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Design, synthesis and molecular modeling of novel *N*-acylhydrazone derivatives as pyruvate dehydrogenase complex E1 inhibitors

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ABSTRACT

As potential inhibitors of pyruvate dehydrogenase complex E1 (PDHc-E1), a series of 19 1-((4-amino-2-methylpyrimidin-5-yl)methyl)-5-methyl-*N*′-(substituent)benzylidene-1*H*-1,2,3-triazole-4-carbohydrazide **4** has been synthesized and tested for their PDHc-E1 inhibitory activity in vitro. Some of these compounds such as **4a**, **4g**, **4l**, **4o**, **4p**, and **4q** were demonstrated to be effective inhibitors by the bioassay of *Escherichia coli* PDHc-E1. SAR analysis indicated that the PDHc-E1 inhibitory activity could be further enhanced by optimizing the substituted groups in the parent compound. Molecular modeling study with compound **4o** as a model was performed to evaluate docking. The results of modeling study suggested a probable inhibition mechanism.

1. Introduction

The pyruvate dehydrogenase complex (PDHc) is the key regulatory site in cellular metabolism, in which it catalyzes the irreversible oxidative of pyruvate to acyl CoA.[1] Therefore, it has been reported as a promising target for antimicrobials,[2] agrochemicals,[3] and age-related diseases.[4] The pyruvate dehydrogenase complex is comprised of three different enzymes and a number of cofactors. Pyruvate dehydrogenase complex E1 component (PDHc-E1, EC 1.2.4.1) is the initial member of PDHc, which catalyzes the first step of multistep process. Accordingly, blocking the activity of PDHc-E1 will inactivate the PDHc.

There had been some reports about synthesis and inhibition of PDHc-E1 inhibitors as analogs of substrate (pyruvate).[5–9] Currently, some compounds as analogs of PDHc-E1 cofactor, thiamine diphosphate (ThDP), had been chemically synthesized and also reported as effective inhibitors against *Escherichia coli* (*E. coli*) PDHc-E1 in vitro (Fig. 1), but no reports about the study on their antifungal or antibacterial activities in vivo.[10–13] We expected to find a potential fungicide or bactericide by designing inhibitor against PHDc-E1 in microorganisms. On the basis of our study to design plant PHDc-E1 inhibitors as practical herbicide,[14,15] we made an attempt to design novel PHDc-E1 inhibitors as potential fungicide or bactericide. We recently had got preliminary progress for finding an effective *E. coli* PDHc-E1 inhibitor **I** with antifungal activity through structure-based rational design (Fig. 1, compound **I**).[16,17] Compound **I** was designed based on the characteristics of active site occupied by ThDP in *E. coli* PDHc-E1. These results encouraged us to further design and study *E. coli* PDHc-E1 inhibitors.

In order to find additional PDHc-E1 inhibitors, structurally diversified inhibitors should be investigated. In the present work, the synthesis strategy focused on the *N*-acylhydrazone moiety ($R^1CONHN{=}CHR^2$) scaffold due to its well-known biological activities.[18–21] *N*-Acylhydrazone moiety was introduced into the compound **I** to form a new structural class **4** as potential *E. coli* PDHc-E1 inhibitors (Fig. 2). Different from the study on PHDc-E1 inhibitors reported in literatures, an integration study including structure-based rational design by molecular docking, synthesis, and bioassay for PDHc-E1 inhibition were carried out in this work. Considering the binding site of diphosphate of ThDP in the active site of *E. coli* PDHc-E1, various chemical groups as Ar were incorporated into the parent compounds **4** to investigate their preliminary structural–activity relationship.

Herein, we describe the synthesis of nineteen 1-((4-amino-2-methylpyrimidin-5-yl)methyl)-5-methyl-*N*′-(substituent)benzylidene-1*H*-1,2,3-triazole-4-carbohydrazides **4a**–**s**. These 19 title compounds were tested for their inhibition activity against *E. coli* PDHc-E1. Interaction between mode of inhibitor and target PDHc-E1 was also explored by molecular docking study to identify the critical binding sites of the target PDHc-E1.

2. Chemistry

The synthetic strategy followed for the preparation of the target compounds (**4a**–**s**) is depicted in Scheme 1. The

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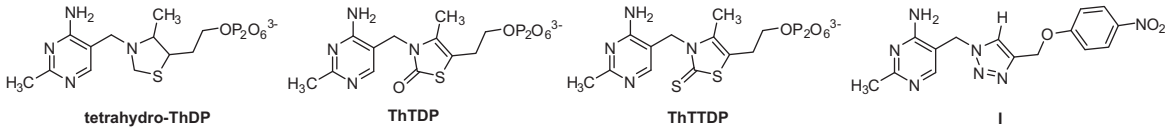


Figure 1. Structures of known PDHc-E1 inhibitors.

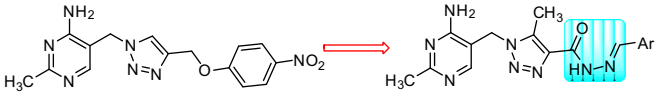
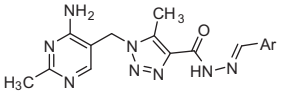


Figure 2. Design of the new *N*-acylhydrazone derivatives **4**.

