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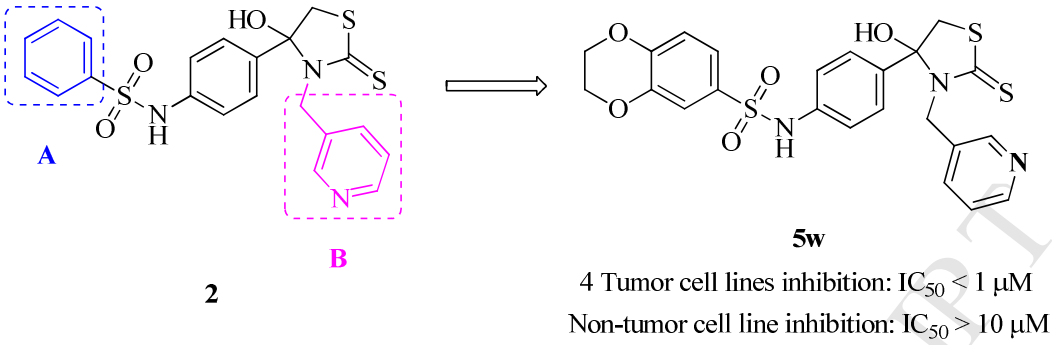
Graphical abstract



Discovery and structure-activity relationship of novel 4-hydroxy-thiazolidine-2-thione derivatives as tumor cell specific pyruvate kinase M2 activators

Ridong Li [a, b, e], Xianling Ning [a, e], Shuo Zhou[f], Zhiqiang Lin [a], Xingyu Wu [a, d], Hong Chen [a, c], Xinyu Bai [a, d], Xin Wang [b], Zemei Ge [b], Runtao Li [b, *], Yuxin Yin [a, b, c, d, *]

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Abstract

Pyruvate kinase M2 isoform (PKM2) is a crucial protein responsible for aerobic glycolysis of cancer cells. Activation of PKM2 may alter aberrant metabolism in cancer cells. In this study, we discovered a 4-hydroxy-thiazolidine-2-thione compound **2** as a novel PKM2 activator from a random screening of an in-house compound library. Then a series of novel 4-hydroxy-thiazolidine-2-thione derivatives were designed and synthesized for screening as potent PKM2 activators. Among these, some compounds showed higher PKM2 activation activity than lead compound **2** and also exhibited significant anti-proliferative activities on human cancer cell lines at nanomolar concentration. The compound **5w** was identified as the most potent antitumor agent, which showed excellent anti-proliferative effects with IC_{50} values from 0.46 μM to 0.81 μM against H1299, HCT116, Hela and PC3 cell lines. **5w** also showed less cytotoxicity in non-tumor cell line HELF compared with cancer cells. In addition, Preliminary pharmacological studies revealed that **5w** arrests the cell cycle at the G2/M phase in HCT116 cell line. The best PKM2 activation by compound **5t** was rationalized through docking studies.

Keywords:
PKM2 activators; 4-Hydroxy-thiazolidine-2-thione derivatives; Anti-tumor activity; Structure-activity relationship

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1. Introduction

Many cancer cells exhibit elevated glucose uptake and lactate production, regardless of oxygen availability. This phenomenon, known as aerobic glycolysis or the Warburg effect, facilitates tumor cell growth [1]. Pyruvate kinase (PK), which catalyzes the final rate-limiting step of glycolysis and converts phosphoenolpyruvate (PEP) to pyruvate, has been recognized as a key regulator of this metabolic pathway [2]. PK comprises a total of four isoforms, including PKM1, PKM2, PKL and PKR, which have different kinetic properties and tissue distributions. The PKL and PKR isoform are encoded by *Pkrl* gene and expressed in the liver and red blood cells respectively. The PKM1 and PKM2 isoform are encoded by *Pkm* gene and differ at only 23 amino acid residues [3]. In spite of their high sequence similarity, PKM1 and PKM2 have different catalytic and regulatory properties. PKM1 is a constitutively active tetrameric enzyme, which is found in most normal adult tissues. However, PKM2 alternates between a high-activity tetramer form and a low-activity dimer form in normal tissue, while exhibiting a distinctive tendency to exist as a dimer in cancer cells [4]. Dimeric PKM2 has low enzymatic activity, which supports cell growth by increasing glycolytic intermediates necessary for biosynthetic processes, and thus promotes cancer cell proliferation [5]. Highly enzymatic PKM2 can be formed by the binding of allosteric activators like fructose-1,6-biphosphate (FBP) and serine [6]. In light of these findings, small molecule PKM2 activators, which induce tetramer formation of PKM2, are expected to suppress tumor formation *in vitro* and *in vivo*, providing a novel anti-cancer therapeutic strategy [7].

Currently, several PKM2 activators with different scaffolds have been reported, including a series of diarylsulfonamides (**a**, Fig.1) [8], thieno[3,2-b]pyrrole[3,2-d]pyridazinones (**b**, Fig.1) [9], 2-oxo-*N*-aryl-1,2,3,4-tetrahydroquinoline-6-sulfonamides (**c**, Fig.1) [10], quinoline sulfonamides (**d**, Fig.1) [11], 1-(sulfonyl)-5-(arylsulfonyl)indolines (**e**, Fig.1) [12], imidazolethiazolo pyridopyrimidinones (**f**, Fig.1) [13] and 3-(trifluoromethyl)-1H-pyrazole-5-carboxamides (**g**, Fig.1) [14]. Recently, Y. Matsui et al. reported a structure-guided fragment-linking of 4-(2,3-dichlorobenzoyl)-1-methyl-pyrrole-2-carboxamide as a PKM2 activator (**h**, Fig.1) [15].

Though the above compounds show significant PKM2 activation activity, they have no significant anti-proliferative effects on tumor cell lines. The possible reason is that these agents activate PKM2 by binding at the same site as FBP but are not released after interaction with phosphotyrosine, which led to no potency against tumor cell lines [16]. Fortunately, we found two novel scaffolds of PKM2 activators (Fig.2, **1a** and **2**) with potent anti-proliferative activities from a random screening of an in-house small molecule library (more than 1000 compounds) on the basis of the established PKM2 model. Encouraged by these results, the optimization of compound **1a** has been carried out and the more potential compound **1b** as PKM2 activators with significant anti-proliferative effects on tumor cell lines was identified in our previous work [17]. Here, we describe the identification of potent compound **5w** as PKM2 activator with excellent anti-proliferative effects and lower toxicity from compound **2**

and the structure-activity relationships (SAR).

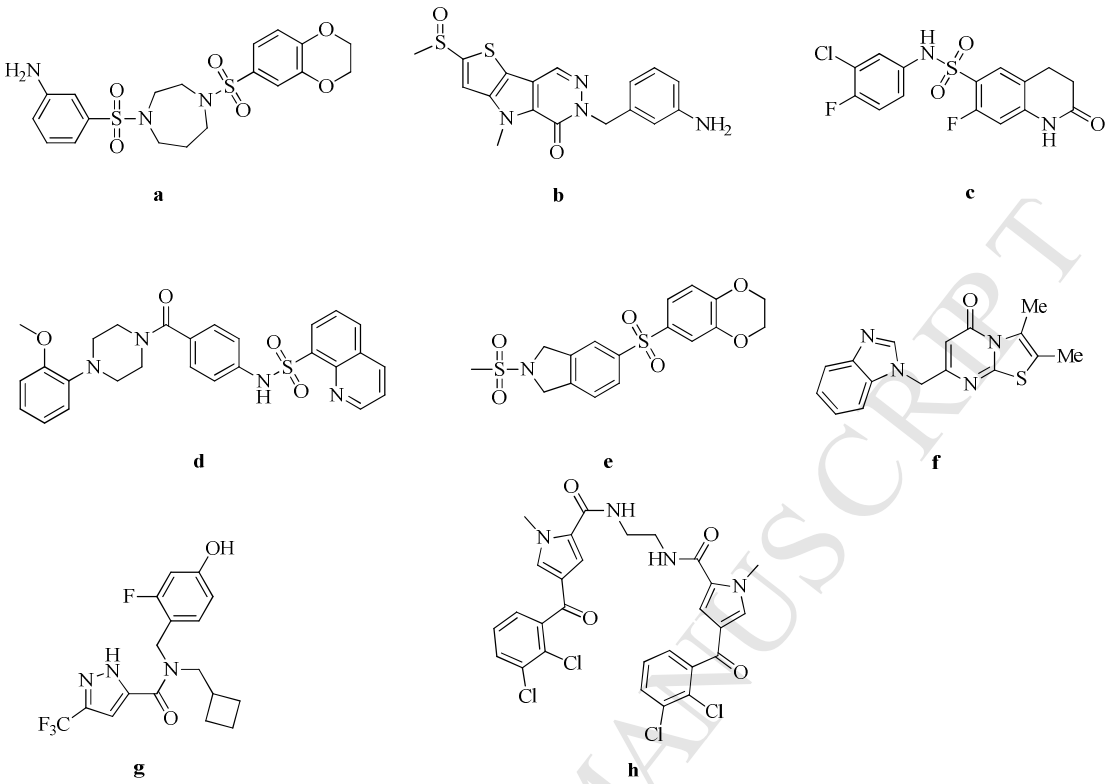


Fig. 1. Structures of several known PKM2 activators.

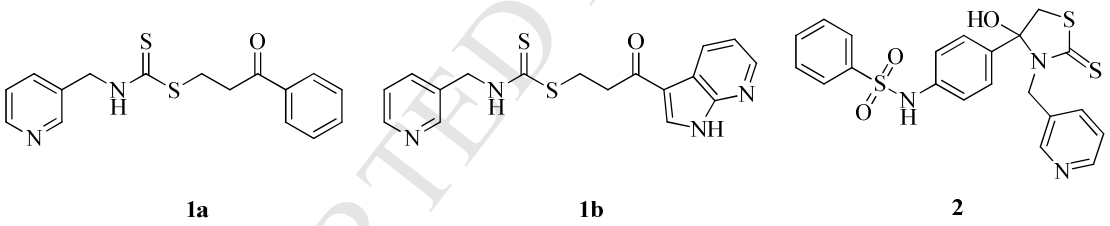


Fig. 2. Structures of compound **1a, 1b** and **2**.

2. Results and discussion

The SAR study of compound **2** was carried out in two aspects based on its structure. At first, the phenyl group (called region A) was modified with various substituted aromatic and heterocyclic rings, and the pyridin-3-ylmethyl moiety (called region B) remained intact. Afterwards, with the most favored substitution at region A, region B was modified with different derivatives of pyridin-3-ylmethyl (Fig. 3).

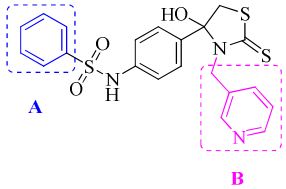
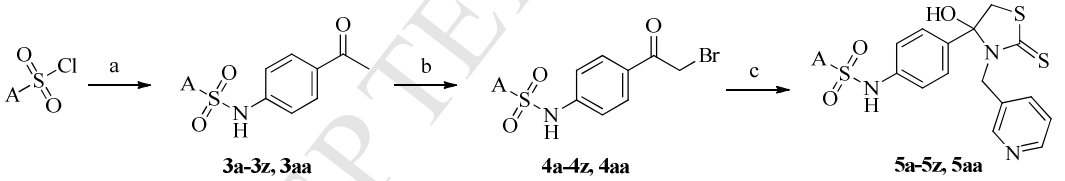


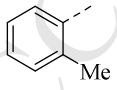
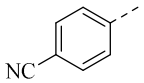
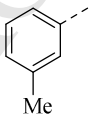
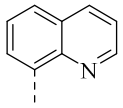
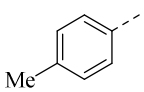
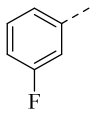
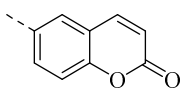
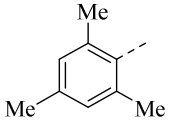
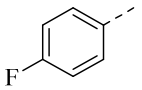
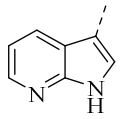
Fig. 3. Optimization strategy of lead compound **2**.

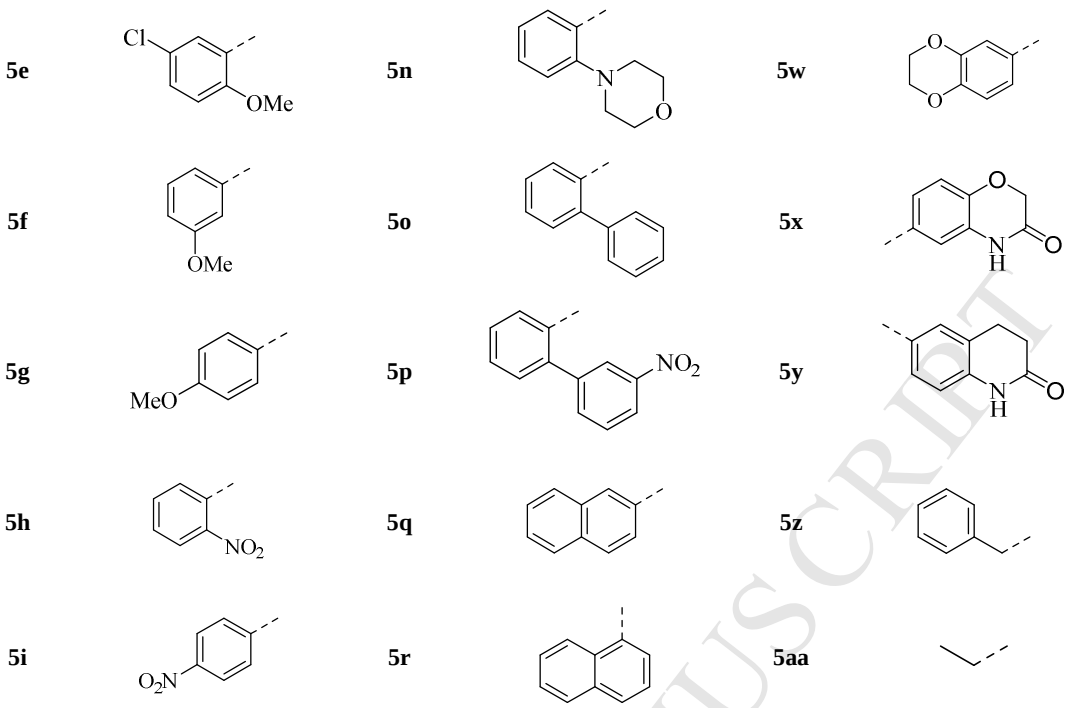
For the structural optimization of region **A**, a number of substituted phenyl groups (**5a-5p**) were selected to replace the phenyl group of compound **2** to examine the electronic or steric effects of substituents attached to the phenyl ring on the PKM2 activation activity. Furthermore, the phenyl ring was also modified with various heterocyclic rings (**5q-5y**) to evaluate the effect of different heterocycles on the bioactivity. As a comparison, in order to investigate the influence of arylalkyl and alkyl group on the activation activity, we used benzyl group (**5z**) and methyl group (**5aa**) to replace the phenyl group.

The modification of region **A** is mainly outlined in Scheme **1**. A variety of substituted sulfonyl chlorides, which were commercially available or prepared by literature procedures [18-20], reacted with 4-aminoacetophenone using pyridine as base in THF to provide the corresponding key intermediates **3a-3z** or **3aa**. The intermediates **3a-3z** or **3aa** were respectively treated with phenyltrimethylammonium tribromide in THF to afford the corresponding bromides **4a-4z** or **4aa**. Without further purification, **4a-4z** or **4aa** reacted with pyridin-3-ylmethanamine and carbon disulfide using triethylamine as base in acetone to generate the target compounds **5a-5z** or **5aa**.

