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Original Article

Synthesis and inhibitory activity of *N*-acetylpyrrolidine derivatives on α-glucosidase and α-amylase

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Abstract

Background and purpose: Carbohydrate hydrolysis enzymes including α-glucosidase and α-amylase are related to type 2 diabetes mellitus. The inhibiting of these enzymes might use for type 2 diabetes mellitus treatment.
Experimental approach: *N*-substituted-acetylpyrrolidine linked with -benzyl- (*N*-(benzyl)-2-acetylpyrrolidine (**4a**)) and -tosyl- (*N*-(tosyl)-2-acetylpyrrolidine (**4b**)) were synthesized and evaluated for their pharmaceutical properties against α-glucosidase and α-amylase and free radical scavenging activity. The structures of **4a** and **4b** were determined through spectral studies (^{1}H-NMR).
Findings / Results: Both compounds **4a** and **4b** had highest inhibitory potential on α-glucosidase with the IC_{50} values of 0.52 ± 0.02 and 1.64 ± 0.08 mM, respectively. The kinetic investigation of **4a** and **4b** against α-glucosidase and α-amylase were functioned in mixed type inhibition. Moreover, both compounds are more likely to bind with the free enzyme than the enzyme-substrate complex based on the $K_i < K_i'$ on the α-glucosidase and α-amylase enzymes. Regarding the free radical scavenging, **4a** had a higher capacity than **4b** with IC_{50} values of 1.01 ± 0.010 mM for **4a** and 1.82 ± 0.048 mM for **4b**.
Conclusion and implications: Our results indicated that a derivative of *N*-substitute-acetylpyrrolidine had high potential to inhibit α-glucosidase and α-amylase, and their free radical scavenging properties might be applied to the therapeutic care of patients with type 2 diabetes mellitus.

Keywords: α-Glucosidase and α-amylase inhibitory activity; Diabetes type 2; Type 2 diabetes mellitus, *N*-acetylpyrrolidine.

INTRODUCTION

Diabetes mellitus is a chronic disorder related to a high blood glucose level (hyperglycemia) (1). The blood glucose level is controlled by the starch-hydrolyzing enzyme (2). The long chain of oligosaccharide undergoes hydrolysis by α-amylase to short chain carbohydrate and disaccharides, and finally, the glucose is released from disaccharides by α-glucosidase (3). Therefore, the inhibition of the starch-hydrolyzing enzyme might cause a reduction in the blood glucose level. Many researchers have reported the inhibitors of α-glucosidase and α-amylase, which is obtained from both natural inhibitors and synthetic inhibitors (4-7). Currently, diabetes is treated by inhibiting the starch-hydrolyzing enzyme with commercial inhibitors such as acarbose, miglitol, and voglibose (8-10). However, the use of commercial inhibitors for diabetes therapy involves many side effects, such as allergic reactions, diarrhea, and abnormal liver function (11,12). Thus the candidate of the new starch-hydrolyzing enzyme inhibitor without side effects from natural and synthetic sources might be important.

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N-acetylpyrrolidine and its derivatives have been reported to function in biological activity and pharmaceutical activity. Strobilurin derivatives containing pyrrolidine-2,4-dione have shown fungicidal activity against *Rhizoctonia solani*, *Botrytis cinerea,* and *Fusarium graminearum* (13). A reduction in hyperglycemia after diabetes type 2 diabetes treatment by dipeptidyl peptidase IV (DPP-IV) which inhibits 1-[[(3-hydroxy-1-adamantyl) amino]acetyl]-2-cyano-(*S*)-pyrrolidine has been reported (14). Moreover, some more derivatives (oxopyrrolidine) have shown anti-Alzheimer's properties by acting as acetyl cholinesterase inhibitors (15). Thus, the new candidate of *N*-acetylpyrrolidine derivatives might be important for pharmaceutical activity.

The benzyl group and its derivatives have also shown to have biological activity related to diabetes properties. One report showed that benzyl 2H-chromenones has α-amylase inhibitory properties and radical scavenging activities (16). Additionally, 5-[4-(1-methylcyclohexylmethoxy)benzyl]-thiazolidine-2,4-dione is effective to decrease hyperglycemia and improve insulin sensitivity in *in vivo* assays (17). The α-glucosidase inhibitory activity and free radical scavenging properties have been affected by benzyl-substituted flavones (18). In this study, we synthesized *N*-acetylpyrrolidine linked with -benzyl- and -tosyl- (Fig. 1) to investigate the new α-glucosidase and α-amylase inhibitor for further application in diabetes therapy.

MATERIAL AND METHODS

Material

All the chemicals and enzymes including acarbose, *p*-nitrophenyl-α-glucopyranoside (4-*p*NPG), amylose from potato, α-glucosidase from *Saccharomyces cerevisiae,* and α-amylase from *Aspergillus oryzae,* were obtained from Sigma-Aldrich (St. Louis, MO, USA).

General procedure for preparation of N-substituted-2-acetylpyrrolidine (4a-b)

Preparation of *N*-substituted-2-acetylpyrrolidine **4a-b** was achieved according to the route shown in Scheme 1. *N*-substitution of pyrrolidine-2-carboxylic acid **1** (*L*-proline) was performed using benzyl chloride or tosyl chloride in basic condition followed by esterification with methanol *via* the transformation from acid chloride, which gave the *N*-(substituted)-2-(carbomethoxy) pyrrolidine **3a-b**. *N*-(substituted)-2-acetylpyrrolidine **4a** and **4b** were produced by the Grignard reaction of compound **3a-b** with methyl magnesium iodide.