Table 1
Structures and IC_{50} values against *E. coli* PDHc-E1 of compounds **4a–s**



Compd	Ar	IC_{50} (μM)
4a	4-Cl–Ph	2.35 ± 2.35
4b	2-Cl–Ph	13.64 ± 0.38
4c	2,4-2Cl–Ph	NT[a]
4d	2,3-2Cl–Ph	NT[a]
4e	3,4-2Cl–Ph	NT[a]
4f	2-Br–Ph	53.83 ± 3.63
4g	3-NO_2-4-Cl–Ph	1.71 ± 0.08
4h	4-CH_3–Ph	50.30 ± 2.50
4i	4-F–Ph	8.00 ± 0.48
4j	3-CH_3–Ph	20.88 ± 1.41
4k	3,4-OCH_2O–Ph	14.46 ± 1.02
4l	4-Br–Ph	1.89 ± 0.38
4m	H–Ph	110.40 ± 2.36
4n	2-OH–Ph	11.64 ± 1.35
4o	4-NO_2–Ph	0.65 ± 0.10
4p	4-OH–Ph	3.43 ± 0.52
4q	4-CF_3–Ph	1.92 ± 0.54
4r	3-OH–Ph	23.35 ± 1.77
4s	2-Pyridine	22.76 ± 1.27

[a] NT = not tested.

5-azidomethyl-2-methylpyrimidine-4-ylamine **1** was prepared readily from thiamine hydrochloride according to literature.[22] It was converted into compound **2** with ethyl acetoacetate in the presence of potassium carbonate, using DMF as solvent. The carbohydrazide **3** was easily synthesized via the hydrazinolysis of the ethoxycarbonyl group by treatment with hydrazine hydrate in refluxing ethanol. Finally, the new *N*-acylhydrazone compounds (**4a–s**) were prepared in good yields by condensing compound **3** with a variety of commercially available aromatic aldehydes in ethanol at reflux.

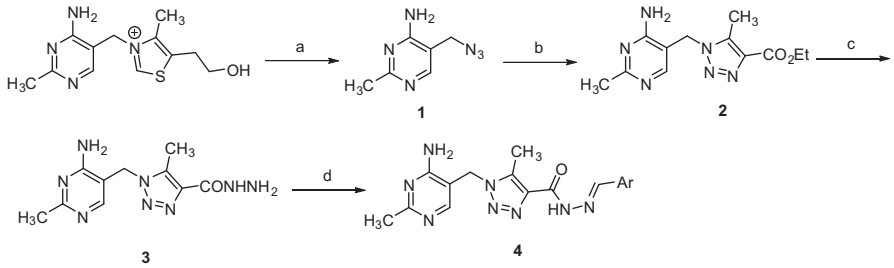
The structural assignments to new compounds **4a–s** were based on their elemental analysis and spectral data (^1H, ^{13}C NMR, and MS). The ^1H NMR spectra of all the compounds showed two singlet for three ($2CH_3$) protons at δ (2.31–2.33, and 2.26–2.58, respectively), another singlet for two (CH_2) protons at δ (5.40–5.43), a singlet for two (NH_2) protons at δ (6.88–6.91), and a singlet of imine (CH=N) proton at δ (8.42–9.02). A detailed analysis of the ^1H NMR spectra of these new compounds **4a–s** showed only one N–H signal, which was deduced as the (*E*)-diastereomer.[23] ^{13}C NMR spectra of all these compounds were taken in DMSO-d_6 and the signal obtained further confirmed the proposed structures. The mass spectrum of all the target compounds is in agreement with the molecular formula. The spectral values for all the compounds and C, H, N analyses are given in the experimental section.

3. Results and discussion

3.1. In vitro inhibition of *E. coli* PDHc E1

All the synthesized compounds **4a–s** were subjected to be evaluated for their inhibitory activity against PDHc-E1 from *E. coli*. The IC_{50} values are summarized in Table 1. It was observed that most of the compounds showed moderate to potent inhibitory activity with IC_{50} values in the range of 14.46–0.65 μM. Compound **4o** was the most potent inhibitor in this series with the activity of IC_{50} at 0.65 ± 0.10 μM, followed by **4g**, **4l**, **4q**, **4a**, and **4p**.

To explore the structure–activity relationship (SAR) of these new *N*-acylhydrazone compounds, the modifications are focused on the right terminal Ar, in which the benzene ring locates in the binding site of diphosphate of ThDP at the active site of PDHc-E1. Compound **4m** with unsubstituted benzene ring as Ar showed the lowest activity (IC_{50} = 110.40 ± 2.36 μM). After replacing the benzene ring with a 2-pyridine ring as Ar led to compound **4s**, we noticed a fivefold increase in **4s**'s inhibitory activity. This suggested that the nitrogen atom on pyridine moiety in the structure of **4s** may interact with the binding site of the receptor. We also noticed that the introduction of a variety of substituent groups would increase the inhibitory activity against *E. coli* PDHc-E1. In this case, compounds with electron-withdrawing groups on the benzene ring as Ar seem to be more effective than these



Scheme 1. Reagents and conditions (a) NaN_3, Na_2SO_3, H_2O, 60–65 °C, 6 h, 63%; (b) ethyl acetoacetate, DMF, 80 °C, 8 h, 70%; (c) $N_2H_4{\cdot}H_2O$, EtOH, reflux, 5 h, 85%; (d) Ar-CHO, EtOH, reflux, 5 h, 29–96%.