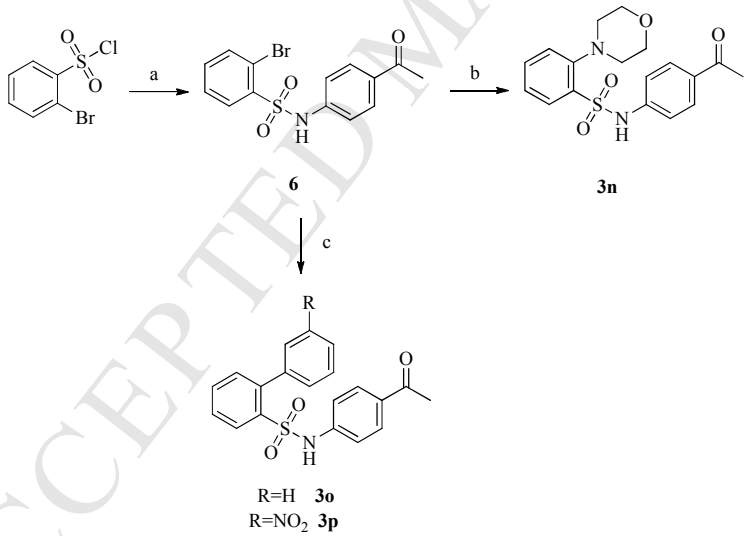
The synthetic routes of intermediates **3n-3p** have one more step than other intermediates **3a-3m**. The routes are specifically depicted in Scheme **2**. 2-Bromobenzenesulfonyl chloride was treated with 4-aminoacetophenone and pyridine in THF to obtain compound **6**. The bromine moiety of **6** was replaced by morpholine in DMSO at 100°C, catalyzed by CuI, L-proline and K_2CO_3, to afford the intermediate **3n** [21]. Compound **6** reacted with phenylboronic acid or 3-nitrophenylboronic acid using $PdCl_2(dppf)CH_2Cl_2$ and K_2CO_3 as catalysts in refluxing 1,4-dioxane and H_2O to respectively provide the intermediate **3o** or **3p** [22].



Compd.	A	Compd.	A	Compd.	A
5a		**5j**		**5s**	
5b		**5k**		**5t**	
5c		**5l**		**5u**	
5d		**5m**		**5v**	



Scheme 1. Synthesis of **5a-5z** and **5aa**. Reagents and conditions: (a) 4-Aminoacetophenone, Py, THF, rt; (b) Phenyltrimethylammonium tribromide, THF; (c) Pyridin-3-ylmethanamine, CS_2, Et_3N, Acetone.



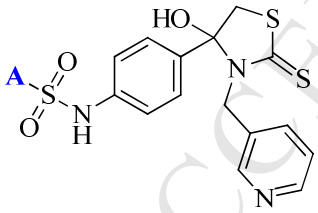
Scheme 2. Synthetic routes for the intermediate **3o-3p** and **3n**. Reagents and conditions: (a) 4-Aminoacetophenone, Py, THF, rt; (b) Morpholine, CuI, L-proline, K_2CO_3, DMSO, 100 °C;(c) Phenylboronic acid or 3-Nitrophenylboronic acid, $PdCl_2(dppf)CH_2Cl_2$, K_2CO_3, 1,4-dioxane, H_2O, reflux.

The PKM2 activation activity of target compounds (**5a-5z**, **5aa**) was evaluated with a fluorescent PK-LDH coupled assay as previously reported [17]. The lead compound **2** was used as the positive control. The results expressed as AC_{50} are shown in Table 1. Part of target compounds exhibited moderate to excellent PKM2 activation activity. Among them, some compounds, such as **5t** and **5y** with AC_{50} values of 0.52 μM and 0.74 μM respectively, displayed more potent activity than the positive control compound **2** (AC_{50} = 2.96 μM). Different substituents in benzene ring markedly

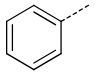
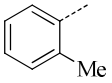
influenced PKM2 activation activity. Introduction of an ortho methyl on the benzene ring (**5a**, AC_{50} = 1.19 μM) increased the activity compared to that of lead compound **2**. On the contrary, methyl at meta and para-position of the benzene ring led to a slight decrease and a dramatic loss in potency (**5b**, AC_{50} = 6.25 μM; **5c**, AC_{50} > 20 μM). Introducing a meta or para methoxy on the benzene ring (**5f** and **5g**) also resulted in slight reduction of activity. In addition, introduction of multi-substituents (**5d** and **5e**), electron-withdrawing groups (**5h-5m**) and large steric hindrance groups (**5n-5p**) on the benzene ring led to a complete loss of activity. These results suggested that both the electronic and steric effects of the substituents at benzene rings play important roles in generating the PKM2 activation activity. Replacing the benzene ring of lead compound **2** with naphthalene ring (**5q** and **5r**, AC_{50} > 20 μM) and benzyl (**5z**, AC_{50} = 7.24 μM) respectively resulted in significant and slight reduction of activity. The different heterocyclic structures at region A also showed great impact on the PKM2 activation activity. Unlike naphthalene ring, N-containing bicyclic ring replacements such as quinoline (**5t**, AC_{50} = 0.52 μM), azaindole (**5v**, AC_{50} = 1.41 μM) and 3,4-dihydroquinolin-2(1H)-one ring (**5y**, AC_{50} = 0.74 μM) resulted in a significant improvement of activity, with the exception of 2H-benzo[b][1,4]oxazin-3(4H)-one (**5x**, AC_{50} > 20 μM). These results showed that the hydrogen atom of these three heterocycles might forms an important hydrogen bonding interaction with the target protein. When the phenyl group was replaced with 2,3-dihydrobenzo[b][1,4]dioxine and thiophene ring, the corresponding compounds (**5w**, AC_{50} = 3.14 μM; **5s**, AC_{50} = 1.47 μM) exhibited similar or more potent activity comparing with the lead compound **2**. However, compounds with coumarin (**5u**) showed complete loss of activity (AC_{50} > 20 μM). In addition, replacing the benzene ring of lead compound **2** with methyl also resulted in significant loss in potency (**5aa**, AC_{50} > 20 μM).

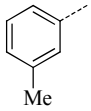
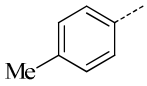
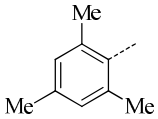
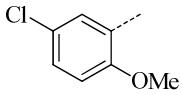
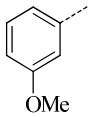
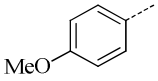
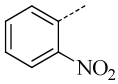
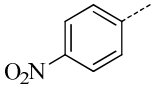
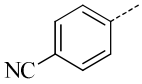
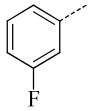
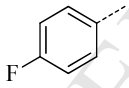
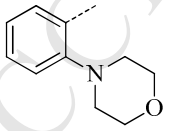
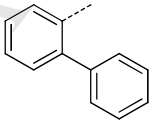
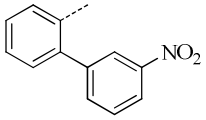
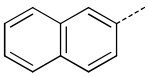
Table 1

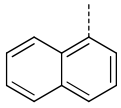
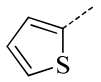
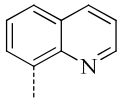
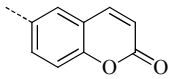
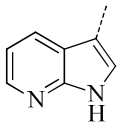
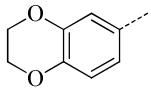
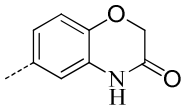
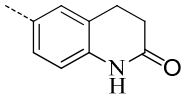
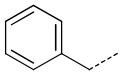
PKM2 activation activities and anti-proliferative activities of target compounds modified at region **A**.



5a-5z, 5aa

Compd.	A	Enzymatic activity AC_{50}[a] (μM)	Antiproliferative activity IC_{50}[b] (μM)			
		PKM2	H1299	HCT116	Hela	PC3
2		2.96±0.57	1.44±0.22	1.70±0.25	0.45±0.04	0.99±0.17
5a		1.19±0.25	1.63±0.23	2.41±0.03	0.55±0.03	1.58±0.29

5b		6.25±0.90	1.68±0.20	1.73±0.16	1.14±0.08	1.11±0.10
5c		>20	1.20±0.15	2.18±0.14	0.44±0.07	0.94±0.17
5d		>20	1.75±0.68	1.65±0.44	0.94±0.19	1.33±0.08
5e		>20	1.62±0.15	3.69±0.24	1.11±0.03	4.51±0.95
5f		5.95±0.92	0.93±0.06	2.62±0.75	0.59±0.04	0.93±0.08
5g		4.69±0.32	0.96±0.10	1.75±0.13	0.48±0.02	1.07±0.24
5h		>20	1.75±0.15	2.30±0.02	0.61±0.04	3.87±0.49
5i		>20	1.22±0.06	2.72±0.14	0.26±0.02	1.31±0.28
5j		>20	1.47±0.58	1.80±0.33	0.77±0.08	4.11±0.25
5k		>20	1.45±0.12	1.18±0.03	0.55±0.04	2.68±0.46
5l		>20	1.14±0.01	4.11±0.69	0.48±0.02	2.19±0.31
5m		>20	1.31±0.12	3.50±0.02	0.57±0.02	1.40±0.02
5n		>20	1.24±0.18	3.45±0.60	1.33±0.11	8.62±0.73
5o		>20	2.70±0.14	6.11±0.51	1.10±0.12	2.81±0.05
5p		>20	2.96±0.20	3.89±0.27	1.40±0.04	3.26±0.14
5q		>20	0.81±0.04	1.65±0.08	0.46±0.04	1.52±0.06

5r		>20	1.53±0.07	2.61±0.09	0.63±0.08	1.69±0.09
5s		1.47±0.29	1.23±0.17	1.44±0.25	0.42±0.03	1.25±0.26
5t		0.52±0.14	2.43±0.33	2.11±0.26	0.84±0.11	2.35±0.76
5u		>20	1.65±0.49	1.41±0.04	0.69±0.10	2.40±0.48
5v		1.41±0.47	2.00±0.21	1.55±0.33	0.76±0.02	1.26±0.08
5w		3.14±0.55	0.69±0.08	0.81±0.03	0.46±0.05	0.66±0.07
5x		>20	2.12±0.11	1.84±0.31	0.78±0.08	1.95±0.29
5y		0.74±0.15	1.65±0.25	1.64±0.10	0.71±0.11	4.29±0.21
5z		7.24±1.02	1.55±0.26	3.32±0.29	1.26±0.03	2.44±0.31
5aa		>20	1.25±0.37	1.56±0.30	0.86±0.09	2.20±0.39
Taxol (nM)	----	----	7.83±1.15	1.37±0.64	6.42±0.70	4.57±0.47

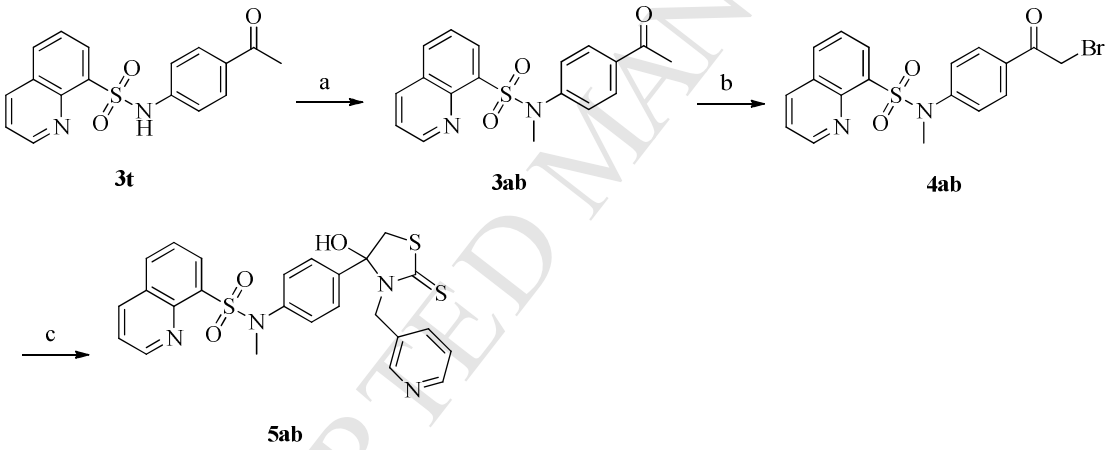
[a] AC_{50} values were determined using enzymatic PKM2 activity assay.

[b] Dose-response curves were determined at five concentrations. The IC_{50} values are the concentrations needed to inhibit cell growth by 50% as determined from these curves.

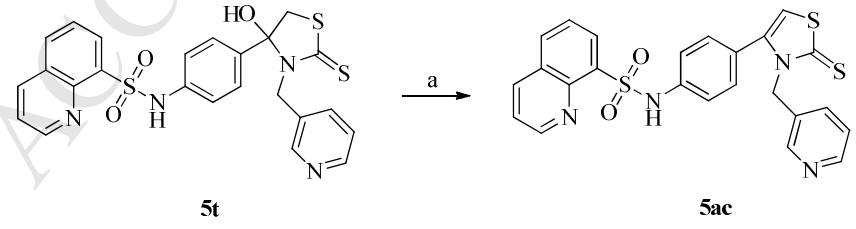
To detect the efficacy of target compounds as anti-tumor agents, we evaluated the antiproliferative activity of the synthesized compounds against four PKM2 high expression human tumor cell lines derived from human lung cancer (H1299), colon cancer (HCT116), cervical cancer (Hela) and prostate cancer (PC3). The well-known anticancer drug paclitaxel was used as the positive control. The results of this evaluation are also shown in Table 1. The IC_{50} values of tested compounds against different tumor cells ranged between 0.26 and 8.62 μM. Among them, Hela cells were the most sensitive to these compounds with most IC_{50} value in the nanomolar range. Those compounds with better enzymatic activity also have significant antiproliferative activity. In particular, compound **5w** showed the greatest anti-proliferative activity towards these cell lines ranging from 0.46 to 0.81 μM, which is also potent at enzyme level. Structure-activity relationship (SAR) analysis showed that the steric hindrance of substituents on the benzene ring (**5o** and **5p**) result in 2-fold or 3-fold decrease in anti-proliferative activity against four cell lines comparing with lead compound **2**. Different structural units in region **A** showed great

impact on the anti-proliferative activities against PC3 cell line. Replacement of the phenyl moiety with 2-methoxy-5-chlorophenyl (**5e**, 4.51 μM), 2-nitrophenyl (**5h**, 3.87μM), 4-cyanophenyl (**5j**, 4.11 μM), 2-morpholinylphenyl (**5n**, 8.62 μM) and 3,4-dihydroquinolin-2(1H)-one (**5y**, 4.29 μM) led to obvious decrease in potency against PC3 cell line in comparison with compound **2** (0.99 μM).

In view of its excellent PKM2 activation activity, compound **5t** was selected to evaluate the SAR of this scaffold in depth. In order to investigate the role of NH in sulfonamide fragment, we converted the NH to NMe and generated the compound **5ab**. The design of dehydroxyl compound **5ac** was to examine the effect of hydroxyl group in 4-hydroxy-thiazolidine-2-thione moiety. The synthetic routes for target compounds **5ab** and **5ac** are respectively depicted in Schemes **3** and **4**. The front synthesized intermediate **3t** served as a starting material was treated with MeI using Cs_2CO_3 as catalyst in DMF to obtain compound **3ab** with high yields. Then **3ab** was bromized and treated with pyridin-3-ylmethanamine and carbon disulfide with the same synthetic process as **5a**-**5z** or **5aa** to afford the target compound **5ab**. Compound **5t** was dehydrated in refluxing MeOH and 0.5% HCl for 24 h to generate the target compound **5ac** according to the literature method [23].



Scheme 3. Synthesis of compound **5ab**. Reagents and conditions: (a) MeI, Cs_2CO_3, DMF, rt; (b) Phenyltrimethylammonium tribromide, THF, rt; (c) Pyridin-3-ylmethanamine, CS_2, Et_3N, Acetone, rt.



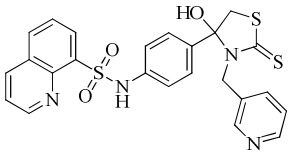
Scheme 4. Synthesis of compound **5ac**. Reagents and conditions: (a) 0.5% HCl, MeOH, reflux.

The PKM2 activation activities and anti-proliferative activities of compounds **5t**, **5ab** and **5ac** were listed in Table 2. Methyltion of the NH to NMe (**5ab**) resulted in a complete loss in enzyme potency, but retained the cellular activity. This suggested that hydrogen atom in the sulfonamide is critical for achieving potent PKM2

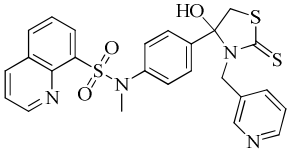
activation activity. The dehydration product **5ac** of **5t** exhibited the complete loss of both enzymatic and cellular activities. This phenomenon showed that the 4-hydroxyl group in the 4-hydroxy-thiazolidine-2-thione derivatives is the key factor to maintain the enzymatic and cellular activities.

