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ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ietp20

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To cite this article: Yan Zhou, Yizhen Guo & Kin Yip Tam (2022) Targeting glucose metabolism to develop anticancer treatments and therapeutic patents, Expert Opinion on Therapeutic Patents, 32:4, 441-453, DOI: 10.1080/13543776.2022.2027912

To link to this article: https://doi.org/10.1080/13543776.2022.2027912

EXPERT OPINION ON THERAPEUTIC PATENTS
2022, VOL. 32, NO. 4, 441–453
https://doi.org/10.1080/13543776.2022.2027912

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REVIEW

Targeting glucose metabolism to develop anticancer treatments and therapeutic patents

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ABSTRACT

Introduction: One of the most distinctive hallmarks of cancer cells is increased glucose consumption for aerobic glycolysis, which is called the Warburg effect. In recent decades, extensive research has been carried out to exploit this famous phenomenon, trying to detect promising targetable vulnerabilities in altered metabolism to fight cancer. Targeting aberrant glucose metabolism can perturb cancer malignant proliferation and even induce programmed cell death.

Areas covered: This review covered the recent patents which focused on targeting key glycolytic enzymes, including hexokinase, pyruvate dehydrogenase kinases, and lactate dehydrogenase for cancer treatment.

Expert opinion: Compared with the conventional cancer treatment, specifically targeting the well-known Achilles heel, the Warburg effect has attracted considerable attention. Although there is still no single glycolytic agent for clinical cancer treatment, the combination of glycolytic inhibitor with conventional anticancer drugs or the combined use of multiple glycolytic inhibitors are being investigated extensively in recent years, which could emerge as attractive anticancer strategies.

ARTICLE HISTORY
Received 4 October 2021
Accepted 7 January 2022

KEYWORDS
Cancer treatment patent; cancer glucose metabolism; Warburg effect; hexokinase 2 (HK2); pyruvate dehydrogenase kinase (PDK); lactate dehydrogenase A (LDHA); glycolytic inhibitor

1. Introduction

Most of the non-proliferating cells in our bodies undergo oxidative phosphorylation (OXPHOS) to generate adenosine triphosphate (ATP). In contrast, Otto Warburg found that many cancer cells favor glycolysis rather than much more effective OXPHOS even with plenty of oxygen and undamaged mitochondria. This phenomenon, which was observed in the early 1920s, was later called aerobic glycolysis or Warburg effect [1] (Figure 1(a)). One major advantage of aerobic glycolysis in cancer cells is generating precursors for building vital macromolecules and biomass for supporting cancer survival and rapid proliferation [2]. In recent decades, researchers have once again raised a wave of interest in studying cancer metabolism, especially glucose metabolism, with the aim of developing promising cancer therapies. There are several key enzymes that play pivotal roles in the aberrant glucose metabolism in cancer cells. So far, there is still no commercial anticancer drug that specifically interferes with altered cancer metabolism. Considering the obvious side effects of carpet-bombing traditional chemotherapy, precisely targeting critical aerobic glycolytic enzymes provides exciting opportunities for potential cancer therapy [3,4].

As shown in Figure 1(b), cancer cells promote the first irreversible step of glycolysis by accelerating glucose uptake, as well as upregulating hexokinases (HKs) including HK1 and HK2. The facilitation of this critical step subtly prevents glucose from escaping from the cell through glucose transporters. Most notably, HK2, which binds to the outer membrane of mitochondria, plays a crucial role in cancer cells by cooperating with four pivotal partners including plasma membrane glucose transporter (Glut), the mitochondrial voltage-dependent anion channel (VDAC), the ATP synthase located in the inner mitochondrial membrane and the adenine nucleotide translocator that transports the ATP to the VDAC-HK2 complex [5]. In addition to these delicate collaborations that greatly facilitate glycolysis and subsequent biosynthesis, HK2 is essential to help cancer cells evade mitochondrial-induced apoptosis by binding to VDAC [6].

As the final product of glycolysis, pyruvate stands at the intersection of two fate pathways, which can be simplified as [7]: (1) reversible conversion to lactate by the lactate dehydrogenase (LDH) in cytosol, and (2) transport into mitochondrial TCA cycle where it will be oxidized to acetyl-CoA and CO_2 by pyruvate dehydrogenase (PDH) which can be negatively modulated by pyruvate dehydrogenase kinases (PDKs) upregulation. The hypoxia-inducible factor-1α (HIF1α), which commonly presents in solid cancer, is reported to induce the overexpression of PDK and LDH [8]. Since the reversible reactions catalyzed by LDH led to the formation of lactic acid, cancer cells often overexpressed monocarboxylate transporters (MCTs) to prevent intercellular acidification [9]. Targeting LDH has recently emerged as one of the most promising anticancer strategies.

This review summarizes the recently disclosed patents, which focuse on targeting critical glycolytic enzymes including HK2, PDKs, and lactate dehydrogenase A (LDHA) for fighting

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Article highlights

- Aberrant glucose metabolism signified by elevated aerobic glycolysis is one of the most important hallmarks of cancer.
- HK2, PDK, and LDHA are three key enzymes, which are often associated with aberrant glucose metabolism.
- Extensive investigations have been undertaken to develop novel inhibitors of HK2, PDK, and LDHA in anticancer research.
- Recent patents reporting the development of these novel metabolic inhibitors are reviewed.

cancer. Particular focus is placed on these new inhibitors patented in the years between 2015 and 2021. Moreover, the claimed derivatives, prodrug, and drug combination with other anticancer agents are included as appropriate. All the materials used were obtained from the public domain, including Google Patent and Espacenet. By reviewing the recent progress in research exploiting vulnerabilities of these enzymes, it is expected to provide specific knowledge and insights for further anticancer drug development targeting Warburg effect.

2. HK2 inhibitors

Hexokinase catalyzes the phosphorylation of glucose into glucose-6-phosphate (G6P) by transferring a phosphate group from ATP to glucose, which is the first committed and rate-limiting step of glycolysis [10]. Among its four isoforms, Hexokinase 2 (HK2) is the uppermost isoform in insulin-sensitive tissues, such as heart, skeletal muscle, and adipose tissues in mammal [11]. It has also been found to be upregulated in multiple types of solid tumors, exhibiting enhanced aerobic glycolysis [10,11]. In addition to its basic role in glycolysis, HK2 also affects many important cellular processes including cellular growth and survival by direct molecular-molecular or functional interactions with the Akt/mTOR pathways [12]. Excitingly, the differences in expression level of HK2 between cancer cells and normal cells may indicate huge potential for inhibiting HK2 as therapeutic strategies to preferentially kill cancer cells.

