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Synthesis, Bioactivity and SAR study of *N′*-(5-substituted -1,3,4-thiadiazol-2-yl)-*N*-cyclopropylformyl-thioureas as ketol-acid reductoisomerase Inhibitors

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Abstract

Ketol-acid reductoisomerase (KARI; EC 1.1.1.86) catalyzes the second common step in branched-chain amino acid biosynthesis. The catalyzed process consists of two steps, the first of which is an alkyl migration from one carbon atom to its neighboring atom. The likely transition state is a cyclopropane derivative, thus a new series of cyclopropanecarbonyl thiourea derivatives were designed and synthesized involving a one-pot phase transfer catalyzed reaction. Rice KARI inhibitory activity of these compounds were evaluated and the 5-butyl substituted (**3e**) and 3-pyridinyl substituted (**3n**) compounds reached 100% at 100μg·mL^{-1}. Structure-activity relationship shows that longer chain derivatives had higher KARI inhibitory activity. Meanwhile substitution of the 4-position of the benzene ring had higher KARI inhibitory activity than that of the 2 and 3-position. Auto-Dock was used to predict the binding mode of **3n**. This was done by analyzing the interaction of compound **3n** with the active sites of the available spinach KARI. This was in accord with the results analyzed by the frontier molecular orbital theory.

Keywords: *Cyclopropane derivatives, Auto-Dock, KARI activity, thiourea, inhibition, Ketol-acid reductoisomerase*

Introduction

Microorganisms and plants contain numerous enzymes that some of which are potential targets for designing bioactive compounds such as antibiotics and herbicides. Enzymes involved in the biosynthesis of the branched chain amino acids are one such example. The success of these herbicides (sulfonyl-ureas [1], imidazolinones [2], and so on) which target the first enzyme (acetohydroxyacid synthase) has stimulated research into inhibitors of other enzymes in the pathway, including the second enzyme in the common pathway [3], ketol-acid reductoisomerase (KARI; EC 1.1.1.86). But there are no commercial herbicides targeting KARI yet, only HOE 704 [4], IpOHA [5] and CPD analogs [6–7] are reported as potent competitive inhibitors of the enzyme *in vitro* (Scheme 1). Additionally, Grandoni et al. [8] found HOE 704 [4], IpOHA [5] can inhibit *tuberculosis* much better than ATCC35801, so these branched chain amino acids inhibitors became novel anti- *tuberculosis* medicine hopefully.

The reaction catalyzed by KARI is shown in Scheme 1 which consists of two steps [9–10], an alkyl migration followed by a NADPH dependent reduction. Both steps require a divalent metal ion, such as Mg^{2+}, Mn^{2+} or Co^{2+}, but the alkyl migration is highly specific for Mg^{2+}.

A transition state being a cyclopropane is postulated and mimicked by Gerwick et al. [11] They showed

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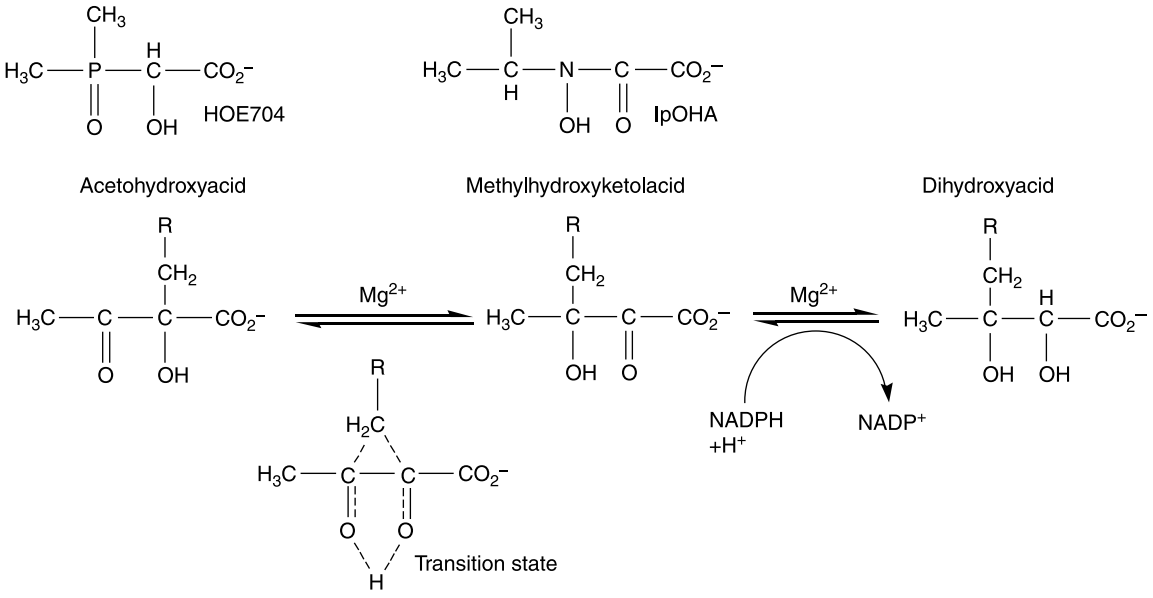