Table 2

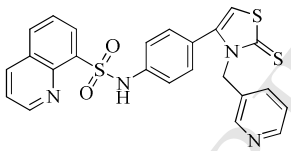
PKM2 activation activities and anti-proliferative activities of compounds **5t**, **5ab** and **5ac**.



5t



5ab



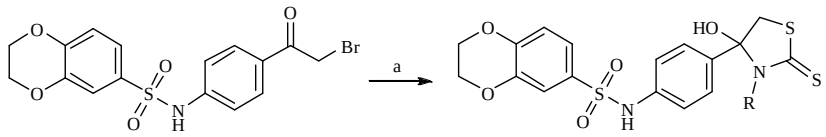
5ac

Compd.	Enzymatic activity AC_{50}[a] (μM)	Antiproliferative activity IC_{50}[b] (μM)			
	PKM2	H1299	HCT116	Hela	PC3
5t	0.52±0.14	2.43±0.33	2.11±0.26	0.84±0.11	2.35±0.76
5ab	>20	2.47±0.87	2.32±0.17	0.93±0.10	6.15±0.78
5ac	>20	>20	>20	>20	>20
Taxol (nM)	----	7.83±1.15	1.37±0.64	6.42±0.70	4.57±0.47

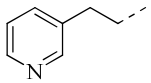
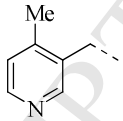
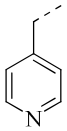
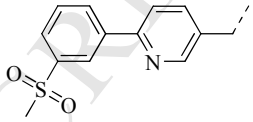
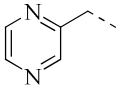
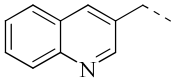
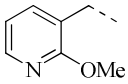
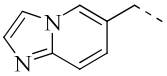
[a] AC_{50} values were determined using enzymatic PKM2 activity assay.

[b] Dose-response curves were determined at five concentrations. The IC_{50} values are the concentrations needed to inhibit cell growth by 50% as determined from these curves.

In view of the above results, we found that different structural units at region **A** and the hydrogen atom in the sulfonamide showed great impact on the PKM2 activation activity, and the 4-hydroxyl group in 4-hydroxy-thiazolidine-2-thione moiety is the key pharmacophore for these kind of compounds. Compound **5w** not only has potent PKM2 activation activity, but also exhibits the highest inhibitory against the four tested cancer cell lines among all the target compounds modified at region A. Therefore, compound **5w** represented a new starting point for further optimization. The pyridin-3-ylmethyl moiety in region **B** was modified while maintaining 1,4-benzodioxane as region **A**. To investigate whether the length of carbon linker between the 4-hydroxy-thiazolidine-2-thione scaffold and the pyridine ring might affect the activation activity, compound **5ad** was synthesized. The pyridin-3-ylmethyl moiety was modified with pyridin-4-ylmethyl (**5ae**) to evaluate the influence of the position of nitrogen atom in pyridine ring on the bioactivity. Replacement of pyridine fragment of compound **5w** with various pyridine derivatives (**5af**, **5aj** and **5ak**) to determine whether the pyridine unit has an effect on the activity. To study the effect of pyridine moiety in more detail, we synthesized compounds **5ag**-**5ai** with various substituents in the pyridine ring. The synthesis of target compounds **5ad**-**5ak** is outlined in Scheme 5. The front intermediate compound **4w** reacted with carbon disulfide and various amines, which were commercially available or prepared by literature procedures [24, 25], using triethylamine as base in acetone to afford the target compounds **5ad**-5**ak**.



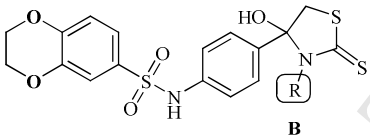
4w **5ad-5ak**

Compd.	R	Compd.	R
5ad		**5ah**	
5ae		**5ai**	
5af		**5aj**	
5ag		**5ak**	

Scheme 5. Synthesis of **5ad-5ak**. Reagents and conditions: (a) RNH_2, CS_2, Et_3N, Acetone, rt.

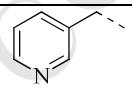
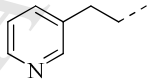
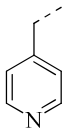
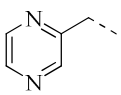
Table 3

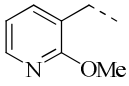
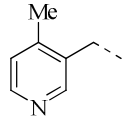
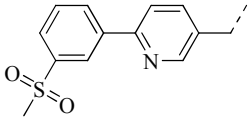
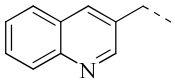
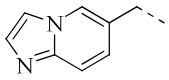
PKM2 activation activities and anti-proliferative activities of target compounds modified at region B.



B

5ad-5ak

Compd.	R	Enzymatic activity AC_{50}[a] (μM)	Antiproliferative activity IC_{50}[b] (μM)			
		PKM2	H1299	HCT116	Hela	PC3
5w		3.14±0.55	0.69±0.08	0.81±0.03	0.46±0.05	0.66±0.07
5ad		>20	1.28±0.14	1.04±0.01	0.62±0.01	2.16±0.45
5ae		>20	0.75±0.02	0.66±0.01	0.47±0.01	0.70±0.04
5af		2.15±0.39	1.72±0.06	0.85±0.03	0.55±0.01	0.92±0.20

5ag		>20	0.83±0.03	0.74±0.01	0.41±0.01	1.13±0.01
5ah		>20	0.51±0.01	0.41±0.09	0.34±0.01	1.53±0.08
5ai		17.61±2.19	1.02±0.04	0.92±0.02	0.51±0.02	1.35±0.28
5aj		1.75±0.33	1.81±0.14	1.28±0.01	0.55±0.01	1.28±0.13
5ak		7.07±1.46	0.79±0.01	0.77±0.01	0.45±0.01	1.33±0.02

[a] AC_{50} values were determined using enzymatic PKM2 activity assay.

[b] Dose-response curves were determined at five concentrations. The IC_{50} values are the concentrations needed to inhibit cell growth by 50% as determined from these curves.

As shown in Table 3, increasing the length of carbon linker between the pyridine scaffold and the 4-hydroxy-thiazolidine-2-thione scaffold from 1 to 2 carbon atoms resulted in a significant decrease in PKM2 activation activity (AC_{50} > 20 μM). Movement of nitrogen to the para position led to dramatic reduction in potency for 4-pyridyl analogue **5ae** (AC_{50} > 20 μM) as compared to compound **5w**. This indicated that the position of nitrogen atom in the pyridine ring is critical for achieving potent PKM2 activation activity. Introduction of different substituents (**5ag-5ai**) in pyridine ring of compound **5w** significantly decreased the activity (AC_{50} of **5ag** and **5ah** > 20 μM, AC_{50} of **5ai** = 17.61μM). This suggested that introducing substituents into the pyridine ring is unfavorable for the improvement of activity. The target compound **5af** (AC_{50} = 2.15 μM) and **5aj** (AC_{50} = 1.75 μM) respectively with pyrazin-2-ylmethyl and quinolin-3-ylmethyl showed more potent activity than compound **5w** (AC_{50} = 3.14 μM). The imidazo[1,2-a]pyridin-6-ylmethyl (**5ak**, AC_{50} = 7.07 μM) instead of pyridin-3-ylmethyl of compound **5w** slightly reduced the activation activity. These results suggested that there is still more optimization space for region B. Anti-proliferative activities of the target compounds modified at region B were also evaluated in the same four cancer cell lines (H1299, HCT116, Hela and PC3). The results in Table 3 showed that most of the modifications at region B have no obvious impact on the antiproliferative activities. In other words, region A of this series of compounds is more crucial for antitumor activity.

To further explore the selectivity of target compounds against cancer cells, they were also tested in HELF cells derived from normal human embryonic lung fibroblast cells. As seen in Table 4, some tested compounds showed higher cytotoxicity in cancer cells (H1299) than normal cells (HELF). The results indicate that target compounds have highly safety index. This is consistent with our design objective, pharmacological activation of PKM2 enables cancer cells to restore the metabolism of normal cells, which has no side effects on normal cells.

Table 4

Anti-proliferative activities of selected target compounds against H1299 cancer cell line and non-tumor cell line HELF.

Compd.	IC_{50} (μM)[a]	
	H1299	HELF
5f	0.93±0.06	6.90±0.60
5g	0.96±0.10	8.63±0.52
5q	0.81±0.04	8.05±0.26
5w	0.69±0.08	11.85±0.83
5ae	0.75±0.02	8.31±1.47
5ag	0.83±0.03	10.44±1.35
5ah	0.51±0.01	7.21±0.54
5ak	0.79±0.01	4.91±0.62

[a] Dose-response curves were determined at five concentrations. The IC_{50} values are the concentrations needed to inhibit cell growth by 50% as determined from these curves.

According to the optimization results of region **A** and region **B**, we found an interesting phenomenon. Some compounds display excellent anti-proliferative activities in spite of their poor PKM2 activation activities. PKM2 was reported to translocate from the cytoplasm into the nucleus of cancer cells to interact with Bub3 and MCL2, which is required for cell division in tumors [26]. In order to identify whether these compounds have an effect on cell cycle distribution, compound **5w** was chosen to investigate the effect of these 4-hydroxy-thiazolidine-2-thione derivatives on the cell cycle of HCT-116 cells. Exposure of HCT116 cells to compound **5w** for 8 h resulted in an interference with cell cycle distribution. As shown in Fig. 4, treatment of **5w** caused blockage of the cell cycle in the G2/M phase. Compared with control, when HCT116 cells were treated with increasing concentrations (1.25 μM, 2.5 μM and 5 μM) of **5w**, the percentage of cells in the G2/M phase increased from 21.28% to 63.21%, and the percentages of cells in S and G0/G1 phase decreased concomitantly. Our results suggest that this series of compounds may display antitumor activity by inhibiting PKM2 to enter nucleus to induce cell cycle arrest.

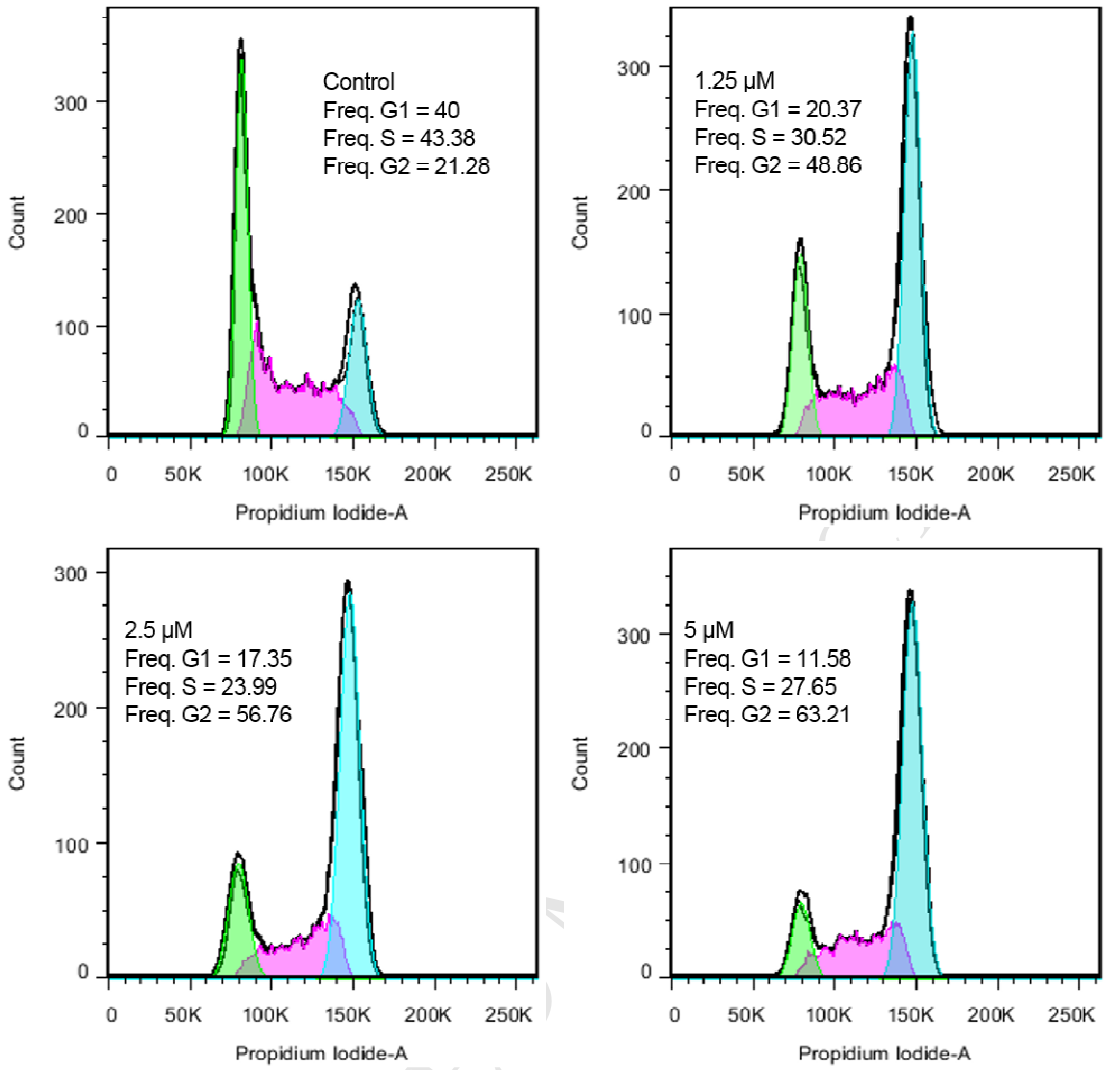


Fig. 4. Compound **5w** induced cell cycle arrest in G2/M phase of HCT116 cells in a dose-dependent manner.

To get further insight into the binding profile and reveal the structure–activity relationship of this series of compounds, molecular docking studies were performed. Compound **5t** was chosen as the representative compound as it has the lowest AC_{50} value. Both R and S configurations of **5t** were calculated, based on the estimated binding energy and possible binding modes, R configuration was considered to be active. The predicted optimal binding mode is shown in Fig. 5.

Compound **5t** was tightly fitted into the small cave same as where other reported PKM2 activators located. The quinoline ring was inside the hydrophobic pocket surrounded by F26, M30, Q393, L394 and L398. The 4-methylphenyl group of compound **5c** will be sterically unfavorable due to the existence of nearby E397 and leads to the loss of activation activity. Replacing the sulfonamide hydrogen with a methyl group will also clash with the phenyl side chain of F26 thus eliminates its activity. The hydroxyl group is crucial as it can form a hydrogen bond with the backbone oxygen of L353. Removal of this hydroxyl group in **5t** will not only delete this important interaction but also significantly change the conformation of the thiazolidine-2-thione ring and the attached pyridine ring. The pyridine nitrogen will

also build a hydrogen bond with the side chain NH of Q393, changing the position of the nitrogen will likely lower the strength of this hydrogen bond and lower the activity.

