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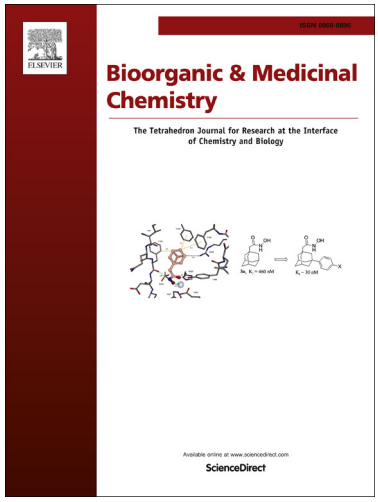

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PII: S0968-0896(14)00247-8
DOI: http://dx.doi.org/10.1016/j.bmc.2014.04.003
Reference: BMC 11498

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 10 March 2014
Revised Date: 2 April 2014
Accepted Date: 4 April 2014

Please cite this article as: He, J-B., Ren, Y-L., Sun, Q-S., You, G-Y., Zhang, L., Zou, P., Feng, L-L., Wan, J., He, H-W., Design, synthesis and molecular docking of amide and urea derivatives as *Escherichia coli* PDHc-E1 inhibitors, *Bioorganic & Medicinal Chemistry* (2014), doi: http://dx.doi.org/10.1016/j.bmc.2014.04.003

Design, synthesis and molecular docking of amide and urea derivatives as *Escherichia coli* PDHc-E1 inhibitors

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Abstract: By targeting the ThDP binding site of *E. coli* PDHc-E1, two new "open-chain" classes of *E. coli* PDHc-E1 inhibitors, amide and urea derivatives, were designed, synthesized, and evaluated. The amide derivatives of compound **6d**, with 4-NO_2 in the benzene ring, showed the most potent inhibition of *E. coli* PDHc-E1. The urea derivatives displayed more potent inhibitory activity than the corresponding amide derivatives with the same substituent. Molecular docking studies confirmed that the urea derivatives have more potency due to the two hydrogen bonds formed by two NH of urea with Glu522. The docking results also indicate it might help us to design more efficient PDHc-E1 inhibitors that could interact with Glu522.

Keywords: PDHc-E1 inhibitors, amide and urea derivatives, molecular docking

1. Introduction

The pyruvate dehydrogenase complex (PDHc) is an exquisite machine that catalyzes the irreversible oxidative of pyruvate to acetyl CoA.[1] The fundamental reactions of this complex are carried out by three enzymatic components: pyruvate dehydrogenase (E1), dihydrolipoamide acetyltransferase (E2), and dihydrolipoamide dehydrogenase (E3).[2] The pyruvate dehydrogenase complex E1 component (PDHc-E1) catalyzes the first inreversible step of the multistep process, using thiamine diphosphate (ThDP) (Figure **1**) and Mg^{2+} as cofactors.[3-5] Therefore, the PDHc-E1 has been reported as promising target for herbicide[6-8] and fungicide.[9]

So far, a number of studies on small molecular inhibitors of PDHc-E1 have been reported, such as pyruvate analogs[10-12] and phosphonate analogs of pyruvate.[6] Specially, the ThDP analogs have been studied greatly,[13-16] for their high binding affinities against PDHc-E1 and more modification sites at ThDP. However, because of the structural complexity and highly charged pyrophosphate, there are no commercial potential inhibitors of ThDP analogs that occupy the binding site of ThDP in PDHc-E1. In the present, it is an utmost need for the development of practical small molecules as inhibitors of PDHc-E1.

In our early efforts to design novel PDHc-E1 inhibitors, series of novel PDHc-E1 inhibitors (Figure **2**) were reported[17-19] and showed potent *Escherichia coli* (*E. coli*) PDHc-E1 inhibitory activity and antifungal activity[18]. Structure-activity relationship (SAR) indicated the introduction of iodine into the 5-position of 1,2,3-triazole of title compounds could further increase the inhibitory activity against *E. coli* PDHc-E1 and antifungal activity[18]. These results convinced us that the linker plays a vital role in the biological activity of these compounds. Aiming to explore more optimizing linkages, the amide, which has been reported as bioisostere of 1,4-substituted 1,2,3-triazole,[20, 21] is introduced to the structure **I** as a "open-chain"[22] linker. Furthermore, the amide was replaced by urea for its ability to simultaneously donate two hydrogen bonds compared with amide. Therefore, two new structural classes of PDHc-E1 inhibitors were formed (Figure **3**).

Herein, the chemical synthesis of these new amide and urea derivatives as *E. coli* PDHc-E1 inhibitors is described in details. The inhibitory activities on *E. coli* PDHc-E1 are presented along with their structure-activity relationship (SAR) analysis as follows. We also performed molecular docking studies leading to identify the critical binding sites of the target PDHc-E1.

2. Chemistry

The synthesis of amide derivatives (**6a-6n**) was depicted in Scheme **1**. The critical intermediate of 5-(aminomethyl)-2-methylpyrimidin-4-amine (**2**) was synthesized by Pd/C-catalyzed reduction of 5-(azidomethyl)-2-methylpyrimidin-4-amine (**1**), which was prepared readily from thiamine hydrochloride according to literature.[23] The substituted phenoxyacetic acids **3** were prepared by condensation of corresponding substituted phenols with chloroacetic acid in the presence of sodium hydroxide. However, the yield of the reaction of chloroacetic acid with strong electron-withdrawing substituted phenols in the presence of sodium hydroxide was very poor. Therefore, the substituted phenoxyacetic acids **5** were prepared in satisfactory yields by reaction of corresponding substituted phenols with ethyl bromoacetate in the presence of K_2CO_3 in DMSO followed by

alkaline hydrolysis.[7] The final compounds **6a-6n** were synthesized easily by coupling reaction of compound **2** with mixed anhydrides, which were generated from substituted phenoxyacetic acid **3** and **5** in THF at -5 ^{o}C by the addition of ethyl chloroformate in the presence of triethylamine.

The synthesis of urea derivatives **8a-8e** was depicted in Scheme **2**. Acyl azides were prepared from NaN_3 with mixed anhydries, which could be easily generated from acid **3** and **5** with ethyl chloroformate. Then acyl azides undergo Curtius rearrangement[24] in toluene to give isocyanates, which were trapped by amine **2** leading to the formation of urea derivatives **8a-8e** in 57-82% yields.

