

Phosphoglycerate Dehydrogenase (PHGDH) inhibitors: a comprehensive review 2015-2020

ABSTRACT

The phosphoglycerate dehydrogenase (PHGDH), a metabolic enzyme involved in the serine synthetic pathway (SSP), appears to play a central role in supporting cancer growth and proliferation. PHGDH is a dehydrogenase whose expression in cancers was first demonstrated in 2010. Because its silencing allows a significant reduction in tumor proliferation, it appears to be a promising target in the development of new anti-cancer agents.

Areas covered: In this review, we will detail PHGDH inhibitors that were reported since 2015. These compounds will be ranked according to their chemical class and their site of action. Representative examples of each series will be presented as well as their inhibitory potency *in vitro* and/or *in vivo*. Finally, their most significant biological effects will be detailed.

Expert opinion: Currently, and despite significant efforts, the search for PHGDH inhibitors has not yet led to the development of compounds that can be used therapeutically. The available inhibitors have either too weak inhibitory potency or limited selectivity. Therefore, it seems crucial, given the importance of this enzyme in the progression of cancer but also in other pathologies, to pursue the development of new chemical series.

Keywords: Serine synthetic pathway, targeted therapies, cancer metabolism, Phosphoglycerate Dehydrogenase

1. INTRODUCTION

Among the different strategies to tackle cancer in a potentially more effective and less aggressive way for cancer patients, the last decade witnessed a renew of interest in targeting the very specific metabolism of cancer cells.^{1,2} Hence, cancer cells and their microenvironment reprogram their metabolism in order to optimize their proliferation under nutrient limiting conditions. Understanding these changes and vulnerabilities that they confer to cancer cells is of critical importance, as it can lead to development of new targeted therapies.³ Otto Warburg was the first to demonstrate that tumor cells displayed increased rates of aerobic glycolysis compared to normal cells.⁴ This process allows these cells to produce, in a very fast way, biosynthetic intermediates required for efficient growth.⁵

The serine synthetic pathway (SSP) is a particularly important branch of glycolysis (**Figure 1**). It consists of three consecutive enzymes, phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase 1 (PSAT-1) and phosphoserine phosphatase (PSPH). This biosynthetic pathway is responsible for the production of a non-essential amino acid, serine, from the glycolytic intermediate 3-Phosphoglyceric acid (3-PG) but also for the production of an equimolar amount of reduced nicotinamide adenine dinucleotide (NADH) and α -ketoglutarate (α -KG). In brief, the SSP is involved in diverse cellular processes including methylation reactions, antioxidant production, lipid head group modifications and nucleotide metabolism.⁶

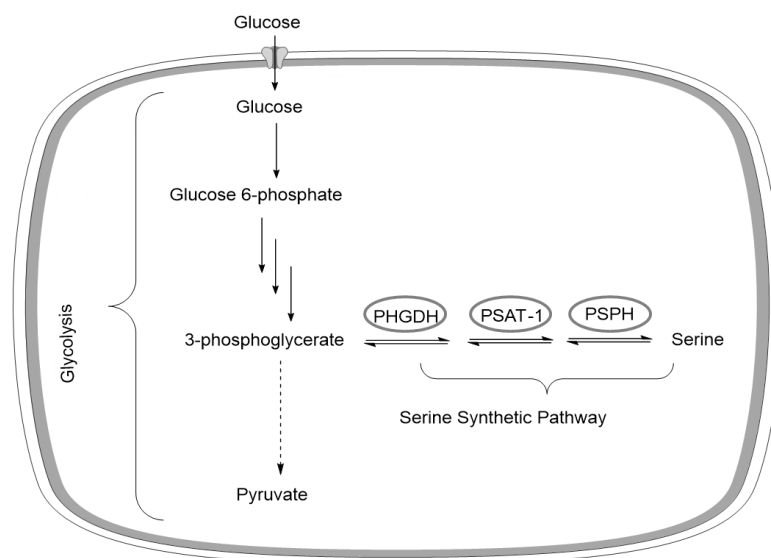


Figure 1: Overview of glycolysis and of the serine synthetic pathway (SSP) with its three constitutive enzymes phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase (PSAT-1) and phosphoserine phosphatase (PSPH).

In 1984, pioneering works of Snell led to the identification of SSP overexpression in rats' hepatomas, suggesting the potential involvement of this pathway in tumor development.⁷ However, it was not until the early 2010's and Pollari's works to eventually consider this significance in human cancer.⁸ An enhanced serine production in highly metastatic breast cancer cell line (MDA-MB-231-SA) was demonstrated in comparison with the less invasive parental cell line, which is mainly related to overexpression of the first two SSP enzymes (PHGDH and PSAT-1). These findings were followed by the discovery of PHGDH overexpression in melanoma and in 70% of estrogen receptor (ER)-negative breast cancers by the Vander Heiden and Sabatini groups, respectively.^{9,10} This research has also highlighted the deleterious impact of silencing this enzyme on tumor proliferation, thus opening new therapeutic perspectives.

In the last years, various studies have shown that PHGDH may play a role in cancer proliferation due to its central role in serine production, although the molecular mechanism involving PHGDH remains poorly understood.^{6,11,12} Serine is used as source of one-carbon unit for *de novo* purine and deoxythymidine synthesis that are necessary for DNA replication during proliferation.¹³ Serine is also involved in the production of glutathione, NADPH and hypotaurine,¹⁴⁻¹⁶ three antioxidants that enable cancer cells to resist the stress associated with aberrant proliferation. Also, PHGDH can stabilize the forkhead box protein M1 (FOXO1) (FOXO1), an oncogenic factor involved in tumor invasion, proliferation and initiation.^{17,18} Finally, PHGDH is also, to a lesser extent, partly responsible for the reduction of α -ketoglutarate to the oncometabolite D-2-hydroxyglutarate (D-2HG).¹⁹

Currently, overexpression of PHGDH has been reported in several types of cancer including breast,¹⁰ pancreatic,²⁰ thyroid,²¹ lung,²² melanoma²³ and glioma¹⁷ cancers to name a few examples.

PHGDH therefore appears to be an interesting target for the development of new anti-cancer agents in the field of tumor metabolism and the last years witnessed the discovery of various series of PHGDH inhibitors that are the subject of this review.

2. INHIBITION OF PHGDH

Over the last 5 years, no less than 12 series of inhibitors were described in several patents or in various peer-reviewed publications. The following paragraphs will detail the lead compounds in each of these series, their activity on isolated enzyme as well as their mode of action when reported.

The most significant biological effects (cellular and *in vivo*) will also be detailed to provide a better understanding of the potential of these compounds.

2.1 NAD COMPETITIVE INHIBITORS

Targeting the NAD binding motif (Rossman fold), common to all the dehydrogenase enzyme family, and further expending these scaffolds into the 3-PG substrate pocket appears as a viable strategy to develop competitive inhibitors.²⁴ In the following sections, we will detail the efforts made by four distinct research groups in identifying such particular inhibitors since August 2016.

