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Mechanism, kinetics, and antimicrobial activities of 2-hydroxy-1-naphthaldehyde semicarbazone as a new jack bean urease inhibitor

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A new inhibitor of jack bean urease, 2-hydroxy-1-naphthaldehyde semicarbazone (HNDSC), was synthesized and its inhibitory mechanism and kinetics with respect to jack bean urease were investigated. HNDSC inhibited the activity of jack bean urease, with the inhibitor concentration leading to 50% activity loss (IC_{50}) of 0.032 ± 0.004 mM. Kinetic analyses showed that HNDSC is a reversible and competitive inhibitor of jack bean urease. Microscopic rate constants were

obtained by the progress-of-substrate-reaction method. The results obtained from inhibitory kinetic and fluorescence titration assay methods showed very good agreement that one molecule of HNDSC binds the active unit of the jack bean urease. The inhibition mechanism and kinetic studies indicate that HNDSC could be a candidate for the development of new urease inhibitors. Its antibacterial activities, evaluated against *Escherichia coli*, *Bacillus subtilis*, and *Staphyloccocus aureus*, were highest against *E. coli*.

Introduction

Urease (urea amidohydrolase, E.C 3.5.1.5) is a nickel-containing enzyme that catalyzes the hydrolysis of urea into ammonia and carbamate in a variety of plants, bacteria, algae, fungi, soil, and invertebrates.[1-3] High concentrations of ammonia arising from urease catalysis are responsible for negative effects in agriculture and human health. In agriculture, hydrolysis of urea by soil urease may result in volatilization of nitrogen and plant damage due to alkalinity and ammonia toxicity. Medically, bacterial ureases are important virulence factors for the pathogenesis of many clinical conditions such as hepatic coma, pyelonephritis, peptic ulceration, stomach cancer, and the formation of infection-induced urinary stones.[4] Thus, the control of urease activity is important for counteracting its deleterious effects. The use of urease inhibitors is an effective strategy for controlling urease activity. [5, 6]

In general, jack bean urease can be inhibited by urea analogs,[7] phosphoroamide,[8-10] heavy metal ions,[11] quinones,[12,13] boric acid,[14-16] and bismuth compounds.[17] Two new sphingolipids,[18] bi(2-fluorobenzylaminoethyl)amine and its copper(II), cobalt(III) complexes[19] have shown potent inhibition ability against jack bean urease. However, most inhibitors cannot be put into practical use because of their weak inhibition ability or safety concerns. Further research for

new jack bean urease inhibitors with desirable properties are ongoing. Schiff base derivatives have attracted much attention because of their versatile coordination with different metal ions, which may possess antimicrobial,[20,21] antitumor,[22,23] anti-inflammatory,[24] antimalarial,[25] and antifungal[26] properties. In addition, some new Schiff base analogs[27-29] have proven to be effective inhibitors of jack bean urease, including 3-nitro- and 4-(dimethylamino)-benzylidene thiosemicarbazides. Although the kinetics of inhibition of jack bean urease have been described,[15,30,31] there is no report about these kinetics of Schiff base derivatives. Here, we present a new inhibitor of jack bean urease, 2-hydroxy-1-naphthaldehyde semicarbazone (HNDSC; see **Scheme 1**). We investigated the inhibitory ability, inhibition mechanism, and kinetics of HNDSC on jack bean urease. As a Schiff base derivative, it has a much stronger inhibitory effect on jack bean urease than does boric acid. In addition, some Schiff base derivative may exhibit bacteriostatic activity. Protein–drug interaction is a topic of strong interest in fields of medicine, chemistry, and biology. Because of the pharmacokinetic effects on drugs bound to proteins, the pharmaceutical may implement their functionality mainly by virtue of their mutual interaction between pharmaceutical and protein. As a result, there is an increasing interest in the study for the mechanism/kinetics between pharmaceutical and protein.[32,33] The study of new urease inhibitors is essential not only for basic research on jack bean urease biochemistry but also for the possible development of a highly required therapy for jack bean urease-mediated bacterial infections.

Experimental

Chemicals and materials

Jack bean urease, Sigma type III, with specific activity 57 U/mg protein was used (Sigma Chemical Co.). 2-Hydroxy-1-naphthaldehyde was purchased from HBC Chem. Inc. (United States). Semicarbazone hydrochloride was from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Phenylphosphorodiamidate was purchased from 9Ding Chemistry Co. Ltd. (Shanghai, China). N-(n-butyl) thiophosphoric triamide was from J&K Scientific Ltd. The purity of these compounds was greater than 99%. Dimethyl sulfoxide (DMSO) was obtained from Aldrich (St. Louis, MO, USA). *Escherichia coli, Bacillus subtilis,* and *Staphyloccocus aureus* were collected from a colony preserved at −80 °C at the Biochemistry Laboratory, School of Life Science in Fuyang Normal College (China). Urea (substrate) and other reagents were local products of analytical grade. Doubly deionized water was used throughout. Ultraviolet absorption were recorded on a UV-8000A spectrophotometer (Beijing Purkinje General Corporation, China). Fluorescence spectra were measured with a FluoroMax-4 fluorescence spectrophotometer (Horiba, Japan) equipped with a water-thermostatted cell holder.

