Role of Enzyme Inhibitors in Cancer Therapy

Subjects: Chemistry, Medicinal

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The Warburg effect describes a change in the glucose metabolism in cancer cells, consuming excess glucose and converting it into lactate independently of the presence of oxygen. During this process, a wide variety of enzymes can modify their expression and activity to contribute to the mechanism of deregulated cancer metabolism. Therefore, the modulation of enzymes regulating aerobic glycolysis is a strategy for cancer treatment. Although numerous enzymes play a role in regulating aerobic glycolysis, hexokinase 2 (HK2), pyruvate dehydrogenase kinase (PDK), pyruvate kinase (PK), and lactate dehydrogenase (LDH) are worth mentioning.

Keywords: Warburg effect ; aerobic glycolysis ; drug discovery ; cancer

1. Introduction

In 1926, Otto Heinrich Warburg described for the first time the change in glucose metabolism in cancer cells, which consumes excess glucose and converts it into lactate independently of the presence of oxygen, naming this process aerobic glycolysis or the Warburg effect [1][2]. This discovery was first associated with damage to the mitochondria and the oxidative phosphorylation (OXPHOS) chain, considering that this mitochondrial damage could be the cause of cancer [3]. However, after years of controversy and research, the correct function of the mitochondria and an adequate level of oxygen was shown in cancer cells. Aerobic glycolysis not only is an adaptation of hypoxia. It is also a metabolic reprogramming in the early stage of carcinogenesis independent of the exposition of oxygen, which results in the loss of function of tumor suppressors, altered signaling pathways, and oncogene activation, generating proliferation and malignant progression [4]. The use of the Warburg effect in non-transformed cells with pro-proliferative signals demonstrated that it is a metabolic strategy for cell proliferation [1]. Aerobic glycolysis provides some advantages to the cell, such as the faster regeneration of ATP than the tricarboxylic acid (TCA) cycle. Despite a lower adenosine triphosphate (ATP) production, the increment in the glucose consumption compensates for it in addition to lactate, a key molecule in the progression of tumors [5], whose production and shuttle to the extracellular matrix involves establishing a favorable emerging tumor microenvironment (TME).

The reduction in or disablement of mitochondria produces the alkalinization of the cytoplasm due to a decrease in carbon dioxide (CO_2) secretion that reacts with water (H_2O) and produces carbonic acid [3]. On the contrary, the acidification of the TME by the excretion of lactate derived in a deregulated pH gradient favors tumor progression, hence reducing the T-cell activity [6], evasion of apoptosis, migration, chemoresistance, etc. [3][6].

During this process, a wide variety of enzymes, transcription factors, and transporters modify their expression and activity to contribute to the mechanism of deregulated cancer metabolism. Mutations in these genes can be the cause of their deregulated activity. It has been described that the oncogene C-MYC is one of the most relevant genes in the regulation of the gene expression implicated in glucose, glutamine, and fatty acid metabolism.

2. Target Enzymes and the Reported Inhibitors

2.1. HK2 Modulators

Since HK2 is the first enzyme implicated in the Warburg effect, several molecules have been reported to be HK2 modulators. Within this group, there is a great variety of molecular structures, which have been grouped into six different groups: small molecules, polyphenols, oxygen and sulfur heterocycles, metal complexes, miscellanea, and compounds in combination with other therapies.

2.1.1. Small Molecules

Different authors provided evidence that the HK2 inhibitor 3-bromopyruvic acid (compound **one** in **Figure 1**) significantly inhibited the viability and growth of colon cancer cells, prompting apoptosis via the mitochondrial apoptosis signaling

pathway [7]. The 3-bromopyruvic acid inhibitor has been demonstrated to be a potent HK2 inhibitor in other cell lines, including 8505C (human anaplastic thyroid cancer cells) [8], HCC143 (triple-negative breast cancer), MCF-7 (non-triple-negative breast cancer) [9], and Panc-2 (pancreatic cancer cells) [10]. These findings showed the capacity of 3-bromopyruvic acid for inhibiting HK2, turning it into a strategy to develop new treatments for several types of cancer.

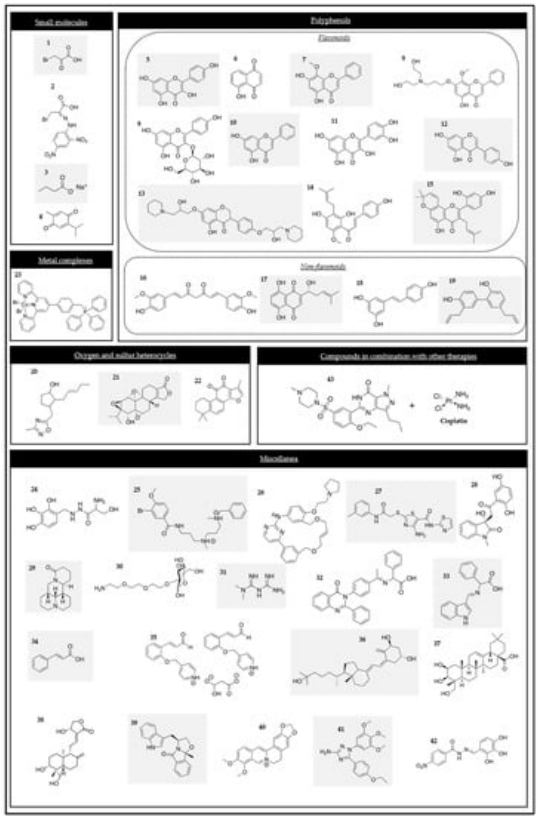

Figure 1. Chemical structures of the HK2 modulators.

Owing to all the evidence regarding the potent anti-cancer activity of 3-bromopyruvate, some researchers have synthesized and studied the anti-cancer activity of several 3-bromopyruvate derivatives. Specifically, the cytotoxic activity of novel hydrazone derivatives, such as compound **two** (**Figure 1**), were assessed on colon, breast, lung, and liver cancer cell lines. The results showed that these derivatives presented a higher HK2 affinity than 3-bromopyruvate, converting them into suitable candidates for developing potent HK2 inhibitors [11].

Sodium butyrate (compound **three** in **Figure 1**), a gut microbiota metabolite, inhibited glycolysis in hepatocellular carcinoma (HCC) cells [12]. This outcome suggested that sodium butyrate inhibited the expression of HK2 and subsequently downregulated aerobic glycolysis and the proliferation of HCC cells along with the induction of apoptosis via the C-MYC pathway.

