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Rational design, synthesis and biological evaluation of 1,3,4-oxadiazole pyrimidine derivatives as novel pyruvate dehydrogenase complex E1 inhibitors

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Abstract

On the basis of previous study on 2-methylpyrimidine-4-ylamine derivatives **I**, further synthetic optimization was done to find potent PDHc-E1 inhibitors with antibacterial activity. Three series of novel pyrimidine derivatives **6**, **11** and **14** were designed and synthesized as potential *E. coli* PDHc-E1 inhibitors by introducing 1,3,4-Oxadiazole-thioether, 2,4-disubstituted-1,3-thiazole or 1,2,4-triazol-4-amine-thioether moiety into lead structure **I**, respectively. Most of **6**, **11** and **14** exhibited good inhibitory activity against *E. coli* PHDc-E1 (IC_{50} 0.97 to 19.21 μM) and obvious inhibitory activity against cyanobacteria (EC_{50} 0.83 to 9.86 μM). Their inhibitory activities were much higher than that of lead structure **I**. **11** showed more potent inhibitory activity against both *E. coli* PDHc-E1 (IC_{50} < 6.62 μM) and cyanobacteria (EC_{50} < 1.63 μM) than that of **6**, **14** or lead compound **I**. The most effective compound **11d** with good enzyme-selectivity exhibited most powerful inhibitory potency against *E. coli* PDHc-E1 (IC_{50} = 0.97 μM) and cyanobacteria (EC_{50} = 0.83 μM). The possible interactions of the important residues of PDHc-E1 with title compounds were studied by molecular docking, site-directed mutagenesis, and enzymatic assays. The results indicated that **11d** had more potent inhibitory activity than that of **14d** or **I** due to its 1,3,4-Oxadiazole moiety with more binding position and stronger interaction with Lsy392 and His106 at active site of *E.coli* PDHc-E1.

Keywords antibacterial activity, PDHc-E1 inhibitor, cyanobacteria, 1,3,4-Oxadiazole

1. Introduction

The frequent application of bactericides with same or limited mode of action has led to the wide spread evolution of resistance.[1-3] It is, therefore, necessary to develop efficient bactericides with novel structure or mode of action to overcome microbial disease.

The 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.[4-5] The overall reaction of oxidative decarboxylation can be simply exhibited in **Fig. 1**.

Fig.1 Insert Here

The complex (PDHc) is comprised of three different enzyme components (E1, E2 and E3) and a number of cofactors.[6] Pyruvate dehydrogenase complex E1 component (PDHc-E1, EC1.2.4.1) is the initial member of PDHc, which catalyzes the first step of multistep process, under condition of using thiamine diphosphate (ThDP) and Mg^{2+} as cofactors.[7–9] Particularly PDHc-E1 catalyzes the first irreversible step among multistep process which is catalyzed by PDHc. Hence blocking the activity of PDHc-E1 is the best way to inactivate the PDHc. ThDP plays an important role in the enzyme reaction and the catalysis mechanism.[10] Therefore, we selected *E. coli* PDHc-E1 as the target pattern of bacterium to design new cofactor ThDP analogs as inhibitors of PDHc-E1 with bactericidal activity.

Certain ThDP analogs have been reported as efficient inhibitors of PDHc-E1 (such as ThTDP, ThTTDP, and triazole-ThDP in **Fig. 2**).[11-15] However there were few reports about their bactericidal or fungicidal activity, due to their complex structure with highly charged pyrophosphate, poor bioavailability[16] and poor enzyme-selective inhibition between

microorganisms and mammals.[12]

Aiming at the aforementioned problems, a series of 2-methylpyrimidine-4-ylamine derivatives containing 1,2,3-triazole ring and substituted benzene ring **I**, had been firstly chemically synthesized in our laboratory. **II**, **III**, **IV** and **V** were further synthesized as ThDP analogs by the modification of **I** (**Fig. 2**).[17–21] Some of them were demonstrated to be effective inhibitors of *E. coli* PDHc-E1 with moderate antifungal and antibacterial activity. These findings encouraged us to further find useful PDHc-E1 inhibitors out with antibacterial or antifungal activity by further optimization of lead structure **I**.

Fig.2 Insert Here

The high charge of the pyrophosphate moiety in ThDP has been replaced by the low charge of the substituted phenoxy group in **I**. The analysis of molecular docking indicated that the substituted phenoxy ("A and B part") moiety could occupy the binding site of pyrophosphate in active site of *E. coli* PDHc-E1. However, unliked the interaction of oxygen atom of pyrophosphate with amino acid residues, the oxygen atom of ether bond ("A part") in **I** could not form hydrogen bond with any amino acid residue in active site of *E. coli* PDHc-E1.[17] It was thought that inhibitory potency against *E. coli* PDHc-E1 should be increased by enhancing the interaction of the "A or B part" with PDHc-E1. Therefore, the scaffold of **I** was kept, further optimization focused on "A or B part". In this work, 1,3,4-Oxadiazole-thioether moiety as "A part" was introduced into **I** to replace phenoxy-ether bond and produce novel 2-methyl-4-amino-5-((4-(((5-substituted-pheny-1,3,4-oxadiazol-2-yl)thio)methyl)-*1H*-1,2,3-triazol-1-yl)methyl)pyrimidine **6**. Moreover, 2,4-disubstituted-1,3-thiazole group as "B part" was further introduced into the parent structure **6** to replace substituted benzene ring and form

novel 2-methyl-4-amino-5-((4-(((5-(2,4-disubstituted-thiazol-5-yl)-1,3,4-oxadiazol-2-yl)thio) methyl)-*1H*-1,2,3-triazol-1-yl)methyl)pyrimidine **11** (**Fig. 3**). On the other hand, considering that 1,3,4-Oxadiazole-thioether or 1, 3-thiazole moiety could be as hydrogen bond receptor which would benefit the interaction of the "A and B part" with PDHc-E1, some 1,3,4-Oxadiazole and 1,3-thiazole derivatives also showed excellent antibacterial activity.[22-25] Therefore these novel ThDP analog, 1,3,4-Oxadiazole pyrimidine derivatives **6** and **11** are expected to be good *E.coli* PDHc-E1 inhibitors with bactericidal activity.

Fig. 3 Insert Here

In order to examine the effect of the structure of "A part" on inhibitory activity, 1,3,4-Oxadiazole-thioether moiety in pyrimidine derivatives **11** was replaced with 1,2,4-triazol-thioether moiety to give novel 2-methyl-4-amine-5-((4-(((5-(2,4-disubstituted-thiazol) -*4H*-1,2,4-triazol-4-amine-2-yl)thio)methyl)-*1H*-1,2,3-triazol-1-yl)methy l)pyrimidine derivatives **14** (**Fig. 3**).

Herein, we report the synthesis of new 1,3,4-Oxadiazole pyrimidine derivatives **6**, **11** and 1,2,4-triazolyl pyrimidine derivatives **14**. Their enzyme inhibition, antibacterial and antifungal activity were examined. The interaction mode of some title compounds with *E.coli* PDHc-E1 was studied by molecular docking method, probable inhibition mechanism was discussed. The enzyme-selectivity of representative compounds between pig heart and *E. coli* PDHc-E1 was also examined.

2. Chemistry

The synthetic route of **6a-l**, **11a-d** and **14a-d** is depicted in **Scheme 1**.

Scheme 1. Insert Here

Vitamin B1 or thiamine hydrochloride as starting material was used to prepare 5-azido-methyl-2-methylpyrimidine-4-ylamine **1**, according to the literature method.[26] **1** is the key intermediate for the preparation of title compounds **6a-l**, **11a-d** and **14a-d**. The title compounds could be synthesized by a five-step sequence starting from starting material. Various substituted ethyl benzoate **2** reacted with hydrazine hydrate in ethanol to produce corresponding hydrazide **3**. Under same condition, 2,4-disubstituted-1,3-thiazole-5-ethyl formate **7** could be converted into corresponding hydrazide **8**.

The preparation of 5-substituted-phenyl-1,3,4-Oxadiazole-2-thiol **4** was achieved by the reaction of hydrazide derivatives **3** with carbon disufide under strong basic conditions followed by acidification with dilute hydrochloric acid. Under same condition, 5-(2,4-disubstituted -thiazol-5-yl)-1,3,4-Oxadiazole-2-thiol **9**[27] could be obtained from hydrazide derivatives **8** using carbon disulfide. 4-Amino-5-(2,4-disubstituted-thiazol-5-yl)-*4H*-1,2,4-triazole-3-thiol **12** was prepared by the cyclization of hydrazide derivatives **8** , in which **8** was firstly converted into the corresponding potassium dithiocarbamate and further cyclized with hydrazine hydrate. The key intermediate, **5**, **10** or **13** with terminal alkynes, was prepared via reaction of 3-bromopropyne with corresponding substituted thiol **4**, **9** or **12** respectively in refluxing acetone with K_2CO_3 as base. The 1,2,3-triazol ring in the skeleton of title compounds **6**, **11** and **14** could be constructed by applying 'click chemistry'. In our present work, **6a-l**, **11a-d** and **14a-d** were synthesized by the 1,3-dipolar cycloaddition of **1** with substituted-prop-2-yn-1-thioethers **5**, **10**, or **13** respectively using CuI as catalyst in the presence of Et_3N and THF. All synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR and mass spectrometry (HR-EIMS).