Synthesis of 2-hydroxy-1-naphthaldehyde semicarbazone (HNDSC)

Semicarbazide hydrochloride was reacted with 2-hydroxy-1-naphthaldehyde in ethanol under reflux for 3 h to give HNDSC (**scheme 1**). The resulting mixture was filtered and washed with ethanol several times. Crystals of HNDSC were obtained by slow evaporation of ethanol solution in air for 5 days, m. p. 305–306 °C; Anal. calcd. (%) for C_{12} $H_{11}O_2N_3$: C, 62. 87; H, 4.84; N, 18. 33. Found (%): C, 62. 72; H, 4.92; N, 18. 37; ^{1}H NMR (400 MHz, DMSO -d_6) δ: 10.23 (s, 1H), 8.87 (s, 1H), 8.35 (d, 1H), 7.82 (d, 2H), 7.54 (t,1H), 7.36 (t,1H), 7.17 (d,1H), 6.39(s,2H); IR (KBr) ν/cm^{-1}: 3452, 3348, 1668, 1603, 1435, 1190, 933.

Enzymatic activity assay

The activity assay of jack bean urease was performed according to the literature cited.[34] Enzymatic activity was defined as the amount of product catalyzed per minute by 1 mg enzyme substrate under certain conditions. The activity of uninhibited urease was defined as the control activity of 100% and IC_{50} was defined as the inhibitor concentration leading to 50% loss of activity. Urea was employed as a substrate for the activity assay. The solution for the activity assay contained different concentrations of inhibitor and certain concentrations of urease in NaH_2PO_4–Na_2HPO_4 buffer (pH 7.4). The reaction was initiated by the addition of quantitative urea solution to mixed phosphate buffer solutions of urease and inhibitor. It was performed at 25 °C for some time and then was monitored by measurement of the ammonia concentration by the phenol–hypochlorite method.[35] The inhibition type was determined according to the Lineweaver−Burk plot, and the inhibition constant was obtained by second plots of the apparent K_m/V_m or $1/V_m$ against the inhibitor concentration.

Determination of inhibitory rate constants

The progress-of-substrate-reaction method described by Tsou[36] was used to determine the reaction rate constants of jack bean urease inhibited by HNDSC. To perform the measurement, solutions (1.0 mL) for the activity assay were prepared as mixtures of 16 μg/mL urease with 22 mM NaH_2PO_4–Na_2HPO_4 buffer (pH 7.4) and different concentrations of inhibitor. The reaction was initiated by the addition of 0.67 μmol urea solution to mixed phosphate buffer solutions of urease and inhibitor. After reaction at 25 °C for different times, 2.0 mL phenol solution and 3.0 mL hypochlorite sodium solution were added to the reaction system. Absorption at 625 nm was recorded using a UV-8000A spectrophotometer. The time course of the hydrolysis of the substrate (urea) at different inhibitor concentrations levels showed that at each concentration of inhibitor, the rate of the hydrolysis of urea decreased with time until a straight line was

approached (Fig. 4a). It can be observed that curves approach straight lines with 61 minutes reaction duration, which suggests the reaction has nearly reach saturated status to obtain the definite data information for the established kinetic model. The appearance showed that it was a reversible reaction at lower concentrations of HNDSC with residual activity. This reaction can be written as **Scheme 2**, where S, E, P and Y represent substrate, enzyme, product, and inhibitor (HNDSC). ES and EY are the respective complexes. k_{+0} and k_{-0} are rate constants for the formation and dissociation, respectively, of the ES complex. The deduced kinetic model in detail are listed in the electronic supplementary information (ESI†).

Antimicrobial assay

Antibacterial assays were performed in tryptone beef extract agar at pH 7.4, with an inoculum of 1.5×10^8 CFU/mL, and the improved agar well diffusion method was employed to evaluate the antimicrobial activity of HNDSC.[37,38] Briefly, culture medium was inoculated with the indicated microorganism by spreading bacterial inoculum in the medium. Wells (5.5 mm in diameter) were punched in the agar and filled with HNDSC at different concentrations. Control wells, containing neat DMSO (negative control) and the standard antibiotic streptomycin sulfate (1000 U/mL) for the tested bacteria (positive control), were run in parallel in the same plate. Bacteria were incubated at 37 °C for 18 h. Antimicrobial activity was assessed as the diameter of the zone of inhibition of the respective drug.

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were tested by broth macrodilution methods.[39] Briefly, serial twofold dilutions of the test compounds were prepared in DMSO and 30 μL of each dilution was added to 3.0 mL of the above medium with the same inoculum of 1.5×10^8 CFU/mL and under the same cultural conditions. After the cultures were incubated at 37 °C for 24 h, MIC was determined as the

lowest concentration of the test compound that supported no visible growth. The minimum bactericidal concentration (MBC) was determined as follows. After the determination of the MIC, 100-fold dilutions with drug-free medium from each tube showing no turbidity were incubated at 37 °C for 24 h. The MBC was the lowest concentration of the test compound that showed no visible growth in drug-free cultivation.

