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Design, synthesis and biological evaluation of novel 2-methylpyrimidine-4-ylamine derivatives as inhibitors of *Escherichia coli* pyruvate dehydrogenase complex E1

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ABSTRACT

As potential inhibitors of *Escherichia coli* pyruvate dehydrogenase complex E1 (PDHc E1), a series of novel 2-methylpyrimidine-4-ylamine derivatives were designed based on the structure of the active site of PDHc E1 and synthesized using 'click chemistry'. Their inhibitory activity in vitro against PDHc E1 and fungicidal activity were examined. Some of these compounds such as **3g**, **3l**, **3n**, **3o**, and **5b** demonstrated to be effective inhibitors of PDHc E1 from *E. coli* and exhibited antifungal activity. SAR analysis indicated that both, the inhibitory potency against *E. coli* PDHc E1 and the antifungal activity of title compounds, could be increased greatly by optimizing substituent groups in the compounds. The structures of substituent group in 5-position on the 1,2,3-triazole and 4-position on the benzene ring in title compounds were found to play a pivotal role in both above-mentioned biological activities. Amongst all the compounds, compound **5b** with iodine in the 5-position of 1,2,3-triazole and with nitryl group in the 4-position of benzene ring acted as the best inhibitor against PDHc E1 from *E. coli*. It was also found to be the most effective compound with higher antifungal activity against *Rhizoctonia solani* and *Botrytis cinerea* at the dosage of 100 μg mL^{-1}. Therefore, in this study, compound **5b** was used as a lead compound for further optimization.

1. Introduction

Pyruvate dehydrogenase complex (PDHc) plays a pivotal role in cellular metabolism catalyzing the oxidative decarboxylation of pyruvate and the subsequent acetylation of coenzyme A (CoA) to acetyl-CoA.[1–4] The complex is comprised of three different enzymes components 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 the multistep process, using thiamine diphosphate (ThDP) (Fig. 1) and Mg^{2+} as cofactors.[5–7] Therefore, it has been studied clearly that the ThDP plays an important role in the enzyme reaction and the catalysis mechanism.[8]

A systematic study for the rational design for the potential use of a novel effective inhibitor of PDHc E1 as herbicide has been previously carried out in our laboratory for several years.[9–12] Some of 1-(substituted phenoxyacetoxy)alkylphosphonates (such as HW02) were successfully found to be effective inhibitors of PDHc E1 from plants and showed potential utility as herbicide.[13] These results encouraged further exploration and research of new inhibitors of PDHc E1 possessing bactericidal or fungicidal activity based on the structure of PDHc E1 in the microorganism.

Due to the important role of fungicide in modern agriculture, it is essential to develop efficient fungicide with novel structures or modes of action to overcome fungicide resistance. In order to find a novel fungicide or antifungal activity compound based on the design of inhibitor of PDHc E1, *Escherichia coli* PDHc E1 was selected as the target pattern of microorganism. Considering the importance of ThDP in the metabolic process of pyruvate, this research also focuses on designing novel ThDP analogs as inhibitors of PDHc E1 in *E. coli*. Through literature review, it was noticed that some ThDP analogs (Fig. 2) have been chemically synthesized and were reported as effective inhibitors of *E. coli* PDHc E1.[14–17] Although these analogs of ThDP showed potent inhibition against *E. coli* PDHc E1, they exhibit no potential utility as fungicide due to their complex structure with highly charged pyrophosphate. These complex structures are unsuitable for good bioavailability.[18] Moreover, there was no report about their bactericidal or fungicidal activity.

Aiming at the above-mentioned problems, both the pyrophosphate and the thiazolium ring moiety in ThDP were replaced by substituted benzene ring and 1,2,3-triazole ring, respectively. It

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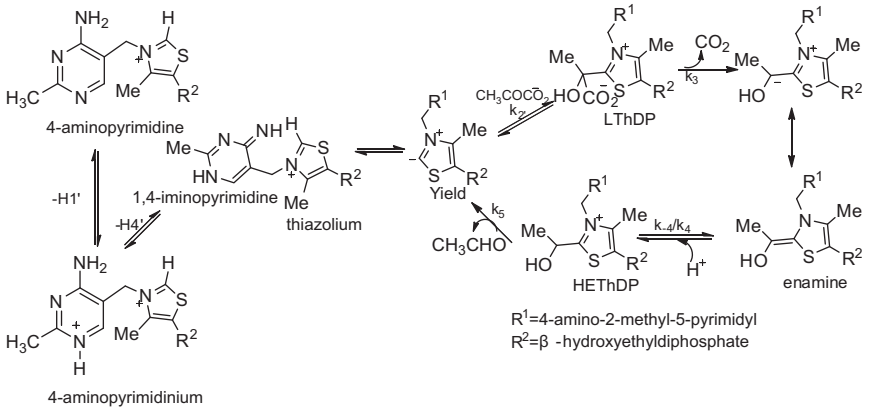


Figure 1. Mechanism of pyruvate dehydrogenase complex (E1) component.

