

Journal Pre-Proof

A Fragment-Like Approach to PYCR1 Inhibition

Kirsty Milne, Jianhui Sun, Esther A. Zaal, Jenna Mowat, Patrick H.N. Celie, Alexander Fish, Celia R. Berkers, Giuseppe Forlani, Fabricio Loayza-Puch, Craig Jamieson, Reuven Agami

PII: S0960-894X(19)30503-7
DOI: <https://doi.org/10.1016/j.bmcl.2019.07.047>
Reference: BMCL 26588

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 19 June 2019
Revised Date: 23 July 2019
Accepted Date: 24 July 2019

Please cite this article as: Milne, K., Sun, J., Zaal, E.A., Mowat, J., Celie, P.H.N., Fish, A., Berkers, C.R., Forlani, G., Loayza-Puch, F., Jamieson, C., Agami, R., A Fragment-Like Approach to PYCR1 Inhibition, *Bioorganic & Medicinal Chemistry Letters* (2019), doi: <https://doi.org/10.1016/j.bmcl.2019.07.047>

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2019 Published by Elsevier Ltd.



A Fragment-Like Approach to PYCR1 Inhibition

Kirsty Milne^a, Jianhui Sun^b, Esther A. Zaal^c, Jenna Mowat^a, Patrick H. N. Celie^{b,d}, Alexander Fish^{b,d}, Celia R. Berkers^{c,e}, Giuseppe Forlani^f, Fabricio Loayza-Puch^{b,g*}, Craig Jamieson^{a*}, and Reuven Agami^{b,g*}

^aDepartment of Pure and Applied Chemistry, Thomas Graham Building, University of Strathclyde, Glasgow, G1 1XL;

^bH5 Division of Oncogenomics, Oncode Institute, The Netherlands Cancer Institute, 121 Plesmanlaan, 1066 CX, Amsterdam, The Netherlands; ^cBiomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research, Utrecht University, Padualaan 8, 3584 CH, Utrecht, The Netherlands; ^dNKI Protein Facility, Division of Biochemistry, The Netherlands Cancer Institute, 121 Plesmanlaan, 1066 CX, Amsterdam, The Netherlands; ^eDepartment of Biochemistry and Cell Biology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 2, 3584 CM, Utrecht, The Netherlands; ^fDepartment of Life Science and Biotechnology, University of Ferrara, 44121 Ferrara, Italy;

^gDepartment of Molecular Genetics, Erasmus MC, Rotterdam University, The Netherlands

KEYWORDS: inhibitor, proline modulation, oncology, tool compound, fragment-based drug discovery

GRAPHICAL ABSTRACT



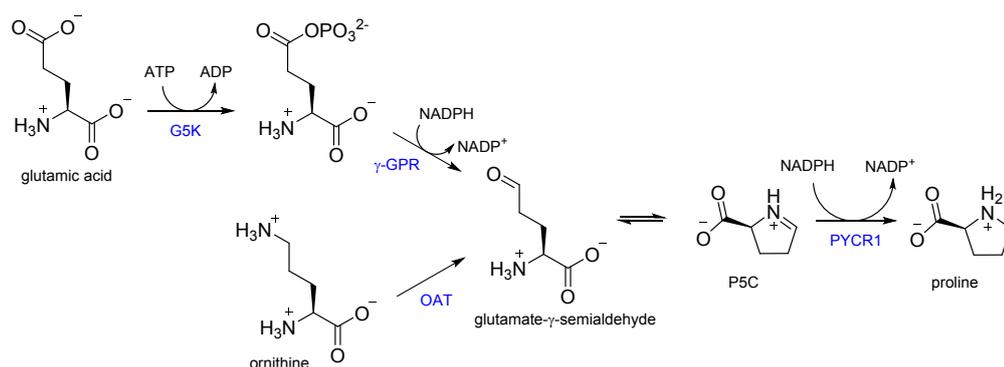
ABSTRACT: Pyrroline-5-carboxylate reductase 1 (PYCR1) is the final enzyme involved in the biosynthesis of proline and has been found to be upregulated in various forms of cancer. Due to the role of proline in maintaining the redox balance of cells and preventing apoptosis, PYCR1 is emerging as an attractive oncology target. Previous PYCR1 knockout studies led to a reduction in tumor growth. Accordingly, a small molecule inhibitor of PYCR1 could lead to new treatments for cancer, and a focused screening effort identified pargyline as a fragment-like hit. We report the design and synthesis of the first tool compounds as PYCR1 inhibitors, derived from pargyline, which were assayed to assess their ability to attenuate the production of proline. Structural activity studies have revealed the key determinants of activity, with the most potent compound (**4**) showing improved activity *in vitro* in enzyme (IC₅₀ = 8.8 μM) and pathway relevant effects in cell-based assays.

