PAPER

Check for updates

Open-chain thiamine analogues as potent inhibitors of thiamine pyrophosphate (TPP)-dependent enzymes†

Alex H. Y. Chan, [ID] ‡ Terence C. S. Ho‡ and Finian J. Leeper [ID] *

A common approach to studying thiamine pyrophosphate (TPP)-dependent enzymes is by chemical inhibition with thiamine/TPP analogues which feature a neutral aromatic ring in place of the positive thiazolium ring of TPP. These are potent inhibitors but their preparation generally involves multiple synthetic steps to construct the central ring. We report efficient syntheses of novel, open-chain thiamine analogues which potently inhibit TPP-dependent enzymes and are predicted to share the same binding mode as TPP. We also report some open-chain analogues that inhibit pyruvate dehydrogenase E1-subunit (PDH E1) and are predicted to occupy additional pockets in the enzyme other than the TPP-binding pockets. This opens up new possibilities for increasing the affinity and selectivity of the analogues for PDH, which is an established anti-cancer target.

Introduction

Thiamine pyrophosphate (TPP)-dependent enzymes encompass a diverse range of catalytic activities but they all require the coenzyme TPP as its ylide **1** to catalyse the cleavage and formation of bonds adjacent to the carbonyl group of the acyl-donor substrate. For example in pyruvate dehydrogenase complex E1-subunit (PDH E1) an acetyl group is transferred from a donor (pyruvate) to an acceptor (lipoamide) through a ping–pong mechanism *via* intermediates **2–4** (Fig. 1a).[1] Using thiamine/TPP analogues as small-molecule inhibitors has been a well-established approach to studying and/or manipulating cellular pathways involving TPP-dependent enzymes.[2–20] The structures of three such inhibitors **5–7** are shown in Fig. 1b.[2,14,15] They all possess a neutral central ring to capture the strong stabilising interactions between the enzyme and the catalytically active high-energy TPP ylide **1**.[7] However the need to make the central ring often lengthens the synthesis considerably. For this reason, many previous studies have focussed on an easily synthesised triazole as the central ring.[19,20] In this paper we question the need for a central ring by synthesising "open-chain" thiamine/TPP analogues.

Some open-chain thiamine analogues that inhibit TPP-dependent enzymes in cell-based studies have been reported,[4] but they were operating as prodrugs: a ring-forming step (and enzymic pyrophosphorylation) was required for enzyme inhibition. He *et al.* have replaced the central ring with urea[17] and *N*-acylhydrazone[18] groups and these compounds showed potent inhibition of PDH E1 from *Escherichia coli* (and two other bacteria) but much less inhibition of mammalian PDH E1.[18] Our focus in this paper is on inhibition of mammalian PDH E1 as recent papers have shown that certain cancers over-express PDH E1[21–23] and a small-molecule inhibitor of PDH E1 suppresses development of one of these types of cancer in a mouse model.[23] Devimistat (CPI-613), a lipoic acid derivative which targets PDH E1, shows strong antitumor activity against several cancers[24] and has progressed into Phase III clinical trials.[25,26] However, the inhibitors used in these studies were relatively unselective: fluoropyruvate[23] inhibits a wide range of TPP-dependent enzymes, while devimistat[24–26] targets all α-ketoacid dehydrogenases. There is, therefore, a need to develop selective inhibitors that are more easily synthesised than **6**, for example.

We report herein open-chain thiamine analogues that bind in the TPP binding site and potently inhibit TPP-dependent enzymes. Some of our analogues are predicted by molecular docking to occupy alternative parts of the active site that are not involved in binding TPP. We believe that if TPP analogues could be designed that occupy all the available binding pockets, then they would be extremely potent and selective inhibitors of PDH E1.

Yusuf Hamied Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK. E-mail: fjl1@cam.ac.uk

†Electronic supplementary information (ESI) available: Methods and results for enzyme assays and computational docking; synthetic methods, compound characterisation and NMR spectra. See DOI: https://doi.org/10.1039/d3ob00884c
‡Contributed equally.