compounds with electron-donating alkyl counterparts as Ar. The inhibitory activity is also definitely related to the position of substituent groups at the benzene ring. Among the active compounds (**4n**, **4p**, and **4r**) with a hydroxyl group at the benzene ring, the best compound was *para*-OH (**4p**), followed by *ortho*-OH (**4n**), and *meta*-OH (**4r**). When substituted with the halogen groups, the activity sequence is *para*-Cl (**4a**) > *ortho*-Cl (**4b**), *para*-Br (**4l**) > *ortho*-Br (**4f**). These results suggest that the 4-position group on the benzene ring is favorable for the PDHc-E1 inhibitory activity. When comparing the PDHc-E1 inhibitory activity of compounds **4a**, **4i**, and **4l**, the activity sequence is Br > Cl > F, which indicates the larger substituent is better. Base on this SAR analyses, we modified compound **4** by introducing a larger electron-withdrawing groups such as nitryl (compound **4o**, IC_{50} = 0.65 ± 0.10 μM) and trifluoromethyl (compound **4q**, IC_{50} = 1.92 ± 0.54 μM) groups on the 4-position of benzene ring as Ar. As we expected, inhibitory activity of both compounds **4o** and **4q** against *E. coli* PDHc-E1were greatly increased.

3.2. Molecular docking studies

To explore the interaction mode of *N*-acylhydrazone compounds with the active site of PDHc-E1, several molecular docking simulation studies were carried out by using the SURFLEX module of SYBYL package.[16] Based on the in vitro inhibition results, we selected compound **4o**, which was found to be best PDHc-E1 inhibitor (IC_{50} = 0.65 ± 0.10 μM) at our present work, as ligand example.

As shown in Figure 3, compound **4o** is bound in the active site of PDHc-E1, which had been reported as the ThDP-binding pocket, with the 'V' conformation.[16] On the right side of the 'V' conformation, it was observed that the pyrimidine ring of compound **4o** displayed a strong π–π stacking with the side chain ring of Phe602. Also observed was that, three hydrogen bonds participate in binding the aminopyrimidine ring. The 4-amino group of the pyrimidine ring forms a hydrogen bond with the main chain oxygen of Val192. Other two key hydrogen bonding interaction are made by the N1′ and N2′ of the pyrimidine ring with the residues Glu571 and Met194, respectively. On the middle of the 'V' conformation, the triazole in compound **4o** forms two hydrogen bond with the residues Glu522 and His640. It should be noted that the binding modes of the right and middle of the 'V' conformation were in perfect agreement with the results in recently publication.[16] However, on the left side of the 'V' conformation, the docking results indicate that the nitryl group on benzene ring not only can form two strong hydrogen bonds with Gly231 and Glu232, but also coordinate with the Mg^{2+} in the active site. It should be noticed that the nitrogen atom of the imine group of the *N*-acylhydrazone moiety establishes a hydrogen bond with His106.

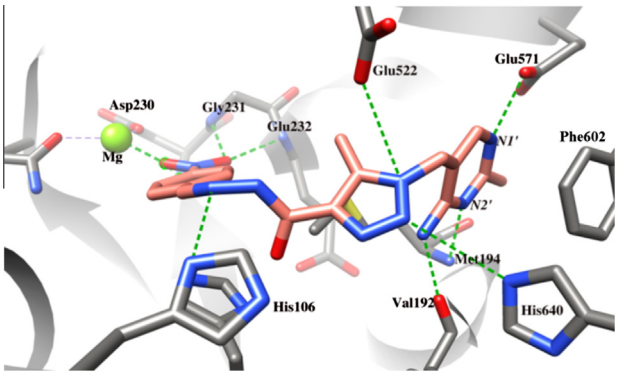


Figure 3. Optimal binding model for compound **4o** into active site of PDHc-E1 from *E. coli* docked by SURFLEX module, ligand and some key residues are shown in stick, hydrogen bonds are shown in dashed lines.

These docking results provided us a reasonable explanation for why compound **4o** had the highest PDHc-E1 inhibitory activity and some hints for future optimization synthetic directions.

4. Conclusion

We have designed and synthesized a series of 19 1-((4-amino-2-methylpyrimidin-5-yl)methyl)-5-methyl-*N*′-(substituent)benzylidene-1*H*-1,2,3-triazole-4-carbohydrazide as *E. coli* PDHc-E1 inhibitors. Among the group of 19 compounds, compounds **4a**, **4g**, **4l**, **4o**, **4p**, and **4q** were found to be very effective inhibitors of *E. coli* PDHc-E1, with IC_{50} values ranging from 0.65 to 3.43 μM. Compound **4o**, with 4-NO_2 in the benzene ring as Ar, was the best inhibitor of *E. coli* PDHc-E1 among the group. The molecular docking results revealed that the nitro group on compound **4o** not only could form two strong hydrogen bonds with Gly231 and Glu232, but also could form a coordinate-bond with the Mg^{2+}, which in turn seems to be important for enhancing its inhibitory potency. The SAR analyses and modeling study by molecular docking suggested a probable inhibition mechanism. These results provided us important hints for future optimization to design even better PDHc-E1 inhibitors.