Pyrroline-5-carboxylate reductase 1 (PYCR1) is the final enzyme involved in the biosynthesis of proline from both glutamic acid and ornithine, as outlined in Scheme 1.¹ Glutamic acid is firstly phosphorylated by glutamate 5-kinase (G5K), before being dephosphorylated by gamma-glutamyl phosphate reductase (γ -GPR) to produce glutamate- γ -semialdehyde, which exists in an equilibrium with pyrroline-5-carboxylate (P5C). Ornithine is also transformed to the same intermediate through the action of ornithine amino transferase (OAT). P5C is then finally reduced to proline by PYCR1 using the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor, although *in vitro* nicotinamide adenine dinucleotide (NADH) can also serve as a co-factor.

Proline is essential for protein synthesis and plays a role in the secondary structure of proteins.² This amino acid and its derivatives are also the main residues found in collagen, the most abundant protein found within the body.³ However, it also plays a role in maintaining the redox balance of cells through a process known as the proline cycle, outlined in **Figure 1**.³⁻⁵

*corresponding authors: C. Jamieson (craig.jamieson@strath.ac.uk); R. Agami; (r.agami@nki.nl); F. Loayza-Puch (f.loayza-puch@dkfz-heidelberg.de);

Current address (F. Loayza-Puch): Translational Control and Metabolism, Deutsches rebsforschungszentrum Im Neuenheimer, Feld 280, 69120 Heidelberg



Scheme 1: The proline biosynthesis pathway (adapted from reference 1).

P5C is regenerated in the mitochondria by the oxidation of proline by proline dehydrogenase (PRODH), generating adenosine triphosphate (ATP), in the process. Outside of the mitochondria, P5C can be reconverted to proline. This produces a molecule of nicotinamide adenine dinucleotide phosphate (NADP⁺) which is available for use by the pentose-phosphate pathway. The pentose-phosphate pathway will eventually produce ribose-5-phosphate (R-5-P), which can be used to synthesize nucleotides or undergo further transformations to eventually reach fructose-6-phosphate (F-6-P), that is able to produce ATP through glycolysis. These three processes have an essential role in the survival and proliferation of cells.⁶⁻⁸ The pentose-phosphate pathway also reduces NADP⁺ back to NADPH which then supports the disulfide reduction system via thioredoxin reductase and glutathione reductase, minimising production of reactive oxygen species (ROS), again contributing to cell survival.

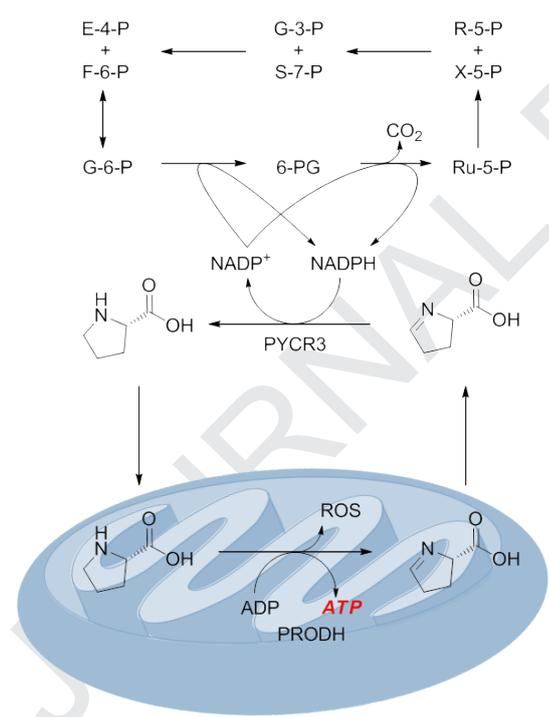


Figure 1: The proline cycle and pentose-phosphate pathway (adapted from reference 2).

In healthy cells, these processes are highly regulated and essential for maintaining normal function.⁵ However, in certain cancers; such as breast, prostate and some lung and skin cancers, PYCR1 is found to be upregulated.⁹⁻¹⁵ This leads to higher levels of proline and exacerbated effects of the proline cycle, with the cells effectively using this method to increase cell survival.^{5,15} If a method of reducing or inhibiting PYCR1 could be discovered, it could provide a new means of treating cancer.

We and others have reported studies in both breast cancer¹² and human prostate¹⁴ cell lines showing that PYCR1 knockout causes phenotypic changes in the cell. In the prostate cancer studies, this resulted in an increase in cell cycle arrest and apoptosis *in vitro*, while in the breast cancer studies a reduction in tumor size *in vivo* was observed. These experiments have shown that modulating PYCR1 directly affects the survival rate of some cancers, validating PYCR1 as an emerging oncology target.

While omission of a gene is a useful tool in its own right, the process is complex and careful selection of vectors and delivery vehicles are required to minimize inflammatory and off-target effects, which can make the process lengthy and expensive.¹⁶⁻¹⁸ Furthermore, gene therapy is not yet an officially approved treatment for any disease and until more is known about the human genome, this is not likely to be approved in the near future.¹⁹ A more tractable approach would utilize a small molecule tool compound, potentially leading to a new approved therapy for cancer treatment.