3. Results and discussion

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

The synthesized amide and urea derivatives (**6a-6n** and **8a-8e**) were evaluated for their inhibitory activities against PDHc-E1 from *E. coli*. The IC_{50} values were summarized in Table **1**. It was observed that most compounds exhibited good inhibitory activity (IC_{50} < 20 μM). The structure-activity relationships (SAR) in these compounds were investigated by introducing substituents on the benzene ring, and changing the linker with amide and urea linkages.

We started our work focusing on the amide linkage. As shown in Table **1**, it was noticed that the inhibitory activity is highly related to the nature of the substituents on benzene ring, and electron-withdrawing groups are beneficial for PDHc-E1 inhibitory activity. Comparing the data of compounds **6b**, **6k**, **6l**, and **6m**, the activity sequence is 2-Cl-4-NO_2 (**6l**) > 2-Cl-4-F (**6k**) > 2,4-diCl (**6b**) > 2-CH_3-4-Cl (**6m**), which indicates the stronger of the electron-withdrawing groups, the more potent inhibitory activity. We also notice that compound **6d**, with 4-NO_2 on the benzene ring, possesses the most potent inhibitory activity with IC_{50} value of 3.58 ± 0.52 μM. Based on the recently publication,[17] we suppose that the nitryl group on benzene ring presented a special case as it can form hydrogen bonds and coordinate bond with Mg^{2+} in the active site. It should be noted that compound **6d** (IC_{50} =

3.58 ± 0.52 μM) showed slightly improved inhibitory activity, compared with compound **6l** (IC_{50} = 4.90 ± 0.81 μM). This suggests that the 2-position group on benzene ring is not good for the PDHc-E1 inhibitory activity because of the steric effect. Compared with compound **6h** (2-Br), compound **6i** (2-Cl) showed more potent inhibitory activity, which can be explained that compound **6i** (2-Cl) has less steric hindrance effect. In conclusion, the amide derivatives **6a-6n** exhibited higher inhibitory activity than structure **I**.[18]

Encouraged by the improved potency of compounds **6a-6n** versus structure **I**, we continued our optimization effort by changing the amide linkage with urea linkage. As shown in Table **2**, all the compounds **8a-8e** displayed improved inhibitory activity versus the corresponding amide derivatives with the same substituents. We notice that compound **8e** (4-Cl, IC_{50} = 3.67 ± 0.38 μM) exhibited the same potency with compound **6d** (4-NO_2), and showed 3-folds increase than compound **6c**. The results suggest that the urea linkage should play an important role in the PDHc-E1 inhibitory activity. We also tried to synthesize the urea derivative with 4-NO_2 in the benzene ring, but failed.

Based on the aforementioned results, the "open-chain" linkers, amide and urea linkages, do have a beneficial effect on the *E. coli* PDHc-E1 inhibition. Compared to 1,2,3-triazole derivatives, the "open-chain" linker derivatives can be easily functionalized into more new classes of inhibitors.

3.2 Molecular docking studies

To explore the interaction modes of amide and urea derivatives with the active site of PDHc-E1, several molecular docking simulation studies were carried out by using SURFLEX module of SYBYL package version. Based on the *in vitro* inhibition results, we selected compounds **6d** and **8e**, our best PDHc-E1 inhibitors in present study (IC_{50} = 3.58 ± 0.52 μM and 3.67 ± 0.38 μM, respectively), as ligand examples.

The binding modes of compound **6d** and **8e** were shown in Figure **4A** and **4B**, respectively. As depicted in Figure **4A**, 4-aminopyrimidine moiety formed two hydrogen bonds with residues Val192 and Glu571, and π-π stacking

interaction with Phe602. For benzene ring part, the nitryl group can form three hydrogen bonds with Gly231, Asn260, and Lys392, furthermore, the coordination of nitryl group with Mg^{2+} in the active site also plays an important role in increasing the bind interaction. The binding modes of these two parts, 4-aminopyrimidine and benzene ring, were in perfect agreement with the recently publication.[17] The amide, was used as the bioisostere of 1,4-substituted 1,2,3-triazole, can form a strong hydrogen bond with Glu522 which is an important residue in the stabilization of the enzyme-bound LThDP.[25, 26] We also investigated how the two NH of urea bind in the active site of *E. coli* PDHc-E1. Figure **4B** showed the binding mode of compound **8e**, it can be seen that the binding mode of 4-aminopyrimidine of compound **8e** is almost same to those of compound **6d**, but the two NH of urea really formed two strong hydrogen bonds with Glu522. Interestingly, the oxygen atom of phenoxy also formed a hydrogen bong with Leu264, which maybe because the chain length is increased by introduction of urea. The docking results provided us a reasonable explanation for why compound **8e** has more potent *E. coli* PDHc-E1 inhibitory activity. The docking results also provide us a new method to design new PDHc-E1 inhibitors that can interact with Glu522 based on amide and urea linkages.

4. Conclusion

In the present study, two new "open-chain" classes of *E. coli* PDHc-E1 inhibitors, amide and urea derivatives, were designed and synthesized. SAR analyses indicated that the inhibitory potency against *E. coli* PDHc-E1 of title compounds could be increased by introducing amide and urea linkages. The amide derivatives with 4-NO2 in the benzene ring exhibited much more inhibitory potency against *E. coli* PDHc-E1 than other compounds. Moreover, optimizing the amide linker with urea linker, the *E. coli* PDHc-E1 inhibitory activity could be further increased. Molecular docking was performed to study the inhibitor-PDHc-E1 protein interactions. Analysis of compounds **6d**'s and **8e**'s binding modes in the active binding site demonstrated that the two hydrogen bonds, formed by two NH of urea with Glu522, really plays an important role in increasing the inhibitory potency of urea

derivatives against *E. coli* PDHc-E1. Furthermore, the docking results also give us a new direction to design new PDHc-E1 inhibitors that can interact with Glu522. The above-mentioned results of SAR analysis and molecular docking study may allow the rational design of more efficient PDHc-E1 inhibitors.

