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α-Hydroxyketones as inhibitors of urease

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Abstract—A variety of α-hydroxyketones (**1**–**13**) and α-diketones (**14**–**20**) were evaluated for their effect on the jack bean urease. Of 13 α-hydroxyketones (**1**–**13**) tested, 2,2′-thenoin (**10**) ($IC_{50}=0.18$ mM), furoin (**9**) ($IC_{50}=0.36$ mM), 2-hydroxy-1-phenylethanone (**5**) ($IC_{50}=0.47$ mM) and acetol (**1**) ($IC_{50}=2.9$ mM) showed potent inhibitory activity against the enzyme, comparable with hydroxyurea ($IC_{50}=0.1$ mM). The inhibitory effects were completely blocked by 2-mercaptoethanol or dithiothreitol. A nickel ion influenced the inhibitory effects of **5** and **9** in a dose-dependent manner, but not of **1** and **10**. On the other hand, the corresponding α-diketones such as 2,2′-thenil (**20**), furil (**19**) and PhCOCHO (**14**) exhibited little or no ability to inhibit the urease. We have demonstrated for the first time that some α-hydroxyketone derivatives show urease inhibitory activity, possibly by binding to cysteinyl residues in the active site.

1. Introduction

Urease (urea amidohydrolase; E.C.3.5.1.5) is a nickel-containing enzyme that catalyzes the hydrolysis of urea to produce ammonia and carbamate.[1,2] Urease is widely distributed in a variety of algae, bacteria, fungi and plants. The structure, number and type of subunits, molecular weight and amino acid sequence of urease depend on its origin. Apart from these differences, the amino acid sequences of the active sites and the mechanism of enzyme activity are the same. A recent topic is an urease in *Helicobacter pylori* (*H. pylori*), which is now accepted as a major cause of peptic ulcers.[2,3] *H. pylori* is characterized by very high urease activity which may act as a virulence or survival factor.

The study of urease inhibition has medical, environmental and agronomic significance. For example, urease serves as a virulence factor in pathogens that are responsible for the development of kidney stones, pyelonephritis, peptic ulcers, and other medical complications.[2] Current efforts are focused on the discovery of novel urease inhibitors against *H. pylori* urease. Therefore, urease inhibitors have recently attracted much attention as potential new anti-ulcer drugs. Urease inhibitors can be broadly classified into two categories: (1) substrate-like inhibitors such as hydroxyurea[4] and hydroxamic acids[5]; (2) mechanism-based inhibitors such as phosphorodiamidates[6,7] and imidazoles such as proton pump inhibitors of rabeprazole,[8] lansoprazole[9] and omeprazole.[10]

α-Hydroxyketones or acyloins are frequently encountered as structural subunits in many natural products.[11,12] Recent examples are farnesyltransferase inhibitors, kurasoin A and B,[13] and antitumor antibiotics, olivomycin A and chromomycin A_3.[14] Recently disclosed synthetic α-hydroxyketones have been used as inhibitors of amyloid-β (Aβ) protein production[15] and inducers of apoptosis in oral tumors.[16]

α-Hydroxyketones [compounds containing the-COCH(OH)- functionality] are structurally related to acetohydroxamic acid ($CH_3CONHOH$) (AHA) and hydroxyurea ($NH_2CONHOH$) (HU) identified as potent urease inhibitors.[3] It seemed reasonable to assume that α-hydroxyketones might likewise inhibit urease. We herein report the discovery of novel urease inhibitors.

2. Experimental

The following chemicals were obtained from each indicated company: acetol (**1**), acetoin (**2**), 3-hydroxy-3-methyl-2-butanone (**4**), 2-hydroxy-1-phenylethanone (**5**), benzoin (**8**), furoin (**9**), 2,2′-thenoin (**10**), phenylglyoxal (**14**), 1-phenyl-1,2-propanedione (**15**), phe-

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nylglyoxylic acid (**17**) and methyl phenylglyoxylate (**18**), hydroxyurea (HU) (Tokyo Kasei Co. Ltd, Japan); 2-hydroxycyclohexanone (**13**) (Aldrich Chemical Co. Inc, Milwaukee, USA), 3,3,3-trifluoro-1-phenyl-1,2-propanedione hydrate (**16**) furil (19), 2,2′-thenil (**20**) and acetohydroxamic acid (AHA) (Aldrich Chemical Co. Inc, Milwaukee, USA); 2-mercaptoethanol (2-ME)(Kanto Chemical Co. Inc. Tokyo, Japan); and dithiothreitol (DTT)(Invitrogen Corp., Carlsbad, CA, USA).