In order to identify a chemical starting point, a commercially available library of pharmaceutically active compounds (LOPAC®1280, Sigma Aldrich)²⁰ was screened against PYCR1. Based on this screening campaign, pargyline was identified as a fragment-like hit (**1**, Figure 2). Pargyline had a modest IC₅₀ of 198 μM, however, displayed an encouraging ligand efficiency (LE) of 0.42.²¹

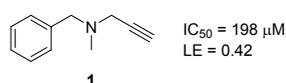
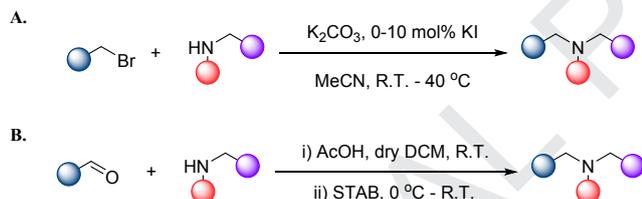


Figure 2: Structure and activity of pargyline.

These considerations, coupled with its low molecular weight, made it an attractive fragment-like hit and a number of analogues were prepared in order to assess the structure activity relationship (SAR). Starting from the appropriate amine, the target analogues were synthesized *via* alkylation or reductive amination with the corresponding bromide or aldehyde, respectively, as outlined in **Scheme 2**.



Scheme 2: A: Alkylation conditions; bromide (0.25 mmol), amine (0.25 mmol), acetonitrile (0.8 M), with potassium iodide added for less activated bromides. B: reductive amination conditions; aldehyde (3 mmol), amine (1 mmol), acetic acid (1 mmol), dry dichloromethane (0.05 M) then sodium triacetoxyborohydride (3 mmol).

The modular structure of pargyline makes it amenable to targeted elaboration of three principal regions: the benzyl, *N*-methyl and propargyl groups. As no crystallographic information of the binding site pargyline occupies within PYCR1 was available, a stepwise approach was adopted in order to assess the impact of changing these substituents on enzyme inhibition using the *in vitro* compound screening assay (see Supplementary Material).

Initially, changes to the benzyl group were examined. Due to their relative abundance in pharmaceutically active compounds, the presence of halogens in various positions around the phenyl ring of the benzyl group was assessed first (**Figure 3A**).²² Pleasingly almost all of the compounds were found to be more active than pargyline at PYCR1, with the exception of the 4-fluoro, 2-chloro and 2-bromo derivatives (**2**, **8** and **9**, respectively) which showed reduced activity. It was also found that the influence on activity was greatest when the halogen was in the 4-position (**3-5**) and weakest in the 2-position (**7** and **9**), with the 3-position being between the two potencies (**6** and **7**). The size of the halogen in the 4-position was also found to have an effect with a general increase in potency observed moving down the group (**2-5**), with the optimum being the 4-bromo system, which was very similar in potency to the 4-iodo moiety, with both of these surpassing the 4-chloro derivative.

In order to follow up on these observations, a number of different functional groups on the benzyl ring were studied, as shown in **Figure 3B**. A range of electron donating and withdrawing groups were analyzed in various positions around the ring. Again, the 4-position was favored with a similar pattern of activity being observed with the nitro group (**14-16**) as with the halogens. Unfortunately, none of the compounds matched the potency of compound **4**, with no clear preference for electron donating (**10-12**) or withdrawing groups (**13-18**) noted from this study.

With more potent analogues observed with increasing size of halogen, it was reasoned that larger groups on the benzyl ring could also result in an increase in potency. Due to the similarities in volume of iodine and phenyl moieties^{23,24} some biphenyls (**19** and **20**) were prepared by Suzuki coupling of compound **4** (See Supplementary Material). Unfortunately, these analogues were less active than both pargyline and compound **4**, suggesting that there are other properties beyond the size of substituent contributing to the increase in potency. This could be linked to a halogen bonding effect, where the strength of an interaction increases with the size of the halogen atom, as observed with analogues **2-4**.²²