2.1.1 *Indole derivative 3.*

In 2016, AstraZeneca reported the first series of orthosteric PHGDH inhibitors based on an indole scaffold (**Figure 2**).²⁵ The discovery strategy of this compound is based on a high throughput screening (HTS) of a library of fragments by crystal soaking. The screening led to the identification of numerous fragments, including the hit **1**, which is characterized by a benzothiophene scaffold binding in the NAD-binding site and which demonstrates an interesting ligand efficiency (LE = 0,39). This fragment was then extended to improve its interaction with the Rossman fold and the 3-PG pocket leading to the development of **2**, a more potent inhibitor with still excellent ligand efficiency (LE = 0,37). Successive rounds of optimisations finally led to a series of indole derivatives presenting submicromolar inhibition. The Lead compound in this series, the indole **3**, exhibits a K_d of 0.18 μ M and a good LE of 0,29. The formation of a key hydrogen bond between the carboxylate of **3** and the Arg236 residue of PHGDH contributed to the high inhibitory potency of this compound.

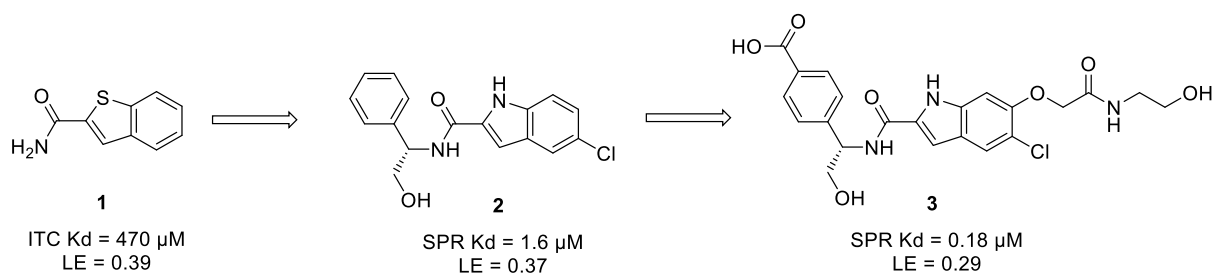


Figure 2: Strategy followed by AstraZeneca to develop the first series of orthosteric PHGDH inhibitor.

2.1.2 AstraZeneca analogues **5** (**1-15**).

More recently, Mullarky *et al.* initially characterized the mode of action of the indole derivative **3** reported earlier by Astra Zeneca with the aim to discover more potent compounds.²⁶ The reversible and competitive profile of this compound was confirmed following competition experiments as well as X-ray diffraction. Their analysis revealed the important role of a hydrogen bond between the amide of **4** and the carboxylate function of Asp175. The importance of the lipophilicity of the indole skeleton was also highlighted. Mapping of these essential interactions led to the development of a novel indole analogue with improved PHGDH inhibition such as compound **5** comprising an oxetane function and characterized by a PHGDH inhibitory potency of 15 nM that is more than 10-fold improvement compared to that of the parent compound. Compound **5** also demonstrated a reduction of serine synthesis in cell-based settings. Finally, although **5** was shown to act in a NAD-competitive manner, it also exhibits a good selectivity towards PHGDH compared to a small library of other dehydrogenases. A prodrug strategy was applied by the authors in order to further increase the cellular efficiency of compound **5** (**Figure 3**). The resulting esterified prodrug (**6**) showed promising effects on proliferation reduction of PHGDH-dependent tumor lineage (Carney cells) but could not be used *in vivo* because of a very low plasma stability.

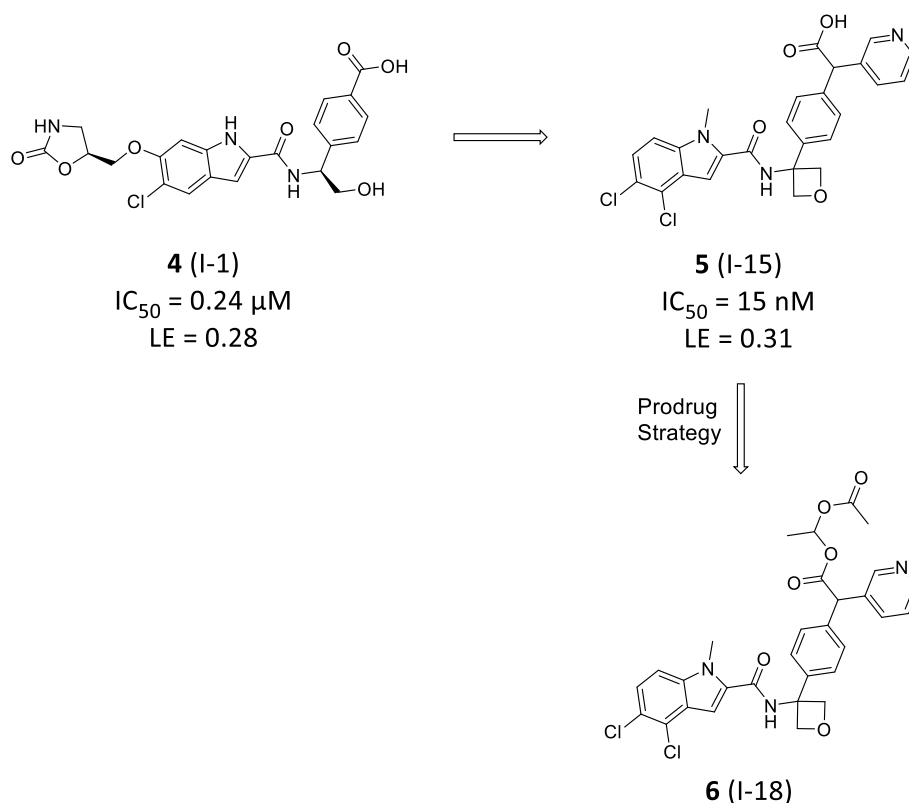


Figure 3: Strategy followed by Mullarky et al. to obtain NAD competitive inhibitors.

2.1.3 Boehringer indole derivative **9 (BI-4924)**.

Using a combined strategy of high throughput screening (HTS) and fragment-based screening (FBS) the team of Weinstabl at Boehringer reported the discovery of very similar indole-based analogues as those initially reported by Astra Zeneca and further optimized by Mullarky **(Figure 4)**.²⁷ The HTS strategy led to the identification of the promising hit compound **7** whereas FBS, notably using biophysical methods such as saturation-transfer difference nuclear magnetic resonance (STD NMR) and microscale thermophoresis (MST), led to the discovery of the pyrazole hit compound **8**. Based on the X-ray crystal structures of each compound with PHGDH, a convergent approach followed by different pharmacomodulations led to **9 (BI-4924)**. This compound exhibits a single-digit nanomolar PHGDH inhibitory potency and was confirmed as a NAD-competitive inhibitor with a high selectivity for PHGDH compared to other dehydrogenases. In addition, it is characterized by excellent microsomal stability. A prodrug strategy was also preconized to increase cell-permeability leading to the esterified analogue **10** which was shown to significantly inhibit *de novo* serine synthesis in several cell models. Unfortunately, as for the compounds developed by Mullarky et al., the weak plasma stability of this compound did not allow its use *in vivo*.²⁸