Results and Discussion

Effect of HNDSC on the activity of jack bean urease

The relationship between residual enzyme activity and HNDSC concentrations is shown in **Fig. 1**, which reveals that the effect of HNDSC on jack bean urease activity was concentration-dependent. When the concentration of HNDSC reached 0.045 mM, enzyme activity was inhibited by 58%. Residual enzyme activity rapidly decreased with increasing concentrations of HNDSC. The IC_{50} value was determined to be 0.032 ± 0.004 mM by the fitting curve. Deduced from the formula of competitive inhibitor $(K_m+c_s)K_i/K_m$, the IC50 value is 0.034 mM, which is consistent with experimental value 0.032 ± 0.004 mM. The ammonia generated by urease in the absence of urea as concentrations of HNDSC increased from 0 to 0.2 Mm. This result showed that the amount of ammonia was unchanged; thus, indicating that HNDSC is not an alternative substrate for the urease molecule.

The IC_{50} values of several common inhibitors were investigated under nearly the same conditions (**Table 1**) because inhibitory activities can be well evaluated by IC_{50} values of the inhibitors. **Table 1** indicates that the inhibitory activity of HNDSC is comparable to that of acetohydroxamic acid but is smaller than those of PPD or NBPT.

The inhibition constant (K_i) for HNDSC binding to the free enzyme was obtained as 0.030 ± 0.002 mM (inset, **Fig. 2**). The ratio K_m/K_i is commonly employed for measuring the effectiveness of competitive inhibitors: the higher the ratio, the stronger is the inhibitory action on the enzyme.[43] The ratios for several competitive inhibitors studied are listed in **Table 1**, from which it is evident that HNDSC is an effective inhibitor with activity much stronger than that of F^- ion, boric acid, or 2-mercaptoethanol.

Inhibition mechanism of HNDSC on the activity of jack bean urease

The hydrolysis of urea by jack bean urease followed Michaelis–Menten kinetics under the conditions employed in this study. The kinetic parameters K_m and V_m determined from the Lineweaver–Burk plot (**Fig. 2**, curve 1) were 5.23 ± 0.04 mM and 45.36 ± 0.12 μmol NH_3 min^{-1} mg^{-1}, respectively (**Table 2**), which are well consistent with reported values.[44] **Fig. 3** shows the relationship between enzymatic activity and concentration of HNDSC. The straight lines passing through the origin indicate that the inhibition mechanism of HNDSC on jack bean urease is a reversible reaction. The presence of HNDSC did not reduce the amount of enzyme but only weakened its activity.

In the Michaelis−Menten plot, plots of $1/v$ against 1/[S] gave a family of straight lines with the same positive intercept on the y-axis (**Fig. 2**), indicating that HNDSC is a competitive inhibitor of jack bean urease. The result suggested that the inhibitor binds only to the free enzyme rather than to an enzyme–substrate complex.

Microscopic rate constants of jack bean urease inhibition by HNDSC

The plot of product concentration against time is a fundamental principle to study the kinetic study. Therefore, the time-varying product concentrations during urea hydrolysis in the presence of different HNDSC concentrations are shown in **Fig. 4a**. At each concentration of

HNDSC, the rate decreased with time until a straight line was approached whose slope decreased with increasing HNDSC concentration. The results suggested that inhibited jack bean urease retained residual activity, according to Tsou's method,[36] indicating that the formation of an EY complex was also a reversible reaction.

According to Equation: $\ln([P]_{calc} - [P]_t) = \text{constant} - (A[Y] + B)t$ (ESI†), plots of $\ln([P]_{calc} - [P]_t)$ against t gave a series of straight lines with slopes of $-(A[Y] + B)$ (**Fig. 4b)**. Similarly, plots of the slopes against HNDSC concentrations gave straight lines at a fixed substrate concentration. The apparent reverse rate constant B (k_{-0}) can be obtained from the intercept on the y-axis, and the value of k_{-0} is shown in **Table 2**.

Fig. 5 shows the kinetic courses of the reaction at different substrate concentrations in the presence of 10 μM HNDSC. As shown in **Fig. 5a**, the curves approach straight lines when t is sufficiently large. Both the initial rate and the slope of the asymptote increase with substrate concentration. Similarly, plots of $\ln([P]_{calc} - [P]_t)$ against t give a family of straight lines at different concentrations of the substrate with slopes of $-(A[Y] + B)$ in **Fig. 5b**.[45]

From the equation: $\frac{1}{A} = \frac{1}{k_{+0}K_m}[S] + \frac{1}{k_{+0}}$ (ESI†), a plot of $1/A$ against [S] gives a straight line with $1/(K_m k_{+0})$ as the slope of the straight line and $1/k_{+0}$ as the intercept on the y-axis (**Fig. 6**). From the slope or the intercept of the straight line, the microscopic rate constant, k_{+0}, can be obtained separately (**Table 2**).

According to the equation: $\frac{A}{v} = \frac{k_{+0}K_m}{V_m}\frac{1}{[S]}$ (ESI†), a plot of A/v against 1/[S] gives a straight line with a slope of $K_m k_{+0}/V_m$, passing through the origin (**Fig. 6**). From the slope, the microscopic rate constant, k_{+0}, can also be obtained and shown in **Table 2**.[46]

From the results in **Table 2**, we can see that the forward microscopic inhibitory rate constant (k_{+0}) obtained from the plot of $1/A$ against [S] (**Fig. 6**) and the plot of A/v against 1/[S] (the inset of **Fig. 6**) were almost identical (7.71×10^{-3}, 7.65×10^{-3}, and 7.64×10^{-3} $mM^{-1} \cdot S^{-1}$). This similarity indicates that the established inhibition kinetics model is appropriate.