HK1 and HK2 bind to mitochondria through the interaction with VDAC and play an important role in the stability of the mitochondrial environment [12]. The upregulation of HK2 protein levels is required to maintain a high glycolytic environment in aggressive cancer cells. About 80% of HK2 is related to mitochondria through interaction with VDAC in cancer cells. The mitochondrial-bound HK2 can obtain the ATP required for glucose phosphorylation to accelerate the glycolysis pace [13]. The interaction between VDAC and HK2 on the outer mitochondrial membrane (OMM) down-regulates the apoptotic activity by inhibiting the translocation of cytochrome c to cytoplasm [14]. VDAC increases the connection between HK2 and the mitochondria of cancer cells [15]. VDAC has the privilege to obtain mitochondrial ATP and regulate the opening of mitochondrial permeability transition pores, leading to the resistance of cancer cells to apoptosis. The transfer of HK2 from the cytoplasm to OMM in cancer cells, coupled with its important role in the glycolytic pathway, makes HK2 a promising target for the development of anticancer treatments [16].

Modulating the activities of HK2 can be loosely defined as inhibiting the kinase activity or decreasing its expression [14]. Accordingly, the current HK2 inhibitors can be classified as: siRNA targeting HK2 [17], shRNA targeting HK2 [18], miRNA targeting HK2 [19], HK2 antibody [20], and chemical inhibitors, such as 3-bromopyruvate, benserazide, and 2-deoxyglucose [14,21]. Herein, attention is focused mainly on the chemical inhibitors of HK2 that have been evaluated in cancer research for the past 6 years (see Figure 2).

Indazole derivatives (**1–1**) have been investigated for cancer treatment since 1970's [16]. One of the most famous ones, lonidamine (**1-1-1**), is an approved drug in a few countries in Europe for several types of human cancer treatment. **1-1-1** is capable of inhibiting of mitochondria bounded HK2 and then reducing ATP generation [22]. Mark Matteucci and his team found that **1-1-1** and its analogs (**1-1-2, 1-1-3**) with high aqueous solubility were useful in treating or preventing cancer, benign prostatic hyperplasia, macular degeneration, and prostatic intraepithelial neoplasia, or for use as an antispermatigenic agent with effective doses around 380.00 μM/mL in cells [23]. In addition to that, the effective dose of **1-1-1** was relatively safe for combination therapy [24].

2-Deoxyglucose (**1–2**) is an analogue of glucose [25]. It can inhibit the absorption of glucose by forming 2-deoxy-D-glucose-6-phosphate (2-DG6P), inhibit the glycolysis of cancer cells, and thereby inhibit the growth of cancer cells [26,27]. In the range of millimolar concentration, **1–2** can cause ATP consumption and lead to cell death, especially for cancer cells with mitochondrial respiratory defects and hypoxia [28]. However, animal studies have shown that **1–2**, as a single agent, has no obvious therapeutic effect on mice in the dose range of 500–2000 mg/mL. In the clinics, it is mainly used in combination with other anticancer drugs to treat cancer [29–31]. In 2006, Theodore J. Lampidis' team came up with a method of anticancer therapy in human by using **1–2** as a glycolytic inhibitor to inhibit the glycolytic pathway. In particular, **1–2** exhibited natural selective toxicity toward cancer cells that mainly created ATP for energy anaerobically, which increased the efficiency of traditional chemotherapeutic and radiation anticancer therapy [32].

Metformin (**1–3**) is widely used in the treatment of diabetes, mainly used as a first-line oral therapy for type 2 diabetes. In recent years, more and more studies have shown that **1–3** is a potential anticancer drug that can be used to prevent and treat a variety of cancers [30,33,34]. Research by Salani et al. showed that **1–3** could inhibit the enzymatic function of HK1 and HK2 in Calu-1 cells, significantly reducing glucose uptake and intensifying mitochondrial depolarization, thereby attenuating cell proliferation [35]. In 2016, Chen and his team came up with the invention of a preparative method and the application of N-2-deoxyglucosyl dimethyldiguanide (**1–4**) for anticancer therapy. This new compound was obtained by recrystallization of 2-deoxyglucose and metformin hydrochloride at a mole ratio of 3:1–1:3. They claimed that **1–4** not only retained the original effect of **1–2** but also inhibited serum lactic dehydrogenase [30].