1-Hydroxy-1-phenyl-2-propanone (**3**)[17] was obtained by the catalytic reduction of compound (**15**). 2-Hydroxy-1-phenyl-1-propanone (**6**) was prepared from 2-bromo-1-phenyl-1-propanone according to the literature.[18] 3,3,3-Trifluoro-2-hydroxy-1-phenyl-1-propanone (**7**), 1,1,1-trifluoro-2-hydroxy-3-octanone (**11**) and 6-benzamide-1,1,1-trifluoro-2-hydroxy-3-hexanone (**12**) were previously synthesized.[19]

2.1. Measurement of urease

Jack bean urease was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). The assay mixture, containing 25 μL (4U) of jack bean urease and 25 μL (100 μg) of the test compound, was preincubated for 0.5 or 3 h at room temperature in a 96-well assay plate. After preincubation, 0.2 mL of 100 mM phosphate buffer pH 6.8 containing 500 mM urea and 0.002% phenol red were added and incubated at room temperature. The reaction time was measured by micro plate reader (570 nm), which was required for enough ammonium carbonate to form to raise the pH of the phosphate buffer from 6.8 to 7.7.[20]

3. Results

Thirteen α-hydroxyketones (**1**–**13**) were tested against jack bean urease and showed significantly varied inhibitory activity, depending on the structural features (Fig. 1). To our knowledge, this is the first report on the screening of α-hydroxyketones for their urease inhibitory activity. When the amount of urease was fixed at 4 U (0.25 mL) in the assay buffer, 3 h of preincubation with the compound was needed for maximum inhibition with the inhibitor, whereas about 0.5 h preincubation was adequate for AHA (Fig. 2).

In concentration up to 400 μg/mL, no or only minimal inhibitory activity of most α-hydroxyketones tested could be detected under the assay conditions. As shown in Table 1, the inhibitory activity of the potent compounds was: 2,2′-thenoin (**10**) (IC_{50} = 0.18 mM) < furoin (**9**) (IC_{50} = 0.36 mM) < 2-hydroxy-1-phenylethanone (**5**)(IC_{50} = 0.47 mM) < acetol (**1**)(IC_{50} = 2.9 mM). On the other hand, no or only minimal inhibitory activity of all α-diketones (**14**–**20**) tested could be detected under the

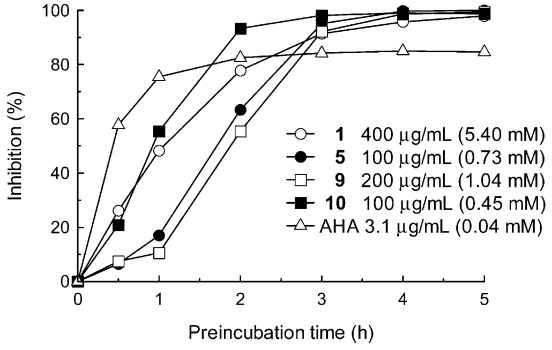
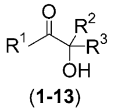


Figure 2. Time course of inhibition of jack bean urease activity by acetohydroxamic acid and some α-hydroxyketones.

Table 1. Inhibition of jack bean urease by some α-hydroxyketones

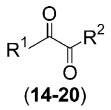
Compd	IC_{50} (mM)	
	0.5 h[a]	3 h[a]
1	—	2.9
5	—	0.47
9	—	0.36
10	1.2	0.18
Hydroxyurea	0.22	0.10
Acetohydroxamic acid	0.017	0.0050

[a] Preincubation time.