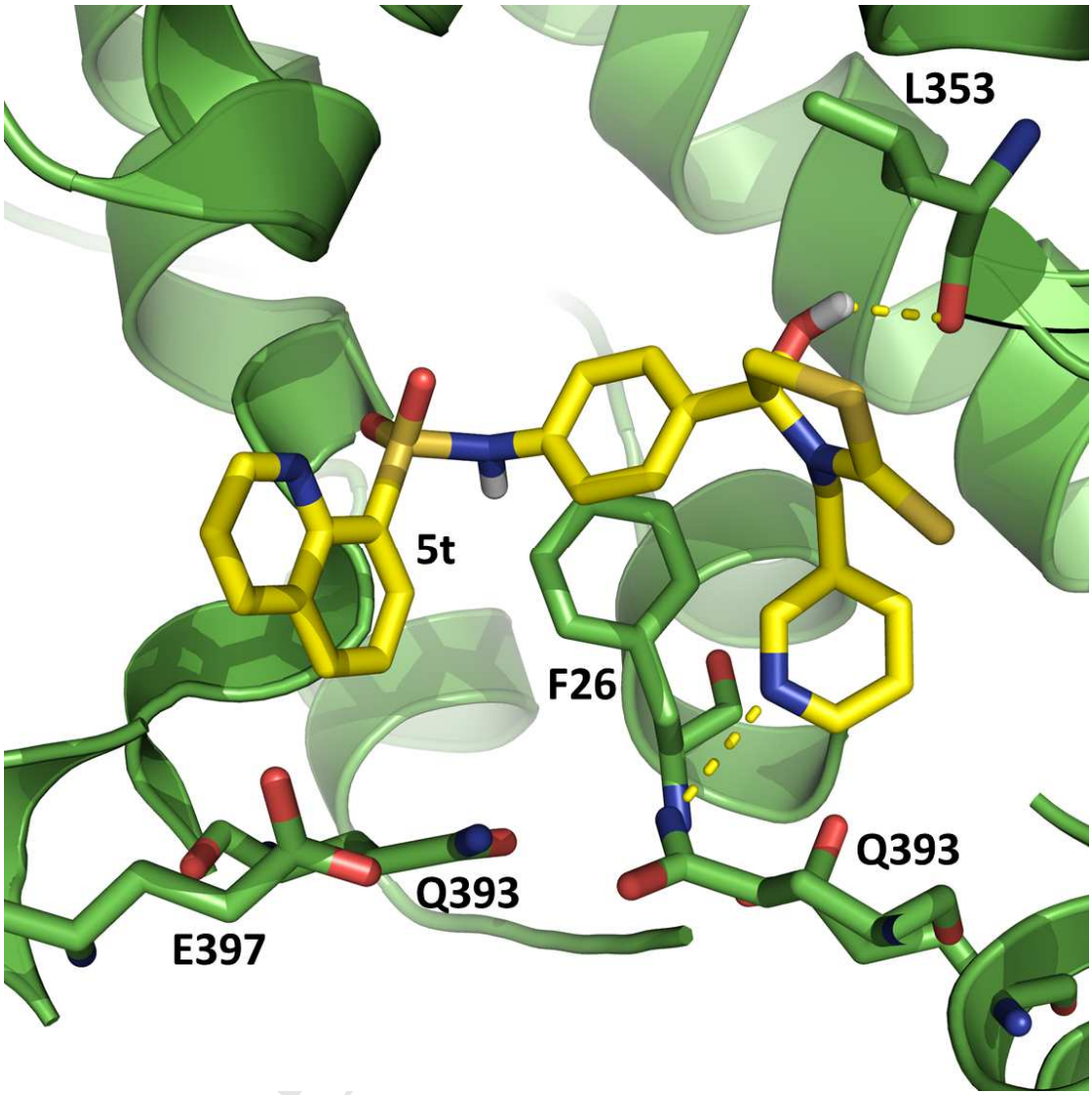


Fig. 5. Predicted binding mode of compound **5t** with PKM2. **5t** is shown in yellow sticks and PKM2 is shown in green cartoon. Key residues are shown in green sticks. Polar interactions are shown in yellow dashed lines.

3. Conclusion

In this paper, we have described the discovery and SAR of a previously undescribed chemical class of PKM2 activators. Systematic structural modification was carried out based on the structure of the lead compound **2**. A series of novel 4-hydroxy-thiazolidine-2-thione derivatives were synthesized and evaluated for their in vitro PKM2 activation activities and anti-tumor activities. Among them, some compounds, such as **5t** and **5y**, showed higher PKM2 activation activity than lead compound **2** and also exhibited significant anti-proliferative activities on human cancer cell lines. The compound **5w** was identified as the most potent antitumor agent,

which inhibited the growth of four types of tumor cells with IC_{50} from 0.46 μM to 0.81 μM. A flow cytometric study showed that compound **5w** delayed cell cycle progression by arresting cells in G2/M phase, which may be due to its ability to prevent PKM2 from entering nucleus. The molecular docking studies revealed the interaction mode between compound **5t** and PKM2, and the results may inspire the design of more potent PKM2 activators. Further mechanism studies of compound **5w** are currently in progress.

4. Experiment

4.1. General

All reagents and solvents were purchased from commercial sources and were used without further purification. Melting points were determined on X4 microscope and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCEIII 400 MHz and 100 MHz spectrometer respectively. High resolution mass spectrum (HRMS) was recorded on a Thermo Scientific Orbitrap Elite MS.

*4.2. General procedure for the synthesis of compounds **2a, 3a-3m**, **3q-3z**, **3aa** and **6***

To a solution of 4-aminoacetophenone (0.68 g, 5 mmol) and pyridine (1.6 ml, 20 mmol) in THF (10 mL) was slowly added the substituted sulfonyl chloride (5 mmol). The reaction mixture was stirred at room temperature for 12 h. Water (50 mL) was added and the mixture was extracted with ethyl acetate (10 mL×3), the combined organic phase was washed with 1N hydrochloric acid (30 mL×2) and water (30 mL×2), dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford the product.

*4.2.1. N-(4-Acetylphenyl)benzenesulfonamide (**2a**)*

Yield 95%. Mp: 130-131°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.86 (dd, *J* = 18.4, 8.4 Hz, 4H), 7.55 (t, *J* = 7.4 Hz, 1H), 7.46 (t, *J* = 7.8 Hz, 2H), 7.21 (d, *J* = 8.8 Hz, 2H), 2.53 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.45, 141.48, 138.98, 133.54, 133.24, 130.12, 129.39, 127.29, 119.09, 26.51.

*4.2.2. N-(4-Acetylphenyl)-2-methylbenzenesulfonamide (**3a**)*

Yield 76%. Mp: 173-174°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.02 (s, 1H), 7.98 (d, *J* = 7.6 Hz, 1H), 7.82 (d, *J* = 8.4 Hz, 2H), 7.53 (t, *J* = 7.2 Hz, 1H), 7.42 – 7.38 (m, 2H), 7.18 (d, *J* = 8.8 Hz, 2H), 2.60 (s, 3H), 2.45 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.37, 142.07, 137.20, 136.79, 133.39, 132.77, 131.54, 129.85, 129.47, 126.47, 116.93, 26.37, 19.63.

*4.2.3. N-(4-Acetylphenyl)-3-methylbenzenesulfonamide (**3b**)*

Yield 91%. Mp: 165-166°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (s, 1H), 7.84 (d, *J* = 8.8Hz, 2H), 7.67 – 7.62 (m, 2H), 7.45 (d, *J* = 5.6 Hz, 2H), 7.21 (d, *J* = 8.0 Hz, 2H), 2.46 (s, 3H), 2.35 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.45, 142.27, 139.94, 139.22, 133.90, 131.91, 129.81, 129.29, 126.83, 123.85, 117.83, 26.40, 20.81.

*4.2.4. N-(4-Acetylphenyl)-4-methylbenzenesulfonamide (**3c**)*

Yield 85%. Mp: 203-204°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.81 (s, 1H), 7.83 (d, *J* = 8.4 Hz, 2H), 7.72 (d, *J* = 8.0 Hz, 2H), 7.37 (d, *J* = 8.0 Hz, 2H), 7.20 (d, *J* = 8.4 Hz,

2H), 2.46 (s, 3H), 2.33 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.43, 143.70, 142.36, 136.44, 131.89, 129.87, 129.80, 126.75, 117.84, 26.37, 20.95.

*4.2.5. N-(4-Acetylphenyl)-2,4,6-trimethylbenzenesulfonamide (**3d**)*

Yield 47%. Mp: 223-225°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (s, 1H), 7.82 (d, *J* = 8.0 Hz, 2H), 7.06 – 7.03 (m, 4H), 2.61 (s, 6H), 2.45 (s, 3H), 2.22 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.35, 142.50, 142.34, 138.72, 133.43, 131.96, 131.32, 129.88, 116.57, 26.35, 22.36, 20.37.

*4.2.6. N-(4-Acetylphenyl)-5-chloro-2-methoxybenzenesulfonamide (**3e**)*

Yield 71%. Mp: 206-208°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.79 (s, 1H), 7.85 – 7.81 (m, 3H), 7.66 (dd, *J* = 9.0, 2.6 Hz, 1H), 7.22 (d, *J* = 8.8 Hz, 3H), 3.87 (s, 3H), 2.47 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.47, 155.32, 142.04, 135.01, 131.95, 129.76, 129.43, 127.52, 123.80, 117.82, 115.21, 56.70, 26.40.

*4.2.7. N-(4-Acetylphenyl)-3-methoxybenzenesulfonamide (**3f**)*

Yield 93%. Mp: 151-152°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (s, 1H), 7.84 (d, *J* = 8.8 Hz, 2H), 7.48 (t, *J* = 8.0 Hz, 1H), 7.39 (d, *J* = 7.6 Hz, 1H), 7.33 (d, *J* = 1.6 Hz, 1H), 7.24 – 7.18 (m, 3H), 3.78 (s, 3H), 2.47 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.50, 159.42, 142.18, 140.48, 132.10, 130.70, 129.82, 118.94, 118.73, 118.08, 111.72, 55.61, 26.41.

*4.2.8. N-(4-Acetylphenyl)-4-methoxybenzenesulfonamide (**3g**)*

Yield 86%. Mp: 178-179°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.77 (d, *J* = 2.4 Hz, 1H), 7.84 (d, *J* = 8.4 Hz, 2H), 7.79 (dd, *J* = 8.8, 2.0 Hz, 2H), 7.22 (dd, *J* = 8.6, 2.6 Hz, 2H), 7.08 (d, *J* = 8.8 Hz, 2H), 3.79 (s, 3H), 2.47 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.46, 162.70, 142.48, 131.81, 130.82, 129.82, 129.00, 117.75, 114.58, 55.67, 26.40.

*4.2.9. N-(4-Acetylphenyl)-2-nitrobenzenesulfonamide (**3h**)*

Yield 89%. Mp: 153-155°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.33 (s, 1H), 8.06 (dd, *J* = 7.6, 1.6 Hz, 1H), 8.02 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.90 – 7.84 (m, 4H), 7.24 (d, *J* = 8.8 Hz, 2H), 2.49 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.53, 147.89, 141.22, 135.04, 132.83, 132.50, 131.09, 129.96, 129.89, 124.90, 118.38, 26.46.

*4.2.10. N-(4-Acetylphenyl)-4-nitrobenzenesulfonamide (**3i**)*

Yield 86%. Mp: 191-192°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.18 (s, 1H), 8.39 (dd, *J* = 6.8, 2.0 Hz, 2H), 8.07 (dd, *J* = 6.8, 2.0 Hz, 2H), 7.86 (dd, *J* = 6.8, 2.0 Hz, 2H), 7.24 (dd, *J* = 6.8, 2.0 Hz, 2H), 2.48 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.50, 150.04, 144.55, 141.43, 132.55, 129.93, 128.30, 124.86, 118.57, 26.45.

*4.2.11. N-(4-Acetylphenyl)-4-cyanobenzenesulfonamide (**3j**)*

Yield 97%. Mp: 207-208°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.12 (s, 1H), 8.08 (d, *J* = 8.4 Hz, 2H), 7.99 (d, *J* = 8.4 Hz, 2H), 7.86 (d, *J* = 8.8 Hz, 2H), 7.22 (d, *J* = 8.4 Hz, 2H), 2.48 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.50, 143.22, 141.50, 133.69, 132.49, 129.92, 127.43, 118.49, 117.47, 115.73, 26.45.

*4.2.12. N-(4-Acetylphenyl)-2-fluorobenzenesulfonamide (**3k**)*

Yield 56%. Mp: 181-182°C. ^{1}H NMR (400 MHz, DMSO-D6) δ 11.22 (s, 1H), 7.94 (td, *J* = 7.6, 1.2 Hz, 1H), 7.84 (d, *J* = 8.8 Hz, 2H), 7.74 – 7.69 (m, 1H), 7.45 – 7.38 (m, 2H), 7.21 (d, *J* = 8.8 Hz, 2H), 2.46 (s, 3H).

*4.2.13. N-(4-acetylphenyl)-3-fluorobenzenesulfonamide (**3l**)*

Yield 92%. Mp: 146-147°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.02 (s, 1H), 7.88 (d, *J* = 8.8 Hz, 2H), 7.73 – 7.64 (m, 3H), 7.54 (s, 1H), 7.27 (dd, *J* = 8.4, 2.4 Hz, 2H), 2.49 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.48, 161.70 (d, *J* = 249.1 Hz), 141.82, 141.29 (d, *J* = 6.7 Hz), 132.33, 131.94, 129.89, 123.00, 120.54 (d, *J* = 22.0 Hz), 118.33, 113.91, 113.79 (d, *J* = 24.5 Hz), 26.41.

*4.2.14. N-(4-Acetylphenyl)-4-fluorobenzenesulfonamide (**3m**)*

Yield 88%. Mp: 139-140°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.96 (s, 1H), 7.97 – 7.93 (m, 2H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.45 – 7.41 (m, 2H), 7.27 (d, *J* = 8.8 Hz, 2H), 2.49 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 196.46, 164.53 (d, *J* = 252.1 Hz), 142.07, 135.66, 132.19, 129.88, 129.81, 118.18, 116.71 (d, *J* = 22.8 Hz), 26.39.

*4.2.15. N-(4-Acetylphenyl)naphthalene-2-sulfonamide (**3q**)*

Yield 95%. Mp: 198-199°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.05 (d, *J* = 11.6 Hz, 1H), 8.61 (d, *J* = 5.6 Hz, 1H), 8.18 – 8.11 (m, 2H), 8.02 – 8.00 (m, 1H), 7.88 – 7.82 (m, 3H), 7.71 – 7.65 (m, 2H), 7.30 (t, *J* = 9.2 Hz, 2H), 2.43 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.38, 142.22, 136.21, 134.37, 131.96, 131.53, 129.82, 129.76, 129.32, 129.19, 129.16, 128.30, 127.83, 121.82, 117.93, 26.35.

*4.2.16. N-(4-Acetylphenyl)naphthalene-1-sulfonamide (**3r**)*

Yield 83%. Mp: 239-240°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.30 (s, 1H), 8.73 (d, *J* = 8.4 Hz, 1H), 8.33 (d, *J* = 7.2 Hz, 1H), 8.25 (d, *J* = 8.0 Hz, 1H), 8.08 (d, *J* = 8.0 Hz, 1H), 7.79 – 7.75 (m, 3H), 7.69 – 7.65 (m, 2H), 7.16 (d, *J* = 8.8 Hz, 2H), 2.41 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.31, 142.01, 134.82, 133.79, 131.56, 130.30, 129.77, 129.20, 128.39, 127.26, 127.11, 124.51, 123.97, 117.01, 26.32.

*4.2.17. N-(4-Acetylphenyl)thiophene-2-sulfonamide (**3s**)*

Yield 73%. Mp: 149-151°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.03 (s, 1H), 7.95 – 7.89 (m, 3H), 7.69 – 7.68 (m, 1H), 7.29 (dd, *J* = 8.8, 2.4 Hz, 2H), 7.14 (t, *J* = 4.4 Hz, 1H), 2.50 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.55, 141.96, 139.66, 133.93, 133.03, 132.28, 129.84, 127.80, 118.26, 26.47.

*4.2.18. N-(4-Acetylphenyl)quinoline-8-sulfonamide (**3t**)*

Yield 84%. Mp: 257-258°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.79 (s, 1H), 9.13 (dd, *J* = 4.0, 1.6 Hz, 1H), 8.52 – 8.47 (m, 2H), 8.30 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.78 – 7.69 (m, 4H), 7.20 (d, *J* = 8.8 Hz, 2H), 2.39 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.35, 151.56, 142.64, 142.39, 137.00, 134.89, 134.63, 132.56, 131.55, 129.50, 128.46, 125.65, 122.70, 117.61, 26.31.

4.2.19. N-(4-Acetylphenyl)-2-oxo-2*H*-chromene-6-sulfonamide *(**3u**)*

Yield 80%. Mp: 190-191°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.02 (s, 1H), 8.33 (d, *J* = 2.4 Hz, 1H), 8.19 (d, *J* = 9.6 Hz, 1H), 7.98 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.85 (d, *J* = 8.8 Hz, 2H), 7.58 (d, *J* = 8.8 Hz, 1H), 7.25 (d, *J* = 8.8 Hz, 2H), 6.63 (d, *J* = 9.6 Hz, 1H), 2.47 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.48, 159.06, 156.05, 143.35, 141.90, 135.15, 132.15, 129.89, 129.53, 127.84, 119.06, 118.12, 117.99, 117.95, 26.43.