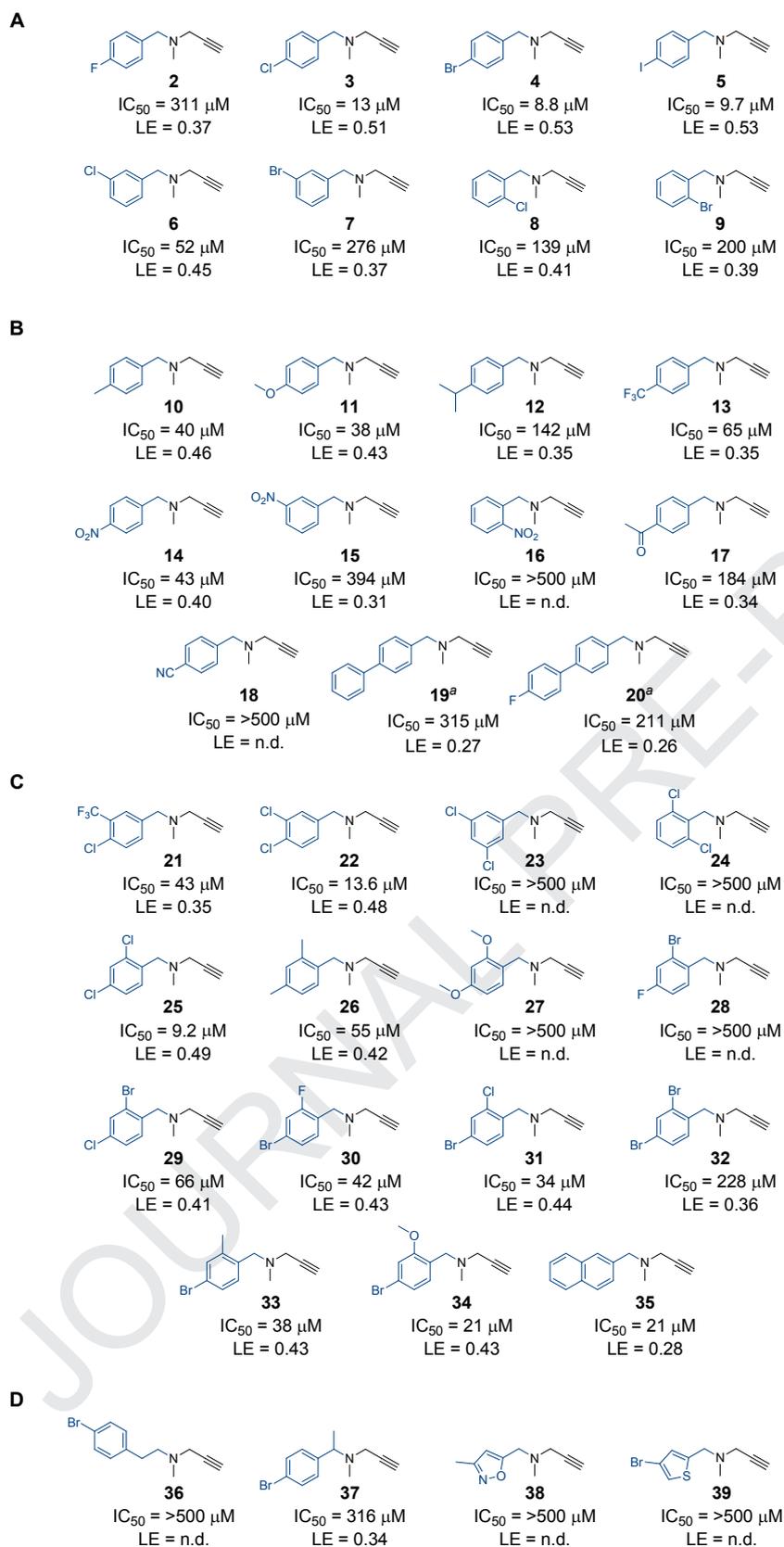


Figure 3: A: SAR of halogen substitutions (blue). B: SAR of non-halogen substitutions (blue). C: SAR of disubstitutions (blue). D: SAR of non-benzyl substitutions (blue). ^a prepared by Suzuki-Miyaura cross-coupling.

Compound **4** was found to have the highest potency, also improving the LE, showing that the presence of a bromine atom on the 4-position of the benzyl group may be beneficial to binding.

With the previous exemplars only representing a single substitution on the benzyl group, it was decided to incorporate a variety of disubstituted analogues as shown in **Figure 3C**. Initially, this was probed using dichlorobenzyl moieties due to their synthetic availability. In comparison to compound **3**, having substitution of the 3- and 4-positions resulted in a compound with a similar potency to the monosubstituted 4-position, while the 3,5- and 2,6- derivatives (compounds **23** and **24**, respectively) were inactive.

Exchanging the 3-chloro of compound **22** for a trifluoromethyl group (**21**) resulted in a less active compound, again suggesting the importance of halogens. However, the 2,4-dichloro species **25** had a much greater potency than compound **3** and a similar potency to compound **4**. This was surprising as a monosubstituted compound in the 2-position was found to be the least active regioisomer in the initial halogen screen (**7** and **9**). This increase in potency could be due to an extra interaction at the, as yet unknown, binding site of the enzyme which complements the interaction at the 4-position. Other disubstituted modifications (**26** – **34**) and naphthyl (**25**) resulted in lower levels of activity.

The final modifications to the benzyl group involved exchanging it for a completely different functional handle to assess its necessity for activity, as outlined in **Figure 3D**. Homologation (**36**) and exchange of the 6-membered benzyl group for 5-membered heterocycles (**38** and **39**) resulted in compounds that were inactive, indicating that the benzyl group is essential for activity. Incorporation of a branched methyl in the benzyl position (**37**) reduced the activity of the compound, suggesting that there may be a steric clash at the binding site.

With the 4-bromobenzyl moiety identified as the optimal group for PYCR1 inhibition, and this motif was carried on throughout the rest of the SAR exploration.