Scheme 1. Reaction catalyzed by KARI, the known inhibitors of HOE704 and IpOHA are the analogies of Acetohydroxyacid and Methylhydroxyketolacid respectively.

that cyclopropane-1,1-dicarboxylate (CPD) can inhibit *Escherichia coli* KARI. They also showed that application of CPD to various plant tissues caused the accumulation of the substrate 2-acetolactate; which data strongly suggest that the CPD can inhibit the activity of KARI *in vivo* [6].

The first step in the KARI catalyzing process involves an alkyl migration from one carbon atom to its neighboring atom. The likely transition state is a cyclopropane derivative. Also Halgand et al. [12] found that 1,2,3-thiadiazole can inhibit KARI effectively using high throughput screening. By the way, all these inhibitors contain C=O, P=O, S=O and other groups. For this reason, some new cyclopropane derivatives contain C=O, C=S and 1,3,4-thiadiazole were synthesized in our laboratory (Scheme 2).

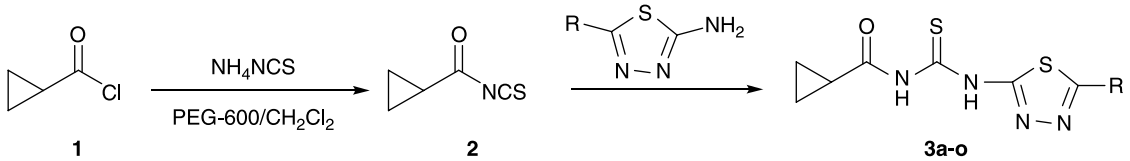
Experimental

Instruments

Melting points were determined using an X-4 melting apparatus and were uncorrected. Infrared spectra were recorded on a Bruker Equinox55 spectrophotometer as potassium bromide tablets. ^{1}H NMR spectra were measured on a Bruker AC-P500 instrument (300 MHz) using tetramethylsilane as an internal standard and deuterochloroform as solvent. Mass spectra were recorded on a Thermo Finnigan LCQ Advantage LC/mass detector instrument. FTMS were determined by Ionspec FT-MS 7.0T.

Synthesis of compounds

The title compounds were synthesized according to the route shown in Scheme 2, and the yields were not optimized. To a solution (25 mL) of cyclopropanecarboxylic acid (7.50 mmol) was added thionyl chloride (30 mmol) and the mixture was refluxed for 2 h to give acid chloride. Powdered ammonium thiocyanate (1.14 g, 15 mmol), cyclopropanecarbonyl chloride (1.04 g, 10 mmol), PEG-600 (0.18 g, 3% with respect to ammonium thiocyanate) and methylene chloride (25 ml) were placed in a dried round-bottomed flask containing a magnetic stirrer bar and stirred at room temperature for 1 h. Then 2-amino-5-substituted-1,3,4-thiadiazoles (4.5 mmol) in methylene dichloride (10 mL) was added dropwise over



R = CH_3, C_2H_5, n-Pr, iso-Pr, n-Bu, Ph, *o*-CH_3Ph, *m*-CH_3Ph, *o*-ClPh, *p*-ClPh, *o*-FPh, *p*-NO_2Ph, *p*-OCH_3Ph, 3-Py, furan,

Scheme 2. Synthetic route for compounds **3a**–**3o**.

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0.5 h, and the mixture was stirred for 1 ~ 2 h while monitored by TLC. The corresponding products precipitated immediately. The product was filtered, washed with water to remove inorganic salts, dried, and recrystallized from DMF-EtOH-H_2O, afforded a light yellow solid.

