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Synthesis of pyrrothiamine, a novel thiamine analogue, and evaluation of derivatives as potent and selective inhibitors of pyruvate dehydrogenase†

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Inhibition of thiamine pyrophosphate (TPP)-dependent enzymes with thiamine/TPP analogues that have the central thiazolium ring replaced with other rings is well established, but a limited number of central rings have been reported. We report a novel analogue, pyrrothiamine, with a central pyrrole ring. We further develop pyrrothiamine derivatives as potent and selective inhibitors of pyruvate dehydrogenase, which might have anti-cancer potential.

Thiamine pyrophosphate (TPP)-dependent enzymes have diverse activities and include pyruvate dehydrogenase E1-subunit (PDH E1), pyruvate decarboxylase (PDC), oxoglutarate dehydrogenase E1-subunit (OGDH E1) (Fig. 1).[1,2] Individual TPP-dependent enzymes differ in substrate binding and chemistry, but they all need the coenzyme TPP **2** for activity and possess a highly similar TPP-binding pocket (key interactions summarized in Fig. 2a).[1–7] The use of small molecule inhibitors to inhibit these enzymes for cellular studies and for medicinal or agrochemical purposes has long been of interest, and a common strategy lies in the use of thiamine/TPP analogues having a neutral central ring, in place of the thiazolium ring, to capture the strong stabilising interactions between the enzyme and the catalytically active high-energy TPP ylide **3** (Fig. 2a).[8–25] Many derivatives with a central triazole ring **4** and modifications on the hydroxyethyl tail have been described in the literature (Fig. 2b).[18–25] Although other central rings have also been reported - thiophene (deazathiamine) **6**,[8] furan **7**,[13] benzene[8] and pyrazole[16] - they have received much less attention than triazole **4** probably because of their longer synthetic routes. Comparison between TPP analogues clearly indicates that the choice of central rings directly impacts potency (Fig. 2c). In this study, we develop a novel thiamine analogue, pyrrothiamine **16** with a central pyrrole ring.

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Pyrrothiamine **16** was synthesised *via* a Paal–Knorr reaction between primary amine **14** and 1,4-dicarbonyl **12** (Scheme 1a). The 1,4-dicarbonyl **12** was prepared from 2-acetylbutyrolactone **8** by alkylation with allyl bromide to give racemic lactone **9**, then hydrolysis and decarboxylation to alcohol **10**. An equilibrium between acyclic alcohol **10** and its cyclic hemiacetal complicated the purification and analysis of the NMR spectra, so **10** was benzoylated to ester **11**. Oxidative cleavage of the alkene was implemented in two-steps *via* a 1,2-diol by Sharpless dihydroxylation followed by sodium periodate cleavage, giving aldehyde **12** in 80% yield over two steps. The amine **14** was prepared by hydrogenating azide **13**, in turn obtained in a single-step reaction[13] from thiamine hydrochloride. Aldehyde **12** was then coupled with amine **14** to yield the protected pyrrole **15**, which was then hydrolysed to give pyrrothiamine **16**.

Pyrrothiamine **16** was not expected to be a potent inhibitor *per se*, as it lacks any group to occupy the pyrophosphate pocket or interact with the Mg^{2+}. We attempted to make its pyrophosphate **18** in the same way as we had done for other thiamine analogues.[8,13] However the first step, formation of the tosylate **17**, failed. We suspect that **17** may have formed but was unstable, due to the electron-rich pyrrole ring assisting S$_N$1 displacement of tosylate by nucleophiles. Although pyrroTPP **18** would be expected to be very potent inhibitor of TPP-dependent enzymes, it would not be useful in cellular studies and/or medicinal applications as the polyanionic pyro-
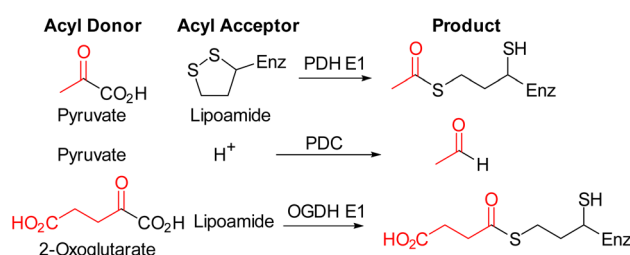