The binding characteristics of HNDSC with jack bean urease

According to the equation: $[P]_{calc}\text{-}[P]_t = \frac{A[Y]v}{(A[Y]+B)^2} e^{-(A[Y]+B)t}$ (ESI†), plots of $\ln([P]_{calc} - [P]_t)$ against t gave a family of straight lines at different concentrations of HNDSC with slopes of $-(A[Y] + B)$ (**Fig. 4b**). The $(A[Y] + B)$ are the apparent rate constants of inhibition. The value of $(A[Y] + B)$ was denoted by k. The results show that the values of k increase as HNDSC concentrations increase. The relationship between k and the inhibitor concentration [Y] can be written as follows:[47]

$$\log k = \log k_I + n \log [Y] \quad (1)$$

Thus, a plot of log k against log [Y] gives a straight line with the slope equal to n (**Fig. 4c**), which is equal to the number of molecules of inhibitor reacting with each active unit of the enzyme to produce an inactive enzyme–inhibitor complex. The obtained result $n = 0.962$ shows that only one molecule of HNDSC binds the active unit of the jack bean urease to destroy its activity. HNDSC is capable of binding jack bean urease to form a stable complex.

This bonding characteristic of enzyme-inhibitor complex deduced by kinetic model was further confirmed by virtue of fluorescence titration assay. Fluorescence titration assays (**Fig. 7**) showed that the fluorescence of jack bean urease could be quenched by HNDSC and that increasing the concentration of HNDSC resulted in a gradual decrease in fluorescence intensity of jack bean urease. The emission peak of the native jack bean urease is located at 337 nm, suggesting that the tryptophan (Trp) residues in the enzyme are partly protected from water.

Trp is known for its fluorescent characteristic and high sensitivity to the polarity of its environment.[32] In **Fig. 7**, a slight blue shift of jack bean urease fluorescence emission suggests that the conformational changes induced by the interaction of HNDSC with jack bean urease may lead to increases in polarity around the Trp residues. The results show that the binding of the HNDSC molecule to the active site of jack bean urease not only leads to the loss of activity but also results in a change in microenvironment of Trp residues.

To further confirm the formation of the enzyme-inhibitor complex, the fluorescence quenching data were analyzed using the Stern–Volmer equation:

$$F_0/F = 1 + k_q\tau_0 [Y] = 1 + K_{SV} [Y] \quad (2)$$

where F_0 and F are the fluorescence intensities in the absence and presence of quencher, respectively, k_q is the biomolecular quenching constant, τ_0 is the average fluorescence lifetime of biomolecular without addition of quencher which is considered as 10^{-8} s, [Y] is the concentration of quencher, and K_{SV} is the Stern–Volmer quenching constant. The values of K_{SV} and k_q are equal to 2.40×10^4 L·mol^{-1} and 2.40×10^{12} L·mol^{-1}·s^{-1}, respectively. A value of k_q greater than 2.0×10^{10} L·mol^{-1}·s^{-1} indicates that the quenching process is static and that the fluorescence-quenching mechanism of jack bean urease by HNDSC was initiated by an enzyme–inhibitor complex.

The binding constant (K_a) and the number of binding sites (n) were estimated from fluorescence titration studies, using the plot of log $(F_0 - F)/F$ versus log $(1/([Y] - [P](F_0 - F)/F_0))$ which is based on the equation:

$$\log (F_0 - F)/F = n \log Ka + n \log(1/([Y] - [P](F_0 - F)/F_0)) \quad (3)$$

where F_0 and F are the fluorescence intensity of urease in absence and presence of HNDSC, respectively, [Y] and [P] are the total HNDSC concentration and the total urease concentration.

From a plot of log $(F_0–F)/F$ against log $(1/ ([Y]–[P](F_0–F)/F_0))$, the n and K_a can be obtained. The K_a and n for HNDSC associated with urease were 6.44×10^4 $L \cdot mol^{-1}$ and 1.091, respectively. The correlation coefficient was 0.9985, indicating that the interaction between HNDSC and urease agrees well with the site-binding model described by Eq. 3. The finding with the n value of approximately 1 further supports the formation of an enzyme–inhibitor complex, which is in good agreement with that of the kinetic study.

The antimicrobial activity of HNDSC

The antimicrobial activities of HNDSC on *E. coli*, *B. subtilis*, and *S. aureus* are shown in **Fig. 8** and **Table 3**. HNDSC inhibited the proliferation of the three different bacteria to different extents. The results of the broth dilution assay are presented in **Table 3**. The antimicrobial activity against *E. coli* was highest, with MIC of 156 μg/mL and MBC of 312 μg/mL.