5. Experimental

5.1. Chemistry

Melting points (mp) were measured on an electrothermal melting point apparatus and were uncorrected. ^{1}H NMR spectra were recorded in DMSO-d_6 solution on a Varian Mercury-Plus 600 spectrometer at 600 MHz, ^{13}C NMR spectra were recorded in DMSO-d_6 solution on a Varian Mercury-Plus 400 spectrometer at 100 MHz and chemical shifts were recorded in parts per million (ppm) with TMS as the internal reference. Mass spectra (MS) were run on a QTRAP LC/MS/MS system (API2000; Applied Biosystems, Foster City, CA, USA) or a TraceMS 2000 organic mass spectrometry, and signals were given in *m*/*z*. Elemental analyses (EA) were measured on a Vario ELIII CHNSO elemental analyzer. Unless otherwise noted, reagents were purchased from commercial suppliers and used without further purification. Intermediate **1** was synthesized according to the existing methods.[22]

5.2. Preparation of ethyl 1-((4-amino-2-methylpyrimidin-5-yl)methyl)-5-methyl-1*H*-1,2,3-triazole-4-carboxylate (2)

A solution of 5-azidomethyl-2-methylpyrimidine-4-ylamine (4.92 g, 30 mmol), ethyl acetoacetate (7.80 g, 60 mmol) and K_2CO_3 (7.28 g, 60 mmol) in DMF (60 mL) was stirred at 80 °C for 8 h. After this, the reaction mixture was poured into cold water and the resulting precipitate was filtered out affording compound **2** (5.79 g, 70%) as a white solid. Mp >260 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 1.27 (t, 3H, *J* = 7.2 Hz, CH_3), 2.32 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 4.27 (q, 2H, *J* = 7.2 Hz, CH_2), 5.36 (s, 2H, CH_2), 6.85 (s, 2H, NH_2), 7.80 (s, 1H, pyrimidine CH).

5.3. Preparation of 1-((4-amino-2-methylpyrimidin-5-yl)methyl)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (3)

To a solution of compound **2** (5.52 g, 20 mmol) in ethanol (80 mL) was added 20 mL of hydrazine monohydrate (80%). The reaction mixture was maintained under reflux for 5 h. The reaction mixture was then concentrated under reduced pressure and the resulting solid was collected by filtration, washed with cold water and dried to give the desired compound **3** (4.45 g, 85%) as a white solid. Mp >260 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.30 (s, 3H,

CH_3), 2.47 (s, 3H, CH_3), 4.41 (s, 2H, NH_2), 5.35 (s, 2H, CH_2), 6.85 (s, 2H, NH_2), 7.78 (s, 1H, pyrimidine CH), 9.59 (s, 1H, NH).

5.4. General procedure for preparation of 1-((4-amino-2-methylpyrimidin-5-yl)methyl)-*N*-(substituented)benzylidene-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide derivatives (4a–s)

To a solution of compound **3** (1 mmol) in ethanol (10 mL) was added appropriate aromatic aldehyde (1.2 equiv). The reaction mixture was stirred for 3 h at reflux, and then the mixture was poured into cold water and the resulting precipitate was collected by filtration, washed with CH_2Cl_2 (10 mL), and dried in the atmospheric pressure to give the desired compounds **4a**–**s**.

5.4.1. ((4-Amino-2-methylpyrimidin-5-yl)methyl)-*N*′-(4-chlorobenzylidene)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (4a)

White solid, yield: 76%, mp >260 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.32 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 5.42 (s, 2H, CH_2), 6.88 (s, 2H, NH_2), 7.52–7.53 (d, 2H, J = 8.4 Hz, Ar-H), 7.71–7.72 (d, 2H, J = 8.4 Hz, Ar-H), 7.84 (s, 1H, pyrimidine CH), 8.53 (s, 1H, CH═N); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 8.46, 24.92, 45.10, 107.30, 128.67, 128.94, 133.39, 134.45, 137.39, 137.65, 146.59, 154.52, 157.38, 161.69, 166.45; ESI-MS m/z: 385 $(M+1)^+$; Anal. Calcd for $C_{17}H_{17}ClN_8O$ (%): C, 53.06; H, 4.45; N, 29.12. Found: C, 52.89; H, 4.76; N, 28.83.

5.4.2. 1-((4-Amino-2-methylpyrimidin-5-yl)methyl)-*N*′-(2-chlorobenzylidene)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (4b)

White solid, yield: 77%, mp >260 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.31 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 5.42 (s, 2H, CH_2), 6.88 (s, 2H, NH_2), 7.44–7.46 (m, 2H, Ar-H), 7.52 (t, 1H, J = 7.2 Hz, Ar-H), 7.84 (s, 1H, pyrimidine CH), 8.01 (d, 1H, J = 7.2 Hz, Ar-H), 8.98 (s, 1H, CH = N); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 8.50, 24.73, 45.08, 107.40, 126.86, 127.55, 129.93, 131.40, 131.83, 133.31, 137.37, 137.80, 144.04, 153.93, 157.53, 161.79, 166.19; ESI-MS m/z: 385 $(M+1)^+$; Anal. Calcd for $C_{17}H_{17}ClN_8O$ (%): C, 53.06; H, 4.45; N, 29.12. Found: C, 52.91; H, 4.56; N, 28.76.