Thymoquinone (compound **four** in **Figure 1**), an active component from *Nigella sativa*, showed antioxidant, antimicrobial, antidiabetic, and anti-inflammatory effects, among others. It has also been extensively studied against cancer. Recent studies have evaluated its role in cancer metabolism. Thymoquinone was demonstrated to inhibit glycolytic metabolism in colorectal cancer cell lines because it works as a HK2 inhibitor via the modulation of the PI3K/AKT axis [13].

2.1.2. Polyphenols

- Flavonoid phenolic compounds

Several polyphenolic compounds have been reported to be potent HK2 inhibitors. Flavone derivatives are widely distributed in nature and have antioxidant, antiangiogenic, and antineoplastic activities. Furthermore, some flavone derivatives have been demonstrated to be HK2 inhibitors. This capacity could convert them into anti-cancer drugs [14][15][16].

Kaempferol (compound **five** in **Figure 1**), one of the active ingredients of traditional Chinese medicine, has been demonstrated to interfere with the cell cycle, angiogenesis, and the capacity of metastasis in tumor cells. It also produces apoptosis. Moreover, the studies by Zheng et al., proved that kaempferol inhibited metastasis by blocking aerobic glycolysis in melanoma cells, since the binding of HK2 and a voltage-dependent anion (VDAC1) on the mitochondria was disrupted [17].

Juglone (compound **six** in **Figure 1**), another natural compound, has shown an anti-cancer activity. Hu and co-workers proved that juglone suppressed OXPHOS and glycolysis through the inhibition of HK, phosphofructokinase (PFK), and PK activity in prostate cancer [18].

Another example of a flavonoid compound is wogonin (compound **seven** in **Figure 1**), which is extracted from *Scutellariae radix*. It has shown a variety of properties in HT144 melanoma cells, such as an anti-inflammatory effect. This compound also inhibited cell proliferation, colony formation, and tumor growth in the mentioned cells and decreased the activities of HK, PFK, and PK [19].

FV-429 (compound **eight** in **Figure 1**), a derivative of wogonin and a glycolysis inhibitor, was considered to be a promising anti-cancer compound. Extensive research into its mechanism of action (MOA) has revealed that it induced glycolysis inhibition and apoptosis in human prostate cancer cells by downregulating the androgen receptor (AR)-AKT-HK2 signaling network. This evidence reinforces that FV-429 is a promising candidate for the treatment of advanced prostate cancer [20].

Astragalin (compound **nine** in **Figure 1**), commonly found in a variety of food components, was previously demonstrated to be cytotoxic on human leukemia cells. Recently, it has also been demonstrated to suppress the proliferation of HCC cells. It suppresses the expression of HK2 through the upregulation of miR-125b [21].

Chrysin (compound **10** in **Figure 1**), a natural flavone found in plant extracts and widely used in Chinese medicine, has been reported to be a glycolysis inhibitor. It has been proved that when HCC cells are exposed to chrysin, the HK2 expression is decreased, producing cell apoptosis. Chrysin is a promising candidate for novel therapy for HCC [22].

Quercetin (compound **11** in **Figure 1**), a bio-active flavonoid, has an anti-tumor effect on HCC. Thus, researchers wanted to define the underlying mechanism of this effect. They found that similar to the previous flavones, quercetin lowered the protein levels of HK2 and suppressed the AKT/mTOR pathway within HCC cells [23].

Genistein (compound **12** in **Figure 1**) is a natural isoflavone known for its numerous health advantages, including anti-tumor effects. Its effect on HIF-1α and glycolysis in HCC was still unclear, so it has been studied. Genistein has been demonstrated to inhibit aerobic glycolysis and induce mitochondrial apoptosis in HCC cells by directly downregulating HIF-1α, therefore inactivating GLUT1 and HK2 [24]. One derivative of the mentioned compound, 5-hydroxy-7-(2-hydroxy-3-(piperidin-1-yl)propoxy)-3-(4-(2-hydroxy-3-(piperidin-1-yl)propoxy)phenyl)-4*H*-chromen-4-one, called gen-27 (compound **13** in **Figure 1**), is a newly synthesized isoflavone which has been demonstrated to inhibit the proliferation of HCC cells as well as prevent the development of colitis-derived cancer. It has also been demonstrated that gen-27 inhibited glycolysis in human breast cancer cells by decreasing the HK2 expression, leading to the induction of apoptosis [25].

Xanthohumol (2′,4′,4-trihydroxy-6′-methoxy-3′-prenylchalcone) (compound **14** in **Figure 1**) is an attractive compound due to its multiple pharmacological activities. It has been demonstrated that xanthohumol suppressed colorectal cancer cells via the suppression of HK2 and glycolysis. Thus, it could be considered an interesting new anti-tumor agent [26].

Morusin (compound **15** in **Figure 1**) is a compound that is known for its antioxidant anti-inflammatory, antiangiogenic, antimigratory, and apoptotic effects. Recently, it has been demonstrated that it has an anti-tumor activity in HCC. Particularly, it has been studied in Huh7 and Hep3B cells. Morusin has been shown to attenuate the expression of p-AKT, p-mTOR, c-Myc, HK2, PKM2, and LDHA in those cancer cell lines. These findings provide evidence that Morusin exhibits an anti-tumor effect in HCC and has the potential to be a potent dietary anti-cancer candidate [27].

- Non-flavonoid phenolic compounds

Several studies have reported that curcumin (compound **16** in **Figure 1**), a natural polyphenolic pigment extracted from the *Curcuma aromatica salisb*, has significant pharmacological actions, including a chemo-preventive efficacy. It has been demonstrated that curcumin could be a potent drug for the treatment of colorectal cancers. Curcumin downregulated the expression and activity of HK2 in HTC116 and HT29 cells, and it also induced a dissociation of HK2 from the mitochondria, resulting in mitochondrial-mediated apoptosis [28].

Shikonin (SK) (compound **17** in **Figure 1**), an active naphthoquinone that is also present in natural sources in traditional Chinese herbal medicine, is an inhibitor of PKM2 in various cancers. Further studies have been performed with this product. For example, its mechanism has been studied in esophageal squamous cell carcinoma where it has been determined that it not only suppressed the expression of PKM2, but also the expression of HK2 and GLUT1 [29].