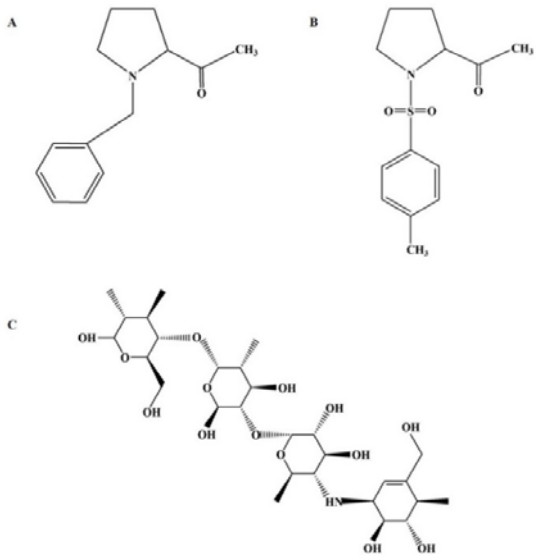
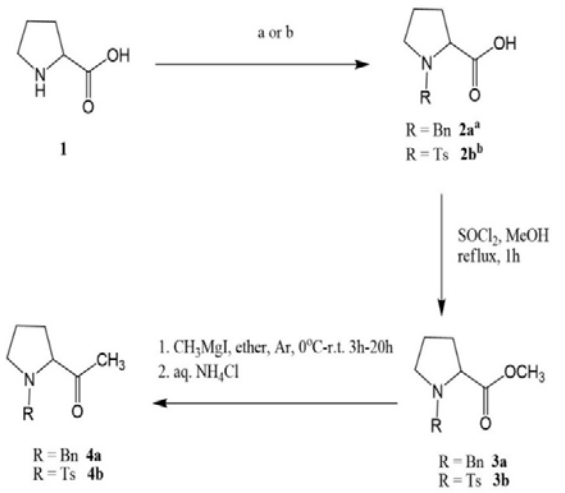


Fig. 1. Structure of (A) *N*-(benzyl)-2-acetylpyrrolidine (**4a**); (B) *N*-(tosyl)-2-acetylpyrrolidine (**4b**); and (C) acarbose.



Scheme 1. Preparation of *N*-substituted-2-acetylpyrrolidine (**4a-b**). DMF, *N*,*N*-dimethylformamide; a, 1. KOH, DMF, 0 °C, 10 min; 2. BnCl, r.t., 3 h; b, 1. 2M NaOH, 0 °C and 2. TsCl, 0 °C, 30 min, then 2M NaOH, room tempreture, 24 h.

N-benzylpyrrolidine-2-carboxylic acid (2a) (19)

Potassium hydroxide (0.63 g, 11 mmol) was added to a stirred solution of pyrrolidine-2-carboxylic acid **1** (*L*-proline, 1.00 g, 8.6 mmol) in *N*,*N*-dimethylformamide (9.0 mL) at 0 °C. After 20 min under argon atmosphere, benzyl chloride (3 mL, 26 mmol) was added dropwise to a stirred solution at 0 °C. The reaction mixture was stirred at room temperature for 3 h. Then, the reaction mixture was poured into ice water and was extracted with diethyl ether (3 × 20 mL). The organic layers were combined, washed with brine solution, dried with anhydrous Na_2SO_4, and then concentrated under reduced pressure to give the crude product, which was purified by column chromatography (silica gel, Hexane:EtOAc, 4:1) to give *N*-benzyl pyrrolidine-2-carboxylic acid **2a** as a pale yellow oil (0.60 g, 34% yield); proton nuclear magnetic resonance (^{1}H-NMR) ($CDCl_3$) {lit.(20)}: δ 1.70-2.20 (m, 3H), 2.35-2.50 (m, 1H), 3.05 (dd, J = 18.9, 9.0 Hz, 1H), 3.32 (dd, J = 18.9, 9.0 Hz, 1H), 3.55-4.00 (dd, J = 108, 24 Hz, 1H), 5.10 (sd, J = 3 Hz, 2H), 7.20-7.40 (m, 5H, Ar).

N-tosyl pyrrolidine-2-carboxylic acid (2b) (21)

Pyrrolidine-2-carboxylic acid **1** (*L*-proline, 1.00 g, 8.6 mmol) was dissolved in aqueous 2.0 M NaOH (8.6 mL) at 0 °C. The *p*-toluenesulfonyl chloride (1.60 g, 8.4 mmol) was added to a stirred solution. After 30 min; the aqueous 2.0 M NaOH (0.5 mL) was added to a stirred solution. After 24 h, the solution was washed with diethyl ether (2 × 100 mL) the aqueous layers were combined with aqueous 2.0 M NaOH (20 mL) and acidified to pH 1.0 with concentrated HCl. The aqueous layer was extracted with ethyl acetate (3 × 30 mL) and the organic layers were combined, washed with brine, dried with anhydrous Na_2SO_4, and then concentrated under reduced pressure to produce *N*-tosyl pyrrolidine-2-carboxylic acid **2b** as a pale yellow oil (2.14 g, 94% yield); ^{1}H-NMR ($CDCl_3$){lit.(22)}:δ = 1.93-2.14 (m, 4 H), 2.44 (s, 3 H), 3.23-3.29 (m, 1 H), 3.49-3.55 (m, 1 H), 4.18-4.28 (dd, J = 8.4, 4.4 Hz, 1 H), 7.33-7.35 (d, J = 8.4 Hz, 2H), 7.76-7.78 ppm (d, J = 8.0 Hz, 2H), 8.15 ppm (brs, 1H, OH).

General procedure for the preparation of N-(substituted)-2-(carbomethoxy) pyrrolidine (3a-b) (23)

A solution of *N*-(substituted) pyrrolidine-2-carboxylic acid (30 mmol) in dry methanol (30 mL) was cooled to 0 °C. The $SOCl_2$ (33 mmol) was added dropwise to the reaction mixture. The solution was stirred for 1 h under refluxing conditions and then allowed to cool to room temperature. The solvent was removed *in vacuo*, producing *N*-(substituted)-2-(carbomethoxy) pyrrolidine.