*N′-(5-methyl-1,3,4-thiadiazol-2-yl)-N-cyclopropyformyl-thiourea(**3a**)*. Light yellow crystals, yield 84.3%, m.p.248-250°C; ^{1}HNMR($CDCl_3$)δ: 1.04-1.19(m, 4H, cyclopropane-CH_2), 2.17(s,1H, cyclopropane-CH), 2.677(s, 3H,CH_3); IR/cm^{-1}: 3161 (N-H), 1680 (C═O), 1296 (C═S); ESI-MS: 182.08 $[M - H_2NCS]^-$; FT-MS for $C_8H_{10}N_4OS_2$: found 182.0395, calcd. 182.0394.

*N′-(5-ethyl-1,3,4-thiadiazol-2-yl)-N-cyclopropyformyl-thiourea(**3b**)*. Light yellow crystals, yield 88.9%, m.p.199-200°C; ^{1}HNMR($CDCl_3$)δ: 1.05-1.19(m, 4H, cyclopropane-CH_2), 1.39(t, 2H, CH_2), 2.26(s, 1H, cyclopropane-CH)3.03(d, 3H,CH_3); IR/cm^{-1}: 3161 (N-H), 1687 (C═O), 1304 (C═S); ESI-MS: 198.13 $[M - H_2NCS]^-$; FT-MS for $C_9H_{12}N_4OS_2$: found 255.0384, calcd. 255.0380.

*N′-(5-n-propyl-1,3,4-thiadiazol-2-yl)-N-cyclopropyformyl-thiourea(**3c**)*. Light yellow crystals, yield 91.2%, m.p.176-178°C; ^{1}HNMR($CDCl_3$)δ: 1.03-1.06(m, 4H, cyclopropane-CH_2),1.18(t, 3H,CH_3), 1.72-1.83 (m, 2H,CH_2), 2.17(s,1H, cyclopropane-CH), 2.98(t, 3H, CH_2); IR/cm^{-1}: 3169 (N-H), 1687 (C═O), 1304 (C═S); ESI-MS: 212.19 $[M - H_2NCS]^-$; FT-MS for $C_{10}H_{14}N_4OS_2$: found 210.0704, calcd. 210.0707.

*N′-(5-iso-propyl-1,3,4-thiadiazol-2-yl)-N-cyclopropyformyl-thiourea(**3d**)*. Light yellow crystals, yield 88.6%, m.p.173-174°C; ^{1}HNMR($CDCl_3$)δ: 1.02-1.29 (m, 4H, cyclopropane-CH_2),1.41(d, 6H,CH_3), 2.17 (s,1H, cyclopropane-CH), 4.37(m, 1H, CH); IR/cm^{-1}: 3169 (N-H), 1687 (C═O), 1311 (C═S); ESI-MS: 210.16[M − H_2NCS]; FT-MS for $C_{10}H_{14}N_4OS_2$: found 210.0704, calcd. 210.0707.

*N′-(5-butyl-1,3,4-thiadiazol-2-yl)-N-cyclopropyformyl-thiourea(**3e**)*. Light yellow crystals, yield 82.6%, m.p.115-117°C; ^{1}HNMR($CDCl_3$)δ: 0.95 (t, 3H, CH_3), 1.03-1.18(m, 4H, cyclopropane-CH_2),1.37-1.46(m, 2H,CH_2), 1.68-1.78(m, 2H,CH_2),2.17(s,1H, cyclopropane-CH), 2.87-3.03(m, 2H, CH_2); IR/cm^{-1}: 3161 (N-H), 1680 (C═O), 1304 (C═S); ESI-MS: 284.19 $[M - H]^-$; FT-MS for $C_{10}H_{14}N_4OS_2$: found 283.0693, calcd. 283.0693.

*N′-(5-phenyl-1,3,4-thiadiazol-2-yl)-N-cyclopropyformyl-thiourea(**3f**)*. Light yellow crystals, yield 93.1%, m.p. > 270°C; ^{1}HNMR($CDCl_3$)δ: 1.11-1.27(m, 4H, cyclopropane-CH_2), 2.17(s, 1H, cyclopropane-CH), 7.96-8.45(m, 5H,C_6H_5); IR/cm^{-1}: 3161 (N-H), 1680 (C═O), 1304 (C═S); ESI-MS: 305.18 $[M + H]^+$; FT-MS for $C_{13}H_{12}N_4OS_2$: found 303.0383, calcd. 303.0380.