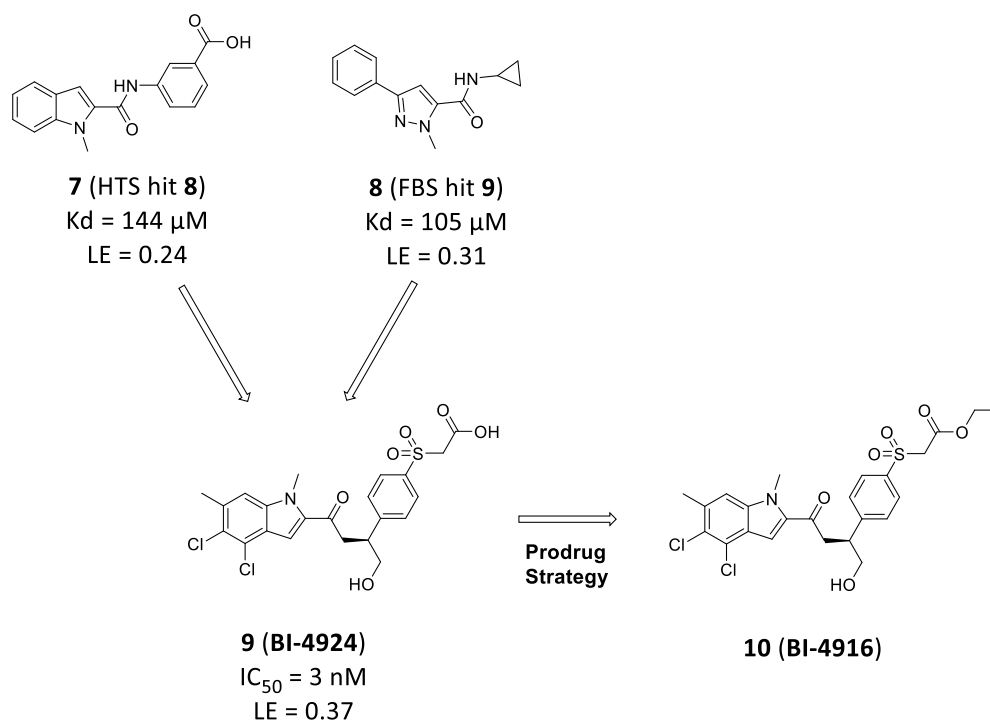


Figure 4: Strategy followed by Weinstabl et al. to obtain **10 (BI-4916)**.

2.1.4 Raze indole derivatives (**11-16**).

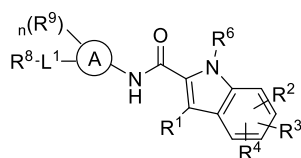
Using the same indole scaffold, Raze Therapeutics recently patented a new series of orthosteric PHGDH inhibitors.²⁹⁻³¹

Figure 5 depicts the most representative compounds within this patent. The different derivatives reported allow to highlight several modifications of the general scaffold.

Some of these modifications seem essential for the activity of the compound. First, the sulfonamide linker in L1 and the piperidine cycle in A cannot be replaced without significantly reducing the PHGDH inhibitory potential of the compounds as exemplified with the cyclobutane **11** and the cyclohexane derivative **12**. It also appears that compounds show better inhibition when the aromatic ring is dichloro-substituted.

Other positions seem to tolerate more chemical diversity. The piperidine ring can be substituted by a nitrile (**14**) or an amide (**13**) without affecting the activity. The indole nitrogen can be demethylated while retaining the inhibition of the target (**16**). Finally, the R8-position also tolerates a large chemical diversity as exemplified with the pyrazine **15**.

Unfortunately, the lack of chemical homogeneity of the reported compounds does not allow to establish more detailed SARs.



Scaffold of the patented series

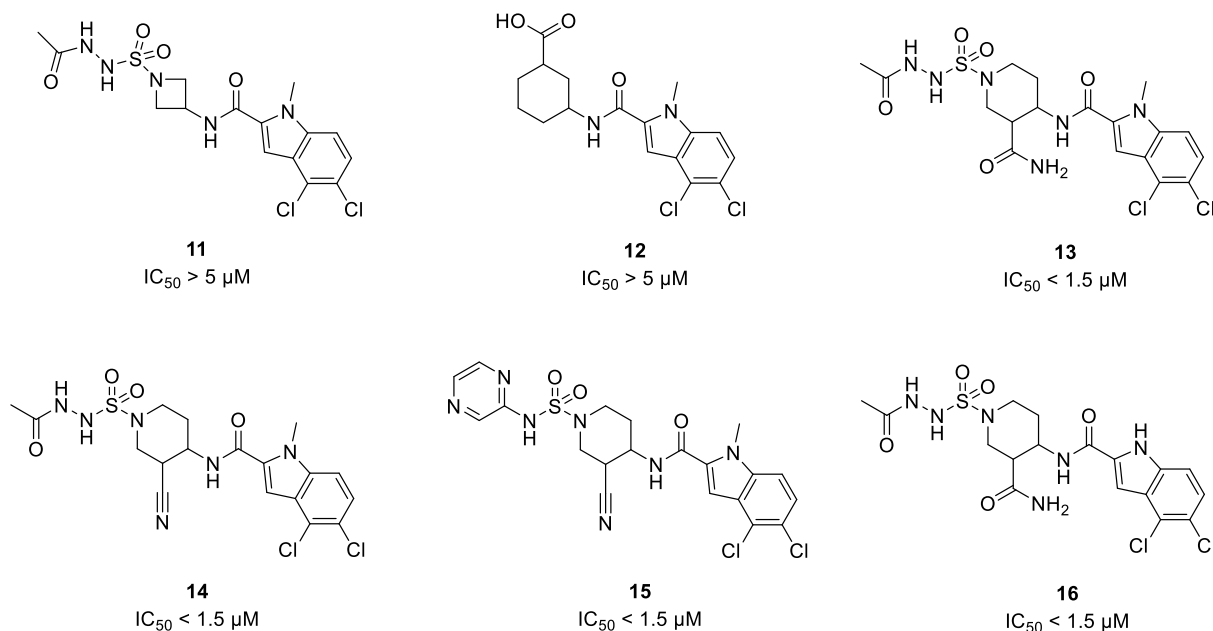


Figure 5: Examples of PHGDH inhibitors reported by Mainolfi et al.

2.2 NON-COMPETITIVE INHIBITORS

Different strategies were also applied to identify allosteric inhibitors of PHGDH. Despite the lack of a complete structure of human PHGDH, several research groups have been able to identify different series of allosteric compounds that will be detailed in the following sections.

2.2.1 Disulfiram.

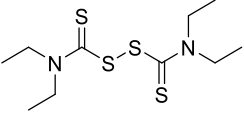
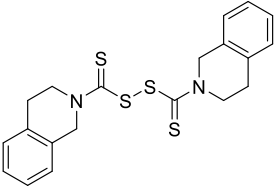
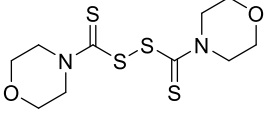
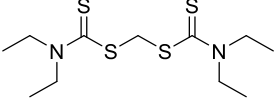
The first evidence that disulfiram (**17**), a well-known aldehyde dehydrogenase inhibitor commercially available under the name Antabuse, was also a potent PHGDH inhibitor came from the Cantley lab in 2016. However, in this very first report no specific data regarding its mode of action were given.³²

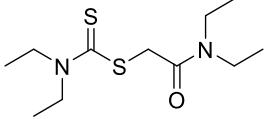
In 2019, our group reported a preliminary structure-activity study around **17** which highlighted the crucial role of the central symmetric bis(dithiocarbamate) motif for the PHGDH inhibition

(Table 1).³³ Indeed, the replacement of this linker by a thioacetamido carbamodithioate, or a methylene dicarbamidodithioate led to poorly active compounds (**20** and **21**). Moreover, we also demonstrated that the diethylamino side chains of this molecule did not seem to be required for PHGDH inhibition (**18** and **19**). Further mechanistic experiments to understand these results led to the demonstration that **17** acts with a covalent allosteric mechanism, probably involving a **disulfide bond** with a specific PHGDH cysteine residue that we have identified to be the Cys116 by mass spectrometry. In fact, the oxidation of Cys116 leads to a disruption of the oligomerization state of the protein, which changes from an active tetramer to an inactive dimer.