N-(benzyl)-2-(carbomethoxy) pyrrolidine (3a)

The following general procedure was employed: treatment of *N*-benzyl pyrrolidine-2-carboxylic acid **2a** (0.56 g, 2.7 mmol) with $SOCl_2$ (0.3 mL, 4.1 mmol) in dry methanol (3 mL) gave the *N*-(benzyl)-2-(carbomethoxy)pyrrolidine **3a** as a white solid (0.52 g, 88% yield); NMR ($CDCl_3$){lit.(24)}: δ 1.70-2.20 (m, 3 H), 2.35-2.45 (m, 1H), 3.03 (dd, J = 8.5, 6.2 Hz. 1 H), 3.24 (dd, J = 8.6, 6.3 Hz, 1H), 3.57-3.98 (dd, J = 117, 24 Hz, 1H), 3.64 (s, 3 H), 5.10 (sd, J = 3 Hz, 2H), 7.20-7.45 (m, 5 H).

N-(tosyl)-2-(carbomethoxy) pyrrolidine (3b)

The following general procedure was employed: treatment of *N*-tosyl pyrrolidine-2-carboxylic acid **2b** (0.50 g, 1.8 mmol) with $SOCl_2$ (0.4 mL, 4.9 mmol) in dry methanol (2 mL). Then, the reaction mixture was concentrated under reduced pressure and the residue was stored in an ice chest overnight. Then, the residue was poured into an aqueous solution of potassium carbonate (50% w/v, 3.0 mL) at 0 °C, which produced *N*-(tosyl)-2-(carbomethoxy) pyrrolidine **3b** as a yellow oil (0.36 g, 71% yield); NMR ($CDCl_3$){lit.(25)}: δ 1.70-1.85 (m, 1H), 1.90-2.10 (m, 3H), 2.43 (s, 3H), 3.30 (ddd, J = 4.5, 7.1, 9.5 Hz, 1H), 3.50 (ddd, J = 4.7, 7.2, 9.8 Hz, 1H), 3.75 (s, 3H), 4.32 (dd, J = 4.6, 8.0 Hz, 1H), 7.32 (d, J = 8.2 Hz, 2H), 7.80 (d, J = 8.2 Hz, 2H).

General procedure for the preparation of N-(substituted)-2-acetylpyrrolidine (4a-b) (26)

The Grignard reagent was prepared from magnesium turnings (120 mmol) and methyl iodide (120 mmol) in dry ether (120 mL) under reflux with an argon atmosphere for 1 h. The solution of *N*-(substituted)-2-(carbomethoxy) pyrrolidine (40 mmol) in dry ether (20 mL) was cooled in an ice bath, which was added to freshly prepared methyl magnesium iodide at 0 °C and stirred continuously at 0 °C to room temperature for 20 h. The supernatant was cautiously poured into a stirred ice-cold aqueous ammonium chloride solution. The viscous residue remaining in the reaction flask was triturated with the ice-cold mixture. The clear organic and aqueous layers were vigorously stirred for 20 min at ambient temperature. The organic layer was isolated, and the aqueous phase was extracted with ether (2 × 20 mL). The ether extracts were combined and dried with anhydrous Na_2SO_4. The drying agent was filtered, and the solvent was removed *in vacuo* without heating to produce *N*-(substituted)-2-acetylpyrrolidine.

N-(benzyl)-2-acetylpyrrolidine (4a)

The following general procedure was employed: treatment of *N*-(benzyl)-2-(carbomethoxy) pyrrolidine **3a** (0.36 g, 1.6 mmol) with freshly prepared methyl magnesium iodide to give *N*-(benzyl)-2-acetylpyrrolidine **4a** as a pale yellow oil (0.29 g, 91% yield); NMR ($CDCl_3$){lit.(27)}: 1.29 (s, 3H), 1.70-2.00 (m, 3H), 2.32-2.49 (m, 1H), 3.03 (dd, J = 8.3, 12.0 Hz, 1H), 3.31 (dd, J = 8.3, 12.1 Hz, 1H), 3.51-3.98 (dd, J = 108, 15 Hz, 1H), 5.10 (sd, J = 3 Hz, 2H), 7.15-7.41 (m, 5H).

N-(tosyl)-2-acetylpyrrolidine (4b)

The following general procedure was employed: treatment of *N*-(tosyl)-2-(carbomethoxy) pyrrolidine (3b) (0.10 g, 0.35 mmol) with freshly prepared methyl magnesium iodide to give *N*-(tosyl)-2-acetylpyrrolidine (**4b**) as a pale yellow oil (0.083 g, 88% yield); NMR ($CDCl_3$){lit.(28)}: 0.90-1.74 (m, 4H), 1.25 (s, 3H), 2.40 (s, 3H), 3.10-3.28 (m, 1H), 3.50-3.62 (m, 2H), 7.25 (d, J = 9.2 Hz, 2H), 7.68 (d, J = 9.2 Hz, 2H).

α-Glucosidase assay

The α-glucosidase inhibitory properties of each compound were measured using a spectrophotometric method with some modification of a procedure described in a previous study (7). The samples (**4a** and **4b**) were prepared in 50 mM sodium phosphate buffer, pH 6.8. In the 100 µL mixture reaction, 10 µL of different concentrations of samples (final concentration of 0.04 to 8.00 mM), 5 µL of 0.25 mM 4-*p*NPG, and 5 µL of 0.05 mg/mL α-glucosidase were included. The reaction was incubated at 37 °C for 30 min. Then, the enzyme activity was stopped with 100 µL of 0.5 M Na_2CO_3. The release of the *p*-nitrophenolate group was monitored with a spectrophotometer at 405 nm. The inhibitory activity of each compound on α-glucosidase was calculated from the concentration that inhibited 50% of the enzyme activity (IC_{50}). Acarbose was used as the standard inhibitor. The percentage of inhibition of samples and acarbose on α-glucosidase was calculated using equation below. The IC_{50} values of the samples and acarbose were calculated using the Grafit 5.0 computer program (Erithacus Software, Horley, UK).

$$\text{Inhibition (\%)} = \frac{A - B}{A} \times 100$$

where, A is the activity of the enzyme without the sample solution and B is the activity of the enzyme with the sample solution.