Resveratrol (compound **18** in **Figure 1**) is a small polyphenol that has demonstrated both chemo-preventive and chemotherapeutic effects in cancer. In 2016, the capacity of resveratrol for impairing metabolism by inhibiting the expression of HK2 mediated by the Akt signaling pathway was published for the first time, demonstrating its anti-tumor effect on human non-small cell lung cancer (NSCLC) [30].

Honokiol (HNK) (compound **19** in **Figure 1**), a naturally occurring phenolic compound derived from Magnolia, is frequently used for its anti-inflammatory and antioxidant properties.

2.1.3. Oxygen and Sulfur Heterocycles

Several oxygen and sulfur heterocycles have been demonstrated to be HK2 inhibitors. The natural product methyl jasmonate is one of the compounds that blocks HK2 activity in cancer cells. Studies have shown that methyl jasmonate suppressed the proliferation of cells and triggered cell death in various human cancer cell lines. In view of these results, the methyl jasmonate derivative, compound **20** (**Figure 1**), was studied as an effective novel HK2 inhibitor in glioblastoma cells [31].

Triptolide (compound **21** in **Figure 1**), a natural diterpenoid epoxide derived from a traditional Chinese herb, presents multiple biological activities, including anti-inflammatory, immunologic suppression, and potent anti-cancer effects. Several studies have been carried out to study its mechanism among different cancers.

Tanshinone IIA (Tan IIA) (compound **22** in **Figure 1**), the main component of *Salvia miltiorrhiza*, is a known active compound that exhibits anti-tumor properties.

2.1.4. Metal Complexes

[Cu(ttpy-tpp)Br_2]Br], abbreviated as CTB, (compound **23** in **Figure 1**) is a novel copper II complex containing tri-phenyl-phosphonium that targets the mitochondria. CTB inhibits aerobic glycolysis and tumor acidity by reducing the activity of HK2 in hepatoma cells through the dissociation of HK2 from the mitochondria [32].

2.1.5. Miscellanea

Benserazide (BEN) (compound **24** in **Figure 1**) was identified as a selective HK2 inhibitor after a structure-based virtual ligand screening of a library of 2924 US Food and Drug Administration (FDA)-approved drugs and a nutraceutical database. The molecular docking results showed that BEN adopted an extended conformation in which the pyrogallol group of the molecule occupied the binding site of the substrate glucose in HK2. BEN was specifically bound to HK2 as a competitive inhibitor.

High-throughput virtual screening is a widely used strategy for searching for new anti-cancer molecules. By using this method, several chemical scaffolds have been identified to be promising HK2 inhibitors. Among them, compound **25** (**Figure 1**) was highlighted due to its outstanding activity in tumor cell suppression and its capacity to be solubilized in aqueous environments.

Other HK2 inhibitors have been identified using structured-based virtual screening. The most promising compounds, meaning those with low binding energies, were selected for cytotoxicity tests and evaluated in different cancer cells. Particularly, the most promising compounds acting as HK2 inhibitors were 4244-3659 and K611-0094 (compounds **27** and **28** in **Figure 1**). The results indicated that the stable binding of the compounds to the receptor was predominantly influenced by hydrogen bonding [33].

Matrine (compound **29** in **Figure 1**) is a pleiotropic alkaloid from Chinese traditional medicine. It has been demonstrated that this compound could have a possible effect on the suppression of cell proliferation and the induction of apoptosis caused by HK2 depletion and a reduction in C-MYC binding to the HK2 gene in the chronic myeloid leukemia K562 and acute myeloid leukemia HL-60 cell lines.

A novel glucose analog with modifications in carbon 2 (C2), called 2-(2-[2-(2-aminoethoxy)ethoxy]ethoxy)-D-glucose (compound **30** in **Figure 1**), was synthesized as a new strategy for cancer treatment, taking advantage of the increment of glucose consumption in cancer cells. This compound inhibited HK binding in its active site [34].

Metformin (compound **31** in **Figure 2**), a widely used anti-hyperglycemic drug, has also shown important anti-cancer properties. It has been demonstrated that metformin can affect glucose metabolism. It decreases the ^{18}F-fluorodeoxyglucose (FDG) uptake by the direct inhibition of the enzymatic activity of HK2 and HK1 and mimics glucose-6-phosphate G6P in NSCLC Calu-1 cells.

New amino acid Schiff bases of quinazolinones and indoles have been studied as anti-cancer agents. In silico studies predicted their ability to inhibit mitochondrial NADH ubiquinone oxidoreductase (complex I) by targeting the AMPK/mTOR signaling pathway and inhibiting HK. Two compounds (**32** and **33** in **Figure 1**) were the most promising ones in accordance with the docking analysis and were selected for the in vitro analyses.

Cinnamon bark extract (compound **34** in **Figure 1**) has received special attention due to the wide variety of pharmacological activities reported for this species. It has been used in traditional medicines for diabetes, inflammation, arthritis, and cancer. It has been reported that cinnamon bark extract exerted a suppressive effect on the metastatic dissemination of cancer cells, reducing both cell motility and invasion in MDA-MB-231 human breast cancer cells as it decreased the expression of HK2 [35]. Owing to the demonstrated activity of cinnamon bark extract, other derivatives have been studied against cancer. Among them, the cinnamaldehyde derivative (CB-PIC) (compound **35** in **Figure 1**), a major component of cinnamon, possesses a well-known anti-cancer activity.

Calcitriol (compound **36** in **Figure 1**), the biologically active form of vitamin D, has been reported to prevent cancer progression by reducing cell proliferation, increasing cell differentiation, and inhibiting angiogenesis. Mechanistically, calcitriol reduced the expression of cyclin D1, C-MYC, GLUT1, and the key glycolytic enzymes HK2 and LDHA. It suppressed glycolysis and cell growth in human colorectal cells [36].

Centella asiática and *Andrographis peniculata* are two ethnomedicinal anti-cancer herbs. They have been studied with the aim of improving cancer therapeutics. Particularly, bayogenin (compound **37** in **Figure 1**) and andrographolide (compound **38** in **Figure 1**) were extracted from *Centella asiática* and *Andrographis peniculta*, respectively. Both compounds were docked against HK2, a drug-likeness prediction was performed, and the docked complexes were subjected to molecular dynamics simulations.

The SLMP53-1 (compound **39** in **Figure 1**) p53 activation by (*S*)-tryptophanol-derived oxazoloisoindolinone has showed that it regulated glycolysis by particularly downregulating the key glycolytic enzymes HK2, GLUT1, and PFKFB3.

chanism in tumor metabolism was not known. Recently, its molecular mechanism regulating the Warburg effect in ovarian cancer cells has been studied. Those studies demonstrated that berberine inhibited the Warburg effect by inhibiting the expression of HK2 in ovarian cancer cells [37].