*N′-(5-(2-methyl-phenyl)-1,3,4-thiadiazol-2-yl)-N-cyclopropyformyl-thiourea(**3g**)*. Light yellow crystals, yield 90.5%, m.p.201-203°C; ^{1}HNMR($CDCl_3$)δ: 1.07-1.25(m, 4H, cyclopropane-CH_2), 2.21(s,1H, cyclopropane-CH), 2.57(s, 3H,CH_3), 7.30 (t, J = 7.00 Hz, 2H,C_6H_4), 7.37 (d, J = 7.84 Hz, 1H, C_6H_4), 7.63 (d, J = 7.84 Hz, 1H, C_6H_4); IR/cm^{-1}: 3161 (N-H), 1687 (C═O), 1289 (C═S); ESI-MS: 317.10 $[M - H]^-$; FT-MS for $C_{14}H_{14}N_4OS_2$: found 318.0609, calcd. 318.0604.

*N′-(5-(3-methyl-phenyl)-1,3,4-thiadiazol-2-yl)-N-cyclopropyformyl-thiourea(**3h**)*. Light yellow crystals, yield 84.8%, m.p.219-220°C; ^{1}HNMR($CDCl_3$)δ: 1.10-1.25(m, 4H, cyclopropane-CH_2), 2.31(s,1H, cyclopropane-CH), 2.42(s, 3H,CH_3), 7.28 (d, J = 6.32 Hz, 1H,C_6H_4), 7.36 (t, J = 7.36 Hz, 1H, C_6H_4), 7.70 (d, J = 8.67 Hz, 2H, C_6H_4); IR/cm^{-1}: 3169 (N-H), 1680 (C═O), 1304 (C═S); ESI-MS: 317.04 $[M - H]^-$; FT-MS for $C_{14}H_{14}N_4OS_2$: found 318.0608, calcd. 318.0604.

*N′-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)-N-cyclopropyformyl-thiourea(**3i**)*. Light yellow crystals, yield 88.2%, m.p.171-173°C; ^{1}HNMR($CDCl_3$)δ: 1.08-1.26(m, 4H, cyclopropane-CH_2), 1.99(s,1H, cyclopropane-CH), 7.45 (d, J = 8.36 Hz, 2H,C_6H_4), 7.86 (d, J = 7.36 Hz, 2H, C_6H_4); IR/cm^{-1}: 3169 (N-H), 1680 (C═O), 1296 (C═S); ESI-MS: 336.98 $[M - H]^-$; FT-MS for $C_{13}H_{11}ClN_4OS_2$: found 339.0130, calcd. 339.0136.

*N′-(5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl)-N-cyclopropyformyl-thiourea(**3j**)*. Light yellow crystals, yield 91.5%, m.p.198-199°C; ^{1}HNMR($CDCl_3$)δ: 1.09-1.25(m, 4H, cyclopropane-CH_2), 2.27(s,1H, cyclopropane-CH), 7.40 (d, J = 5.31 Hz, 2H,C_6H_4), 7.52 (t, J = 7.29 Hz, 1H, C_6H_4), 8.11 (d, J = 7.71 Hz, 1H, C_6H_4); IR/cm^{-1}: 3154 (N-H), 1680 (C═O), 1318 (C═S); ESI-MS: 336.96 $[M - H]^-$; FT-MS for $C_{13}H_{11}ClN_4OS_2$: found 339.0140, calcd. 339.0136.

*N′-(5-(2-florophenyl)-1,3,4-thiadiazol-2-yl)-N-cyclopropyformyl-thiourea(**3k**)*. Light yellow crystals,

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yield 89.6%, m.p. > 270°C; ^{1}HNMR($CDCl_3$)δ: 1.10-1.26(m, 4H, cyclopropane-CH_2), 2.30(s,1H, cyclopropane-CH), 7.29 (d, J = 7.08 Hz, 2H,C_6H_4), 7.46 (d, J = 6.6 Hz, 1H, C_6H_4), 8.23 (t, J = 7.43 Hz, 1H, C_6H_4); IR/cm^{-1}: 3255 (N-H), 1687 (C═O), 1296 (C═S); ESI-MS: 321.00 [M − H]$^-$; FT-MS for $C_{13}H_{11}FN_4OS_2$: found 323.0426, calcd. 323.0438.