α-Amylase assay

The α-amylase activity was determined using a modification of previous methods (29). The reaction was mixed with 10 µL of different concentrations of samples (final concentration of 0.05 to 11.00 mM), 10 µL of 0.05 mg/mL amylose, and 10 µL of 0.05 mg/mL of α-amylase in 20 mM sodium phosphate buffer, pH 6.8. This mixed solution was incubated at 37 °C for 30 min and then the reaction was stopped in boiling water for 5 min. The amount of glucose was determined with a peroxidase-glucose oxidase assay. The reaction was terminated by adding 100 µL of couple enzyme (peroxidase-glucose oxidase

enzyme) and 100 µL of 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid and then further incubated at 37 °C for 30 min. The absorbance was monitored using spectrophotometry at 475 nm. The α-amylase inhibition was calculated as percentage inhibition using aforementioned equatione. IC_{50} values were calculated in the same way as for α-glucosidase.

Kinetic study

The kinetic analysis (inhibition type) of each enzyme was determined from Lineweaver-Burk and Dixon plots. The inhibition constants (K_i and K_i') were determined from slope (from Linweaver-Burk plot) *vs* I for K_i and $1/V_{max}$ *vs* I for K_i'. The inhibition type of each enzyme was maintained at the same concentration as the reaction above, while the concentrations of **4a** and **4b** were 0.30 to 0.60 mM. The 4-*p*NPG and amylose were used as substrates for α-glucosidase and α-amylase, respectively, and acarbose was used as the standard inhibitor.

DPPH assay

The antioxidant activity was determined using a modification of a method used in a previous study (30). The samples (**4a** and **4b**) were dissolved in MeOH and then added to 1 mM DPPH solution (100 µL) to give the final concentration of 0.04 to 11.00 mM. The reaction mixture was incubated at room temperature under dark conditions. The absorbance of the final product was measured at 517 nm with a UV/Vis spectrophotometer (AOE Instruments, Shanghai, China).

Statistical analysis

The inhibition percentage and IC_{50} data were expressed as standard division of mean (means ± SD). One-way analysis of variance (ANOVA) was used to investigate the statistical significance. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Chemistry

The *N*-substituted-2-acetylpyrrolidine **4a** and **4b** samples were prepared from commercial pyrrolidine-2-carboxylic acid **1** (L-proline), which was used as the starting material. The preparation was started from the preparation of *N*-substituted pyrrolidine-2-carboxylic acid **2a-b**. The pyrrolidine-2-carboxylic acid **1** underwent protection of the amino group with benzyl chloride (19) and *p*-toluene sulfonyl chloride (21) under basic conditions, providing *N*-benzylpyrrolidine-2-carboxylic acid **2a** at 34% w/w yield after purification by column chromatography and *N*-tosylpyrrolidine-2-carboxylic acid **2b** at 94% w/w yield. The *N*-substituted pyrrolidine-2-carboxylic acid **2a-b** then underwent one-pot esterification using a procedure *via* conversion of the carboxyl group to acid chloride by refluxing with thionyl chloride in dry methanol, affording *N*-(substituted)-2-(carbomethoxy) pyrrolidine **3a-b** at an excellent yield (23). Following the Grignard protocol (26), reaction of *N*-substituted pyrrolidine-2-carboxylic acid **2a-b** with methyl magnesium iodide in ether at 0 °C to room temperature led to the transformation of methyl ester moiety to methyl ketone, which provided the *N*-substituted-2-acetylpyrrolidine **4a-b** at an excellent yield without purification. The structures of these synthesized compounds were characterized by their ^{1}H-NMR spectral data according to a previous study.

Enzyme inhibition and antioxidant activity of 4a and 4b

The inhibitory potential of **4a** and **4b** with the inhibition percentage and IC_{50} values are shown in Table 1. Samples **4a** and **4b** were presented to inhibit α-glucosidase and α-amylase with inhibition percentages of 40.27 ± 0.80 and 32.31 ± 0.29 for α-glucosidase and 28.93 ± 0.57 and 30.11 ± 0.78 for α-amylase. The IC_{50} values of both samples against α-glucosidase and α-amylase were showed of 0.52 ± 0.02 mM and 1.64 ± 0.08 mM for α-glucosidase and 2.72 ± 0.09 mM and 3.21 ± 0.65 mM for α-amylase. The inhibition type and constant (*K*i and *K*i′) of **4a** and **4b** on α-glucosidase and α-amylase are shown in Table 2 and Figs. 2-5. The inhibition type of both samples were presented in mixed type inhibition which the same as standard inhibitor acarbose.

Table 1. Inhibitory potential of **4a** and **4b** on α-glucosidase and α-amylase. Values represent the mean ± SD, n = 3

Samples	Inhibition at 0.02 mM (%)		IC_{50} values (mM)	
	α-Glucosidase	α-Amylase	α-Glucosidase	α-Amylase
4a	40.27 ± 0.80^{b}	28.93 ± 0.57^{b}	0.52 ± 0.02^{b}	3.21 ± 0.65^{a}
4b	32.31 ± 0.29^{c}	30.11 ± 0.78^{b}	1.64 ± 0.08^{a}	2.72 ± 0.09^{a}
Acarbose	71.34 ± 1.10^{a}	61.68 ± 1.14^{a}	0.16 ± 0.01^{c}	0.22 ± 0.002^{b}

The different superscript letters indicating significant difference among mean values within a same column ($P < 0.05$).

Table 2 . Kinetic parameters of **4a** and **4b.**

Samples	α-Glucosidase			α-Amylase		
	K_i (mM)	K_i' (mM)	Type	K_i (mM)	K_i' (mM)	Type
4a	0.29	2.44	Mixed type	0.23	2.35	Mixed type
4b	0.23	5.61	Mixed type	0.27	0.71	Mixed type
Acarbose	0.09	0.41	Mixed type	0.06	0.27	Mixed type

