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Synthesis, bioactivity, theoretical and molecular docking study of 1-cyano-*N*-substituted-cyclopropanecarboxamide as ketol-acid reductoisomerase inhibitor

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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 stages, the first of which is an alkyl migration from one carbon atom to its neighbouring atom. The likely transition state is a cyclopropane derivative, thus a series of new cyclopropane derivatives, such as 1-cyano-*N*-substituted-cyclopropanecarboxamide, were designed and synthesized. Their structures were verified by ^{1}H NMR, FTIR spectrum, MS and elemental analysis. The K_i values of active compounds **2**, **4b** against rice KARI were 95.30 ± 13.71, 207.9 ± 21.99 μM, respectively. The X-ray crystal structure of compound **4a** was also determined. Auto-Dock was used to predict the binding mode of **4a**. This was done by analyzing the interaction of the compounds **4a** with the active sites of spinach KARI. This result was in accord with the result analyzed by the frontier molecular orbital theory.

Plants and most micro-organisms have biosynthetic ability which allows them to survive on relatively simple nutrients. For this reason, plants and microorganisms contain numerous enzymes that are potential targets for designing bioactive compounds such as herbicides and antibiotics. Enzymes involved in the biosynthesis of the branched chain amino acids are one such example. Isoleucine and valine are synthesized in a parallel set of four reactions while an extension of the valine pathway results in leucine.[1]

This pathway is the target for the sulfonylureas,[2] the imidazolinones[3] and a variety of other herbicides,[1] which all inhibit the first enzyme, acetohydroxyacid synthase. The success of these herbicides has stimulated research into inhibitors of other enzymes in the pathway, including the second enzyme in the common pathway,[4] ketol-acid reductoisomerase (KARI; EC 1.1.1.86), and two enzymes in the leucine extension.[5,6] The reaction catalyzed by KARI is shown in Scheme 1 which consists of two steps,[7,8] 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+}. HOE 704[9] and IpOHA[10] are potent competitive inhibitors of the enzyme (Scheme 1).

A transition state being a cyclopropane is postulated and mimicked by Gerwick et al.[11] They showed 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; in vivo data strongly suggest that the CPD can inhibit the activity of KARI Scheme 1.[12]

The first step in the KARI catalyzed process involves an alkyl migration from one carbon atom to its neighboring atom. The likely transition state is a cyclopropane derivative. For this reason, some new cyclopropane derivatives were synthesized in our laboratory (Scheme 2).

Biological studies revealed that some of these compounds inhibit ketol-acid reductoisomerase in vivo effectively.

Keywords: Cyclopropanecarboxamide; Synthesis; Structure; Molecular docking; Theoretical study; KARI.

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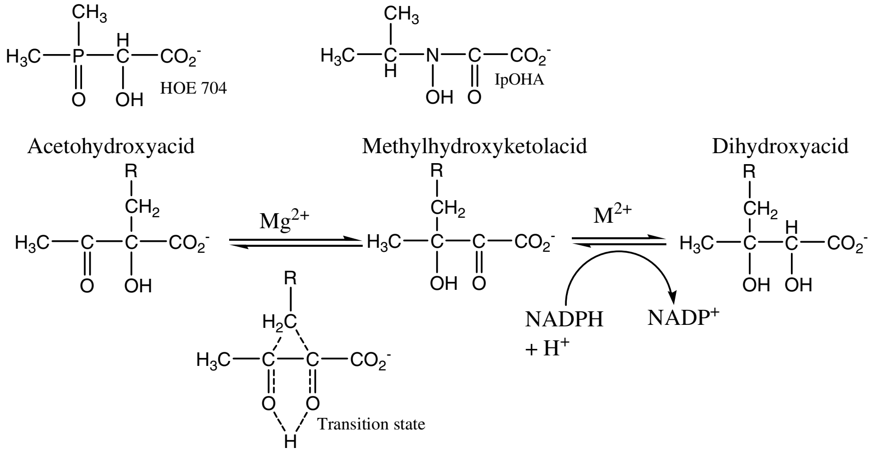
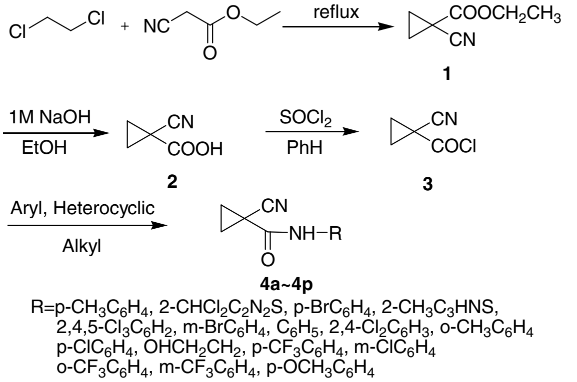


Scheme 1. Reaction catalyzed by KARI.