*N′-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)-N-cyclopropyformyl-thiourea(**3l**)*. Light yellow crystals, yield 92.6%, m.p. 174-175°C; ^{1}HNMR($CDCl_3$)δ: 1.07-1.14(m, 4H, cyclopropane-CH_2), 2.17(s,1H, cyclopropane-CH), 7.48 (d, J = 3.53 Hz, 2H, C_6H_4), 7.91 (d, J = 2.35 Hz, 1H, C_6H_4); IR/cm^{-1}: 3198 (N-H), 1689 (C═O), 1302 (C═S); ESI-MS: 348.00 [M − H]$^-$; FT-MS for $C_{13}H_{11}N_5O_3S_2$: found 348.0225, calcd. 348.0231.

*N′-(5-(4-methoxphenyl)-1,3,4-thiadiazol-2-yl)-N-cyclopropyformyl-thiourea(**3m**)*. Light yellow crystals, yield 91.1%, m.p. 235-238°C; ^{1}HNMR($CDCl_3$)δ: 1.07-1.24(m, 4H, cyclopropane-CH_2), 2.24(s,1H, cyclopropane-CH), 3.87(s, 3H, CH_3), 7.00 (d, J = 8.84 Hz, 2H,C_6H_4), 7.84 (d, J = 8.81 Hz, 2H, C_6H_4); IR/cm^{-1}: 3161 (N-H), 1694 (C═O), 1304 (C═S); ESI-MS: 332.95 [M − H]$^-$; FT-MS for $C_{14}H_{14}N_4O_2S_2$: found 334.0588, calcd. 334.0552.

*N′-(5-(3-pyridinyl)-1,3,4-thiadiazol-2-yl)-N-cyclopropyformyl-thiourea(**3n**)*. Light yellow crystals, yield 89.9%, m.p. 216-218°C; ^{1}HNMR($CDCl_3$)δ: 1.09-1.25(m, 4H, cyclopropane-CH_2), 2.21(s,1H, cyclopropane-CH), 6.55 (d, J = 1.30 Hz, 2H, C_5H_4N), 7.04 (d, J = 3.17 Hz, 1H, C_5H_4N), 7.56 (s, 1H, C_5H_4N); IR/cm^{-1}: 3155 (N-H), 1682 (C═O), 1302 (C═S); ESI-MS: 304.00 [M − H]$^-$; FT-MS for $C_{12}H_{11}N_5OS_2$: found 306.0462, calcd. 306.0477.

*N′-(5-furan-1,3,4-thiadiazol-2-yl)-N-cyclopropyformyl-thiourea(**3o**)*. Light yellow crystals, yield 94.5%, m.p. >270°C; 1HNMR(CDCl3)δ: 1.09-1.24(m, 4H, cyclopropane-CH2), 2.17(s,1H, cyclopropane-CH), 6.56 (dd, J_{ab} = 1.75 Hz, J_{ac} = 1.71 Hz, 1H, C_4H_3O), 7.03 (d, J = 3.31 Hz, 1H, C_4H_3O), 7.58 (d, J = 0.97 Hz, 1H, C_4H_3O); IR/cm^{-1}: 3161 (N-H), 1680 (C═O), 1302 (C═S); ESI-MS: 293.75 [M − H]$^-$; FT-MS for $C_{11}H_{10}N_4O^2S_2$: found 294.0270, calcd. 294.0240.

Theoretical Calculations and DOCK

The structure of N′-(5-(3-pyridinyl)-1,3,4-thiadiazol-2-yl)-N-cyclopropyformyl –thiourea(**3n**) was selected as the initial structure, while HF/6-31G (d,p) [13], DFT-B3LYP/6-31G (d,p) [14–15] and MP2/6-31G (d,p) [16–18] methods in Gaussian 03 package [19] were used to optimize the structure of **3n**. Vibration analysis showed that the optimized structures were in accordance with the minimum points on the potential energy surfaces. All the convergent precisions were the system default values, and all the calculations were carried out on the Nankai Stars supercomputer at Nankai University.