The second group assessed was the *N*-methyl moiety as outlined in **Figure 4**. There was markedly less tolerance in this group than with the benzyl group with only four analogues showing any measurable activity against the enzyme. Carbon chains longer than an ethyl group (**42** and **43**) were found to be inactive as were branched moieties (**44** and **45**). Indeed, of the alkyl substituents only ethyl (**41**) was active, albeit less so than the methyl substituent of compound **4**. Having no substituent on the nitrogen core (**40**) also resulted in a less potent compound. Larger groups such as 4-bromo benzyl (**47**) and isoxazolyl (**46**) were moderately active. However, the higher molecular weight of these compounds, resulted in a lower LE. Larger substituents such as **48** were found not to be tolerated. Tethering the *N*-group to the benzyl group was also not tolerated with the isoindoline and tetrahydroisoquinoline (**49** and **50**, respectively) both being inactive. This could be due to the molecules being constrained in the wrong conformation for binding to the target. The final changes assessed were to the nature of the nitrogen core, with the introduction of an amide (**51**) or sulfonamide (**52**) in the place of a basic tertiary amine. Both of these were inactive suggesting that a basic amine is necessary for activity.

The last modifications examined were alterations to the propargyl moiety, as outlined in **Figure 5**. As with the *N*-methyl group, there was limited tolerance in changes to this group. Fully reducing (**55**) or completely removing the propargyl group (**53**) resulted in inactive derivatives, while reduction to the propenyl analogue (**54**) drastically reduced activity. Introduction of a benzyl (**57**) and cyclopropyl group (**58**) also resulted in inactive compounds and suggests the alkyne component of the molecule is required. Homologation (**56**) and incorporation of a branched methyl to the propargyl unit (**61**) resulted in a considerable reduction in potency. In the case of the branched analogue, this could be due to a steric restriction in the binding site, however further cements the need for a propargyl amine for optimum potency. Finally, the terminal alkyne was exchanged for internal alkynes **59** and **60**. Pleasingly, these compounds retained some degree of potency which decreased when the size of the capping group increased. However, they remained less potent than compound **4**, which again could be attributed to a steric constraint. It should be noted that further structural data would be required to corroborate this hypothesis.

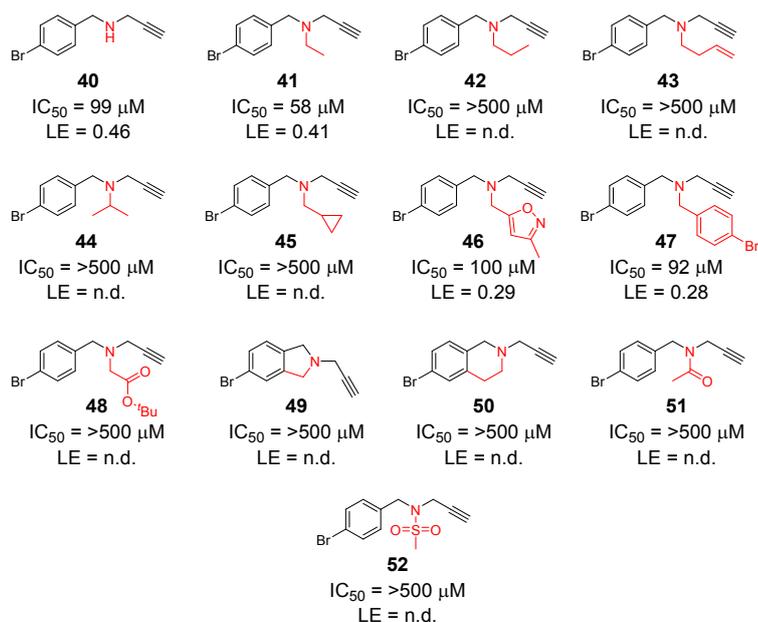


Figure 4: Structure and activity of alterations to the *N*-methyl group (red).

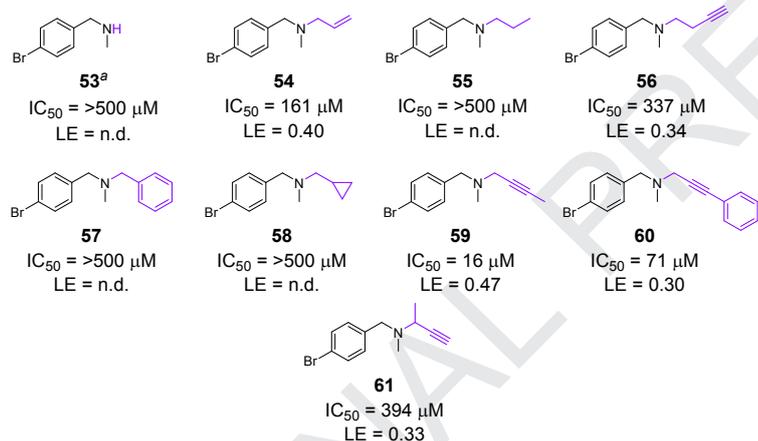


Figure 5: Structure and activity of changes to the propargyl group (purple). ^a Commercially available feedstock

Compound **4** was found to be the most active of all the analogues synthesized and accordingly was advanced for further biological evaluation as the lead compound.