5.4.3. 1-((4-Amino-2-methylpyrimidin-5-yl)methyl)-*N*′-(2,4-dichlorobenzylidene)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (4c)

White solid, yield: 62%, mp >260 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.31 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 5.42 (s, 2H, CH_2), 6.88 (s, 2H, NH_2), 7.53 (d, 1H, J = 8.4 Hz, Ar-H), 7.72 (s, 1H, Ar-H), 7.84 (s, 1H, pyrimidine CH), 7.99 (d, 1H, J = 8.4 Hz, Ar-H), 8.94 (s, 1H, CH═N), 12.40 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 8.52, 24.04, 44.96, 107.64, 128.02, 129.36, 130.95, 132.29, 133.95, 134.99, 137.29, 137.96, 142.95, 151.95, 157.51, 162.07, 165.26; ESI-MS m/z: 419 $(M+1)^+$; Anal. Calcd for $C_{17}H_{16}Cl_2N_8O$ (%): C, 48.70; H, 3.85; N, 26.73. Found: C, 48.75; H, 3.90; N, 26.40.

5.4.4. 1-((4-Amino-2-methylpyrimidin-5-yl)methyl)-*N*′-(2,3-dichlorobenzylidene)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (4d)

White solid, yield: 76%, mp >260 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.32 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 5.42 (s, 2H, CH_2), 6.88 (s, 2H, NH_2), 7.46 (t, 1H, J = 7.8 Hz, Ar-H), 7.72 (d, 1H, J = 7.2 Hz, Ar-H), 7.84 (s, 1H, pyrimidine CH), 7.97 (d, 1H, J = 8.4 Hz, Ar-H), 9.02 (s, 1H, CH = N), 12.43 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 8.56, 22.90, 44.74, 108.07, 125.44, 128.43, 131.10, 131.47, 132.38, 134.28, 137.27, 138.14, 143.82, 148.56, 157.55, 162.54, 163.71; ESI-MS m/z: 419 $(M+1)^+$; Anal. Calcd for $C_{17}H_{16}Cl_2N_8O$ (%): C, 48.70; H, 3.85; N, 26.73. Found: C, 48.26; H, 3.80; N, 26.44.

5.4.5. 1-((4-Amino-2-methylpyrimidin-5-yl)methyl)-*N*′-(3,4-dichlorobenzylidene)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (4e)

White solid, yield: 76%, mp >260 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.32 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 5.42 (s, 2H, CH_2), 6.88 (s, 2H, NH_2), 7.68 (d, 1H, J = 7.8 Hz, Ar-H), 7.72 (d, 1H, J = 8.4 Hz, Ar-H), 7.84 (s, 1H, pyrimidine CH), 7.91 (s, 1H, Ar-H), 8.51 (s, 1H, CH═N), 12.23 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 8.50, 24.48, 45.04, 107.47, 126.96, 128.35, 131.11, 131.74, 132.19, 135.29, 137.31, 137.85, 145.24, 153.24, 157.50, 161.89, 165.86; ESI-MS m/z: 419 $(M+1)^+$; Anal. Calcd for $C_{17}H_{16}Cl_2N_8O$ (%): C, 48.70; H, 3.85; N, 26.73. Found: C, 48.36; H, 3.72; N, 26.54.

5.4.6. 1-((4-Amino-2-methylpyrimidin-5-yl)methyl)-*N*′-(2-bromobenzylidene)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (4f)

White solid, yield: 77%, mp >260 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.32 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 5.42 (s, 2H, CH_2), 6.88 (s, 2H, NH_2), 7.36 (t, 1H, J = 7.8 Hz, Ar-H), 7.46–7.48 (t, 1H, J = 7.2 Hz, Ar-H), 7.68 (d, 1H, J = 8.4 Hz, Ar-H), 7.84 (s, 1H, pyrimidine CH), 7.98 (d, 1H, J = 7.8 Hz, Ar-H), 8.92 (s, 1H, CH = N), 12.39 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 8.49, 24.50, 45.00, 107.47, 123.63, 127.24, 128.06, 131.66, 133.33, 137.38, 137.80, 146.36, 153.26, 157.53, 161.88, 165.87; ESI-MS m/z: 429 $(M+1)^+$; Anal. Calcd for $C_{17}H_{17}BrN_8O$ (%): C, 47.56; H, 3.99; N, 26.10. Found: C, 47.89; H, 4.12; N, 25.69.

5.4.7. 1-((4-Amino-2-methylpyrimidin-5-yl)methyl)-*N*′-(4-chloro-3-nitrobenzylidene)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (4g)

White solid, yield: 81%, mp 208–209 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.31 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 5.41 (s, 2H, CH_2), 6.89 (s, 2H, NH_2), 7.84 (d, 1H, J = 9.0 Hz, Ar-H), 7.84 (s, 1H, pyrimidine CH), 7.99 (d, 1H, J = 7.8 Hz, Ar-H), 8.34 (s, 1H, Ar-H), 8.56 (s, 1H, CH═N), 12.35 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 8.48, 25.22, 45.20, 107.15, 123.48, 125.78, 131.58, 132.24, 135.11, 137.24, 137.90, 144.45, 147.84, 155.48, 157.57, 161.58, 166.91; ESI-MS m/z: 430 $(M+1)^+$; Anal. Calcd for $C_{17}H_{16}ClN_9O_3$ (%): C, 47.50; H, 3.75; N, 29.33. Found: C, 47.50; H, 4.06; N, 29.66.