A novel tubulin inhibitor, 5-(4-ethoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1H-1,2,4-triazol-3-amine (YAN) (compound **41** in **Figure 1**) effectively suppressed glycolysis in both A549 and A549/Taxol cells. The inhibition was achieved by downregulating the expression of HK2, LDHA, and GLUT1 and it was able to block the mitochondrial binding of HK2 in A549 and A549/Taxol cells.

In 2020, benitrobenzerazide (compound **42** in **Figure 1**) (BZNZ) was reported as a novel selective HK2 inhibitor with EC_{50} values in the nanomolar range. BZNZ induced cell apoptosis in the SW 1990 cells pancreatic cancer cell line. Its capacity was proved by the authors both for in vitro and in vivo activity.

2.1.6. Compounds in Combination with Other Therapies

Sildenafil (compound **43** in **Figure 1**) has been demonstrated to enhance the cytotoxic effect of cisplatin by the induction of apoptosis. Previous studies have reported the promising chemo-sensitizing potential of the phosphodiesterase 5 inhibitor sildenafil in breast, colon, prostate, glioma, and lung tumors.

2.2. PDK Modulators

2.2.1. Small Molecules

The small molecule dichloroacetate (DCA) (compound **44** in **Figure 2**) has been proposed as an anti-cancer drug. For several decades, this metabolic modulator has been employed in the treatment of lactic acidosis and inherited mitochondrial diseases. The efficacy of DCA in cancer therapy has been associated with the Warburg effect. The salts of DCA selectively target cancer cells shifting their metabolism from aerobic glycolysis to OXPHOS by the inhibition of PDK.

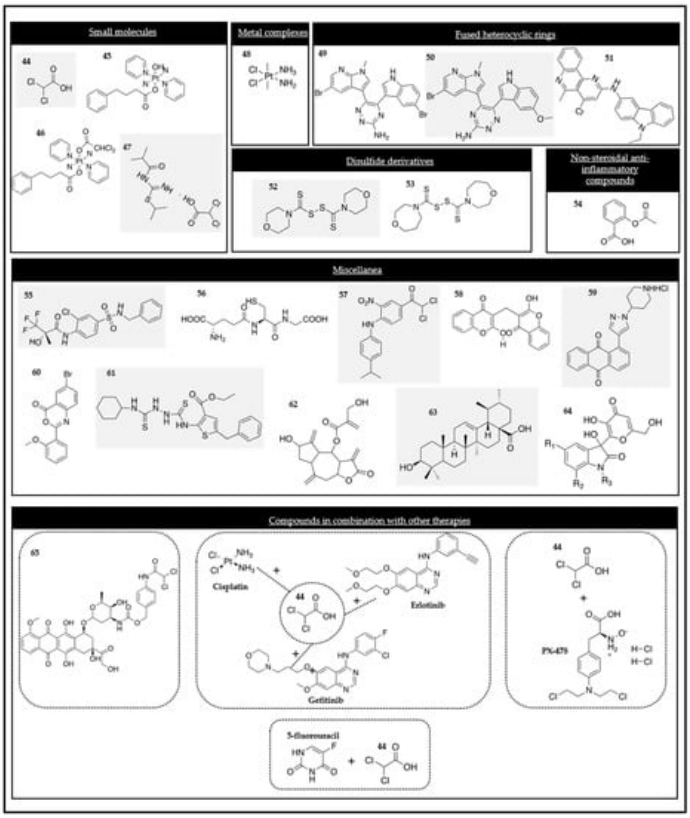

Figure 2. Chemical structures of the PDK modulators.

Some prodrugs have also been described to be PDK inhibitors. These were Pt (IV) prodrugs (compounds **45** and **46** in **Figure 2**) with bio-active axial ligands that have been designed to selectively target cancer cells and attack several cellular components at the same time, which can result in an enhanced anti-cancer potenc

Another compound, named T1084 (1-isobutanoyl-2-isopropylisothiourea dichloroacetate) (compound **47** in **Figure 2**), incorporated the nitric oxide synthase (NOS) inhibiting fragment, 1-isobutanoyl-2isopropylisothiourea, and the PDK inhibiting fragment, DCA, into the molecular structure. This multifunctionality enabled T1084 to concurrently exert two distinct pharmacological actions, anti-angiogenesis and hypoxia-oriented cytotoxicity, that could potentially enhance its anti-tumor effects.

2.2.2. Metal Complexes

The novel platinum (Pt) (IV) complex *c,c,t*-[Pt(NH_3)$_2$$Cl_2$($C_{10}H_{15}N_2O_3S$)($C_2HO_2Cl_2$)] (DPB) (compound **48** in **Figure 2**), containing DCA as one of the axial ligands in the structure, was synthetized and evaluated as an anti-tumor agent. DCA interfered in the mitochondrial energy metabolism after its reduction, activated PDH due to the inhibition of PDK, depolarized the mitochondrial membrane potential, and promoted ROS. This novel complex induced apoptosis after the inhibition of both glycolysis and glucose oxidation, thus highlighting the interest in these types of drugs [38].

2.2.3. Fused Heterocyclic Rings

To find novel classes of potential PDK inhibitors, 1,2,4-amino triazine derivatives (compounds **49** and **50** in **Figure 2**) were reported to be promising candidates for inhibiting the PDK expression. Despite the different candidates that were synthetized and evaluated, compounds 49 and 50 must be emphasized [39]. These novel compounds demonstrated a promising ability to inhibit the enzymatic activity of PDK1 and PDK4.

The 2-amino substituted 2,4-dichlorobenzo[h]naphthyridine derivatives, concretely compound **51** (**Figure 2**), have been proposed to be PDK1 inhibitors.

2.2.4. Disulfide Derivatives

Some disulfide derivatives have demonstrated a capacity to inhibit PDK1. JX06 (compound **52** in **Figure 2**) has been identified as a selective covalent inhibitor of PDK1. This compound formed a disulfide bond with its thiol group, showing a cysteine residue localized in the hydrophobic pocket of the PDK1 enzyme. This bond induced conformation changes in arginine 286 through Van der Waals forces and hindered access of ATP to this binding pocket, blocking the PDK1 enzymatic activity. As a result, this finding explained the mechanism of inhibition of PDK1 by the JX06 compound and its derivatives [40].