Hence, these results are a first step towards novel PHGDH inhibitors acting along an original mechanism of action and reinforce the idea of repurposing Disulfiram in cancer therapy.

Table 1: PHGDH inhibition (IC₅₀) of representative disulfiram analogues **17-21**. Under bracket: 95% confidence interval.

Compound	Structure	PHGDH inhibition (IC ₅₀ , μ M)
17 (Disulfiram)		0.59 [0.37-0.95]
18		0.38 [0.29-0.50]
19		0.21 [0.18-0.25]
20		45.86 [29.15-72.16]

21		> 1 mM
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2.2.2 Thiophene derivative 22 (CBR-5884).

Another series of allosteric covalent PHGDH inhibitors **were** also identified and patented by the Cantley group in 2016.^{32,34,35} This series of molecules obtained following an HTS on the purified enzyme from a library of more than 800,000 compounds and are characterized by a substituted thiophene scaffold. Some of the most representative derivatives of this series are depicted in **Figure 6**.

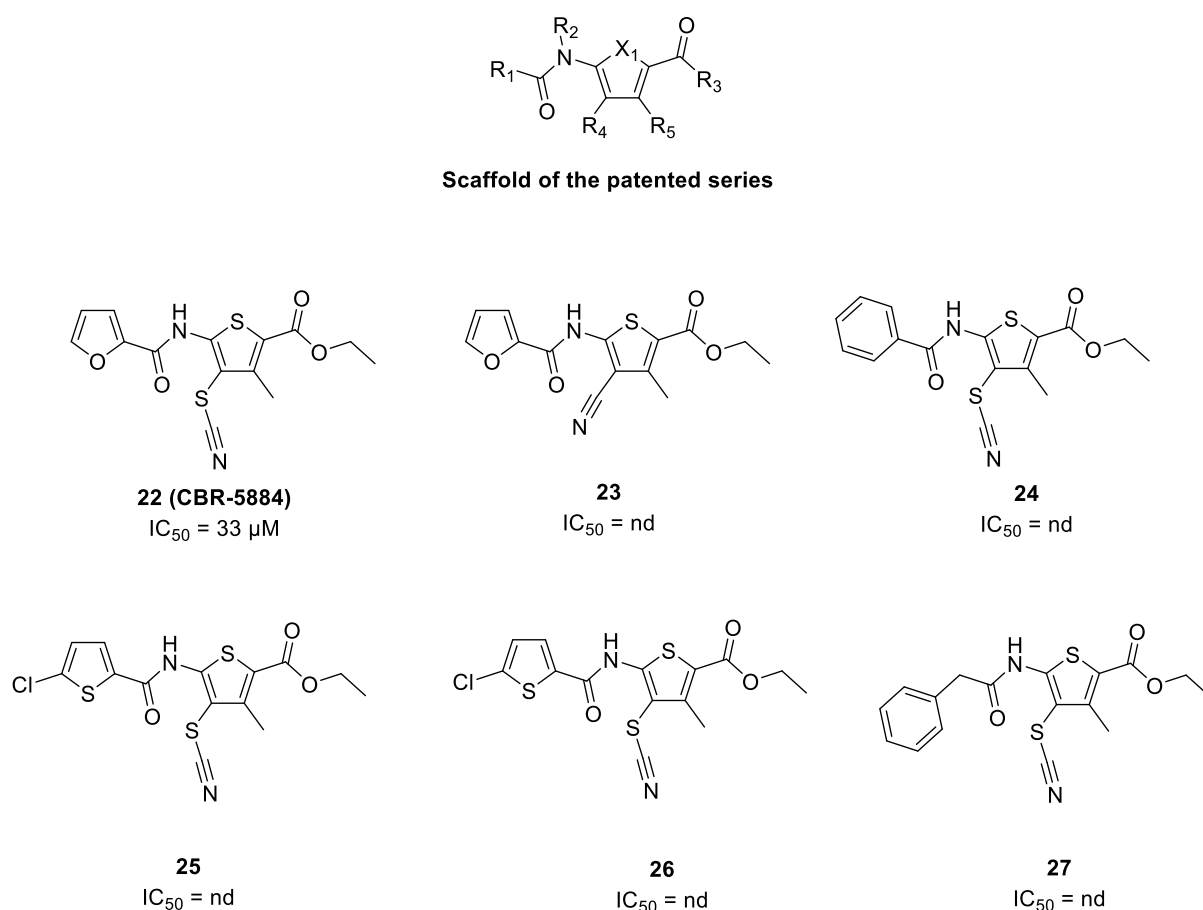


Figure 6: Example of structures of PHGDH inhibitors reported by Mullarky et al.

These compounds, **despite most of them exhibit** an electrophilic thiocyanate moiety that can be targeted by sulfhydryl groups of cysteine residues, have demonstrated a good selectivity on PHGDH versus other NAD-dependent dehydrogenases. Unfortunately, compound **22 (CBR-**

5884) is the only compound whose inhibitory potential and mode of action was reported in this series.

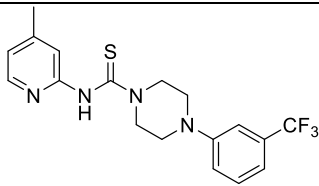
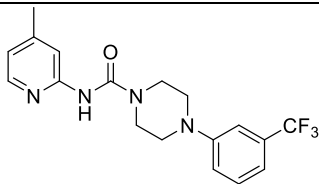
The authors demonstrated that this compound has a non-competitive mode of action with respect to both substrates as well as an efficacy increasing with an increased incubation time, hence probably indicating a covalent allosteric mode of action. However, the site of action of this molecule remains undefined. Interestingly, similar to disulfiram, this compound seems to disrupt the PHGDH oligomerization state.

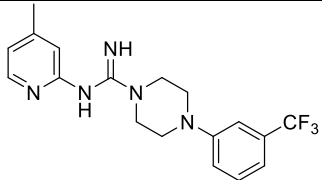
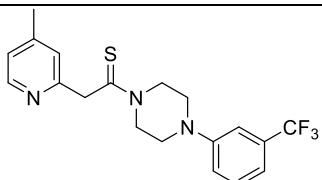
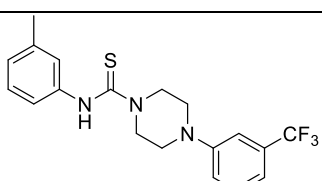
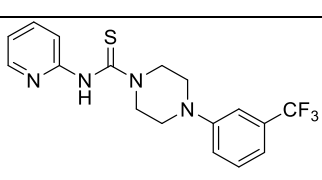
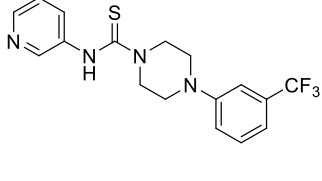
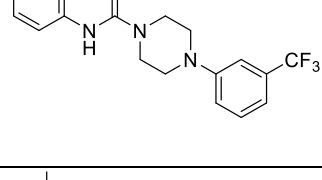
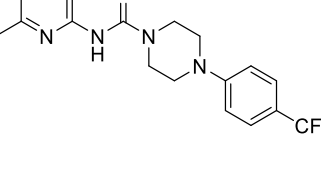
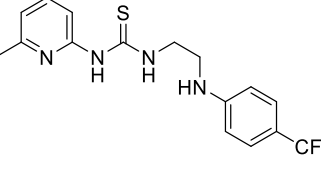
Finally, cell-based experiments using this compound demonstrated its potential to reduce *de novo* serine synthesis by around 30% and selectively inhibit the proliferation of different cancer lines overexpressing PHGDH without affecting cancer cells that did not overexpress the enzyme.

