Accepted Manuscript

Discovery of novel naphthoquinone derivatives as inhibitors of the tumor cell specific M2 isoform of pyruvate kinase

Xianling Ning, Hailong Qi, Ridong Li, Yunqiao Li, Yan Jin, Michael A. McNutt, Junyi Liu, Yuxin Yin

PII: S0223-5234(17)30518-4

DOI: 10.1016/j.ejmech.2017.06.064

Reference: EJMECH 9559

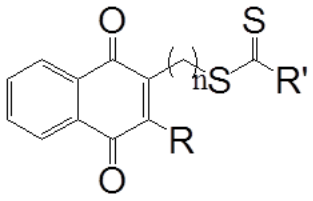
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 23 May 2017

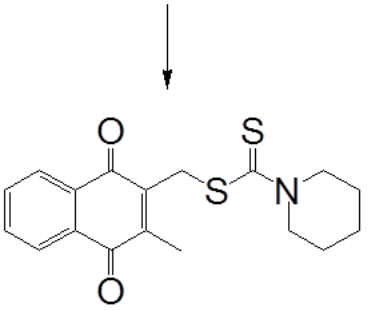
Revised Date: 27 June 2017

Accepted Date: 28 June 2017

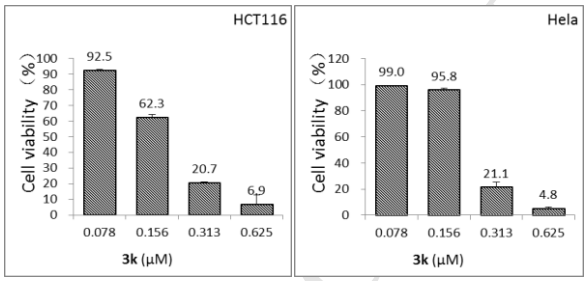
Please cite this article as: X. Ning, H. Qi, R. Li, Y. Li, Y. Jin, M.A. McNutt, J. Liu, Y. Yin, Discovery of novel naphthoquinone derivatives as inhibitors of the tumor cell specific M2 isoform of pyruvate kinase, *European Journal of Medicinal Chemistry* (2017), doi: 10.1016/j.ejmech.2017.06.064.



PKM2 inhibitors: 3a-3w, 4a-4d, 6a-6d



3k



IC_{50} = 0.18 μM

IC_{50} = 0.29 μM

Discovery of novel naphthoquinone derivatives as inhibitors of the tumor cell specific M2 isoform of pyruvate kinase

Xianling Ning[a, d], Hailong Qi[a, b], Ridong Li[a, d], Yunqiao Li[a, c], Yan Jin[a, c], Michael A. McNutt[a, b, c], Junyi Liu[e] and Yuxin Yin[a, b, c*]

[a]*Institute of Systems Biomedicine, School of Basic Medical Sciences, Peking University Health Science Center, Beijing, China.*

[b]*Peking-Tsinghua Center for Life Sciences, Peking University Health Science Center, Beijing, China.*

[c]*Department of Pathology, School of Basic Medical Sciences, Peking University Health Science Center, Beijing, China.*

[d]*Department of Pharmacology, School of Basic Medical Sciences, Peking University Health Science Center, Beijing, China.*

[e]*State Key Laboratory of Natural and Biomimetic Drugs, Department of Chemical Biology, School of Pharmaceutical Sciences, Peking University Health Science Center, Beijing, China.*

ABSTRACT

Pyruvate kinase M2 (PKM2) is a rate-limiting enzyme of the glycolytic pathway which is highly expressed in cancer cells. Cancer cells rely heavily on PKM2 for anabolic and energy requirements, and specific targeting of PKM2 therefore has potential as strategy for cancer therapy. Here, we report the synthesis and biologic evaluation of novel naphthoquinone derivatives as selective small molecule inhibitors of PKM2. Some target compounds, such as compound **3k**, displayed more potent PKM2 inhibitory activity than the reported optimal PKM2 inhibitor shikonin. The well performing compound **3k** also showed nanomolar antiproliferative activity toward a series of cancer cell lines with high expression of PKM2 including HCT116, Hela and H1299 with IC_{50} values ranging from 0.18-1.56 μM. Moreover, compound **3k** exhibited more cytotoxicity on cancer cells than normal cells. The identification of novel potent small molecule inhibitors of PKM2 not only offers candidate compounds for cancer therapy, but also provides a tool with which to evaluate the function of PKM2 in depth.

Keywords:

Pyruvate kinase M2, PKM2 inhibitor, Naphthoquinone derivatives, Antiproliferative activity

1. Introduction

Metabolic reprogramming is one of the hallmarks of highly proliferative cancer cells [1, 2]. Evidence suggests pyruvate kinase M2 (PKM2) makes a significant contribution to cancer metabolism, especially aerobic glycolysis [3, 4]. Pyruvate kinase converts phosphoenolpyruvate (PEP) to pyruvate and is expressed in four isoforms in different tissues [5]. The isoforms PKL, PKR and PKM1 are mainly

* Corresponding author

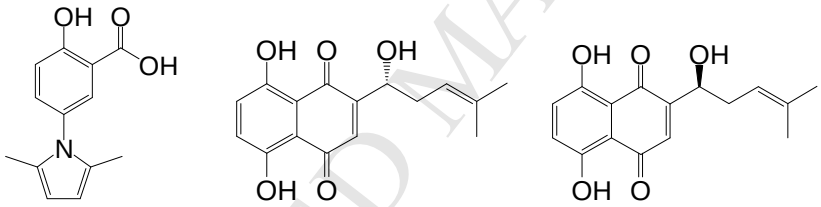
Email address: yinyuxin@hsc.pku.edu.cn

[键入文字]

expressed in normal tissues. However, PKM2 is preferentially expressed in various tumors [6, 7]. Selective targeting of PKM2 therefore offers an opportunity to target cancer cell metabolism and with reduced the side effects of therapy [8-10].

PKM2 exists in two catalytically different states and converts between high- and low-activity state. The state with high catalytic activity contributes to catabolic metabolism to produce energy. The state with low catalytic activity is conducive to anabolic metabolism to support cell growth. This regulation of PKM2 activity may provide tumor cells with the flexibility to adapt to different microenvironments [11]. Therefore, agonists or inhibitors of PKM2 may inhibit tumorigenesis by locking PKM2 into an on or off state.

There have been a number of studies of PKM2 agonists of in recent years [12-14]. However, PKM2 inhibitors have been less well studied. Christofk et al. report that PKM2 knockdown by RNA interference or displacement of PKM2 with PKM1 dramatically reduces the ability of human tumor cell lines to form tumor in nude mice [6]. Compound 3 (Fig. 1) is an effective PKM2 inhibitor that was found in a high-throughput screen which can inhibit PKM2 activity at millimolar concentrations in H1299 cells. However, compound 3 also has an inhibitory effect on the activity of PKL and PKR [15]. Shikonin (Fig.1) and its its enantiomeric isomer alkannin also inhibit PKM2 activity in cancer cells, and their inhibitory effects are much stronger than compound 3 [16].



Compound 3 Shikonin Alkannin

Fig. 1. Structures of known PKM2 inhibitors

In order to further improve the PKM2 inhibitory activity and selectivity of shikonin, we designed and synthesized a series of novel naphthoquinone derivatives (Fig. 2) that preserve the naphthoquinone skeleton and introduce diverse dithiocarbamate moieties. The dithiocarbamate moiety which replaces the alkyl chain of shikonin may provide more hydrogen bond receptors or donors to enhance the interaction of proteins and small molecules. At the same time, the dithiocarbamate moiety is well known as a molecular scaffold which possesses diverse biologic activity, such as anti-fungal, anti-bacterial and anti-tumor effects, which is conducive to increasing antitumor activity of target compounds [17, 18].

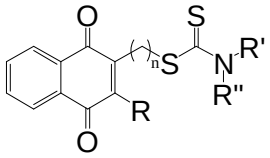


Fig. 2. The structure of target compounds

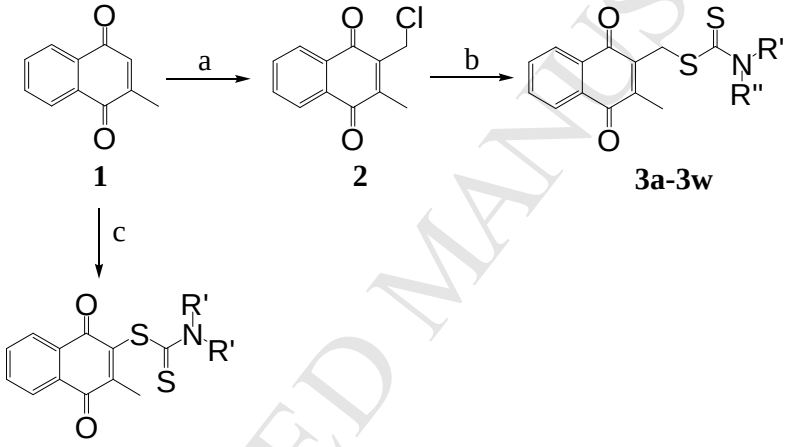
In this study we describe the synthesis, PKM2 inhibitory activity, PKM2 selectivity and cytotoxicity of a series of novel naphthoquinone derivatives. Some of the compounds we obtained, such as **3k**, show higher inhibitory activity for PKM2, and

display antiproliferative activity which is more potent than shikonin.