(**1-13**)

	R^1	R^2	R^3
1	CH_3	H	H
2	CH_3	CH_3	H
3	CH_3	C_6H_5	H
4	CH_3	CH_3	CH_3
5	C_6H_5	H	H
6	C_6H_5	CH_3	H
7	C_6H_5	CF_3	H
8	C_6H_5	C_6H_5	H
9	2-Furyl	2-Furyl	H
10	2-Thenyl	2-Thenyl	H
11	C_5H_{11}	CF_3	H
12	$C_6H_5CONH(CH_2)_3$	CF_3	H
13	-$(CH_2)_4$-		H



(**14-20**)

	R^1	R^2
14	C_6H_5	H
15	C_6H_5	CH_3
16	C_6H_5	CF_3
17	C_6H_5	OH
18	C_6H_5	OCH_3
19	2-Furyl	2-Furyl
20	2-Thenyl	2-Thenyl

Figure 1. Chemical structures of α-hydroxyketones (**1**–**13**) and α-diketones (**14**–**20**).

assay conditions in concentrations up to 400 μg/mL (data not shown). Inhibitory data for AHA and HU are also included in this Table 1.

The inhibitory effect of α-hydroxyketones was prevented by the addition of sulfhydryl compounds, such as 2-mercaptoethanol (2-ME) and dithiothreitol (DTT) (Table 2). 2-ME prevented the inhibition of urease activity by potent α-hydroxyketones in a concentration-dependent manner when the inhibitor was preincubated with urease solution containing 2-ME (Fig. 3). On the other hand, the inhibitory activity of AHA and HU was not affected by 2-ME or DTT (Table 2).

When $NiCl_2$ was added to the preincubation media, it restored the inhibiton of urease activity by AHA and HU under assay conditions (Table 3). Among four potent α-hydroxyketones, inhibitory activity of two compounds **5** and **9** was prevented in a dose-dependent manner (Fig. 4). However, activity of two other com-

Table 2. Effects of additives on the inhibitory activity of some α-hydroxyketones against jack bean urease

Compd	Additives	IC_{50} (mM)	
		0.5[a]	3 h[a]
1	none	—	2.2
	+2-ME (0.2 mM)	—	>5.4
	+DTT (0.2 mM)	—	>5.4
5	none	—	0.44
	+2-ME (0.4 mM)	—	>2.2
	+DTT (0.4 mM)	—	>2.2
9	none	—	0.27
	+2-ME (0.4 mM)	—	>2.0
	+DTT (0.4 mM)	—	>2.0
10	none	—	0.18
	+2-ME (0.4 mM)	—	>1.7
	+DTT (0.4 mM)	—	>1.7
Hydroxyurea	none	0.22	—
	+2-ME (0.4 mM)	0.25	—
	+DTT (0.4 mM)	0.30	—
Acetohydroxamic acid	none	0.039	—
	+2-ME (0.4 mM)	0.043	—
	+DTT (0.4 mM)	0.055	—

[a] Preincubation time.

Table 3. Effects of nickel ion on the inhibitory activity of some α-hydroxyketones against jack bean urease

Compd	$NiCl_2$	IC_{50} (mM)	
		0.5[a]	3 h[a]
1	none	—	2.0
	+4 mM	—	1.4
5	none	—	0.48
	+4 mM	—	>3.0
9	none	—	0.40
	+4 mM	—	1.9
10	none	—	0.20
	+4 mM	—	0.19
Hydroxyurea	none	0.10	0.10
	+4 mM	0.64	0.26
Acetohydroxamic acid	none	0.020	0.0050
	+4 mM	0.29	0.027

[a] Preincubation time.