Based on the scaffold of JX06, new disulfide-based PDK1 inhibitors have been synthesized. Among all the new novel compounds, one of them, compound **53** (**Figure 2**) was shown to inhibit PDK1 more potently than JX06. Furthermore, it was more selective with PDK1 and induced notable alterations in the glucose metabolic pathways within A549 cells by reducing ECAR and increasing the ROS levels.

2.2.5. Nonsteroidal Anti-Inflammatory Drugs

It was found that aspirin (compound **54** in **Figure 2**) attenuated glycolysis by suppressing PDK1. The study identified a novel role and regulatory mechanism of PDK1 in breast cancer stem cells (BCSC) reprogramming. It was concluded that PDK1 regulated by the HIF-1 pathway was required for BCSC self-renewal reprogramming, which could be blocked by aspirin. This provides a promising strategy for breast cancer therapy [41].

2.2.6. Miscellanea

Numerous works have highlighted the new therapeutic approach of PDK inhibitors for the treatment of numerous pathologies, such as diabetes, lactic acidosis, cerebro-vascular and cardiovascular diseases, cancer, hypertension, and neurodegenerative diseases. Therefore, many authors have developed novel PDK inhibitors. The *N*-(4-(*N*-alkyl/aralkylsulfamoyl)phenyl)-2-methylpropanamides (compound **55** in **Figure 2**) were synthesized and studied.

α-Lipoic acid (compound **56** in **Figure 2**) is a natural coenzyme in the mitochondrial PDH complex commonly known as an antioxidant. It has been studied in cancer research. A-lipoic acid has inhibitory effects on the proliferation and migration of cells and demonstrated a proapoptotic effect in the NSCLC A549 and PC9 cell lines.

The compound 2,2-Dichloro-1-(4-((4-isopropylphenyl)amino)-3-nitrophenyl)ethan-1-one, namely XB-1 (compound **57** in **Figure 2**), was discovered as a new PDK inhibitor.

Dicumarol (compound **58** in **Figure 2**) is a member of a family of coumarins with various pharmacological properties, including anti-inflammatory and anti-cancer activities. It was demonstrated that dicumarol inhibited the PDK1 activity with an IC_{50} of 19.42 μM in SKOV3.

New compounds have been designed and synthesized specifically to be PDK inhibitors. Among them, compound **59** (**Figure 2**) was a potent novel compound. Particularly, it was predicted and later verified that it binds to the lipoyl-binding site and interrupts intermolecular interactions with the E2-E3bp subunits of PDC.

The limited number of reported PDK inhibitors motivated several authors to continue discovering novel small molecules that can serve as PDK inhibitors. High-throughput screening is a commonly used strategy in these cases and has led to the discovery of two novel compounds (compounds **60** and **61** in **Figure 2**) that inhibited cancer cell proliferation and colony formation in A549 cells.

Hemistepsin A (compound **62** in **Figure 2**) is a sesquiterpene lactone isolated from *Hemistepta lyrata* Bunge. It has been reported as a PDK1 inhibitor in colorectal cancer cells. Firstly, through computational simulation and biochemical assays, it was demonstrated that Hemistepsin A directly binds to the lipoamide-binding site of PDK1.

Ursolic acid (compound **63** in **Figure 2**), a triterpenoid widely found in food, has been investigated on adult T-cell leukemia cells. It was demonstrated that it inhibits the proliferation of adult cells due to the inhibition of PDK, AKT, RSK1, and TOR [42].

A group of synthetic compounds formed by the aldolic reaction of isatins and kojic acid have been studied as inhibitors of PDK. This new family of compounds, 3-hydroxy-3-[3-hydroxy-6-(hydroxymethyl)-4-oxo-4H-pyran-2-yl] indolin-2-ones (compound **64** in **Figure 2**), have been proven to have different biomedical applications, such as anticonvulsant, anti-inflammatory, and anti-cancer effects.

2.2.7. Compounds in Combination with Other Therapies

Particularly, a binary prodrug named PDOX (compound **65** in **Figure 2**) was designed and investigated. It consisted of a combination of DCA (PDK inhibitor) and the anti-cancer drug doxorubicin (DOX). The prodrug was activated selectively by cancer-associated esterase to deliver DCA and DOX.

The PDK inhibitor DCA (compound **44**) has also been explored in lung cancer in combination with commercial therapeutic drugs, particularly A549 and LNM35. Al-Azawi and co-workers demonstrated that DCA significantly enhanced the anti-cancer effect of cisplatin, gefitinib, and erlotinib. The additive effects on the inhibition of the mentioned cell lines due to the synergistic effect were shown.

Alongside the drug combination approach, new evidence for the synergism between the PDK inhibitor DCA and the HIF-1α inhibitor PX-478 was found. The group demonstrated that the combination of DCA and PX-478 produced synergistic effects in colorectal, lung, breast, cervical, liver, and brain cancer cell lines.

2.3. PK Modulators

2.3.1. Small Molecules

Small molecules include molecules that activate the enzyme, along with others that can trigger PKM2 and promote different mechanisms that inhibit tumor growth.

TEPP-46 and DASA-58 (compounds **66** and **67** in **Figure 3**), two allosteric activators, were studied in five breast cancer cell lines to investigate the potential effect of PKM2 when it is activated. The studies showed that the molecules were able to increase the activity of the enzyme in those cancer cells without affecting the overall cell survival and that they were also able to reduce an intracellular glucose sensor (TXNIP levels) [43].

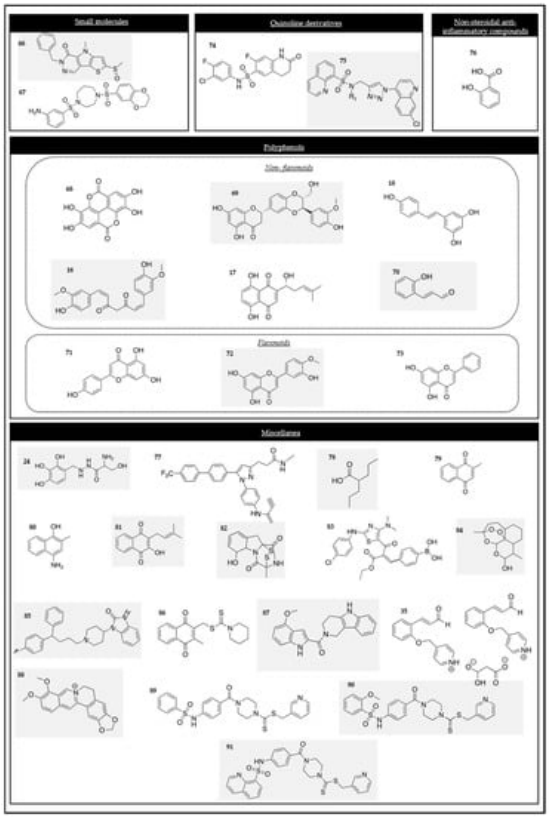

Figure 3. Chemical structures of the PK modulators.