*4.2.20. N-(4-Acetylphenyl)-1H-pyrrolo[2,3-b]pyridine-3-sulfonamide (**3v**)*

Yield 31%. Mp: 151-153°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.67 (s, 1H), 10.45 (s, 1H), 8.35 (d, *J* = 4.8 Hz, 2H), 8.25 – 8.17 (m, 3H), 7.71 (d, *J* = 5.6 Hz, 1H), 7.45 – 7.43 (m, 1H), 7.28 – 7.18 (m, 3H), 7.09 – 7.00 (m, 3H), 4.71 (d, *J* = 15.6 Hz, 1H),

4.43 (d, *J* = 15.6 Hz, 1H), 3.61 (d, *J* = 7.6 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.65, 148.98, 148.04, 147.67, 144.62, 138.69, 135.25, 134.95, 132.25, 131.84, 127.56, 126.54, 122.70, 118.07, 117.63, 115.57, 111.97, 99.95, 46.33, 42.37. HRMS m/z: calcd for $C_{22}H_{20}N_5O_3S_3$ $[M+H]^+$: 498.0728; found: 498.0723.

*4.2.21. N-(4-Acetylphenyl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamide (**3w**)*

Yield 78%. Mp: 161-162°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.81 (s, 1H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.35 – 7.30 (m, 2H), 7.24 (dd, *J* = 8.4, 6.4 Hz, 2H), 7.02 (dd, *J* = 8.4, 1.6 Hz, 1H), 4.28 (d, *J* = 2.8 Hz, 4H), 2.47 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.46, 147.51, 143.38, 142.43, 131.86, 131.63, 129.87, 120.44, 117.73, 115.71, 64.38, 64.05, 26.39.

4.2.22.

*N-(4-Acetylphenyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazine-6-sulfonamide (**3x**)*

Yield 97%. Mp: 277-279°C. ^{1}H NMR (400 MHz, DMSO- d_6) δ 10.99 (s, 1H), 10.86 (s, 1H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.40 – 7.36 (m, 2H), 7.20 (d, *J* = 8.8 Hz, 2H), 7.08 (d, *J* = 8.4 Hz, 1H), 4.66 (s, 2H), 2.48 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.48, 164.13, 146.77, 142.25, 132.75, 131.91, 129.85, 127.72, 122.19, 117.83, 116.71, 114.12, 66.64, 26.42.

*4.2.23. N-(4-Acetylphenyl)-2-oxo-1,2,3,4-tetrahydroquinoline-6-sulfonamide (**3y**)*

Yield 91%. Mp: 250-251°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.76 (s, 1H), 10.46 (s, 1H), 7.84 (d, *J* = 8.8 Hz, 2H), 7.66 – 7.62 (m, 2H), 7.20 (d, *J* = 8.8 Hz, 2H), 6.94 (d, *J* = 8.0 Hz, 1H), 2.93 (t, *J* = 7.6 Hz, 2H), 2.49 – 2.45 (m, 5H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.43, 170.29, 142.64, 142.43, 131.98, 131.69, 129.82, 126.54, 126.51, 124.37, 117.56, 115.11, 29.68, 26.40, 24.39.

*4.2.24. N-(4-Acetylphenyl)-1-phenylmethanesulfonamide (**3z**)*

Yield 85%. Mp: 192-193°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.36 (s, 1H), 7.92 (d, *J* = 8.4 Hz, 2H), 7.36 – 7.4 (m, 3H), 7.27 (d, *J* = 8.4 Hz, 4H), 4.58 (s, 2H), 2.53 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.49, 143.11, 131.40, 130.95, 129.96, 129.30, 128.46, 128.37, 117.13, 57.17, 26.45.

*4.2.25. N-(4-Acetylphenyl)methanesulfonamide (**3aa**)*

Yield 66%. Mp: 155-156°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1H), 7.94 (d, *J* = 8.8 Hz, 2H), 7.29 (d, *J* = 8.8 Hz, 2H), 3.11 (s, 3H), 2.53 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.53, 143.00, 131.65, 129.99, 117.50, 39.87, 26.47.

*4.2.26. N-(4-Acetylphenyl)-2-bromobenzenesulfonamide (**6**)*

Yield 92%. Mp: 198-200°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.22 (s, 1H), 8.17 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.83 (d, *J* = 8.4 Hz, 3H), 7.63 – 7.53 (m, 2H), 7.18 (d, *J* = 8.8 Hz, 2H), 2.45 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.41, 141.53, 137.83, 135.61, 134.95, 131.94, 131.80, 129.82, 128.41, 119.17, 117.20, 26.39.

*4.3. The procedure for the synthesis of N-(4-Acetylphenyl)-2-morpholinobenzenesulfonamide (**3n**)*

To a solution of compound **6** (0.36 g, 1 mmol) in DMSO (10ml) was added morpholine (0.26 g, 3 mmol), CuI (19 mg, 0.1 mmol), L-proline (23 mg, 0.2 mmol) and K_2CO_3 (0.42 g, 3 mmol). The mixture was heated 24 h at 100 °C *under argon* followed by cooling to room temperature. Water (50 mL) was added and the mixture

was extracted with ethyl acetate (10 mL×3), the combined organic phase was washed with brine (30 mL×2), dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography (eluent: petroleum ether/ethyl acetate = 2:1) to provide **3n** as a yellow solid (0.16 g, 44%). Mp: 216-217°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.27 (s, 1H), 8.01 (d, *J* = 7.6 Hz, 1H), 7.78 (d, *J* = 8.4 Hz, 2H), 7.63 (t, *J* = 7.8 Hz, 1H), 7.49 (d, *J* = 8.0 Hz, 1H), 7.35 (t, *J* = 7.6 Hz, 1H), 7.12 (d, *J* = 8.8 Hz, 2H), 3.86 (s, 4H), 2.80 (s, 4H), 2.43 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.36, 151.96, 142.30, 134.94, 134.34, 131.36, 131.21, 129.72, 125.36, 124.54, 116.87, 65.76, 53.65, 26.35.

*4.4. General procedure for the synthesis of compounds **3o-3p***

To a solution of compound **6** (1.24 g, 3.5 mmol) in 1,4-dioxane (20ml) was added substituted phenylboronic acid (5.25 mmol), $PdCl_2(dppf)CH_2Cl_2$ (0.29 g, 0.35 mmol), K_2CO_3 (1.45 g, 10.5 mmol) and 0.5ml H_2O. The mixture was reflux *under argon* for 24 h followed by cooling to room temperature. Water (100 mL) was added and the mixture was extracted with ethyl acetate (15 mL×3), the combined organic phase was washed with brine (30 mL×2), dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography (eluent: petroleum ether/ethyl acetate = 3:1) to provide the product.

*4.4.1. N-(4-Acetylphenyl)-[1,1'-biphenyl]-2-sulfonamide (**3o**)*

Yield 81%. Mp: 187-189°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (d, *J* = 7.6 Hz, 1H), 7.75 (d, *J* = 8.4 Hz, 2H), 7.60 – 7.42 (m, 5H), 7.30 – 7.24 (m, 3H), 6.75 (d, *J* = 8.8 Hz, 2H), 6.08 (s, 1H), 2.49 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.84, 140.87, 140.71, 138.59, 137.30, 133.22, 132.73, 132.58, 130.23, 129.89, 129.51, 128.80, 128.19, 128.04, 117.15, 26.41.

*4.4.2. N-(4-Acetylphenyl)-3'-nitro-[1,1'-biphenyl]-2-sulfonamide (**3p**)*

Yield 63%. Mp: 195-196°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.52 (s, 1H), 8.31 (dd, *J* = 8.2, 1.4 Hz, 1H), 8.17 (dd, *J* = 7.4, 1.4 Hz, 1H), 7.98 (t, *J* = 1.8 Hz, 1H), 7.79 (d, *J* = 8.8 Hz, 2H), 7.74 – 7.69 (m, 3H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.40 – 7.37 (m, 1H), 6.91 (d, *J* = 8.8 Hz, 2H), 2.46 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.36, 147.13, 141.59, 139.81, 138.39, 137.39, 135.76, 133.38, 132.80, 131.52, 129.71, 129.48, 129.42, 129.16, 123.58, 122.95, 116.93, 26.36.

*4.5. The procedure for the synthesis of N-(4-Acetylphenyl)-N-methylquinoline-8-sulfonamide **(3ab)***

To a solution of compound **3t** (0.93 g, 2.85 mmol) in DMF (10ml) was added Cs_2CO_3 (1.86 g, 5.70 mmol) and MeI (0.45 g, 3.14 mmol). The reaction mixture was stirred at room temperature for 19 h. Water (50 mL) was added and the mixture was extracted with ethyl acetate (15 mL×3), the combined organic phase was washed with water (50 mL×3), dried over anhydrous Na_2SO_4 and concentrated to afford **3ab** as a yellow solid (0.87 g, 89%), Mp: 127-128 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.05 (dd, *J* = 4.2, 1.4 Hz, 1H), 8.52 – 8.49 (m, 1H), 8.43 (d, *J* = 7.2 Hz, 1H), 8.30 – 8.28 (m, 1H), 7.79 – 7.67 (m, 4H), 7.34 (d, *J* = 8.8 Hz, 2H), 3.66 (s, 3H), 2.45 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.66, 151.63, 145.42, 143.00, 136.94, 135.78, 134.67, 133.32, 132.90, 128.90, 128.54, 125.70, 122.68, 122.13, 38.15, 26.48.

*4.6. General procedure for the synthesis of compounds **2**, **5a-5z** and **5aa-5ab***

To a solution of compound **2a**, **3a-3z** and **3aa-3ab** (4.5 mmol) in THF (10ml) was added phenyltrimethylammonium tribromide (1.69 g, 4.5 mmol). The reaction mixture was stirred at room temperature for 12 h. Water (50 mL) was added and the mixture was extracted with ethyl acetate (10 mL×3), the combined organic phase was washed with water (30 mL×2), dried over anhydrous Na_2SO_4 and concentrated to afford the intermediates **2b**, **4a-4z** and **4aa-4ab**. To a solution of pyridin-3-ylmethanamine (0.49 g, 4.5 mmol) in acetone (10ml) was added Et_3N (0.91 g, 9 mmol). The reaction mixture was stirred for 5 min and was added CS_2 (0.52 g, 6.75 mmol) to continuously stir for 30 min. The intermediates **2b**, **4a-4z** and **4aa-4ab** were added respectively and this mixture was stirred at room temperature for 4 h. Water (50 mL) was added and the mixture was extracted with ethyl acetate (10 mL×3), the combined organic phase was washed with brine (30 mL×2), dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography to provide the product.

4.6.1.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)benzenesulfonamide (**2**)*

Yield 27%. Mp: 129-130°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.51 (s, 1H), 8.32 (d, *J* = 3.2 Hz, 2H), 7.81 – 7.78 (m, 3H), 7.64 – 7.56 (m, 3H), 7.49 (d, *J* = 7.6 Hz, 1H), 7.26 (d, *J* = 7.2 Hz, 2H), 7.14 – 7.05 (m, 3H), 4.71 (d, *J* = 15.6 Hz, 1H), 4.49 (d, *J* = 15.6 Hz, 1H), 3.64 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.87, 148.93, 147.78, 139.64, 138.26, 135.66, 135.27, 133.05, 132.24, 129.39, 126.82, 126.64, 122.82, 118.99, 99.84, 46.35, 42.40. HRMS m/z: calcd for $C_{21}H_{20}N_3O_3S_3$ $[M+H]^+$: 458.0667; found: 458.0655.

4.6.2.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-2-methylbenzenesulfonamide (**5a**)*

Yield 46%. Mp: 176-177°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.62 (s, 1H), 8.31 (d, *J* = 2.8 Hz, 2H), 7.93 – 7.91 (m, 1H), 7.73 (s, 1H), 7.53 (t, *J* = 7.0 Hz, 1H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.39 (t, *J* = 6.8 Hz, 2H), 7.22 (d, *J* = 8.8 Hz, 2H), 7.11 (dd, *J* = 7.6, 4.8 Hz, 1H), 7.01 (d, *J* = 8.8 Hz, 2H), 4.69 (d, *J* = 15.2 Hz, 1H), 4.47 (d, *J* = 15.2 Hz, 1H), 3.62 (s, 2H), 2.58 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.78, 148.89, 147.72, 138.19, 137.71, 136.76, 135.21, 135.02, 133.13, 132.69, 132.22, 129.26, 126.74, 126.42, 122.76, 117.65, 99.83, 46.30, 42.31, 19.67. HRMS m/z: calcd for $C_{22}H_{22}N_3O_3S_3$ $[M+H]^+$: 472.0823; found: 472.0823.

4.6.3.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-3-methylbenzenesulfonamide (**5b**)*

Yield 41%. Mp: 154-155°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, *J* = 1.6 Hz, 1H), 8.04 – 8.03 (m, 1H), 7.72 – 7.63 (m, 3H), 7.72 – 7.63 (m, 4H), 7.10 (d, *J* = 8.8 Hz, 2H), 7.03 (dd, *J* = 7.8, 5.0 Hz, 1H), 5.09 (d, *J* = 15.2 Hz, 1H), 4.31 (d, *J* = 15.2 Hz, 1H), 3.66 (d, *J* = 12.0 Hz, 1H), 3.55 (d, *J* = 12.4 Hz, 1H), 2.36 (s, 3H). ^{13}C NMR (100

MHz, $CDCl_3$) δ 196.82, 148.64, 146.93, 139.60, 139.27, 138.08, 138.00, 136.51, 134.16, 133.52, 129.18, 127.64, 127.08, 124.48, 123.64, 120.62, 100.77, 46.90, 43.59, 21.51. HRMS m/z: calcd for $C_{22}H_{22}N_3O_3S_3$ $[M+H]^+$: 472.0823; found: 472.0823.

4.6.4.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-4-methylbenzenesulfonamide (**5c**)*

Yield 46%. Mp: 132-133°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.42 (s, 1H), 8.32 (s, 2H), 7.75 (s, 1H), 7.67 (d, *J* = 7.6 Hz, 2H), 7.49 (d, *J* = 8.0 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 2H), 7.25 (d, *J* = 8.24 Hz, 2H), 7.13 (dd, *J* = 7.6, 4.8 Hz, 1H), 7.05 (d, *J* = 8.0 Hz, 2H), 4.71 (d, *J* = 15.2 Hz, 1H), 4.47 (d, *J* = 15.6 Hz, 1H), 3.64 (s, 2H), 2.34 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.82, 148.91, 147.74, 143.36, 138.36, 136.81, 135.50, 135.24, 132.21, 129.77, 126.74, 126.66, 122.77, 118.84, 99.83, 46.33, 42.36, 20.96. HRMS m/z: calcd for $C_{22}H_{22}N_3O_3S_3$ $[M+H]^+$: 472.0823; found: 472.0811.

4.6.5.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-2,4,6-trimethylbenzenesulfonamide (**5d**)*

Yield 46%. Mp: 181-183°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.44 (d, *J* = 4.4 Hz, 1H), 8.35 – 8.32 (m, 2H), 7.76 – 7.74 (m, 1H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.24 (dd, *J* = 8.8, 2.8 Hz, 2H), 7.12 (dd, *J* = 8.0, 4.8 Hz, 1H), 7.03 (d, *J* = 3.6 Hz, 2H), 6.94 – 6.91 (m, 2H), 4.73 (dd, *J* = 15.4, 3.0 Hz, 1H), 4.46 (d, *J* = 15.6 Hz, 1H), 3.63 (s, 2H), 2.58 (s, 6H), 2.22 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.69, 148.95, 147.74, 142.19, 138.64, 138.42, 135.24, 134.91, 133.91, 132.24, 131.91, 126.76, 122.78, 117.63, 99.91, 46.35, 42.38, 22.44, 20.40. HRMS m/z: calcd for $C_{24}H_{26}N_3O_3S_3$ $[M+H]^+$: 500.1136; found: 500.1137.