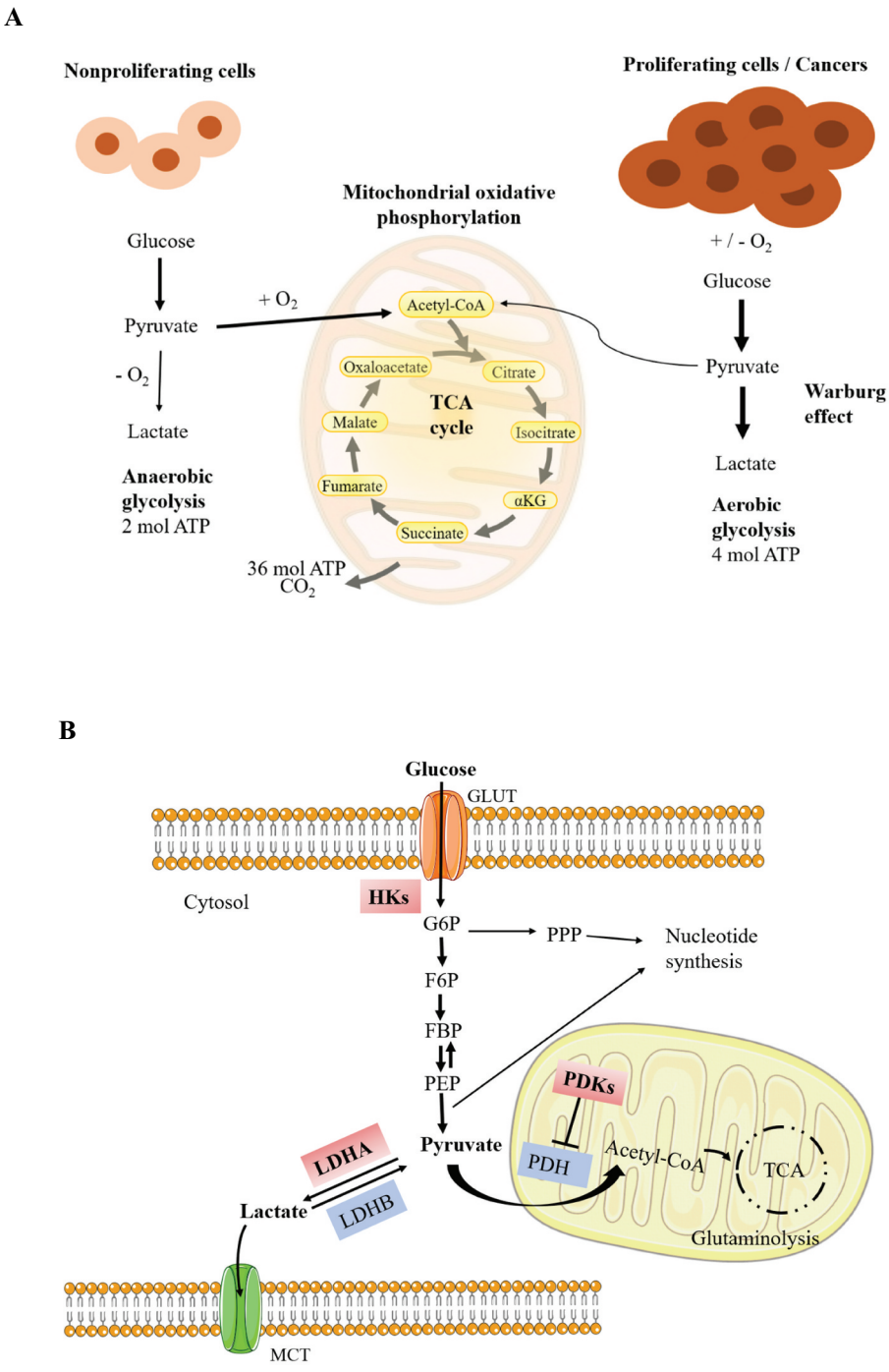

Figure 1. Overview of glucose metabolism in cancer cells. a. Schematic of OXPHOS, anaerobic glycolysis and aerobic glycolysis. In an oxygen-rich environment, normal nonproliferating cells mainly conduct oxidative phosphorylation. Firstly, cells perform glycolysis which generate pyruvate from glucose. Second, most pyruvate transport to mitochondria to carry out TCA cycle and eventually produce abundant of ATP with a lite lactate. While in a low oxygen environment, normal cells primarily carry out glycolysis. Conversely, proliferating normal tissues or cancer cells favor glycolysis even when exposed to plenty of oxygen. b. Schematic depiction of critical glycolysis pathways in cancer. Cancer metabolic reprogramming has several key steps which are controlled by a variety of glycolytic enzymes. This schematic briefly outlines the critical steps and enzymes of glucose metabolism in cancer cells. GLUT, glucose transporter; HK, hexokinase; G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; FBP, fructose bisphosphate; PEP, phosphoenolpyruvate; PPP, pentose phosphate pathway; LDHA, lactate dehydrogenase A; LDHB, lactate dehydrogenase B; MCT, monocarboxylate transporter; PDK, pyruvate dehydrogenase kinase; PDH, pyruvate dehydrogenase.

In 2016, Adnan M.M. Mjalli's team reported the use of substituted pyran derivatives or substituted fused oxazoline derivative compounds (**1–5** or **1–6**) and the associated pharmaceutical compositions as HK2 inhibitors at relatively low dosage in enzyme assay (mostly ranging from 3.00 nM to 4.60 μM). It was shown that these preparations were able to be used for cancer therapy, fungal infection therapy, and anti-angiogenesis therapy. In this invention, **1–5** or **1–6** were reported to induce tumor-specific oxidative stress cell death, increasing the production of reactive oxygen species in tumor cells by inhibition of glycolysis and NADPH synthesis [36].

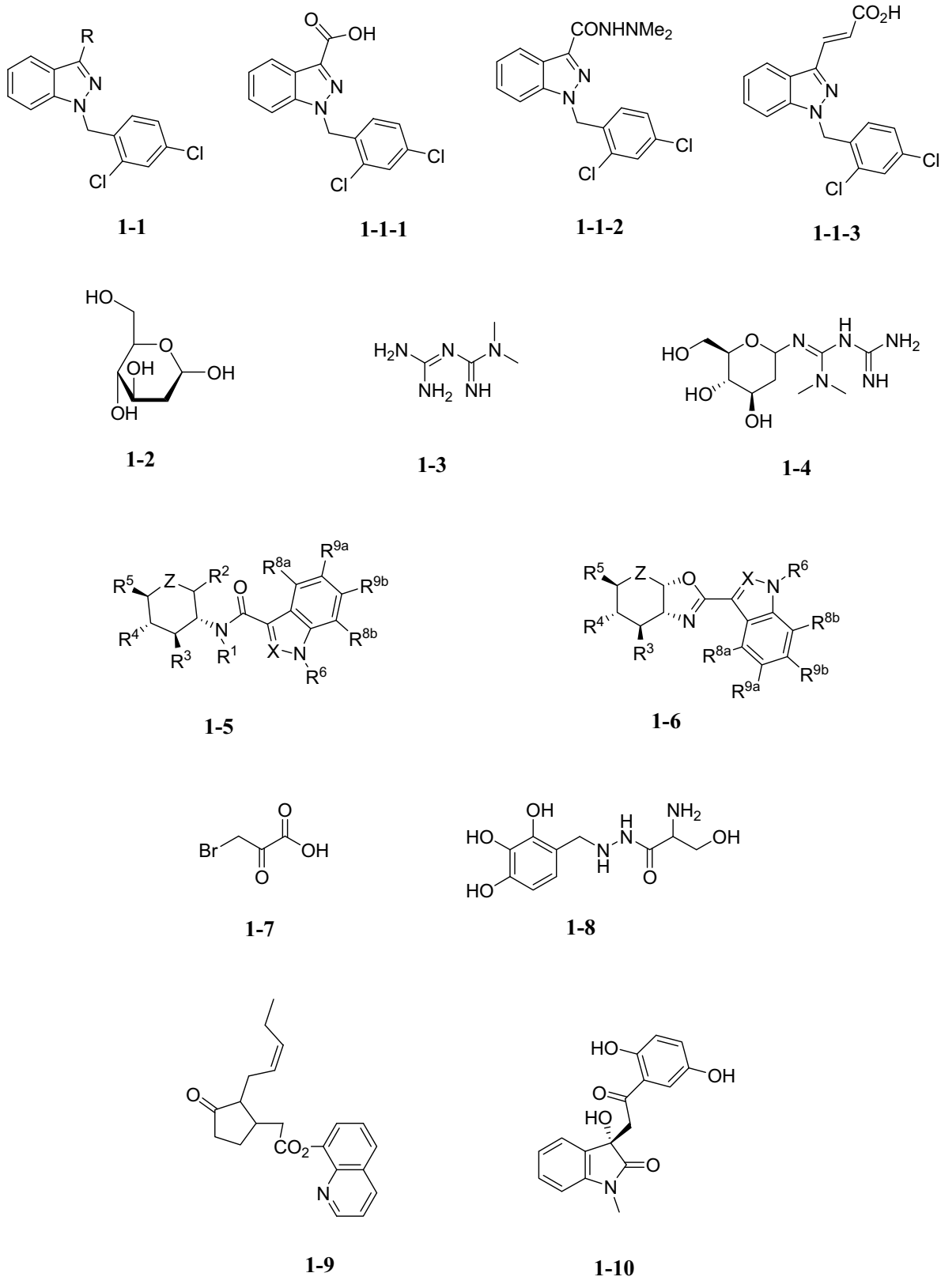

Figure 2. Chemical structures of some HK2 inhibitors. **1–5**, **1–6**: Z = O, N, NR*, NC(O)R*; X = N, C7; R* = alkyl, alkenyl, alkynyl, aryl, aralkyl, cycloalkyl, (cycloalkyl) alkyl, heteroaryl, heteroaralkyl, heterocyclyl, (heterocyclyl)alkyl, haloalkyl, alkoxy, haloalkoxy.