5. Experiments

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 obtained at 400 MHz or 600 MHz, in $CDCl_3$ or DMSO-d_6 solution on a Varian Mercury-Plus 400 or 600 sepctrometer and chemical shifts were recorded in parts per million (ppm) with TMS as the internal reference. Mass spectra (MS) were run on a QTRSP 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. All the reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel G plates at 254 nm under a UV lamp. 5-(azidomethyl)-2-methylpyrimidin-4-amine (**1**) was synthesized according to the existing method.[23] Substituted phenoxyacetic acid **3** and **5** were prepared according to the methods previously reported.[7]

5.2. 5-(aminomethyl)-2-methylpyrimidin-4-amine (2)

To a solution of **1** (4.0 g) in methanol (100 mL) was added 10% Pd/C (0.4 g), then the flask was fit tightly over with a balloon filled with hydrogen gas. The mixture was stirred at room temperature for 12 h. The reaction mixture was filtered through a pad of Celite and the solvent were evaporated under reduced pressure to afford compound **2** as white solid (3.34 g, 99%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.85 (s, 2H, NH_2), 2.48 (s, 3H, CH_3), 3.93 (s, 2H, CH_2), 6.04 (s, 2H, NH_2), 7.92 (s, 1H, pyrimidine CH).

5.3. General procedure for preparation of compounds 6a-6n

To a solution of corresponding substituted phenoxyacetic acid **3** or **5** (1.5 mmol) and Et_3N (0.16 g, 1.6 mmol) in THF (15 mL) was added ethyl chlorocarbonate (0.17 g, 1.6 mmol) in THF (2 mL) slowly at -5 ^oC. After the addition was complete, the cold mixture was stirred for an additional 15 min. A solution of **2** (0.22 g, 1.6 mmol) in DMF (2 mL) was added dropwise while the temperature was kept at -5 ^oC. After the addition was complete, the mixture was stirred at room temperature for 12 h. It was poured into water (30 mL), and the precipitate was collected by filtration and dried in the atmospheric pressure. Recrystallization with appropriate solvent afforded the desired solid compounds **6a-6n**.

5.3.1. N-((4-amino-2-methylpyrimidin-5-yl)methyl)-2-phenoxyacetamide (6a)

White solid, yield: 92%, mp 120-121; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.47 (s, 3H, CH_3), 4.38 (d, 2H, J = 9.6 Hz, CH_2), 4.53 (s, 2H, CH_2), 5.95 (s, 2H, NH_2), 6.89 (d, 1H, J = 12.0 Hz, Ar-H), 7.03 (t, 2H, J = 10.8 Hz, Ar-H), 7.12 (s, 1H, NH), 7.31 (t, 2H, J = 12.0 Hz, Ar-H), 7.98 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 25.17, 36.45, 66.85, 110.63, 114.77, 121.30, 129.51, 154.94, 157.60, 161.52, 165.64, 168.59. EI-MS *m/z* (%): 273.3 (M^++1, 3.62), 272.2 (M^+, 35.15). Anal. Calcd. For $C_{14}H_{16}N_4O_2$: C, 61.75; H, 5.92; N, 20.58. Found: C, 61.48; H, 5.82; N, 20.64.

5.3.2. N-((4-amino-2-methylpyrimidin-5-yl)methyl)-2-(2,4-dichlorophenoxy)acetamide (6b)

Light yellow solid, yield: 67%, mp 201-203; ^1H NMR (DMSO-d_6, 600 MHz): δ 2.29 (s, 3H, CH_3), 4.10 (s, 2H, CH_2), 4.68 (s, 2H, CH_2), 6.73 (s, 2H, NH_2), 7.04 (s, 1H, Ar-H), 7.35 (s, 1H, Ar-H), 7.60 (s, 1H, Ar-H), 7.89 (s, 1H, CH), 8.48 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 25.12, 36.49, 67.88, 110.49, 115.50, 122.65, 125.29, 128.03, 129.41, 152.41, 154.70, 161.51, 165.69, 167.83. EI-MS *m/z* (%): 342.2 (M^++2, 10.06), 340.2 (M^+, 10.13). Anal. Calcd. For $C_{14}H_{14}Cl_2N_4O_2$: C, 49.28; H, 4.14; N, 16.42. Found: C, 49.78; H, 4.29; N, 16.74.

5.3.3. N-((4-amino-2-methylpyrimidin-5-yl)methyl)-2-(4-chlorophenoxy)acetamide (6c)

White solid, yield: 92%, mp 192-194; ^1H NMR ($CDCl_3$, 600 MHz): δ 2.47 (s, 3H, CH_3), 4.38 (d, 2H, J = 7.2 Hz,

155 CH_2), 4.50 (s, 2H, CH_2), 5.94 (s, 2H, NH_2), 6.83 (d, 2H, *J* = 9.0 Hz, Ar-H), 7.09 (s, 1H, NH), 7.26 (d, 2H, *J* = 9.0

156 Hz, Ar-H), 7.98 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 25.15, 36.45, 67.14, 110.58, 116.55, 125.09,

157 129.28, 154.88, 156.45, 161.54, 165.73, 168.37. EI-MS *m/z* (%): 308.3 (M^++2, 5.16), 307.3 (M^++1, 2.87), 306.3

158 (M^+, 17.32),. Anal. Calcd. For $C_{14}H_{15}ClN_4O_2$: C, 54.82; H, 4.93; N, 18.26. Found: C, 54.46; H, 5.28; N, 17.91.

159 **5.3.4. N-((4-amino-2-methylpyrimidin-5-yl)methyl)-2-(4-nitrophenoxy)acetamide (6d)**

160 Yellow solid, yield: 79%, mp 239-241; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.29 (s, 3H, CH_3), 4.11 (s, 2H, CH_2),

161 4.73 (d, 2H, *J* = 4.8 Hz, CH_2), 6.71 (s, 2H, NH_2), 7.16 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.23 (d, 2H, *J* = 9.0 Hz,

162 Ar-H),7.89 (s, 1H, CH), 8.68 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 25.13, 36.46, 67.14, 110.49, 115.36,

163 125.82, 141.33, 154.76, 161.50, 162.79, 165.73, 167.72. EI-MS *m/z* (%): 318.2 (M^++1, 3.87), 317.3 (M^+, 25.46).

164 Anal. Calcd. For $C1_4H_{15}N_5O_4$: C, 52.99; H, 4.76; N, 22.07. Found: C, 52.68; H, 4.38; N, 19.78.

165 **5.3.5. N-((4-amino-2-methylpyrimidin-5-yl)methyl)-2-(p-tolyloxy)acetamide (6e)**

166 White solid, yield: 57%, mp 172-174; ^{1}H NMR ($CDCl_3$, 600 MHz): δ 2.29 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 4.38 (d,

167 2H, *J* = 6.6 Hz, CH_2), 4.49 (d, 2H, *J* = 7.2 Hz, CH_2), 5.95 (s, 2H, NH_2), 6.78 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.09 (d, 3H,

168 *J* = 8.4 Hz, Ar-H + NH), 7.99 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 20.08, 25.14, 36.44, 67.11, 110.66,

169 114.63, 129.86, 130.11, 154.91, 155.53, 161.57, 165.70, 168.81. EI-MS *m/z* (%): 286.2 (M^+, 3.62). Anal. Calcd.