4.6.6.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-5-Chloro-2-methoxybenzenesulfonamide (**5e**)*

Yield 27%. Mp: 121-122°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.33 (s, 1H), 8.31 (d, *J* = 6.0 Hz, 2H), 7.73 (s, 2H), 7.66 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.24 (t, *J* = 8.0 Hz, 3H), 7.13 (dd, *J* = 7.8, 5.0 Hz, 1H), 7.05 (d, *J* = 8.4 Hz, 2H), 4.70 (d, *J* = 15.6 Hz, 1H), 4.46 (d, *J* = 15.6 Hz, 1H), 3.85 (s, 3H), 3.63 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.80, 155.29, 148.86, 147.69, 138.00, 135.60, 135.18, 134.65, 132.15, 129.13, 128.04, 126.63, 123.67, 122.71, 118.95, 115.12, 99.81, 56.57, 46.29, 42.33. HRMS m/z: calcd for $C_{22}H_{21}ClN_3O_4S_3$ $[M+H]^+$: 522.0383; found: 522.0367.

4.6.7.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-3-methoxybenzenesulfonamide (**5f**)*

Yield 64%. Mp: 91-92°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, *J* = 1.6 Hz, 1H), 8.05 (dd, *J* = 4.9, 1.3 Hz, 1H), 7.73 (d, *J* = 8.0 Hz, 1H), 7.44 – 7.33 (m, 5H), 7.15 – 7.04 (m, 4H), 5.10 (d, *J* = 15.1 Hz, 1H), 4.31 (d, *J* = 15.1 Hz, 1H), 3.66 (d, *J* = 12.6 Hz, 1H), 3.57 (d, *J* = 12.2 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.87, 160.02, 148.69, 146.99, 140.50, 138.10, 137.90, 136.70, 133.53, 130.41, 127.12, 123.66, 120.90, 119.58, 119.47, 112.17, 100.75, 55.83, 46.90, 43.60. HRMS m/z: calcd for C22H22N3O4S3 $[M+H]^+$: 488.0772; found:

488.0759.

4.6.8.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-4-methoxybenzenesulfonamide (**5g**)*

Yield 53%. Mp: 125-126°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.37 (s, 1H), 8.33 (d, *J* = 2.8 Hz, 2H), 7.77 – 7.72 (m, 3H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.26 (d, *J* = 8.8 Hz, 2H), 7.14 (dd, *J* = 7.6, 4.8 Hz, 1H), 7.10 – 7.04 (m, 4H), 4.72 (d, *J* = 15.2 Hz, 1H), 4.48 (d, *J* = 15.6 Hz, 1H), 3.80 (s, 3H), 3.64 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.84, 162.51, 148.94, 147.77, 138.50, 135.41, 135.28, 132.24, 131.23, 128.90, 126.77, 122.82, 118.75, 114.49, 99.88, 55.67, 46.37, 42.41. HRMS m/z: calcd for $C_{22}H_{22}N_3O_4S_3$ $[M+H]^+$: 488.0772; found: 488.0772.

4.6.9.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-2-nitrobenzenesulfonamide (**5h**)*

Yield 29%. Mp: 102-104°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.94 (s, 1H), 8.32 – 8.30 (m, 2H), 8.01 (dd, *J* = 8.6, 1.4 Hz, 2H), 7.90 – 7.85 (m, 2H), 7.80 (s, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.30 (d, *J* = 8.8 Hz, 2H), 7.14 (dd, *J* = 8.0, 4.8 Hz, 1H), 7.08 (d, *J* = 8.8 Hz, 2H), 4.71 (d, *J* = 15.2 Hz, 1H), 4.51 (d, *J* = 15.6 Hz, 1H), 3.65 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.89, 148.91, 147.87, 147.77, 137.30, 136.32, 135.29, 134.77, 132.74, 132.24, 131.50, 129.86, 126.93, 124.81, 122.82, 119.44, 99.78, 46.33, 42.34. HRMS m/z: calcd for $C_{21}H_{19}N_4O_5S_3$ $[M+H]^+$: 503.0518; found: 503.0503.

4.6.10.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-4-nitrobenzenesulfonamide (**5i**)*

Yield 43%. Mp: 154-155°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.81 (s, 1H), 8.40 (d, *J* = 8.4 Hz, 2H), 8.31 (d, *J* = 4.0 Hz, 1H), 8.24 (s, 1H), 8.02 (d, *J* = 8.7 Hz, 2H), 7.82 (s, 1H), 7.54 (d, *J* = 7.6 Hz, 1H), 7.30 (d, *J* = 8.4 Hz, 2H), 7.16 (dd, *J* = 8.0, 4.8 Hz, 1H), 7.07 (d, *J* = 8.4 Hz, 2H), 4.68 (d, *J* = 15.6 Hz, 1H), 4.52 (d, *J* = 15.6 Hz, 1H), 3.65 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.00, 149.93, 148.85, 147.76, 144.91, 137.48, 136.41, 135.35, 132.23, 128.25, 127.03, 124.77, 122.83, 119.68, 99.72, 46.32, 42.39. HRMS m/z: calcd for $C_{21}H_{19}N_4O_5S_3$ $[M+H]^+$: 503.0518; found: 503.0505.

4.6.11.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-4-cyano-benzenesulfonamide (**5j**)*

Yield 42%. Mp: 105-107°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.73 (s, 1H), 8.32 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.27 (s, 1H), 8.07 (d, *J* = 8.4 Hz, 2H), 7.93 (d, *J* = 8.4 Hz, 2H), 7.79 (s, 1H), 7.52 (d, *J* = 7.6 Hz, 1H), 7.28 (d, *J* = 8.8 Hz, 2H), 7.15 (dd, *J* = 8.0, 4.8 Hz, 1H), 7.04 (d, *J* = 8.8 Hz, 2H), 4.68 (d, *J* = 15.6 Hz, 1H), 4.51 (d, *J* = 15.6 Hz, 1H), 3.65 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.96, 148.83, 147.72, 143.54, 137.52, 136.30, 135.31, 133.59, 132.22, 127.37, 126.97, 122.81, 119.55, 117.58, 115.49, 99.70, 46.29, 42.35. HRMS m/z: calcd for $C_{22}H_{19}N_4O_3S_3$ $[M+H]^+$: 483.0619; found: 483.0600.

4.6.12.

*N-(4-(4-hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-2-fluorobenzenesulfonamide (**5k**)*

Yield 36%. Mp: 92-93°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H), 8.32 (d, *J* = 8.0 Hz, 2H), 7.89 (t, *J* = 7.6 Hz, 1H), 7.77 (s, 1H), 7.74 – 7.69 (m, 1H), 7.49 – 7.46 (m, 1H), 7.43 – 7.37 (m, 2H), 7.27 (d, *J* = 8.4 Hz, 2H), 7.14 – 7.07 (m, 3H), 4.72 (d, *J* = 15.6 Hz, 1H), 4.49 (d, *J* = 15.6 Hz, 1H), 3.64 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.81, 158.15 (d, *J* = 254.9 Hz), 148.92, 147.71, 137.77, 135.98 (d, *J* = 8.6 Hz), 135.70, 135.24, 132.20, 130.36, 127.18 (d, *J* = 13.7 Hz), 126.77, 125.02 (d, *J* = 3.4 Hz), 122.73, 118.57, 117.35 (d, *J* = 20.7 Hz), 99.80, 46.32, 42.34. HRMS m/z: calcd for $C_{21}H_{19}FN_3O_3S_3$ $[M+H]^+$: 476.0573; found: 476.0560.

4.6.13.

*N-(4-(4-hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-3-fluorobenzenesulfonamide (**5l**)*

Yield 41%. Mp: 119-120°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.60 (s, 1H), 8.33 (s, 2H), 7.81 (s, 1H), 7.65 – 7.60 (m, 3H), 7.52 (d, *J* = 7.2 Hz, 2H), 7.31 (d, *J* = 8.4 Hz, 2H), 7.16 – 7.08 (m, 3H), 4.73 (d, *J* = 15.6 Hz, 1H), 4.53 (d, *J* = 15.2 Hz, 1H), 3.66 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.88, 161.67 (d, *J* = 249.0 Hz), 148.93, 147.72, 141.64 (d, *J* = 6.6 Hz), 137.84, 136.13, 135.30, 132.23, 131.76 (d, *J* = 8.0 Hz), 126.86, 122.91 (d, *J* = 2.5 Hz), 122.75, 120.22 (d, *J* = 21.3 Hz), 119.44, 113.69 (d, *J* = 24.2 Hz), 99.78, 46.33, 42.38. HRMS m/z: calcd for $C_{21}H_{19}FN_3O_3S_3$ $[M+H]^+$: 476.0573; found: 476.0570.

4.6.14.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-4-fluorobenzenesulfonamide (**5m**)*

Yield 27%. Mp: 93-94°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.50 (s, 1H), 8.33 – 8.30 (m, 2H), 7.85 (dd, *J* = 8.6, 5.0 Hz, 2H), 7.78 (s, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.41 (t, *J* = 8.8 Hz, 2H), 7.28 (d, *J* = 8.8 Hz, 2H), 7.15 (dd, *J* = 8.0, 4.8 Hz, 1H), 7.05 (d, *J* = 8.4 Hz, 2H), 4.71 (d, *J* = 15.6 Hz, 1H), 4.51 (d, *J* = 15.6 Hz, 1H), 3.65 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.88, 164.34 (d, *J* = 251.7 Hz), 148.88, 147.70, 138.01, 135.90, 135.25, 132.18, 129.72, 129.62, 126.79, 122.74, 119.23, 116.51 (d, *J* = 22.8 Hz), 99.75, 46.30, 42.35. HRMS m/z: calcd for $C_{21}H_{19}FN_3O_3S_3$ $[M+H]^+$: 476.0573; found: 476.0566.

4.6.15.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-2-morpholinobenzenesulfonamide (**5n**)*

Yield 26%. Mp: 116-118°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.83 (s, 1H), 8.32 – 8.27 (m, 2H), 7.93 (d, *J* = 6.8 Hz, 1H), 7.70 (s, 1H), 7.63 (t, *J* = 7.6 Hz, 1H), 7.48 (t, *J* = 8.0 Hz, 2H), 7.33 (t, *J* = 7.6 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 2H), 7.15 – 7.12 (m, 1H), 6.97 (d, *J* = 8.4 Hz, 2H), 4.64 (d, *J* = 15.6 Hz, 1H), 4.45 (d, *J* = 15.6 Hz, 1H), 3.82 (t, *J* = 4.4 Hz, 4H), 3.61 (d, *J* = 2.0 Hz, 2H), 2.80 (t, *J* = 4.2 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.16, 151.84, 148.72, 148.37, 147.64, 138.39, 135.23, 134.88, 134.60, 132.14, 130.82, 126.61, 125.26, 124.41, 122.79, 117.89, 99.76, 65.80, 53.63, 46.26, 42.38. HRMS m/z: calcd for $C_{25}H_{27}N_4O_4S_3$ $[M+H]^+$: 543.1194; found: 543.1191.

4.6.16.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-[1,1'-biphenyl]-2-sulfonamide (**5o**)*

Yield 42%. Mp: 110-112°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 8.24 – 8.22 (m, 2H), 7.93 (dd, *J* = 5.0, 1.4 Hz, 1H), 7.69 (d, *J* = 8.0 Hz, 1H), 7.59 (td, *J* = 7.6, 1.2 Hz, 1H), 7.52 (td, *J* = 7.6, 1.2 Hz, 1H), 7.46 – 7.43 (m, 3H), 7.37 – 7.34 (m, 2H), 7.30 – 7.28 (m, 3H), 7.00 (dd, *J* = 7.8, 5.0 Hz, 1H), 6.78 (d, *J* = 8.8 Hz, 2H), 5.13 (d, *J* = 15.2 Hz, 1H), 4.19 (d, *J* = 15.2 Hz, 1H), 3.66 (d, *J* = 12.0 Hz, 1H), 3.51 (d, *J* = 12.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.59, 148.61, 146.79, 140.92, 138.74, 137.99, 137.70, 137.33, 135.84, 133.40, 132.99, 132.74, 130.05, 129.57, 128.72, 128.18, 128.04, 126.79, 123.48, 118.64, 100.82, 46.84, 43.49. HRMS m/z: calcd for $C_{27}H_{24}N_3O_3S_3$ $[M+H]^+$: 534.0980; found: 534.0979.

4.6.17.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-3'-nitro-[1,1'-biphenyl]-2-sulfonamide (**5p**)*

Yield 62%. Mp: 121-123°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.20 (s, 1H), 8.32 – 8.27 (m, 3H), 8.13 – 8.10 (m, 1H), 8.04 (d, *J* = 2.0 Hz, 1H), 7.76 (s, 1H), 7.75 – 7.69 (m, 3H), 7.61 (d, *J* = 7.6 Hz, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.41 – 7.39 (m, 1H), 7.21 (d, *J* = 8.8 Hz, 2H), 7.12 (dd, *J* = 8.0, 4.8 Hz, 1H), 6.80 (d, *J* = 8.4 Hz, 2H), 4.68 (d, *J* = 15.6 Hz, 1H), 4.55 (d, *J* = 15.6 Hz, 1H), 3.65 (d, *J* = 4.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.99, 148.83, 147.71, 147.13, 140.04, 138.37, 137.80, 137.76, 135.76, 135.23, 134.97, 133.13, 132.76, 132.30, 129.38, 129.26, 129.09, 126.75, 123.68, 122.85, 122.78, 117.54, 99.73, 46.27, 42.36. HRMS m/z: calcd for $C_{27}H_{23}N_4O_5S_3$ $[M+H]^+$: 579.0831; found: 579.0839.

*4.6.18. N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl) naphthalene-2-sulfonamide (**5q**)*

Yield 34%. Mp: 152-153°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.58 (s, 1H), 8.49 (s, 1H), 8.28 (s, 1H), 8.21 (d, *J* = 4.0 Hz, 1H), 8.16 (d, *J* = 8.0 Hz, 1H), 8.11 (d, *J* = 8.8 Hz, 1H), 8.02 (d, *J* = 8.0 Hz, 1H), 7.79 (d, *J* = 8.8 Hz, 1H), 7.72 – 7.64 (m, 3H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.22 (d, *J* = 8.8 Hz, 2H), 7.09 (d, *J* = 8.8 Hz, 2H), 7.01 (dd, *J* = 8.0, 4.8 Hz, 1H), 4.66 (d, *J* = 15.6 Hz, 1H), 4.42 (d, *J* = 15.6 Hz, 1H), 3.60 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.74, 148.88, 147.61, 138.17, 136.57, 135.65, 135.17, 134.26, 132.14, 131.54, 129.53, 129.24, 129.00, 127.94, 127.80, 127.69, 126.70, 122.64, 121.91, 118.97, 99.76, 46.25, 42.27. HRMS m/z: calcd for $C_{25}H_{22}N_3O_3S_3$ $[M+H]^+$: 508.0823; found: 508.0806.

*4.6.19. N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl) naphthalene-1-sulfonamide (**5r**)*

Yield 46%. Mp: 112-113°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.94 (s, 1H), 8.72 (d, *J* = 8.4 Hz, 1H), 8.33 – 8.23 (m, 4H), 8.09 (d, *J* = 8.4 Hz, 1H), 7.78 – 7.74 (m, 2H), 7.70 – 7.65 (m, 2H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.19 (d, *J* = 8.8 Hz, 2H), 7.03 – 6.98 (m, 3H), 4.69 (d, *J* = 15.6Hz, 1H), 4.44 (d, *J* = 15.6 Hz, 1H), 3.59 (d, *J* = 7.6 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.68, 148.81, 147.56, 138.20, 135.37, 135.12, 134.57, 134.39, 133.83, 132.31, 130.01, 129.16, 128.26, 127.43, 127.08, 126.72, 124.61, 124.24, 122.77, 117.80, 99.85, 46.31, 42.30. HRMS m/z: calcd for

$C_{25}H_{22}N_3O_3S_3$ [M+H]$^+$: 508.0823; found: 508.0808.