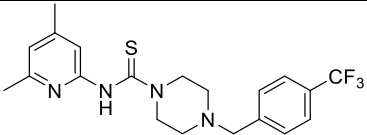
2.2.3 Piperazine-1-thioamide derivatives **38** (**NCT-503**).

The same year, using an HTS strategy similar to the one developed by the Cantley group, the Sabatini team published and secured (WO2016115463A1) a new series of low micromolar inhibitors based on a 2-pyridinyl-N-(4-aryl)piperazine-1-carbothioamides scaffold (**Table 2**).^{13,36,37}

Table 2: PHGDH inhibition (IC₅₀) of representative piperazine-1-thioamide analogues **28-38**

Compound	Structure	PHGDH inhibition (IC ₅₀ , μM)
28		15.3
29		> 57 μM

30		> 57 μM
31		> 57 μM
32		> 57 μM
33		6.8
34		8.9
35		14
36		4.3
37		9.7

38		2.5
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Various chemical modifications of this series were performed to explore the SARs. Briefly, these modifications demonstrated the main role of the thiourea function, as replacement of this moiety by a urea **29**, guanidine **30** or thioamide **31** led to less potent PHGDH inhibitors. Rohde *et al.* then demonstrated that the pyridine side-chain was also important for PHGDH inhibition as its replacement by a phenyl led to a poorly active compound (**32**). It also appears that the position of the nitrogen atom of this pyridine is optimal in the 2-position (**33**) compared to the 3- (**34**) and 4-position (**35**). Finally, although removal of the ring of the piperazine led to a decrease in inhibition (**37**), other modifications around this piperazine moiety underlined the importance of a *para*-substituted phenyl notably with a trifluoromethyl group (**38**) to attain high PHGDH inhibition.

This lead **38** molecule is characterized with an IC₅₀ of 2.5 μM, a good solubility (25 μM in water) and promising microsomal stability (>30min). An investigation of its mode of action revealed a non-competitive and reversible inhibition. Moreover, mutagenesis experiments suggested that this compound would interact with the cysteine (Cys234) of the Rossman fold.

The selective inhibition of tumor proliferation on PHGDH overexpressing lines in vitro and in vivo by this compound appears to be related to a significant decrease in intracellular serine production. Additionally, a decreased incorporation of carbons from both extracellular and intracellular serine into nucleotides is seen.

2.2.4 α -ketothioamide inhibitors.

In 2017, our group also reported a series of PHGDH inhibitors based on a α -ketothioamide scaffold.^{38–40} A detailed pharmacomodulation of the hit compound **42** was undertaken and led to interesting structure-activity relationships (**Figure 7**). At the left side (part A) of the molecule, only aryl groups led to potent PHGDH inhibitors whereas introduction of alkyl groups in this position resulted in inactive derivatives. Substitutions on the aryl moiety are only tolerated in the *para*-position with the chlorinated derivative **54** being the most potent of the α -ketothioamide series. Then, except for the 4-methylpiperidine analogue **56**, it was clear that modulating part C (right-side amino moiety) of the molecule decreased or abolished the

inhibitory potency. Regarding the influence of the linker, only replacement of the α -ketothioamide (part B) with a thioamide (**43**), or a sulphonamide (**45**) led to equally potent PHGDH inhibitors whereas all other modulations led to inactive derivatives ($IC_{50} > 150 \mu M$). Interestingly, with the thioamide and sulphonamide linkers, the introduction of a chlorine in the *para*-position, again, improved the inhibitory potency compared to the non-substituted analogue (**50** and **51**).

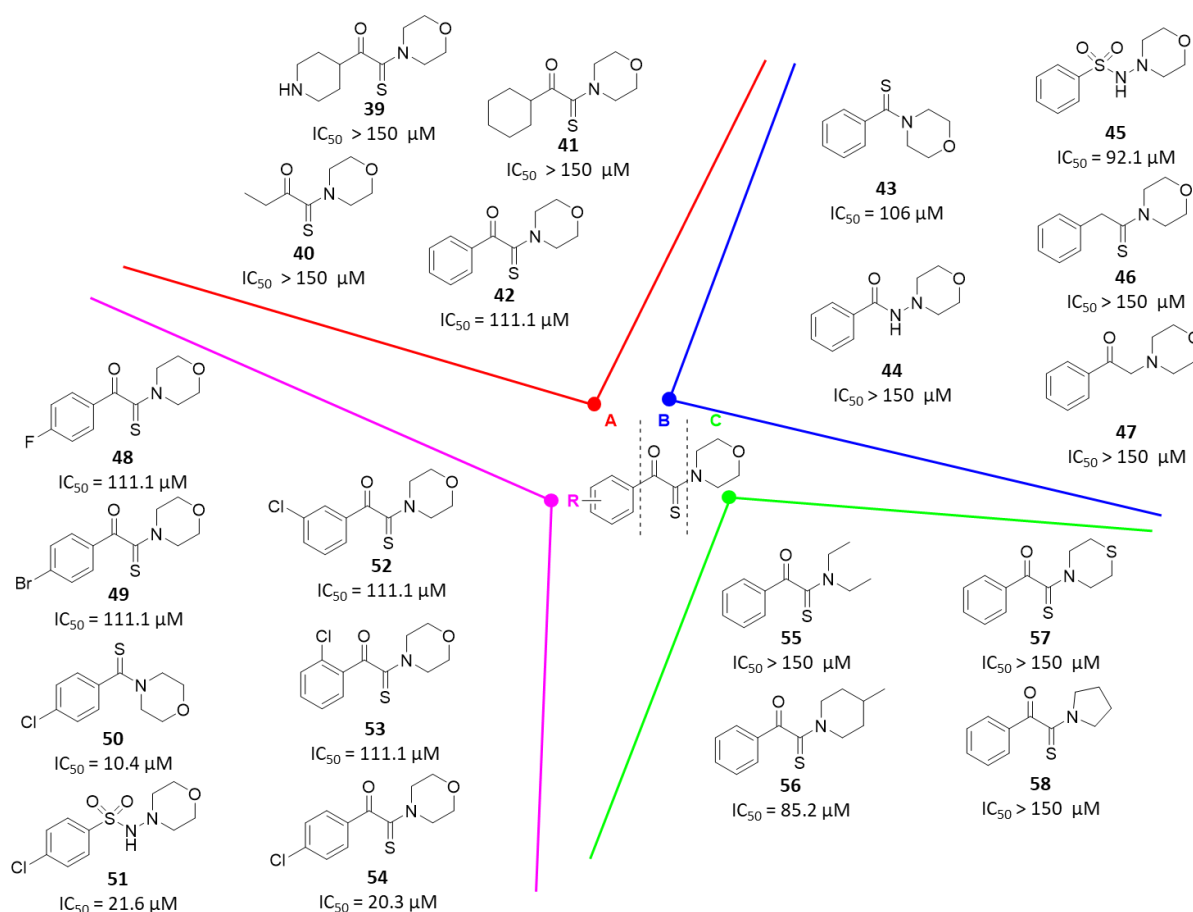


Figure 7: Overview of the structure–activity relationships of the α -ketothioamide scaffold.