170 For $C_{15}H_{18}N_4O_2$: C, 62.92; H, 6.34; N, 19.57. Found: C, 62.81; H, 6.62; N, 19.47.

171 **5.3.6. N-((4-amino-2-methylpyrimidin-5-yl)methyl)-2-(4-bromophenoxy)acetamide (6f)**

172 White solid, yield: 63%, mp 209-210; ^{1}H NMR ($CDCl_3$, 600 MHz): δ 2.48 (s, 3H, CH_3), 4.38 (d, 2H, *J* = 7.2 Hz,

173 CH_2), 4.50 (s, 2H, CH_2), 5.91 (s, 2H, NH_2), 6.78 (d, 1H, *J* = 9.0 Hz, Ar-H), 7.02 (s, 1H, NH), 7.41 (d, 2H, *J* = 9.6

174 Hz, Ar-H), 7.99 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 25.16, 36.43, 67.03, 110.56, 112.78, 117.07,

175 132.16, 154.87, 156.90, 161.51, 165.70, 168.31. EI-MS *m/z* (%): 352.1 (M^++2, 10.61), 350.2 (M^+, 11.10). Anal.

176 Calcd. For $C_{14}H_{15}BrN_4O_2$: C, 47.88; H, 4.31; N, 15.95. Found: C, 47.58; H, 4.39; N, 15.57.

5.3.7. N-((4-amino-2-methylpyrimidin-5-yl)methyl)-2-(4-methoxyphenoxy)acetamide (6g)

White solid, yield: 67%, mp 177-179; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.48 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 4.38 (d, 2H, *J* = 10.8 Hz, CH_2), 4.48 (s, 2H, CH_2), 5.93 (s, 2H, NH_2), 6.84 (d, 2H, *J* = 12.6 Hz, Ar-H), 7.03 (d, 2H, *J* = 10.8 Hz, Ar-H), 7.26 (s, 1H, NH), 7.99 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 25.16, 36.44, 55.37, 67.72, 110.68, 114.62, 115.83, 151.64, 153.96, 154.98, 161.58, 165.74, 168.93. EI-MS *m/z* (%): 302.3 (M^+, 13.45). Anal. Calcd. For $C_{15}H_{18}N_4O_3$: C, 59.59; H, 6.00; N, 18.53. Found: C, 59.55; H, 6.43; N, 18.71.

5.3.8. N-((4-amino-2-methylpyrimidin-5-yl)methyl)-2-(2-bromophenoxy)acetamide (6h)

White solid, yield: 63%, mp 196-198; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.48 (s, 3H, CH_3), 4.41 (d, 2H, *J* = 10.2 Hz, CH_2), 4.57 (s, 2H, CH_2), 5.89 (s, 2H, NH_2), 6.86 (d, 1H, *J* = 7.2 Hz, Ar-H), 6.93 (t, 1H, *J* = 7.2 Hz, Ar-H), 7.30 (d, 1H, J = 6.8 Hz, Ar-H), 7.55 (d, 1H, J = 8.0 Hz, Ar-H), 8.04 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 25.16, 36.47, 67.91, 110.52, 111.30, 122.89, 128.95, 133.11, 154.15, 154.74, 161.50, 165.70, 168.07. EI-MS *m/z* (%): 352.1 (M^++2, 6.39), 350.2 (M^+, 5.11). Anal. Calcd. For $C_{14}H_{15}BrN_4O_2$: C, 47.88; H, 4.31; N, 15.95. Found: C, 48.24; H, 4.70; N, 16.36.

5.3.9. N-((4-amino-2-methylpyrimidin-5-yl)methyl)-2-(2-chlorophenoxy)acetamide (6i)

White solid, yield: 65%, mp 188-190; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.47 (s, 3H, CH_3), 4.38 (d, 2H, *J* = 9.6 Hz, CH_2), 4.50 (s, 2H, CH_2), 5.92 (s, 2H, NH_2), 6.83 (d, 2H, *J* = 4.4 Hz, Ar-H), 7.07 (s, 1H, NH), 7.26 (t, 2H, *J* = 4.0 Hz, Ar-H), 7.98 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 25.18, 36.48, 67.16, 110.65, 116.62, 125.14, 129.34, 154.89, 156.49, 161.58, 165.78, 168.46. EI-MS *m/z* (%): 308.2 (M^++2, 6.53), 306.2 (M^+, 22.59). Anal. Calcd. For $C_{14}H_{15}ClN_4O_2$: C, 54.82; H, 4.93; N, 18.26. Found: C, 54.57; H, 5.28; N, 18.35.

5.3.10. N-((4-amino-2-methylpyrimidin-5-yl)methyl)-2-(4-fluorophenoxy)acetamide (6j)

White solid, yield: 79%, mp 165-167; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.48 (s, 3H, CH_3), 4.38 (d, 2H, *J* = 10.2 Hz, CH_2), 4.49 (s, 2H, CH_2), 5.93 (s, 2H, NH_2), 6.83-6.86 (m, 2H, Ar-H), 7.00 (t, 2H, *J* = 12.6 Hz, Ar-H), 7.07 (s, 1H,

NH), 7.99 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 25.15, 36.43, 67.49, 110.60, 115.78, 116.01, 116.13, 116.21, 153.92, 154.85, 161.51, 165.69, 168.54. EI-MS *m/z* (%): 291.3 (M^++1, 3.99), 290.2 (M^+, 28.62). Anal. Calcd. For $C_{14}H_{15}FN_4O_2$: C, 57.92; H, 5.21; N, 19.30. Found: C, 58.24; H, 5.54; N, 18.98.

5.3.11. N-((4-amino-2-methylpyrimidin-5-yl)methyl)-2-(2-chloro-4-fluorophenoxy)acetamide (6k)

White solid, yield: 71%, mp 167-169; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.48 (s, 3H, CH_3), 4.40 (d, 2H, *J* = 9.6 Hz, CH_2), 4.54 (s, 2H, CH_2), 5.91 (s, 2H, NH_2), 6.84-6.87 (m, 1H, Ar-H), 6.93-6.96 (m, 1H, Ar-H), 7.14-7.19 (m, 2H, Ar-H + NH), 8.03 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 25.13, 36.49, 68.34, 110.51, 114.75, 115.40, 115.48, 117.11, 122.28, 122.39, 150.0, 154.77, 161.51, 165.70, 168.01. EI-MS *m/z* (%): 326.3 (M^++2, 2.97), 324.2 (M^+, 13.84). Anal. Calcd. For $C_{14}H_{14}ClFN_4O_2$: C, 51.78; H, 4.35; N, 17.25. Found: C, 51.98; H, 3.98; N, 17.65.