Conclusions

Inhibition by HNDSC of jack bean urease was studied by inhibitory mechanism, kinetics, and fluorescence titration assay. HNDSC exhibited high inhibitory activity against jack bean urease. The inhibitory activity was much stronger than those of F^- ion, boric acid, and 2-mercaptoethanol, with an IC_{50} of 0.032 ± 0.004 mM. HNDSC was found to be a reversible, competitive inhibitor of jack bean urease, with the binding of a single molecule of HNDSC to the active unit of the jack bean urease being sufficient to destroy urease activity. The inhibition constant (K_i) was 0.030 ± 0.002 mM. The forward microscopic rate constants (k_{+0}), obtained in three different ways, were 7.71×10^{-3}, 7.65×10^{-3}, and 7.64×10^{-3} $mM^{-1} \cdot S^{-1}$. The reverse microscopic rate constant (k_{-0}) was 1.77×10^{-3} S^{-1}. The data fit well with those expected of reversible competitive inhibition, indicating that the established inhibition kinetics model is

appropriate. HNDSC can also play a potent role in the pharmaceutical application as exhibited bactericidal action against *E. coli, B. subtilis,* and *S. aureus*. These results provide a basis for developing a new, effective, and safe inhibitor of jack bean urease.

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Abbreviations

DMSO, dimethyl sulfoxide; HNDSC, 2-hydroxy-1-naphthaldehyde semicarbazone; PPD, phenylphosphorodiamidate; NBPT, N-(n-butyl) thiophosphoric triamide; IC_{50}, the inhibitor concentration leading to 50% activity loss; CFU, colony-forming unit; K_i, equilibrium constant of the inhibitor combining with the free enzyme; k_{+0}, forward microscopic rate constant; k_{-0}, reverse microscopic rate constant; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.

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Figure Captions

Fig. 1 Inhibition by HNDSC on jack bean urease. Conditions were a 1.0 mL assay system containing 22 mM NaH_2PO_4–Na_2HPO_4 buffer (pH 7.4), 0.67 mM of urea, 6 μg/mL of urease and different concentrations of HNDSC at 25 °C for 15 min. The label in the figure represents absolute error.

Fig. 2 Lineweaver−Burk plots for inhibition by HNDSC of jack bean urease. The assay conditions were as described for **Fig. 1**, except that the concentration of urease was 16 μg/mL. The concentrations of HNDSC for curves 1−5 were 0, 0.01, 0.02, 0.03, and 0.04 mM, respectively. The inset shows a plot of K_m against concentration of HNDSC to determine the inhibition constant (K_i). The label in the figure represents absolute error.

Fig. 3 Determination of the mechanism of HNDSC inhibition of urease. The assay conditions were as described for **Fig. 1**, except that the concentration of urease. The concentrations of HNDSC for curves 1−3 were 5, 15, and 30 μM, respectively.

Fig. 4 Course of inhibition of enzyme in different concentrations of HNDSC and 16 μg/mL urease. The assay conditions were as described for **Fig. 2**. (**a**) Substrate reaction course. The concentrations of HNDSC for curves 0−3 were 0, 0.015, 0.030, and 0.045 mM, respectively. (**b**) Semilogarithmic plot of $\ln([P]_{calc} - [P]_t)$ against time. Data are taken from curves 1–3 in (**a**). (**c**) Plot of k against Y.

Fig. 5 Course of reaction at different substrate concentrations in the presence of 0.025 mM HNDSC and 6 μg/mL urease. **(a)** Substrate reaction course. The concentrations of urea for curves 1−4 were 0.54, 1.09, 1.63, and 2.18 mM, respectively. (**b**) Semilogarithmic plot of $\ln([P]_{calc} - [P]_t)$ against time. Data are taken from curves 1–4 in (**a**).

Fig. 6 The plot of 1/*A* against [S]. The inset shows a plot of *A*/*v* against 1/[S].

Fig. 7 (**a**) Fluorescence emission spectra of urease in absence and presence of HNDSC at 29 °C. The urease concentration was at 1.0 μM and the concentrations of HNDSC were 0, 2.0, 4.0, 8.0, 12.0, 16.0, 20.0, 24.0, 28.0, and 32.0 μM from top to bottom. pH =7.4, λ_{ex}=280 nm. (**b**) Plots of $\log(F_0 - F)/F$ against $\log (1/([Y] - (F_0 - F)\,[P]/F_0))$.

Fig. 8 Antimicrobial activity of HNDSC at different concentrations. The concentrations of HNDSC in dishes 1–5 were 10, 5, 2.5, 1.25 and 0.625 mg/mL, respectively. (**a**) positive control with 1000 U/mL of streptomycin sulfate for bacterium; (**b**) negative control with DMSO.