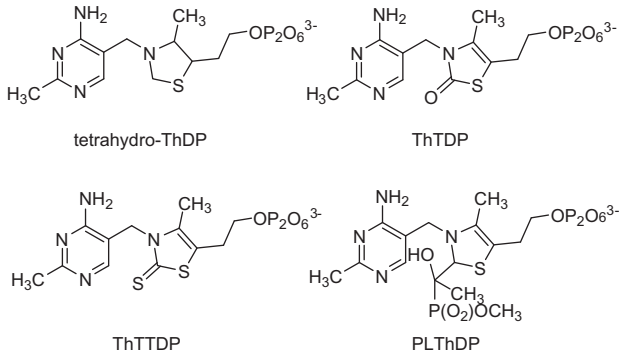


Figure 2. Structures of ThDP analogs.

has been verified that some hit compounds with aminopyrimidine, triazole and benzene ring moiety could be effective in occupying the ThDP-binding pocket of PDHc E1 and also may be bound in a pocket of PDHc-E1 by using structure-based molecular docking methods.[19] Therefore, a series of novel 2-methylpyrimidine-4-ylamine derivatives **3** and **5** as potential inhibitors of *E. coli* PDHc E1 were designed based on the structure of ThDP and properties of the active site of PDHc E1. The design rationality of title compounds **3** and **5** was further verified by later experiments.

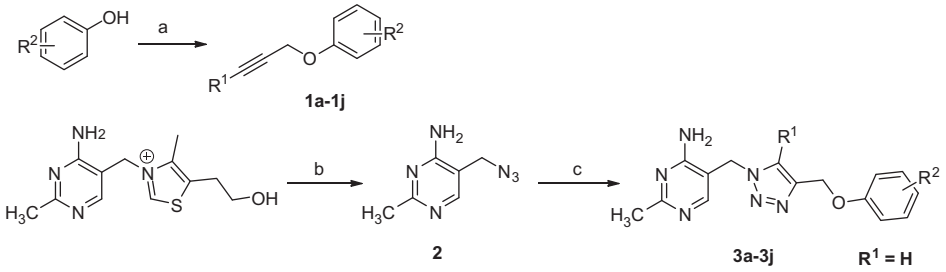
In this paper, we reported the synthesis of 12 novel 1,2,3-triazole containing 2-methylpyrimidine-4-ylamine derivatives **3** and **5** using click chemistry. All these compounds were tested for their inhibition against PDHc E1 from *Escherichia coli*. The title compounds, including some previously reported analogous compounds, were further evaluated on the basis of their practicality, antifungal properties and structure–activity relationships.

2. Chemistry

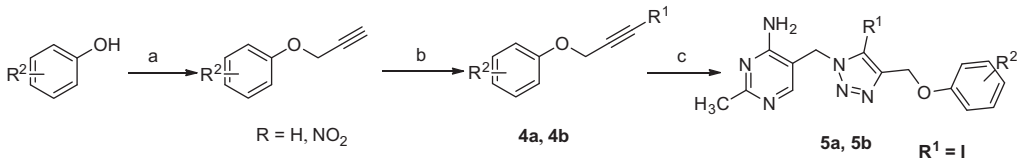
Triazole-based pyrophosphate analogs of ThDP had been reported by Leeper and co-workers,[20] followed their method, the synthetic route employed to obtain the title compounds **3a–3j** is depicted in Scheme 1. The 10 terminal alkynes **1a–1j** were synthesized from the 3-bromopropyne with corresponding substituted phenol in refluxing acetone with K_2CO_3 as base. The 5-azidomethyl-2-methylpyrimidine-4-ylamine **2** was prepared readily from thiamine hydrochloride according to the literature method as described.[20] The Cu-catalyzed 1,3-dipolar cycloaddition reaction[21] was introduced to assemble the final target compounds **3a–3j**. A combination of copper(II) sulfate/sodium ascorbate was utilized in situ to prepare the copper(I) species, and a 'click chemistry' was achieved in 12–24 h at room temperature.

General synthetic procedure for compounds **5a** and **5b** is shown in Scheme 2. A one-pot, two-stage sequence was used to synthesize 5-iodotirazoles **5a** and **5b**. The two terminal alkynes were treated with *N*-iodomorpholine[22] catalyzed with copper(I) iodide (CuI) within 60 min, the corresponding 1-iodoalkynes (**4a** and **4b**) could be purified by filtration through a pad of neutral alumina in excellent yield. The 1-iodoalkynes and azide **2** were catalyzed by copper(I) iodide-triethylamine (TEA) in THF, and gave 5-iodotriazoles **5a** and **5b** in excellent yield.