The first study considered was levels of whole cell proline. As the inhibition of PYCR1 should prevent the formation of proline, compound **4** should lower the concentration of intracellular proline. Thus, a human breast cancer cell line (SUM-159-PT) was incubated with both 100 μM pargyline and compound **4** at concentrations of 1, 5, 20 and 100 μM , respectively. The cells were lysed and the quantity of proline (Pro) and essential amino acids were acquired by LCMS. As outlined in **Figure 6a**, pargyline is a modest inhibitor of PYCR1 with approximately 50% reduction in proline levels at a concentration of 100 μM , in comparison to the control, with no effect on the quantities of the other amino acids measured (**Figure S1**, Supplementary Material), with no effects on cellular toxicity apparent at this concentration. Pleasingly, a similar effect is shown with compound **4**, with approximately 50 % reduction in proline shown at 1 μM and no effect on the other amino acid levels. Furthermore, a concentration dependent response was observed with the proline levels decreasing with increasing concentration of compound **4** until 100 μM , where it then increases. The increase in proline levels could be attributed to the limited solubility of compound **4** in the buffer at higher concentrations. In order to show if the lower levels of proline are a result of PYCR1 inhibition, we performed a ^{13}C -glutamine tracer experiment. By culturing cells with $[\text{U-}^{13}\text{C}]$ -glutamine we followed the incorporation of ^{13}C from glutamine, via glutamate, in proline (M+5), **Figure 6b**. After 24 hours incubation with compound **4**, less proline M+5 is present compared to the controls. These data further show that lower levels of proline can be attributed to significant inhibition of synthesis from glutamine.

All of the above data indicates that both pargyline and compound **4** not only inhibit PYCR1, but are also inducing a measurable, pathway-relevant, biological response. To the best of our knowledge this represents the first time such a phenotypic response been observed using small molecules as PYCR1 inhibitors.

In an additional study, two breast cancer cell lines, MDA-MB-231 and SUM-159-PT, were incubated with and without the presence of compound **4** and the number of cells monitored as a measure of the percentage confluence as shown in **Figure 7**. Both cell lines have relatively high PYCR1 levels, and depend on PYCR1 activity for proliferation and tumor formation.¹²

The control experiment, without compound **4**, was run both with and without exogenous proline. Here, there was no difference between the two experiments with both types of cell showing that exogenous proline had no effect on the growth of the cells. However, when compound **4** was dosed at 10 μM with no exogenous proline, the number of cells was significantly reduced in both cases, with a 40% reduction in the MDA-MD-231 cells and a 30% reduction in the SUM159PT cell line; the effect of compound **4** was negated by supplementing proline to the medium. This demonstrated unequivocally that compound **4** is having a phenotypic effect on the cell cycle of both cell types. As normal growth is observed in the presence of exogenous proline it may be reasoned that compound **4** is diminishing the levels of proline within the cell and causing the retardation in growth. As compound **4** is derived from pargyline it does retain residual monoamine-B activity ($\text{IC}_{50} = 0.3 \mu\text{M}$), however, these experiments indicated that the phenotypic effects observed are pathway relevant driven through inhibition of PYCR1. Having stated this, the effects of the compound as an inhibitor at the related PYCR2 and PYCR3 enzymes cannot be excluded at present.

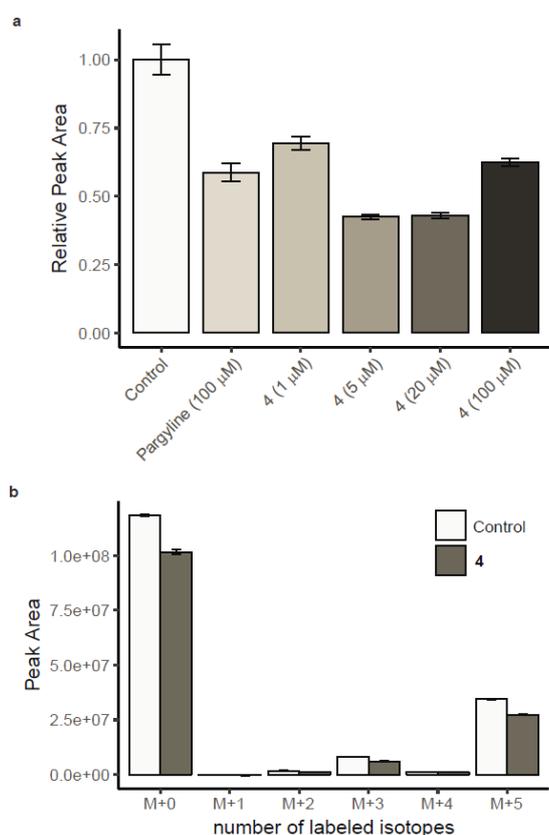


Figure 6. (a) Results of the LC-MS based amino acid assay, showing the quantity of proline present in lysed human breast cancer cells (SUM-159-PT) after incubation with pargyline and compound **4**. (b) Results of the glutamine flux study showing lower levels of M+5 proline after incubation with compound **4** (5 μM).