Fig. 1

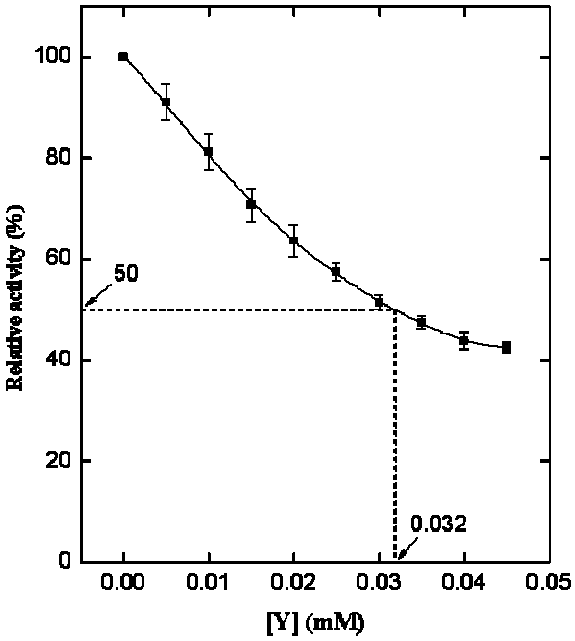


Fig. 2

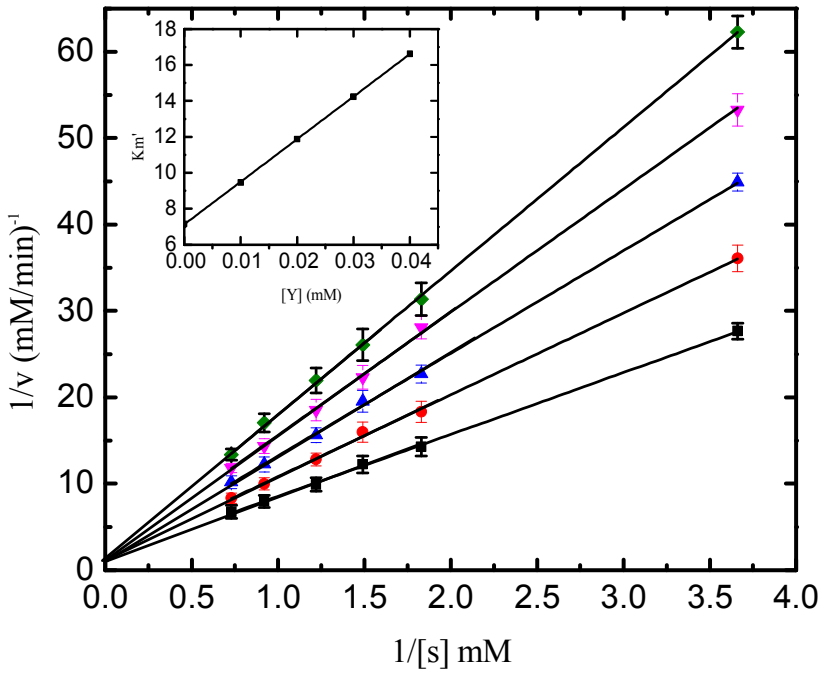


Fig. 3

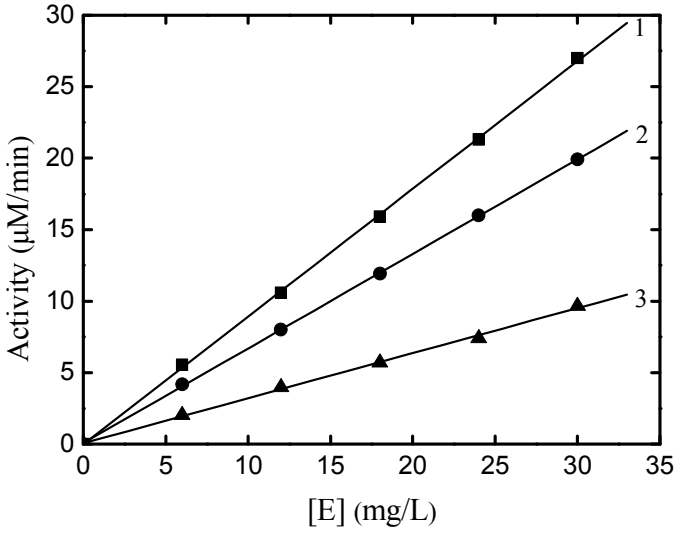


Fig. 4

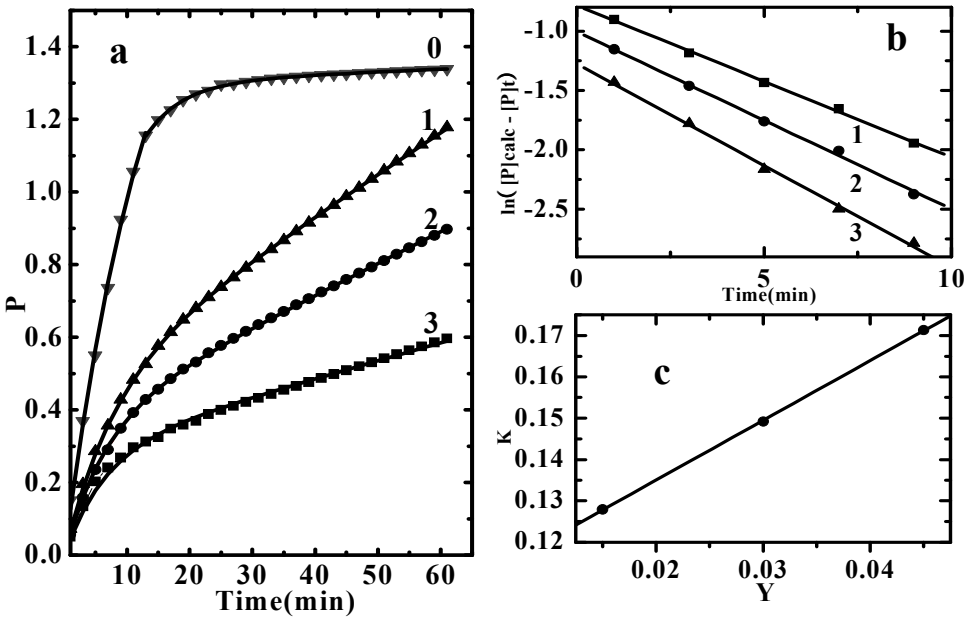


Fig. 5