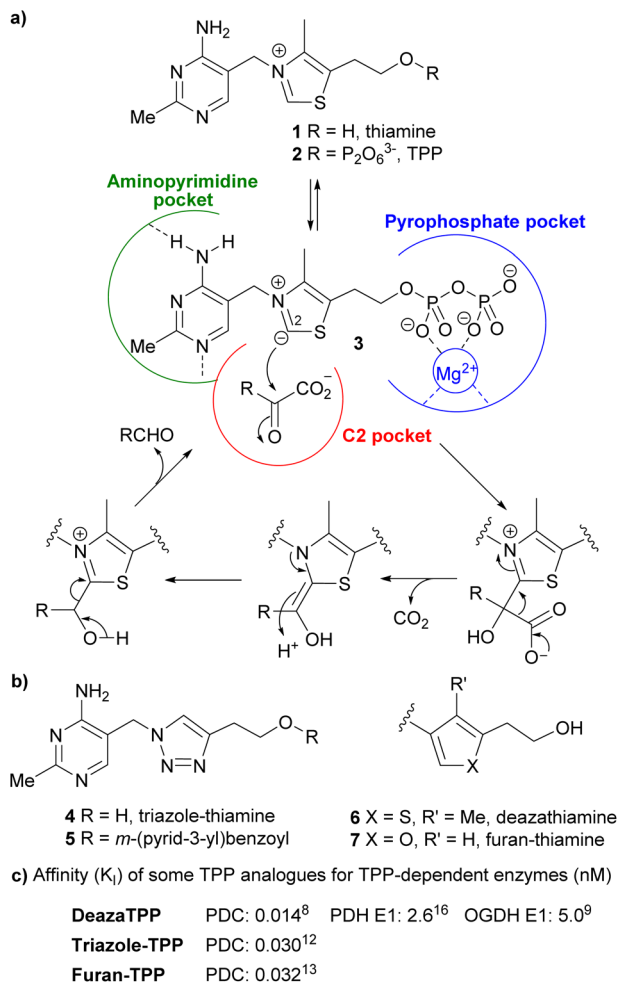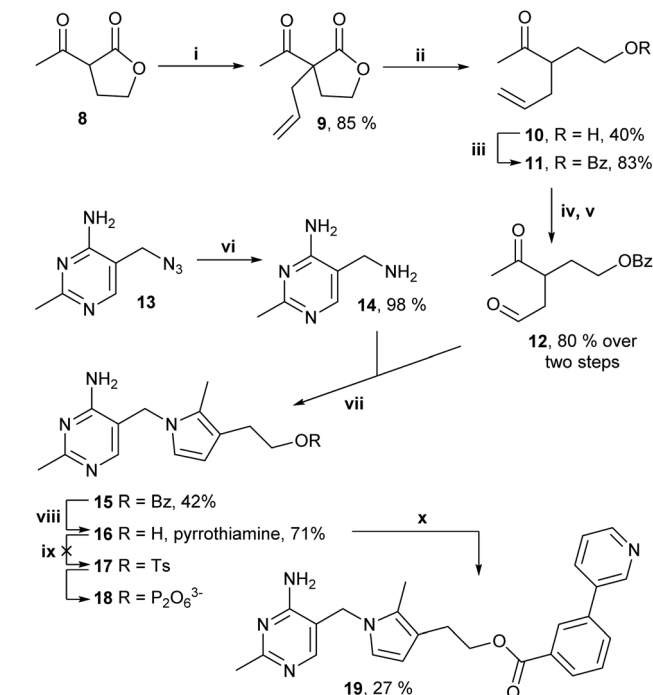**Fig. 1** Reactions catalysed by the enzymes studied in this work.

Fig. 2 (**a**) Binding mode of the catalytically active TPP ylide **3** in the TPP pocket and reaction mechanism for PDC. (**b**) Structures of thiamine/TPP analogues. (**c**) Inhibitory data of some TPP analogues.

Scheme 1 Synthesis of pyrrothiamine **16** and its ester **17**. *Reagents and conditions*: (i) NaH, THF, allyl bromide, RT; (ii) LiOH monohydrate, THF, H$_2$O, 40 °C; (iii) benzoyl chloride, pyridine, 40 °C; (iv) AD-mix-α, methanesulfonamide, *t*-BuOH, H$_2$O, RT; (v) NaIO$_4$, acetone, H$_2$O, RT; (vi) H$_2$(g), 10% Pd/C, MeOH, RT; (vii) DMF, 45 °C; (viii) K$_2$CO$_3$, MeOH, 30 °C; (ix) TsCl, pyridine; (x) *m*-(pyrid-3-yl)benzoic acid, DCC, DMAP, DMF, RT.