This journal is © The Royal Society of Chemistry 2023

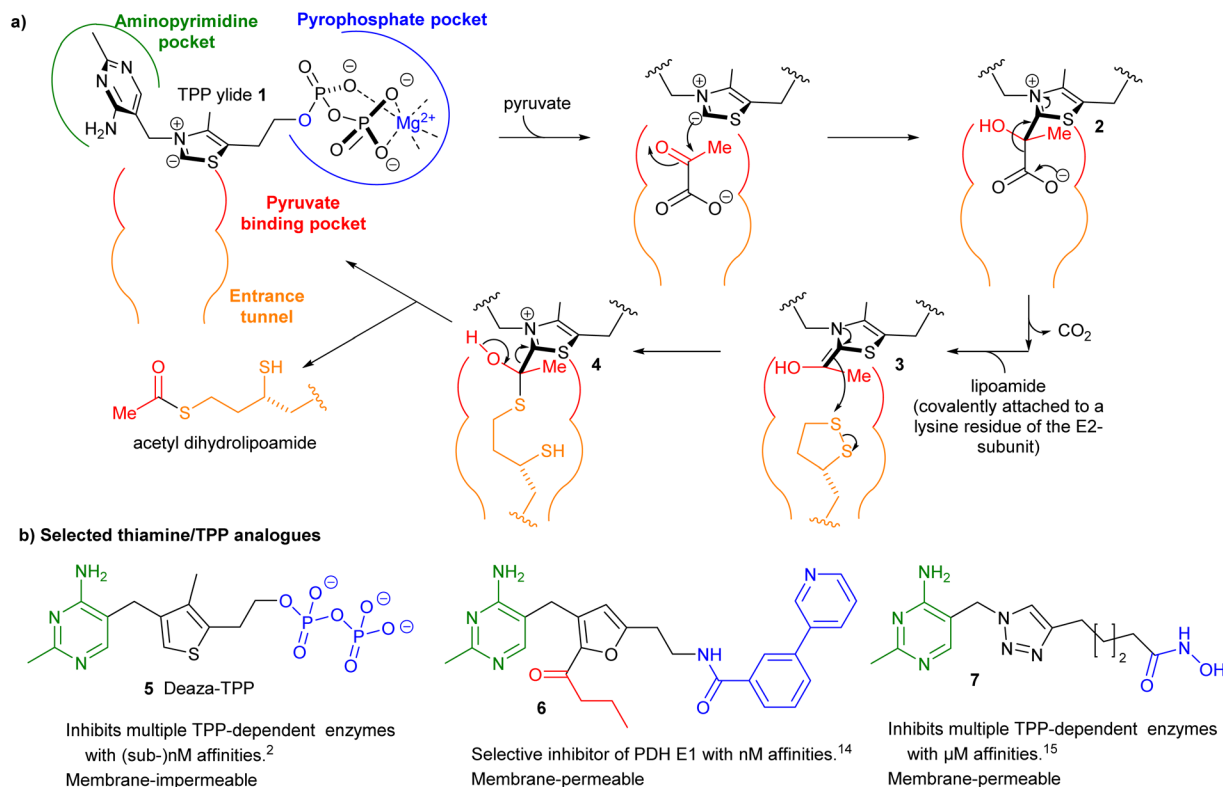*Org. Biomol. Chem.*, 2023, **21**, 6531–6536 | **6531**

Fig. 1 (a) Schematic depiction of the active site and mechanism of PDH E1. The relative position of the binding pockets in the active site is outlined. (b) Selected examples of thiamine/TPP analogues. The coloured substructures of the selected examples indicate the pockets that they occupy.

Results and discussion

Our exploration of ring-opened TPP analogues was inspired by the conventional triazole-bearing compounds **12a–c**. Bis-pyrimidines **12a–c** were prepared *via* coupling of amine **9** with alkynoic acids **10a–c** and then copper-catalysed alkyne–azide cycloaddition (CuAAC) between the resulting alkynes **11a–c** and azide **8**[12] (Scheme 1). Varying lengths of alkynes **10** were
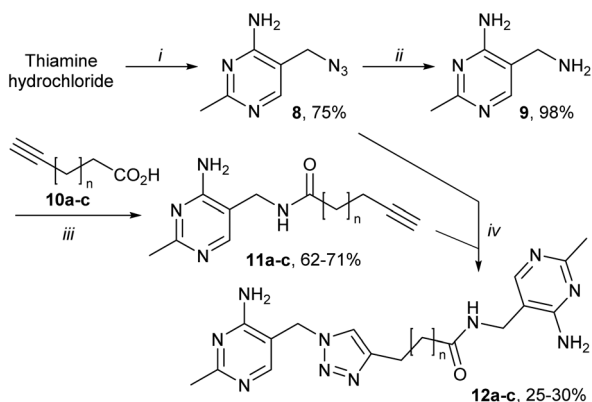
Scheme 1 Synthesis of bis-pyrimidines **8a–c**. Reagents and conditions: (i) NaN_3, Na_2SO_3, H_2O, 65 °C; (ii) $H_2(g)$, 10% Pd/C, MeOH, RT; (iii) **10a–c**, DCC, DMAP, DMF, RT; (iv) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, *t*-BuOH, H_2O, 40 °C. For all compounds: **a** n = 1, **b** n = 2, **c** n = 3.