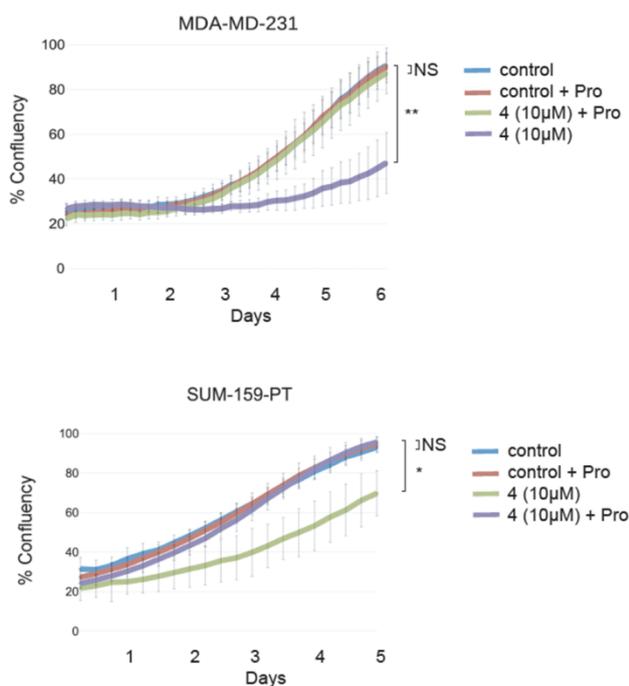


Figure 7: Results of the cell proliferation assay for both MDA-MB-321 and SUM159PT breast cancer cell lines. Controls were performed both with and without exogenous proline showing normal growth in both cell lines. Compound **4** (10 μ M) showed a 40% and 30% reduction in the MDA-MB-231 and SUM159PT cells, respectively. Cell growth was completely rescued in the presence of exogenous proline.

In conclusion, over 60 potential small molecule inhibitors of PYCR1 were synthesized in order to probe the SAR around PYCR1 inhibition. Of all the synthesized analogues, compound **4** (termed Proline Production Inhibitor-1, ProPI-1) was identified as the most potent in the PYCR1 inhibition assay with an IC_{50} of 8.8 μ M, approximately 20 times more potent than pargyline, with the most efficient binding as evidenced by a LE of 0.53. As a result of this PPI-1 was taken forward as a lead into a series of pathway-relevant biological tests and, was found to significantly reduce the levels of proline within a breast cancer cell line and reduce cell proliferation in two different breast cancer cell lines – the first time this has been achieved using a small molecule as a PYCR1 inhibitor. Further work is ongoing to obtain an X-ray crystal structure of ProPI-1 and PYCR1, as well as further target validation through demonstration of efficacy *in vivo*.

ACKNOWLEDGEMENTS

GlaxoSmithKline/Engineering and Physical Sciences Research Council UK (KM); The Dutch Cancer Society KWF 2017-11037 (RA). The National Mass Spectrometry Facility at Swansea University for provision of HRMS data.

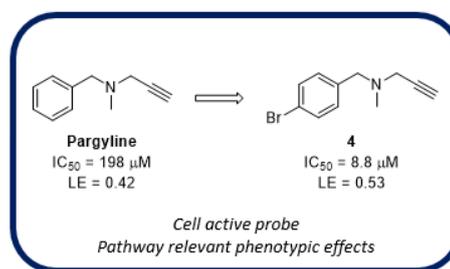
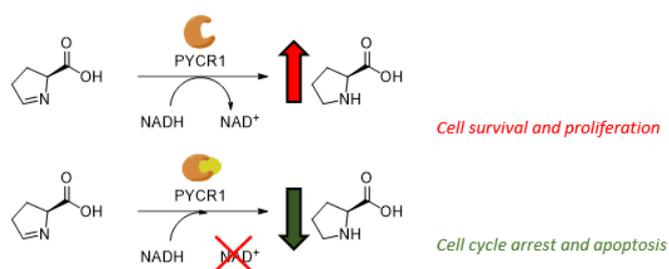
SUPPLEMENTARY MATERIAL

Experimental procedures and characterization data of all pargyline analogues and protocol for PYCR1 testing.

REFERENCES AND NOTES

- Christensen, E. M.; Patel, S. M.; Korasick, D. A.; Campbell, A. C.; Krause, K. L.; Becker, D. F.; Tanner, J. J. *J. Biol. Chem.* **2017**, *292*, 7233.
- Deber, C. M.; Brodsky, B.; Rath, A. In *Encyclopedia of Life Sciences*; John Wiley & Sons, Ltd: Chichester, UK, **2010**.
- Phang, J. M.; Donald, S. P.; Pandhare, J.; Liu, Y. *Amino Acids* **2008**, *35*, 681.
- Liang, X.; Zhang, L.; Natarajan, S. K.; Becker, D. F. *Antioxid. Redox Signal.* **2013**, *19*, 998.
- Tanner, J. J.; Fendt, S. M.; Becker, D. F. *Biochemistry* **2018**, *57*, 3433.
- Song, S.; Jacobson, K. N.; McDermott, K. M.; Reddy, S. P.; Cress, A. E.; Tang, H.; Dudek, S. M.; Black, S. M.; Garcia, J. G. N.; Makino, A.; Yuan, J. X.-J. *Am. J. Physiol. - Cell Physiol.* **2015**, *310*, ajpcell.00092.2015.
- Lane, A. N.; Fan, T. W.-M. *Nucleic Acids Research*. Oxford University Press **2015**, 2466.
- Kim, J. H.; Nam, B.; Choi, Y. J.; Kim, S. Y.; Lee, J. E.; Sung, K. J.; Kim, W. S.; Choi, C. M.; Chang, E. J.; Koh, J. S.; Song, J. S.; Yoon, S.; Lee, J. C.; Rho, J. K.; Son, J. *Cancer Res.* **2018**, *78*, 4482.