*4.6.20. N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl) thiophene-2-sulfonamide (**5s**)*

Yield 24%. Mp: 149-150°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.64 (s, 1H), 8.35 (s, 2H), 7.92 (d, *J* = 4.8 Hz, 1H), 7.81 (d, *J* = 4.4 Hz, 1H), 7.59 (s, 1H), 7.52 (d, *J* = 7.2 Hz, 1H), 7.33 – 7.31 (m, 2H), 7.18 – 7.13 (m, 4H), 4.74 (d, *J* = 15.6 Hz, 1H), 4.53 (d, *J* = 15.2 Hz, 1H), 3.67 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.93, 148.93, 147.80, 140.06, 138.01, 136.02, 135.29, 133.52, 132.61, 132.24, 127.71, 126.85, 122.86, 119.30, 99.84, 46.39, 42.44. HRMS m/z: calcd for $C_{19}H_{18}N_3O_3S_4$ [M+H]$^+$: 464.0231; found: 464.0224.

*4.6.21. N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl) quinolone-8-sulfonamide (**5t**)*

Yield 24%. Mp: 135-136°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1H), 9.13 (dd, *J* = 4.4, 1.6 Hz, 1H), 8.54 – 8.49 (m, 1H), 8.41 (d, *J* = 7.6 Hz, 1H), 8.29 (s, 2H), 8.23 – 8.22 (m, 1H), 4.66 (d, *J* = 15.6 Hz, 1H), 4.39 (d, *J* = 15.6 Hz, 1H), 3.56 (d, *J* = 3.2 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.68, 151.50, 148.83, 147.60, 142.76, 138.43, 137.00, 135.36, 135.18, 134.34, 132.17, 132.09, 129.66, 128.48, 126.44, 125.72, 123.49, 122.67, 118.65, 99.84, 46.28, 42.29. HRMS m/z: calcd for $C_{24}H_{21}N_4O_3S_3$ [M+H]$^+$: 509.0776; found: 509.0781.

4.6.22.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-2-oxo-2H-chromene-6-sulfonamide (**5u**)*

Yield 38%. Mp: 136-137°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.62 (s, 1H), 8.29 – 8.15 (m, 3H), 8.16 (d, *J* = 9.6 Hz, 1H), 7.94 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.80 (s, 1H), 7.54 (t, *J* = 8.0 Hz, 2H), 7.27 (d, *J* = 8.8 Hz, 2H), 7.14 – 7.07 (m, 3H), 6.58 (d, *J* = 10.0 Hz, 1H), 4.69 (d, *J* = 15.6 Hz, 1H), 4.49 (d, *J* = 15.6 Hz, 1H), 3.63 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.94, 159.15, 155.95, 148.85, 147.68, 143.42, 137.98, 135.99, 135.48, 132.32, 129.61, 127.75, 126.93, 122.86, 119.36, 119.02, 117.84, 99.83, 46.37, 42.44. HRMS m/z: calcd for $C_{24}H_{20}N_3O_5S_3$ [M+H]$^+$: 526.0565; found: 526.0549.

4.6.23.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-1H-pyrrolo [2,3-b]pyridine-3-sulfonamide (**5v**)*

Yield 31%. Mp: 151-153°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.67 (s, 1H), 10.45 (s, 1H), 8.35 (d, *J* = 4.8 Hz, 2H), 8.25 – 8.17 (m, 3H), 7.71 (d, *J* = 5.6 Hz, 1H), 7.45 – 7.43 (m, 1H), 7.28 – 7.18 (m, 3H), 7.09 – 7.00 (m, 3H), 4.71 (d, *J* = 15.6 Hz, 1H), 4.43 (d, *J* = 15.6 Hz, 1H), 3.61 (d, *J* = 7.6 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.65, 148.98, 148.04, 147.67, 144.62, 138.69, 135.25, 134.95, 132.25, 131.84, 127.56, 126.54, 122.70, 118.07, 117.63, 115.57, 111.97, 99.95, 46.33, 42.37. HRMS m/z: calcd for $C_{22}H_{20}N_5O_3S_3$ [M+H]$^+$: 498.0728; found: 498.0723.

4.6.24.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamide (**5w**)*

Yield 18%. Mp: 102-103°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.37 (s, 1H), 8.33 (d,

J = 2.8 Hz, 2H), 7.77 (s, 1H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.27 – 7.24 (m, 4H), 7.15 (dd, *J* = 7.6, 4.8 Hz, 1H), 7.06 – 7.00 (m, 3H), 4.72 (d, *J* = 15.6 Hz, 1H), 4.47 (d, *J* = 15.6 Hz, 1H), 4.28 (d, *J* = 4.4 Hz, 4H), 3.64 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.80, 148.90, 147.73, 147.27, 143.30, 138.40, 135.46, 135.30, 132.24, 132.00, 126.78, 122.81, 120.30, 118.73, 117.65, 115.60, 99.87, 64.37, 64.04, 46.34, 42.36. HRMS m/z: calcd for $C_{23}H_{22}N_3O_5S_3$ $[M+H]^+$: 516.0722; found: 516.0707.

4.6.25.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazine-6-sulfonamide (**5x**)*

Yield 37%. Mp: 143-144°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.00 (s, 1H), 10.47 (s, 1H), 8.33 – 8.31 (m, 2H), 7.79 (s, 1H), 7.53 (d, *J* = 7.6 Hz, 1H), 7.34 – 7.33 (m, 2H), 7.27 (d, *J* = 8.8 Hz, 2H), 7.17 – 7.15 (m, 1H), 7.06 (dd, *J* = 8.8, 6.4 Hz, 3H), 4.73 (d, *J* = 15.6 Hz, 1H), 4.67 (s, 2H), 4.49 (d, *J* = 15.6 Hz, 1H), 3.65 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.90, 164.21, 148.92, 147.77, 146.61, 138.34, 135.63, 135.43, 133.18, 132.33, 127.66, 126.85, 122.90, 122.17, 118.95, 116.69, 114.21, 99.91, 66.70, 46.41, 42.45. HRMS m/z: calcd for $C_{23}H_{21}N_4O_5S_3$ $[M+H]^+$: 529.0674; found: 529.0681.

4.6.26.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-2-oxo-1,2,3,4-tetrahydroquinoline-6-sulfonamide (**5y**)*

Yield 46%. Mp: 185-187°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.50 (s, 1H), 10.41 (s, 1H), 8.35 (s, 2H), 7.82 (s, 1H), 7.66 – 7.62 (m, 2H), 7.55 (d, *J* = 7.6 Hz, 1H), 7.30 (d, *J* = 8.4 Hz, 2H), 7.18 – 7.10 (m, 3H), 6.99 (d, *J* = 8.4 Hz, 1H), 4.77 (d, *J* = 15.6 Hz, 1H), 4.52 (d, *J* = 15.6 Hz, 1H), 3.67 (s, 2H), 2.96 (t, *J* = 7.4 Hz, 2H), 2.50 (t, *J* = 7.6 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.86, 170.41, 149.00, 147.80, 142.46, 138.55, 135.41, 132.49, 132.33, 126.83, 126.52, 124.32, 122.87, 118.79, 115.17, 99.96, 46.44, 42.49, 29.82, 24.51. HRMS m/z: calcd for $C_{24}H_{23}N_4O_4S_3$ $[M+H]^+$: 527.0881; found: 527.0870.

4.6.27.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-1-phenylmethanesulfonamide (**5z**)*

Yield 34%. Mp: 78-79°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.98 (s, 1H), 8.35 – 8.33 (m, 1H), 8.27 (s, 1H), 7.83 (s, 1H), 7.65 (d, *J* = 8.0 Hz, 1H), 7.37 – 7.35 (m, 4H), 7.33 (s, 1H), 7.27 – 7.23 (m, 3H), 7.11 (d, *J* = 8.4 Hz, 2H), 4.69 (q, *J* = 15.6 Hz, 2H), 4.45 (s, 2H), 3.76 (d, *J* = 12.0 Hz, 1H), 3.67 (d, *J* = 12.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.06, 148.78, 147.66, 138.94, 135.60, 135.04, 132.48, 130.99, 129.46, 128.42, 128.26, 126.96, 123.00, 118.27, 99.75, 56.93, 46.27, 42.44. HRMS m/z: calcd for $C_{22}H_{22}N_3O_3S_3$ $[M+H]^+$: 472.0823; found: 472.0823.

*4.6.28. N-(4-(4-hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl) methanesulfonamide (**5aa**)*

Yield 27%. Mp: 171-173°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.89 (s, 1H), 8.34 (dd, *J* = 4.8, 1.2 Hz, 1H), 8.26 (d, *J* = 0.8 Hz, 1H), 7.85 – 7.84 (m, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.34 (d, *J* = 8.8 Hz, 2H), 7.22 (dd, *J* = 7.4, 5.0 Hz, 1H), 7.14 – 7.12 (m, 2H), 4.69 (q, *J* = 15.4 Hz, 2H), 3.71 (q, *J* = 12.4 Hz, 2H), 3.00 (s, 3H). ^{13}C NMR (100 MHz,

DMSO-d_6) δ 195.95, 148.94, 147.77, 138.90, 135.51, 135.42, 132.41, 126.98, 122.93, 118.85, 99.76, 46.28, 42.42, 39.38. HRMS m/z: calcd for $C_{16}H_{18}N_3O_3S_3$ $[M+H]^+$: 396.0510; found: 396.0496.

4.6.29.

*N-(4-(4-Hydroxy-3-(pyridin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-N-methylquinoline-8-sulfonamide (**5ab**)*

Yield 39%. Mp: 106-108°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.04 (dd, *J* = 4.2, 1.8 Hz, 1H), 8.54 (dd, *J* = 8.4, 1.6 Hz, 1H), 8.32 – 8.28 (m, 4H), 7.80 (s, 1H), 7.73 – 7.69 (m, 2H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.22 (d, *J* = 8.8 Hz, 2H), 7.15 (dd, *J* = 7.8, 4.6 Hz, 1H), 7.10 (d, *J* = 8.8 Hz, 2H), 4.68 (d, *J* = 15.6 Hz, 1H), 4.46 (d, *J* = 15.6 Hz, 1H), 3.63 (d, *J* = 1.6 Hz, 2H), 3.54 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.80, 151.50, 148.84, 147.69, 143.14, 141.87, 137.55, 136.92, 136.36, 135.36, 134.37, 132.88, 132.31, 128.60, 126.25, 125.71, 124.18, 122.87, 122.62, 99.70, 46.25, 42.22, 38.94. HRMS m/z: calcd for $C_{25}H_{23}N_4O_3S_3$ $[M+H]^+$: 523.0932; found: 523.0921.

4.7. The procedure for the synthesis of N-(4-(3-(Pyridin-3-ylmethyl)-2-thioxo-2,3-dihydrothiazol-4-yl)phenyl)quinoline-8-sulfonamide ***(5ac)***

A mixture of **5t** (0.20 g, 0.39 mmol) and 5% hydrochloric acid (20ml) in methanol (4 mL) was heated under reflux for 24 h. The reaction mixture was cooled to room temperature and was extracted with ethyl acetate (15 mL×3), the combined organic phase was washed with water (30 mL×2), dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by recrystallization (petroleum ether and ethyl acetate) to provide **5ac** as a yellow solid (0.15, 77%). Mp: 253-254 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.50 (s, 1H), 9.11 (d, *J* = 3.2 Hz, 1H), 8.52 (d, *J* = 8.0 Hz, 1H), 8.43 (d, *J* = 6.8 Hz, 1H), 8.40 – 8.28 (m, 2H), 7.92 (s, 1H), 7.77 – 7.69 (m, 2H), 7.13 – 7.04 (m, 6H), 6.94 (s, 1H), 5.29 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.74, 151.47, 148.45, 147.85, 143.28, 142.66, 139.35, 136.98, 134.99, 134.46, 134.17, 132.28, 130.95, 130.08, 128.43, 125.62, 124.84, 123.22, 122.64, 118.61, 110.04, 47.58. HRMS m/z: calcd for $C_{24}H_{19}N_4O_2S_3$ $[M+H]^+$: 491.0670; found: 491.0660.

*4.8. General procedure for the synthesis of compounds **5ad-5ak***

To a solution of compound **3w** (0.70 g, 2.1 mmol) in THF (10ml) was added phenyltrimethylammonium tribromide (0.79 g, 2.1 mmol). The reaction mixture was stirred at room temperature for 12 h. Water (50 mL) was added and the mixture was extracted with ethyl acetate (10 mL×3), the combined organic phase was washed with water (30 mL×2), dried over anhydrous Na_2SO_4 and concentrated to afford the intermediate **4w**. To a solution of different pyridin-3-ylmethanamine derivatives (2.1 mmol) in acetone (10ml) was added Et_3N (0.43 g, 4.2 mmol). The reaction mixture was stirred for 5 min and was added CS_2 (0.24 g, 3.15 mmol) to continuously stir for 30 min. The intermediate **4w** were added respectively and this mixture was stirred at room temperature for 4 h. Water (50 mL) was added and the mixture was extracted with ethyl acetate (10 mL×3), the combined organic phase was washed with brine (30 mL×2), dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography to provide the product.

4.8.1.

*N-(4-(4-Hydroxy-3-(2-(pyridin-3-yl)ethyl)-2-thioxothiazolidin-4-yl)phenyl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamide (**5ad**)*

Yield 52%. Mp: 101-102°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.42 (s, 1H), 8.40 (dd, *J* = 4.6, 1.4 Hz, 1H), 8.19 (d, *J* = 1.6 Hz, 1H), 7.78 (s, 1H), 7.42 – 7.38 (m, 3H), 7.28 – 7.23 (m, 3H), 7.19 (d, *J* = 8.8 Hz, 2H), 6.90 (d, *J* = 8.0 Hz, 1H), 4.23 – 4.17 (m, 4H), 3.69 – 3.56 (m, 3H), 3.29 (td, *J* = 12.8, 5.2 Hz, 1H), 2.79 (td, *J* = 12.4, 4.8 Hz, 1H), 2.39 (td, *J* = 12.4, 4.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.16, 149.33, 147.71, 147.23, 143.24, 138.51, 136.18, 135.86, 133.90, 131.79, 126.67, 123.61, 120.28, 119.19, 117.55, 115.61, 99.68, 64.28, 63.95, 46.79, 42.44, 30.47. HRMS m/z: calcd for $C_{24}H_{24}N_3O_5S_3$ $[M+H]^+$: 530.0878; found: 530.0874.

4.8.2.

*N-(4-(4-Hydroxy-3-(pyridin-4-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamide (**5ae**)*

Yield 28%. Mp: 198-199°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.39 (s, 1H), 8.33 (d, *J* = 6.0 Hz, 2H), 7.76 (s, 1H), 7.29 – 7.24 (m, 4H), 7.09 – 7.00 (m, 5H), 4.69 (d, *J* = 16.4 Hz, 1H), 4.45 (d, *J* = 16.4 Hz, 1H), 4.29 (d, *J* = 4.0 Hz, 4H), 3.68 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.06, 148.98, 147.32, 145.46, 143.34, 138.47, 135.37, 131.96, 126.87, 122.29, 120.32, 118.68, 117.68, 115.61, 99.81, 64.39, 64.07, 47.72, 42.40. HRMS m/z: calcd for $C_{23}H_{22}N_3O_5S_3$ $[M+H]^+$: 516.0722; found: 516.0705.

4.8.3.

*N-(4-(4-Hydroxy-3-(pyrazin-2-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamide (**5af**)*

Yield 24%. Mp: 89-91°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.37 (s, 1H), 8.44 – 8.38 (m, 3H), 7.78 (s, 1H), 7.34 (d, *J* = 8.8 Hz, 2H), 7.23 (dd, *J* = 4.2, 2.2 Hz, 2H), 7.05 – 6.99 (m, 3H), 4.75 (d, *J* = 16.4 Hz, 1H), 4.59 (d, *J* = 16.4 Hz, 1H), 4.29 (d, *J* = 4.8 Hz, 4H), 3.72 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.51, 151.48, 147.29, 143.31, 142.76, 138.40, 135.39, 131.95, 126.94, 120.29, 118.74, 117.66, 115.61, 99.62, 64.39, 64.06, 48.58, 42.62. HRMS m/z: calcd for $C_{22}H_{21}N_4O_5S_3$ $[M+H]^+$: 517.0674; found: 517.0656.