3. Results and discussion

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

In order to enhance the inhibitory potency against *E. coli* PDHc-E1, lead structure **I** was modified by replacing "A part" with 1,3,4-Oxadiazole-thioether moiety (**Fig.3**). Several 1,3,4-Oxadiazole pyrimidine derivatives **6a-e** were firstly synthesized and their inhibitory potency against *E. coli* PDHc-E1 were evaluated. The IC_{50} values of **6a-e** and **Ia-e**[17] are summarized in **Table 1**. All **6a-e** displayed higher inhibitory activity than that of **Ia-e** with same **R** on the benzene ring. Especially the inhibitory activity of **6e** was 7-folds higher than that of **Ie**. The results showed that the inhibitory potency could be significantly improved by replacing ether bond at "A part" in **I** with 1,3,4-Oxadiazole-thioether moiety. This suggested that the introduction of 1,3,4-Oxadiazole-thioether moiety at "A part" should be much beneficial for enhancing inhibition against *E. coli* PDHc-E1.

Table 1 Insert Here

Further optimization was focused on R of **6**. As shown in **Table 1**, R at 4-position on the benzene ring was favorable to inhibitory activity. Inhibitory activity against *E. coli* PDHc-E1 decreased in the following order: **6a** (R=4-NO_2, IC_{50} = 7.59 μM) > **6c** (R=2-NO_2, IC_{50} = 9.84 μM)> **6i** (R=3-NO_2, IC_{50} = 10.38 μM); **6b** (R=4-Cl, IC_{50} = 7.08 μM) > **6l** (R=2-Cl, IC_{50}=12.39 μM); **6f** (R=4-F, IC_{50} = 9.11 μM) > **6j** (R=2-F, IC_{50} = 19.21 μM). Small size group as R at 4-position of benzene ring seemed to be good for inhibitory activity. For example, **6f** with 4-F as R showed better inhibitory activity (IC_{50} = 9.11 μM) than that of **6j** with 4-CF_3 as R (IC_{50} = 15.87 μM). In the other hand, single substituent was favorable for inhibitory activity, such as **6b** (R=4-Cl, IC_{50}= 7.08 μM) > 6l (R=2-Cl-4-Cl, IC_{50}= 13.18 μM); **6a** (R=4-NO_2, IC_{50}= 7.59 μM) > **6c**

(R=2-Cl-4-NO_2, IC_{50} = 12.80 μM). Above results indicated that the inhibitory activity of **6** was also dependent upon the structure, position and size of **R** on the benzene ring.

6 was further modified by replacing substituted benzene ring with 2,4-disubstituted-1,3-thiazole moiety at "B part" to form a series of 1,3,4-Oxadiazole pyrimidine derivatives **11**. Compared with **6**, the inhibitory activity of **11** was further enhanced. As shown in **Table 1** and **2**, **11a-d** (IC_{50} = 0.97-6.62 μM) displayed better inhibitory activity than **6a-l** (IC_{50} = 7.08-19.21 μM). Among **11a-d**, compounds with CF_3 as R^1 displayed higher inhibitory activity than that of compounds with CH_3 as R^1, irrespective of NH_2 or CH_3NH as R^2. For example, the inhibitory activity of **11d** (R^1=CF_3) was 6-folds higher than that of **11b** (R^1=CH_3). The inhibitory potency against *E. coli* PDHc-E1 could be remarkably enhanced by introducing both 1,3,4-Oxadiazole-thioether and 2,4-disubstituted-1,3 -thiazole moiety to replace both ether bond and benzene ring at "A and B part" in **I**.

Table 2 Insert Here

In order to examine the effect of "A part" on inhibitory activity against *E.coli* PDHc-E1, **11** was modified to produce **14** by replacing 1,3,4-Oxadiazole-thioether moiety in **11** with 1,2,4-triazol-4-amine-thioether moiety as "A part". The inhibitory activity of **14** was shown in **Table 2**. All **14a-d** displayed weaker inhibitory activity than that of corresponding **11a-d** with same R^1 and R^2. Especially **14d** showed inhibitory activity 19-folds lower than that of **11d**. The result showed that the loss of 1,3,4-Oxadiazole moiety led to a decrease in inhibitory activity against *E.coli* PDHc-E1. It indicated that the structure of "A part" had a significant effect on inhibitory activity against *E.coli* PDHc-E1.

3.2. Antibacterial activity

Most reported ThDP analogs as *E. coli* PDHc-E1 inhibitors had no useful antibacterial or antifungal activity. **6**, **11**, and **14** as *E. coli* PDHc-E1 inhibitors were evaluated for their antifungal and antibacterial activity. Interestingly all tested compounds displayed good antibacterial activity, but very weak fungicidal activity. As shown in **Fig. 4**. **6**, **11**, and **14** exhibited obvious inhibitory potency against cyanobacteria at 10 μM. Especially **6b**, **6c**, **6e**, **6f**, **6g**, **6h**, **6i**, **11a-d**, and **14a-c** could 90-99% control cyanobacteria, but **I** only showed <30% inhibitory potency.

Fig.4 Insert Here

EC_{50} values of **6**, **11**, **14** and **I** were further evaluated their inhibitory potency against cyanobacteria. The structure and EC_{50} of **6**, **11**, **14** and **I** are listed in **Tables 1** and **2**. **6**, **11**, and **14** exhibited obvious inhibitory potency against cyanobacteria with EC_{50} ranging from 0.83 to 9.86 μM. However **I** with EC_{50} > 50 μM. According to the data in **Tables 1** and **2**, we see that the inhibitory potency of some tested compounds against cyanobacteria is related to their inhibition against *E. coli* PDHc-E1. **6**, **11**, and **14** with the IC_{50} values ranging from 0.97 to 19.21 μM against *E. coli* PHDc-E1 could exhibit moderate to good antibacterial activity against cyanobacteria. **I** with much weak inhibitory potency against *E. coli* PHDc-E1 also showed much weak activity against cyanobacteria.

As shown in **Tables 1** and **2**, most **6**, **11** and **14** exhibited much higher inhibitory potency against both *E. coli* PDHc-E1 and cyanobacteria than that of **I**. Especially 1,3,4-Oxadiazole pyrimidine derivatives **11** were much favorable to both enzyme inhibition and antibacterial activity. It could be noticed that **14a-d** containing 1,2,4-triazole moiety as "A part" showed weaker inhibition against both *E. coli* PDHc-E1 and cyanobacteria than that of **11a-d** containing

199 1,3,4-Oxadiazole moiety as "A part". 1,3,4-Oxadiazole pyrimidine derivative **11d** was found to be
200 the most effective compound against *E. coli* PDHc-E1 (IC_{50} = 0.97 μM), and against
201 cyanobacteria with EC_{50} value of 0.83 μM. However 1,2,4-triazole pyrimidine derivative **14d**
202 showed poor activity against *E. coli* PDHc-E1 (IC_{50} = 15.67□μM), and against cyanobacteria with
203 EC_{50} value of 9.86 μM.

204 Above observation showed that both enzyme inhibition and antibacterial activity could be
205 greatly enhanced by introducing both 1,3,4-Oxadiazole-thioether and
206 2,4-disubstituted-1,3-thiazole moiety at "A and B part" to replace both ether bond and benzene
207 ring in **I**. These results suggested that 1,3,4-Oxadiazole-thioether and 1,3-thiazole moiety as "A
208 and B part" in structure **11** would be much beneficial for enhancing the interaction of **11** with *E.*
209 *coli* PDHc-E1 by forming hydrogen bond. Although most **6**, **11** and **14** exhibited obvious
210 antibacterial activity against cyanobacteria, they had no significant fungicidal activity against
211 fungus. As shown in **Table 3**, most **6**, **11**, and **14** showed < 60% inhibitory potency against *A.*
212 *solani, R. solani, B. cinerea and C. lagenarium*. It showed that most **6**, **11**, and **14** could
213 selectively inhibit bacterium due to their good inhibition against PDHc-E1 from *E. coli*.