2. Results and discussion

2.1.Chemistry

To study the structure activity relationship (SAR) of target compounds, we synthesized the compounds **3a-3w**, **4a-4d** and **6a-6d**. The synthetic routes for the preparation of 2-dithiocarbamate-3-methyl substituted naphthoquinones (**3a-3w, 4a-4d**) are shown in Scheme **1**. Commercially available 2-Methyl-1,4-naphthoquinone **1** was used as a starting material and reacted with formaldehyde in the presence of dry hydrogen chloride in a cold mixed solvent of H_2O and acetic acid to provide 2-methyl-3- chloromethyl-1,4-naphthoquinone **2**. The target compounds **3a-3w** were obtained by reaction of the intermediate **2** with carbon disulfide (CS_2) and different amines using THF as a solvent which gave good yields. The target compounds **4a-4d** were obtained by reaction of compound **1** with CS_2 and amines in CH_3CN by one step with moderate yields.

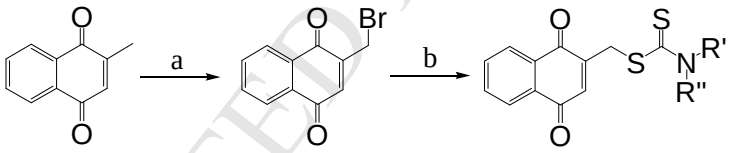


Compd.	
3a	$N(Et)_2$
3b	$N(n\text{-}Pr)_2$
3c	$N(n\text{-}Bu)_2$
3d	$N(CH_2CH_2OH)_2$
3e	$N(allyl)_2$
3f	1-morpholinyl
3g	1-thiamorpholinyl
3h	1-(2,6-dimethyl)morpholinyl
3i	1-thiazolidinyl
3j	1-pyrrolidinyl
3k	1-piperidinyl
3l	1-(4-methyl)piperazinyl
3m	1-(4-isopropyl)piperazinyl
3n	1-(4-acetyl)piperazinyl
3o	NCH_3cyclohexyl

3p	$NHCH_3$
3q	$NHCH_2$-2-Py
3r	$NHCH_2$-3-Py
3s	$NHCH_2$-4-Py
3t	$NHCH_2Ph$
3u	NH-1-piperidinyl
3v	NH-1-(4-methyl)piperazinyl
3w	$NHCH_2CH_2N(Et)_2$
4a	$N(n\text{-}Pr)_2$
4b	1-morpholinyl
4c	1-piperidinyl
4d	1-(4-acetyl)piperazinyl

Scheme 1. Synthesis of 2-dithiocarbamate-3-methyl substituted naphthoquinones. Reagents and conditions: (a) formaldehyde, HCl, HAc, H_2O, 0 ℃, 78.0%; (b) CS_2, amine, THF, rt, 75-98%. (c) CS_2, amine, CH_3CN, rt, 55-70%.

2-Dithiocarbamate-3-hydrogen substituted naphthoquinones (**6a-6d**) were prepared as shown in Scheme **2**. 2-Methyl-1,4-naphthoquinone **1** was used as a starting material, and was brominated with N-bromosuccinimide (NBS) in the presence of a catalytic amount of 2,2-azobisisobutyronitrile (AIBN) in reflux acetic anhydride to give the compound 2-bromomethyl-1,4-naphthoquinone **5**. Compound **5** was treated with CS_2 and different amines to obtain corresponding target compounds **6a-6d** in moderate yields.

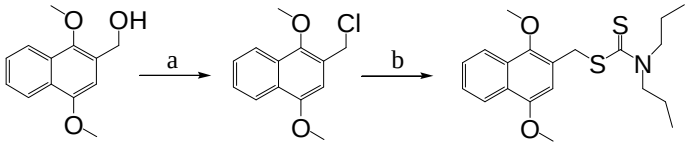


1 **5** **6a-6d**

Compd.	
6a	$N(Et)_2$
6b	1-morpholinyl
6c	1-piperidinyl
6d	1-(N-phenyl)piperazinyl

Scheme 2. Synthesis of 2-dithiocarbamate-3- hydrogen substituted naphthoquinones. Reagents and conditions: (a) NBS, AIBN, acetic anhydride, reflux, 63%; (b) CS_2, amine, CH_2Cl_2, rt, 69-75%.

To further study the SAR of these compounds, the 1,4-dimethoxy-naphthalen derivative **9** was also synthesized (Scheme 3). 1,4-Dimethoxy-2-hydroxymethyl naphthalen **7** was synthesized as previously described [19], and was chloridized with thionyl chloride ($SOCl_2$) in refluxed CH_2Cl_2 to achieve 1,4-dimethoxy-2-chloromethyl naphthalen **8**. Compound **8** was treated with CS_2 and n-propylamine to obtain the corresponding target compound **9**.



7 **8** **9**

Scheme 3. Synthesis of dipropyl-dithiocarbamic acid 1,4-dimethoxy-naphthalen-2-ylmethyl ester

Reagents and conditions: (a) $SOCl_2$, CH_2Cl_2, reflux, 78%; (b) CS_2, di-n-propylamine, CH_2Cl_2, rt, 80 %.

2.2.Biological evaluation

2.2.1. PKM2 inhibition activity by target compounds

After the synthesis of these compounds, the cell-free PKM2 inhibitory activity of target compounds was evaluated with a fluorescent PK-LDH coupled assay as previously reported (Fig. **4**) [15]. Shikonin was used as the positive control. The results expressed as IC_{50} are shown in Table **1**. As shown in Table **1**, most of the compounds exhibited some degree of PKM2 inhibition and some compounds, such as **3k** and **6d**, displayed more potent activity than the positive control shikonin. The amine moiety markedly influenced PKM2 inhibitory activity. Introduction of a long chain amine slightly enhanced inhibitory activity (**3a** vs **3b** vs **3c**). When the n-propyl amine of **3b** was replaced by ethoxyl amine (**3d**) and allyl amine (**3e**), the inhibitory activity was reduced or lost. The chain amines were further altered to various heterocycle amines. The thiamorpholinyl **3g** (IC_{50} = 5.89±1.75 μM) and thiazolidinyl **3i** (IC_{50} = 5.28±0.13 μM) substitution compounds respectively demonstrated greater potency than morpholinyl **3f** (IC_{50} = 15.24±2.72 μM) and pyrrolidinyl **3j** (IC_{50} = 19.73±5.11 μM) substitution compounds. This indicated that introduction of a sulfur atom was conductive to improving PKM2 inhibitory activity. The piperidinyl substitution compound **3k** (IC_{50} = 2.95±0.53 μM) showed slightly stronger potency than the thiamorpholinyl substitution compound **3g** (IC_{50} = 5.89±1.75 μM). In addition, the 4-methylpiperazinyl substitution compound **3l** (IC_{50} = 14.24±2.04 μM) showed low activity, and increasing steric hindrance (**3m** and **3n**) caused loss of activity. Introduction of primary amines (**3p**-**3s**, **3u**-**3w**) reduced their inhibitory activity. Interestingly, compound **3t** (IC_{50} = 2.21±0.60 μM) was an exception which displayed high inhibitory activity, and this compound warrants further study. We further investigated the contribution of the linker of naphthoquinone and dithiocarbamate moieties and the 3-substituent in naphthoquinone. It was found that n is 0 or 1 in target compounds rarely influenced the potency of inhibitory activity (**3b** vs **4a**, **3k** vs **4c**, **3n** vs **4d**). Removal of the 3-methyl substituent in the naphthoquinone results in a 2-4-fold increase in IC_{50} (**3a** vs **6a**, **3f** vs **6b**, **3k** vs **6c**). In other words, a 3-unsubstitution at the naphthoquinone scaffold significantly improved PKM2 inhibitory potency. The 1, 4-dimethoxy-naphthalen derivative **9** (IC_{50} = 15.66±4.47 μM) exhibited lower potency than the naphthoquinone derivative **3b** (IC_{50} = 6.79±0.54 μM), indicating that the naphthoquinone unit is essential for PKM2 inhibitory activity.

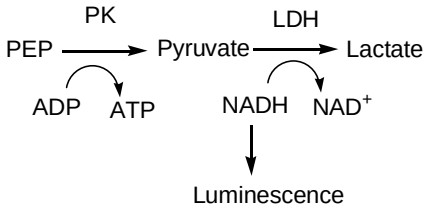
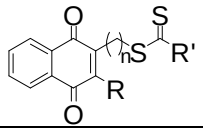


Fig. 4. In vitro a fluorescent PK-LDH coupled assay.