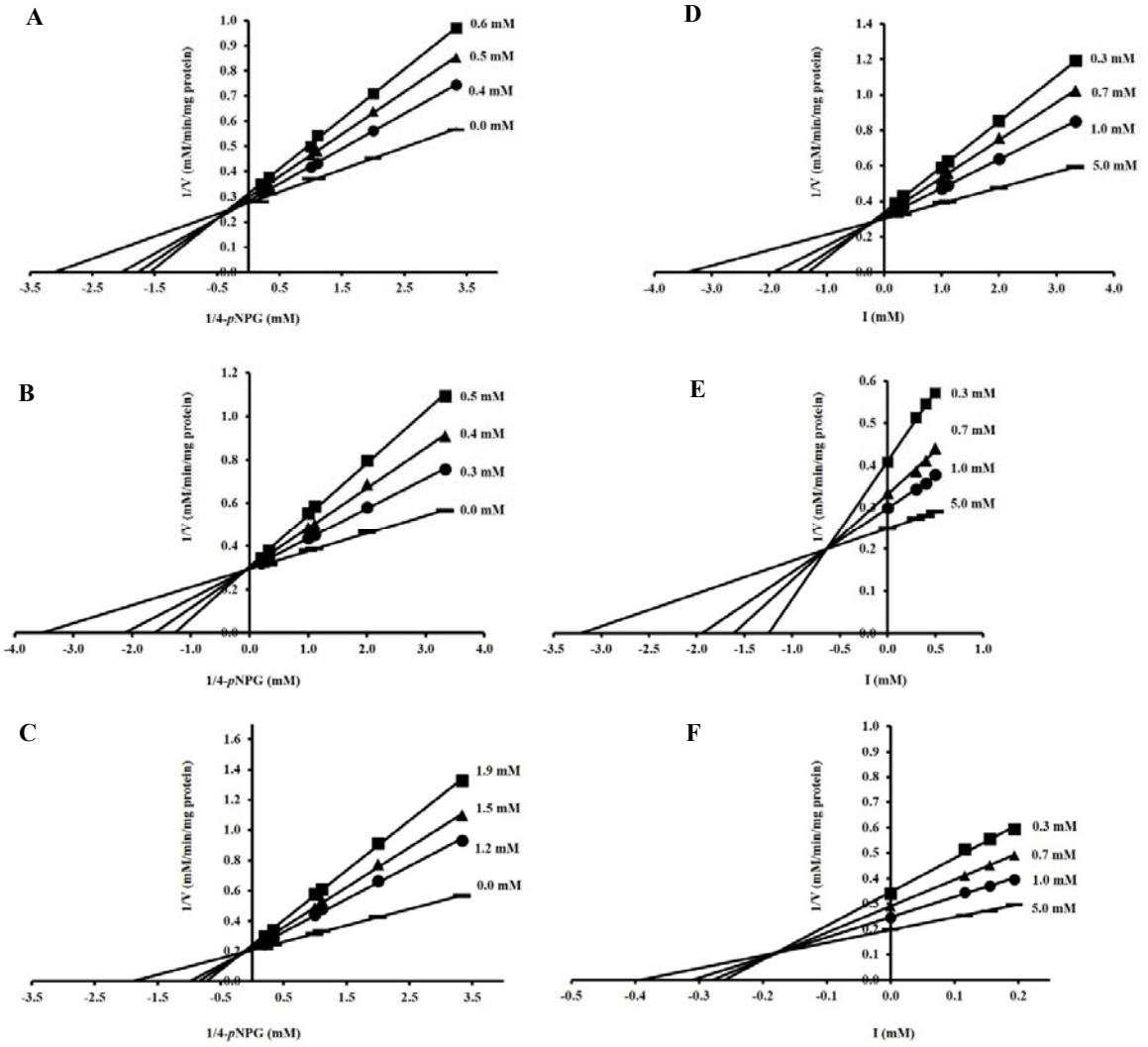


Fig. 2. Lineweaver-Burk plot of (A) **4a**, (B) **4b**, (C) acarbose; and Dixon plots of (D) **4a**, (E) **4b**, and (F) acarbose for α-glucosidase inhibitory activity.