3-Bromopyruvate (**1–7**) [37] is a brominated derivative of pyruvate, which is a strong alkylating agent affecting the free SH groups from cysteine residues in proteins. In view of its strong alkylation properties, **1–7** may affect a variety of enzymes including HK2. In 2008, researchers in Johns Hopkins University found that **1–7** could suppress cancerous tumor growth by inhibiting HK2 activity and affecting ATP generation [38]. Yogesh Rai's team found in 2019 that **1–7** dissociated HK2 from mitochondrial complex and caused enhancement of anti-leukemic drugs sensitization in leukemic cells [15]. In cancer cells with mitochondrial defects or hypoxia, **1–7** induced a large amount of ATP depletion of cancer cells and caused cell death, which enhanced the sensitivity of cancer cells to other anticancer drugs. Although **1–7** was more efficacious than **1–2**, the aqueous solution of **1–7** was unstable and required 100.00 μM *in vitro* to be effective [26,39]. In 2017, Young Hee Ko's team disclosed a group of new cellular energy inhibitors that were **1–7**'s analogs and the associated preparative methods, which were able to serve as anticancer compositions through the inhibition of hexokinase [40]. The inventors claimed that this group of anticancer compositions could conjugate with at least one sugar unit, which stabilized the cellular energy inhibitor by substantially preventing the inhibitor from hydrolysis.

Benserazide [15,41] (**1–8**) is an approved drug in the UK for the treatment of Parkinson's disease. It is a peripheral aromatic L-amino acid decarboxylase inhibitor and is often combined with levodopa in clinical practice. Li et al. used a structure-based virtual ligand screening method to screen the FDA-approved drug database and found that **1–8** was a selective HK2 inhibitor [42] (Enzyme inhibition IC_{50}: 5.52 ± 0.17 μM). As a clinically used drug, **1–8** has clear pharmacokinetics, pharmacodynamics, and low toxicity, which greatly facilitates the development of **1–8** and its derivatives as anti-tumor drugs. Moreover, **1–8** was found to reduce the glucose uptake rate, lactate production, and intracellular ATP levels of cancer cells, as well as depolarize the cancer cell mitochondrial membrane potential leading to apoptosis. These results suggested **1–8** might be a very promising anticancer drug candidate.

Jasmonates (a plant stress hormones family) and some of their synthetic derivatives exhibited anticancer effects both *in vitro* and *in vivo* [43,44]. O Fingrut and E Flescher found in 2002 that jasmonic acid (JA) and methyl jasmonate (MJ) induced apoptosis in lymphoblastic leukemia cells and suppressed cell proliferation, as well as tumor growth in various human cancer cell lines [45]. In 2018, Vered Behar's team reported methods of using HK2/mitochondria-detaching compounds, including jasmonate derivatives and piperazine derivatives, as well as pharmaceutical compositions for treating, inhibiting, or suppressing a HK2-expressing cancer [14]. Among these compounds, it was found that one of the Jasmonate derivatives (**1–9**) inhibited colony formation and decreased cell viability of CTCL tumor cells in a dose-dependent manner (0.50–3.00 μM). In addition to that, **1–9** was reported to be a selective HK2 inhibitor with minimal effect on HK1.

In 2019, a team from University of Lanzhou has developed a compound LY2019-001 (**1–10**) [46], inhibiting HK2 enzyme at 17.50 μM. Moreover, **1–10** and its derivatives were found to inhibit the growth of cervical cancer cells (HeLa), liver cancer cells (HepG2), and lung cancer cells (A549).

3. PDKs inhibitors

Pyruvate dehydrogenase complex (PDC) is a 9.5 million Da multi-enzyme complex located in mitochondrial matrix and consisting of four major enzyme components: pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2), dihydrolipoamide dehydrogenase (E3), and E3-binding protein (E3BP), as well as the two kinds of dedicated regulatory enzymes: PDKs and pyruvate dehydrogenase phosphatase [47]. The detailed structure and function of PDC have been well reviewed [48]. As an important gatekeeper enzyme that links pyruvate to the TCA cycle, PDC catalyzes the conversion of pyruvate to acetyl-CoA coupled with the reduction of NAD^+ to NADH. The modulation of PDC activities depends on the reversible phosphorylation and dephosphorylation [49]. Phosphorylation of E1α component, regardless of which one of the three serine residues, is enough to switch off PDC activity. Thus, phosphorylation of PDC by PDKs will downregulate its activity, and subsequently reduce the flux of pyruvate into the TCA cycle. In human, phosphorylation of PDC is catalyzed by any of four isoforms of pyruvate dehydrogenase kinase (PDK1-4) which are expressed differently in specific tissues. In particular, PDK1 is closely associated with cancer malignancy and serves as the only PDK isoform that could phosphorylate all serine sites of PDC [50]. To sum up, inhibiting PDKs has been one of the recognized strategies to fight against cancer by increasing OXPHOS and reversing Warburg effect.

The four binding sites for PDK inhibitors including pyruvate site, lipoamide site, nucleotide site, and allosteric site, as well as the small molecular inhibitor development history till 2015 have been elaborated nicely by a recent literature review [51]. The known molecular mechanisms of PDKs activities in regulating tumor malignancy and possible strategies to target PDKs have also been summarized recently [52]. In this section, the recent patent contributions focusing on novel PDK inhibitors for cancer treatment will be emphatically reviewed. The chemical structures of representative inhibitors are shown in Figure 3.