5.4.8. 1-((4-Amino-2-methylpyrimidin-5-yl)methyl)-5-methyl-*N*′-(4-methylbenzylidene)-1*H*-1,2,3-triazole-4-carbohydrazide (4h)

White solid, yield: 69%, mp 249–251 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.31 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 5.40 (s, 2H, CH_2), 6.89 (s, 2H, NH_2), 7.27 (d, 2H, J = 7.8 Hz, Ar-H), 7.58 (d, 2H, J = 7.8 Hz, Ar-H), 7.83 (s, 1H, pyrimidine CH), 8.48 (s, 1H, CH═N), 11.96 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 8.45, 21.03, 25.24, 45.17, 107.21, 127.05, 129.44, 131.75, 137.45, 137.53, 139.82, 147.98, 155.46, 157.28, 161.57, 166.89; ESI-MS m/z: 365 $(M+1)^+$; Anal. Calcd for $C_{18}H_{20}N_8O$ (%): C, 59.33; H, 5.53; N, 30.75. Found: C, 59.70; H, 5.52; N, 31.13.

5.4.9. 1-((4-Amino-2-methylpyrimidin-5-yl)methyl)-*N*′-(4-fluorobenzylidene)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (4i)

White solid, yield: 73%, mp >260 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.31 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 5.41 (s, 2H, CH_2), 6.89 (s, 2H, NH_2), 7.29 (d, 2H, J = 7.8 Hz, Ar-H), 7.75 (d, 2H, J = 7.8 Hz, Ar-H), 7.83 (s, 1H, pyrimidine CH), 8.51 (s, 1H, CH═N), 12.03 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 8.45, 25.23, 45.15, 101.58, 105.00, 107.21, 108.46, 123.38, 128.87, 137.40, 137.53, 147.72, 148.01, 149.07, 155.45, 157.22, 161.57, 166.89; ESI-MS m/z: 369 $(M+1)^+$; Anal. Calcd for $C_{17}H_{17}FN_8O$ (%): C, 55.43; H, 4.65; N, 30.42. Found: C, 55.79; H, 5.09; N, 29.90.

5.4.10. 1-((4-Amino-2-methylpyrimidin-5-yl)methyl)-5-methyl-*N*′-(3-methylbenzylidene)-1*H*-1,2,3-triazole-4-carbohydrazide (4j)

White solid, yield: 93%, mp >260 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.31 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 5.41 (s, 2H, CH_2), 6.89 (s, 2H, NH_2), 7.24 (d, 1H, J = 7.8 Hz, Ar-H), 7.33 (d, 2H, J = 7.8 Hz, Ar-H), 7.47 (d, 1H, J = 7.8 Hz, Ar-H), 7.52 (s, 1H, Ar-H), 7.83 (s, 1H, pyrimidine CH), 8.48 (s, 1H, CH=N), 12.01 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 8.46, 20.89, 25.24, 45.17, 107.22, 124.48, 127.39, 128.74, 130.76, 134.42, 137.52, 138.08, 148.03, 155.45, 157.37, 161.58, 166.90; ESI-MS m/z: 365 $(M+1)^+$; Anal. Calcd for $C_{18}H_{20}N_8O$ (%): C, 59.33; H, 5.53; N, 30.75. Found: C, 59.67; H, 5.47; N, 30.51.

5.4.11. 1-((4-Amino-2-methylpyrimidin-5-yl)methyl)-*N*′-(benzo[*d*][1,3]dioxol-5-ylmethylene)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (4k)

White solid, yield: 29%, mp >260 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.31 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 5.40 (s, 2H, CH_2), 6.08 (s, 2H, OCH_2O), 6.88 (s, 2H, NH_2), 6.98 (d, 1H, J = 7.8 Hz, Ar-H), 7.11 (d, 1H, J = 7.8 Hz, Ar-H), 7.27 (s, 1H, Ar-H), 7.83 (s, 1H, pyrimidine CH), 8.42 (s, 1H, CH=N), 11.92 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 8.44, 25.23, 45.15, 101.58, 105.00, 107.21, 108.46, 123.37, 128.87, 137.40, 137.53, 147.72, 148.01, 149.07, 155.45, 157.21, 161.57, 166.89; MS (EI) (m/z, %): 395.15 (M^++1, 2.26), 394.14 (M^+, 22.93); Anal. Calcd for $C_{18}H_{20}N_8O$ (%): C, 54.82; H, 4.60; N, 28.41. Found: C, 54.60; H, 4.61; N, 28.89.

5.4.12. 1-((4-Amino-2-methylpyrimidin-5-yl)methyl)-*N*′-(4-bromobenzylidene)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (4l)

White solid, yield: 84%, mp >260 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.31 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 5.40 (s, 2H, CH_2), 6.89 (s, 2H, NH_2), 7.65 (s, 4H, Ar-H), 7.83 (s, 1H, pyrimidine CH), 8.49 (s, 1H, CH=N), 12.01 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 8.47, 25.24, 45.17, 107.19, 123.26, 128.92, 131.87, 133.73, 137.39, 137.65, 146.70, 155.46, 157.40, 161.57, 166.90; ESI-MS m/z: 431 $(M+3)^+$; Anal. Calcd for $C_{17}H_{17}BrN_8O$ (%): C, 47.56; H, 3.99; N, 26.10. Found: C, 47.72; H, 4.46; N, 26.26.

5.4.13. 1-((4-Amino-2-methylpyrimidin-5-yl)methyl)-*N*′-benzylidene-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (4m)

White solid, yield: 54%, mp >260 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.32 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 5.41 (s, 2H, CH_2), 6.89 (s, 2H, NH_2), 7.44 (t, 3H, J = 8.4 Hz, Ar-H), 7.69 (d, 2H, J = 7.8 Hz, Ar-H), 7.83 (s, 1H, pyrimidine CH), 8.52 (s, 1H, CH=N), 12.02 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 8.44, 25.24, 45.14, 107.18, 127.05, 128.82, 130.01, 134.43, 137.46, 147.90, 155.44, 157.34, 161.55, 166.87; ESI-MS m/z: 351 $(M+1)^+$; Anal. Calcd for $C_{17}H_{18}N_8O$ (%): C, 58.27; H, 5.18; N, 31.98. Found: C, 58.35; H, 5.14; N, 32.01.

