kakkar, *Int. Res. J. Pharm.*, 2011, **1**, 33.
17 J. D. Moore, A. Staniszewska, T. Shaw, J. D'Alessandro, B. Davis, A. Surgenor, L. Baker, N. Matassova, J. Murray, A. Macias, P. Brough, M. Wood, P. C. Mahon, *Oncotarget*, 2014, **5**, 12862.
18 M. Kato, J. Li, J. L. Chuang and D. T. Chuang, *Structure*, 2007, **15**, 992.
19 P. W. Stacpoole, T. L. Kurtz, Z. Han and T. Langaee, *Adv. Drug. Deliv. Rev.*, 2008, **60**, 1478.
20 M. H. Norman, N. Chen, Z. D. Chen, C. Fotsch, C. Hale, N. Han, R. Hurt, T. Jenkins, J. Kincaid, L. B. Liu, Y. L. Lu, O. Moreno, V. J. Santora, J. D. Sonnenberg and W. Karbon, *J. Med. Chem.*, 2000, **43**, 4288.
21 N. Baindur, N. Chadha and M. R. Player, *J. Comb. Chem.*, 2003, **5**, 653.

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Development of dichloroacetamide pyrimidines as pyruvate dehydrogenases kinase inhibitors to reduce cancer cell growth: synthesis and biological evaluation

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The synthesis and biological assays were described herein to firstly identify a novel PDK1 inhibitor.