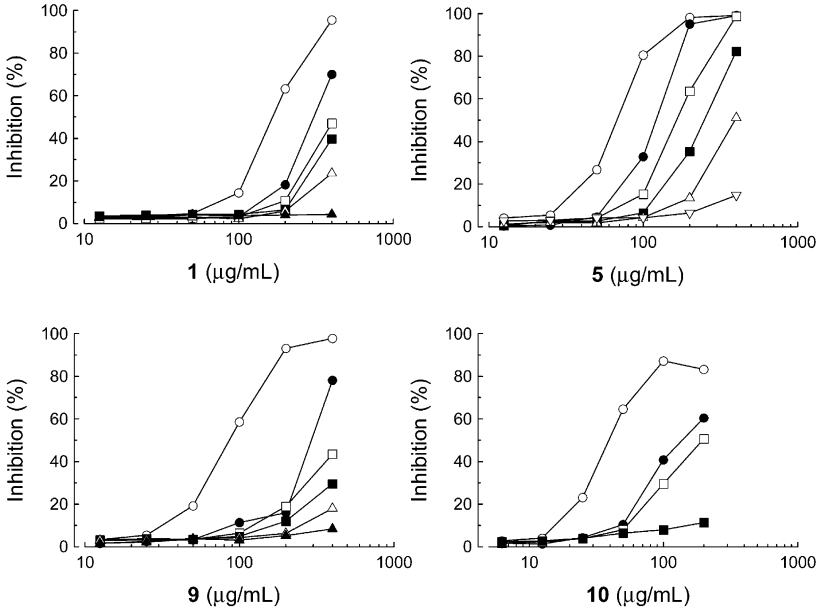


Figure 3. Effect of 2-ME on the inhibition of urease activity by potent α-hydroxyketones 2-ME, at final concentrations of 0 (○), 12.5 (●), 25 (□), 50 (■), 100 (□), 200 (▲) or 400 (□) μM, was added to the urease solutions. Urease activity was assayed as described in Experimental.

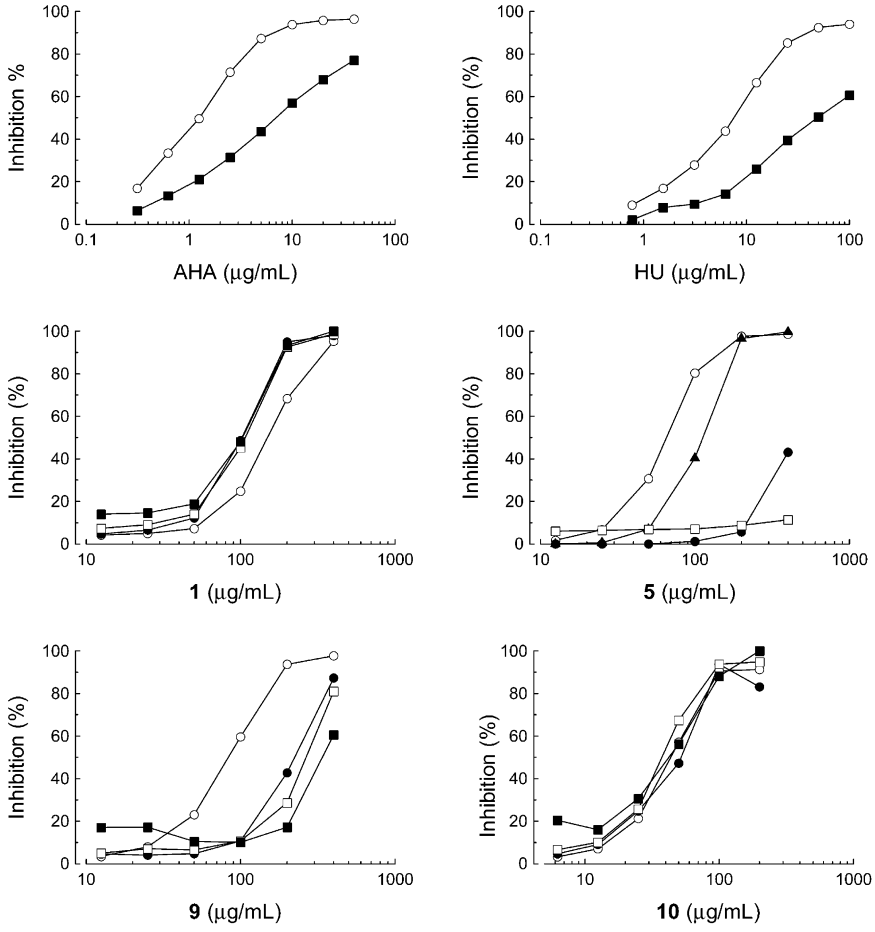


Figure 4. Effects of nickel ion on inhibition of urease activity by acetohydroxamic acid (AHA), hydroxyurea (HU) and α-hydroxyketones. $NiCl_2$, at final concentrations of 0 (○), 0.75 (▲), 1 (●), 2 (□) or 4 (■) mM, was added to the urease solutions.

pounds (**1** and **10**) was not affected in the presence of $NiCl_2$ in concentration up to 4 mM (data not shown).