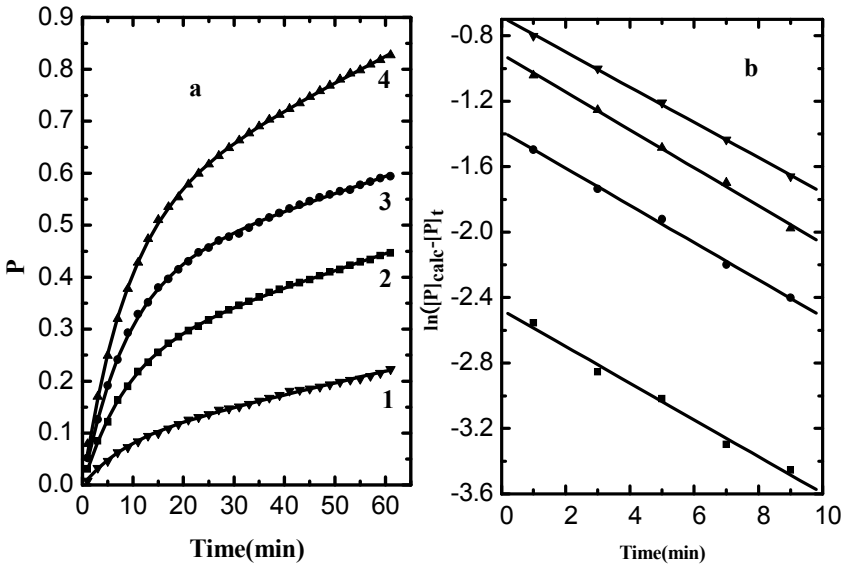


Fig. 6

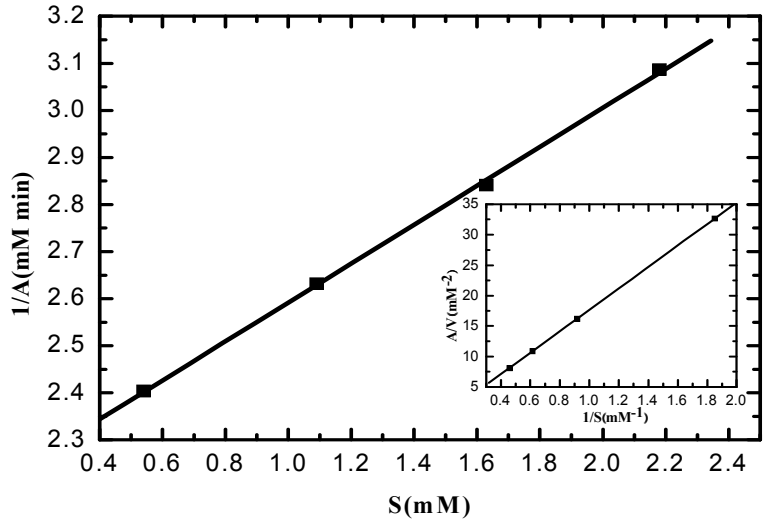


Fig. 7

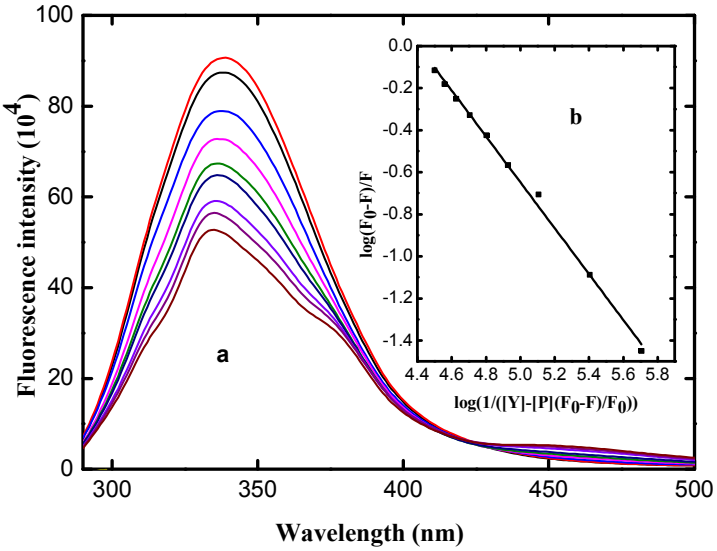
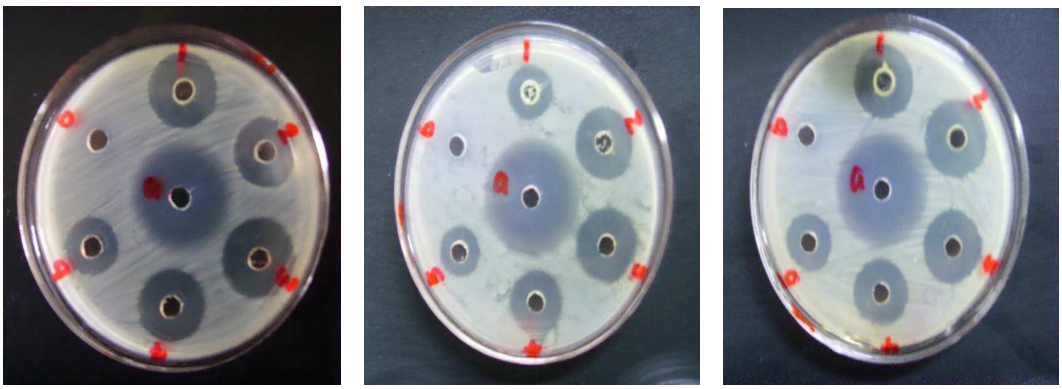
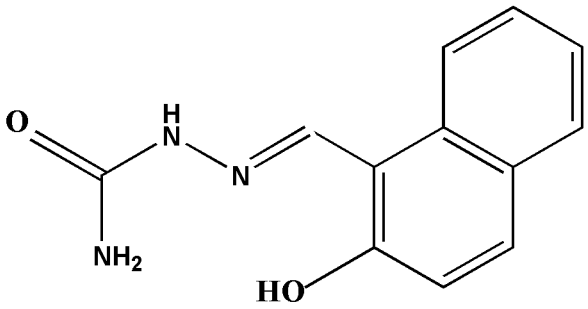