2.3.2. Polyphenolic Compounds

- Non-flavonoids

The evaluation of these phenolic compounds against three binding sites of PKM2 provides insight into how molecule–enzyme binding occurs and functionally how amino residues of PKM2 are important. These compounds have different IC_{50} values and can exert anti-tumor effects in different cancer cell lines.

Ellagic acid (compound **68** in **Figure 3**) is an organic heterotetracyclic compound and is found in numerous fruits and vegetables [44]. Ellagic acid can induce apoptosis in MCF-7 and MDA-MB-231 cancer cells and can induce a reduction in the key glycolytic enzymes, such as PKM2, HK2, and GLUT1, which were measured in MCF-7 and MDA-MB-231 cells. It induced apoptosis in those cancer cell lines with IC_{50} values of 23 µM and 27 µM, respectively [45].

Silibinin (compound **69** in **Figure 3**) is the principal component of silymarin, a standardized extract of *silybum marianum*. It is well known due to its chemo-preventive, anti-angiogenic, and apoptosis inducer properties. It has been identified as a potent PKM2 inhibitor with an IC_{50} value of 0.91 µM. It can reduce PKM2 and the protein in TNBC cells (MDA-MB-231 and BT549 cells) and induce a reduction in the key glycolytic enzymes, such as PKM2, HK2, and GLUT1.

Resveratrol (compound **18** in **Figure 3**) is a natural stilbene and non-flavonoid polyphenol that can be found in cereals, fruits, and vegetables. It has been demonstrated that it possesses antioxidant, anti-inflammatory, anti-tumoral, and

cardioprotective effects.

Curcumin (compound **16** in **Figure 3**) has been studied as an anti-cancer compound. It has been demonstrated that curcumin inhibited the glucose uptake and lactate production in the H1299 (lung), MCF-7 (breast), HeLa (cervical), and PC-3 (prostate) cancer cell lines by downregulating the PKM2 expression via the inhibition of the mTOR-HIF-1α axis [46].

HCA (compound **70** in **Figure 3**) is an active component isolated from cinnamon bark. It inhibits the PKM2 and STAT3 signaling pathways. Its biochemical methods (affinity chromatography, drug affinity, responsive stability assay, etc.) demonstrated that HCA bound directly to PKM2 and decreased the protein kinase activity of the enzyme by decreasing the phosphorylation at the tyrosine 105 residue [47].

- Flavonoids

This section highlights the various flavonoids derived from vegetables and fruits, which have demonstrated antiproliferative effects on human cancer cells.

Apigenin (compound **71** in **Figure 3**) inhibits cancer cell proliferation by triggering cell apoptosis and modulating the cell cycle. It interferes with multiple signaling pathways and protein kinases (PI3K/AKT, MAPK/ERK, JAK/STAT, NF-κB and Wnt/β-catenin) [48]. Apigenin targeted and reduced the PKM2 enzyme expression and activity in colon cancer cells (HCT-116, HT-29, and DLD1) [49].

Other flavones, such as diosmetin and chrysin (compounds **72** and **73** in **Figure 3**), have been studied due to their structural features, showing their capacity to inhibit the PKM2 enzyme. This interruption of glycolysis resulted in a reduction in tumor growth [50].

2.3.3. Quinoline Derivatives

Quinoline derivatives (NZT) have been the subject of syntheses, structure–activity relationships, selectivity, and other properties research. Certain studies focused on the 2-oxo-*N*-aryl-1,2,3,4-tetrahydroquinoline-6-sulfonamide scaffold (compound **74** in **Figure 3**). These studies revealed an EC_{50} value of 90 nM with a significant selectivity over other PK isoforms [51].

In silico studies confirmed that the 8-quinolinesulfonamide derivatives, concretely compound 79 (compound **75** in **Figure 3**), were potential PKM2 modulators. In vitro research studies showed that this compound could reduce the intracellular pyruvate level in A549 lung cancer cells, reduce cell viability, and induce apoptosis [52].

2.3.4. Nonsteroidal Anti-Inflammatory Drugs

Salicylic acid (SA) (compound **76** in **Figure 3**) is the major and active metabolite of aspirin. Both compounds are well-described drugs that can be used without the need of a prescription for lowering fever, reducing inflammation, and relieving low to moderate pain.

2.3.5. Miscellanea

Benserazide (BEN) (compound **24** in **Figure 3**) is currently in clinical use as a co-adjuvant treatment in Parkinson's disease with L-DOPA. It has been demonstrated, first by a structure-based virtual ligand screening in an FDA-approved drug database and then by in vitro and in vivo studies, that BEN was able to direct PKM2 binding, hence blocking its activity. In this way, the aerobic glycolysis concurrent upregulation of OXPHOS can be inhibited in a dose-dependent manner.

N-(4-(3-(3-(methylamino)-3-oxopropyl)-5-(4′-(trifluoromethyl)-[1,1′-biphenyl]-4-yl)-1H-pyrazol-1-yl)phenyl)propiolamide (compound **77** in **Figure 3**) is a novel irreversible PKM2 inhibitor that has demonstrated tumor suppressive effects in different cell lines. This compound can inhibit PKM2 by interacting with CYS326 and CYS317 of the enzyme and the propiolamide electrophile terminus of compound **77**. The inhibition of the enzyme and, as result, the growth suppression of the tumor was tested in PC9 cells where the PKM2 expression was shown to be decreased.

Valproic acid (compound **78** in **Figure 3**) has been found to reduce the PKM2 expression in breast cancer cells, specifically in MCF-7 and MDA-MB-231 cells [53].

Vitamin K compounds, known as VKs, are fat-soluble compounds. VK3 (compound **79** in **Figure 3**) and VK5 (compound **80** in **Figure 3**) have been reported to be adjuvant agents for cancer therapy. Chen et al., demonstrated that these vitamins acted as selective PKM2 inhibitors in Hela cells [54][55].