Table 1

In vitro PKM2 inhibition activity (IC_{50}) of target compounds



Compd.	n	R	R’	IC_{50} ± SD (μM)
3a	1	CH_3	$N(Et)_2$	8.78±1.05
3b	1	CH_3	$N(n\text{-}Pr)_2$	6.79±0.54
3c	1	CH_3	$N(n\text{-}Bu)_2$	4.57±0.37
3d	1	CH_3	$N(CH_2CH_2OH)_2$	> 20
3e	1	CH_3	$N(allyl)_2$	14.11±3.40
3f	1	CH_3	1-morpholinyl	15.24±2.72
3g	1	CH_3	1-thiamorpholinyl	5.89±1.75
3h	1	CH_3	1-(2,6-dimethyl)morpholinyl	11.70±0.67
3i	1	CH_3	1-thiazolidinyl	5.28±0.13
3j	1	CH_3	1-pyrrolidinyl	19.73±5.11
3k	1	CH_3	1-piperidinyl	2.95±0.53
3l	1	CH_3	1-(4-methyl)piperazinyl	14.24±2.04
3m	1	CH_3	1-(4-isopropyl)piperazinyl	> 20
3n	1	CH_3	1-(4-acetyl)piperazinyl	> 20
3o	1	CH_3	NCH_3cyclohexyl	5.08±0.34
3p	1	CH_3	$NHCH_3$	> 20
3q	1	CH_3	$NHCH_2$-2-Py	> 20
3r	1	CH_3	$NHCH_2$-3-Py	> 20
3s	1	CH_3	$NHCH_2$-4-Py	> 20
3t	1	CH_3	$NHCH_2Ph$	2.21±0.60
3u	1	CH_3	NH-1-piperidinyl	> 20
3v	1	CH_3	NH-1-(4-methyl)piperazinyl	> 20
3w	1	CH_3	$NHCH_2CH_2N(Et)_2$	> 20
4a	0	CH_3	$N(n\text{-}Pr)_2$	9.98±0.14
4b	0	CH_3	1-morpholinyl	8.50±2.06
4c	0	CH_3	1-piperidinyl	4.93±1.78
4d	0	CH_3	1-(4-acetyl)piperazinyl	> 20
6a	1	H	$N(Et)_2$	2.58±0.76
6b	1	H	1-morpholinyl	4.23±1.02
6c	1	H	1-piperidinyl	1.74±0.71
6d	1	H	1-(N-phenyl)piperazinyl	1.40±0.41

[键入文字]

9	-	-	-	15.66±4.47
Shikonin	-	-	-	8.82 ± 2.62

2.2.2. PKM2 selectivity of target compounds

Because of the similarity of amino acid sequences in PKM2 and the other isoforms of pyruvate kinase, evaluation of the selectivity for PKM2 of these target compounds was required. Shikonin was used as the positive control. The representative compounds **3k**, **6d** displayed dose-dependent inhibition of PKM2 with less inhibition of PKM1 and PKL like shikonin. The IC_{50} of shikonin for PKM1 and PKL were respectively about 1.5-fold and 4.5-fold higher than that for PKM2 according to the assay results. However, the IC_{50}s of compounds **3k** and **6d** for PKM1 and PKL were respectively about 4-5-fold and 2-3-fold higher than that for PKM2 (Table **2**). Thus, the selectivity for PKM2 of target compounds is close to that of shikonin.

Table 2

PKM2 selectivity of tested compounds on different PK isoforms

Compd.	PKM1 (μM)	IC_{50}(PKM1)/ IC_{50}(PKM2)	PKL (μM)	IC_{50}(PKL)/ IC_{50}(PKM2)
3k	16.71±4.66	5.7	8.20±3.43	2.8
6d	6.26±1.75	4.5	3.80±0.25	2.7
Shikonin	12.96 ± 3.37	1.5	39.25 ± 6.53	4.5

2.2.3. Antiproliferative effects of target compounds

To determine the effectiveness of target compounds as anti-tumor agents, we evaluated the effect of target compounds on cancer cell viability. In vitro cytotoxicity was assessed using three PKM2 high expression human tumor cell lines derived from human colon cancer (HCT116), cervical cancer (Hela) and lung cancer (H1299). The results of this evaluation are presented in Table **3**. Some target compounds reduced cancer cell viability at nanomolar concentrations with showing more pronounced cytotoxicity than the positive control shikonin in MTS reduction assays in human cancer cell. Among these, the most potent compounds were **3a**, **3k** (Fig.**5**) and **3r**, which exhibited IC_{50} values against HCT116 and Hela cells ranging from 0.39 to 0.41 μM, 0.18 to 0.29 μM and 0.18 to 0.38 μM, respectivdy. Introduction of a long chain amine lowered the cytotoxicity of target compounds (**3a** vs **3b** vs **3c**). When the n-propyl amine in **3b** was replaced by ethoxyl amine (**3d**) and allyl amine (**3e**), the inhibitory activity increased slightly. In addition, replacing the chain amines with various cyclic amines, the piperidinyl (**3k**), morpholinyl (**3f**), thiamorpholinyl (**3g**) substitution compounds demonstrated greater potency than pyrrolidinyl (**3j**), thiazolidinyl (**3i**) and piperazinyl (**3l-3n**) substitution compounds. Compound **3r** bearing a 3-pyridylamino substitution exhibited greater cytotoxicity than 2- and 4-pyridylamino substitution. However, replacing 3-pyridylamino (**3r**) with benzylamino (**3t**) reduced its activity. In addition, the deletion of a 3-methyl substituent in the naphthoquinone resulted in a decrease of cytotoxicity of the target compounds in cell lines (**3b** vs **6a** and **3k** vs **6c**). When naphthoquinone and

dithiocarbamate moieties were linked by a methylene group (n = 1), the cytotoxicity was higher than that by a direct connect (n = 0). The oxidization state of derivative **3b** exhibited greater potency than the reducing state of derivative **9**. Thus the naphthoquinone moiety is very important for inhibitory activity.

These results show that a weak correlation exists between antiproliferative effects and PKM2 inhibitory activity. The discrepancies between them may be due to the different properties of these compounds such as cell penetration which is important in the cellular assay. It is also possible that target compounds have other mechanisms by which tumor cells are targeted. However, some compounds which were tested showed high PKM2 inhibitory potency with close to nanomolar activity, which supports a ditect enzyme interaction mechanism. In addition, we found that fructose-1,6-bisphosphate (FBP) which is a well endogenic allosteric activator of PKM2, reduced the inhibitory activity and relieve the cytotoxicity of tested PKM2 inhibitors compounds **3a** and **3k** (Table **4**), which also suggests that PKM2 is the direct target of compounds which were tested.

Table 3

In vitro cytotoxicity of target compounds

	$IC_{50} \pm SD^a$ (μM)		
Compd.	HCT116	Hela	H1299
3a	0.41±0.13	0.39±0.08	0.89±0.19
3b	6.00±1.12	12.08±2.20	4.23±1.24
3c	10.94±2.45	18.53±3.14	6.41±1.25
3d	2.23±0.51	4.80±0.84	1.95±0.99
3e	2.17±0.37	1.16±0.66	3.20±0.17
3f	0.80±0.17	0.95±0.39	1.23±0.51
3g	0.89±0.32	0.32±0.11	1.19±0.44
3h	1.10±0.33	0.87±0.92	1.47±0.67
3i	1.75±0.15	1.96±0.95	3.28±1.21
3j	1.46±0.32	0.88±0.64	1.64±0.73
3k	0.18±0.16	0.29±0.11	1.56±0.25
3l	2.84±1.14	1.57±0.17	2.44±0.14
3m	1.97±0.77	2.19±1.37	4.06±2.51
3n	2.08±0.26	3.52±1.94	4.68±0.44
3o	10.61±3.14	13.55±3.27	11.27±1.26
3p	4.59±1.30	8.36±3.19	5.39±0.67
3q	5.42±1.75	6.77±2.59	4.49±0.33
3r	0.18±0.05	0.38±0.13	1.08±0.25
3s	0.59±0.23	0.50±0.31	0.67±0.17
3t	1.72±0.76	1.30±1.07	4.84±0.89
3u	7.36±3.20	10.42±4.75	4.37±0.12

[键入文字]

3v	2.00±1.52	3.92±1.35	> 20
3w	3.37±0.86	5.78±2.07	3.97±0.77
4a	4.30±1.20	> 20	15.59±5.93
4b	2.28±1.19	2.14±0.24	3.27±1.24
4c	3.55±2.02	1.29±1.23	10.18±3.32
4d	4.95±1.08	10.61±3.68	6.61±0.52
6a	1.82±0.36	0.51±0.29	1.90±0.11
6b	3.68±1.61	2.39±0.04	5.69±3.10
6c	2.37±0.31	0.92±0.20	2.06±0.09
6d	> 20	> 20	8.71±2.07
9	> 20	> 20	> 20
Shikonin	1.84±0.18	2.45±0.81	1.88±0.15

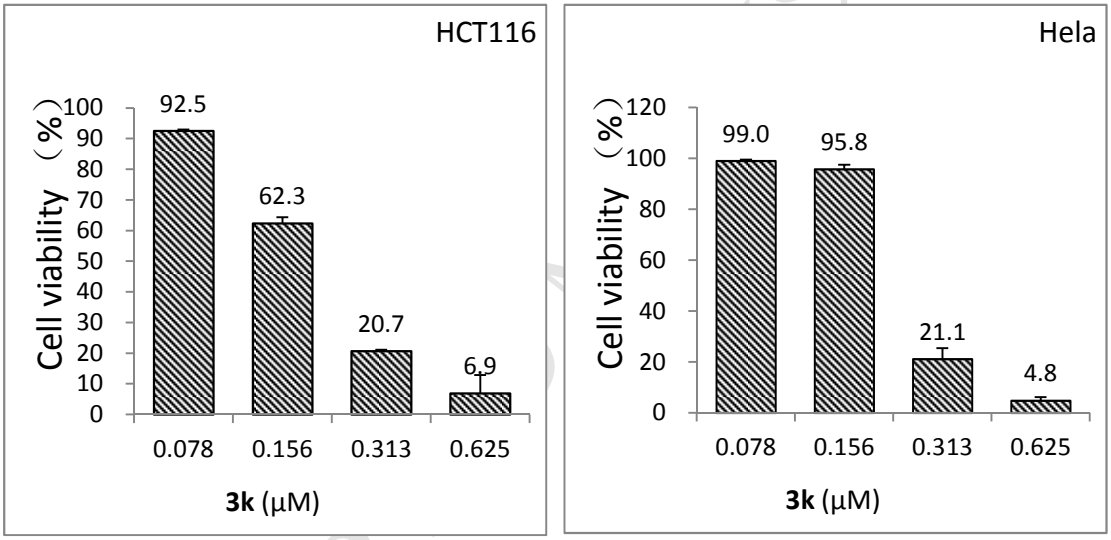


Fig. 5. Compound **3k** reduces the tumor cell viability in a dose depend manner. Cells were treated with increasing concentrations of the PKM2 inhibitor compound **3k**. Cell viability was measured using MTS.