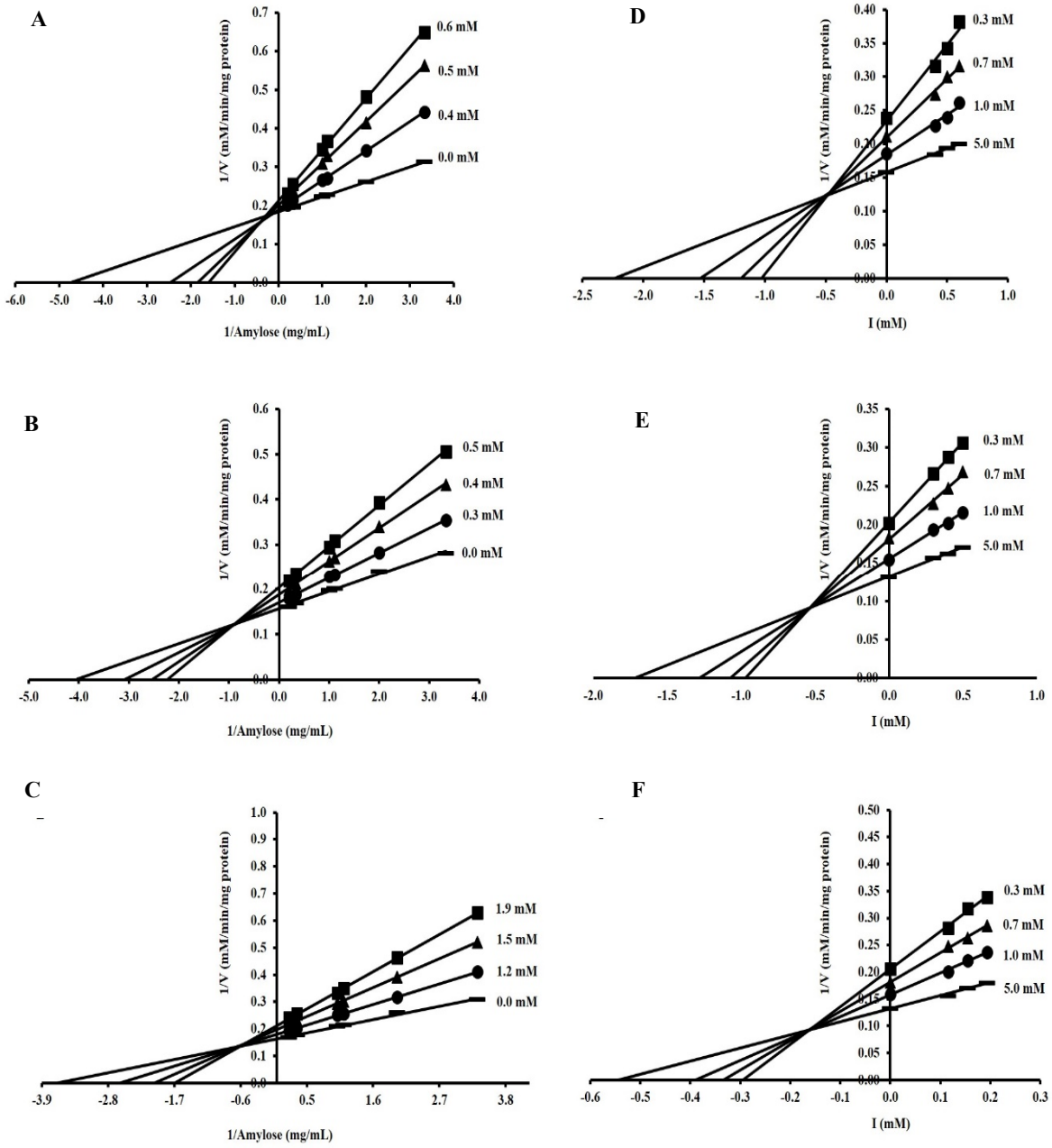


Fig. 3. Lineweaver-Burk plot of (A) **4a**, (B) **4b**, (C) acarbose; and Dixon plots of (D) **4a**, (E) **4b,** and (F) acarbose for α-amylase inhibitory activity.

Table 3. Antioxidant potential of **4a** and **4b.** Values represent the mean ± SD, n = 3 .

Samples	Inhibitory activity at 0.5 mM (%)	IC_{50}) mM (
4a	41.62 ± 0.64^{a}	1.01 ± 0.010^{b}
4b	34.28 ± 0.73^{b}	1.82 ± 0.048^{a}

The different superscript letters indicating significant difference among mean values within a same column ($P < 0.05$).

The antioxidant potentials of **4a** and **4b** are shown in Table 3. The results reveal that the IC_{50} values were low for compound **4a** (1.01 ± 0.010 mM) and high for compound **4b** (1.82 ± 0.048 mM), indicating that the antioxidant capacity among the compounds is significantly higher ($P < 0.05$) in compound **4a**.

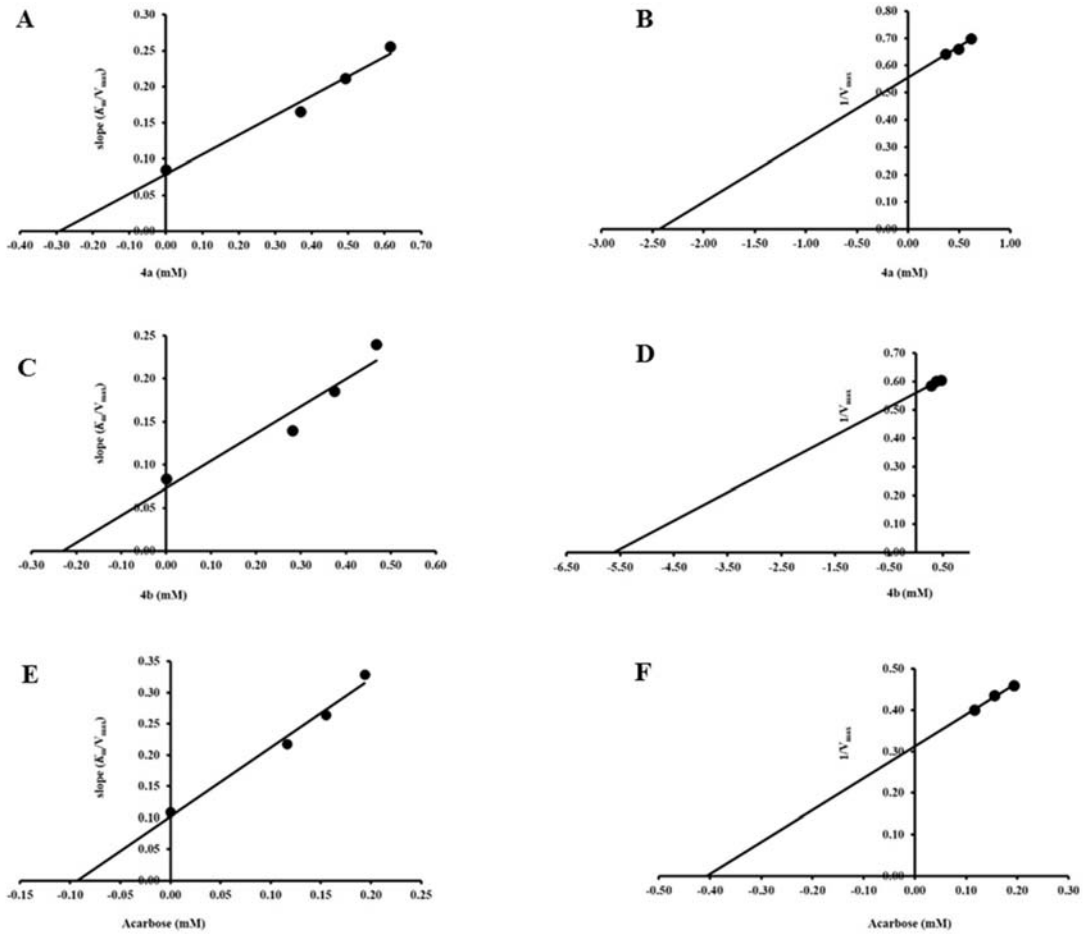


Fig. 4. Secondary plot of slope (K_m/V_{max}) *vs* [I] for determination of K_i and secondary plot of $1/V_{max}$ *vs* [I] for determination of K_i' of (A and B) **4a**, (C and D) **4b**, and (E and F) acarbose for α-glucosidase inhibitory activity.