5.3.12. N-((4-amino-2-methylpyrimidin-5-yl)methyl)-2-(2-chloro-4-nitrophenoxy)acetamide (6l)

Light yellow solid, yield: 57%, mp 201-202; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.49 (s, 3H, CH_3), 4.43 (d, 2H, *J* = 9.6 Hz, CH_2), 4.68 (s, 2H, CH_2), 5.84 (s, 2H, NH_2), 6.99 (d, 1H, *J* = 13.8 Hz, Ar-H), 7.05 (s, 1H, NH), 8.05 (s, 1H, CH), 8.19 (d, 1H, *J* = 10.2 Hz, Ar-H), 8.34 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 25.16, 36.60, 67.85, 110.43, 113.77, 122.05, 124.39, 125.48, 141.14, 154.71, 158.61, 161.51, 165.79, 167.22. EI-MS *m/z* (%): 353.2 (M^++2, 4.66), 352.2 (M^++1, 2.89), 351.2 (M^+, 18.48). Anal. Calcd. For $C_{14}H_{14}ClN_5O_4$: C, 47.80; H, 4.01; N, 19.91. Found: C, 47.61; H, 4.31; N, 20.05.

5.3.13. N-((4-amino-2-methylpyrimidin-5-yl)methyl)-2-(4-chloro-2-methylphenoxy)acetamide (6m)

White solid, yield: 83%, mp 188-189; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.29 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 4.17 (d, 2H, *J* = 5.4 Hz, CH_2), 4.62 (s, 2H, CH_2), 6.81 (s, 2H, NH_2), 6.90 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.23 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.31 (s, 1H, Ar-H), 7.94 (s, 1H, CH), 8.48 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 15.95, 25.14, 36.49, 67.42, 110.57, 113.21, 124.61, 126.35, 128.74, 130.07, 154.66, 161.49, 165.64, 168.46. EI-MS *m/z* (%): 322.1 (M^++2, 3.29), 321.1 (M^++1, 3.62), 320.2 (M^+, 35.15). Anal. Calcd. For $C_{15}H_{17}ClN_4O_2$: C, 56.16; H,

5.34; N, 17.47. Found: C, 56.01; H, 5.44; N, 17.54.

5.3.14. N-((4-amino-2-methylpyrimidin-5-yl)methyl)-2-(4-cyanophenoxy)acetamide (6n)

White solid, yield: 71%, mp 232-233; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.28 (s, 3H, CH_3), 4.09 (d, 2H, J = 4.2 Hz, CH_2), 4.67 (s, 2H, CH_2), 6.72 (s, 2H, NH_2),7.11 (d, 2H, J = 7.8 Hz, Ar-H), 7.79 (d, 2H, J = 8.4 Hz, Ar-H), 7.87 (s, 1H, CH), 8.66 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 25.16, 36.43, 66.83, 103.58, 110.51, 115.86, 119.04, 134.20, 154.79, 161.01, 161.504, 165.73, 167.88. EI-MS *m/z* (%): 298.16 (M^++1, 6.34), 297.1 (M^+, 41.81). Anal. Calcd. For $C_{15}H_{15}N_5O_2$: C, 60.60; H, 5.09; N, 23.56. Found: C, 60.59; H, 4.95; N, 23.54.

5.4. General procedure for preparation of compounds 8a-8e

To a solution of corresponding substituted phenoxyacetic acid **3** or **5** (4 mmol) and Et_3N (0.48 g, 4.8 mmol) in acetone (15 mL) was added ethyl chlorocarbonate (0.51 g, 4.8 mmol) in acetone (5 mL) at -5 ^{o}C. After the addition was complete, the cold mixture was stirred for an additional 15 min. A solution of sodium azide (0.52 g, 8 mmol) in water (2 mL) was added over 5 min while the temperature was kept at -5 ^{o}C. The mixture was stirred for 30 min longer at this temperature, poured into ice water (50 mL), and shaken with toluene (30 mL x 3). The combined toluene extracts were dried over $MgSO_4$. The toluene solution was heated cautiously at 70 ^{o}C for 1 h, and then the solution was cooled to room temperature, which was used for next step directly.

To the solution of isocyanate **7** in toluene was added compound **2** (0.21 g, 1.5 mmol) in DMF (2 mL) at 0 ^{o}C over 5 min, and then the solution was stirred at room temperature. After 4 h, the toluene was removed under reduced pressure to give a residue. The residue was poured into cold water (30 mL), and the precipitate was collected by filtration and dried to afford the desired compounds **8a-8e**.

5.4.1. 1-((4-amino-2-methylpyrimidin-5-yl)methyl)-3-(phenoxymethyl)urea (8a)

White solid, yield: 82%, mp 158-160; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.27 (s, 3H, CH_3), 4.01 (d, 2H, J = 6.0 Hz, CH_2), 5.09 (d, 2H, J = 6.6 Hz, CH_2), 6.71 (s, 1H, NH), 6.82 (s, 2H, NH_2), 6.91-6.94 (m, 3H, Ar-H), 7.26 (t, 2H,

J = 7.2 Hz, Ar-H), 7.33 (t, 1H, J = 6.6 Hz, NH), 7.84 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 25.17, 37.18, 69.34, 111.96, 115.25, 120.73, 129.40, 129.49, 154.43, 156.86, 157.72, 161.72, 165.62. EI-MS *m/z* (%): 287.35 (M^+, 0.50). Anal. Calcd. For $C_{14}H_{17}N_5O_2$: C, 58.52; H, 5.96; N, 24.37. Found: C, 58.39; H, 5.80; N, 24.23.