The chemical structures of all the target compounds (Table 1) were confirmed by recording their ^{1}H NMR, ^{13}C NMR, mass spectra and elementary analysis. The ^{1}H NMR spectrum of all the compounds showed a singlet for three (CH_3) protons at δ (2.30–2.33), another two singlets for two (OCH_2 and CH_2) protons at δ (5.10–5.47 and 5.35–5.52, respectively), and a singlet for two (NH_2) protons at δ (5.59–5.60 for $CDCl_3$ and 5.59–5.98 for DMSO-d_6, respectively). Interestingly, it must be noted that one proton (pyrimidine-H) disappeared for compounds **5a** and **5b**, but on the

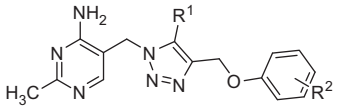


Scheme 1. Reagents and conditions: (a) 3-Bromopropyne, K_2CO_3, acetone, reflux, 5 h; (b) NaN_3, Na_2SO_3, H_2O, 60–65 °C, 6 h; (c) **1a**–**1j**, sodium ascorbate, $CuSO_4{\cdot}5H_2O$, *t*-BuOH/H_2O (2:1), rt, 12–24 h.



Scheme 2. Reagents and conditions: (a) 3-Bromopropyne, K_2CO_3, acetone, reflux, 5 h; (b) *N*-iodomorpholine, CuI, THF, rt, 1 h; (c) **2**, CuI, THF, Et_3N, rt, 6 h.

Table 1
Structures and IC_{50} values against PDHc E1 inhibitory activities of derivatives **3a–3o** and **5a–5b**



Compounds	R^1	R^2	IC_{50}[a] (μM)
3a	H	4-OCH_3	81.62
3b	H	2-NO_2	36.29
3c	H	4-Cl	26.44
3d	H	3-CF_3	32.73
3e	H	2-Cl-5-CH_3	28.99
3f	H	2-Cl-4-F	43.32
3g	H	2,4-2NO_2	11.35
3h	H	4-CH_2CO_2Me	101.77
3i	H	2,4-2Cl	18.74
3j	H	3-CH_3-4-Cl	26.20
3k	H	4-CO_2Et	28.75
3l	H	4-NO_2	8.80
3m	H	H	55.15
3n	H	2-Cl-4-NO_2	6.88
3o	H	4-COOH	15.10
5a	I	H	19.56
5b	I	4-NO_2	5.33

[a] The values were estimated statistically by origin 7.0 software using a personal computer.

basis of the above-mentioned data, it can be conclude that the structures of compounds **5a** and **5b** have been identified correctly. ^{13}C NMR spectra of all the compounds were taken in DMSO-d_6 and the obtained signal further verified the proposed structures. All the compounds showed a signal at δ (25.2–25.4), which is due to the presence of methyl carbon (2-methylpyrimidine). Two methylene carbons displayed signals at 46.7–48.0 and 60.3–63.6, respectively. The mass spectrum of all the compounds showed molecular ion peak at m/z = [M+1], which is in agreement with the molecular formula. The spectral values for all the compounds and C, H, N analyses are given in the experimental part.

3. Results and discussion

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

All the synthesized compounds **3a–3j** and **5a–5b**, including previously reported analogous compounds **3k–3o**,[19] were evaluated for their inhibitory activity against PDHc E1 from *Escherichia coli*. The IC_{50} values are summarized in Table 1. It was observed that many compounds exhibited good inhibitory activity (IC_{50} <30 μM). A simple structure–activity relationship analysis showed that the PDHc E1 inhibitory potency closely related to the substituent group R^2 on the benzene ring.

It was noticed that the compounds with electron-donating substituent as R^2 on the benzene ring had very weak inhibitory activity against PDHc E1 from *Escherichia coli*, while the presence of electron-withdrawing groups as R^2 slightly improved the inhibitory activity. However, the inhibitory activity was highly dependent upon both, the structure and position of substituent R^2 on the benzene ring. Comparing the data in Table 1, the compounds **3** with 2-Cl, 4-NO_2 and 4-NO_2 as R^2 on the benzene ring showed a great promotive effect to the inhibitory activity followed by 2,4-2NO_2, such as **3n** and **3l** with 2-Cl, 4-NO_2 and 4-NO_2 as R^2 had IC_{50} values of 6.88 and 8.80 μM against *E. coli* PDHc E1, respectively. However, compound **3h** with 4-CH_2CO_2Me as R^2 showed the lowest inhibitory activity (IC_{50} = 101.77 μM). It showed that the more bulky substituent at 4-position of benzene ring was not favorable for the PDHc E1 inhibitory activity. We found that 4-NO_2 could significantly enhance the inhibitory activity against the PDHc-E1. In conclusion, all the compounds with 4-NO_2 in the benzene ring displayed much higher inhibitory potency than other compounds. The relationship between the inhibitory activity and the functional groups in title compounds could be reasonable explained by jointly using molecular docking and MD simulation. For instance, the recent result analyses of representative compound **3n** with 2-Cl-4-NO_2 as R^2 has revealed that the aminopyrimidine ring could interact with the active site of PDHc-E1 by strong π–π and hydrogen bonds, and the triazole could form two hydrogen bond with the residues Glu522 and His640, respectively. Moreover, the nitryl group on benzene ring presented a special case as it not only formed three hydrogen bonds with Gly231, Asn260 and Lys392, but also coordinated with the Mg^{2+} in the active site. Furthermore, it appears that the benzene group appears formed π–π stacking with the residue His106.[19] Using the aforementioned results, a powerful inhibitor can be designed by focusing on enhancing its direct interactions with the key residues present in the active site of PDHc-E1.