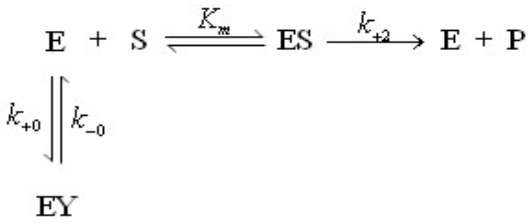
Fig. 8



I: *Escherichia coli* II: *Staphylococcus aureus* III: *Bacillus subtilis*



Scheme 1 Chemical structure of HNDSC.



Scheme 2 The kinetic model of the inhibition.

Table 1 Kinetic constants of Jack Bean Urease in the absence and presence of inhibitors

inhibitor	buffer pH	IC_{50} (mM)	K_m (mM)	K_i (mM)	K_m/K_i	reference
PPD	22 mM phosphate pH 7.4	12.11×10^{-6}	5.24	3.41×10^{-6}	1.54×10^{6}	this work
NBPT	22 mM phosphate pH 7.4	0.0026	5.24	0.0016	3275	this work
acetohydro-xamic acid	22 mM phosphate pH 7.4	0.030	5.23	0.029	180.3	this work
HNDSC	22 mM phosphate pH 7.4	0.032	5.23	0.030	174.3	this work
boric acid	22 mM phosphate pH 7.0	__	7.1	0.19	37.4	(40)
boric acid	20 mM phosphate pH 7.4	0.22	5.11	0.18	28.4	(15)
boric acid	22 mM phosphate pH 7.4	0.20	5.23	0.16	32.7	this work
F^- ion	22 mM phosphate pH 7.0	__	7.1	1.0	7.1	(41)
F^- ion	22 mM phosphate pH 7.4	5.20	5.23	1.03	5.08	this work
2-ercapto-ethanol	10μM HEPES pH 7.1	__	2.1	0.72	2.92	(42)

Table 2 Kinetic parameters and microscopic inhibitory rate constants of the Jack bean Urease by HNDSC (22 mM NaH_2PO_4–Na_2HPO_4 buffer, pH = 7.4)

K_m (mM)	V_m (μmolNH_3 min^{-1} mg^{-1})	K_i (mM)	k_{+0} (× 10^{-3} mM^{-1} S^{-1})	k_{-0} (× 10^{-3} S^{-1})
			7.71[a]	
5.23± 0.04	45.36 ± 0.12	0.030 ± 0.002	7.65[b]	1.77[d]
(n = 6)	(n = 6)		7.64[c]	

[a] From the slope of the straight line of 1/*A* against [S] (**Fig. 6**).

[b] From the intercept of the straight line of 1/*A* against [S] (**Fig. 6**).

[c] From the slope of the straight line of *A*/v against 1/[S] (the inset of **Fig. 6**).

[d] From the intercept of the straight line of K against [Y] (**Fig. 4c**).

Table 3. Antimicrobial activity of HNDSC

bacteria	MIC (μg/mL)	MBC (μg/mL)	concentration (mg/mL)						
			a[a]	b[b]	10	5	2.5	1.25	0.625
Staphylococcus aureus	156	156	+++[c]	-[f]	+[e]	++[d]	++	+	+
Escherichia coli	156	312	+++	–	++	++	++	++	+
Bacillus subtilis	312	625	+++	–	++	++	++	++	+

a positive control with 1000 U/mL of streptomycin sulfate for bacterium.

b negative control with DMSO.

c +++, antimicrobial zone is above 22 mm in diameter.

d ++, antimicrobial zone is between 17 and 22 mm.

e +, antimicrobial zone is less than 17 mm.

f −, no inhibition

Graphical abstract

A new inhibitor of jack bean urease, 2-hydroxy-1-naphthaldehyde semicarbazone, was synthesized and employed to investigate inhibitory mechanism of HNDSC on jack bean urease by kinetic and fluorescence titration assay, and its antibacterial activities were also investigated.