5.4.2. 1-((4-amino-2-methylpyrimidin-5-yl)methyl)-3-((4-methylphenoxy)methyl)urea (8b)

White solid, yield: 57%, mp 172-174 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.22 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 4.02 (d, 2H, J = 6.0 Hz, CH_2), 5.06 (d, 2H, J = 6.6 Hz, CH_2), 6.68 (s, 1H, NH), 6.80 (s, 2H, NH_2), 6.83 (s, 2H, Ar-H), 7.06 (t, 2H, J = 6.0 Hz, Ar-H), 7.27 (s, 1H, NH), 7.85 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 20.11, 25.14, 37.13, 69.45, 111.96, 115.19, 129.40, 129.82, 154.37, 154.70, 157.72, 161.71, 165.57. EI-MS *m/z* (%): 301.49 (M^+, 0.86). Anal. Calcd. For $C_{15}H_{19}N_5O_2$: C, 59.79; H, 6.36; N, 23.24. Found: C, 59.60; H, 6.47; N, 24.43.

5.4.3. 1-((4-amino-2-methylpyrimidin-5-yl)methyl)-3-((4-methoxyphenoxy)methyl)urea (8c)

White solid, yield: 67%, mp 177-179 °C; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.27 (s, 3H, CH_3), 3.68 (s, 3H, CH_3), 4.01 (d, 2H, J = 6.0 Hz, CH_2), 5.01 (d, 2H, J = 7.2 Hz, CH_2), 6.67 (s, 1H, NH), 6.83 (d, 4H, J = 7.8 Hz, Ar-H + NH_2), 6.87 (d, 2H, J = 8.4 Hz, Ar-H), 7.27 (t, 1H, J = 6.6 Hz, NH), 7.84 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 25.15, 37.15, 55.34, 70.02, 222.99, 114.60, 116.57, 150.74, 153.62, 154.37, 157.75, 161.72, 165.59. EI-MS *m/z* (%): 317.26 (M^+, 0.40). Anal. Calcd. For $C15H_{19}N_5O_3$: C, 56.77; H, 6.03; N, 22.07. Found: C, 56.93; H, 5.95; N, 21.91.

5.4.4. 1-((4-amino-2-methylpyrimidin-5-yl)methyl)-3-((4-fluorophenoxy)methyl)urea (8d)

White solid, yield: 79%, mp 165-167; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 2.27 (s, 3H, CH_3), 4.01 (d, 2H, J = 6.0 Hz, CH_2), 5.07 (d, 2H, J = 6.6 Hz, CH_2), 6.76 (s, 1H, NH), 6.80 (s, 2H, NH_2), 6.96 (d, 2H, J = 8.4 Hz, Ar-H), 7.08 (t, 2H, J = 8.4 Hz, Ar-H), 7.36 (t, 1H, J = 6.6 Hz, NH), 7.84 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 25.13, 37.13, 70.03, 111.91, 115.71, 115.86, 116.70, 116.76, 153.17, 154.40, 155.87, 157.68, 161.68, 165.58. EI-MS *m/z*

(%): 306.51 (M^+, 0.75). Anal. Calcd. For $C_{14}H_{16}FN_5O_2$: C, 55.08; H, 5.28; N, 22.94. Found: C, 55.17; H, 5.34; N, 22.77.

5.4.5. 1-((4-amino-2-methylpyrimidin-5-yl)methyl)-3-((4-chlorophenoxy)methyl)urea (8e)

Yellow solid, yield: 67%, mp 192-194; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.27 (s, 3H, CH_3), 4.01 (d, 2H, J = 6.0 Hz, CH_2), 5.09 (d, 2H, J = 7.2 Hz, CH_2), 6.73 (s, 1H, NH), 6.82 (s, 2H, NH_2), 6.97 (d, 2H, J = 9.0 Hz, Ar-H), 7.30 (d, 2H, J = 8.4 Hz, Ar-H), 7.35 (t, 1H, J = 6.6 Hz, NH), 7.84 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 25.17, 37.18, 69.83, 111.91, 117.07, 124.49, 129.24, 154.43, 155.73, 157.64, 161.71, 165.63. EI-MS *m/z* (%): 321.20 (M^+, 0.65). Anal. Calcd. For $C_{14}H_{16}ClN_5O_2$: C, 52.26; H, 5.01; N, 21.77. Found: C, 52.60; H, 5.45; N, 21.44.

5.5. 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 isopropyl β-D-1-thiogalactopyranoside (IPTG) for 7 h at 25 °C before harvesting. Purification of the fusion protein was carried out using a maltose-binding protein (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[27] 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 N. Nemeria,[14] 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,

287 0.8 mM 2,6-DCPIP, 7.1 μM enzyme and different concentration of inhibitors. The reaction mixtures were
288 incubated for 3 min at 37 °C, then added different concentration of ThDP (ranging from 0 to 200 μM) to initial
289 reaction. To determine the inhibitor concentration of synthesized compounds at 50% inhibition (IC_{50}), initial rate
290 data taken at saturating substrate, fixed effectors, and systematically varied inhibitor concentrations were fit to Hill
291 equation, $V = V_0 - (V_0 - V_\infty)/((IC_{50}/I)^n + 1)$,[28] Where V, V_0, and V_∞ are the velocity, maximum velocity (at I = 0),
292 and the limiting velocity (at I saturating); n is the Hill coefficient associated with the inhibitor; and IC_{50} is the
293 inhibition concentration of synthesized compounds at 50% inhibition. All kinetic data were fit to the
294 Growth/sigmoidal model from origin 7.0 software. One unit of activity is defined as the amount of 2,6-DCPIP
295 reduced (μmol/min/mg of PDHc-E1).

296 **5.6. Molecular docking**

297 For docking purposes, the crystallographic coordinates of the PDHc-E1 with bound ThDP from *E. coli* (PDB code:
298 1L8A) were obtained from Brookhaven Data Bank. Hydrogen atoms were added to the structure allowing for
299 appropriate ionization at physiological pH. The protonated state of several important residues, such as His106,
300 His142, Tyr599, Glu751 and His640, were adjusted by using SYBYL7.3 (Tripos, St. Louis, USA) in favor of
301 forming reasonable hydrogen bond with the ligand. Molecular docking analysis was carried out by the SURFLEX
302 module of SYBYL package to explore the interaction model for the active site of PDHc-E1 with its ligand. All
303 atoms located within the range of 6.5 Å from any atom of the cofactor ThDP were selected into the active site, and
304 the corresponding amino acid residue was, therefore, involved into the active site if only one of its atoms was
305 selected. Other default parameters were adopted in the SURFLEX-docking calculations. All calculations were
306 performed on a CCNUGrid-based computational environment (CCNUGrid website
307 http://www.202.114.32.71:8090/ccnu/chem/platform.xml).