We have noticed that the introduction of halogens sometimes can lead to an improved activity profile.[23] Owing to the larger van der Waals radius (with 2.15 Å) and total size (with 4.29 $Å^3$) of the C–I bond compared with that of C–H bond (with 1.20 Å and 2.29 $Å^3$, respectively),[23] the introduction of iodine would be better to satisfy the steric requirement at the receptor site. So, the H and 4-NO_2 as R^2 were kept constant, while further optimization of the design was achieved by the introduction of iodine in the 5-position of 1,2,3-triazole of compounds **3**. Compounds **5a** and **5b** were synthesized to investigate the effect of introduction of iodine on the inhibitory activity of PDHc E1. As shown in Table 1, compounds **5a** and **5b** with **I** as R^1 (IC_{50} = 19.56 μM and 5.33 μM, respectively) showed higher enzyme inhibitory activity than that of the corresponding compounds **3m** and **3l** with **H** as R^1 (IC_{50} = 55.15 μM and 8.80 μM, respectively). Based on the results, it can be concluded that the 5-position of 1,2,3-triazole plays an important role in the PDHc E1 inhibitory activity. Additionally, the inhibitory activity could be further enhanced by introducing **I** in the 5-position of 1,2,3-triazole of compounds. Once again, it was also found that 4-NO_2 could significantly enhance the inhibitory activity of PDHc-E1, irrespective of **H** or **I** as R^1. The compounds with 4-NO_2 in the benzene ring displayed much higher inhibitory potency than other compounds. Furthermore, the compound **5b** with iodine as R^1 in the 5-position of 1,2,3-triazole and with nitryl group as R^2 in the 4-position of benzene ring was found to be the best inhibitor against PDHc E1 from *E. coli*.

Table 2
The antifungal activities of some compounds **3** and **5a–5b** at 100 μg mL^{-1}

Compounds	R^1	R^2	Inhibition rate (%)					
			G. zeae[a]	*R. solani*[a]	*B. cinerea*[a]	*A. solani*[a]	*A. alternate Keissler*[a]	*C. orbiculare*[a]
3c	H	4-Cl	51	23	73	44	52	50
3g	H	2,4-2NO_2	48	0	54	0	0	42
3n	H	2-Cl-4-NO_2	0	31	44	20	36	57
3o	H	4-COOH	21	0	67	33	57	42
3m	H	H	0	0	53	25	43	40
5a	I	H	59	96	88	57	23	45
3l	H	4-NO_2	0	24	37	24	33	43
5b	I	4-NO_2	68	97	84	66	59	35
Difenoconazol			98	100	96	100	100	97

[a] *G. zeae*, *Gibberella zeae*; *R. solani*, *Rhizoctonia solani*; *B. cinerea*, *Botrytis cinerea*; *A. solani*, *Alternaria solani*; *A. alternate Keissler*, *Alternaria alternate Keissler*; *C. orbiculare*, *Colletotrichum orbicul*.

3.2. Fungicidal activity

In order to examine the practicality of title compounds, some of the compounds with higher enzyme inhibitory activity were chosen from groups **3** and **5** to further evaluate their antifungal activity against *G. zeae*, *R. solani*, *B. cinerea*, *A. solani*, *A. alternate Keissler*, and *C. orbiculare*. The results in Table 2 showed that most of the tested compounds **3** did not display obvious fungicidal activity, except compounds **3c** and **3o**, which showed fair fungicidal activity against *B. cinerea*. It should be noted that the compounds **3m** and **3l** had weak fungicidal activity (<60% inhibitory rate) against all types of fungi at 100 μg mL^{-1}. However the corresponding compounds **5a** and **5b** with **I** as **R**^{1} in the 5-position of the 1,2,3-triazole ring exhibited significantly better fungicidal activity than that of compounds **3m** and **3l**. Compounds **5a** and **5b** exhibited good to excellent fungicidal activity against *B. cinerea* and *R. solani*, especially compounds **5b** also displayed obvious fungicidal activity against *G. zeae* and *A. solani* at the dosage of 100 μg mL^{-1}. The test results indicated that the fungicidal activity could be significantly enhanced by introducing iodine in the 5-position of the 1,2,3-triazole ring.