Scheme 2. Synthesis route for compounds **4a**–**p**.

The 1-cyan-1-cyclopropane carboxylic acid, prepared from 1,2-dichloroethane and ethyl cyanacetate was cyclized for 16 h at refluxing temperature. In order to optimize the reaction time, microwave assisted irradiation was applied which shortened the reaction time to 40 min. Compound **3** reacted with substituted anilines, heterocyclic amine or alkyl amines in the presence of inorganic base to yield substituted cyclopropanecarboxamides as shown in Scheme 2.[13]

The KARI activities in vitro of these compounds were determined.[14] The results for compound, 1, 2 and compounds **4a**–**p** are summarized in Table 1.

It was found from Table 1 that compounds **2**, **4b**, **4h**, **4i**, **4j** and **4k** have favourable inhibitory activity against KARI. The data given in Table 1 indicated that the change of substituent at phenyl ring affects the KARI activity. When the benzene ring is substituted by CF_3 group, the compounds generally have no KARI bioactivity, as **4l**, **4n**, **4o**. While for heterocyclic and alkane substituents, their inhibitory activities increase for **4b** and **4k**. For the compounds, **2**, **4a** and **4b**, further bioassay was conducted and their K_i values against KARI were 95.30 ± 13.71, >300 and 207.9 ± 21.99 μM, respectively. Hence, these identified cyclopropane derivatives could be useful for further optimization work in finding the potential KARI inhibitors.

Table 1. Inhibition rate (%) of compounds **4a**–**p** against rice KARI at 200 ppm in vitro

Compound	R	KARI activity
1		0
2		100
4a	p-$CH_3C_6H_4$–	61.21
4b	2-$CHCl_2C_2N_2S$–	100
4c	p-BrC_6H_4–	32.23
4d	2-CH_3C_3HNS–	69.81
4e	2,4,5-$Cl_3C_6H_2$–	0
4f	m-BrC_6H_4–	17.25
4g	C_6H_5–	77.23
4h	2,4-$Cl_2C_6H_3$–	97.04
4i	o-$CH_3C_6H_4$–	100
4j	p-ClC_6H_4–	93.92
4k	$OHCH_2CH_2$–	98.92
4l	p-$CF_3C_6H_4$–	0
4m	m-ClC_6H_4–	0
4n	o-$CF_3C_6H_4$–	0
4o	m-$CF_3C_6H_4$–	0
4p	p-$OCH_3C_6H_4$–	3.95

In order to study the structure–activity relationship, the single-crystal structure of **4a** was determined[15] by X-ray crystallography[16] as illustrated in Figure 1 in which three C–N bond lengths C(5)–N(2), C(6)–N(2) and N(1)–C(1) are 0.135, 0.142, and 0.114 nm, respectively, which are all longer than that of 0.134 nm in the single heterocycle ring.[17] In **4a**, the bond length of C(1)–N(1) is 0.1396 nm, which is longer than the double C–N bond.[18] Based on the computal results by Gaussian,[19,20] it was seen that DFT, HF and MP2 have good coherence with the crystal diffraction, for example it can be observed that C(2)–C(5) > C(1)–C(2) > N(2)–C(6) > N(2)–C(5) >O(1)–C(5) > N(1)–C(1) in crystal structure, which is accordance with the order of C(2)–C(5) >C(1)–C(2) > N(2)–C(6) > N(2)–C(5) > O(1)–C(5) > N(1)–C(1) in all calculation structures.

According to the frontier molecular orbital theory, HOMO and LUMO are two most important factors which affect the bioactivities of compounds. HOMO has the priority to provide electrons, while LUMO accepts electrons first.[21,22] Thus, study on the frontier