5.4.14. 1-((4-Amino-2-methylpyrimidin-5-yl)methyl)-*N*′-(2-hydroxybenzylidene)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (4n)

White solid, yield: 67%, mp >260 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.32 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 5.41 (s, 2H, CH_2), 6.90 (s, 2H, NH_2), 6.91 (t, 2H, J = 8.4 Hz, Ar-H), 7.29 (s, 1H, Ar-H), 7.45 (d, 1H, J = 7.8 Hz, Ar-H), 7.84 (s, 1H, pyrimidine CH), 8.69 (s, 1H, CH=N), 12.03 (s, 2H, NH+OH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 8.46, 25.42, 45.18, 107.14, 116.47, 118.58, 119.34, 129.91, 131.32, 137.04, 137.81, 148.96, 155.48, 157.17, 157.56, 161.56, 166.88; ESI-MS m/z: 367 $(M+1)^+$; Anal. Calcd for $C_{17}H_{18}N_8O_2$ (%): C, 55.73; H, 4.95; N, 30.58. Found: C, 55.91; H, 4.94; N, 30.84.

5.4.15. 1-((4-Amino-2-methylpyrimidin-5-yl)methyl)-5-methyl-*N*′-(4-nitrobenzylidene)-1*H*-1,2,3-triazole-4-carbohydrazide (4o)

Light yellow solid, yield: 79%, mp >260 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.33 (s, 3H, CH_3), 2.60 (s, 3H, CH_3), 5.43 (s, 2H, CH_2), 6.90 (s, 2H, NH_2), 7.86 (s, 1H, pyrimidine CH), 7.96 (d, 2H, J = 8.4 Hz, Ar-H), 8.32 (d, 2H, J = 8.4 Hz, Ar-H), 8.65 (s, 1H, CH=N), 12.37 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 8.50, 25.22, 45.19, 107.15, 124.08, 127.94, 137.24, 137.94, 140.77, 145.42, 147.79, 155.48, 157.59, 161.57, 166.90; MS (EI) (m/z, %): 395.16 (M^+, 3.37); Anal. Calcd for $C_{17}H_{17}N_9O_3$ (%): C, 51.64; H, 4.33; N, 31.88. Found: C, 51.99; H, 4.33; N, 32.37.

5.4.16. 1-((4-Amino-2-methylpyrimidin-5-yl)methyl)-*N*′-(4-hydroxybenzylidene)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (4p)

White solid, yield: 71%, mp >260 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.33 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 5.42 (s, 2H, CH_2), 6.83–6.84 (d, 2H, J = 8.4 Hz, Ar-H), 6.88 (s, 2H, NH_2), 7.52–7.53 (d, 2H, J = 8.4 Hz, Ar-H), 7.84 (s, 1H, pyrimidine CH), 8.42 (s, 1H, CH=N), 9.94 (s, 1H, OH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 8.50, 25.26, 45.22, 107.32, 115.81, 125.49, 128.97, 137.37, 137.68, 148.40, 155.47, 157.23, 159.47, 161.65, 166.98; MS (EI) (m/z, %): 367.24 (M^++1, 2.24), 366.21 (M^+, 11.73); Anal. Calcd for $C_{17}H_{18}N_8O_2$ (%): C, 55.73; H, 4.95; N, 30.58. Found: C, 55.55; H, 5.38; N, 30.26.

5.4.17. 1-((4-Amino-2-methylpyrimidin-5-yl)methyl)-5-methyl-*N*′-(4-(trifluoromethyl)benzylidene)-1*H*-1,2,3-triazole-4-carbohydrazide (4q)

White solid, yield: 96%, mp >260 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.32 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 5.41 (s, 2H, CH_2), 6.88 (s, 2H, NH_2), 7.81 (d, 2H, J = 7.8 Hz, Ar-H), 7.84 (s, 1H, pyrimidine CH), 7.90 (s, 2H, J = 7.8 Hz, Ar-H), 8.60 (s, 1H, CH=N), 12.24 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 8.48, 25.22, 45.19, 107.17, 122.78, 125.73, 127.62, 137.32, 137.80, 138.43, 146.15, 155.48, 157.53, 161.58, 166.90; MS (EI) (m/z, %): 418.26 (M^+, 4.31); Anal. Calcd for $C_{18}H_{17}F_3N_8O$ (%): C, 51.67; H, 4.10; N, 26.78. Found: C, 51.55; H, 4.38; N, 27.06.