DISCUSSION

Based on the inhibition percentage, the inhibition activity of **4a** and **4b** on α-glucosidase had higher potential than α-amylase. However, the inhibition percentages of both **4a** and **4b** were lower than the standard inhibitor acarbose by approximately 2.21 times on α-glucosidase and 2.13 times on α-amylase. Based on the IC_{50} values of these compounds, the inhibitory activity on α-glucosidase and α-amylase was consistent with the inhibition percentage. **4a** had the highest inhibitory effect on α-glucosidase inhibitory activity with IC_{50} values of 0.52 ± 0.02 mM, whereas **4b**, with IC_{50} values of 1.64 ± 0.08 mM, had also higher potential on α-glucosidase inhibitory activity than α-amylase inhibitory activity but still less potential than **4a** on α-glucosidase inhibitory activity. The lowest inhibitory activity was obtained from **4a** on α-amylase inhibitory activity with IC_{50} values of 3.21 ± 0.65 mM and approximately 1.20 times less potential than **4b** on α-amylase inhibitory activity and less potential than **4b** and **4a** on α-glucosidase inhibitory activity by approximately 2.0 times and 6.2 times, respectively. A previous researcher also reported the inhibitory potential of compound **9a/b** (N-benzyl-benzimidazolyl) on α-glucosidase and α-amylase. The compound **9b** with a chloro substitution at position 4 of the N-benzyl ring had higher potential on α-glucosidase than compound **9a** without chloro substitution (6). Our results also showed the high potential of compound **4a** (N-(benzyl)-2-acetylpyrrolidine) on α-glucosidase, which is consistent with previous reports.

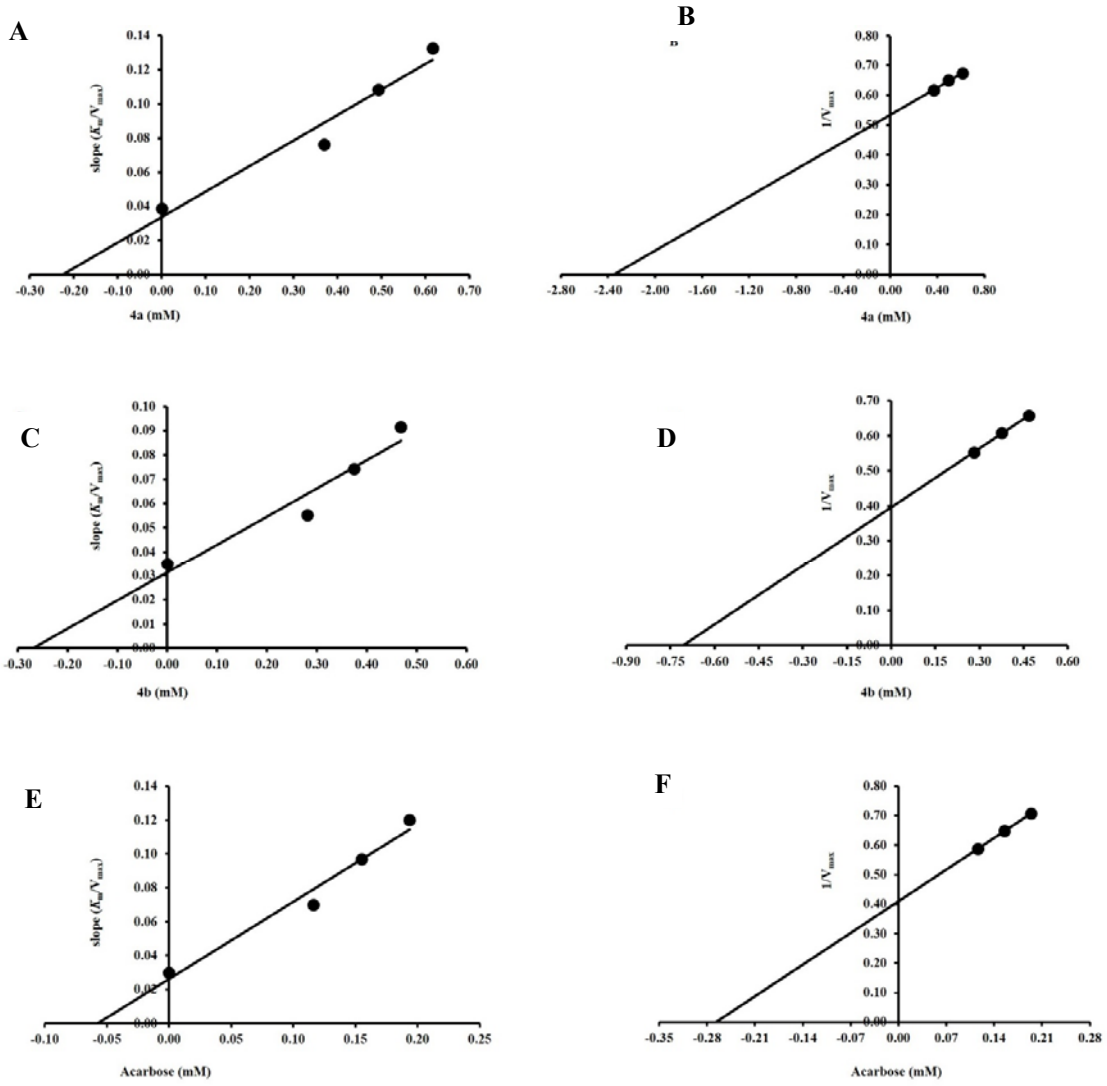


Fig. 5. Secondary plot of slope (K_m/V_{max}) *vs* [I] for determination of K_i and secondary plot of $1/V_{max}$ *vs* [I] for determination of K_i' of (A and B) **4a**, (C and D) **4b**, and (E and F) acarbose for α-amylase inhibitory activity.