To give a deeper understanding of PHGDH inhibition by α -ketothioamide compounds, we next investigated the mechanism-of-action of **42** and **54**. The analysis revealed the irreversible and non-competitive profile for both inhibitors. The mechanism of action of these compounds on PHGDH was further detailed using a photoactivatable diazirine chemical probe.⁴¹ Interestingly, this led to the identification of a previously unknown allosteric site on PHGDH delineated by the ⁵²³QHVTEAFQFHF⁵³³ C-terminal peptide sequence, that is part of the PHGDH ACT domain, as the target binding site.

Of note, a study of related α -ketoamides targeting the HCV Protease suggested that these compounds act irreversibly through the formation of a covalent bond with a serine moiety. One can probably hypothesized that a similar mechanism involving, for instance, a histidine residue on the PHGDH ACT domain account for the irreversible properties of α -ketothioamides on PHGDH (**Figure 8**).

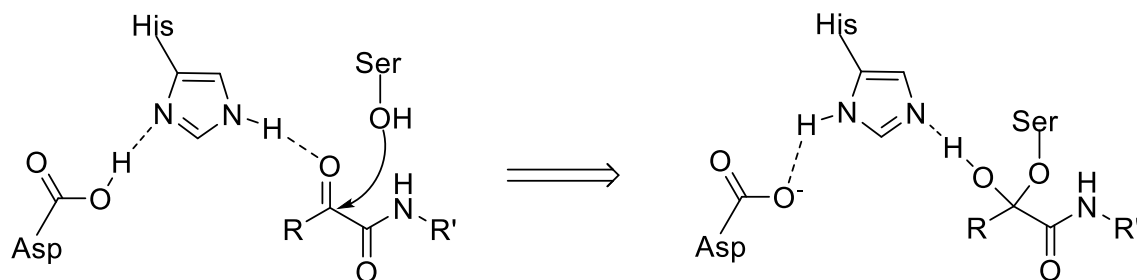


Figure 8: Suggested mechanism of action of α -ketoamide inhibitors on HCV Protease.⁴²

Interestingly, PHGDH engagement in cells investigated by cellular thermal shift assay (CETSA) allowed to demonstrate the interaction between our inhibitors and the target but also confirm our SARs. Moreover, this series of compounds demonstrated a promising selective reducing of the cellular proliferation of tumor cell lines expressing PHGDH (BT-20, Siha, HL-60, MDA-MB-468) without showing any effect on cell lines characterized with a low expression of PHGDH (MDA-MB-231, HL-60 sPHGDH) or on fibroblast (Bj-5ta).

2.2.5 Pyrazole-5-carboxamide derivatives (59-72).

In 2016, a patent from Raze Therapeutics highlighted another series of PHGDH inhibitors. This series is based on the general scaffold depicted in **Figure 9**.⁴³

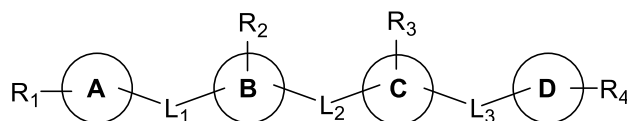
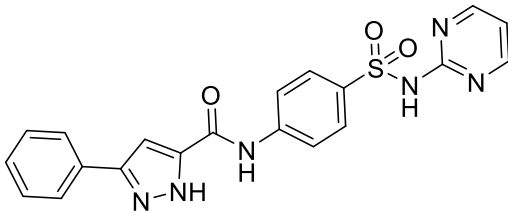
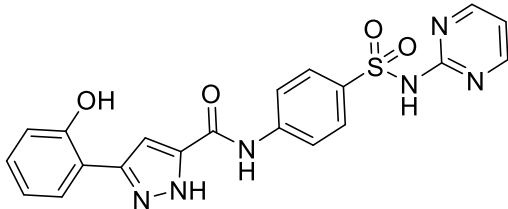
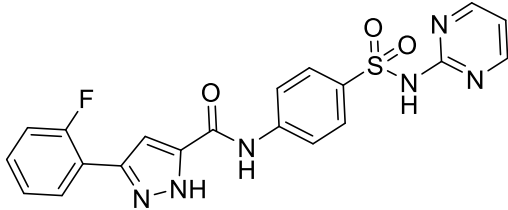
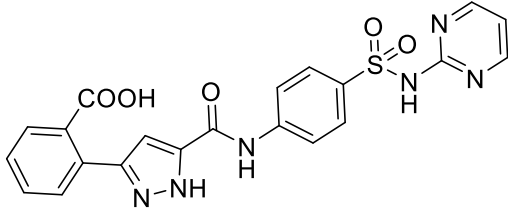
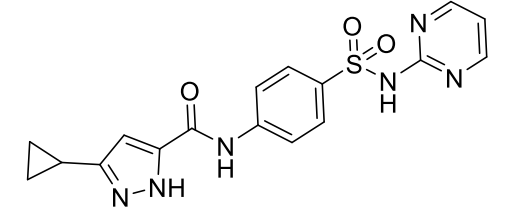


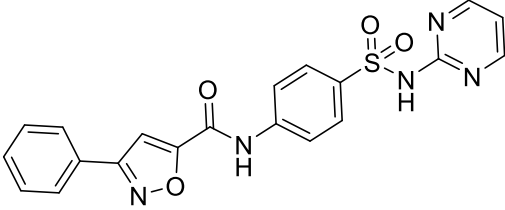
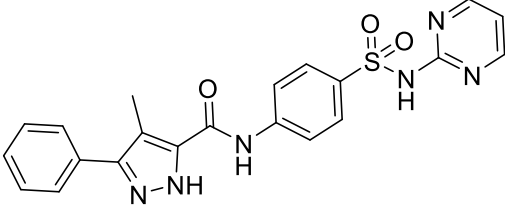
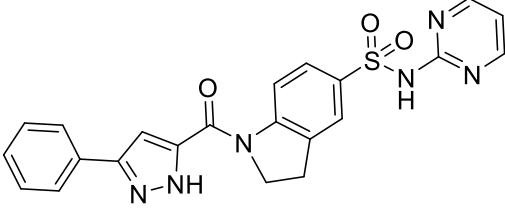
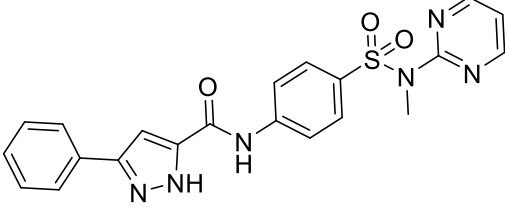
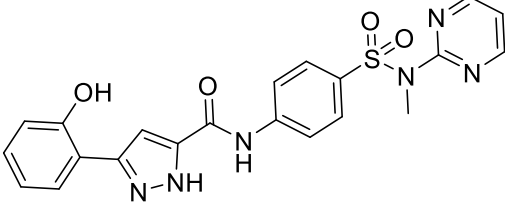
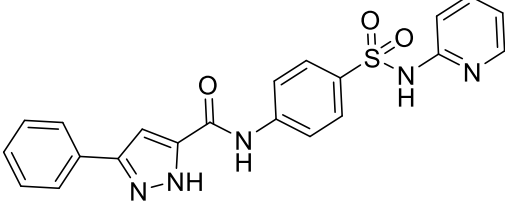
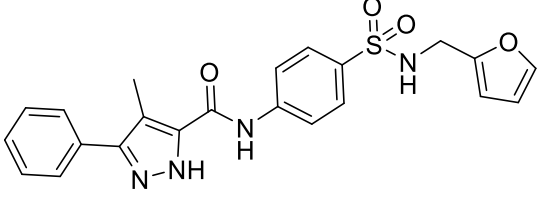
Figure 9: General scaffold of the Raze inhibitors.