3.1. DCA based small molecular inhibitors

Dichloroacetate (DCA (**2–1**)), one of the best-known inhibitors of PDKs, has been clinically evaluated and found to be well tolerated in patients with relatively moderate toxicity [53]. As an allosteric pan-PDK inhibitor, albeit nonspecific, abundant studies indicated that by inhibiting PDKs, **2–1** showed anticancer effect in numerous cancers including glioblastoma, prostate, colorectal, and breast cancers both *in vitro* and *in vivo* [54–57]. The possible underlying mechanisms of this inhibitor may be summarized as [47,54,58,59]: (1) depolarizes the mitochondrial inner membrane electrochemical gradient and decreases membrane potential in cancer cells; (2) reverses the Warburg effect and restores mitochondrial OXPHOS function; (3) decreases cellular lactic acid production and elevates large amounts of reactive oxygen species; (4) promotes cancer cell apoptosis, accompanied by cytochrome c release. In addition, **2–1** has been found to exert synergistic anticancer effects when combined with several cancer drugs including Vemurafenib [60], Gefitinib or Erlotinib [61], and Metformin

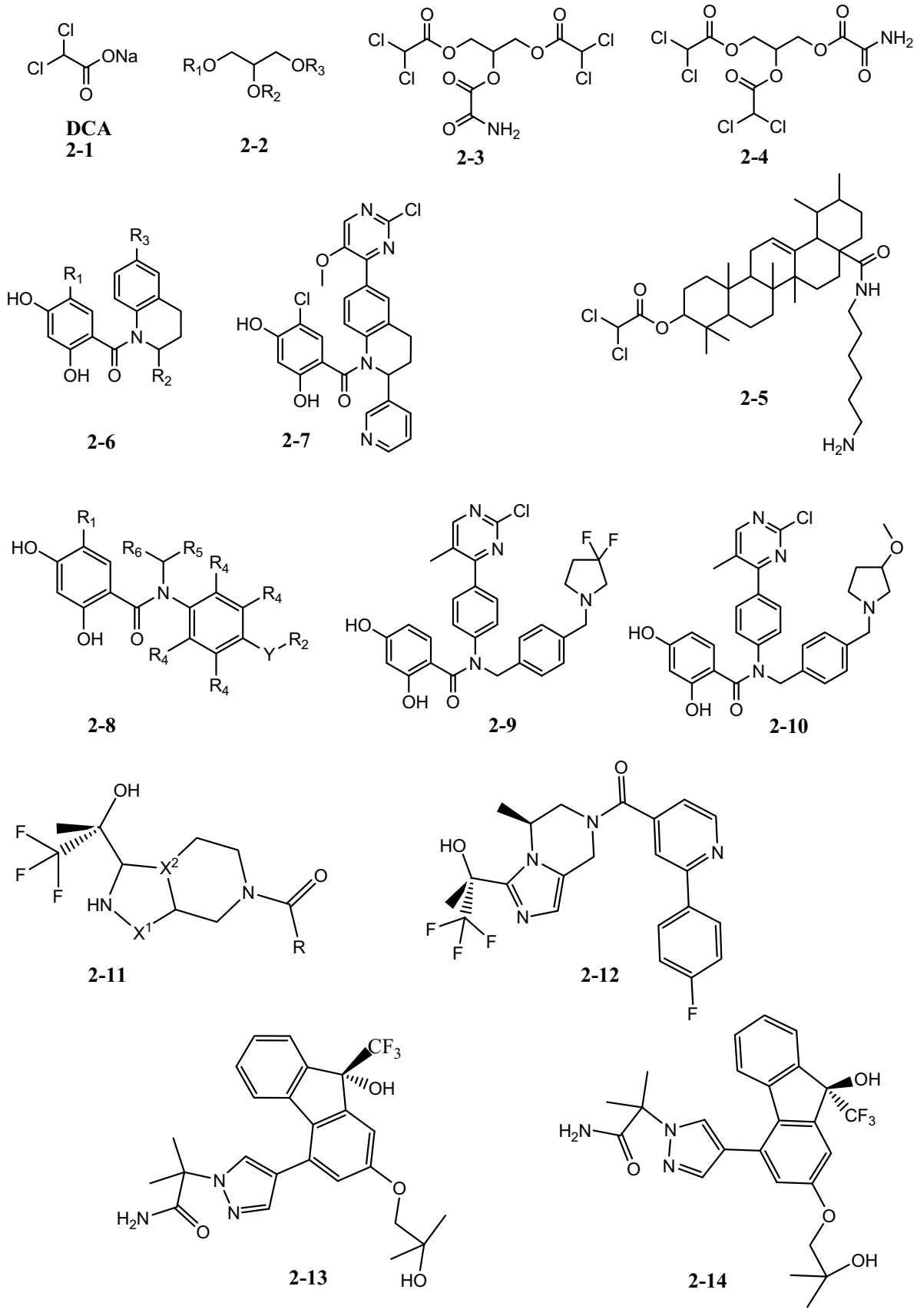

Figure 3. Chemical structures of some PDK inhibitors.