Table 4

In vitro cytotoxicity of **3a** and **3k** in the presence of FBP (100 μM)

	IC_{50} ± SD[a] (μM) (H1299)	
FBP	+	-
3a	1.58±0.33	0.89±0.19
3k	2.01±0.16	1.56±0.25

2.2.4. Cytotoxicity of some target compounds against normal cells

To further explore the selectivity of target compounds against cancer cells, they were also tested in BEAS-2B cells derived from normal human bronchial epithelial cells. As seen in Table **5**, the tested compounds showed higher cytotoxicity in cancer cells (H1299) than normal cells (BEAS-2B). The selectivity for tumor cells of target compounds was superior to that of shikonin. The results indicate that target

[键入文字]

compounds have highly safety index.

Table 5

In vitro safety index of target compounds (BEAS-2B)

Compd.	IC_{50} ± SD[a] (μM)	safety index
3a	15.09±0.54	17.0
3g	10.54±1.80	8.6
3k	18.46±1.04	11.8
6c	19.31±1.79	9.4
Shikonin	5.36±0.69	2.9

3. Conclusions

In this study we have described the identification and characterization of a previously undescribed chemical class of PKM2 inhibitors. These novel naphthoquinone derivatives act as PKM2 inhibitors, and show high inhibitory responses that are more potent than shikonin and have selectivity similar to shikonin. These compounds lock PKM2 into a low activity conformation which forces alteration of cancer cell metabolism that is less metabolically flexible than metabolism in the normal state. Moreover, some of these target compounds show higher antiproliferative effects than shikonin. However, there are unexpected results in the enzyme and cytotoxicity experiments. The absence of correlation between PKM2 inhibitory activity and in vitro cytotoxicity of the target compounds suggests that the cells are probably using other mechanisms to activate these compounds, except in the case of PKM2 inhibitory activity. However, there is no doubt that PKM2 is a target of these synthesized naphthoquinone derivatives, through the IC_{50} of some compounds which inhibit PKM2 activity is closed to nanomolar concentration range.

PKM2 has been identified as a major contributor not only of metabolic reprogramming as for pyruvate kinases but also in direct regulation of gene expression as a protein kinase in cancer cells [20-22]. Upon EGFR activation, PKM2 translocates from the cytoplasm into the nucleus of cancer cells where it activates β-catenin to induce CCDN1 and c-Myc expression and upregulate GLUT1 and lactate dehydrogenase A (LDHA) [21]. Upregulation of these glycolysis genes increases glucose consumption and lactate production, and subsequently promotes tumorigenesis [23].Therefore, inhibition of PKM2-coactivated tumor gene transcription and glycolysis gene expression are also potential tumor treatment strategies. An attempt will be made to determine whether the mechanism works for the synthesized naphthoquinone derivatives. If so, the mechanism also can explain the SAR discrepancy of enzyme activity and cytotoxicity of target compounds.

Further investigation will also focus on structural studies of PKM2 protein and small molecule inhibitor complex, which may lead to identification of compounds with higher effectiveness. In any case, the findings presented here establish a foundation for the development of PKM2-targeted anticancer therapies.

4. Experimental section

4.1. General. Reactions were monitored by thin layer chromatography (TLC) on precoated silica gel F_{254} plates. Detection was by iodine vapor staining and UV light irradiation (UV lamp, model UV-IIB). Column chromatography was carried out with Silica gel H (200-300 mesh or 500 mesh). Melting points were determined using an X_4-type apparatus and left uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE III-400 spectrometer, Chemical shifts δ in ppm with Me_4Si as an internal standard, coupling constants J in Hertz. High resolution mass spectrum (HRMS) was recorded on a Thermo Scientific Orbitrap Elite MS. Unless otherwise stated, all reagents were purchased from commercial sources. When necessary, reagents were purified and dried by standard methods. Organic solutions were dried over anhydrous sodium sulfate.

*4.2. Procedure for preparation of 2-chloromethyl-3-methyl-[1, 4] naphthoquinone (**2**)*

The 2-methyl-1,4-naphthaquinone (**1**) (2 g, 11.6 mmol) in glacial acetic acid (20 mL) was taken in a 100 mL round-bottomed flask, and 36% aqueous formaldehyde (7 mL) was added. The reaction solution was cooled in ice-water. Dry hydrogen chloride passed in for 1 h. The solution turned red, and was then kept at room temperature for 24 h. The reaction mixture was poured on ice and extracted with ethyl acetate. The combined organic fractions were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification of the crude residue by column chromatography (petroleum ether/ethyl acetate) afforded compound **2** (yellow solid). The yield of this reaction was 78.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13-8.18 (m, 2H, Ar*H*), 7.76-7.78 (m, 2H, Ar*H*), 4.64 (s, 2H, CH_2S), 2.35 (s, 3H, C=CCH_3).

*4.3. General procedure for preparation of dithiocarbamic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3**)*

Carbon disulfide (120 μL, 2 mmol) and amine (2 mmol) were added to THF (5 mL) and the resulting solution was stired for 30 minutes. 2-Chloromethyl-3-methyl-[1, 4] naphthoquinone (**2**) (220 mg, 1 mmol) was added in portions at frequent intervals. The reaction mixture was then kept at room temperature until compound **2** was absent (as evaluated by TLC). The reaction mixture was concentrated in vacuo, diluted with H_2O, and extracted with CH_2Cl_2. The combined organic fractions were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification of the crude residue by column chromatography (petroleum ether/ CH_2Cl_2) afforded the compound of interest.

*4.3.1. Data for diethyl-dithiocarbamic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3a**):* yellow solid (89.6%); mp 155-156 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11-8.14 (m, 2H, Ar*H*), 7.73-7.74 (m, 2H, Ar*H*), 4.65 (s, 2H, CH_2S), 4.06 (q, 2H, NCH_2), 3.73 (q, 2H, NCH_2), 2.37 (s, 3H, C=CCH_3), 1.30 (t, 6H, 2CH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.49, 184.81, 183.95, 146.42, 141.66, 133.67, 133.63, 132.19, 131.92, 126.49, 49.92, 46.80, 33.85, 13.70, 12.53, 11.57. HR-MS (ESI^+) m/z: 334.0936 $[M+H]^+$. Found: 334.0932 $[M+H]^+$.

*4.3.2. Data for dipropyl-dithiocarbamic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3b**):* yellow solid (86.0%); mp 80-81 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10-8.14 (m, 2H, Ar*H*), 7.72-7.74 (m, 2H, Ar*H*), 4.63 (s, 2H, CH_2S), 3.93 (t, 2H, NCH_2), 3.61 (t, 2H, NCH_2), 2.35 (s, 3H, C=CCH_3), 1.70-1.82 (m, 4H, 2CH_2CH$_3$), 0.92-0.98 (m, 6H, 2CH$_2$CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.02, 184.79, 183.89, 146.48, 141.64, 133.62, 132.19, 131.93, 126.49, 57.25, 54.43, 33.92, 20.70, 19.62, 13.66, 11.18. HR-MS (ESI$^+$) m/z: 362.1249 [M+H]$^+$. Found: 362.1244 [M+H]$^+$.

*4.3.3. Data for dibutyl-dithiocarbamic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3c**)*: yellow liquid (90.5%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11-8.15 (m, 2H, Ar*H*), 7.73-7.75 (m, 2H, Ar*H*), 4.63 (s, 2H, CH_2S), 3.98 (t, 2H, NCH_2), 3.64 (t, 2H, NCH_2), 2.36 (s, 3H, C=CCH_3), 1.67-1.75 (m, 4H, 2CH_2CH$_2$CH$_3$), 1.32-1.41 (m, 4H, 2CH$_2$CH_2CH$_3$), 0.93-1.00 (m, 6H, 2CH$_2$CH$_2$CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.80, 184.81, 183.88, 146.48, 141.67, 133.60, 132.21, 131.97, 126.48, 55.46, 52.60, 33.93, 29.69, 29.38, 26.36, 20.12， 13.81， 13.65. HR-MS (ESI$^+$) m/z: 390.1562 [M+H]$^+$. Found: 390.1558 [M+H]$^+$.

*4.3.4. Data for bis-(2-hydroxy-ethyl)-dithiocarbamic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3d**):* yellow solid (82.4%); mp 91-92 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10-8.12 (m, 2H, Ar*H*), 7.73-7.75 (m, 2H, Ar*H*), 4.61 (s, 2H, CH_2S), 3.98-4.33 (m, 8H, 2NCH_2CH_2), 2.78 (s, 2H, 2OH), 2.35 (s, 3H, C=CCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.91, 184.62, 183.85, 146.68, 141.64, 133.78, 133.72, 132.13, 131.83, 126.56, 126.50, 60.65, 59.35, 57.57, 57.52, 34.19, 13.69. HR-MS (ESI$^+$) m/z: 366.0834 [M+H]$^+$. Found: 366.0830 [M+H]$^+$.