All docking procedures were done in NanKai Stars supercomputer at Nankai University. The automated molecular docking calculations were carried out using AutoDock 3.05. The AUTOTORS module of AutoDock defined the active torsions for each docked compound. The active sites of the protein were defined using AutoGrid centered on the IpOHA in the crystal structure. The grid map with 60 × 60 × 60 points centered at the center of mass of the KARI and a grid spacing of 0.375 Å was calculated using the AutoGrid program to evaluate the binding energies between the inhibitors and the protein. The Lamarckian genetic algorithm (LGA) was used as a searching method. Each LGA job consisted of 50 runs, and the number of generation in each run was 27000 with an initial population of 100 individuals. The step size was set to 0.2 Å for translation and 5° for orientation and torsion. The maximum number of energy evaluations was set to 1500000. Operator weights for cross-over, mutation, and elitism were 0.80, 0.02, and 1, respectively. The docked complexes of the inhibitor-enzyme were selected according to the criterion of interaction energy combined with geometrical and electronic matching quality.

KARI assay

Cloning, expression and purification of rice KARI. The KARI resultant expression plasmid was obtained from Professor Ronald G. Duggleby's lab, and was used to transform *Escherichia coli* BL21(DE3) cells. The methods of expression and purification of rice KARI are according to the reference [6].

Enzyme and protein assays. Gerwick et al. [11] reported that the inhibition of *Escherichia coli* KARI is time-dependent. KARI activity was measured by following the decrease in A_{340} at 30°C in solutions containing 0.2 mM NADPH, 1 mM $MgCl_2$, substrate 2-acetolactate and inhibitors as required, in 0.1 M phosphate buffer, pH 8.0. Inhibitors was preincubated with enzyme in phosphate buffer at 30°C for 10 min before the reaction was started by adding the substrate combining with NADPH and $MgCl_2$. Protein concentrations were estimated using the bicinchoninic acid method [20] and protein purity was assessed by SDS-PAGE [21]. The yield of

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recombinant rice KARI from a 30 culture was 50 mg with a specific activity (measured with saturating 2-acetolactate) of 1.17 U/mg. The 2-acetolactate was prepared according to reference [22].

Results and discussion

Synthesis

One-pot synthesis method was used in this process. Cyclopropanecarbonyl chloride was treated with ammonium thiocyanate, 3% PEG-600 as the solid-liquid phase-transfer catalyst to afford the intermediate **2**, which was not isolated but reacted with the 2-amino-5-substituted-1,3,4- thiadiazoles to give the target compounds (Scheme 2). It can easily react with NH_4SCN to form complex [PEG-600-NH_4^+]SCN^-, which makes it possible for SCN^- to readily react with cyclopropanecarbonyl chloride. With the enhancement of the ion exchange between inorganic salt and organic solution, PEG-600 efficiently facilitated this heterogeneous solid-liquid two-phase reaction. As a result, PEG-600 can fasten the NH_4^+ effectively [23]. Besides, the catalyst PEG-600 is inexpensive, relatively nontoxic, highly stable and easily available, making this method more applicable.

In addition, the method of synthesis of 2-amino-5-substituted-1,3,4-thiadiazoles was studied. Several procedures are available for the one-step synthesis of 2-amino-5-substituted-1,3,4-thiadiazole derivatives [24].

KARI activity

The KARI inhibitory activities of the title compounds were tested at 100μg·mL^{-1}; a known inhibitor, cyclopropane-1,1-dicarboxylic acid(CPD), was selected as a control. The results are shown in Table I where it is seen that some of these compounds inhibit ketol-acid reductoisomerase *in vitro* effectively, such as **3e** and **3n**. The KARI activities of these two compounds are similar to those of other cyclopropane compounds which were synthesized in our lab [7a]. For example, compound **3n** can inhibit KARI to reach 100% at 100μg·mL^{-1}, also compound 1-cyano-N-*o*-tolylcyclopropanecarboxamide can inhibit KARI effectively at the same level [7a]. Meanwhile the two compounds displayed as good activity as the known inhibitor CPD at 100μg·mL^{-1}.

Structure-activity relationship

The structure-activity relationship can be summarized from the data given in Table I which indicate that the change of substituent affects the KARI activity. The compounds that were substituted at the 4-position of the phenyl ring had higher potency against KARI than that of the 2- and 3- substituted position. With the longer chain compound for alkane substituted, their inhibitory activities increased up to **3e**. The heterocyclic substituent also can enhance the activity, such as **3m**. Hence, these identified cyclopropane derivatives could be useful for further optimization work in finding potential KARI inhibitors.