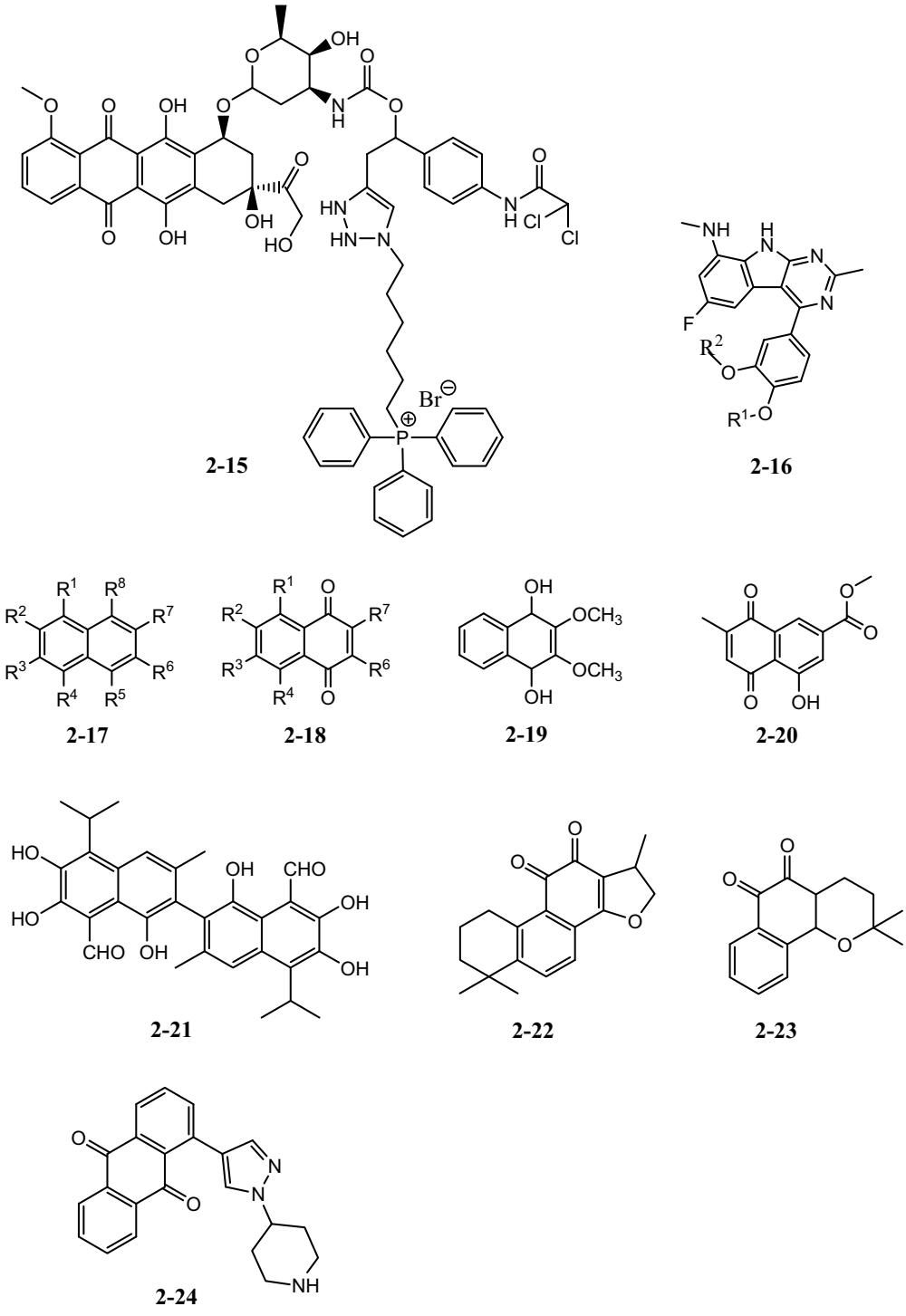

Figure 3. Continued.

[62]. Previously, several clinical trials with **2–1** exploiting either alone or in combination have been launched for various cancer treatments. Unfortunately, the further clinical application of **2–1** is quite limited due to its weak inhibitory ability, unsatisfactory low potency, and high-dose requirement. Further notice, based on existing **2–1** studies, many other derivatives with improved efficacy were synthesized to selectively inhibit activities of PDKs [51].

In 2015, inventors of the South Dakota Board of Regents reported a series of novel prodrugs (**2–2**) which could suppress tumor growth and induce apoptosis *via* synergistically inhibiting PDK and LDH [63]. The key designing scheme of these prodrugs was to use glycerol and ester bonds to conjugate **2–1** with oxamate, which was able to inhibit LDH. As exemplified by **2–3** and **2–4**, glycerol served as a linker for **2–1** and oxamate *via* ester bonds to form prodrugs. The

compounds were tested on a human papillomavirus-positive head and neck squamous cell carcinomas mouse model. The final data showed that the prodrugs exhibited stronger anticancer effect, reduced lactate secretion, and elevated OXPHOS compared with the treatments using **2–1** and oxamate alone. Further data suggested that the sensitivity of tumor to cisplatin/radiation therapy was also enhanced by the compounds, which quickly released **2–1** and oxamate in mice. This application implied the promising anticancer effect by dual inhibition of PDK and LDH activities.

Similarly, Chen et al. disclosed compounds, which were ursolic acid-glycolysis inhibitor-DCA conjugates with the anticancer effects by double targeting cell apoptosis resistance and metabolism in cancer [64]. The representative compound **2–5** was able to dramatically decrease the lactate and ATP productions in cancer cells. The compound treatment inhibited cell proliferation in a dose-dependent manner in MCF-7, B16F10, RL95-2, and 4T1 cell lines with IC_{50} of 3.9 μM, 10.3 μM, 7.7 μM, and 7.2 μM, respectively.

3.2. Quinoline derivatives and amide-containing inhibitors

Brough et al. synthesized more than two hundred tetra-hydro-iso-quinoline compounds (**2–6**) as novel PDK inhibitors with functions to suppress cancer cell proliferation [65]. The Phospho-(Ser293) E1a MSP ELISA Assay and the PDK1 DELFIA immunoassay, which measures phosphorylation of E1 by PDK1, were used to test the PDK1 inhibitory activities of the synthesized compounds. Several compounds such as **2–7** showed potent effects with PDK1 enzyme inhibition IC_{50} of 1.0 μM to 10.0 μM.

Soon after, the same team disclosed a series of new resorcinol N-aryl amide (NAA) compounds (**2–8**), as PDK inhibitors [66]. The new batch of compounds such as **2–9** and **2–10** were more potent with IC_{50} of PDK1 inhibition ranging from 0.10 μM to 1.0 μM.

In 2019, researchers in Japan disclosed the nitrogen-containing heterocyclic amide compounds (**2–11)** exhibiting PDKs inhibitory activities [67]. *In vitro* PDK activity inhibitory assay demonstrated that the most potent **2–12** exerted 88% PDK1 inhibition and 81% PDK2 inhibition at 3.0 nM. One year later, researchers in the same institution disclosed the synthetic method for a pyrazole-amide compound that also exhibited PDK inhibitory effect [68]. **2–13** and **2–14** both showed potent PDK1 and PDK2 inhibitory activities with enzyme inhibition IC_{50} lower than 5.0 nM.

3.3. Conjugated compounds and others

In 2020, a conjugate compound **2–15** which contained a doxorubicin (Dox) moiety, a DCA subunit, and a tri-phenylphosphonium (TPP) mitochondrial targeting group was reported [69]. As an anthracycline antibiotic, Dox has been used to treat various cancers. With the aid of the positively charged TPP group, the DCA moiety was designed to be susceptible to enzymatic-induced release in mitochondria to exert PDK inhibitory effects. Once activated by carboxylesterase, which led to the release of the DCA moiety and the Dox moiety, the anticancer prodrug **2–15** was found to simultaneously alter the glucose metabolism, suppress tumor growth, and help overcome drug resistance in the resistant cell-line xenograft (MCF7/Dox) models.

Researchers at Idorsia Pharmaceuticals Ltd disclosed an invention of pyrimido[4,5-*b*]indolyl derivatives (**2–16**) which could inhibit PDK1 and modulate immunometabolism [70]. More than 20 compounds were tested by the PDK1 fluorescence polarization assay, which demonstrated potent inhibitory effects against PDK1 with IC_{50} arranging from 60 to 200 nM. However, further evaluations on pharmacokinetic, pharmacological, and toxicological properties were needed.

3.4. PDK4 inhibitors

It is also worth noting that pyruvate dehydrogenase kinase 4 (PDK4) has emerged to exhibit multiple novel functions in addition to the metabolic role in cancer cells. Recent studies revealed that Pdk4-/- mice exhibited enhanced hepatocyte proliferation and similar phenomena, which were also reflected in PDK4 knockdown hepatocellular carcinoma cells, suggesting PDK4 also plays a potential role in cell cycle regulation [71]. Moreover, another study revealed that PDK4 stood at the checkpoint to determine the hepatocyte extrinsic apoptosis mediated by NF-κB/TNF [72]. Together with other studies related to PDK4 inhibition and apoptosis in breast cancer [73], lung and colorectal cancer [74], PDK4 inhibitors appear to play a pivotal role in triggering cell death in some specific cancers.

