Electronic supplementary information (ESI)

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

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Based on the derivation of the kinetic equations,[s1] the product formation can be described as follows:

$$[\mathrm{P}]_t = \frac{vB}{A[\mathrm{Y}]+B}t + \frac{A[\mathrm{Y}]v}{(A[\mathrm{Y}]+B)^2}(1-e^{-(A[\mathrm{Y}]+B)t}) \quad (1)$$

$$A = \frac{k_{+0}K_\mathrm{m}}{K_\mathrm{m}+[\mathrm{S}]} \quad (2)$$

$$B = k_{-0} \quad (3)$$

where $[\mathrm{P}]_t$ represents the concentration of the product at time t, which is the reaction time, [S] and [Y] are the concentrations of the substrate and inhibitor, respectively, A and B are the apparent rate constant of inactivation, v is the initial reaction rate in the absence of the inhibitor, and $v = V_\mathrm{m} \times [\mathrm{S}]/(K_\mathrm{m} + [\mathrm{S}])$, is the Michaelis–Menten equation.[s2] When t is sufficiently large, the curves become straight lines and the product concentration is defined as $[\mathrm{P}]_{\mathrm{calc}}$:

$$[\mathrm{P}]_{\mathrm{calc}} = \frac{Bv}{A[\mathrm{Y}]+B}t + \frac{A[\mathrm{Y}]v}{(A[\mathrm{Y}]+B)^2} \quad (4)$$

Combining Eqs. (1) and (4) yields

$$[\mathrm{P}]_{\mathrm{calc}}\text{-}[\mathrm{P}]_t = \frac{A[\mathrm{Y}]v}{(A[\mathrm{Y}]+B)^2}e^{-(A[\mathrm{Y}]+B)t} \quad (5)$$

$$\ln([\mathrm{P}]_{\mathrm{calc}} - [\mathrm{P}]_t) = \mathrm{constant} - (A[\mathrm{Y}]+B)t \quad (6)$$

where $[\mathrm{P}]_{\mathrm{calc}}$ is the product concentration to be expected from the straight-line portions of the curves as calculated from Eq. (4) and $[\mathrm{P}]_t$ is the product concentration actually observed at time t. Plotting $\ln([\mathrm{P}]_{\mathrm{calc}} - [\mathrm{P}]_t)$ against t gives a series of straight lines at different concentrations of inhibitor with slopes of $-(A[\mathrm{Y}] + B)$. A secondary plot of the slopes against [Y] gives a straight line. The apparent forward

and reverse rate constants, A and B, can be obtained from the slope and intercept of this line. The value of B equals that of the microscopic rate constant k_{-0}.

From Eq. (2), we have

$$\frac{1}{A}=\frac{1}{k_{+0}K_{\mathrm{m}}}[S]+\frac{1}{k_{+0}} \qquad (7)$$

A plot of $1/A$ against [S] gives a straight line with $1/(k_{+0}K_{\mathrm{m}})$ as the slope and $1/k_{+0}$ as the intercept on the y-axis.

Combining Eq. (2) and the Michaelis–Menten equation gives

$$\frac{A}{v}=\frac{k_{+0}K_{\mathrm{m}}}{V_{\mathrm{m}}}\frac{1}{[S]} \qquad (8)$$

A plot of A/v against 1/[S] gives a straight line with slope $K_{\mathrm{m}}k_{+0}/V_{\mathrm{m}}$ that passes through the origin, indicating that the inhibitor is a competitive inhibitor of the enzyme. As K_{m} and V_{m} are determined from measurements of the substrate reaction in the absence of HNDSC at different substrate concentrations, the rate constant k_{+0} is readily obtained.

Determination of inhibitory rate constants

The progress-of-substrate-reaction method was used to determine the reaction rate constants of jack bean urease inhibited by some inhibitors according to the literature.[s3] The corresponding results are shown in Table 1. The detailed experiment of three inhibitors of acetohydroxamic acid, boric acid and F^- ion are completely same with the HNDSC.

The solution (1.0 mL) for the activity assay contained different concentrations of inhibitor (PPD or NBPT) and 16 μg/mL urease in 22 mM NaH_2PO_4–Na_2HPO_4 buffer (pH 7.4). The reaction was initiated by the addition of 0.67 μmol urea solution to mixed phosphate buffer solutions of urease and inhibitor. It was carried out at 25℃

for 15 minute, 2.0 mL phenol solution together with 3.0 mL hypochlorite sodium solution were added into the mixture.

The solution (1.0 mL) for the assay contained fixed concentrations of inhibitor (The concentrations of PPD were 0, 1, 4 and 8 nM, espectively) and 16 μg/mL urease in 22 mM NaH_2PO_4–Na_2HPO_4 buffer (pH 7.4). The reaction was initiated by the addition of different concentrations of urea solution to mixed phosphate buffer solutions of urease and inhibitor. The concentrations of urea were 0.273, 0.546, 0.670, 0.819, 1.09 and 1.365 mM, respectively.

The solution (1.0 mL) for the assay contained fixed concentrations of inhibitor (the concentrations of NBPT were 0, 0.001, 0.002, 0.003 and 0.004 mM, respectively) and 16 μg/mL urease in 22 mM NaH_2PO_4–Na_2HPO_4 buffer (pH 7.4). The reaction was initiated by the addition of different concentrations of urea solution to mixed phosphate buffer solutions of urease and inhibitor. The concentrations of urea were 0.273, 0.546, 0.670, 0.819, 1.09 and 1.365 mM, respectively.

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