214 **Table 3 Insert Here**

215 **Table 4 Insert Here**

216 Some reported ThDP analogs as PDHc-E1 inhibitors not only had powerful inhibition potency
217 against *E. coli* PDHc-E1, but also had powerful inhibition potency against human PDHc-E1,
218 because they were had poor enzyme-selective inhibition between mammals and bacteria.[28] In this
219 work, **11a-d** with good inhibition against *E. coli* PDHc-E1 (IC_{50} = 0.97-6.62 μM) were selected to
220 test their inhibition against Pig heart PDHc-E1. As shown in **Table 4**, **11a-d** showed <30%

inhibitory potency against pig heart PDHc-E1. These findings showed that **11a-d** had better enzyme-selective inhibition between microorganism and mammal, possibly acting as specific inhibitors against bacteria.

3.3. Analyses of the interaction between inhibitors and *E.coil* PDHc-E1

In order to understand the inhibition of **11** and **14** against *E. coli* PDHc-E1, the interaction mode of **11** and **14** with active site of *E.coli* PDHc-E1 was further explored. The molecular docking simulation of several compounds was carried out by using the SURFLEX module of SYBYL package.[16] 1,3,4-Oxadiazole pyrimidine derivative **11d** as the best PDHc-E1 inhibitor and its corresponding 1,2,4-triazolyl pyrimidine derivative **14d** were selected to analyse their interaction with *E. coil* PDHc-E1 by molecular docking.

The binding mode of **11d** or **14d** with amino acid residues is shown in **Fig. 5 A** and **B**, respectively. As shown in **Fig. 5**, **11d** or **14d** with a 'V' conformation can occupy the ThDP-binding pocket and bind in the active site of *E.coli* PDHc-E1. On the right side of the 'V' conformation, the 4-aminopyrimidine ring of **11d** or **14d** displays a π－π stacking with the side chain ring of Phe602，also forms hydrogen bonds with Met194, Glu571 and Val192, which is similar to the interactions of ThDP or lead structure **I** with corresponding amino acid residues. For the left side of the 'V' conformation of **11d** or **14d**, trifluoromethyl group as R^1 on the 1,3-thiazole ring of **11d** or **14d** has an interaction with Lys392 in the active site of *E.coli* PDHc-E1 by forming hydrogen bond.

Fig. 5 Insert Here

In order to confirm the prediction of molecular docking, site-directed mutagenesis and enzymatic assays were further performed. As shown in **Fig. 6**, the IC_{50} value of **11d** against mutants M194A (19.05 μM), E571A (5.02 μM), V192A (33.17 μM), K392A (20.44 μM) or F602A

(5.08 μM) was about 18 times, 4 times, 33 times, 20 times or 4 times higher than its value against wild-type PDHc-E1 (0.97 μM), respectively. These results suggest that the interaction between **11d** and Met194, Glu571, Val192, Lys392 or Phe602 by forming hydrogen bond plays an important role in the binding of **11d** with *E.coli* PDHc-E1.

Fig. 6 Insert Here

It was very interesting to explore the different of the binding mode of **I**, **11** and **14** by comparing with "A part" in their parent structures. No hydrogen bond between any amino acid residues and the oxygen atom of ether bond as "A part" at the middle of the 'V' conformation of **I** was observed by molecular docking in our previous study.[15] However the two nitrogen atoms (N-N) and oxygen atom (C-O-C) of 1,3,4-Oxadiazole moiety as "A part" at the middle of the 'V' conformation of compound **11d** could form two strong hydrogen bond with Lsy392 and a strong hydrogen bond with His106 , respectively (**Fig. 5A**). As shown in **Fig. 5B**, the two nitrogen atoms (N-N) of 1,2,4-triazolyl moiety in pyrimidine derivative **14d** is turned 180 degree comparing with that of 1,3,4-Oxadiazole moiety in pyrimidine derivative **11d**. In this case, the two nitrogen atoms (N-N) of 1,2,4-triazolyl moiety in **14d** only form a hydrogen bond with His106, but no other hydrogen bond between 1,2,4-triazolyl moiety and Lsy392 or any amino acid residues was observed.

Further site-directed mutagenesis and enzymatic assays showed that the IC_{50} values of **11d** against the mutants K392A (20.44 μM) or H106A (11.61 μM) was about 20 times or 11 times higher than its value against wild-type PDHc-E1 enzyme (0.97 μM) (**Fig. 6**). These results also revealed that the interaction between **11d** and Lsy392 or His106 by forming hydrogen bond had a significant contribution for its inhibitory activity against *E.coli* PDHc-E1.

Above observation indicated that 1,3,4-Oxadiazole moiety as "A part" in **11** was most

267 favorable to enzyme inhibition against *E. coli* PDHc-E1 due to more binding position and stronger
268 interaction with the active site of *E.coli* PDHc-E1. However 1,2,4-triazolyl moiety or ether bond
269 as "A part" had fewer binding position or no interaction with amino acid residues, which led to
270 weak enzyme inhibition of **14** or **I**. These results provided us a reasonable explanation for why
271 1,3,4-Oxadiazole pyrimidine derivatives **11** had more potent inhibitory activity against *E.coli*
272 PDHc-E1 than that of 1,2,4-triazolyl pyrimidine derivatives **14** or **I**.

273 **4. Conclusion**

274 On the basis of lead structure **I**, 1,3,4-Oxadiazole pyrimidine derivatives **6a-l**, **11a-d** and
275 1,2,4-triazolyl pyrimidine derivatives **14a-d** as potential *E. coli* PDHc-E1 inhibitors were designed
276 and synthesized. As novel ThDP analogs, all **6a-l**, **11a-d** and **14a-d** with the IC_{50} values ranging
277 from 0.97 to 19.21 μM against *E. coli* PHDc E1 could exhibit moderate to good inhibitory potency
278 against cyanobacteria (EC_{50} = 0.83-9.86 μM). However **I** with much weaker inhibition against *E.*
279 *coli* PHDc-E1 showed no inhibitory activity against cyanobacteria. The inhibitory potency of
280 compounds against both *E. coli* PDHc-E1 and cyanobacteria could be greatly increased by
281 replacing the ether bond of **I** with 1,3,4-Oxadiazole-thioether, 2,4-disubstituted-1,3-thiazole or
282 1,2,4-triazol-4-amine-thioether moiety as "A and B part". **11** with 1,3,4-Oxadiazole-thioether and
283 2,4-disubstituted-1,3-thiazole moiety as "A and B part" showed more potent inhibitory activity
284 against both *E. coli* PDHc-E1 and cyanobacteria than that of **6**, **14** or **I**. The most effective
285 compound **11d** (IC_{50} = 0.97 μM) exhibited not only much stronger inhibition against *E. coli*
286 PDHc-E1 than that of corresponding 1,2,4-triazolyl pyrimidine **14d** (IC_{50} = 15.67 μM), but also
287 displayed much higher inhibitory potency (EC_{50} = 0.83 μM) against cyanobacteria than that of
288 **14d** (EC_{50} = 9.86 μM). The above findings showed that there was some correlation between

289 enzyme inhibition and antibacterial activity.

290 Binding mode analysis revealed that **11d** displayed much powerful interaction by forming
291 hydrogen bond between 1,3,4-Oxadiazole moiety and Lsy392, His106 at active site of *E.coli*
292 PDHc-E1. The site-directed mutagenesis and enzymatic assays further proved that the interaction
293 between 1,3,4-Oxadiazole moiety in **11d** with Lsy392 or His106 had a significant contribution for
294 its inhibitory activity against *E.coli* PDHc-E1. It suggested that **11d** had more potent inhibitory
295 activity against *E.coli* PDHc-E1 and bacterium than that of **14d** or lead compound **I** due to
296 1,3,4-Oxadiazole moiety as a "A part" with more binding position and stronger interaction with
297 important amino acid residues than that of **14d** or **I**. These results elucidated that
298 1,3,4-Oxadiazole-thioether and 2,4-disubstituted-1,3-thiazole moiety as "A and B part" were much
299 beneficial than substituted ether bond in the parent structure **I** for enhancing both inhibition
300 against *E. coli* PDHc-E1 and cyanobacteria. **11a-d** with weak inhibition against pig PDHc-E1
301 acted as special inhibitor selectively against *E. coli* PDHc-E1 and bacterium. Therefore, the
302 skeleton of 1,3,4-Oxadiazole pyrimidine derivatives **11** could be as the novel lead structure of
303 bactericide for further optimization.

304 **5. Experimental**

305 **5.1 Chemistry**

306 Melting points (mp) were measured on an electrothermal melting point apparatus and were
307 uncorrected. ^{1}H NMR spectra were recorded at 400 MHz, in DMSO-d_6 solution on a Varian
308 Mercury-Plus 400 spectrometer and chemical shifts were recorded in parts per million (ppm) with
309 TMS as the internal reference. High-resolution electron impact mass spectra (HR-EIMS) were
310 recorded under electron impact (70 eV) condition using a MicroMass GCT CA 055 instrument.