used to find the optimum linker length to allow one pyrimidine ring to bind in the aminopyrimidine pocket and one in the pyrophosphate pocket. Tested on porcine PDH E1, the longest bis-pyrimidine (**12c**) had the highest potency of the three (Table 1). *In silico* studies showed the expected binding mode of **12c** in the TPP pocket (Fig. 2a and S2†) but also a second possible binding mode (with slightly lower docking score) in which the pyrimidine–CH_2–amide motif occupies the binding region of TPP's pyrimidine–CH_2–thiazolium (Fig. 2b and S2†). This alerted us to the possibility that open-chain analogues could be effective inhibitors. *In silico* docking studies also suggested that the aminopyrimidine ring in the aminopyrimidine pocket provided far more binding than the aminopyrimidine ring in the pyrophosphate pocket (Fig. S3†). In a previous paper[14] we tested the effect of changing the substituents on the aminopyrimidine ring, but any change weakened the binding, so in the current study we kept the substituents unchanged.

To test the hypothesis, we replaced pyrimidine–CH_2–triazole of bis-pyrimidines **12a–c** with HOOC–CH_2–triazole (**14a–c**) because a carboxylate group might interact ionically with the Mg^{2+} in the pyrophosphate pocket (Fig. 2b). Unfortunately, **14a–c**, synthesised in one step from **11a–c** and azidoacetic acid **13** (Scheme 2), were much weaker inhibitors than **12a–c** (Table 1). Given that the second aminopyrimidine of **12c** binds well in the pyrophosphate pocket, a benzyl group was introduced into **14a–c**. Carboxylates **18a–c** were synthesised from

6532 | *Org. Biomol. Chem.*, 2023, **21**, 6531–6536

This journal is © The Royal Society of Chemistry 2023

Organic & Biomolecular Chemistry

Paper

Table 1 Summary of inhibitory activities on PDH E1

Compounds	Inhibition[a,b](%) [Compound] : [TPP]			$IC_{50}^{a,c}$ (µM)	vs. TPP[d]
	5 : 1	1 : 1	1 : 5		
12a	53 ± 2	20 ± 2	<15	42 ± 5	0.24
12b	73 ± 3	32 ± 2	<15	20 ± 4	0.50
12c	82 ± 3	48 ± 2	18 ± 4	12 ± 1	0.83
14a	27 ± 4	ND	ND	ND	ND
14b	33 ± 3	ND	ND	ND	ND
14c	28 ± 5	ND	ND	ND	ND
17a	52 ± 2	18 ± 2	ND	ND	ND
17b	61 ± 3	22 ± 3	ND	ND	ND
17c	54 ± 4	19 ± 4	ND	ND	ND
18a	44 ± 3	<15	<15	ND	ND
18b	52 ± 2	20 ± 4	<15	ND	ND
18c	35 ± 4	<15	<15	ND	ND
21a	60 ± 2	23 ± 2	<15	ND	ND
21b	72 ± 3	32 ± 2	<15	ND	ND
21c	80 ± 2	45 ± 3	<15	ND	ND
22a	>90	67 ± 2	27 ± 3	4.9 ± 0.6	2.0
22b	>90	77 ± 3	35 ± 2	3.8 ± 0.5	2.6
22c	80 ± 3	51 ± 4	20 ± 3	9.5 ± 0.7	1.1
23a	ND	52 ± 3	ND	ND	ND
23b	ND	60 ± 2	ND	ND	ND

[a] Data are the means of measurements in three technical replicates. [b] Percentage inhibition determined for compounds at 50 µM with [TPP] = 10 µM (for 5 : 1); at 10 µM with [TPP] = 10 µM (for 1 : 1); at 10 µM with [TPP] = 50 µM (for 1 : 5). [c] IC_{50} values (µM ± SEM) determined at [TPP] = 10 µM (refer to Fig. S1† for IC_{50} curves). [d] Affinity of the compound relative to that of TPP (= [TPP]/IC_{50}). ND, not determined.
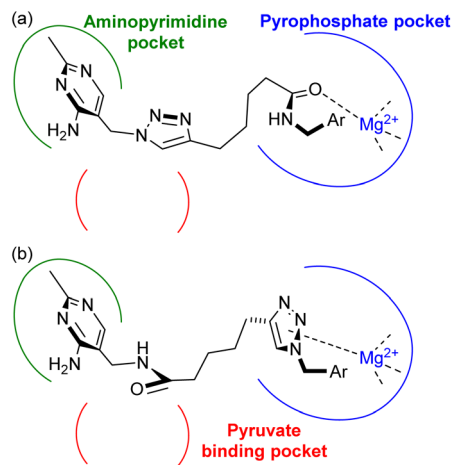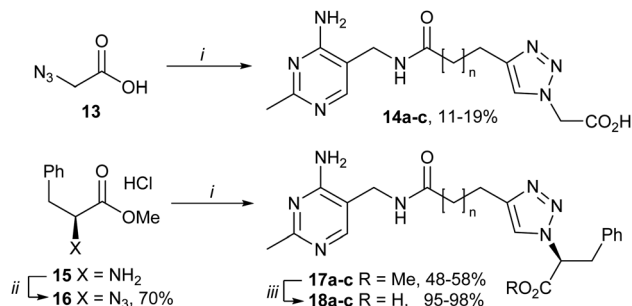

Fig. 2 Schematic depiction of the two predicted binding modes of bis-pyrimidine 8c in the TPP pocket of PDH E1 (from Fig. S2†). Ar = 4-amino-2-methylpyrimidin-5-yl. The active site is viewed from the same angle as in Fig. 1a.