Lapachol (compound **81** in **Figure 3**), a compound derived from *Tabebuia impetiginosa*, has been shown to have a greater affinity compared to other related natural products, such as shikonin, which was demonstrated using computer-aided design.

Glyotoxin (compound **82** in **Figure 3**) bound directly and strongly to PKM2 and suppressed its activity. Moreover, it exhibited a dose-dependent inhibition of glycolytic activity, resulting in a reduced glucose consumption and lactate production within the U87 human glioma cell line. The inhibition of PKM2 activity further led to the suppression of STAT3 phosphorylation. These studies demonstrated the potential of gliotoxin as a treatment for glioma by targeting cancer metabolism [56].

Boronic acid has the ability to activate the PKM2 enzyme, leading to redox metabolism in oral cancer cells. Consequently, a novel class of boronic acid derivatives was developed. Particularly, the PKM2 activity of compound **83** (**Figure 3**) was tested in CAL-27 cells. It was found that compound **83** activated PKM2 with an AC50 of 25 nM [57].

Dihydroartemisinin (DHA) (compound **84** in **Figure 3**), also known as artenimol, is an active metabolite of the anti-malarial drug artemisinin. DHA induces a pro-inflammatory cell death called pyroptosis. It has been shown to downregulate the PKM2 expression and induce cell death in the esophageal squamous cell carcinoma (ESCC) cells Eca109 and EC9706 at 24 h. DHA has the potential to be a therapeutic agent for ESCC by downregulating the PKM2 expression, activating the caspase-8/3-GSDME axis, and mediating tumor cell pyroptosis [58].

The antipsychotic medication Pimozide (compound **85** in **Figure 3**) acts on the signaling pathway and promotes the effect of p53, leading to a decrease in the expression of PKM2. Pimozide has demonstrated strong anti-breast cancer effects both in vitro and in vivo.

Compound **86** (**Figure 3**) is a specific inhibitor of the PKM2 enzyme. Investigations on the anti-cancer effects of this inhibitor were conducted using MTT and colony formation assays in SK-OV-3 cells. Compound **86** was found to induce AMPK activation, which is associated with suppressing the tumor progression. The inhibition of PKM2 by compound **86** affected the Warburg effect and led to autophagic cell death.

NPD10084 (compound **87** in **Figure 3**) has demonstrated an antiproliferative activity against colorectal cancer cells both in vitro and in vivo. In this study, PKM2 was identified as a potential target protein for this compound. Interestingly, NPD10084 disrupted the protein–protein interactions between PKM2 and β-catenin or STAT3, leading to the suppression of the downstream signaling pathways [59].

The cinnamaldehyde derivative (CB-PIC) (compound **35** in **Figure 3**), the major component of cinnamon, has been found to inhibit the migration of H1299 lung tumor cells in scratch and transwell assays. This compound was directly and specifically bound to recombinant PKM2, resulting in a reduction in its catalytic activity. The cells with PKM2 knockdown demonstrated a significantly reduced migration compared to the control cells when subjected to glucose and oxygen deprivation. The anti-cancer mechanism of compound **35** was investigated in human HCC cells in relation to STAT3 signaling.

In addition, a literature-based phytochemical analysis of *Mangifera indica* identified a total of 94 compounds, which were docked against three binding sites of PKM2 to identify the potential PKM2 inhibitors. Among these compounds, berberine (compound **88** in **Figure 3**), isolated from *Coptis* and *Hydrastis canadensis*, was found to inhibit the PKM2 activity, showing antiproliferative effects in HCT116 and HeLa cells.

A novel sulfonamide compound with a pyridin-3-ylmethyl 4-(benzoyl)piperazine-1-carbodithioate moiety has been identified as a potent activator of PKM2. A series of analogs based on the lead compound **89** (**Figure 3**) were designed and synthetized. Among these analogs, compounds **90** and **91** (**Figure 3**) demonstrated higher PKM2 activation activities. Furthermore, they exhibited more significant antiproliferative effects against human tumor cell lines.

2.4. LDH Enzyme Modulators

2.4.1. Small Molecules

Sodium oxamate (compound **92** in **Figure 4**) exerted a competitive inhibition of human LDHA in vitro with a Ki of 136.3 uM in two NPC cancer cell lines. However, this derivative presented several problems related to a low and limited selectivity, weak potency, and poor cellular uptake [60]. The studies showing the MOA [61] stated that compound **92** could promote cell apoptosis via the downregulation of the CDK1/cyclin B1 pathway, which ultimately provoked the inhibition of LDHA.

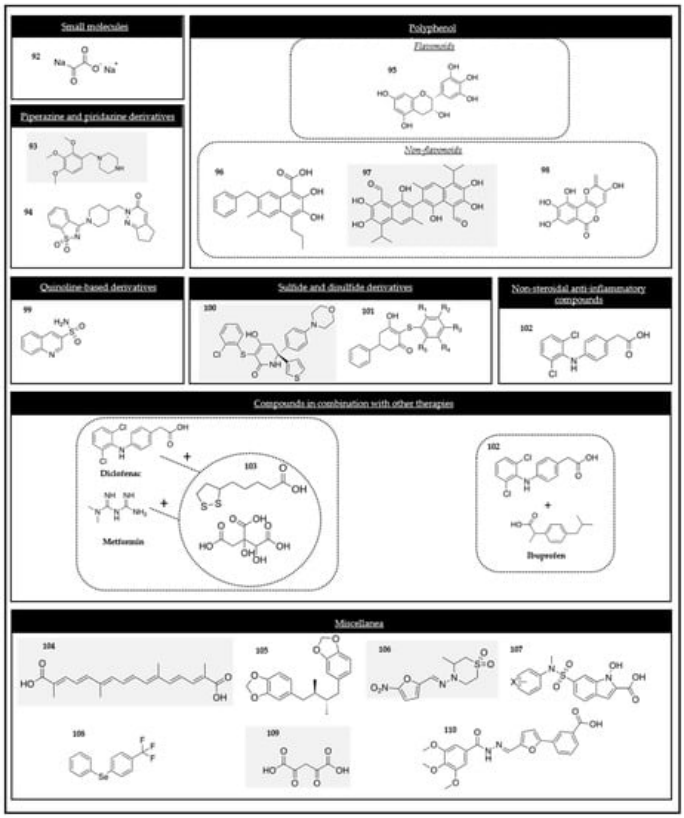

Figure 4. Chemical structures of the LDH modulators.