*4.3.5. Data for diallyl-dithiocarbamic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3e**):* yellow solid (75.0%); mp 95-96 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10-8.12 (m, 2H, Ar*H*), 7.72-7.75 (m, 2H, Ar*H*), 5.81-5.91(m, 2H, 2C*H*=CH$_2$)，5.20-5.28 (m, 4H, 2CH=CH_2), 4.68 (m, 2H, NCH_2), 4.65 (s, 2H, CH_2S), 4.31 (m, 2H, NCH_2), 2.35 (s, 3H, C=CCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.95, 184.72, 183.83, 146.51, 141.44, 133.65, 133.62, 132.19, 131.92, 130.98, 130.92, 130.31, 126.48, 118.74, 56.86, 53.71, 34.25, 13.65. HR-MS (ESI$^+$) m/z: 358.0936 [M+H]$^+$. Found: 358.0934 [M+H]$^+$.

*4.3.6. Data for morpholine-4-carbodithioic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3f**):* yellow solid (86.4%); mp 132-133 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11-8.14 (m, 2H, Ar*H*), 7.74-7.76 (m, 2H, Ar*H*), 4.68 (s, 2H, CH_2S), 4.32 (m, 2H, NCH_2), 3.99 (m, 2H, NCH_2), 3.78 (m, 4H,CH_2CH_2), 2.38 (s, 3H, C=CCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.44, 184.71, 183.93, 146.54, 141.26, 133.76, 133.71, 132.15, 131.86, 126.56, 126.50, 66.26, 66.17, 53.63, 33.70, 13.74. HR-MS (ESI$^+$) m/z: 348.0728 [M+H]$^+$. Found: 348.0727 [M+H]$^+$.

*4.3.7. Data for thiomorpholine-4-carbodithioic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3g**):* yellow solid (88.4%); mp 157-158 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12-8.13 (m, 2H, Ar*H*),

7.73-7.75 (m, 2H, Ar*H*), 4.67 (s, 2H, CH_2S), 4.55 (t, 2H, NCH_2), 4.36 (t, 2H, NCH_2),2.77 (s, 4H, CH_2SCH_2), 2.37 (s, 3H, C=CCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.78, 184.68, 183.91, 146.54, 141.27, 133.74, 133.69, 132.16, 131.87, 126.54, 126.49, 60.19, 54.19, 33.92, 27.27, 13.71. HR-MS (ESI^+) m/z: 340.0500 $[M+H]^+$. Found: 364.1151 $[M+H]^+$.

*4.3.8. Data for 2,6-dimethyl-morpholine-4-carbodithioic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3h**):* yellow solid (76.5%); mp 141-142 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11-8.14 (m, 2H, Ar*H*), 7.73-7.76 (m, 2H, Ar*H*), 5.48 (m, 1H, NCH_2CH), 4.68 (s, 2H, CH_2S), 4.42 (m, 1H, NCH_2CH), 3.67 (t, 2H, NCH_2), 2.83 (t, 2H, NCH_2), 2.37 (s, 3H, C=CCH_3), 1.25 (d, 3H, CHCH_3), 1.24 (d, 3H, CHCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.87, 184.70, 183.97, 146.52, 141.33, 133.75, 133.69, 132.16, 131.86, 126.55, 126.49, 71.33, 71.30, 33.65, 18.56, 13.71. HR-MS (ESI^+) m/z: 376.1041 $[M+H]^+$. Found: 376.1039 $[M+H]^+$.

*4.3.9. Data for thiazolidine-3-carbodithioic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3i**): y*ellow solid (76.3%); mp 148-149 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11-8.14 (m, 2H, Ar*H*), 7.73-7.76 (m, 2H, Ar*H*), 5.07 (s, 1H, NCH_2S), 4.70 (s, 1H, NCH_2S), 4.67 (s, 2H, CH_2S), 4.35 (t, 1H, NCH_2CH_2S), 3.98 (t, 1H, NCH_2CH_2S), 3.21 (t, 1H, NCH_2CH_2S),3.14 (t, 1H, NCH_2CH_2S), 2.38 (s, 3H, C=CCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.87, 184.80, 183.98, 146.43, 141.25, 133.78, 133.71, 132.16, 131.83, 126.56, 126.49, 56.61, 52.58, 33.82, 31.18, 13.78. HR-MS (ESI^+) m/z: 350.0343 $[M+H]^+$. Found: 350.0339 $[M+H]^+$.

*4.3.10. Data for pyrrolidine-1-carbodithioic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3j**):* yellow solid (91.0%); mp 171-172 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11-8.12 (m, 2H, Ar*H*), 7.72-7.74 (m, 2H, Ar*H*), 4.68 (s, 2H, CH_2S), 3.97 (t, 2H, NCH_2), 3.64 (t, 2H, NCH_2), 2.38 (s, 3H, C=CCH_3), 2.05-2.12 (m, 2H, $CH_2CH_2CH_2CH_2$), 1.97-2.03 (m, 2H, $CH_2CH_2CH_2CH_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.67, 184.80, 184.02, 146.18, 141.82, 133.64, 133.60, 132.20, 131.92, 126.48, 126.45, 55.37, 50.52, 33.19, 26.11, 24.25, 13.72. HR-MS (ESI^+) m/z: 332.0779 $[M+H]^+$. Found: 332.0777 $[M+H]^+$.

*4.3.11. Data for piperidine-1-carbodithioic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3k**)*: yellow solid (88.0%); mp 146-147 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10-8.12 (m, 2H, Ar*H*), 7.72-7.74 (m, 2H, Ar*H*), 4.66 (s, 2H, CH_2S), 4.25 (m, 4H, CH_2NCH_2), 2.37 (s, 3H, C=CCH_3), 1.72 (m, 6H, CH_2CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.53, 192.77, 184.77,183.94, 146.38, 141.68, 133.63, 133.60, 132.20, 131.94, 126.47, 53.47, 51.41, 33.90, 25.74, 24.22, 13.66. HR-MS (ESI^+) m/z: 346.0936 $[M+H]^+$. Found: 346.0932 $[M+H]^+$.

*4.3.12. Data for 4-methyl-piperazine-1-carbodithioic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3l**)*: yellow solid (97.2%); mp 124-125 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11-8.12 (m, 2H, Ar*H*), 7.72-7.75 (m, 2H, Ar*H*), 4.66 (s, 2H, CH_2S), 4.37 (m, 2H, S=CNCH_2), 3.96 (m, 2H, S=CNCH_2), 2.52 (m, 4H, CH_2NCH_2), 2.37 (s, 3H, NCH_3), 2.35 (s, 3H, C=CCH_3). ^{13}C

NMR (100 MHz, $CDCl_3$) δ 195.86, 184.72, 183.93, 146.47, 141.41, 133.70, 133.66, 132.17, 131.89, 126.52, 126.48, 54.34, 45.56, 33.86, 13.71. HR-MS (ESI^+) m/z: 361.1045 $[M+H]^+$. Found: 361.1035 $[M+H]^+$.

*4.3.13. Data for 4-isopropyl-piperazine-1-carbodithioic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3m**)*: yellow solid (75.7%); mp 157-158 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.11-8.12 (m, 2H, Ar*H*), 7.72-7.75 (m, 2H, Ar*H*), 4.66 (s, 2H, CH_2S), 4.37 (m, 2H, S=CNCH_2), 3.93 (m, 2H, S=CNCH_2), 2.76 (m, 1H, NC*H*), 2.61 (m, 4H, CH_2NCH_2), 2.37 (s, 3H, NCH_3), 1.07 (s, 3H, CHCH_3), 1.06 (s, 3H, CHCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.35, 184.75, 183.94, 146.45, 141.48, 133.69, 133.64, 132.18, 131.90, 126.51, 54.35, 48.13, 33.78, 18.39, 13.71. HR-MS (ESI^+) m/z: 389.1358 $[M+H]^+$. Found: 389.1348 $[M+H]^+$.

*4.3.14. Data for 4-acetyl-piperazine-1-carbodithioic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3n**)*: yellow solid (92.8%); mp 157-158 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.10-8.12 (m, 2H, Ar*H*), 7.73-7.75 (m, 2H, Ar*H*), 4.66 (s, 2H, CH_2S), 4.12-4.18 (m, 4H, CH_2NCH_2), 3.75 (t, 2H, S=CNCH_2), 3.62 (t, 2H, S=CNCH_2),2.36 (s, 3H, O=CCH_3), 2.15 (s, 3H, C=CCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.75, 184.64, 183.90, 169.30, 146.59, 141.06, 133.81, 133.74, 132.12, 131.82, 126.56, 126.50, 45.20, 40.56, 33.91, 21.36, 13.75. HR-MS (ESI^+) m/z: 389.0994 $[M+H]^+$. Found: 389.0991 $[M+H]^+$.

*4.3.15. Data for cyclohexyl-methyl-dithiocarbamic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3o**)*: yellow solid (75.0%); mp 128-129 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.11-8.12 (m, 2H, Ar*H*), 7.72-7.74 (m, 2H, Ar*H*), 4.66 (s, 2H, CH_2S), 3.17-3.42 (d, 3H, NCH_3), 2.36 (s, 3H, C=CCH_3), 1.11-1.87 (m, 11H, NC*H*CH_2CH_2CH_2CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.02, 184.75, 183.90, 146.53, 146.50, 141.60, 133.63, 133.60, 132.21, 131.96, 126.48, 62.92, 62.12, 37.75, 33.91, 33.69, 30.22, 29.42, 25.47, 25.17, 13.65. HR-MS (ESI^+) m/z: 374.1249 $[M+H]^+$. Found: 374.1247 $[M+H]^+$.