L-phenylalanine methyl ester **15** *via* azide **16**, then CuAAC with **11a–c** and final hydrolysis of esters **17a–c** (Scheme 2). This benzyl-substituted series (**18a–c**) was consistently more potent than **12a–c** (Table 1). Computational docking suggested that this was because the phenyl ring occupies a relatively hydrophobic extension of the pyrophosphate pocket (Fig. 3). However, esters **17a–c** had inhibitory potencies as high as car-

Scheme 2 Synthesis of carboxylates **14a–c** and **18a–c**. Reagents and conditions: (i) **11a–c**, CuSO$_4$·5H$_2$O, sodium ascorbate, DMF, 40 °C; (ii) triflyl azide, CuSO$_4$·5H$_2$O, DIPEA, MeOH, DCM, RT; (iii) KOH, THF, H$_2$O, RT. For all compounds: **a** *n* = 1, **b** *n* = 2, **c** *n* = 3.

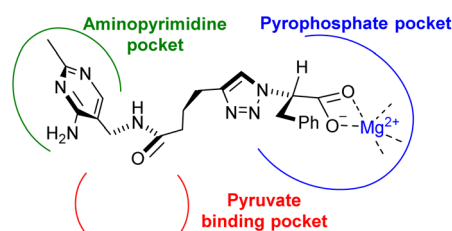**Fig. 3** Schematic depiction of the predicted binding modes of carboxylate 18b in the TPP pocket of PDH E1 (from Fig. S4†). The active site is viewed from the same angle as in Fig. 1a.

boxylates **18a–c** (Table 1), suggesting that the ionic interactions between the carboxylate group and the Mg^{2+} ion did not compensate for the energy penalty of desolvation.

To address this issue, the anionic carboxylate was replaced by an uncharged metal binding group (MBG), hydroxamate.[27] Hydroxamates **22a–c** were prepared from L-phenylalanine *via* azides **19** and **20**, then CuAAC with alkynes **11a–c** and final debenzylation (Scheme 3). These hydroxamates **22a–c** were much stronger inhibitors than the bis-pyrimidines **12a–c** or the carboxylates **18a–c**, with affinity **22b** > **22a** > **22c** (Table 1).
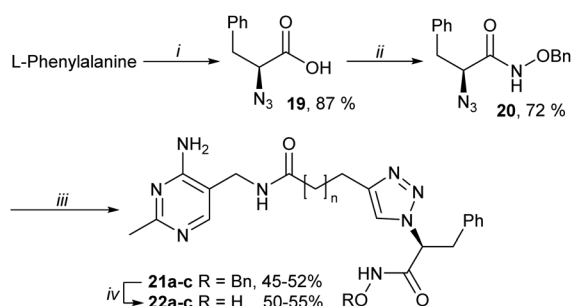
Scheme 3 Synthesis of hydroxamates **22a–c**. Reagents and conditions: (i) triflyl azide, CuSO$_4$·5H$_2$O, K$_2$CO$_3$, MeOH, H$_2$O, RT; (ii) CDI, NH$_2$OBn·HCl, THF, DMF, RT; (iii) **11a–c**, CuSO$_4$·5H$_2$O, sodium ascorbate, *t*-BuOH, H$_2$O, 40 °C; (iv) BCl$_3$, DCM, RT. For all compounds: **a** *n* = 1, **b** *n* = 2, **c** *n* = 3.

This journal is © The Royal Society of Chemistry 2023

Org. Biomol. Chem., 2023, **21**, 6531–6536 | **6533**

Insights into the binding mode of **22a–c** were obtained through *in silico* studies. As expected, the ionic interactions of **18a–c** have been replaced by a bidentate interaction between the hydroxamate MBG and the Mg^{2+} but the phenyl group, instead of being positioned in the pyrophosphate pocket, as with **18a–c** (Fig. 3), was predicted to flip back towards the amide linkage, occupying a region close to where the 4-Me group of TPP normally resides, with the amide linkage protruding into the pyruvate binding pocket (as depicted in Fig. 4a). This supported the pharmacophore of thiamine/TPP analogues that the binding region of the central neutral ring (mimicking the ylide of TPP) highly favours non-polar groups.[1–3,7,16,28]

The TPP-competitive nature of **22a–c** was confirmed as the observed potency decreased with increasing [TPP] (Table 1). A full IC$_{50}$ determination was conducted at 10 μM TPP for **12a–c** and **22a–c** (Fig. S1†) and showed that **22a** and **22b** bound 2.0 and 2.6 times tighter than TPP. Given the reported K_M of TPP = 50 nM,[29] the calculated K_I values of **17a** and **17b** for PDH E1 (using [TPP]/IC$_{50}$ = $K_{M(\text{TPP})}/K_I$) were in the low-nanomolar range (**17a**: 25 nM; **17b**: 19 nM).