The reported clinical candidates such as AZD7545 and Nov3r potently inhibit PDK1-3 with IC_{50} in the nanomolar range, while dramatically promote PDK4 activity [75]. Unlike other PDK isoenzymes, PDK4 exists in a semi-active state, which could be one of the obstacles in developing PDK4 inhibitor [49,76].

In 2015, scientists disclosed a series of PDK4 inhibitors based on structure **2–17** or **2–18** [77]. Using PDK4 enzyme inhibitory assay, the inventors identified two representative and potent compounds (**2–19** and **2–20**) with the PDK4 enzyme inhibition IC_{50} of 9.0 μM. On another related invention, several novel compounds, namely, **2–21, 2–22,** and **2–23**, were reported to exhibit potent PDK4 inhibitory activities with IC_{50} of 4.0 μM, 11.0 μM and 3.0 μM respectively [78]. These compounds also inhibited the colony formation of HeLaS3 cancer cells in soft agar at 3.0 μM, which is in the similar order of magnitude in terms of the inhibition against PDK4 enzyme.

In 2020, scientists in Korea disclosed an anthraquinone derivative (**2–24**) which allosterically inhibited PDK4 activity [79]. This novel PDK4 inhibitor showed a more potent inhibitory effect against PDK4 than previous inhibitors *in vitro* with an IC_{50} value of 84 nM. Yet, the allosteric binding mode was identified by molecular docking study only. Additionally, the follow-up mechanistic studies revealed that **2–24** exhibited anticancer effect by inducing apoptosis and cell cycle arrest in HCT116 and RKO cells. Moreover, it exhibited good metabolic stability with reasonable pharmacokinetic profile in mouse models.

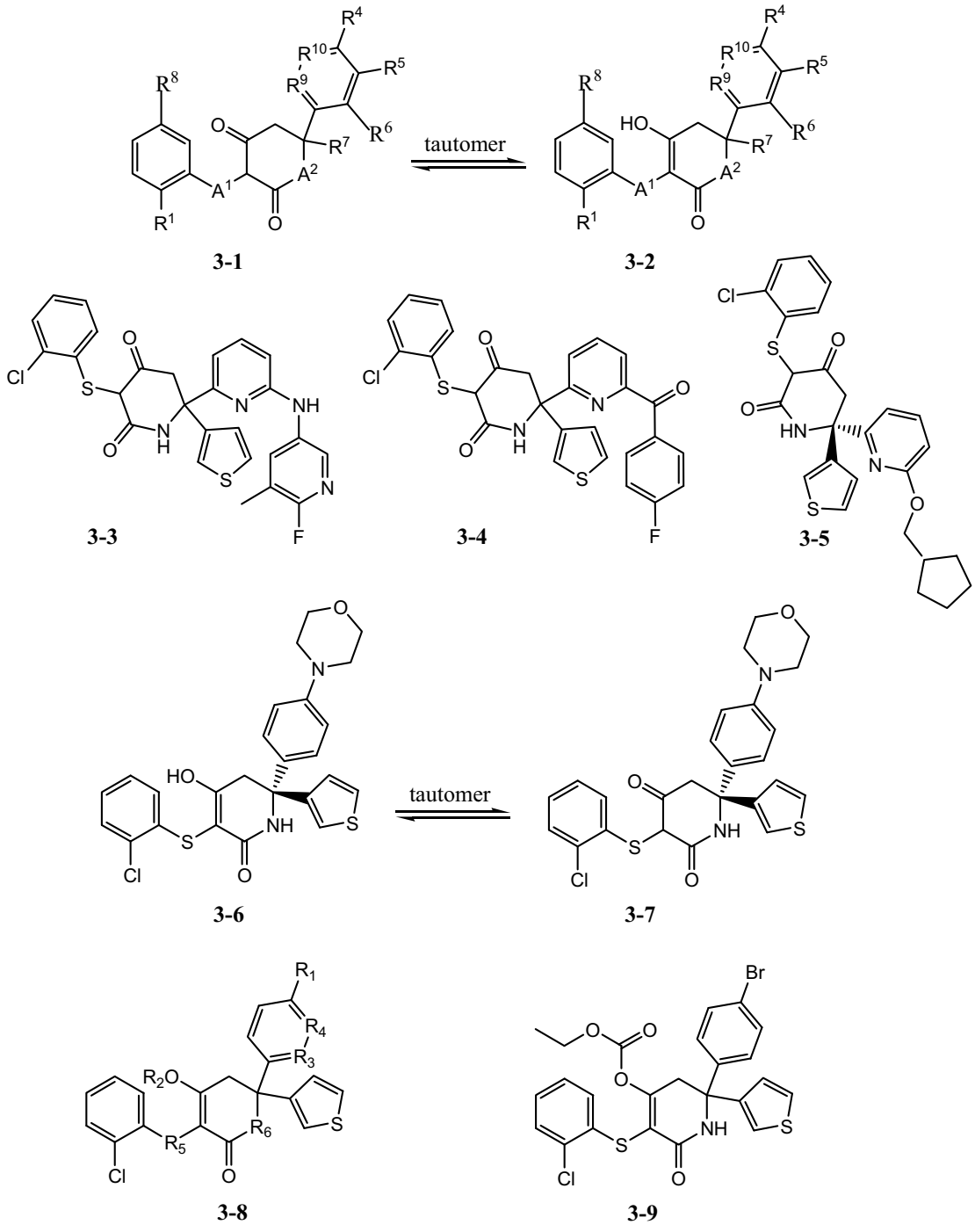

Figure 4. Chemical structures of some LDHA inhibitors.

4. LDHA inhibitors

In many cancer cells, the generation of lactate, which is catalyzed by LDH, is often enhanced. There are four *LDH* genes: *LDHA, LDHB, LDHC,* and *LDHD*. Only *LDHA, LDHB* and *LDHC* are L isomers which are capable of producing the major enantiomer L-lactate in vertebrates. The *LDHC* is a testis-specific gene. LDHA and LDHB, which respectively known as M and H subunits, can form homo- or hetero-tetramers while the others can only form homo-tetramers. The tetrameric LDH isoenzymes LDH1-5 consist of different ratios of M and H subunits. In particular, LDHA has a higher affinity for pyruvate than LDHB. Therefore, LDHA is an important glycolytic enzyme in cancer cells which catalyzes the conversion of pyruvate to lactate coupled with the conversion of NADH to NAD+. Elevated LDHA has been detected in various human cancers and has been recognized as an emerging anticancer target given its remarkable role in cancer glucose metabolism as well as other potential roles such as apoptosis, cancer invasion, metastasis, angiogenesis, and immune surveillance escape [80–82]. In

addition, humans lacking LDHA due to genetic deficiency are generally normal except for exertional myoglobinuria during exercise, suggesting the side effects of LDHA inhibition could be manageable. The two structural binding sites for developing LDHA inhibitors and several reported LDHA inhibitors such as oxamate, gossypol, galloflavin, NHI, and FX-11 for fighting cancer have been well summarized in a recent review [83]. Although the outcomes related to LDHA target were relatively few and no clinical application to date so far, some promising results have been reported in the last five years. Herein, this section is focused on analyzing important patented inventions disclosing LDHA inhibitors in recent years (see Figure 4).