2.4.2. Pyridazine and Piperazine

Trimetazidine (TMZ) (compound **93** in **Figure 5**) is a drug indicated exclusively in adults as additional therapy for the symptomatic treatment of patients with stable angina pectoris. Silica-induced pulmonary fibrosis studies in rats were done to explore the effects of this drug over the LDH activity. The results showed that the derivative of compound **93** was able to reduce and normalize the levels of the enzyme in bronchoalveolar lavage fluid (BALF) [62]. TMZ can be used alone or in combination with antineoplastic drugs, such as gemcitabine or abraxane, to reduce cell viability and induce apoptosis in human pancreatic cancer cells [63].

Pyridazine derivatives exhibited potent anti-cancer activity and were synthesized due to structure-based virtual screening studies. Compound **94** (**Figure 5**) exerted its effect on multiple cancer lines: Med1-MB (a cell line obtained from Sonic Hedgehog medulloblastoma), CRC HCT116, SW620, lung cancer A549, and pancreatic PANC-1 cancer cell lines.

2.4.3. Polyphenols

- Flavonoids

Epigallocatechin (EGCG) (compound **95** in **Figure 4**) is one of the main compounds of green tea and a compound found in the medical plant *Spatholobus suberectus*, which is commonly used in China for the treatment of cancer-related blood stasis.

- Non-flavonoids

FX11 (3-dihydroxy-6-methyl-7-(phenylmethyl)-4-propylnaphthalene-1-carboxylic acid) (compound **96** in **Figure 4**) is a synthetic molecule that was discovered using an HITS assay. FX11 is structurally related to gossypol and is well known to be a sensitizer in chemotherapy-resistant tumor cells [64][65][66].

Gossypol (compound **97** in **Figure 4**) is a natural aldehyde derived from cotton seed and it can inhibit several cancer cell lines, such as melanoma, lung, breast, cervix, and leukemia.

Galloflavin (compound **98** in **Figure 4**), a synthetic derivative of gallic acid, binds to LDHA, but it is also able to block LDHB without competing with substrates and cofactors. The cytotoxic effects were demonstrated by this analog in various human breast cancer cells (MCF-7, triple-negative MDA-MB-321, and tamoxifen-resistant MCF-7tam cell lines) [64][67].

2.4.4. Quinoline-Based Derivatives

Compound **99** (**Figure 4**) a quinoline 3-sulfonamide derivative, is a selective and competitive LDHA inhibitor, competing with NADH. This derivative can exert its effect in multiple cell lines, including hepatocellular and breast carcinomas [68].

2.4.5. Sulfide and Disulfide Derivatives

GNE-140 (compound **100** in **Figure 4**), a synthetic molecule discovered using an HITS assay, was tested in MDA-MB-231 and HCC143 cells in vitro. GNE-140 has been demonstrated to block LDH [69].

Several substituted 3-hydroxy-mercaptocyclo-2-enone compounds, especially compound **101** (**Figure 4**), have been identified as potent novel class LDH inhibitors using the HITS approach. Compound **101** can bind to the active site of LDH where pyruvate usually binds, hence limiting and competing with the substrate binding.

2.4.6. Nonsteroidal Anti-Inflammatory Compounds

Diclofenac (compound **102** in **Figure 4**) is commonly used to reduce inflammation and alleviate pain. Apart from its classical role as a cyclooxygenase (COX) inhibitor, diclofenac has exhibited potent anti-cancer effects. Studies were conducted to investigate whether diclofenac could induce cancer cell death in HeLa cells through the production of ROS.

2.4.7. Compounds in Combination with Other Therapies

Different concentrations of METABLOC (compound **103** in **Figure 4**), a combination of hydroxycitrate and lipoic acid, was used in combination with diclofenac and metformin in LL/2 lung carcinoma cells. Cisplatin was used as a positive control. The effects of METABLOC appeared to be enhanced when high-dose metformin was used.

In another study, the combination of diclofenac (**102** in **Figure 4**) and ibuprofen was examined. Both drugs are NSAIDs associated with anti-tumoral effects in glioma cells. However, ibuprofen showed a stronger inhibition of cell growth compared to diclofenac. Both drugs were able to decrease STAT3 phosphorylation. Interestingly, diclofenac led to a decreased C-MYC expression and a subsequent reduction in the LDHA activity.

2.4.8. Miscellanea

Crocetin (compound **104** in **Figure 4**) is a 20-carbon dicarboxylic acid diterpenoid that was obtained using chemical synthesis. Its LDH inhibition properties have been tested against two cancer cell lines (A549 and HeLa) [70][71].

Machillin A (compound **105** in **Figure 4**), a natural product found mainly in *Machilus thunbergii*, *Iryanthera lancifolia*, and *Magnolia sinica*, has been demonstrated to reduce tumor growth by inhibiting LDHA. It is a competitive inhibitor, which blocks the NAD binding site [70][72].

Nifurtimox (compound **106** in **Figure 4**), a drug used for treating parasitic protozoan *Trypanosoma cruzi* infections, has also been reported to present cytotoxic properties in the neuroblastoma cell lines LA-N-1, IMR-32, LS and SK-N-SH. Nifurtimox can achieve a reduction in the LDH enzyme activity [73].

N-hydroxyndole-based inhibitors have been demonstrated to be small, competitive, and LDHA isoform-selective inhibitors. The derivative of compound **107** (**Figure 4**) can compete against pyruvate and NADH. SAR studies confirmed the importance of the OH-COOH pharmacophore, since the enzyme active site will usually have a gap for lactate (hydroxyl group) and another for pyruvate (alpha-keto acid group) [74].

Selenobenzenes, such as (1-(phenylseleno)-4-(trifluoromethyl)benzene (PSTMB) (compound **108** in **Figure 4**), demonstrated their ability to decrease tumor growth in several tumor cell lines (NCI-H460, MCF-7, Hep3B, A375, HT-29, and LLC).

Oxaloacetate (OAA) (compound **109** in **Figure 4**) is a competitive inhibitor of human LDHA and has been found to be regulated by PKM2 activity. An elevated PKM2 activity can promote the de novo synthesis of OAA through glutaminolysis, leading to the inhibition of LDHA in cancer cells.

N-acylhydrazone derivatives have been studied as LDHA inhibitors. For this purpose, a virtual screening procedure was conducted. Afterwards, chemical modifications were made to the selected compounds to enhance their inhibitory activity. The new molecules demonstrated activity in the micro-molar range. Specifically, compound **110** (**Figure 4**) exhibited a significant effect on lactate production in the Raji human cell line.

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