As a control, O-benzyl hydroxamates **21a–c** were tested and found to be weaker inhibitors than **17a–c**. However, given that the O-benzyl group should greatly diminish the metal-binding capability, the residual activities of **21a–c** were surprisingly high. Therefore, they were subjected to computational docking. Interestingly, completely new binding modes were predicted: the tricyclic terminus was positioned into the substrate binding pockets, instead of the pyrophosphate pocket (Fig. 4b). This novel binding mode is presumably possible because these open-chain analogues have greater conformational flexibility than their ring-bearing counterparts. It also provided a possible explanation for the relative affinities among the series **21a–c**: the longer homologues (**21b** and **21c**) positioned the C-benzyl into a side-pocket off the lipoamide-binding entrance tunnel (Fig. 4b), while the shortest **21a** could not reach this pocket and positioned the C-benzyl group in the pyruvate/lipoamide-binding pockets. In all three compounds the O-benzyl group occupied the entrance tunnel.

Hydroxamates **23a,b**, enantiomers of **22a,b**, were synthesised from D-phenylalanine (as in Scheme 3) and they were only slightly weaker inhibitors than **22a,b** (Table 1). *In silico* studies predicted that the enantiomers shared similar binding modes, with the hydroxamate as the MBG and with the phenyl ring flipping towards the amide linkage (not shown). The relatively low sensitivity of binding to chirality can be attributed to the conformational flexibility of these open-chain analogues.

Hydroxamate **22b** is a drug-like molecule:[30] possessing the neutral hydroxamate as the MBG (unlike TPP's polyanionic pyrophosphate moiety[31]), molecular weight = 438 g mol^{-1}, hydrogen bond (HB) donors = 5, HB acceptors = 8, and calculated log P = 0.6 (calculated using MarvinSketch 21.1). **22b** is also an efficient ligand for PDH E1, with ligand efficiency of 0.34 kcal mol^{-1} per heavy atom.[32] The PDH E1 used throughout the study was commercially available porcine PDH E1, which is a widely accepted alternative to human PDH E1 as they share almost identical sequences (>95%).[14] Taken together, hydroxamate **22b** is expected to be a membrane-permeable, potent inhibitor of human PDH E1. In recent years, evidence has suggested that small molecule inhibitors of PDH E1 may be effective against cancers that over-express the PDH complex (PDHc).[21–24] Thus, we hope to test **22b** on cancer cell lines and to compare its effects with other cellular probes for PDHc.[13–15,23]

It was also of interest to evaluate the activity of **22b** on other TPP-dependent enzymes, such as pyruvate decarboxylase (PDC), pyruvate oxidase (PO) and 2-oxoglutarate dehydrogenase (OGDH) E1. Hydroxamate **22b** displayed modest inhibition of bacterial OGDH E1 and PDC, but little or no activity on bacterial PO or yeast PDC (Table S1†). With bacterial OGDH E1 and PDC, the level of inhibition by **22b** reduced with increasing concentrations of TPP (Table S1†) suggesting a TPP-competitive mode of action.

Comparing the two hydroxamates **22b** and **7**, **22b** is the better inhibitor of PDH E1 (K_I = 19 nM *vs.* 40 nM (ref. 15)), which shows that replacing the central ring of thiamine/TPP analogues **5–7** with an open-chain amide has not lost too much of the binding energy and that is more than compensated by the favourable binding of the C-benzyl group. Furan **6** is 4-5-times tighter binding than **22b** (K_I = 4.2 nM)[14] but that was the result of extensive optimisation of the amide moiety that occupies the pyrophosphate pocket and of the acyl group attached to the furan (at the equivalent position to C-2 of TPP), whereas no optimisation of **22b** has been undertaken.
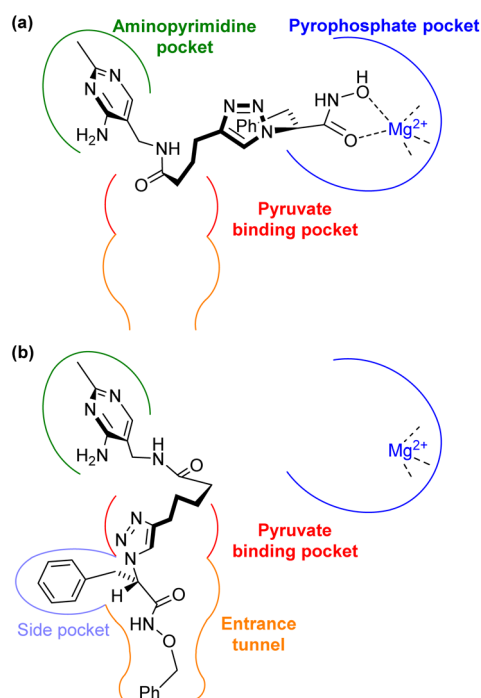