Several inhibitors disclosed in this patent permit to draw some SARs. As described in **Table 3**, the *ortho* substitution of the benzene ring (A) does not seems to have a detrimental effect on the activity (**60-61**) with the exception of the substitution by a carboxylic acid moiety **62**. The replacement of this benzene ring by a **cyclopropane moiety** also seems poorly tolerated (**63**). On the opposite, the pyrazole ring (B) appears to be crucial and does not seem to tolerate any modification without dropping the compound activity (**65**). The cyclisation or the replacement of the amido linker (L2) also lead to a significant decrease in the inhibitory potential (**66**). As

depicted by compounds **67** and **68**, a modification, even minor, of the sulfonamide linker (L3) appears to be poorly tolerated. Finally, the pyrimidine cycle (D) is also central in the inhibition of PHGDH because its removal or replacement by other heterocycles like pyridine (**69**) or furan-2-ylmethyl (**70**) lead to a significant drop in activity.

Table 3. SARs of PHGDH inhibitors reported in the patent WO2016/040449A1.

Compounds	Structure	PHGDH inhibition (IC ₅₀ , μM)
59		< 50
60		< 50
61		< 50
62		> 100
63		> 100

64		> 100
65		> 100
66		> 100
67		> 100
68		> 100
69		50-100
70		> 100

Unfortunately, no biological data **has** been described for these inhibitors, preventing further analysis of this series.

2.2.6 Acyl-hydrazones **71** (**PKUMDL-WQ-2201**) and **72** (**PKUMDL-WQ-2101**).

In 2016, two allosteric sites on PHGDH were computationally identified by Wang and co-workers using a cavity prediction algorithm. The first (site I), sharing at least 5 amino acids with the enzyme active site (Gly 78, Val 79, Asp 80, Asn 81 and Val 82), is located at the interface of the enzyme active site and the Rossmann fold, whereas the second (site II), smaller, was identified in the substrate binding cavity. Virtual screening experiments at these allosteric sites led to the identification of 98 compounds of which 2 compounds, **71** (**PKUMDL-WQ-2201**) and **72** (**PKUMDL-WQ-2101**) (**Figure 10**) were shown to bind PHGDH by surface plasmon resonance (SPR) experiments.⁴⁴ Competition experiments as well as site-directed mutagenesis confirmed the interactions between **72** and site I as well as **71** with site II. In addition, cellular assays demonstrated the ability of these two molecules to selectively reduce the proliferation of cancer lines overexpressing PHGDH as well as their potential to significantly reduce intracellular serine and glycine flux. Interestingly, these two inhibitors were also documented to reduce *in vivo* the growth of tumors overexpressing PHGDH. This series of inhibitors is patented.^{45,46}

2.2.7 Natural product derivative **73** (**Azacocone E**).

Following the screening of a library of 600 natural compounds a fluorescent enzymatic assay, Guo and co-workers identified azacocones C and E (**73**) as PHGDH inhibitors with inhibitory potency in the micromolar range without affecting other NAD⁺-dependent enzymes such as isocitrate dehydrogenase 1 (IDH1) (**Figure 10**).⁴⁷ Further microscale thermophoresis (MST) and cellular engagement (CETSA) experiments confirmed the binding of these two compounds with PHGDH. The inhibition profile of **73** was investigated, and a non-competitive mechanism-of-action regarding the two substrates (3PG and NAD⁺) together with a time-dependent inhibition was revealed. Interestingly, the compounds were shown to bind at an allosteric site distant from the substrate binding sites notably via two crucial hydrogen bonds with the arginines 118 and 134. Compound **73** demonstrated a promising inhibition of cell proliferation of cell lines overexpressing PHGDH that was correlated with apoptosis induction. Tumor cell lines with low PHGDH were not affected, thus the *in cellulo* selectivity of this compound could also be demonstrated.

2.2.8 Natural product derivative **74** (**Ixocarpalactone A**).

Similarly, Zheng *et al.* screened a natural compounds library using an isolated enzyme assay to discover new PHGDH inhibitors. This screening identified **74**, a withanolide derivative from *Physalis ixocarpa* as a potent compound (**Figure 10**).⁴⁸ Binding to the target was subsequently confirmed using MST experiments and competition assays revealed a non-competitive mode of action with respect to both substrates. The investigation of the mode-of-action of this compound, using a computer-based homology model of the full-length human PHGDH, suggested a new allosteric site located near the active site. It appears that **74** would present key steric interactions with the Val301, Pro331 and Ile333 residues and hydrogen bonds with Gln302, Asp305, Thr313 and Pro327 delineating this cavity. Cell-based assays revealed that the compound is not toxic for healthy cells but can induce apoptosis of PHGDH-dependent tumor lines. Compound **74** was also shown to inhibit tumor proliferation in vivo.

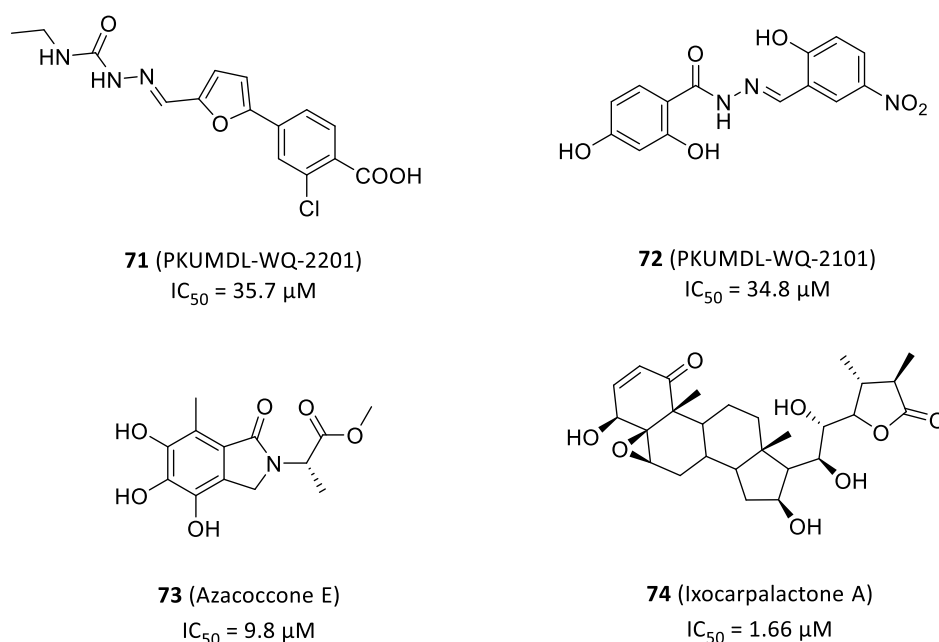


Figure 10: Structure of 3 series of allosteric PHGDH inhibitor.

3. CONCLUSION

Since the publication in 2016 of the first allosteric PHGDH inhibitor (**CBR-5884**) by the Cantley group, numerous inhibitors, acting at allosteric and orthosteric sites **have been** identified and developed.