4. Conclusion

Based on the structure of active site of PDHc E1, where is occupied by ThDP, a series of 2-methylpyrimidine-4-ylamine derivatives as the analogs of ThDP were designed as *E. coli* PDHc E1 inhibitors and synthesized using click chemistry. SAR analyses indicated that the inhibitory potency against *E. coli* PDHc E1 and antifungal activity of title compounds could be increased greatly by optimizing substituent groups in the compounds. All the compounds with 4-NO_2 in the benzene ring displayed much higher inhibitory potency against *E. coli* PDHc E1 than other compounds. Moreover, introduction of iodine into the 5-position of 1,2,3-triazole of title compounds could further increased the inhibitory activity of *E. coli* PDHc E1. However, the obvious antifungal activity of title compound could be achieved by a reasonable combination of both, the substituted benzene ring and substituted 1,2,3-triazole ring moieties in the parent compound. The results of the activity evaluation showed that the 5-position of the 1,2,3-triazole played a pivotal role in both *E. coli* PDHc E1 inhibitory activity and fungicidal activity. Therefore, compound **5b** with **I** as R^1 in the 5-position of 1,2,3-triazole and with 4-NO_2 as R^2 in the benzene ring, acted as the best inhibitor against PDHc E1 from *E. coli* and was found to be the most effective compound with higher antifungal activity against *R. solani* and *B. cinerea* at the dosage of 100 μg mL^{-1}. The above-mentioned results of SAR analyses will be useful for further research on designing more potent *E. coli* PDHc E1 inhibitors with higher antifungal activity, while compounds **5b** will be as used a lead compound for further optimization.

5. Experimental

5.1. Chemistry

Melting points (mp) were measured on an electrothermal melting point apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz or 600 MHz, in $CDCl_3$ or DMSO-d_6 solution on a Varian Mercury-Plus 400 or 600 spectrometer and chemical shifts were recorded in parts per million (ppm) with TMS as the internal reference. Mass spectra (MS) were obtained on a QTRAP LC/MS/MS system (API2000; Applied Biosystems, Foster City, CA, USA), and signals were given in *m/z*. Elemental analysis (EA) was measured on a Vario ELIII CHNSO elemental analyzer. Unless otherwise noted, reagents were purchased from commercial suppliers and used without further purification. Intermediate **2** was synthesized according to the existing methods.[20]

5.2. General procedure for preparation of compounds 3a–3j

A solution of 3-bromopyropyne (0.71 g, 6 mmol), corresponding substituted phenol (5 mmol) and K_2CO_3 (1.38 g, 10 mmol) in acetone (20 mL) was heated under reflux until the reaction was complete based on TLC monitoring. Then the solvent was removed under reduced pressure. The residue was dissolved in water and the aqueous layer was extracted twice with dichloromethane. The combined organic phases were dried with $MgSO_4$ and evaporated at reduced pressure to obtain product, which was used directly for the next step reaction without further purification.

To a stirred solution of 5-azidomethyl-2-methylpyrimidine-4-ylamine **2** (1 mmol, 1.0 equiv) and substituted (prop-2-yn-1-yloxy)benzene **1a–1j** (1.1 mmol, 1.1 equiv) in *tert*-butanol/water (9 mL, 2:1) were added sodium ascorbate (99 mg, 0.5 mmol) and $CuSO_4{\cdot}5H_2O$ (12.5 mg, 0.05 mmol). The reaction mixture was stirred at room temperature for 12–24 h. It was poured into cold water (50 mL), and the precipitate was collected by filtration and dried in the atmospheric pressure. Recrystallization with appropriate solvent afforded the desired solid compounds **3a–3j**.

5.2.1. 5-((4-((4-Methoxyphenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)-2-methylpyrimidin-4-amine (3a)

White solid, yield: 87%, mp 115–117 °C; ^{1}H NMR ($CDCl_3$, 600 MHz): δ 2.30 (s, 3H, CH_3), 3.77 (s, 3H, CH_3), 5.13 (s, 2H, OCH_2), 5.36 (s, 2H, CH_2), 5.60 (s, 2H, NH_2), 6.82–6.83 (d, 2H, J = 9.0 Hz, Ar-H), 6.89–6.90 (d, 2H, J = 8.4 Hz, Ar-H), 7.61 (s, 1H, triazole CH), 8.19 (d, 1H, pyrimidine CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 25.2, 46.8, 55.4, 61.6, 108.3, 114.6, 115.7, 124.5,

143.1, 151.1, 152.1, 153.6, 161.5, 167.1. ESI-MS *m/z*: 327 $(M+1)^+$. Anal. Calcd For $C_{16}H_{18}N_6O_2$: C, 58.88; H, 5.56; N, 25.75. Found: C, 58.95; H, 5.86; N, 25.45.