Unless otherwise noted, reagents were purchased from commercial suppliers and used without further purification. Intermediate 5-(azidomethyl)-2-methylpyrimidine -4-amine **1** was synthesized according to the existing methods.[26]

5.2 General procedure for preparation of 3a-l and 8a-d.

A mixture of substituted ethyl benzoate **2** (10 mmol) and hydrazine hydrate (20 mmol) in ethanol was stirred at room temperature for 3 h and then filtered. The crude product recrystallized from absolute alcohol to give **3a-l**, which were used directly for the next step. Under this same condition, the intermediate compounds **8a-d** were also prepared.

5.3 General procedure for preparation of 4a-l and 9a-d.

Substituted benzohydrazide **3** (5 mmol) was dissolved in a solution of potassium hydroxide (6 mmol) in water (4 mL) and ethanol (20 mL). Carbon disulfide (10 mmol) was then added while stirring and the reaction mixture was heated under reflux for 8 h. The solvents were removed under reduced pressure; the residue was treated with water and then filtered. The filtrate was cooled, and then neutralized to pH 3 using dilute hydrochloric acid and the separated product thiol 4a-l was filtered, washed with water, dried and recrystallised from benzene as yellow crystals, which were used directly for the next step. Under this same condition, the intermediate compounds **9a-d** were also prepared.

5.4 General procedure for preparation of 12a-d.

To a solution of potassium hydroxide (6 mmol) in absolute ethanol (30 mL), 2,4-disubstituted-1,3-thiazole-5-carbohydrazide **8** (5 mmol) and carbon disulphide (10 mmol) were added and the mixture was stired for 16 h. The precipitated potassium dithiocarbazinate was collected by filtering, washed with ethyl acetate and dried under vacuum. The potassium salt was

used in the next step without further purification. A suspension of the potassium salt, water (4.0 mL) and hydrazine hydrate (3 mL) were heated under reflux for 5 h, and then the mixture was diluted with 50 mL water and subsequent acidification with dilute acetic acid gave a white precipitate which was filtered, washed with water and recrystallized from aqueous DMF and obtained **12a-d** as yellow crystals in good yield.

5.5 General procedure for preparation of 5a-l, 10a-d and 13a-d.

A solution of substituted thiol 4 (5 mmol), 3-bromopyropyne (0.71 g, 6 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 residue was dissolved in water (20 mL) and then filtered. The crude product recrystallized from absolute alcohol to give **5a-l**, which were used directly for the next step. Under this same condition, the intermediate compounds **10a-d** and **13a-d** were also prepared.

5.6 General procedure for preparation of target compounds 6a-l, 11a-d and 14a-d.

CuI (0.04g, 0.2 mmol) was added to a stirred solution of 5-azidomethyl-2-methylpyrimidine-4-yla mine **1** (0.33g, 2 mmol) and 2-substituted-phenyl-5-(prop-2-yn-1-ylthio)-1,3,4-oxadiazole **5** (2 mmol) in THF (10mL) followed by Et_3N (0.24g, 2.4 mmol). After overnight stirring at room temperature, the reaction mixture was poured into water (20 mL) and then filtered. The crude products were purified by column chromatography on silica gel and elution with petroleumether/acetone (2:1, v/v) to give the corresponding pure title compounds **6a-l**. Under this same condition, the intermediate compounds **11a-d** and **14a-d** were also prepared.

5.6.1.

2-methyl-4-amine-5-((4-(((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-*1H*-1,2,3-triaz ol-1-yl)methyl)pyrimidine (6a)

Yellow solid; Yield 90%; m.p. 143-145 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.33 (s, 3H, CH_3), 4.67 (s, 2H, SCH_2), 5.45 (s, 2H, CH_2), 7.06 (s, 2H, NH_2), 8.16 (s, 1H, pyrimidine-5-yl-H), 8.18 (s, 2H, Ar-H), 8.37 (d, 2H, Ar-H, J = 6.3 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 25.2, 27.0, 46.9, 108.2, 124.0, 124.7, 127.8, 128.6, 142.3, 149.2, 156.0, 161.5, 164.1, 164.6, 167.3; HR-EIMS (EI) m/z 426.1102 $(M+H)^+$, calcd. for $C_{17}H_{16}N_9O_3S$ m/z = 426.1097.

5.6.2.

2-methyl-4-amine-5-((4-(((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-*1H*-1,2,3-triazol-1-yl)methyl)pyrimidine (6b)

White solid; Yield 93%; m.p. 189-191 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.35 (s, 3H, CH_3), 4.64 (s, 2H, -SCH_2-), 5.45 (s, 2H, CH_2), 7.06 (s, 2H, NH_2), 7.63 (s, 2H, Ar-H), 7.92 (s, 2H, Ar-H), 8.14 (s, 1H, pyrimidine-5-yl-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 25.0, 26.7, 46.9, 107.5, 118.0, 120.3, 124.6, 124.9, 134.6, 147.9, 149.0, 155.0, 156.7, 162.9, 165.2; HR-EIMS (EI) m/z 415.0890 $(M+H)^+$, calcd. for $C_{17}H_{16}ClN_8OS$ m/z = 415.0856.

5.6.3.

2-methyl-4-amine-5-((4-(((5-(2-nitrophenyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-*1H*-1,2,3-triazol-1-yl)methyl)pyrimidine (6c)

Yellow solid; Yield 90%; m.p. 171-173 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.31 (s, 3H, CH_3), 4.67 (s, 2H, -SCH_2-), 5.48 (s, 2H, CH_2), 7.22 (s, 2H, NH_2), 7.89 (s, 2H, Ar-H), 7.98 (s, 1H, pyrimidine-5-yl-H), 8.16 (s, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 24.5, 26.2, 46.2, 107.5, 120.1, 120.6, 123.4, 127.5, 131.3, 133.4, 147.2, 150.2, 150.0, 151.1, 158.8, 165.0, 167.3; HR-EIMS (EI) m/z 426.1102 $(M+H)^+$, calcd. for $C_{17}H_{16}ClN_8OS$ m/z = 426.1097.

5.6.4.

2-methyl-4-amine-5-((4-(((5-(2,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-*1H*-1,2,3-triazol-1-yl)methyl)pyrimidine (6d)

White solid; Yield 91%; m.p. 173-175 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.34 (s, 3H, CH_3), 4.65 (s, 2H, -SCH_2-), 5.46 (s, 2H, CH_2), 7.05 (s, 2H, NH_2), 7.62 (s, 1H, Ar-H), 7.88 (s, 1H, Ar-H), 7.93 (s, 1H, Ar-H), 8.15 (s, 1H, pyrimidine-5-yl-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 25.5, 27.2, 45.1, 105.5, 120.0, 122.2, 125.6, 127.4, 128.4, 133.4, 133.9, 149.9, 151.0, 151.4, 158.7, 164.9, 167.2; HR-EIMS (EI) m/z 471. 0320 $(M+Na)^+$, calcd. for $C_{17}H_{14}Cl_2N_8OSNa$ m/z = 471. 0286.

5.6.5.

2-methyl-4-amine-5-((4-(((5-phenyl-1,3,4-oxadiazol-2-yl)thio)methyl)-*1H*-1,2,3-triazol-1-yl)methyl)pyrimidine (6e)

White solid; Yield 88%; m.p. 138-140 ^{o}C ; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.34 (s, 3H, CH_3), 4.67 (s, 2H, -SCH_2-), 5.48 (s, 2H, CH_2), 7.18 (s, 2H, NH_2), 7.58 (s, 3H, Ar-H), 7.93 (s, 2H, Ar-H), 8.18 (s, 1H, pyrimidine-5-yl-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 23.5, 25.2, 45.2, 106.5, 116.9, 120.0, 122.0, 123.9, 128.2, 146.8, 147.9, 153.8, 155.6, 161.8, 164.1; HR-EIMS (EI) m/z 381.1236 $(M+H)^+$, calcd. for $C_{17}H_{17}N_8OS$ m/z = 381.1246.

5.6.6.

2-methyl-4-amine-5-((4-(((5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-*1H*-1,2,3-triazol-1-yl)methyl)pyrimidine (6f)

White solid; Yield 89%; m.p 132-134 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.34 (s, 3H, CH_3), 4.64 (s, 2H, -SCH_2-), 5.45 (s, 2H, CH_2), 7.05 (s, 2H, NH_2), 7.41 (d, 2H, Ar-H, J = 6.6 Hz), 7.98 (s, 2H, Ar-H), 8.14 (s, 1H, pyrimidine-5-yl-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm):

25.5, 27.2, 45.2, 108.2, 116.0, 119.9, 120.2, 129.4, 129.5, 149.8, 150.9, 156.8, 158.6, 164.8, 166.1, 167.1; HR-EIMS (EI) m/z 399.1151 $(M+H)^+$, calcd. for $C_{17}H_{16}FN_8OS$ m/z = 399.1152.