4. Discussion

As a result of screening to obtain possible lead structures bearing an α-hydroxy carbonyl group, 2,2′-thenoin (**10**) and furoin (**9**) were found to be the most potent inhibitor and equipotent as HU. The inhibitory activities of α-hydroxyketones were dependent on the length of preincubation with the urease. It is indicated that prolonged interaction between the urease and the inhibitors is needed in order to make a stable enzyme-inhibitor complex. Generally, reversible inhibition is characterized by a definite degree of inhibition which is usually attained rapidly.[21] The depression by AHA, a reversible urease inhibitor,[3] rapidly attained the maximal constant degree. α-Hydroxyketones showed the time-dependent inhibition of jack bean urease, and the inhibition may be irreversible. These results agree with published observations that the inhibitory actions of lansoprazole,[9] rabeprazole[22] and α,β-unsaturated ketones[23] were dependent on the length of preincubation with urease.

Ureases are thiol-rich enzymes, and jack bean urease contains in total 15 cysteine residues per subunit. Cystein-592 is recognized to be essential for the activity and is placed in the active site.[24] The interaction of α-hydroxyketones with the sulfhydryl group may be related to their inhibitory influence on urease. Indeed, 2-ME and DTT diminished the activity of potent compounds **1**, **5**, **9** and **10** dose-dependently and even at low concentrations. Similar effects of 2-ME and DTT against the inhibitory effect of rabeprazole on urease activity have been reported.[22] On the other hand, the inhibitory activity of HU and AHA was not influenced by 2-ME or DTT (data not shown).

Thus, α-hydroxyketones are believed to inhibit the enzyme by binding to cysteinyl residues in the active sites. This suggests that α-hydroxyketones may inhibit other SH enzymes, for example, alcohol dehydrogenase, amylase and others. α-Hydroxy carbonyl compounds have been previously shown to be inhibitors of aspartyl protease[25] and are a relatively much less studied class of compounds as inhibitors of various proteolytic enzymes. However, we are unaware of any reports of their use as inhibitors of cysteine proteases. Further study into the effects of α-hydroxyketones on the SH enzymes is needed.

Another feature of urease is the presence of two Lewis acid nickel ions in the active site.[2] Activity of compounds **5** and **9** was reduced in the presence of $NiCl_2$,

but **1** and **10** were not affected. The inhibitory activities of AHA and HU were significantly reduced by the nickel ion. It is supposed that **5**, **9**, HU and AHA bind to the enzyme metallocenter.

The requirements for the α-hydroxy carbonyl moiety on the inhibitory activity against urease by α-hydroxyketone compounds were observed. Among the four methyl ketone derivatives (**1**–**4**) tested, the most potent activity was observed in CH_3COCH_2OH (**1**) only. These observations are in agreement with the phenyl ketone series (**5**–**8**), and $PhCOCH_2OH$ (**5**) showed the most potent inhibition. They define the minimal substitution patterns in the hydroxyketone moiety for obtaining the potent activity. Insertion of the hydroxyketone functionality at the site of enzymatic cleavage may be needed for the inhibition. The active site of urease is located within the cavity or the crevice in the internal territory and is surrounded by hydrophobic amino acid residues of the urease molecule. The hydrophobic character of the R moieties of $RCOCH_2OH$ may play a role in the random walk process of the compounds to the active site and in the hydrophobic binding near the active site of urease. Therefore, phenyl ketone (**5**) is much more potent than the corresponding methyl ketone (**1**).

Some other observations related to the structure–activity relationships are the following: 2,2′-thenoin (**10**) and furoin (**9**) are much more potent than the corresponding α-diketones, 2,2′-thenil (**20**) and furil (**19**). The structural differences between them reside only in the activated ketone functionality. α-Diketones are known to be a well-studied class of activated ketones as inhibitors of cysteine[26] and serine proteases.[27,28] It is speculated that the α-diketones can not insert at the site of enzymatic cleavage in the urease. The log *P* values of **10** (log $P = 1.80$), **9** (log $P = 0.73$), **20** (log $P = 2.84$) and **19** (log $P = 1.73$) were calculated by CLOGP (Pomana College Medical Chemistry Project, Clermont, CA, USA). This demonstrates that the higher activity of α-hydroxyketones (**10** and **9**), compared with α-diketones (**20** and **19**), was not simply caused by the difference in hydrophobicity. Further studies are required to elucidate the mechanism by which α-hydroxyketones exhibit the urease inhibitory activity.

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