5.2.2. 2-Methyl-5-((4-((2-nitrophenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)pyrimidin-4-amine (3b)

Yellow solid, yield: 90%, mp 184–185 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.31 (s, 3H, CH_3), 5.34 (s, 2H, OCH_2), 5.46 (s, 2H, CH_2), 6.95 (s, 2H, NH_2), 7.13–7.15 (t, 1H, J = 7.2 Hz, 7.8 Hz, Ar-H), 7.57–7.58 (d, 1H, J = 8.4 Hz, Ar-H), 7.65–7.68 (d, 1H, J = 7.8 Hz, Ar-H), 7.85–7.87 (d, 1H, J = 7.8 Hz, Ar-H), 8.01 (s, 1H, triazole CH), 8.23 (s, 1H, pyrimidine CH). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 25.2, 46.8, 62.5, 115.1, 116.0, 120.7, 121.1, 121.5, 124.3, 124.7, 125.4, 133.8, 134.7, 139.7, 141.8, 150.6, 161.5. ESI-MS *m/z*: 342 $(M+1)^+$. Anal. Calcd For $C_{15}H_{15}N_7O_3$: C, 52.78; H, 4.43; N, 28.73. Found: C, 52.64; H, 4.36; N, 28.31.

5.2.3. 5-((4-((4-Chlorophenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)-2-methylpyrimidin-4-amine (3c)

White solid, yield: 78%, mp 168–170 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.31 (s, 3H, CH_3), 5.13 (s, 2H, OCH_2), 5.45 (s, 2H, CH_2), 6.95 (s, 2H, NH_2), 7.05–7.06 (d, 2H, J = 9.0 Hz, Ar-H), 7.32–7.34 (d, 2H, J = 9.6 Hz, Ar-H), 8.01 (s, 1H, triazole CH), 8.21 (s, 1H, pyrimidine CH). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 25.3, 46.9, 61.3, 109.1, 116.5, 124.7, 129.3, 142.6, 156.2, 156.9, 161.5, 167.7. ESI-MS *m/z*: 331 $(M+1)^+$. Anal. Calcd For $C_{15}H_{15}ClN_6O$: C, 54.47; H, 4.57; N, 25.41. Found: C, 54.74; H, 4.43; N, 25.17.

5.2.4. 2-Methyl-5-((4-((3-(trifluoromethyl)phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)pyrimidin-4-amine (3d)

White solid, yield: 89%, mp 160–161 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.31 (s, 3H, CH_3), 5.23 (s, 2H, OCH_2), 5.46 (s, 2H, CH_2), 6.96 (s, 2H, NH_2), 7.30–7.31 (d, 1H, J = 7.8 Hz, Ar-H), 7.34–7.36 (t, 2H, J = 8.4 Hz, 12.0 Hz, Ar-H), 7.52–7.53 (d, 1H, J = 7.8 Hz, Ar-H), 8.02 (s, 1H, triazole CH), 8.24 (s, 1H, pyrimidine CH). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 25.2, 46.8, 61.4, 108.2, 111.3, 117.4, 118.9, 123.1, 124.8, 124.9, 130.1, 130.3, 130.5, 130.7, 142.3, 156.4, 158.3, 161.6, 167.2. ESI-MS *m/z*: 365 $(M+1)^+$. Anal. Calcd For $C_{16}H_{15}F_3N_6O$: C, 52.75; H, 4.15; N, 23.07. Found: C, 52.46; H, 4.35; N, 22.94.

5.2.5. 5-((4-((2-Chloro-5-methylphenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)-2-methylpyrimidin-4-amine (3e)

White solid, yield: 80%, mp 184–185 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.30 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 5.20–5.21 (d, 2H, J = 5.4 Hz, OCH_2), 5.46–5.47 (d, 2H, J = 6.0 Hz, CH_2), 6.79 (s, 1H, Ar-H), 6.96 (s, 2H, NH_2), 7.17–7.18 (d, 1H, J = 5.4 Hz, Ar-H), 7.27–7.29 (d, 1H, J = 7.2 Hz, Ar-H), 8.03 (s, 1H, triazole CH), 8.25 (s, 1H, pyrimidine CH). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 20.9, 25.2, 46.8, 61.9, 108.3, 115.0, 118.3, 122.3, 124.8, 129.5, 138.1, 142.4, 153.0, 156.3, 161.5, 167.4. ESI-MS *m/z*: 345 $(M+1)^+$. Anal. Calcd For $C_{16}H_{17}ClN_6O$: C, 55.73; H, 4.97; N, 24.37. Found: C, 55.41; H, 4.99; N, 24.44.

