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1-(sulfonyl)-5-(arylsulfonyl)indoline as activators of the tumor cell specific M2 isoform of pyruvate kinase

Avihai Yacovan *, Rachel Ozeri, Tzofit Kehat, Sima Mirilashvili, Daniel Sherman, Alex Aizikovich, Alina Shitrit, Efrat Ben-Zeev, Nili Schutz, Osnat Bohana-Kashtan, Alexander Konson, Vered Behar, Oren M. Becker

Dynamix Pharmaceuticals, Rehovot 76385, Israel

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ABSTRACT

Cancer cells preferentially use glycolysis rather than oxidative phosphorylation for their rapid growth. They consume large amount of glucose to produce lactate even when oxygen is abundant, a phenomenon known as the Warburg effect. This metabolic change originates from a shift in the expression of alternative spliced isoforms of the glycolytic enzyme pyruvate kinase (PK), from PKM1 to PKM2. While PKM1 is constitutively active, PKM2 is switched from an inactive dimer form to an active tetramer form by small molecule activators. The prevalence of PKM2 in cancer cells relative to the prevalence of PKM1 in many normal cells, suggests a therapeutic strategy whereby activation of PKM2 may counter the abnormal cellular metabolism in cancer cells, and consequently decreased cellular proliferation. Herein we describe the discovery and optimization of a series of PKM2 activators derived from the 2-((2,3-dihydrobenzo[b][1,4] dioxin-6-yl)thio)-1-(2-methyl-1-(methylsulfonyl)indolin-5-yl) ethanone scaffold. The synthesis, SAR analysis, enzyme active site docking, enzymatic reaction kinetics, selectivity and pharmaceutical properties are discussed.

Most cancer cells undergo major metabolic changes to enable their massive proliferation and energy production.[1] Their altered metabolism is represented by increased glucose breakdown and lactate production.[2–5] A key mediator of glycolysis is pyruvate kinase (PK), a rate-limiting enzyme that catalyzes the last step in glycolysis. There are four PK isoforms in mammalian cells[6–10]: the M1 isoform (PKM1) is expressed in many differentiated tissues (skeletal muscle, heart and brain), PKM2 is expressed during embryonic development, PKL and PKR are expressed in liver and erythrocytes, respectively. All four isoforms of PK catalyze the transformation of phosphoenolpyruvate (PEP) and ADP to pyruvate and ATP.[11,12] While PKM2, PKL and PKR are activated by the binding of fructose 1-6-bis-phosphate (FBP) at an allosteric site, PKM1 does not require allosteric activation and is continuously active. Furthermore, the isoform that is over expressed in cancer cells, PKM2[13], facilitates their altered metabolism by switching from a highly active tetramer form to low-activity monomer or dimer form.[13,14] This process is regulated by the upstream glycolytic intermediate, FBP.

The change in the expressed PK isoform, enables cancer cells to balance their use of glucose carbon backbones, whether for ATP production or for biomass generation, including the synthesis of amino acid, nucleotide, and lipid, according to their changing demands.[15] This metabolic difference, between cancer cells and normal cells, provides an attractive new target for cancer therapy. Furthermore, the observed down-regulation of PKM2 activity in cancer cells relative to the high PKM1 activity present in many normal[16] cells suggests a therapeutic strategy whereby activation of PKM2 may perturb cellular metabolism, and consequently decrease cellular proliferation. Thus small molecule PKM2 activators, which stabilize the tetramer form, are expected to affect cancer metabolism, offering a novel anti-cancer therapeutic strategy.

The first small molecules capable of activating PKM2 were reported by the NIH chemical genomics center and include a series of diarylsulfoamides.[17] Since then, very few additional PKM2 activator chemotypes have been reported.[18–20] To identify novel activators with selectivity for PKM2 over the other isoforms we used DynamixFit™ in silico screening technology.[21] Based on available PKM2 crystal structures, a 3D DynamixFit™ model of PKM2 was generated and used to screen for compounds that target the PKM2 dimer interface region.[12] A library of 13,000,000 drug-like compounds (collected from 30 different vendors and continuously updated) was screened in silico against the PKM2 structure. The top 200 selected compounds ("virtual hits") were tested in vitro in an enzymatic PKM2 activation assay. The screening identified 9 novel scaffolds of PKM2 activators[12], including 2-((2,3-dihydrobenzo[b][1,4]dioxin-6-yl)thio)-1-(2-methyl-1-(methylsulfonyl) indolin-5-yl) ethanone, compound **1** (Fig. 1). Here, we describe the

* Corresponding author. Tel.: +972 8 939 6010; fax: +972 8 939 6010.
E-mail address: ayacovan@dynxp.com (A. Yacovan).

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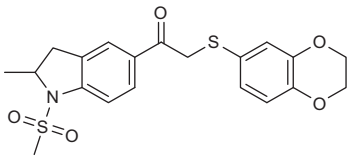


Figure 1. Chemical structure of the PKM2 activator **1**

development and optimization of a series of PKM2 activators derived from **1**.