In 2015, Chen et al. at Genentech Inc., US, developed more than 400 novel piperidinedione derivatives as LDHA inhibitors (**3–1** and its tautomeric form **3–2)** [84]. LDHA enzyme inhibition assay revealed that most compounds exhibited potent LDHA inhibitory effects with nanomolar IC_{50} against LDHA as compared with the positive control compound oxamate, which showed an average enzyme inhibition IC_{50} of 57.2 μM. In particular, three of the representative compounds showed superb potency. It was found that **3–3** and **3–4** exhibited IC_{50} of 2.0 nM against LDHA, while **3–5**, which was the most potent inhibitor in the series, exhibited an impressive enzyme inhibition IC_{50} of 1.0 nM. Unfortunately, the inhibitory effects of these compounds on cancer cells have not been reported.

Subsequently, based on **3–1** and **3–2** disclosed in the previous work, researchers led by Thomas O'Brien at Genentech disclosed a related invention focusing on reducing cellular lactic acid production to develop effective small-molecule LDHA inhibitors as well as their tautomeric forms, stereoisomers, geometric isomers, and pharmaceutically acceptable salts [85]. **3–6** and its tautomer **3–7** exhibited IC_{50} of 22 nM in LDHA enzyme assay. Then its effects on cell viability, cell growth and lactate production were tested on two CHO cell lines. Finally, this concept-proof study showed that **3–6** decreased cell growth and lactate producing in a dose-dependent manner within 10–100 μM range. Soon afterward, the inventor team published a paper specifically describing the follow-up *in vitro* and *in vivo* tumor growth tests of **3–6** [86]. It was shown that **3–6** significantly inhibited LDHA activities in MIA PaCa-2 pancreatic cells and the tumor xenografts. Moreover **3–6** inhibited the biochemical activities of LDHA and LDHB with IC_{50} of 3.0 nM and 5.0 nM, respectively, as well as suppressed cancer cell proliferation, with an IC_{50} of 0.4 μM. Notably, only sustained LDHA inhibition could lead to cancer cell death both *in vitro* and *in vivo*. In 2019, an optimized synthesis scheme of **3–6** was disclosed which provided high yield and low-cost synthetic routes for preparing the key intermediates including a high-purity 6,6-aromatic ring-substituted-2,4-piperidinone [87]. However, the most limiting and challenging aspect of **3–6** may ascribe to the high *in vivo* clearance because of metabolic instability which could prevent its applications in the clinic. More intriguingly, the inventors found that pancreatic cancer cells could develop resistance to LDHA inhibition by increasing OXPHOS, implying LDHA inhibitor combined with OXPHOS inhibitor such as phenformin could exhibit synergistic anticancer effects.

In 2018, inventors disclosed a series of compounds (**3–8** and its stereoisomers) aiming to prevent or treat LDHA mediated diseases such as cancer [88]. **3–9** exhibited selectively LDHA enzyme inhibitory effect with IC_{50} of 1.0 μM to 100 μM while more mild inhibition against LDHB with IC_{50} > 200 μM was observed. Further tests on human breast cancer cell lines MDA-MB-468, MDA-MB-231 and pancreatic cancer cell line MIA PaCa-2 showed that **3–9** inhibited cellular lactate secretion and induced cancer cell apoptosis by activating caspase-3/7 in a dose dependent manner with the concentration ranging from 0.0 to 100 μM.

5. Expert opinion

Compared with the diversity and heterogeneity of gene mutation targets, the widespread and pervasive Warburg effect in cancer continues to be considered as an Achilles heel for potential therapeutic development worldwide. From another perspective, although various novel molecular inhibitors specifically targeting cancer aerobic glycolysis are emerging and well documented in the literature, few of them have been successfully transformed into clinical applications for cancer treatment.

Among HK2, PDK, and LDHA, it was found that the patents associated with the development of novel HK2 inhibitor are much less than the other two enzymes over the last decade. Toxicity of normal cells and high dosage required for the current inhibitors remain the showstopper of targeting HK2 in anticancer therapy. In addition to that, due to the highly conserved structure domain of HKs family, the catalytic pockets of HK1 and HK2 are quite challenging to distinguish. Therefore, selective inhibition of HK2 with limited interfere with other isozymes remains to be resolved. In the future, developing highly effective and selective HK2 inhibitors with decent pharmacokinetic properties based on the dissimilarity between HK1 and HK2, such as sensitivity to G6P inhibition [16], could be one promising research direction.

Although there is still no single HK2 inhibitor for clinical cancer treatment, combination treatment of HK2 inhibitors with other compounds targeting elsewhere in glycolysis at this time could be an ideal solution for these problems because decreasing the dosing concentration of each compound reduces the potential toxicity. Similarly, there are patents reporting the combined use of novel inhibitors for PDK and LDHA, as well as with other known anticancer agents. Indeed, it was found that some of the recent inventions largely focused on various drug combination therapies or prodrugs with the specific aims to enhance the anticancer effects while modulating cancer cell metabolism [63,69,89]. It is expected that drug combinations with, either two metabolic inhibitors or one metabolic inhibitor and one anticancer agent, are likely to exhibit synergistic anticancer effects and the reduced dose administration leads to less systemic toxicity, which may be advantageous in developing anticancer therapies. One promising avenue that could be pursued is well-designed prodrug. For example, the prodrug that links dichloroacetate and oxamate could quickly release DCA and oxamate *in vivo* and surprisingly exerted more potent effects than DCA plus oxamate, with reduced undesirable side effects [63].

Despite the considerable number of recently patented novel metabolic inhibitors that have emerged, the toxicological and pharmacokinetic profiles of the disclosed compounds are recognized as the limiting factors. Undoubtedly, these require further attention and optimization to maximize the chance of success in future clinical evaluations. In addition, research on glycolytic inhibitors is also facing various challenges to break through the constraints of patents to expand on novel chemical spaces for developing new structures, enhance the affinity of binding site inhibitors, improve the specificity of binding site inhibitors, and optimize the selectivity of different enzyme subtypes. It is foreseeable that the development of inhibitors of HK2, PDK, and LDHA still has a long way to go. Based on its great potential in the treatment of cancers and metabolic diseases, the design and development of novel inhibitors targeting aberrant glucose metabolism will remain an attractive topic.

Funding

We thank the financial support from the Science and Technology Development Fund, Macau SAR (File no. 0057/2018/A2) and University of Macau (File no. MYRG2019-00034-FHS).

Declaration of interests

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

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