5.2.6. 5-((4-((2-Chloro-4-fluorophenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)-2-methylpyrimidin-4-amine (3f)

White solid, yield: 78%, mp 169–171 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.31 (s, 3H, CH_3), 5.21 (s, 2H, OCH_2), 5.46 (s, 2H, CH_2), 6.95 (s, 2H, NH_2), 7.18–7.21 (t, 1H, J = 6.6 Hz, 7.8 Hz, Ar-H), 7.35–7.38 (q, 1H, J = 4.8 Hz, Ar-H), 7.43–7.44 (d, 1H, J = 6.0 Hz, Ar-H), 8.01 (s, 1H, triazole CH), 8.23 (s, 1H, pyrimidine CH). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 25.2, 46.8, 62.6, 114.5, 114.6, 115.3, 117.1, 117.3, 122.1, 122.2, 124.8, 142.2, 150.2, 155.1, 156.2, 156.7, 161.5, 167.3. ESI-MS *m/z*: 349 $(M+1)^+$. Anal. Calcd For $C_{15}H_{14}ClFN_6O$: C, 51.66; H, 4.05; N, 24.10. Found: C, 51.18; H, 4.04; N, 24.02.

5.2.7. 5-((4-((2,4-Dinitrophenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)-2-methylpyrimidin-4-amine (3g)

Yellow solid, yield: 50%, mp 210–211 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.30 (s, 3H, CH_3), 5.47 (s, 2H, OCH_2), 5.52 (s, 2H, CH_2), 6.98 (s, 2H, NH_2), 7.80–7.83 (d, 1H, J = 14.4 Hz, Ar-H), 8.27 (s, 1H, pyrimidine CH), 8.51–8.54 (m, 1H, Ar-H), 8.75–8.75 (d, 1H, J = 4.2 Hz, Ar-H). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 25.2, 46.9, 63.6, 108.5, 116.2, 121.2, 125.3, 129.2, 138.7, 139.9, 141.0, 155.2, 155.9, 161.6, 167.3. ESI-MS *m/z*: 387 $(M+1)^+$. Anal. Calcd For $C_{15}H_{14}N_8O_5$: C, 46.63; H, 3.65; N, 29.01. Found: C, 46.45; H, 3.86; N, 28.81.

5.2.8. Methyl 2-(4-((1-((4-amino-2-methylpyrimidin-5-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)acetate (3h)

White solid, yield: 85%, mp 142–143 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.30 (s, 3H, CH_3), 3.59 (s, 5H, CH_2 + CH_3), 5.10 (s, 2H, OCH_2), 5.44 (s, 2H, CH_2), 6.93 (s, 2H, NH_2), 6.96–6.97 (d, 2H, J = 8.4 Hz, Ar-H), 7.16–7.18 (d, 2H, J = 8.4 Hz, Ar-H), 8.01 (s, 1H, triazole CH), 8.20 (s, 1H, pyrimidine CH). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 25.2, 39.9, 46.7, 51.6, 61.0, 108.2, 114.4, 114.6, 124.5, 126.6, 130.9, 142.8, 156.9, 161.5, 167.0, 171.8. ESI-MS *m/z*: 369 $(M+1)^+$. Anal. Calcd For $C_{19}H_{22}N_6O_3$: C, 58.69; H, 5.47; N, 22.81. Found: C, 58.77; H, 5.48; N, 22.67.

5.2.9. 5-((4-((2,4-Dichlorophenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)-2-methylpyrimidin-4-amine (3i)

Yellow solid, yield: 70%, mp 175–177 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ2.31 (s, 3H, CH_3), 5.24 (s, 2H, OCH_2), 5.46 (s, 2H, CH_2), 6.96 (s, 2H, NH_2), 7.38 (s, 2H, Ar-H), 7.57 (s, 1H, Ar-H), 8.01 (s, 1H, triazole CH), 8.25 (s, 1H, pyrimidine CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 25.2, 46.8, 62.3, 108.2, 115.6, 122.5, 124.8, 128.0, 129.3, 142.0, 152.5, 156.3, 161.5, 167.1. ESI-MS *m/z*: 365 $(M+1)^+$. Anal. Calcd For $C_{15}H_{14}Cl_2N_6O$: C, 49.33; H, 3.86; N, 23.01. Found: C, 49.40; H, 4.20; N, 22.89.

5.2.10. 5-((4-((4-Chloro-3-methylphenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)-2-methylpyrimidin-4-amine (3j)

Yellow solid, yield: 74%, mp 142–143 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.28 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 5.11 (s, 2H, OCH_2), 5.45 (s, 2H, CH_2), 6.95 (s, 2H, NH_2), 6.89 (s, 1H, Ar-H), 7.03–7.04 (d, 1H, J = 3.6 Hz, Ar-H), 7.28–7.31 (d, 1H, J = 14.4 Hz, Ar-H), 8.00 (s, 1H, triazole CH), 8.20 (s, 1H, pyrimidine CH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 19.7, 25.2, 46.7, 61.2, 113.8, 117.4, 124.6, 124.9, 129.4, 136.5, 139.9, 140.7, 142.5, 150.2, 156.7, 161.5. ESI-MS *m/z*: 345 $(M+1)^+$. Anal. Calcd For $C_{16}H_{17}ClN_6O$: C, 55.73; H, 4.97; N, 24.37. Found: C, 55.63; H, 4.86; N, 24.39.