Table 1 Summary of inhibitory activity of compounds **16**, **19** and **5** for the three different enzymes

	PDH E1			PDC	OGDH E1
Compounds	IC$_{50}$ (μM ± SEM)^{a,b}	× TPP^c	K_I (nM ± SEM)^d	Inhibition (%)^{a,e}	Inhibition (%)^{a,f}
16	19.7 ± 1.9	0.51	98.5 ± 9.5	22 ± 6	30 ± 5
19	1.2 ± 0.1	8.42	6.0 ± 0.5	<10	<10
5^g	3.0 ± 0.4	3.33	15.0 ± 2.0	Not determined	Not determined

^{a} Data are the means of measurements in three technical replicates. ^{b} IC$_{50}$ values determined at [TPP] = 10 μM. ^{c} Affinity of the inhibitor *versus* that of TPP, *i.e.* [TPP]/IC$_{50}$. ^{d} K_I is based on the previously reported^{27} K_D of TPP = 50 nM using [TPP]/IC$_{50}$ = $K_{D(TPP)}/K_I$. ^{e} Percentage inhibition determined for compounds at 200 μM with [TPP] = 200 μM. ^{f} Percentage inhibition determined for compounds at 40 μM with [TPP] = 40 μM. ^{g} Ref. 27.

phosphate would make it membrane-impermeable.[17,26] To obtain a potentially membrane-permeable inhibitor, we coupled the alcohol of pyrrothiamine **16** to *m*-(pyrid-3-yl) benzoic acid to generate ester **19** (Scheme 1b). This is because our recent study[27] of dozens of triazole-thiamine esters identified **5** as the most potent PDH E1 inhibitor (Table 1). Recent evidence[28–30] has shown that selective PDH E1 inhibitors have anti-cancer potential.

The potency of **16** and **19** against PDH E1 (Table 1) was evaluated in enzyme assays using porcine PDH E1, which is nearly identical (>95%) to human PDH E1 and is a widely used model for it.[3–7,18–24,27,31] Ester **19** is a much better inhibitor (>15-fold) than unesterified **16** and 2.5-fold better than the equivalent triazole analogue **5**. Their TPP-competitive nature was confirmed as the observed potency decreased with increasing [TPP] (Table S1†). The affinity of **19** for PDH E1 was found to be 8.4 times greater than that of TPP and the K_I is in the low-nanomolar range.

Pyrrothiamine ester **15**, an intermediate in the synthesis of **16**, was also tested as an inhibitor under conditions where

[inhibitor] = [TPP]. The percentage inhibition on PDH E1 was found to be 32%, 41% and 78% for compounds **16**, **15** and **19** respectively (Table S1†) so the affinity of **15** is intermediate between the affinities of **16** and **19**. No further enzymatic evaluation was conducted on **15** but the result of this assay implies that both the benzoyl ester and the terminal *m*-pyrid-3-yl ring independently contribute to binding.

To test their selectivity, compounds **16** and **19** were tested on PDC and OGDH E1 because these enzymes process either the same donor or acceptor substrate as PDH E1 (Fig. 1). The assays showed that **16** binds to all three enzymes with similar

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(low) affinity but ester **19** was almost inactive towards PDC and OGDH E1 (Table 1).

Insights into the binding mode were pursued through *in silico* studies: docked using the program GOLD into the active site of human PDH E1, **19** overlays well with TPP (Fig. 3b), thus supporting its TPP-competitive relationship. While the pyrophosphate of TPP interacts with the Mg^{2+}, the bi-aryl tail of **19** has a pi-cation interaction with the Mg^{2+} and occupies a relatively hydrophobic extension of the pyrophosphate pocket, with the nitrogen atom of the pyridine hydrogen-bonded to an arginine residue (Fig. 3d). The binding interactions are shown in Fig. 3e.

When **19** was docked into the active site of OGDH E1, no reasonable docking was found due to substantial intermolecular steric clashes. This suggests that the smaller pyrophosphate pocket of OGDH, relative to PDH, cannot accommodate the bulky bi-aryl ester tail, consistent with earlier findings.[27] In short, the introduction of the bi-aryl ester group of **19** onto the terminus of pyrrothiamine **16** enhanced both binding affinity for PDH E1 (K_I: 6 *vs.* 98 nM) and selectivity over other TPP-dependent enzymes (Table 1), it seems due to steric reasons.