*4.8.4. N-(4-(4-Hydroxy-3-((2-methoxypyridin-3-yl)methyl)-2-thioxothiazolidin-4-yl)phenyl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamide (**5ag**)*

Yield 25%. Mp: 158-159°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.39 (s, 1H), 7.95 (d, *J* = 5.2 Hz, 1H), 7.69 (s, 1H), 7.40 (d, *J* = 7.2 Hz, 1H), 7.29 – 7.22 (m, 4H), 7.05 (d, *J* = 8.4 Hz, 2H), 6.99 (d, *J* = 8.4 Hz, 1H), 6.88 – 6.85 (m, 1H), 4.63 (d, *J* = 16.8 Hz, 1H), 4.31 – 4.28 (m, 4H), 3.73 (s, 3H), 3.69 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.95, 159.81, 147.30, 144.53, 143.34, 138.37, 135.92, 135.39, 131.99, 126.77, 120.29, 118.58, 118.20, 117.63, 116.43, 115.61, 99.96, 64.38, 64.06, 53.03, 43.46, 42.44. HRMS m/z: calcd for $C_{24}H_{24}N_3O_6S_3$ $[M+H]^+$: 546.0827; found: 546.0814.

4.8.5.

*N-(4-(4-Hydroxy-3-((4-methylpyridin-3-yl)methyl)-2-thioxothiazolidin-4-yl)phenyl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamide (**5ah**)*

Yield 23%. Mp: 162-164°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.38 (s, 1H), 8.24 –

8.18 (m, 2H), 7.79 – 7.76 (m, 1H), 7.28 – 7.25 (m, 4H), 7.04 – 7.01 (m, 3H), 6.93 (d, *J* = 4.4 Hz, 1H), 4.71 (d, *J* = 16.0 Hz, 1H), 4.43 (d, *J* = 16.0 Hz, 1H), 4.29 (d, *J* = 3.6 Hz, 4H), 3.69 (s, 2H), 2.00 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.99, 147.63, 147.29, 143.84, 143.32, 138.35, 135.31, 132.01, 130.17, 130.05, 126.78, 124.45, 120.35, 118.61, 117.68, 115.64, 99.90, 64.40, 64.08, 44.16, 42.32, 18.05. HRMS m/z: calcd for $C_{24}H_{24}N_3O_5S_3$ $[M+H]^+$: 530.0878; found: 530.0875.

4.8.6.

*N-(4-(4-Hydroxy-3-((6-(3-(methylsulfonyl)phenyl)pyridin-3-yl)methyl)-2-thioxo-thiazolidin-4-yl)phenyl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamide (**5ai**)*

Yield 27%. Mp: 123-125°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.39 (s, 1H), 8.58 (s, 1H), 8.48 (s, 1H), 8.37 (d, *J* = 8.0 Hz, 1H), 7.98 (d, *J* = 7.2 Hz, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 7.82 (s, 1H), 7.77 (t, *J* = 7.8 Hz, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.31 (d, *J* = 8.8 Hz, 2H), 7.25 (d, *J* = 1.6 Hz, 2H), 7.09 (d, *J* = 8.8 Hz, 2H), 6.99 (d, *J* = 9.2 Hz, 1H), 4.83 (d, *J* = 15.6 Hz, 1H), 4.47 (d, *J* = 15.2 Hz, 1H), 4.27 (d, *J* = 3.2 Hz, 4H), 3.67 (s, 2H), 3.29 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.19, 152.53, 149.17, 147.28, 143.30, 141.60, 139.52, 138.51, 136.73, 135.52, 132.10, 131.98, 131.18, 130.02, 127.20, 126.84, 124.71, 120.31, 119.78, 118.87, 117.65, 115.62, 100.00, 64.36, 64.04, 46.26, 43.49, 42.45. HRMS m/z: calcd for $C_{30}H_{28}N_3O_7S_4$ $[M+H]^+$: 670.0810; found: 670.0794.

4.8.7.

*N-(4-(4-Hydroxy-3-(quinolin-3-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamide (**5aj**)*

Yield 22%. Mp: 117-119°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.37 (s, 1H), 8.74 (s, 1H), 8.00 (s, 1H), 7.96 (d, *J* = 8.4 Hz, 1H), 7.83 (d, *J* = 9.2 Hz, 2H), 7.71 (t, *J* = 7.4 Hz, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.34 (d, *J* = 8.8 Hz, 2H), 7.24 (d, *J* = 8.0 Hz, 2H), 7.09 (d, *J* = 8.8 Hz, 2H), 7.01 – 6.97 (m, 1H), 4.99 (d, *J* = 15.6 Hz, 1H), 4.55 (d, *J* = 15.6 Hz, 1H), 4.27 (d, *J* = 4.4 Hz, 4H), 3.69 (d, *J* = 3.2 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.20, 150.77, 147.28, 146.51, 143.31, 138.52, 135.62, 134.24, 131.99, 129.75, 129.19, 128.58, 127.84, 127.11, 126.82, 126.67, 120.29, 118.87, 117.65, 115.61, 100.09, 64.36, 64.04, 46.69, 42.46. HRMS m/z: calcd for $C_{27}H_{24}N_3O_5S_3$ $[M+H]^+$: 566.0878; found: 566.0865.

4.8.8.

*N-(4-(4-Hydroxy-3-(imidazo[1,2-a]pyridin-6-ylmethyl)-2-thioxothiazolidin-4-yl)phenyl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamide (**5ak**)*

Yield 22%. Mp: 121-123°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.39 (s, 1H), 8.21 (s, 1H), 7.83 – 7.80 (m, 2H), 7.52 (s, 1H), 7.36 (d, *J* = 9.2 Hz, 1H), 7.30 (d, *J* = 8.4 Hz, 2H), 7.26 – 7.25 (m, 2H), 7.09 – 6.99 (m, 4H), 4.78 (d, *J* = 15.2 Hz, 1H), 4.39 (d, *J* = 15.2 Hz, 1H), 4.28 (d, *J* = 4.0 Hz, 4H), 3.67-3.59 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.85, 147.30, 143.70, 143.33, 138.49, 135.52, 133.21, 132.02, 126.82, 125.69, 125.51, 120.95, 120.32, 118.73, 117.68, 115.87, 115.62, 113.11, 100.05, 64.38, 64.06, 46.05, 42.39. HRMS m/z: calcd for $C_{25}H_{23}N_4O_5S_3$ $[M+H]^+$: 555.0831; found: 555.0813.

4.9. PKM2 activity assay

Pyruvate kinase activity was detected with a fluorescent pyruvate kinase-lactate dehydrogenase coupled assay as previously described [17].

4.10. Anti-proliferation activity assay

Cell lines (HCT116, Hela, H1299, PC3 and HELF) were cultured in RPMI 1640 containing 9% fetal bovine serum (FBS) at 37°C in 5% CO_2. Cell viability was detected with the MTS assay (Promega) according to the manufacturer's instructions. Briefly, 3000-10000 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.

4.11. Cell cycle analysis

Cell cycle was detected by flow cytometry according to a previously published method. Briefly, cells were first treated with DMSO or different concentrations of compound **5w** for 12 h and then harvested, washed twice with PBS, and resuspended in 1 mL of PBS. The cells were fixed in 2.5 mL of ice-cold ethanol at -4°C overnight. Then cells were centrifuged to remove the fixing solution and stained with 500 μL of propidium iodide (50 μg/mL, Sigma) containing 0.1% RNase (1 mg/mL, Sigma) for 15 min in dark conditions at room temperature. The cells were then analyzed by flow cytometry (FACSVerse™, BD).

4.12. Molecular modeling and docking

The 3D structure of compound **5t** was generated using OpenBabel [27] and optimized with MMFF94 forcefield. PKM2 structure was extracted from PDB 3ME3 [7]. AutodockVina [28] was used for molecular docking. The original activator binding site in 3ME3 was used as the binding site of **5t**. The binding mode of **5t** was depicted using PyMol.

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References

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

[2] N. Wong, J. De Melo, D. Tang, PKM2, a Central Point of Regulation in Cancer Metabolism, Int J. Cell. Biol. 2013 (2013) 242513.

[3] V. Gupta, R.N. Bamezai, Human pyruvate kinase M2: a multifunctional protein, Protein Sci. 19 (2010) 2031-2044.

[4] W. Yang, Z. Lu, Regulation and function of pyruvate kinase M2 in cancer, Cancer Lett. 339 (2013) 153-158.

[5] Y. C. Yang, T. Y. Cheng, S. M. Huang, C.Y. Su, P.W. Yang, J. M. Lee, C. K. Chen, M. Hsiao, K.T. Hua, M. L. Kuo, Cytosolic PKM2 stabilizes mutant EGFR protein expression through regulating HSP90–EGFR association, Oncogene. 35 (2016) 3387–3398.

[6] W. Yang, Z. Lu, Pyruvate kinase M2 at a glance, J. Cell. Sci. 128 (2015) 1655-1660.

[7] 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, Nat. Chem. Biol. 8 (2012) 839-847.

[8] 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, J. Med. Chem. 53 (2010) 1048-1055.

[9] J.K. Jiang, M.B. Boxer, 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 thieno[3,2-b]pyrrole[3,2-d]pyridazinones as activators of the tumor cell specific M2 isoform of pyruvate kinase, Bioorg. Med. Chem. Lett. 20 (2010) 3387-3393.

[10] M.J. Walsh, K.R. Brimacombe, H. Veith, J.M. Bougie, T. Daniel, W. Leister, L.C. Cantley, W.J. Israelsen, M.G. Vander Heiden, M. Shen, D.S. Auld, C.J. Thomas, M.B. Boxer, 2-Oxo-N-aryl-1,2,3,4-tetrahydroquinoline-6-sulfonamides as activators of the tumor cell specific M2 isoform of pyruvate kinase, Bioorg. Med. Chem. Lett. 21 (2011) 6322-6327.

[11] C. Kung, J. Hixon, S. Choe, K. Marks, S. Gross, E. Murphy, B. DeLaBarre, G. Cianchetta, S. Sethumadhavan, X. Wang, S. Yan, Y. Gao, C. Fang, W. Wei, F. Jiang, S. Wang, K. Qian, J. Saunders, E. Driggers, H.K. Woo, K. Kunii, S. Murray, H. Yang, K. Yen, W. Liu, L.C. Cantley, M.G. Vander Heiden, S.M. Su, S. Jin, F.G. Salituro, L. Dang, Small molecule activation of PKM2 in cancer cells induces serine auxotrophy, Chem. Biol. 19 (2012) 1187-1198.

[12] A. Yacovan, R. Ozeri, T. Kehat, S. Mirilashvili, D. Sherman, A. Aizikovich, A. Shitrit, E. Ben-Zeev, N. Schutz, O. Bohana-Kashtan, A. Konson, V. Behar, O.M. Becker, 1-(sulfonyl)-5-(arylsulfonyl)indoline as activators of the tumor cell specific M2 isoform of pyruvate kinase, Bioorg. Med. Chem. Lett. 22 (2012) 6460-6468.

[13] C. Guo, A. Linton, M. Jalaie, S. Kephart, M. Ornelas, M. Pairish, S. Greasley, P. Richardson, K. Maegley, M. Hickey, J. Li, X. Wu, X. Ji, Z. Xie, Discovery of 2-((1H-benzo[d]imidazol-1-yl)methyl)-4H-pyrido[1,2-a]pyrimidin-4-ones as novel PKM2 activators, Bioorg. Med. Chem. Lett. 23 (2013) 3358-3363.

[14] Y. Xu, X.H. Liu, M. Saunders, S. Pearce, J.M. Foulks, K.M. Parnell, A. Clifford, R.N. Nix, J. Bullough, T.F. Hendrickson, K. Wright, M.V. McCullar, S.B. Kanner, K.K. Ho, Discovery of 3-(trifluoromethyl)-1H-pyrazole-5-carboxamide activators of the M2 isoform of pyruvate kinase (PKM2), Bioorg. Med. Chem. Lett. 24 (2014) 515-519.

[15] 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, Bioorg. Med. Chem. Lett. 25 (2017) 3540-3546.

[16] 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. Davidson, M. 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. Nat.Chem.Biol. 8 (2012) 839-847.

[17] 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, Bioorg. Med. Chem. 23 (2015) 4815-4823.

[18] A.Y. Guan, C.L. Liu, M. Li, Z.N. Li, M.X. Zhang, H. Zhang, Synthesis and bioactivity of novel coumarin derivatives, Nat. Prod. Commun. 6 (2011) 1917-1920.

[19] R.J. Lu, J.A. Tucker, T. Zinevitch, O. Kirichenko, V. Konoplev, S. Kuznetsova, S. Sviridov, J. Pickens, S. Tandel, E. Brahmachary, Y. Yang, J. Wang, S. Freel, S. Fisher, A. Sullivan, J. Zhou, S. Stanfield-Oakley, M. Greenberg, D. Bolognesi, B. Bray, B. Koszalka, P. Jeffs, A. Khasanov, Y.A. Ma, C. Jeffries, C. Liu, T. Proskurina, T. Zhu, A. Chucholowski, R. Li, C. Sexton, Design and synthesis of human immunodeficiency virus entry inhibitors: sulfonamide as an isostere for the alpha-ketoamide group, J. Med. Chem. 50 (2007) 6535-6544.

[20] T. Tashima, H. Murata, H. Kodama, Design and synthesis of novel and highly-active pan-histone deacetylase (pan-HDAC) inhibitors, Bioorg. Med. Chem. 22 (2014) 3720-3731.

[21] D. Ma, Q. Cai, H. Zhang, Mild method for Ullmann coupling reaction of amines and aryl halides, Org. Lett. 5 (2003) 2453-2455.

[22] P. Zhang, J.F. Zuckett, J. Woolfrey, K. Tran, B. Huang, P. Wong, U. Sinha, G. Park, A. Reed, J. Malinowski, S. Hollenbach, R.M. Scarborough, B.Y. Zhu, Design, synthesis, and SAR of monobenzamidines and aminoisoquinolines as factor Xa inhibitors, Bioorg. Med. Chem. Lett. 12 (2002) 1657-1661.

[23] M. Banimustafa, A. Kheirollahi, M. Safavi, S. Kabudanian Ardestani, H. Aryapour, A. Foroumadi, S. Emami, Synthesis and biological evaluation of 3-(trimethoxyphenyl)-2(3H)-thiazole thiones as combretastatin analogs, Eur. J. Med. Chem. 70 (2013) 692-702.

[24] S. Krawczyk, M. Otto, A. Otto, C. Coburger, M. Krug, M. Seifert, V. Tell, J. Molnar, A. Hilgeroth, Discovery of pyridine-2-ones as novel class of multidrug resistance (MDR) modulators: first structure-activity relationships, Bioorg. Med. Chem. 19 (2011) 6309-6315.

[25] H. Jia, G. Dai, J. Weng, Z. Zhang, Q. Wang, F. Zhou, L. Jiao, Y. Cui, Y. Ren, S. Fan, J. Zhou, W. Qing, Y. Gu, J. Wang, Y. Sai, W. Su, Discovery of (S)-1-(1-(Imidazo[1,2-a]pyridin-6-yl)ethyl)-6-(1-methyl-1H-pyrazol-4-yl)-1H-[1,2, 3]triazolo [4,5-b]pyrazine (volitinib) as a highly potent and selective mesenchymal-epithelial transition factor (c-Met) inhibitor in clinical development for treatment of cancer, J. Med. Chem. 57 (2014) 7577-7589.

[26] Y. Jiang, Y. Wang, T. Wang, D.H. Hawke, Y. Zheng, X. Li, Q. Zhou, S. Majumder, E. Bi, D.X. Liu, S. Huang, Z. Lu, PKM2 phosphorylates MLC2 and regulates cytokinesis of tumour cells, Nat.

Commun. 5 (2014) 5566.
[27] N. M. O'Boyle, M. Banck, C. A. James, C. Morley, T. Vandermeersch, G. R. Hutchison, Open Babel: An open chemical toolbox. J Cheminform. 3 (2011) 33.
[28] O. Trott, A. J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem. 31 (2010) 455-461.

Highlights

- Discovery of 4-hydroxy-thiazolidine-2-thione derivatives as PKM2 activators.
- Most compounds showed significant antiproliferative activities.
- Compound **5w** exhibited potent activities against four types of tumor cells at nanomolar concentration.
- Compound **5w** arrests the cell cycle at the G2/M phase in HCT116 cell line.