5.4.18. 1-((4-Amino-2-methylpyrimidin-5-yl)methyl)-*N*′-(3-hydroxybenzylidene)-5-methyl-1*H*-1,2,3-triazole-4-carbohydrazide (4r)

White solid, yield: 80%, mp >260 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.32 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 5.42 (s, 2H, CH_2), 6.83 (d, 1H, J = 7.8 Hz, Ar-H), 6.91 (s, 2H, NH_2), 7.05 (d, 1H, J = 7.8 Hz, Ar-H), 7.16 (s, 1H, Ar-H), 7.25 (t, 1H, J = 7.8 Hz, Ar-H), 7.84 (s, 1H, pyrimidine CH), 8.44 (s, 1H, CH=N), 9.66 (s, 1H, OH), 11.98 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 8.48, 25.25, 45.16, 107.21, 112.70, 117.41, 118.77, 129.91, 135.72, 137.49, 148.06, 155.44, 157.32, 157.68, 161.57, 166.89; MS (EI) (m/z, %): 367.16 (M^++1, 4.29), 366.21 (M^+, 16.29); Anal. Calcd for $C_{17}H_{18}N_8O_2$ (%): C, 55.73; H, 4.95; N, 30.58. Found: C, 55.91; H, 5.20; N, 30.77.

5.4.19. 1-((4-Amino-2-methylpyrimidin-5-yl)methyl)-5-methyl-*N*′-(pyridin-2-ylmethylene)-1*H*-1,2,3-triazole-4-carbohydrazide (4s)

White solid, yield: 83%, mp >260 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.31 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 5.42 (s, 2H, CH_2), 6.91 (s, 2H, NH_2), 7.41 (t, 1H, J = 6.0 Hz, Ar-H), 7.83 (s, 1H, pyrimidine CH), 7.87 (t, 1H, J = 7.8 Hz, Ar-H), 7.94 (d, 1H, J = 7.8 Hz, Ar-H), 8.56 (s, 1H, CH=N), 8.59 (d, 1H, J = 4.2 Hz, Ar-H), 12.36 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 8.51, 25.24, 45.23, 107.19, 119.88, 124.33, 136.83, 137.34, 137.89, 148.18, 149.48, 153.48, 155.48, 157.62, 161.59, 166.92; MS (EI) (m/z, %): 351.20 (M^+, 10.85); Anal.

Calcd for $C_{16}H_{17}N_9O$ (%): C, 54.69; H, 4.88; N, 35.88. Found: C, 54.20; H, 4.94; N, 36.17.

5.5. Assay of *E. coli* PDHc-E1 (in vitro)

The expressing plasmid pMal-C_{2X}-PDHc-E1 was transformed into *E. coli* strain TB1 and inoculated in Luria–Bertani (LB) broth containing 2% glucose and 30 mg/ml ampicillin at 37 °C until reaching a cell density to A600 of 0.6–0.8. Then cells were induced with a final concentration of 0.5 mM IPTG for 7 h at 25 °C before harvesting. Purification of the fusion protein was carried out using a MBP affinity column attached to an AKTA purifier 10 (UPC-F920, GE Healthcare Life Sciences). The concentrations of purified proteins were determined by the method of Bradford[24] using bovine serum albumin (Tiangen) as standard. The final purity (>95%) of the sample was verified by SDS–PAGE and then the purified protein was stored in 50% (v/v) glycerol at −20 °C.

The inhibitory activities of synthesized compounds were measured by the enzymatic assay. PDHc-E1 activity was assayed by a modified method of N. Nemeria,[11] and measured by monitoring the reduction of 2,6-DichloroPhenolindophenol (2,6-DCPIP) at 600 nm using a microplate reader (BioTek Synergy2, USA). The total volume of 100 μL reaction mixture contained 50 mM K_3PO_4, pH 7.2, 2.0 mM sodium pyruvate as substrate, 0.8 mM 2,6-DCPIP, 7.1 μM enzyme and different concentration of inhibitors. The reaction mixtures were incubated for 3 min at 37 °C, then different concentrations of ThDP (ranging from 0 to 200 μM) were added to the initiate reaction. To determine the inhibitor concentration of synthesized compounds at 50% inhibition (IC_{50}), initial rate data taken at saturating substrate, fixed effectors, and systematically varied inhibitor concentrations were fit to Hill equation, $V = V_0 - (V_0 - V_\infty)/((IC_{50}/I)^n + 1)$,[25] Where V, V_0, and V_∞ are the velocity, maximum velocity (at $I = 0$), and the limiting velocity (at I saturating); n is the Hill coefficient associated with the inhibitor; and IC_{50} is the inhibition concentration of synthesized compounds at 50% inhibition. Each experiment was performed at least three times. All kinetic data were fit to the growth/sigmoidal model from origin 7.0 software. One unit of activity is defined as the amount of 2,6-DCPIP reduced (μmol/min/mg of PDHc-E1).

5.6. Molecular docking

For docking purposes, the crystallographic coordinates of the PDHc-E1 with bound ThDP from *E. coli* (PDB code: 1L8A) were obtained from Brookhaven Data Bank. Hydrogen atoms were added to the structure allowing for appropriate ionization at physiological pH. The protonated state of several important residues, such as His106, His142, Tyr599, Glu751 and His640, were adjusted by using SYBYL7.3 (Tripos, St. Louis, USA) in favor of forming reasonable hydrogen bond with the ligand. Molecular docking analysis was carried out by the SURFLEX module of SYBYL package to explore the interaction model for the active site of PDHc-E1 with its ligand. All atoms located within the range of 6.5 Å from any atom of the cofactor ThDP were selected into the active site, and the corresponding amino acid residue was, therefore, involved into the active site if only one of its atoms was selected. Other default parameters were adopted in the SURFLEX-docking calculations. All calculations were performed on a CCNUGrid-based computational environment (CCNUGrid website http://www.202.114.32.71:8090/ccnu/chem/platform.xml).

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