*4.3.16. Data for Methyl-dithiocarbamic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3p**)*: yellow solid (90.7%); mp 106-107°C. ^1H NMR (400 MHz, $CDCl_3$) δ 8.11-8.13 (m, 2H, Ar*H*), 8.04（s, 1H，NH），7.75-7.77 (m, 2H, Ar*H*), 4.40 (s, 2H, CH_2S), 3.29 (s, 3H, NCH_3), 2.33 (s, 3H, C=CCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.22, 185.22, 184.47, 146.40, 141.73, 134.19, 133.81, 132.10, 131.60, 126.71, 126.62, 34.03, 31.09, 13.63. HR-MS (ESI^+) m/z: 292.0466 $[M+H]^+$. Found: 292.0457 $[M+H]^+$.

*4.3.17. Data for Pyridin-2-ylmethyl-dithiocarbamic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3q**)*: yellow solid (72.5%); mp 149-150°C. ^1H NMR (400 MHz, $CDCl_3$) δ 8.54 (d,1H,N*H*), 8.09-8.12 (m, 2H, Ar*H*), 7.73-7.75 (m, 2H, Ar*H*), 7.25-7.73(m,4H,Py*H*),5.02 (d, 2H, CH_2NH), 4.62(s, 2H, CH_2S), 2.38 (s, 3H, C=CCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.13, 184.75, 184.20, 153.96, 148.76, 146.00, 141.70, 137.11, 133.81, 133.69, 132.13, 131.80, 126.55, 126.52, 122.85, 122.22, 51.27, 31.67, 13.72. HR-MS (ESI^+) m/z: 369.0731 $[M+H]^+$. Found: 369.0719 $[M+H]^+$.

*4.3.18. Data for Pyridin-3-ylmethyl-dithiocarbamic acid Data for 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3r**)*: yellow solid

(76.1%); mp 140-141°C. ^{1}H NMR (400 MHz, $CDCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.58 (t,1H,N*H*), 8.48-8.54 (m, 2H, Py*H*), 7.99-8.02 (m, 2H, Ar*H*), 7.84-8.86 (m, 2H, Ar*H*), 7.71-7.73(d,1H,Py*H*), 7.37-7.40(m,1H,Py*H*),4.86 (d, 2H, CH_2NH), 4.49(s, 2H, CH_2S), 2.21 (s, 3H, C=CCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.82, 184.60, 183.79, 149.57, 148.95, 145.79, 141.40, 136.08, 134.64, 134.56, 133.24, 132.13, 131.77, 126.56, 126.44, 124.03, 47.94, 31.34, 13.81. HR-MS (ESI^+) m/z: 369.0731 $[M+H]^+$. Found: 369.0722 $[M+H]^+$.

*4.3.19. Data for Pyridin-4-ylmethyl-dithiocarbamic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3s**):* yellow solid (78.5%); mp 131-132 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (d,1H,N*H*), 8.55-8.57(m, 2H, Ar*H*), 8.11-8.13(m,2H,Py*H*),7.72-7.77(m, 2H, Ar*H*), 7.28(s,2H,Py*H*),5.01(d, 2H, CH_2NH), 4.43(s, 2H, CH_2S), 2.33(s, 3H, C=CCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.96, 185.11, 184.37, 149.99, 146.59, 145.39, 141.42, 134.27, 133.86, 132.07, 131.49, 126.74, 126.56, 122.74, 49.34,31.22, 13.65. HR-MS (ESI^+) m/z: 369.0731 $[M+H]^+$. Found: 369.0722 $[M+H]^+$.

*4.3.20. Data for Benzyl-dithiocarbamic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3t**):* yellow solid (74.8%); mp 113-114 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01-8.12(m, 3H, Ph*H*), 7.73-7.76 (m, 2H, Ar*H*), 7.33-7.38(m, 5H, Ar*H*,N*H*), 4.95 (d, 2H, CH_2NH), 4.44(s, 2H, CH_2S), 2.34 (s, 3H, C=CCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.66, 184.82, 184.51, 146.32, 141.60, 135.93, 134.08, 133.74, 132.09, 131.56, 128.88, 128.46, 128.14, 126.64, 126.60, 51.35, 31.19, 13.65. HR-MS (ESI^+) m/z: 368.078 $[M+H]^+$. Found: 368.077 $[M+H]^+$.

*4.3.21. Data for Piperidin-1-yl-dithiocarbamic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3u**):* yellow solid (90.9%); mp 166-167 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19（s, 1H，NH），8.10-8.12 (m, 2H, Ar*H*), 7.72-7.74 (m, 2H, Ar*H*), 4.51 (s, 2H, CH_2S), 3.18 (m, 4H, CH_2NCH_2), 2.34 (s, 3H, C=CCH_3)， 1.68 (m, 6H, CH_2CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ201.01, 184.80, 183.88, 146.34, 142.05, 133.61, 132.17, 131.94, 126.48, 56.06, 30.51, 25.22, 22.87, 13.59. HR-MS (ESI^+) m/z: 361.1044 $[M+H]^+$. Found: 361.1031 $[M+H]^+$.

*4.3.22. Data for (4-Methyl-piperazin-1-yl)-dithiocarbamic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3v**):* yellow solid (95.9%); mp 162-163 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.11（s, 1H，NH），8.00-8.01 (m, 2H, Ar*H*), 7.85-7.87 (m, 2H, Ar*H*), 4.30 (s, 2H, CH_2S), 2.69-2.79（m,8H，2NCH_2CH_2），2.20 (s, 3H, C=CCH_3), 2.14 (d, 3H, NCH_3). ^{13}C NMR (100 MHz, DMSO) δ 199.97, 183.85, 179.14, 144.78, 143.38, 136.90, 135.12, 134.92, 127.65, 124.81, 122.46, 122.40, 54.30, 53.41, 53.33, 45.50, 13.06. HR-MS (ESI^+) m/z: 376.1153 $[M+H]^+$. Found: 374.1143 $[M+H]^+$.

*4.3.23. Data for (2-Diethylamino-ethyl)-dithiocarbamic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**3w**):* yellow solid (73.3%); mp 149-150 °C. ^{1}H NMR (400 MHz, DMSO) δ 10.41（s, 1H，NH），8.01-8.03 (m, 2H, Ar*H*), 7.85-7.88 (m, 2H, Ar*H*), 4.94 (s, 2H, CH_2S), 3.99(m,2H,NHCH_2), 3.31(m,2H,NHCH_2CH_2), 3.30(q,4H,CH_3CH_2NC$H_2$$CH_3$),2.23 (s,

3H, C=CC*H*$_3$), 1.22 (t, 6H, 2CH$_2$C*H*$_2$). ^{13}C NMR (100 MHz, DMSO) δ 197.34, 184.60, 183.74, 145.89, 141.16, 134.68, 134.60, 132.14, 131.79, 126.58, 126.46, 48.31, 47.19, 41.62, 31.35, 13.80, 8.88. HR-MS (ESI$^+$) m/z: 377.1357 [M+H]$^+$. Found: 377.1348 [M+H]$^+$.

4.4. General procedure for preparation of dithiocarbamic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-yl ester (4)

Carbon disulfide (60 μL, 1 mmol) and amine (1 mmol) were added to CH_3CN (3 mL) and the resulting solution was stirred for 30 minutes. 2-methyl-[1, 4] naphthoquinone (**1**) (110 mg, 0.5 mmol) was added in portions at frequent intervals. The reaction mixture was then kept at room temperature until compound **1** was absent (as evaluated by TLC). The reaction mixture was concentrated in vacuo, diluted with H_2O, and extracted with CH_2Cl_2. The combined organic fractions were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification of the crude residue by column chromatography (petroleum ether/ CH_2Cl_2) afforded the compound of interest.

*4.4.1. Data for dipropyl-dithiocarbamic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-yl ester (**4a**):* yellow solid (65.1%); mp 129-130 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12-8.16 (m, 2H, Ar*H*), 7.73-7.76 (m, 2H, Ar*H*), 3.80-3.89 (m, 4H, C*H*$_2$NC*H*$_2$), 2.40 (s, 3H, C=CC*H*$_3$), 1.97 (m, 2H, C*H*$_2$CH$_3$), 1.80 (m, 2H, C*H*$_2$CH$_3$), 1.08 (m, 3H, CH$_2$C*H*$_3$), 0.95 (m, 3H, CH$_2$C*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.34, 184.09, 180.30, 151.16, 142.25, 133.80, 133.50, 132.15, 127.20, 126.24, 57.37, 56.19, 21.28, 19.71, 15.95, 11.28, 11.19. HR-MS (ESI$^+$) m/z: 348.1092 [M+H]$^+$. Found: 348.1088 [M+H]$^+$.

*4.4.2. Data for morpholine-4-carbodithioic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-yl ester (**4b**):* yellow solid (54.2%); mp 140-141 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13-8.18 (m, 2H, Ar*H*), 7.75-7.78 (m, 2H, Ar*H*), 4.15-4.27 (m, 4H, C*H*$_2$OC*H*$_2$), 3.86-3.87 (m, 4H, C*H*$_2$NC*H*$_2$), 2.41 (s, 3H, C=CC*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.39, 183.85, 180.10, 152.18, 141.55, 133.94, 132.91, 132.11, 127.24, 126.76, 66.26, 66.22, 51.96, 51.07, 16.06. HR-MS (ESI$^+$) m/z: 334.0572 [M+H]$^+$. Found: 334.0568 [M+H]$^+$.