Table I. Inhibition (%) of compounds **3a**–**3o** against rice KARI at 100 ppm *in vitro*.

No	R	KARI
3a	CH_3	0
3b	C_2H_5	0
3c	*n*-Pr	0
3d	*Iso*-Pr	66.18
3e	*n*-Bu	100
3f	C_6H_5	44.91
3g	*o*-$CH_3C_6H_4$	44.96
3h	*m*-$CH_3C_6H_4$	37.30
3i	*p*-ClC_6H_4	82.27
3j	*o*-ClC_6H_4	22.77
3k	*o*-FC_6H_4	38.78
3l	*p*-$NO_2C_6H_4$	32.09
3m	*p*-$OCH_3C_6H_4$	70.34
3n	3-pyridyl	100
3o	furan	18.08
	CPD	100

Theoretical and DOCK

According to the frontier molecular orbital theory, HOMO and LUMO are the two most important factors which affect the bioactivities of compounds. HOMO has the priority to provide electrons, while LUMO accept electrons firstly [25]. Thus a study of

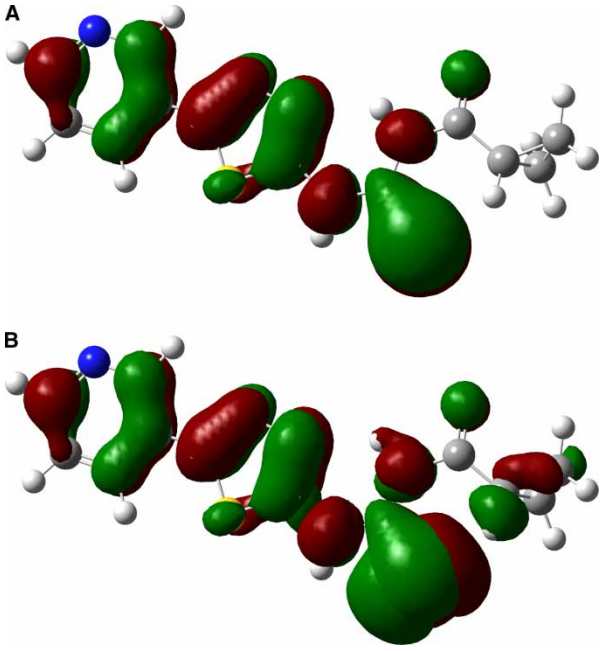

Figure 1. Frontier molecular orbitals of compound **3n**: A. HOMO of compound **3n**; B. HOMO-1 of compound **3n**.

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Table II. Energies of HOMO, HOMO-1 of **3n** and CPD (Hartree).

	3n	CPD
HOMO	−0.33672	−0.32068
HOMO-1	−0.33989	−0.34159

the frontier orbital energy can provide some useful information for the active mechanism. Taking HF results, the HOMO of **3n** is mainly located on the pyridine ring, thiadiazole ring and the thiourea group (Figure 1(A)). On the other hand, the HOMO-1 of **3n** contains the pyridine ring, thiadiazole ring, the thiourea group and the cyclopropane ring (Figure 1(B)). The fact that **3n** has strong affinity suggests the importance of the frontier molecular orbital in the hydrophobic interactions. Meanwhile, the frontier molecular orbital are located on the main groups whose atoms can easily bind with the receptor KARI. This implies that the orbital interaction between **3n** and the rice KARI amino acid residues are dominated by hydrophobic interaction between the frontier molecular orbital.

The energies of HOMO and HOMO-1 of **3n** and CPD are listed in Table II which surprisingly shows that compounds **3n** have similar energies with CPD. This probably is the reason for the good activity of the compound **3n** and CPD.

To make prediction by our frontier molecular orbital model more relevant to the active sites of the enzyme and to describe a probable binding site in the KARI, the compound **3n** was docked into the active sites of spinach KARI.