Fig. 4 Schematic depictions of the predicted binding modes of (a) hydroxamate 22b and (b) O-benzylhydroxamate 21c to PDH E1 (from Fig. S5 & S6). The active site is viewed from the same angle as in Fig. 1a.

6534 | *Org. Biomol. Chem.,* 2023, **21**, 6531–6536

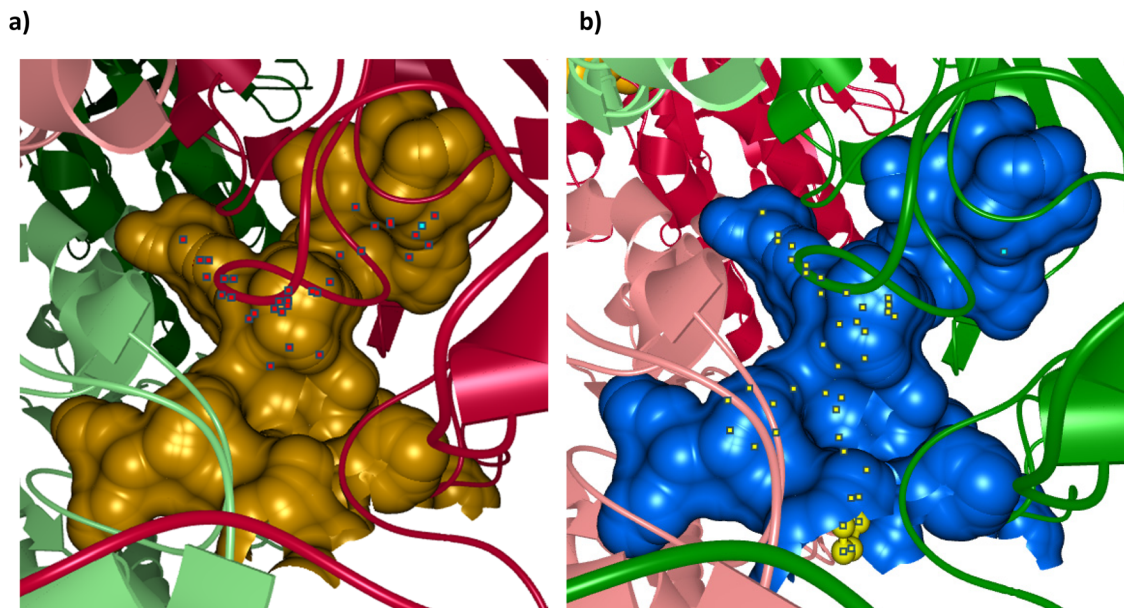This journal is © The Royal Society of Chemistry 2023

a)

b)

Fig. 5 Active site cavities of human PDH E1 (PDB: 6CFO). The protein is an $\alpha_2\beta_2$ tetramer and each chain, in cartoon view, is coloured differently (β chains have the lighter colours). The active sites are viewed from the same angle as in Fig. 1a. (a) The cavity is in gold and bound in the cavity is the adduct between TPP and inhibitor acetyl phosphinate (red dots, part of chain C in 6CFO); the Mg^{2+} ion is a cyan dot. (b) The second cavity (which contained the ligand in chain A in 6CFO) is in blue and bound in the cavity is the docked structure of **21c** (yellow dots). The Mg^{2+} ion is a cyan dot. Images calculated and displayed by Caver Analyser 1.0.[33]

One thing that is clear from our docking studies is that there are further binding sites that can be exploited to improve the affinity of inhibitors other than the TPP-binding sites. We believe **21c** is a relatively good inhibitor because it binds in the pyruvate and lipoamide binding sites and the side-pocket (Fig. 4b), whereas **22b** does not take advantage of these sites but instead has a MBG to bind to the Mg^{2+} in the pyrophosphate pocket (Fig. 4a). This ability to use different binding pockets to get the best binding is only possible because of the flexibility of the open chain analogues. If an inhibitor could be developed that bound well in all the available pockets then it would be very potent indeed. The various pockets in PDH E1 were visualised using the program Caver Analyser 1.0 [33] and Fig. 5 shows the two active site cavities of this $\alpha_2\beta_2$ tetramer. This clearly shows the binding pockets already mentioned, binding the aminopyrimidine, central ring, pyrophosphate, pyruvate and lipoamide/entrance tunnel, plus the side pocket to the left of the entrance tunnel in this view. This side-tunnel seems to be considerably larger than required to accommodate the C-benzyl of **21c** (as shown in Fig. 5b). In addition, there seems to be a further side-pocket to the right of the entrance tunnel, which might also be profitably used in the design of inhibitors.