5.6.7.

2-methyl-4-amine-5-((4-(((5-(4-(trifluoromethyl)phenyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)methyl)pyrimidine (6g)

White solid; Yield 89%; m.p 132-134 ^oC; ^1H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.30 (s, 3H, CH_3), 4.66 (s, 2H, -SCH_2-), 5.43 (s, 2H, CH_2), 7.00 (s, 2H, NH_2), 7.94 (s, 2H, Ar-H), 8.14 (s, 2H, Ar-H), 8.14 (s, 1H, pyrimidine-5-yl-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 25.2, 27.0, 47.5, 108.2, 118.2, 119.9, 124.1, 124.3, 126.6, 129.5, 129.8, 130.0, 130.3, 149.8, 150.9, 156.8, 158.6, 164.8, 167.1; HR-EIMS (EI) m/z 449.1133 $(M+H)^+$, calcd. for $C_{18}H_{16}F_3N_8OS$ m/z = 449.1120.

5.6.8.

2-methyl-4-amine-5-((4-(((5-(3,5-dinitrophenyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-*1H*-1,2,3-triazol-1-yl)methyl)-2-methylpyrimidine (6h)

Yellow solid; Yield 87%; m.p 163-165 ^oC; ^1H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.37 (s, 3H, CH_3), 4.66 (s, 2H, -SCH_2-), 5.48 (s, 2H, CH_2), 7.10 (s, 2H, NH_2), 7.60 (s, 2H, Ar-H), 7.60 (s, 1H, 1,2,3-triazol-1-yl-H), 7.94 (s, 1H, pyrimidine-5-yl-H), 7.94 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 25.5, 27.8, 46.5, 108.5, 118.6, 119.7, 122.6, 127.4, 142.3, 149.2, 151.0, 157.5, 158.9, 164.9, 167.5; HR-EIMS (EI) m/z 471.0950 $(M+H)^+$, calcd. for $C_{17}H_{15}N_{10}O_5S$ m/z = 471.0948.

5.6.9.

2-methyl-4-amine-5-((4-(((5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-*1H*-1,2,3-triazol-1-yl)methyl)pyrimidine (6i)

Yellow solid; Yield 90%; m.p 171-173 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.28 (s, 3H, CH_3), 4.72 (s, 2H, -SCH_2-), 5.51 (s, 2H, CH_2), 7.18 (s, 2H, NH_2), 7.88 (s, 1H, Ar-H), 8.20 (s, 1H, pyrimidine-5-yl-H), 8.36 (s, 1H, Ar-H), 8.43 (s, 1H, Ar-H), 8.62 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 25.1, 27.2, 46.1, 107.6, 119.1, 120.9, 122.1, 127.1, 127.6, 130.1, 145.1, 149.1, 150.0, 155.5, 158.5, 164.2, 167.1; HR-EIMS (EI) m/z 426. 1069 $(M+H)^+$, calcd. for $C_{17}H_{16}N_9O_3S$ m/z = 426. 1097.

5.6.10.

2-methyl-4-amine-5-((4-(((5-(2-fluorophenyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-*1H*-1,2,3-triazol-1-yl)methyl)pyrimidine (6j)

White solid; Yield 90%; m.p 165-167 °C; 1H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.33 (s, 3H, CH_3), 4.64 (s, 2H, -SCH_2-), 5.45 (s, 2H, CH_2), 7.04 (s, 2H, NH_2), 7.42 (d, 2H, Ar-H, J = 21.1 Hz), 7.65 (s, 1H, Ar-H), 7.96 (s, 1H, Ar-H), 8.15 (s, 1H, pyrimidine-5-yl-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 25.1, 27.5, 46.1, 107.3, 116.5, 119.6, 123.2, 125.4, 129.5, 131.5, 149.2, 150.8, 151.4, 157.4, 158.6, 159.4, 164.2, 167.1; HR-EIMS (EI) m/z 399.1143 $(M+H)^+$, calcd. for $C_{17}H_{16}FN_8OS$ m/z = 399.1152.

5.6.11.

2-methyl-4-amine-5-((4-(((5-(2-chloro-4-nitrophenyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-*1H*-1,2,3-triazol-1-yl)methyl)pyrimidine (6k)

Yellow solid; Yield 93%; m.p 162-164 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.28 (s, 3H, CH_3), 4.67 (s, 2H, -SCH_2-), 5.41 (s, 2H, CH_2), 6.91 (s, 2H, NH_2), 8.14 (s, 1H, pyrimidine-5-yl-H), 8.20 (s, 1H, Ar-H), 8.23 (s, 1H, 1,2,3-triazol-1-yl-H), 8.40 (s, 1H, Ar-H), 8.43 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 25.4, 27.8, 47.1, 108.2, 121.5, 124.4, 126.2, 127.1, 130.1,

132.7, 144.4, 149.2, 158.5, 165.5, 164.1, 164.7, 167.5; HR-EIMS (EI) m/z 460.0710 $(M+H)^+$, calcd. for $C_{17}H_{15}ClN_9O_3S$ m/z = 460.0707.

5.6.12.

2-methyl-4-amine-5-((4-(((5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-*1H*-1,2,3-triazol-1-yl)methyl)pyrimidine (6l)

White solid; Yield 86%; m.p 126-128 ^oC; ^1H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.33 (s, 3H, CH_3), 4.65 (s, 2H, -SCH_2-), 5.45 (s, 2H, CH_2), 7.04 (s, 2H, NH_2), 7.53-7.67 (m, 3H, Ar-H), 7.92 (s, 1H, Ar-H), 8.15 (s, 1H, pyrimidine-5-yl-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 25.1, 27.5, 46.1, 107.3, 119.5, 125.3, 126.4, 128.1, 129.7, 131.8, 149.2, 150.8, 150.4, 151.3, 158.3, 164.1, 167.4; HR-EIMS (EI) m/z 415.0831 $(M+H)^+$, calcd. for $C_{17}H_{16}ClN_8OS$ m/z = 415.0856.

5.6.13.

2-methyl-4-amine-5-((4-(((5-(2-amine-4-methylthiazol)-1,3,4-oxadiazol-2-yl)thio)methyl)-*1H*-1,2,3-triazol-1-yl)methyl)pyrimidine (11a)

Gray solid; Yield 85%; m.p 229-230 ^oC; ^1H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.30 (s, 3H, CH_3), 2.36 (s, 3H, thiazol-CH_3), 4.54 (s, 2H, -SCH_2-), 5.42 (s, 2H, CH_2), 6.93 (s, 2H, pyrimidine -4-NH_2), 7.76 (s, 2H, thiazol-2-NH_2), 8.10 (s, 1H, pyrimidine-5-yl-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 14.7, 23.6, 27.8, 46.3, 105.2, 118.3, 119.9, 125.6, 146.1, 149.9, 150.6, 158.6, 164.7, 167.2, 167.8; HR-EIMS (EI) m/z 417.1031 $(M+H)^+$, calcd. for $C_{15}H_{17}N_{10}OS_2$ m/z = 417.1028.

5.6.14.

2-methyl-4-amine-5-((4-(((5-(2-amine-*N*,4-dimethylthiazol)-1,3,4-oxadiazol-2-yl)thio)methyl)-*1H*-1,2,3-triazol-1-yl)methyl)pyrimidine (11b)

Gray solid; Yield 86%; m.p 189-191 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.31 (s, 3H, CH_3), 2.40 (s, 3H, thiazol-CH_3), 2.87 (s, 3H, NCH_3), 4.54 (s, 2H, -SCH_2-), 5.43 (s, 2H, CH_2), 6.95 (s, 2H, pyrimidine-4-NH_2), 8.10 (s, 1H, pyrimidine-5-yl-H), 8.28 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 14.7, 18.5, 22.5, 27.0, 45.2, 105.8, 118.2, 119.9, 125.1, 146.5, 149.8, 150.9, 158.6, 164.5, 164.8, 167.8; HR-EIMS (EI) m/z 431.1190 $(M+H)^+$, calcd. for $C_{16}H_{19}N_{10}OS_2$ m/z = 431.1185.

5.6.15.