Following the pioneering work of Astra Zeneca, a number of orthosteric inhibitors derived from a central indole scaffold were identified. Although the high cellular concentration of NAD (millimolar concentration) hampers the development of potent NAD-competitive inhibitors, the

various compounds that have been reported so far exhibit interesting *in vitro* potencies. Unfortunately, and despite the development of prodrugs to mask poorly druggable functions such as carboxylic acids, these compounds have limited *in vivo* activity.

Allosteric compounds are characterized by a great structural diversity as well as by different modes of action that may involve a disruption of the oligomerization state (**Disulfiram**, **CBR-5884**). These compounds, although presenting more reduced activity than orthosteric inhibitors, show relatively interesting *in cellulo* effects and may even reduce tumor proliferation *in vivo* of specific lines (**NCT-503**, **PKUMDL-WQ-2101**). The Table 4 summarizes the different properties of the developed PHGDH inhibitors.

Table 4. Main properties of reported inhibitors targeting PHGDH.

PHGDH inhibition	Mechanism	Range of inhibition	Comments	References
Indole based derivatives	Reversible	μM to nM	Good potency and selectivity	Fuller, N. <i>et al.</i> ²⁵
	NAD-competitive		Poor bioavailability & probably limited efficacy due to high PHGDH cellular conc.	Mullarky, E. <i>et al.</i> ²⁶ Weinstabl, H. <i>et al.</i> ²⁷ Mainolfi, N. <i>et al.</i> ^{29–31}
	Irreversible		Good potency but probable poor selectivity	Spillier, Q. <i>et al.</i> ³³
	Allosteric		Possible PHGDH oligomeric state disruptor	
Disulfiram analogues		Low μM to high nM		
Thiophene derivatives	Irreversible	μM	Reasonable potency and selectivity	Mullarky, E. <i>et al.</i> ^{28,34}
	Allosteric			

			Possible PHGDH oligomeric state disruptor	
Piperazine-1-thioamide derivatives	Possibly covalent reversible Non-competitive	μM	Moderately potent and selective Would target a Cys residue on the Rossman fold Active <i>in vivo</i>	Pacold, M. <i>et al.</i> ^{13,37} Rohde, J. M. <i>et al.</i> ³⁶
α-ketothioamide derivatives	Irreversible Non-competitive	μM	Moderately potent and selective Target a nucleophilic residue located on the PHGDH ACT-binding domain Possible PHGDH oligomeric state disruptor	Ravez, S. <i>et al.</i> ³⁸ Spillier, Q. <i>et al.</i> ^{41,49}
Pyrazole-5-carboxamide derivatives	Undisclosed	μM	Data not available	Saiah, E. <i>et al.</i> ⁵⁰
Acyl-hydrazones analogues	Reversible Allosteric	μM	Moderately potent Suggested to bind at the interface of the enzyme active site and the Rossman fold	Wang, Q. <i>et al.</i> ^{44,45}

Azacoccone E	Time- dependant inhibition	μM	Low potency	Guo, J. <i>et al.</i> ⁴⁷
	Non- competitive		Some selectivity Suggested allosteric site	
Ixocarpalactone A	Non- competitive	μM	Low potency	Zheng, M <i>et al.</i> ⁴⁸
			Suggested allosteric site	

Although still relatively limited, the search for PHGDH inhibitors is experiencing a strong interest from the scientific community for different reasons. In addition to acting on primary tumor growth, it has recently been shown that PHGDH inhibition can significantly reduce metastatic invasion in the brain.⁵¹ Moreover, there is growing evidence that PHGDH also plays an important role in other diseases such as Alzheimer's disease,⁵² cardiovascular diseases⁵³ and fibrosis.⁵⁴ PHGDH inhibition could thus have broader applications than the development of new anti-cancer treatments.

4. EXPERT'S OPINION

Despite the publication of numerous new PHGDH inhibitors over the past five years, none of these compounds entered clinical trials. This is largely due to a lack of selectivity and/or lack of potency.

On one hand, the different compounds targeting the Rossman fold, although presenting an interesting PHGDH inhibitory potency on isolated enzyme *in vitro* (low nanomolar range for the Boehringer derivatives), failed to translate into *in cellulo* or preclinical outcomes. These compounds must cope with high cellular concentrations of NAD (mM range) as well as the presence of many other dehydrogenases hampering their use.

On the other hand, allosteric PHGDH inhibitors currently show medium to low inhibition on the purified enzyme (μM range). Hence, the absence of a fully-resolved crystallographic structure of human PHGDH prevents the rational development allosteric inhibitors. One of the

most challenging step for the development of allosteric compounds remains the formal identification of new sites of action.

Currently, only crystallographic structures of truncated forms of PHGDH were reported, allowing to visualize the active site but unfortunately not all the allosteric sites described so far.⁵⁵ These crystallographic data as well as the different computational models of the enzyme have nevertheless helped identify no less than 4 new allosteric sites controlling PHGDH activity. The two sites identified by Wang *et al.* are located close to the active site and the cofactor binding site and share some common residues with them (Site I and II).⁴⁴ The allosteric site that binds with Ixocarpalactone A is also located at the back side of the active site.⁴⁸ The last allosteric site described is located on the external surface of PHGDH, away from the active site.⁴⁷ These discoveries pave the way for the development of potent allosteric inhibitors.

Furthermore, it is important to note that these allosteric inhibitors, although less potent than the active-site directed ones, demonstrated significantly greater effects in complex biological models and in particular in *in vivo* settings. This demonstrates the advantage of this type of inhibition over compounds competing with high NAD concentration.

Finally, in order to emphasize that, despite the limited efficacy of the compounds available, the SSP remains a major metabolic pathway in cancer proliferation that would greatly benefit from the development of more potent and selective inhibitors, it is important to remind that PHGDH inhibition can act synergically with other anti-cancer treatments. Indeed, several studies have documented that PHGDH inhibition allows to counteract resistance phenomena to conventional cytotoxic chemotherapies, notably by increasing cells sensitivity to oxidative stress. PHGDH inhibition leads to a decrease of antioxidants production that could lead to deficiencies in DNA repair pathways and cause fatal defects in the affected cells. This is the main reason why Bortezomib (myelomas),⁵⁶ Doxorubicin (ER triple negative breast cancer),⁵⁷ Vemurafenib (BRAF V600E mutated melanoma)⁵⁸ and Erlotinib (lung adenocarcinoma)⁵⁹ have seen the restoration of their antiproliferative effect following PHGDH inhibition with the reference inhibitors (**NCT-503** or **CBR-5884**).

ARTICLE HIGHLIGHTS

- PHGDH, the main enzyme of the serine synthetic pathway, is overexpressed in numerous cancer types and has been reported to play a key role in cancer cell proliferation.
- The first *in vivo* data for PHGDH inhibition was reported in 2016 and spurred the development of PHGDH inhibitors for anti-cancer therapies.
- This article discusses the structure and properties of 8 allosteric and 4 orthosteric series of inhibitors published and/or patented since 2015.
- Representative compounds of each series are detailed with structure-activity relationships, mechanism of action (when available) and limitations.
- Besides the field of cancer, PHGDH inhibitors are promising for the treatment of Alzheimer's disease and cardiovascular diseases.

This box summarizes key points contained in the article.

DECLARATION OF INTEREST

Q. Spillier is a postdoctoral research fellow from Wallonie-Bruxelles International (WBI). The authors have no other 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.

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