Conclusions

We have demonstrated that the synthesis of open-chain thiamine analogues is much easier than the synthesis of analogues with neutral central rings such as deazaTPP **5** and furan **6**. Furthermore, the flexibility of the open chain allows the pendent groups to explore all the available binding sites, instead of just the one site dictated by the conformationally restricted ring. Hydroxamate **22b** is a potent, drug-like inhibitor of TPP-dependent enzymes, particularly mammalian PDH E1, with tighter binding (to PDH E1) than that of TPP. It should be possible to modify **22b** to add groups that bind in the other available pockets identified here and so arrive at an even more potent inhibitor. This may help uncover the roles of PDH E1 in cancer development and lead to a treatment for cancers that rely on PDHc for their growth.

Conflicts of interest

All the authors declare there is no conflict of interest.

Acknowledgements

References

1 R. A. W. Frank, F. J. Leeper and B. F. Luisi, *Cell. Mol. Life Sci.*, 2007, **64**, 892–905.
2 S. Mann, C. Perez Melero, D. Hawksley and F. J. Leeper, *Org. Biomol. Chem.*, 2004, **2**, 1732.
3 K. M. Erixon, C. L. Dabalos and F. J. Leeper, *Org. Biomol. Chem.*, 2008, **6**, 3561.

This journal is © The Royal Society of Chemistry 2023

Org. Biomol. Chem., 2023, **21**, 6531–6536 | **6535**

4   Y. Le Huerou, I. Gunawardana, A. A. Thomas, S. A. Boyd, J. de Meese, W. deWolf, S. S. Gonzales, M. Han, L. Hayter, T. Kaplan, C. Lemieux, P. Lee, J. Pheneger, G. Poch, T. T. Romoff, F. Sullivan, S. Weiler, S. K. Wright and J. Lin, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 505–508.

5   A. A. Thomas, J. De Meese, Y. Le Huerou, S. A. Boyd, T. T. Romoff, S. S. Gonzales, I. Gunawardana, T. Kaplan, F. Sullivan, K. Condroski, J. P. Lyssikatos, T. D. Aicher, J. Ballard, B. Bernat, W. DeWolf, M. Han, C. Lemieux, D. Smith, S. Weiler, S. K. Wright, G. Vigers and B. Brandhuber, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 509–512.

6   A. A. Thomas, Y. Le Huerou, J. De Meese, I. Gunawardana, T. Kaplan, T. T. Romoff, S. S. Gonzales, K. Condroski, S. A. Boyd, J. Ballard, B. Bernat, W. DeWolf, M. Han, P. Lee, C. Lemieux, R. Pedersen, J. Pheneger, G. Poch, D. Smith, F. Sullivan, S. Weiler, S. K. Wright, J. Lin, B. Brandhuber and G. Vigers, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 2206–2210.

7   X. Pei, K. M. Erixon, B. F. Luisi and F. J. Leeper, *Biochemistry*, 2010, **49**, 1727–1736.

8   X. W. A. Chan, C. Wrenger, K. Stahl, B. Bergmann, M. Winterberg, I. B. Müller and K. J. Saliba, *Nat. Commun.*, 2013, **4**, 2060.

9   A. Iqbal, E.-H. Sahraoui and F. J. Leeper, *Beilstein J. Org. Chem.*, 2014, **10**, 2580–2585.

10 F. Rabe von Pappenheim, M. Aldeghi, B. Shome, T. Begley, B. L. de Groot and K. Tittmann, *Nat. Chem. Biol.*, 2020, **16**, 1237–1245.

11 E. Grabowska, M. Czerniecka, U. Czyżewska, A. Zambrzycka, Z. Łotowski and A. Tylicki, *J. Enzyme Inhib. Med. Chem.*, 2021, **36**, 122–129.

12 A. H. Y. Chan, I. Fathoni, T. Ho, K. J. Saliba and F. J. Leeper, *RSC Med. Chem.*, 2022, **13**, 817–821.

13 A. H. Y. Chan, T. C. S. Ho, K. Agyei-Owusu and F. J. Leeper, *Org. Biomol. Chem.*, 2022, **20**, 8855–8858.

14 A. H. Y. Chan, T. C. S. Ho, D. R. Parle and F. J. Leeper, *Org. Biomol. Chem.*, 2023, **21**, 1755–1763.

15 A. H. Y. Chan, T. C. S. Ho, I. Fathoni, R. Pope, K. J. Saliba and F. J. Leeper, *ACS Med. Chem. Lett.*, 2023, **14**, 621–628.