5.3. One-pot, two-stage procedure for preparation of compounds 5a and 5b

Terminal alkynes[19] (1.5 mmol) was dissolved in THF (5 mL) and treated with *N*-iodomorpholine (0.56 g, 1.65 mmol) followed by CuI (0.014 g, 0.075 mmol). The reaction mixture was stirred at room temperature for 60 min, after which time a fine white precipitate had formed. The suspension was poured onto a pad of neutral alumina (20 mL) and the solution was collected under vacuum. The pad was then washed three times with THF (5 mL). This solution was charged with 5-azidomethyl-2-methylpyrimidine-4-ylamine **2** (0.25 g, 1.5 mmol), followed by TEA (0.30 g, 3 mmol) and finally CuI (0.014 g, 0.075 mmol). The reaction mixture was stirred at room temperature for 12 h. The volatile components were removed by evaporation, and the resulting residue was suspended in water and diethyl ether. A precipitate formed upon vigorous stirring and was isolated by filtration, give compounds **5a** and **5b** as a fine yellow powder.

5.3.1. 5-((5-Iodo-4-(phenoxymethyl)-1*H*-1,2,3-triazol-1-yl)methyl)-2-methylpyrimidin-4-amine (5a)

Yellow solid, yield: 75%, mp 169–171 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.32 (s, 3H, CH_3), 5.06 (s, 2H, CH_2), 5.45 (s, 2H, CH_2), 6.97 (s, 2H, NH_2), 7.05 (s, 2H, Ar-H), 7.31 (s, 2H, Ar-H), 7.69 (s, 1H, Ar-H). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 25.27, 48.01, 61.37, 86.15, 107.49, 114.80, 121.16, 129.58, 147.38, 155.09, 158.16, 161.41, 166.86. ESI-MS *m/z*: 423 $(M+1)^+$. Anal. Calcd For $C_{15}H_{15}IN_6O$: C, 42.67; H, 3.58; N, 19.90. Found: C, 42.89; H, 3.59; N, 19.95.

5.3.2. 5-((5-Iodo-4-((4-nitrophenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)-2-methylpyrimidin-4-amine (5b)

Yellow solid, yield: 74%, mp 215–217 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.31 (s, 3H, CH_3), 5.25 (s, 2H, OCH_2), 5.47 (s, 2H, CH_2), 6.92 (s, 2H, NH_2), 7.28–7.29 (d, 2H, J = 7.8 Hz, Ar-H), 8.23–8.27 (d, 2H, J = 8.7 Hz, Ar-H). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 25.4, 48.0, 62.3, 86.6, 107.8, 115.3, 124.5, 125.9, 141.2, 146.5, 158.3, 161.3, 163.2. ESI-MS *m/z*: 468 $(M+1)^+$. Anal. Calcd For $C_{15}H_{14}IN_7O_3$: C, 38.56; H, 3.02; N, 20.99. Found: C, 38.20; H, 3.00; N, 21.35.

5.4. Evaluation of inhibitory activity of PDHc E1

The expressing plasmid pMal-C_{2X}-PDHc-E1 was transformed into *E. coli* stain 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 purify (>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 methods of Nemeria,[15] and measured by monitoring the reduction of 2,6-dichlorophenolindophenol (2,6-DCIP) 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-DCIP, 7.1 μM enzyme and different concentration of inhibitors. The reaction mixtures were incubated for 3 min at 37 °C, then added different concentration of ThDP (ranging from 0 to 200 μM) to initial 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. 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-DCIP reduced (μmol/min/mg of PDHc-E1).

5.5. Antifungal activities assay

The fungicidal activities of a part of compounds **3** and **5a–5b** were tested in vitro against *G. zeae*, *R. solani*, *B. cinerea*, *A. solani*, *A. alternate Keissler*, and *C. orbiculare* and their relative inhibitory ratio (%) had been determined by using the mycelium growth rate method.[26] A set amount of each sample was dissolved in acetone to which a drop of emulsifier, Tween 80, was added. The solution was then diluted in water until it reached the concentrations required. The final concentration of the compounds in the medium was tested at 100 μg mL^{-1}. The solutions (1.5 mL) were mixed rapidly with thawed potato glucose agar culture medium (9 cm) under 50 °C. The mixtures were poured into Petri dished. After the dishes were cooled, the solidified plates were incubated with 5 mm mycelium disk, inverted, and incubated at 28 °C for 48 h. The mixed medium without sample was used as the blank control. Three replicates of each test were carried out. The mycelial elongation radium (mm) of fungi settlements was measured after 48 h of culture. The growth inhibition rates were calculated with the following equation: $I = [(C - T)/C] \times 100\%$. Here, I is the growth inhibition rate (%), C is average diameter of mycelia in the blank control, T is the average diameter of mycelia in the presence of those compounds. The inhibition ratio of those compounds at the dose of 100 μg mL^{-1} was summarized in Table 2.

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