*4.4.3. Data for piperidine-1-carbodithioic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-yl ester (**4c**):* yellow solid (65.4%); mp 139-140 °C; 1H NMR (400 MHz, CDCl3) δ 8.13-8.17 (m, 2H, ArH), 7.74-7.76 (m, 2H, ArH), 4.21 (m, 2H, NCH2), 4.08 (m, 2H, NCH2), 2.41 (s, 3H, C=CCH3), 1.77-1.87 (m, 6H, CH2CH2CH2). 13C NMR (100 MHz, CDCl3) δ 190.63, 184.07, 180.36, 151.21, 141.96, 133.83, 133.53, 133.10, 132.14, 127.22, 126.66, 53.34, 52.44, 26.37, 25.28, 24.03, 15.94. HR-MS (ESI+) m/z: 332.0779 [M+H]+. Found: 332.0775 [M+H]+.

*4.4.4. Data for 4-acetyl-piperazine-1-carbodithioic acid 3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-yl ester (**4d**):* yellow solid (69.5%); mp 119-120 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11-8.18 (m, 2H, Ar*H*), 7.75-7.78 (m, 2H, Ar*H*), 4.18-4.30 (m, 4H, C*H*$_2$NC*H*$_2$), 3.70-3.88 (m, 4H, C*H*$_2$NC*H*$_2$), 2.41 (s, 3H, C=CC*H*$_3$), 2.19 (s, 3H, O=CC*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ183.79, 180.02, 169.38, 152.39, 141.42, 133.96, 132.82, 132.09, 127.24, 126.80, 50.78, 40.69, 29.69,

21.37, 16.10. HR-MS (ESI^+) m/z: 375.0837 $[M+H]^+$. Found: 375.0833 $[M+H]^+$.

*4.5. Procedure for preparation of 2-bromomethyl-[1,4]naphthoquinone (**5**)*

Azodiisobutyronitrile (0.05 g, 0.3 mmol) was added to a well stirred solution of methylnaphthoquinone **1** (1 g, 5.9 mmol) in acetic anhydride (10 mL). N-bromosuccinimide (1.05 g, 5.9 mmol) was then added in portions at frequent intervals. The reaction mixture was refluxed for 20 min. The reaction mixture was poured on ice and extracted with ethyl acetate. The combined organic fractions were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification of the crude residue by column chromatography (petroleum ether/ ethyl acetate) afforded compound **5** (yellow solid). The yield of this reaction was 62.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10-8.18 (m, 2H, Ar*H*), 7.70-7.81 (m, 2H, Ar*H*), 7.13 (s, 1H, C=C*H*), 4.42 (s, 2H, CH_2Br).

4.6. General Procedure for preparation of dithiocarbamic acid 1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (6)

Carbon disulfide (60 μL, 1 mmol) and amine (1 mmol) were added to CH_2Cl_2 (5 mL) and the resulting solution was stirred for 30 minutes. 2-Bromomethyl-[1, 4] naphthoquinone (**5**) (250 mg, 1 mmol) was added in portions at frequent intervals. The reaction mixture was then kept at room temperature until compound **5** was absent (as evaluated by TLC). The reaction mixture was diluted with H_2O, and extracted with CH_2Cl_2. The combined organic fractions were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification of the crude residue by column chromatography (petroleum ether/ ethyl acetate) afforded compound of interest.

*4.6.1. Data for diethyl-dithiocarbamic acid 1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**6a**):* yellow solid (75.3%); mp 118-119 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07-8.14 (m, 2H, Ar*H*), 7.75-7.79 (m, 2H, Ar*H*), 7.23 (s, 1H, C=C*H*), 4.59 (s, 2H, CH_2S), 4.03 (q, 2H, NCH_2), 3.78 (q, 2H, NCH_2), 1.27-1.34 (m, 6H, 2CH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.65, 185.11, 184.76, 146.42, 135.88, 133.90, 133.72, 132.21, 132.08, 126.63,126.20, 50.14, 46.84, 34.42, 12.63, 11.54. HR-MS (ESI^+) m/z: 320.0779 $[M+H]^+$. Found: 320.0770 $[M+H]^+$.

*4.6.2. Data for morpholine-4-carbodithioic acid 1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**6b**)* yellow solid (68.9%); mp 137-138 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07-8.15 (m, 2H, Ar*H*), 7.75-7.78 (m, 2H, Ar*H*), 7.23 (s, 1H, C=C*H*), 4.61 (s, 2H, CH_2S), 4.27 (m, 2H, OCH_2), 4.01 (m, 2H, OCH_2), 3.78 (m, 4H,CH_2NCH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.65, 184.97, 184.66, 145.92, 136.06, 133.97, 133.77, 132.19, 132.03, 126.64, 126.24, 66.25, 66.17, 34.23, 29.67, 22.68. HR-MS (ESI^+) m/z: 334.0572 $[M+H]^+$. Found: 334.0567 $[M+H]^+$.

*4.6.3. Data for piperidine-1-carbodithioic acid 1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**6c**)* yellow solid (70.9%); mp 122-123 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07-8.14 (m, 2H, Ar*H*), 7.74-7.77 (m, 2H, Ar*H*), 7.23 (s, 1H, C=C*H*), 4.59 (s, 2H, CH_2S), 4.03 (m, 2H, CH_2NCH$_2$), 3.78 (m, 2H, CH$_2$NCH_2), 1.27-1.34 (m, 6H, CH_2CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.65, 185.60, 184.78, 146.41, 135.92, 133.90, 133.71, 132.20, 132.08, 126.63, 126.20，

54.62, 53.58, 34.44, 25.97, 25.48, 24.21. HR-MS (ESI^+) m/z: 332.0779 $[M+H]^+$. Found: 332.0770 $[M+H]^+$.

*4.6.4. Data for 4-phenyl-piperazine-1-carbodithioic acid 1,4-dioxo-1,4-dihydro-naphthalen-2-ylmethyl ester (**6d**)*: yellow solid (72.7%); mp 144-145 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08-8.16 (m, 2H, Ar*H*), 7.75-7.78 (m, 2H, Ar*H*), 6.93-7.33 (m, 6H, Ph*H*, C=C*H*), 4.63 (s, 2H, CH_2S), 4.49 (m, 2H, NCH_2), 4.15 (m, 2H, NCH_2), 3.33 (m, 4H,CH_2NCH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.09, 184.71, 183.95, 146.52, 141.34, 133.74, 133.68, 132.18, 131.89, 129.36, 126.55, 126.50, 120.82, 116.44, 48.87, 33.86, 13.74. HR-MS (ESI^+) m/z: 409.1045 $[M+H]^+$. Found: 409.1037 $[M+H]^+$.

*4.7. Procedure for preparation of 2-chloromethyl-1,4-dimethoxy-naphthalene (**8**)*

The compound (1,4-dimethoxy-naphthalen-2-yl)-methanol (**7**) (436 mg, 2 mmol) in CH_2Cl_2 (10 mL) was placed in a 50 mL round-bottomed flask, and thionyl chloride (0.22 mL, 3 mmol) in CH_2Cl_2 (5 mL) was added in portions at frequent intervals, keeping the temperature below 0℃. The reaction mixture was then refluxed for 2 h, and diluted with H_2O, followed by extracted with CH_2Cl_2. The combined organic fractions were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification of the crude residue by column chromatography (petroleum ether/ ethyl acetate) afforded compound **8** (yellow solid). The yield of this reaction was 78.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06-8.28 (m, 2H, Ar*H*), 7.52-7.61 (m, 2H, Ar*H*), 6.79 (s, 1H, C=C*H*), 4.88 (s, 2H, CH_2Cl), 4.03 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3).

*4.8. Procedure for preparation of dipropyl-dithiocarbamic acid 1,4-dimethoxy-naphthalen-2-ylmethyl ester (**9**)*

Carbon disulfide (60 μL, 1 mmol) and dipropyl amine (136μL, 1 mmol) were added to CH_2Cl_2 (5 mL) and the resulting solution was stirred for 30 minutes. 2-chloromethyl-1,4-dimethoxy-naphthalene (**8**) (377mg, 1 mmol) was added in portions at frequent intervals. The reaction mixture was then kept at room temperature until absence of compound **8** (checked by TLC). The reaction mixture was diluted with H_2O, and extracted with CH_2Cl_2. The combined organic fractions were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification of the crude residue by column chromatography (petroleum ether/ ethyl acetate) afforded the compound **9** (white solid). The yield of this reaction was 76.0%; mp 70-71 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47-8.24 (m, 4H, Ar*H*), 6.88 (s, 1H, C=C*H*), 4.80 (s, 2H, CH_2S), 3.97-3.98 (m, 8H, 2OCH_3, NCH_2), 3.62-3.68 (m, 2H, NCH_2), 1.73-1.85 (m, 4H, 2CH_2C$H_2$$CH_3$), 0.91-1.01(m, 6H, 2$CH_2CH_2CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.25, 151.94, 148.11, 128.45, 126.70, 126.40, 125.56, 123.95, 122.43，121.98, 105.54, 62.78, 56.89, 55.72, 54.37, 37.32, 20.70, 19.70, 11.25, 11.19. HR-MS (ESI^+) m/z: 378.1561 $[M+H]^+$. Found: 378.1553 $[M+H]^+$.

4.9. Purification of recombinant pyruvate kinase isoforms

Human cDNA for PKM2 or PKM1 was cloned into pET28a with a N-terminal His tag and purified from *Escherichia coli* strain BL21 (Invitrogen) using Ni-Agarose beads (Qiagen) as described previously.[6, 24] The PKL was purchased from Sino Biological Inc.