The Lineweaver-Burk plots suggest that **4a** worked against both α-glucosidase (from *Saccharomyces cerevisiae*) and α-amylase (from *Aspergillus oryzae*) functions in mixed type inhibition. The **4b** compound against α-glucosidase and α-amylase was also shown in mixed type inhibition. The inhibition type of the standard inhibitor acarbose that retarded both α-glucosidase and α-amylase was also determined to be mixed type inhibition (Figs. 2 and 3). As shown in Figs. 4-6, the *K*i and *K*i′of **4a** were 0.29 and 2.44 mM for α-glucosidase and 0.23 and 2.35 mM for α-amylase, which indicates that it is more likely to bind with a free enzyme than the enzyme-substrate complex. The *K*i values of **4a** on α-glucosidase and α-amylase showed little difference, and the results indicated that it is suitable to bind for the active site of α-amylase. Base on the *K*i and *K*i′ values of **4b**, the results showed that it also more likely to bind with a free enzyme than the enzyme-ubstrate complex. The *K*i and *K*i′of **4b** were 0.23 and 5.61 mM for α-glucosidase and 0.27 and 0.71 mM for α-amylase, respectively. Moreover, the binding efficiency of **4b** with α-glucosidase was shown to be higher than with α-amylase. A previous study showed that the inhibitory activity of

the benzyl group has a function in non-competitive inhibition on α-glucosidase with a *K*i of 18 mM toward maltose (30). Another study showed the α-glucosidase inhibitory activity of *N*-benzyl derivative in a rat model (31). Our results and previous reports suggest that the compounds linked with -benzyl- and -tosyl- have potential inhibitory activity on α-glucosidase and α-amylase.

CONCLUSION

N-acetylpyrrolidine substitute with -benzyl- (**4a**) and -tosyl- (**4b**) was synthesized and investigated for inhibitory activity against diabetes type 2 diabetes α-glucosidase and α-amylase and free radical scavenging. The inhibition percentages values revealed that **4a** and **4b** showed the highest potential inhibitory activity on α-glucosidase with IC_{50} values of 0.52 ± 0.02 and 1.64 ± 0.08 mM, respectively. The kinetic analysis suggests that **4a** and **4b** against α-glucosidase and α-amylase function in mixed type inhibition. The DPPH assays of both compounds showed that **4a** had higher potential for free radical scavenging compared to **4b**.

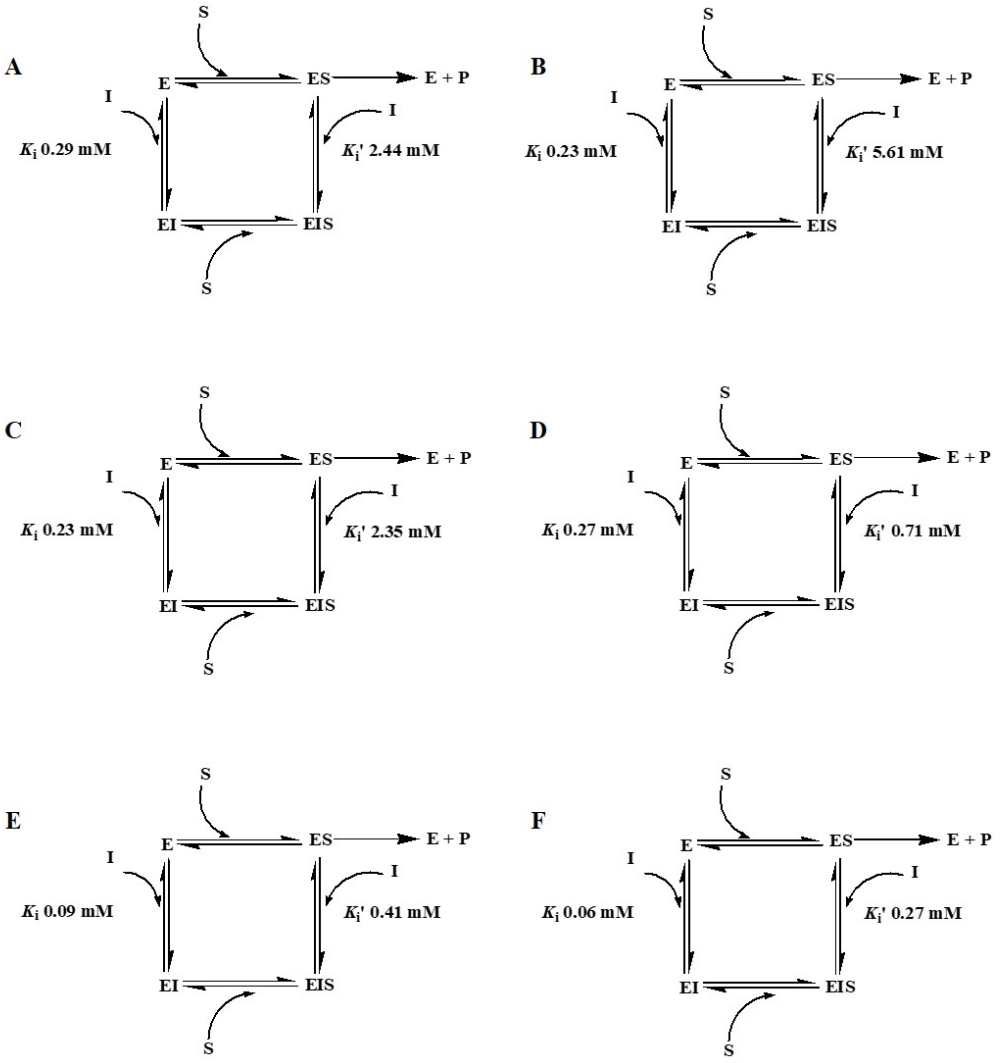


Fig. 6. Proposed inhibitory mechanism of (A and B) **4a** and **4b** against α-glucosidase, (C and D) **4a** and **4b** against α-amylase, and (E and F) acarbose against α-glucosidase and α-amylase. E, Enzyme; S, substrate; P, product; I, inhibitor; ES, enzyme-substrate complex; EI, enzyme-inhibitor complex; ESI, enzyme-substrate-inhibitor complex.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

The experiment and data analysis were provided by S. Sansenya, K. NaNok, C. Winyakul, and W. S. Phutdhawong. English language was checked by S. Sansenya. The manuscript was written by S. Sansenya.

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