Unlike deazaTPP (pyrophosphate of **6**), which has nanomolar (or subnanomolar) affinities for multiple TPP-dependent enzymes (Fig. 1c), ester **19** is selective towards PDH E1 with comparable potency. Furthermore, without the pyrophosphate moiety, ester **19** is likely to be hydrophobic enough to diffuse through membranes in cellular studies. Pyrrole **19** binds 2.5 times tighter to PDH E1 than the equivalent triazole **5** (Table 1) and in addition, pyrrole **13** has a free C2-position (next to the pyrrolic nitrogen) that will allow introduction of substituents at that position. C2-substituents should project into the C2-pocket[10,15,27] where the substrate binding and catalytic groups are located (Fig. 1a).[1–7] It has been verified that exploring the distinctive electronic/steric elements of the C2-pocket can lead to selective inhibition.[3–15,27] Thus, the current pyrrolic scaffold has an additional possibility for functionalisation to enhance its selectivity that triazole **5** does not.

In conclusion, we report herein a novel thiamine analogue – pyrrothiamine **16**, which expands the range of thiamine/TPP analogues. A derivative of **16**, ester **19**, was biochemically and computationally established as a potent and selective PDH E1 inhibitor, which may have anti-cancer potential towards cancers over-expressing PDH.[28–30] The synthesis of **19** is relatively short and could have been two steps shorter if **10** had been protected with the eventual *m*-(pyrid-3-yl)benzoyl group instead of a simple benzoyl group. Our group is hoping to combine C2-substitution and tail modification to prepare compounds selective to transketolase for cancer treatment.[32] Notably, this electron-rich pyrrolic scaffold makes it exceptionally suitable for C2-functionalisation *via* Friedel–Crafts acylation reaction as pyrroles are among the most reactive aromatic rings in electrophilic substitution reactions. We anticipate that this novel thiamine analogue will encourage researchers to shift from the triazole scaffold **4** to pyrrole **16**, as well as thiophene **6** and furan **7**. Not only are these scaffolds (**6**, **7** and **16**) more potent inhibitors than their triazole equivalents, but also their free C2-position allows the introduction of C2-substituents for selectivity (and affinity) improvement.[27]
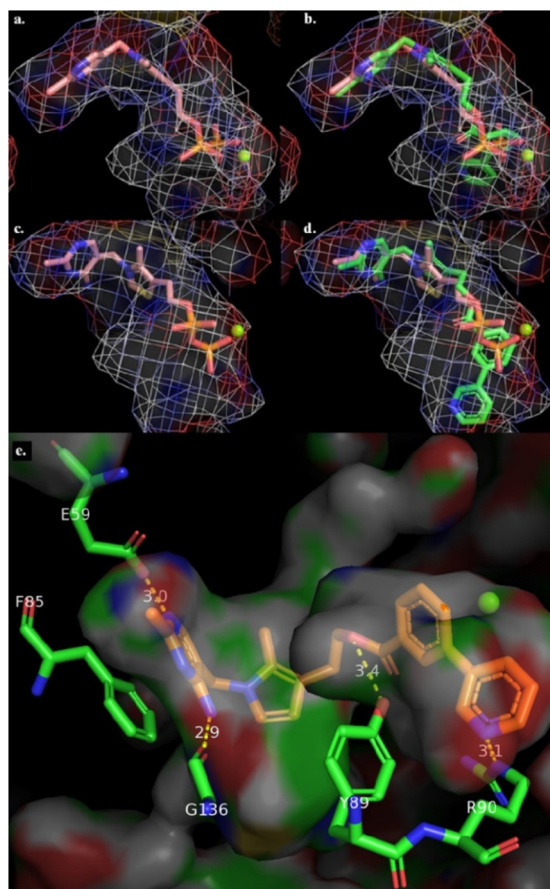

Fig. 3 (a) Binding mode of TPP in human PDH E1 (PDB: 6CFO) showing the V-shaped conformation between the aminopyrimidine and the thiazolium ring. (b) Predicted binding mode of **19** (green atoms) overlayed with TPP as in view (a). (c) Binding mode of TPP showing the coordination between the pyrophosphate moiety and Mg^{2+}. (d) Predicted binding mode of **19** overlayed with TPP as in view (c); (e) interactions between ester **19** and PDH E1 binding pocket shown as surface representation with interactions and distances shown as dashed lines; Mg^{2+} is represented as a yellowish-green sphere.

Author contributions

A. H. Y. C. synthesised all the compounds; T. C. S. H. performed most of the enzyme assays and the molecular docking; K. A.-O. pioneered the synthetic route to **16**; F. J. L. supervised the project. A. H. Y. C., T. C. S. H. and F. J. L. all contributed to writing the paper.

Conflicts of interest

The authors declare no competing financial interest.

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Acknowledgements

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