2-methyl-4-amine-5-((4-(((5-(2-amine-4-trifluoromethylthiazol)-1,3,4-oxadiazol-2-yl)thio)methyl)-*1H*-1,2,3-triazol-1-yl)methyl)pyrimidine (11c)

Gray solid; Yield 81%; m.p 201-202 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.32 (s, 3H, CH_3), 4.58 (s, 2H, -SCH_2-), 5.47 (s, 2H, CH_2), 7.02 (s, 2H, pyrimidine-4-NH_2), 8.12 (s, 1H, pyrimidine-5-yl-H), 8.29 (s, 2H, thiazol-2-NH2); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 14.5, 23.9, 28.5, 47.1, 106.5, 118.9, 119.9, 125.6, 128.7, 128.4, 128.2, 127.9, 146.1, 148.2, 149.9, 158.9, 164.2, 164.7, 167.9; HR-EIMS (EI) m/z 471.0750 $(M+H)^+$, calcd. for $C_{15}H_{14}F_3N_{10}OS_2$ m/z = 471.0746.

5.6.16.

2-methyl-4-amine-5-((4-(((5-(2-amine-*N*-methyl-4-trifluoromethylthiazol)-1,3,4-oxadiazol-2-yl)thio)methyl)-*1H*-1,2,3-triazol-1-yl)methyl)pyrimidine (11d)

Gray solid; Yield 79%; m.p 197-198 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.30 (s, 3H, CH_3), 2.91 (s, 3H, NCH_3), 4.57 (s, 2H, -SCH_2-), 5.43 (s, 2H, CH_2), 6.93 (s, 2H, pyrimidine -4-NH_2), 8.10 (s, 1H, pyrimidine-5-yl-H), 8.76 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 14.7, 20.5, 22.5, 27.0, 45.2, 104.9, 119.3, 120.3, 126.7, 127.8, 128.1, 128.4, 128.6, 148.6,

149.8, 150.9, 158.9, 160.4, 164.8, 167.8; HR-EIMS (EI) m/z 485.0910 $(M+H)^+$, calcd. for $C_{16}H_{16}F_3N_{10}OS_2$ m/z = 485.0902.

5.6.17.

2-methyl-4-amine-5-((4-(((5-(2-amine-4-methylthiazol)-*4H*-1,2,4-triazol-4-amine-2-yl)thio)methyl)-*1H*-1,2,3-triazol-1-yl)methyl)pyrimidine (14a)

Gray solid; Yield 86%; m.p 219-220 ^oC; ^1H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.31 (s, 3H, CH_3), 2.37 (s, 3H, thiazol-CH_3), 4.46 (s, 2H, -SCH_2-), 5.42 (s, 2H, CH_2), 5.99 (s, 2H, 1,2,4-triazol-NH_2), 6.96 (s, 2H, pyrimidine-4-NH_2), 7.23 (s, 2H, thiazol-2-NH_2), 8.10 (s, 1H, pyrimidine-5-yl-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 15.3, 24.3, 27.9, 46.9, 106.3, 118.7, 119.9, 135.5, 149.9, 150.9, 151.3, 155.4, 158.7, 164.2, 167.6; HR-EIMS (EI) m/z 431.1300 $(M+H)^+$, calcd. for $C_{15}H_{19}N_{12}S_2$ m/z = 431.1297.

5.6.18.

2-methyl-4-amine-5-((4-(((5-(2-amine-*N*,4-dimethylthiazol)-*4H*-1,2,4-triazol-4-amine-2-yl)thio)methyl)-*1H*-1,2,3-triazol-1-yl)methyl)pyrimidine (14b)

Yellow solid; Yield 76%; m.p 188-189 ^oC; ^1H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.31 (s, 3H, CH_3), 2.41 (s, 3H, thiazol-CH_3), 2.83 (s, 3H, NCH_3), 4.45 (s, 2H, -SCH_2-), 5.41 (s, 2H, CH_2), 6.00 (s, 2H, 1,2,4-triazol-NH_2), 6.96 (s, 2H, pyrimidine-4-NH_2), 7.75 (s, 1H, 1,2,3-triazol-H), 8.08 (s, 1H, pyramidine-5-yl-H), 8.29 (s, 1H, NH); ^{13}C NMR(100 MHz, DMSO-d_6) δ(ppm): 12.7, 13.5, 21.0, 25.1, 44.8, 105.8, 116.2, 117.9, 131.5, 147.8, 148.9, 149.3, 153.2, 156.6, 162.5, 162.8; HR-EIMS (EI) m/z 445.1450 $(M+H)^+$, calcd. for $C_{16}H_{21}N_{12}S_2$ m/z = 445.1454.

5.6.19.

2-methyl-4-amine-5-((4-(((5-(2-amine-4-trifluoromethylthiazol)-*4H*-1,2,4-triazol-4-amine-2-y

l)thio)methyl)-*1H*-1,2,3-triazol-1-yl)methyl)pyrimidine (14c)

Brown solid; Yield 77%; m.p 169-171 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.32 (s, 3H, CH_3), 4.52 (s, 2H, -SCH_2-), 5.46 (s, 2H, CH_2), 6.02 (s, 2H, 1,2,4-triazol-NH_2), 7.01 (s, 2H, pyrimidine-4-NH_2), 7.82 (s, 2H, thiazol-2-NH_2), 8.13 (s, 1H, pyrimidine-5-yl-H),; ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 14.7, 23.4, 28.9, 47.8, 109.5, 118.9, 116.9, 125.1, 128.5, 128.9, 129.6, 130.3, 151.1, 152.6, 157.2, 160.6, 164.2, 166.7; HR-EIMS (EI) m/z 485.1012 $(M+H)^+$, calcd. for $C_{15}H_{16}F_3N_{12}S_2$ m/z = 485.1014.

5.6.20.

2-methyl-4-amine-5-((4-(((5-(2-amine-*N*-methyl-4-trifluoromethylthiazol)-1,3,4-oxadiazol-2-y l)thio)methyl)-*4H*-1,2,4-triazol-4-amine-2-yl)methyl)pyrimidine (14d)

Yellow solid; Yield 75%; m.p 156-157 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 2.31 (s, 3H, CH_3), 2.89 (s, 3H, NCH_3), 4.48 (s, 2H, -SCH_2-), 5.45 (s, 2H, CH_2), 6.01 (s, 2H, 1,2,4-triazol-NH_2), 6.96 (s, 2H, pyrimidine-4-NH_2), 8.10 (s, 1H, pyrimidine-5-yl-H), 8.32 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 15.3, 22.5, 23.5, 27.6, 47.2, 110.1, 121.3, 126.3, 129.9, 131.9, 132.1, 133.4, 133.6, 150.9, 155.8, 157.3, 159.6, 160.4, 166.6; HR-EIMS (EI) m/z 499.1174 $(M+H)^+$, calcd. for $C_{16}H_{18}F_3N_{12}S_2$ m/z = 499.1171.

5.7. Assay of *E.coli* PDHc-E1(*in vitro*) and site-directed mutagenesis of PDHc-E1

The expressing plasmid pMal-C_{2X}-PDHc E1 was transformed into *E. coli* strain TB1 and inoculated in Luria–Bertani broth containing 2% glucose and 30 mg/ml ampicillin at 37 ℃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℃ 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[29] 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℃. The inhibitory activities of synthesized compounds were measured by the enzymatic assay. PDHc-E1 activity was assayed by a modified method of N. Nemeria[30] and measured by monitoring the reduction of 2,6-DichloroPhenolindophenol (2,6-DCPIP) at 600 nm using a microplate reader (BioTek Synergy 2, 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 ℃, 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, $V = V_0\text{-}(V_0\text{-}V_\infty)/((IC_{50}/I)^n + 1)$.[31] 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).

Site-directed mutagenesis of PDHc E1 was accomplished by the introduction of specific base changes into a double-stranded DNA plasmid, as described previously. DNA encoding of the wild-type PDHc E1 cloned into the pMAL-C_{2x} -PDHc-E1 was used as a template for mutagenesis. The standard PCR mixture contained 50–100 ng of template DNA and 100–200 ng of each

mutagenizing primer. The methylated plasmid was digested with DpnI, and 4 μL of each reaction was used to transform the DH5α competent cells. All mutations were confirmed by DNA sequencing. Verified plasmids containing the desired mutations were transformed into the E. coli TB1 strain. The mutant PDHc E1 proteins were purified in the same manner as the wild-type PDHc E1.

5.8. Molecular docking

For docking purposes, the crystallographic coordinates of the PDHc-E1 with bound ThDP from *E. coli* (PDB code: 1L8A)[32] 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 His142, Tyr177, Glu751, His640 and Met 194, 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 CCNU Grid-based computational environment (CCNUGrid website http://www.202.114.32.71:8090/ccnu/chem/platform.xml).

Acknowledgments

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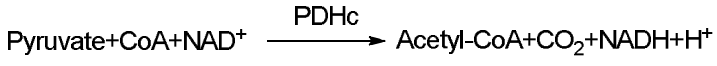


Fig.1 Oxidative decarboxylation catalyzed by PDHc.