308

Acknowledgements

The work was supported in part by the National Basic Research Program of China (2010CB126100), the National Natural Science Foundation of China (No. 20372023, 21172090).

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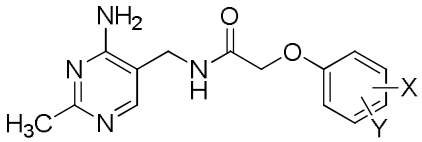
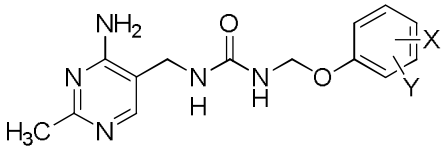
Table 1. Structures and *E. coli* PDHc-E1 inhibitory activities of amide derivatives **6a-6n**



6a-6n

Compounds	X	Y	IC_{50} (μM)
6a	H	H	45.42 ± 4.12
6b	2-Cl	4-Cl	9.45 ± 1.34
6c	H	4-Cl	12.98 ± 1.86
6d	H	4-NO_2	3.58 ± 0.52
6e	H	4-CH_3	21.59 ± 4.89
6f	H	4-Br	11.14 ± 1.07
6g	H	4-OCH_3	16.78 ± 0.95
6h	2-Br	H	60.81 ± 9.08
6i	2-Cl	H	16.55 ± 1.64
6j	H	4-F	17.43 ± 4.96
6k	2-Cl	4-F	7.60 ± 1.84
6l	2-Cl	4-NO_2	4.90 ± 0.81
6m	2-CH_3	4-Cl	21.92 ± 3.44
6n	H	4-CN	13.42 ± 2.02

Table 2. Structures and *E. coli* PDHc-E1 inhibitory activity of urea derivatives **8a-8e**



8a-8e

Compounds	X	Y	IC_{50} (μM)
8a	H	H	12.88 ± 1.33
8b	H	4-CH_3	9.23 ± 0.80
8c	H	4-OCH_3	6.30 ± 0.75
8d	H	4-F	10.33 ± 1.36
8e	H	4-Cl	3.67 ± 0.38

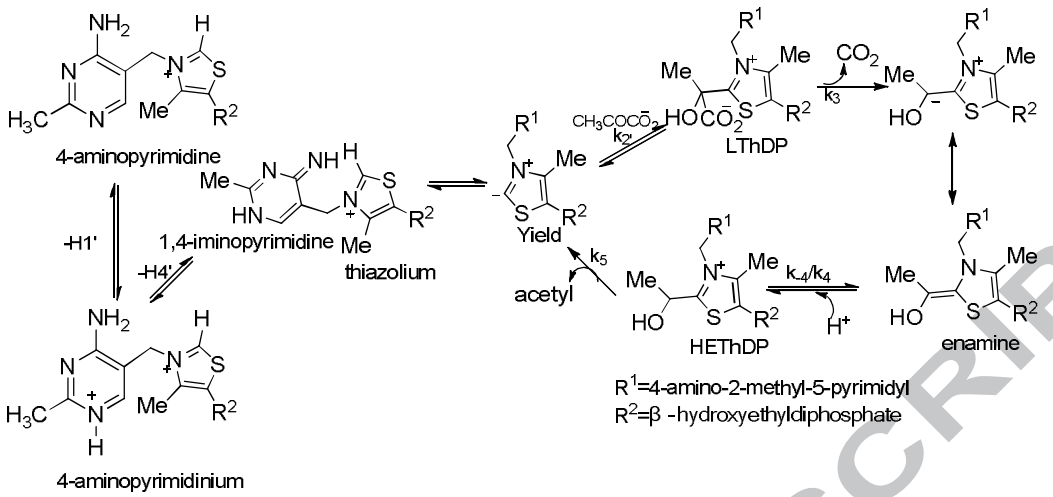


Figure 1. Mechanism of pyruvate dehydrogenase complex (E1) component

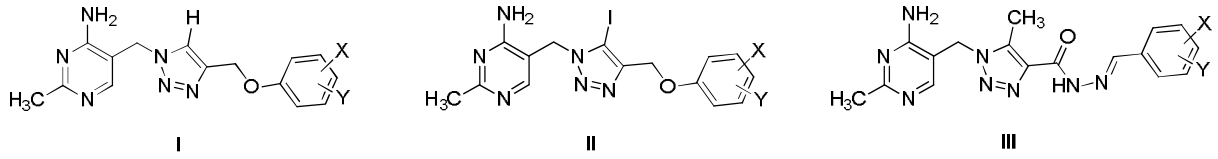


Figure 2. Structures of known PDHc-E1 inhibitors

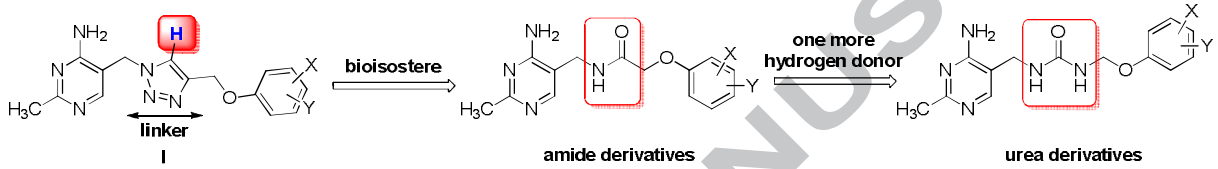


Figure 3. Design of the new amide and urea derivatives as *E. coli* PDHc-E1 inhibitors