4.10. PKM2 activity assay

Pyruvate kinase activity was measured with a fluorescent pyruvate kinase-lactate dehydrogenase coupled assay as described previously [15].All compounds were tested in a kinetic mode by coupling the generation of pyruvate by pyruvate kinase to the depletion of NADH through lactate dehydrogenase. For PKM2, 40 μL of buffer (50 mM Tris–HCl, pH 7.5, 10 mM KCl, 5 mM $MgCl_2$), 1 μL of compound and 5 μL of enzyme solution were dispensed into Corning black solid 96-well plates and incubated for 15 min. 55 μL of substrate mix (final concentration, 0.5 mM PEP, 4.0 mM ADP, 0.12 mM NADH, 0.25 mM FBP and 1 unit LDH) was then added, and the plates were placed in a FlexStation 3 (Molecular Devices), followed by determination of NADH fluorescence at 30 s exposure intervals for 3 to 6 min. Data was collected on the FlexStation 3.

4.11. Cell culture

Cell lines (HCT116, Hela, H1299, BEAS-2B) were cultured in RPMI 1640 containing 9% fetal bovine serum (FBS) at 37°C in 5% CO2.

4.12. Cell viability experiments

Cell viability was detected with the MTS assay (Promega) according to the manufacturer's instructions. Briefly, 5000 cells in per well were plated in 96-well plates. After incubated for 12 h, the cells were treated with different concentration of tested compound or DMSO (as negative control) for 48 h. Then 20 μL MTS was added in per well and incubated at 37°C for 3h. Absorbance of each well was determined by a microplate reader (Flexstation 3) at a 490 nm wavelength. The IC_{50} values were calculated using Prism Graphpad software of the triplicate experiment.

Acknowledgments

This study was supported by the National Key Research and Development Program of China (2016YFA0500302), National Natural Science Foundation of China (81402777) and the China Postdoctoral Science Foundation (2014M560026 and 2015T80028).

References

[1] G. Kroemer, J. Pouyssegur, Tumor cell metabolism: cancer's Achilles' heel, Cancer cell, 13 (2008) 472-482.

[2] T.L. Dayton, T. Jacks, M.G. Vander Heiden, PKM2, cancer metabolism, and the road ahead, EMBO reports, 17 (2016) 1721-1730.

[3] B. Chaneton, E. Gottlieb, Rocking cell metabolism: revised functions of the key glycolytic regulator PKM2 in cancer, Trends in biochemical sciences, 37 (2012) 309-316.

[4] M. Tamada, M. Suematsu, H. Saya, Pyruvate kinase M2: multiple faces for conferring benefits on cancer cells, Clinical cancer research : an official journal of the American Association for Cancer Research, 18 (2012) 5554-5561.

[5] M.S. Jurica, A. Mesecar, P.J. Heath, W. Shi, T. Nowak, B.L. Stoddard, The allosteric regulation of pyruvate kinase by fructose-1,6-bisphosphate, Structure, 6 (1998) 195-210.

[6] H.R. Christofk, M.G. Vander Heiden, M.H. Harris, A. Ramanathan, R.E. Gerszten, R. Wei, M.D. Fleming, S.L. Schreiber, L.C. Cantley, The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth, Nature, 452 (2008) 230-233.

[7] Z.X. Liu, L. Hong, S.Q. Fang, G.H. Tan, P.G. Huang, Z. Zeng, X. Xia, X.X. Wang, Overexpression of pyruvate kinase M2 predicts a poor prognosis for patients with osteosarcoma, Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine, 37 (2016) 14923-14928.

[8] W. Yang, Z. Lu, Pyruvate kinase M2 at a glance, Journal of cell science, 128 (2015) 1655-1660.

[9] C. Wang, J. Jiang, J. Ji, Q. Cai, X. Chen, Y. Yu, Z. Zhu, J. Zhang, PKM2 promotes cell migration and inhibits autophagy by mediating PI3K/AKT activation and contributes to the malignant development of gastric cancer, Scientific reports, 7 (2017) 2886.

[10] Y. Miao, M. Lu, Q. Yan, S. Li, Y. Feng, Inhibition of Proliferation, Migration, and Invasion by Knockdown of Pyruvate Kinase-M2 (PKM2) in Ovarian Cancer SKOV3 and OVCAR3 Cells, Oncology research, 24 (2016) 463-475.

[11] D.Y. Gui, C.A. Lewis, M.G. Vander Heiden, Allosteric regulation of PKM2 allows cellular adaptation to different physiological states, Science signaling, 6 (2013) pe7.

[12] M.B. Boxer, J.K. Jiang, M.G. Vander Heiden, M. Shen, A.P. Skoumbourdis, N. Southall, H. Veith, W. Leister, C.P. Austin, H.W. Park, J. Inglese, L.C. Cantley, D.S. Auld, C.J. Thomas, Evaluation of substituted N,N'-diarylsulfonamides as activators of the tumor cell specific M2 isoform of pyruvate kinase, Journal of medicinal chemistry, 53 (2010) 1048-1055.

[13] D. Anastasiou, Y. Yu, W.J. Israelsen, J.K. Jiang, M.B. Boxer, B.S. Hong, W. Tempel, S. Dimov, M. Shen, A. Jha, H. Yang, K.R. Mattaini, C.M. Metallo, B.P. Fiske, K.D. Courtney, S. Malstrom, T.M. Khan, C. Kung, A.P. Skoumbourdis, H. Veith, N. Southall, M.J. Walsh, K.R. Brimacombe, W. Leister, S.Y. Lunt, Z.R. Johnson, K.E. Yen, K. Kunii, S.M. Davidson, H.R. Christofk, C.P. Austin, J. Inglese, M.H. Harris, J.M. Asara, G. Stephanopoulos, F.G. Salituro, S. Jin, L. Dang, D.S. Auld, H.W. Park, L.C. Cantley, C.J. Thomas, M.G. Vander Heiden, Pyruvate kinase M2 activators promote tetramer formation and suppress tumorigenesis, Nature chemical biology, 8 (2012) 839-847.

[14] Y. Matsui, I. Yasumatsu, T. Asahi, T. Kitamura, K. Kanai, O. Ubukata, H. Hayasaka, S. Takaishi, H. Hanzawa, S. Katakura, Discovery and structure-guided fragment-linking of 4-(2,3-dichlorobenzoyl)-1-methyl-pyrrole-2-carboxamide as a pyruvate kinase M2 activator, Bioorganic & medicinal chemistry, 25 (2017) 3540-3546.

[15] M.G. Vander Heiden, H.R. Christofk, E. Schuman, A.O. Subtelny, H. Sharfi, E.E. Harlow, J. Xian, L.C. Cantley, Identification of small molecule inhibitors of pyruvate kinase M2, Biochemical pharmacology, 79 (2010) 1118-1124.

[16] J. Chen, J. Xie, Z. Jiang, B. Wang, Y. Wang, X. Hu, Shikonin and its analogs inhibit cancer cell glycolysis by targeting tumor pyruvate kinase-M2, Oncogene, 30 (2011) 4297-4306.

[17] R.D. Li, X. Zhang, Q.Y. Li, Z.M. Ge, R.T. Li, Novel EGFR inhibitors prepared by combination of dithiocarbamic acid esters and 4-anilinoquinazolines, Bioorganic & medicinal chemistry letters, 21 (2011) 3637-3640.

[18] Y.C. Duan, Y.C. Zheng, X.C. Li, M.M. Wang, X.W. Ye, Y.Y. Guan, G.Z. Liu, J.X. Zheng, H.M. Liu, Design, synthesis and antiproliferative activity studies of novel 1,2,3-triazole-dithiocarbamate-urea hybrids, European journal of medicinal chemistry, 64 (2013) 99-110.

[19] C. Flader, J. Liu, R.F. Borch, Development of novel quinone phosphorodiamidate prodrugs targeted to DT-diaphorase, Journal of medicinal chemistry, 43 (2000) 3157-3167.

[20] X. Gao, H. Wang, J.J. Yang, X. Liu, Z.R. Liu, Pyruvate kinase M2 regulates gene transcription by acting as a protein kinase, Molecular cell, 45 (2012) 598-609.

[21] W. Yang, Y. Xia, H. Ji, Y. Zheng, J. Liang, W. Huang, X. Gao, K. Aldape, Z. Lu, Nuclear PKM2

regulates beta-catenin transactivation upon EGFR activation, Nature, 480 (2011) 118-122.
[22] Z. Lu, PKM2 functions as a histone kinase, Cell Cycle, 11 (2012) 4101-4102.
[23] W. Yang, Z. Lu, Regulation and function of pyruvate kinase M2 in cancer, Cancer letters, 339 (2013) 153-158.
[24] Y. Zhang, B. Liu, X. Wu, R. Li, X. Ning, Y. Liu, Z. Liu, Z. Ge, Y. Yin, New pyridin-3-ylmethyl carbamodithioic esters activate pyruvate kinase M2 and potential anticancer lead compounds, Bioorganic & medicinal chemistry, 23 (2015) 4815-4823.

Highlights

- The novel dithiocarbamate substituted naphthoquinone derivatives were designed and synthesized.
- All compounds were evaluated for in vitro PKM2 inhibitory activity and antiproliferative activity.
- Compound **3k** showed more potent PKM2 inhibitory activity than the positive control shikonin.
- Compound **3k** exhibited nanomolar antitumor activity toward PKM2 high expression cancer cell lines.