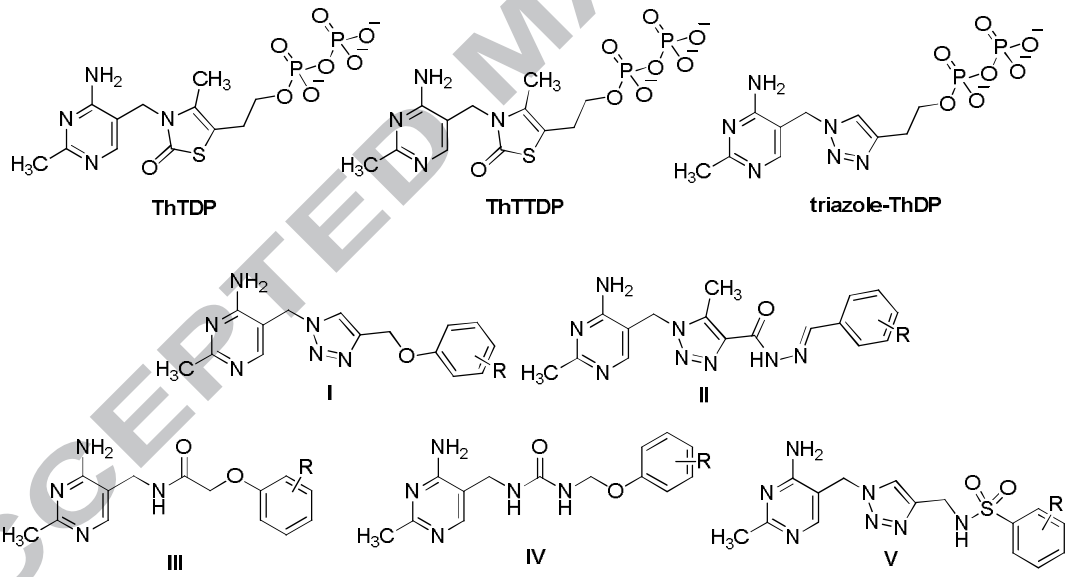


Fig.2 Structures of known PDHc-E1 inhibitors.

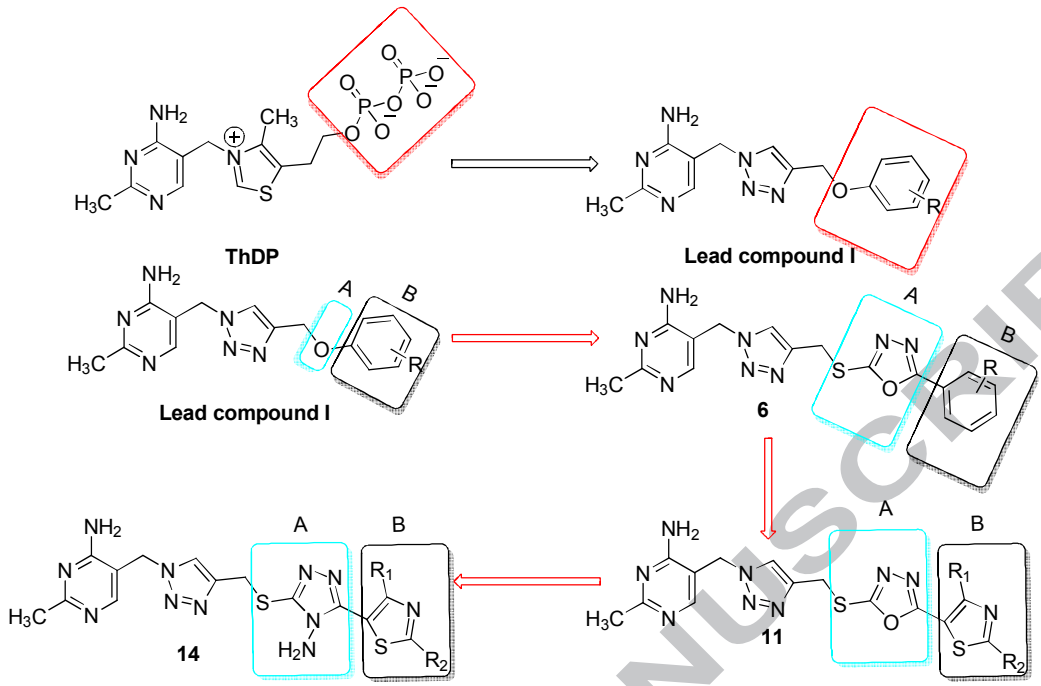


Fig.3 Design of the new 1,3,4-Oxadiazole pyrimidine derivatives **6**, **11** and 1,2,4-triazolyl pyrimidine derivatives **14**

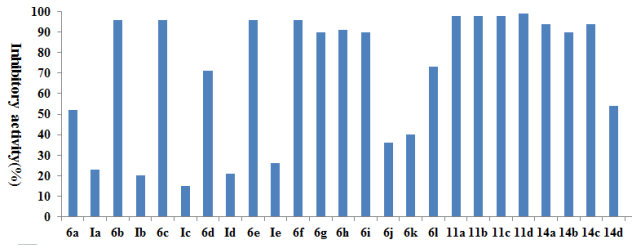


Fig.4 Inhibitory potency (%) of **I**, **6**, **11** and **14** against cyanobacteria at 10μM.

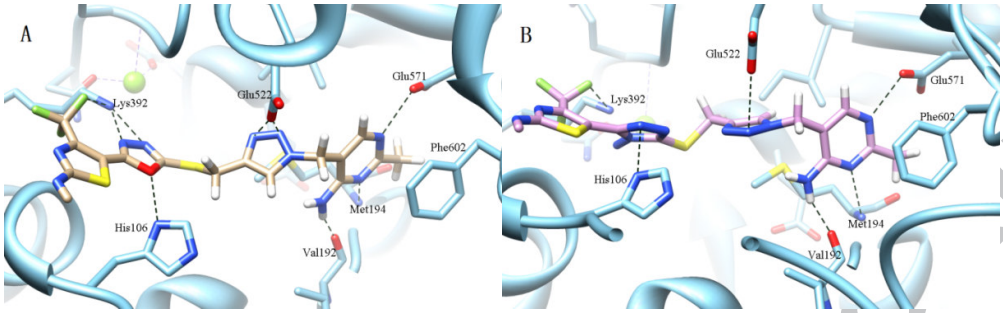


Fig. 5 Optimal binding model for **11d** (A) and **14d** (B) into the active site of *E. coli* PDHc-E1 docked by the SURFLEX module. The ligands and some key residues are presented as a stick model; hydrogen bonds are shown as dashed lines.

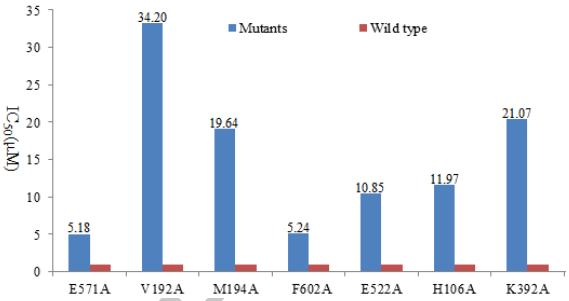
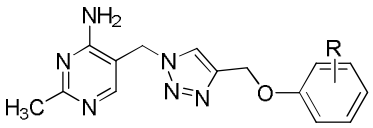


Fig. 6 The IC_{50} values of **11d** against the wild type (WT) and mutants of *E. coli* PDHc-E1. The substrate is pyruvate acid, and the cofactor is ThDP.

Table 1 Structure and inhibitory activity of 1,3,4-Oxadiazole pyrimidine derivatives **6** and **I**



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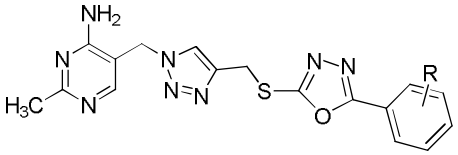

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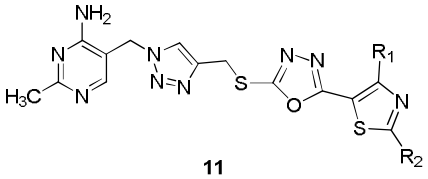
Compd.	R	IC_{50}[a](μM) Inhibitory against *E. coil* PDHc-E1	EC_{50}[b](μM) Inhibitory against cyanobacteria
6a	4-NO_2	7.59±0.37	9.04±1.98
Ia	4-NO_2	8.80±0.35	>50
6b	4-Cl	7.08±0.26	5.25±0.94
Ib	4-Cl	26.44±1.68	>50
6c	2-NO_2	9.84±0.45	4.76±0.31
Ic	2-NO_2	36.29±1.35	>50
6d	2,4-diCl	13.18±0.82	5.99±0.88
Id	2,4-diCl	18.74±1.24	>50
6e	H	7.87±0.35	5.15±0.46
Ie	H	55.15±4.65	>50
6f	4-F	9.11±0.19	5.34±0.69
6g	4-CF_3	15.87±0.53	5.35±0.31
6h	3,5-diNO_2	8.61±0.34	2.33±0.43
6i	3-NO_2	10.38±0.30	5.14±1.11
6j	2-F	19.21±1.57	8.05±1.36
6l	2-Cl	12.39±0.42	2.28±0.18
6k	2-Cl-4NO_2	12.80±0.67	6.37±1.56

[a]IC_{50} (μM) value is defined as the micromolar concentration required for 50% inhibition against PDHc-E1 from *E. coli in vitro*. [b]The inhibitory activity against Synechocystis sp. PCC 6803.