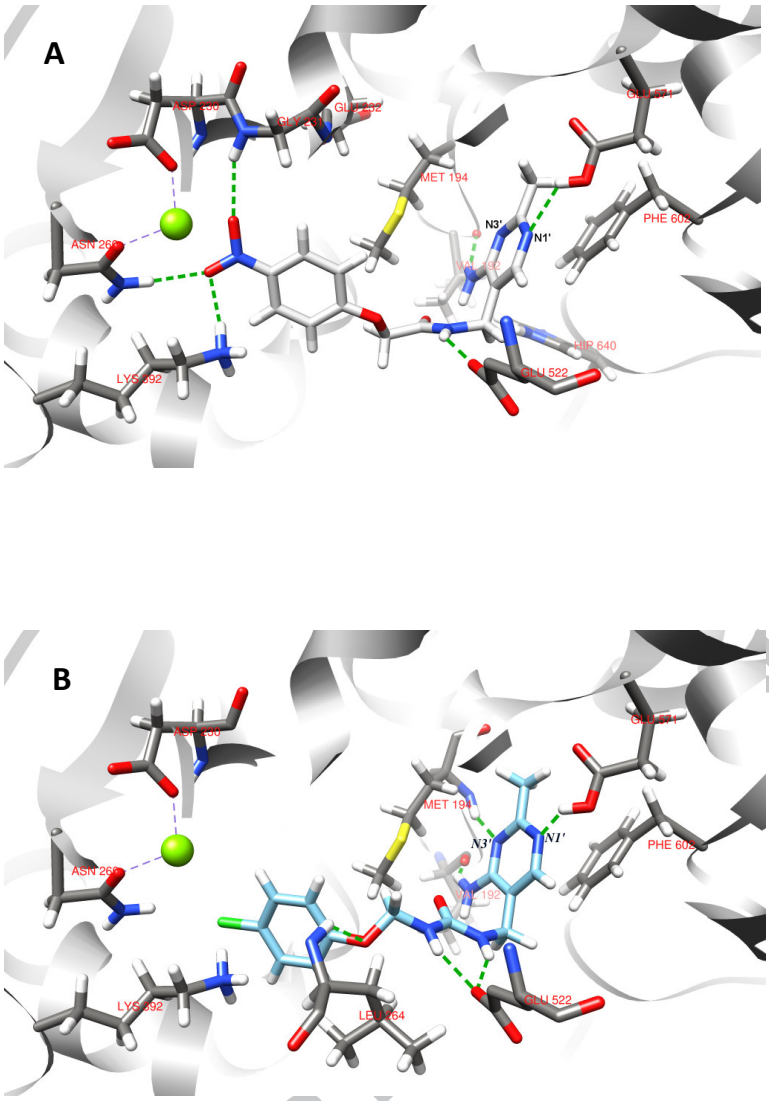
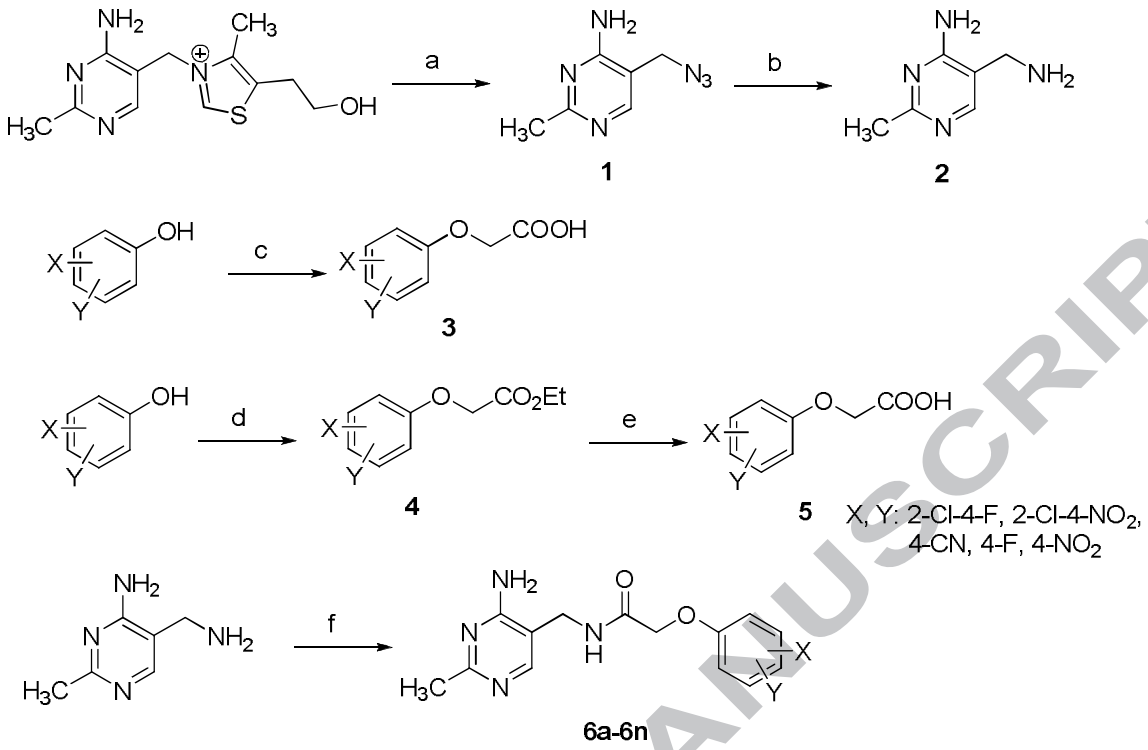
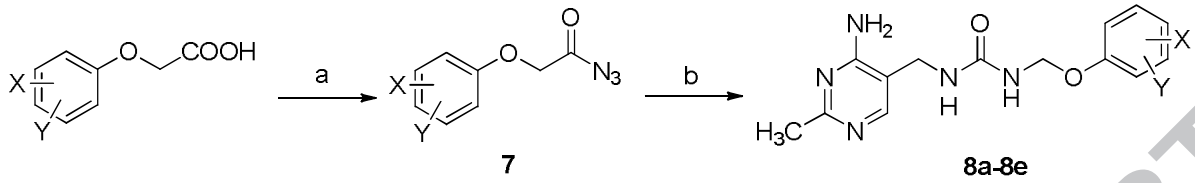


Figure 4. Binding modes of compound **6d** (**A**) and **8e** (**B**) target into active site of *E. coli* PDHc-E1, in which PDHc-E1 is shown in ribbon, ligands and some key residues are shown in stick, both coordination bonds and hydrogen bonds are shown in dashed lines (green).



Scheme 1. Reagents and conditions: (a) NaN_3, Na_2SO_3, H_2O, 60-65 ^oC, 6 h; (b) Pd/C, H_2, methanol, rt; (c) $ClCH_2COOH$, 40% NaOH, 100-110 ^oC, 2-3 h; (d) $BrCH_2CO_2Et$, K_2CO_3, DMSO, 70 ^oC, 5-6 h; (e) 2 N NaOH, acetone, rt, 2-3 h; (f) **3** or **5**, $ClCO_2Et$, Et_3N, THF/DMF, rt, 12 h.



Scheme 2. Reagents and conditions: (a) $ClCO_2Et$, Et_3N, acetone, -5 ^oC, 15 min; then NaN_3, H_2O, -5 ^oC; 1 h; (b) toluene, 70 ^oC, then **2**, DMF, 0 ^oC, 12 h.

Graphical Abstract