16 A. H. Y. Chan, T. C. S. Ho, R. Irfan, R. A. A. Hamid, E. S. Rudge, A. Iqbal, A. Turner, A. K. H. Hirsch and F. J. Leeper, *Bioorg. Chem.*, 2023, **138**, 106602.

17 J.-B. He, L.-L. Feng, J. Li, R.-J. Tao, Y.-L. Ren, J. Wan and H.-W. He, *Bioorg. Med. Chem.*, 2014, **22**, 89–94.

18 H. He, H. Xia, Q. Xia, Y. Ren and H. He, *Bioorg. Med. Chem.*, 2017, **25**, 5652–5661.

19 Y. Zhou, J. Feng, L. Feng, D. Xie, H. Peng, M. Cai and H. He, *J. Agric. Food Chem.*, 2019, **67**, 12538–12546.

20 J. Feng, H. He, Y. Zhou, X. Guo, H. Liu, M. Cai, F. Wang, L. Feng and H. He, *Bioorg. Med. Chem.*, 2019, **27**, 2413–2420.

21 S. M. Davidson, T. Papagiannakopoulos, B. A. Olenchock, J. E. Heyman, M. A. Keibler, A. Luengo, M. R. Bauer, A. K. Jha, J. P. O'Brien, K. A. Pierce, D. Y. Gui, L. B. Sullivan, T. M. Wasylenko, L. Subbaraj, C. R. Chin, G. Stephanopolous, B. T. Mott, T. Jacks, C. B. Clish and M. G. Vander Heiden, *Cell Metab.*, 2016, **23**, 517–528.

22 C. T. Hensley, B. Faubert, Q. Yuan, N. Lev-Cohain, E. Jin, J. Kim, L. Jiang, B. Ko, R. Skelton, L. Loudat, M. Wodzak, C. Klimko, E. McMillan, Y. Butt, M. Ni, D. Oliver, J. Torrealba, C. R. Malloy, K. Kernstine, R. E. Lenkinski and R. J. DeBerardinis, *Cell*, 2016, **164**, 681–694.

23 J. Chen, I. Guccini, D. Di Mitri, D. Brina, A. Revandkar, M. Sarti, E. Pasquini, A. Alajati, S. Pinton, M. Losa, G. Civenni, C. V. Catapano, J. Sgrignani, A. Cavalli, R. D'Antuono, J. M. Asara, A. Morandi, P. Chiarugi, S. Crotti, M. Agostini, M. Montopoli, I. Masgras, A. Rasola, R. Garcia-Escudero, N. Delaleu, A. Rinaldi, F. Bertoni, J. de Bono, A. Carracedo and A. Alimonti, *Nat. Genet.*, 2018, **50**, 219–228.

24 Z. Zachar, J. Marecek, C. Maturo, S. Gupta, S. D. Stuart, K. Howell, A. Schauble, J. Lem, A. Piramzadian, S. Karnik, K. Lee, R. Rodriguez, R. Shorr and P. M. Bingham, *J. Mol. Med.*, 2011, **89**, 1137–1148.

25 P. A. Philip, M. E. Buyse, A. T. Alistar, C. M. S. P. R. Lima, S. Luther, T. S. Pardee and E. Van Cutsem, *Future Oncol.*, 2019, **15**, 3189–3196.

26 T. S. Pardee, S. Luther, M. Buyse, B. L. Powell and J. Cortes, *Future Oncol.*, 2019, **15**, 3197–3208.

27 T. C. S. Ho, A. H. Y. Chan and A. Ganesan, *J. Med. Chem.*, 2020, **63**, 12460–12484.

28 S. Lüdtke, P. Neumann, K. M. Erixon, F. Leeper, R. Kluger, R. Ficner and K. Tittmann, *Nat. Chem.*, 2013, **5**, 762–767.

29 D. A. Walsh, R. H. Cooper, R. M. Denton, B. J. Bridges and P. J. Randle, *Biochem. J.*, 1976, **157**, 41–67.

30 C. A. Lipinski, *Drug Discovery Today: Technol.*, 2004, **1**, 337–341.

31 E. S. Rudge, A. H. Y. Chan and F. J. Leeper, *RSC Med. Chem.*, 2022, **13**, 375–391.

32 A. L. Hopkins, G. M. Keserü, P. D. Leeson, D. C. Rees and C. H. Reynolds, *Nat. Rev. Drug Discovery*, 2014, **13**, 105–121.

33 A. Pavelka, E. Sebestova, B. Kozlikova, J. Brezovsky, J. Sochor and J. Damborsky, *IEEE/ACM Trans. Comput. Biol. Bioinf.*, 2016, **13**, 505–517.