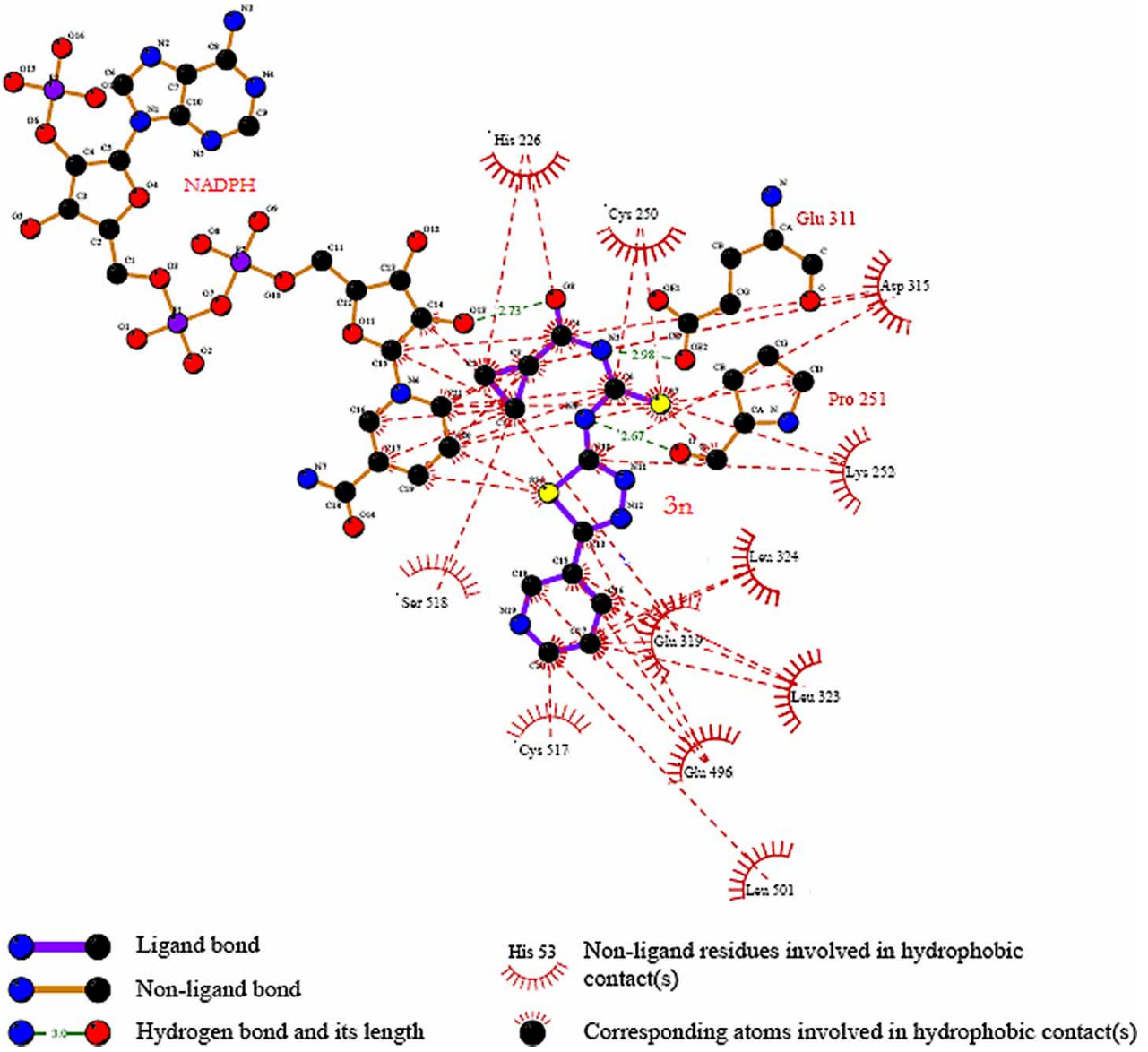

Figure 2. PDB code: 1YVE Binding modes of compound **3n** in the active sites of spinach KARI: hydrogen bond and hydrophobic interaction between **3n** and the rice KARI amino acid residues.

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Visual inspection of the conformation of **3n** docked into the KARI binding site revealed that the phenyl rings are hosted in the pocket of KARI and three hydrogen bonds between the amino groups of **3n** and the carbonyl oxygen of Glu 311, Pro 251 side chain and the NADPH are also observed. Furthermore, the cyclopropane ring and aromatic ring are embedded in a large hydrophobic pocket formed by His 226, Cys 250, Pro 251, Lys 252, Glu 311, Glu 319, Asp 315, Leu 323, Leu 324, Glu 496, Leu 501, Cys 517, Ser 518 and NADPH (Figure 2).

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