Table 2 Structure and inhibitory activity of 1,3,4-Oxadiazole pyrimidine derivatives **11** and 1,2,4-triazolyl pyrimidine derivatives **14**



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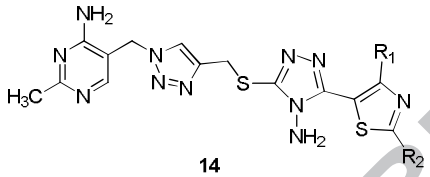

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Compd.	R^1	R^2	IC_{50}[a](μM) Inhibitory against *E. coil* PDHc-E1	EC_{50}[b](μM) Inhibitory against cyanobacteria
11a	CH_3	NH_2	4.36±0.19	1.63±0.46
11b	CH_3	CH_3NH	6.62±0.30	1.51±0.36
11c	CF_3	NH_2	3.94±0.23	1.61±0.39
11d	CF_3	CH_3NH	0.97±0.05	0.83±0.16
14a	CH_3	NH_2	7.02±0.40	2.30±0.30
14b	CH_3	CH_3NH	11.09±0.54	2.99±0.31
14c	CF_3	NH_2	5.03±0.15	3.24±0.51
14d	CF_3	CH_3NH	15.67±0.37	9.86±2.13

[a]IC_{50} (μM) value is defined as the micromolar concentration required for 50% inhibition against PDHc-E1 from *E. coli in vitro*. [b]The inhibitory activity against Synechocystis sp. PCC 6803.

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719 **Table 3** Antifungal activity of **6**, **11** and **14**

Comp d.	Inhibitory potency[a] (%)				Comp d.	Inhibitory potency[a] (%)			
	A.solan i^b	*R.solan* i^b	*B.cinere* a^b	*C.orbicular* e^b		*A.solan* i^b	*R.solan* i^b	*B.cinere* a^b	*C.orbicular* e^b
6a	60	55	45	35	**6k**	35	46	54	38
6b	60	46	45	52	**11a**	56	11	31	24
6c	58	40	54	48	**11b**	44	11	31	26
6e	47	30	60	40	**11c**	53	11	60	25
6f	44	0	60	32	**11d**	41	14	50	36
6g	35	0	45	45	**14a**	29	16	16	26
6h	30	30	41	33	**14b**	25	17	11	16
6i	48	60	59	40	**14c**	21	11	20	35
6j	40	0	28	32	**14d**	21	11	8	28

720 [a]Inhibitory potency (%) against the growth of pathogenic fungi at 100 μg/mL, 0 (no effect), 100% (completely kill).

721 [b]*A. solani, Alternaria solani; R. solani, Rhizoctonia solani; B. cinerea, Botrytis cinerea; C. orbiculare,*

722 *Colletotrichum orbicu*

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736 **Table 4** Inhibition of **11** against *E. coli* and pig PDHc-E1

Compd.	*E. coli* PDHc-E1		Pig PDHc-E1
	IC_{50}[a](μM)	Inhibitory potency[b](%)	Inhibitory potency[b](%)
11a	4.36±0.19	100±1.26	27.15±7.98
11b	6.62±0.30	100±2.36	15.59±0.76
11c	3.94±0.23	100±5.66	28.26±1.52
11d	0.97±0.05	100±3.21	26.07±2.47

737 [a]IC_{50} (μM) value is defined as the micromolar concentration required for 50% inhibition against PDHc-E1 from *E.*
738 *coli in vitro*. [b] Inhibitory potency (%) of compounds against enzyme *in vitro* at 100 μM as average of triplicate.

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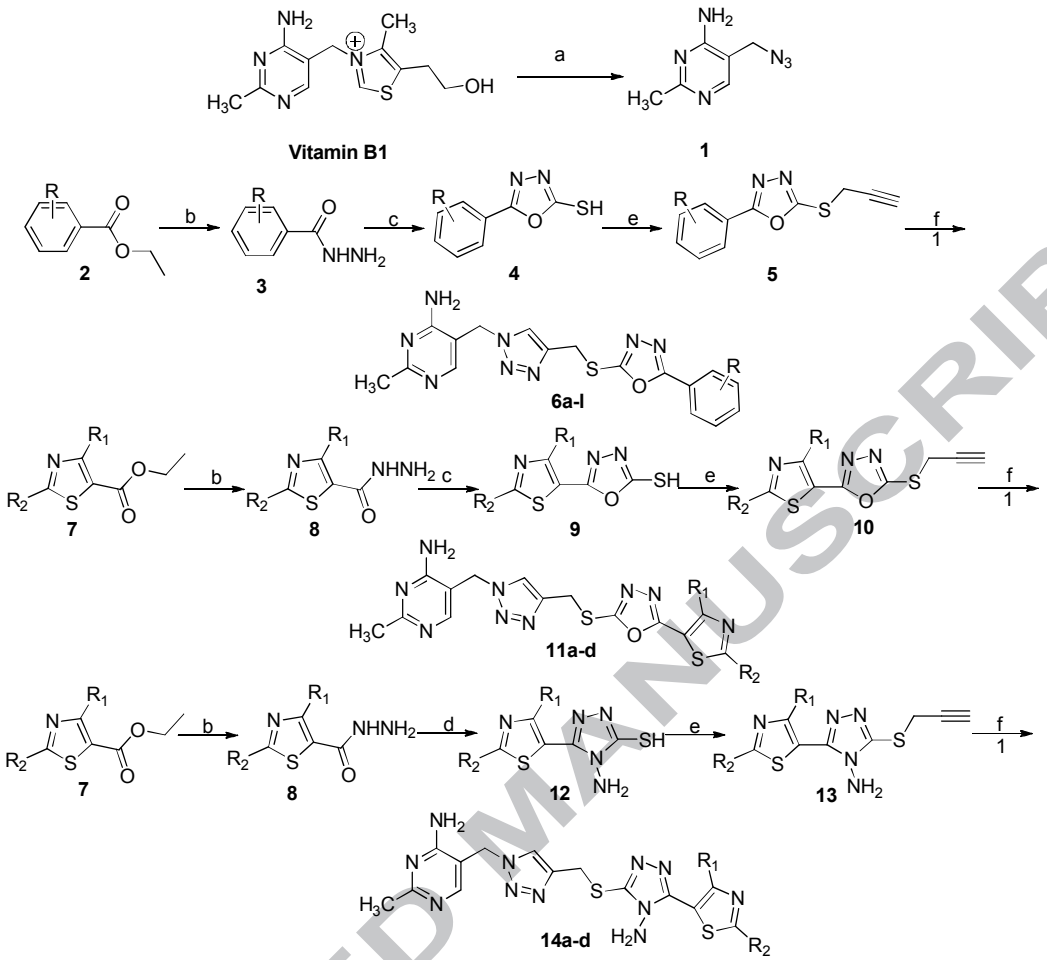

Scheme 1. Reagents and conditions (a) NaN_3, Na_2SO_3, H_2O, 60 – 65℃ 56%; (b) $NH_2NH_2.H_2O$, EtOH, reflux; (c) CS_2, KOH, EtOH reflux; HCl; (d) CS_2, KOH, EtOH reflux; $NH_2NH_2.H_2O$, reflux; (e) $BrCH_2CCH$, K_2CO_3, acetone, reflux, 1h; (f) CuI, Et_3N, THF, rt, 10 – 15 h;

Graphic abstract for

Rational design, synthesis and biological evaluation of 1,3,4-oxadiazole pyrimidine derivatives as novel pyruvate dehydrogenase complex E1 inhibitors

1,3,4-Oxadiazole pyrimidine derivative **11d** exhibited most powerful inhibitory potency against *E. coli* PDHc-E1 (IC_{50} = 0.97 μM) and cyanobacteria (EC_{50} = 0.83 μM).