9. Craze, M. L.; Cheung, H.; Jewa, N.; Coimbra, N. D. M.; Soria, D.; El-Ansari, R.; Aleskandarany, M. A.; Wai Cheng, K.; Diez-Rodriguez, M.; Nolan, C. C.; Ellis, I. O.; Rakha, E. A.; Green, A. R. *Br. J. Cancer* **2018**, *118*, 258.
10. Cai, F.; Cai, F.; Miao, Y.; Miao, Y.; Liu, C.; Liu, C.; Wu, T.; Wu, T.; Shen, S.; Shen, S.; Su, X.; Su, X.; Shi, Y.; Shi, Y. *Oncol. Lett.* **2018**, *15*, 731.
11. Ding, J.; Kuo, M.-L.; Su, L.; Xue, L.; Luh, F.; Zhang, H.; Wang, J.; Lin, T. G.; Zhang, K.; Chu, P.; Zheng, S.; Liu, X.; Yen, Y. *Carcinogenesis* **2017**, *38*, 519.
12. Loayza-Puch, F.; Rooijers, K.; Buil, L. C. M.; Zijlstra, J.; Oude Vrielink, J. F.; Lopes, R.; Ugalde, A. P.; Van Breugel, P.; Hofland, I.; Wesseling, J.; Van Tellingen, O.; Bex, A.; Agami, R. *Nature* **2016**, *530*, 490.
13. De Ingeniis, J.; Ratnikov, B.; Richardson, A. D.; Scott, D. A.; Aza-Blanc, P.; De, S. K.; Kazanov, M.; Pellecchia, M.; Ronai, Z.; Osterman, A. L.; Smith, J. W. *PLoS One* **2012**, *7*, e45190.
14. Zeng, T.; Zhu, L.; Liao, M.; Zhuo, W.; Yang, S.; Wu, W.; Wang, D. *Med. Oncol.* **2017**, *34*, 27.
15. Cai, F.; Miao, Y.; Liu, C.; Wu, T.; Shen, S.; Su, X.; Shi, Y. *Oncol. Lett.* **2017**, *15*, 731.
16. Selkirk, S. M. *Postgraduate Medical Journal.* **2004**, 560.
17. Kang, X.; He, W.; Huang, Y.; Yu, Q.; Chen, Y. *J. Assist. Reprod. Genet.* **2016**, *33*, 581.
18. Liang, P.; Xu, Y.; Zhang, X.; Ding, C.; Huang, R.; Zhang, Z.; Lv, J.; Xie, X.; Chen, Y.; Li, Y.; Sun, Y.; Bai, Y.; Songyang, Z.; Ma, W.; Zhou, C.; Huang, J. *Protein Cell* **2015**, *6*, 363.
19. Petropoulos, S.; Edsgård, D.; Reinius, B.; Deng, Q.; Panula, S. P.; Codeluppi, S.; Plaza Reyes, A.; Linnarsson, S.; Sandberg, R.; Lanner, F. *Cell* **2016**, *165*, 1012.
20. LOPAC@1280 Sigma-Aldrich
<https://www.sigmaaldrich.com/catalog/product/sigma/lo3300?lang=en®ion=GB> (accessed Oct 5, 2018).
21. Chen, H.; Engkvist, O.; Kogej, T. In *The Practice of Medicinal Chemistry: Fourth Edition*; Academic Press, **2015**, 379.
22. Ford, M. C.; Ho, P. S. *J. Med. Chem.* **2016**, *59*, 1655.
23. Batsanov, S. S. *Inorg. Mater.* **2001**, *37*, 871.
24. Stepan, A. F.; Subramanyam, C.; Efremov, I. V.; Dutra, J. K.; O'Sullivan, T. J.; Dirico, K. J.; McDonald, W. S.; Won, A.; Dorff, P. H.; Nolan, C. E.; Becker, S. L.; Pustilnik, L. R.; Riddell, D. R.; Kauffman, G. W.; Kormos, B. L.; Zhang, L.; Lu, Y.; Capetta, S. H.; Green, M. E.; Karki, K.; Sibley, E.; Atchison, K. P.; Hallgren, A. J.; Oborski, C. E.; Robshaw, A. E.; Sneed, B.; O'Donnell, C. J. *J. Med. Chem.* **2012**, *55*, 3414.



Highlights

- higher levels of protein are found in certain types of cancer
- this is attributed to overexpression of a key protein involved in proline biosynthesis
- focused screening has for the first time identified a small fragment-like inhibitor (ProPI-1) of this protein target

- **mechanistic studies show ProPI-1 can attenuate proline biosynthesis in relevant cell types**
- **phenotypic studies reveal that ProPI-1 can control cell proliferation in two different breast cancer cell types**

JOURNAL PRE-PROOF