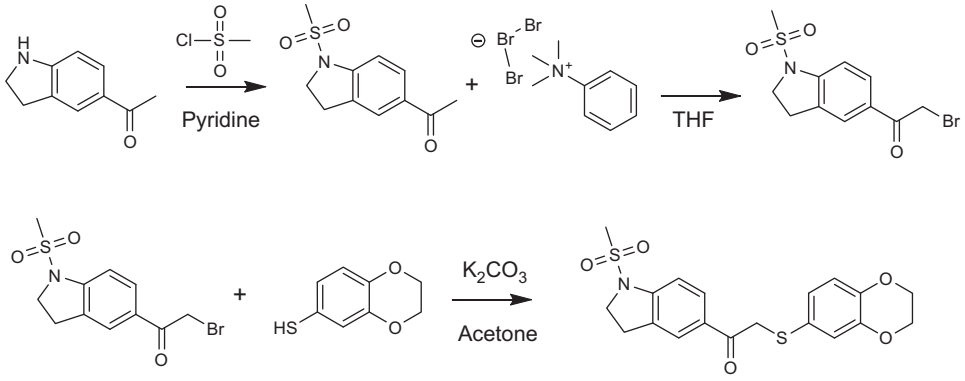
The cell-free enzymatic assay of PKM2 activity was performed as described (Supplementary data). Using this assay for PKM2 activators, compound **1** (as a racemic mixture) was found to have an AC_{50} = 550 nM and a maximal activation of 164% relative to the enzyme's basal activity level, an activation level that is comparable to that achieved in this same assay by the natural activator FBP (AC_{50} = 80 nM, max. activity 148%). Resynthesis of this hit was achieved according to the methodology presented in Scheme 1. The synthetic route selected for the chemical synthesis of **1** was based on an assembly of literature procedures.

At the first stage the indoline-ethanone derivative was dissolved in THF in the presence of pyridine, then an equimolar amount of methanesulfonyl chloride was added with stirring. The reaction mixture was stirred at 40 °C for about 5 h to give the desired sulfonamide product. Then, the sulfonamide ketone was submitted to a bromination reaction in THF using a bromination agent, for example, phenyltrimethylammonium tribromide (Jacques reagent), yielding the mono-brominated sulfonamide.[22] Finally, base-mediated coupling was performed with the commercially available 2,3-dihydrobenzo[b][1,4]dioxine-6-thiol in acetone, to give the desired product, compound **1**.

Given that compound **1**, discovered using in silico screening, is commercially available, additional close analogs of **1** were also purchased and screened in vitro for their PKM2 activation potency. Results are presented in Table 1. All tested analogs showed activity between 0.5–4 μM. Notably, altering the alkoxy groups as R^1 substituents with di-fluoro (**5**) decreased the activity. Cyclic moieties such as indoline (**1**) and tetrahydroquinoline (**2**) were more potent than the acyclic sulfone amide (**6**). The AC_{50} range of these analogs suggests that this scaffold is susceptible to further optimization.

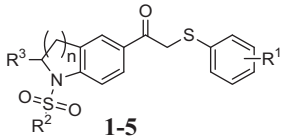
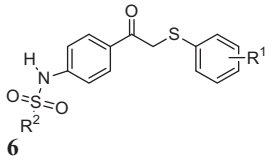
The next step for validating compound **1** as a lead, and moving towards the optimization stage, was to test its cell permeability (Caco-2 assay) and in vitro metabolic stability (in human liver microsoms, hLM). These tests (Table 2) showed that although **1** demonstrated good Caco-2 permeability with no efflux (efflux ratio <1), it suffered from poor metabolic stability in human liver microsoms ($t_{1/2}$ = 3.4 min). Analogs **3** and **6** demonstrated similarly poor metabolic stability in human liver microsoms.

At this stage it was clear that structural modifications were necessary in order to address this issue and generate metabolically stable compounds that would be suitable for further optimization. We began by looking at the importance of the thioethanone group as the linker between the two aryl moieties of compound **1** (Table 3). As structural analysis suggested that shorter linkers could still maintain key binding interactions, four different linkers



Scheme 1. General synthetic methodology used for compound **1**.

Table 1
SAR of 1-Aryl-2-(arylthio)ethanone

	#	R^1	R^2	R^3	n	hPKM2 AC_{50}[a] (μM)	hPKM2 Max Activity[b] (%)
 **1-5**	**1**	3,4-ethylendioxy	Me	Me[c]	1	0.55	164
	2	3,4-ethylendioxy	Me	H	2	1.3	107
	3	3,4-propylendioxy	Et	H	1	0.51	149
	4	3-methoxy	Et	H	1	2.4	110
	5	2,4-difluoro	Et	H	1	4.1	171
 **6**	**6**	3,4-ethylendioxy	Me	—	—	2.5	140

[a] AC50 values were determined using enzymatic PKM2 activity assay (Supplementary data).
[b] Max Activation represents the maximal% activation of the enzymatic activity above its basal level (where 0% is enzyme activity in the absence of compound).
[c] Racemic mixture.