Original Article

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Microwave-assisted synthesis, *in silico* studies and *in vivo* evaluation for the antidiabetic activity of new brominated pyrazoline analogs

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ABSTRACT

Introduction: Pyrazolines have played a crucial role in the drug discovery researches and have been used widely as important pharmacophores or intermediate for the synthesis of bioactive compounds. **Objective:** The aim of this work is to explore the potential of new brominated pyrazoline analogs as antidiabetic. **Materials and Methods:** Synthesis of brominated pyrazoline analogs has been conducted under microwave irradiation, *in silico* studies were performed using AutoDock 1.5.4 software packages and nanoscale MD Program 2.9, and the animal used in the *in vivo* evaluation is male albino mice (*Mus musculus* L.). **Results:** The *in silico* studies for antidiabetic activity showed that compound 3a is the most active compound and furthermore it can be developed as new active agents for antidiabetic. The *in vivo* evaluation showed that compound 3a has a good ability to increase the percentage of change in blood glucose level and weight loss prevention, decreasing of the drinking water and also decreasing of the urine volume, significantly ($P < 0.05$) compared with negative control with dosage of 25, 50, and 100 mg/kg of the body weight. The oral administrations of compound 3a with dosage 50 and 100 mg/kg of body weight did not show the significant difference ($P > 0.05$) in the ability to increase the percentage of change in the loss of weight prevention compared with the glibenclamide. Then, the oral administrations of compound 3a with a dosage of 25, 50, and 100 mg/kg of body weight did not show the significant difference ($P > 0.05$) in the ability to decrease the drinking and urine volume compared with the glibenclamide. In addition, the oral administration of compound 3a also did not show the significant effect ($P > 0.05$) on change in the relative weight of organs of treated diabetic mice, compared to the normal control in the given dosages. **Conclusion:** This strategy reflects a logical progression for early-stage drug discovery that can be used to successfully identify drug candidates for antidiabetic agents.

Keywords: Antidiabetic activity, brominated pyrazolines, *in vivo* evaluation, microwave-assisted synthesis, molecular docking studies, molecular dynamic simulation

INTRODUCTION

Diabetes mellitus is a complex metabolic syndrome. It is caused by the abnormality of the carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin.[1] It is a major of human health concern in the world over, a common and very prevalent disease affecting to the citizens in the world. Assuming that age-specific prevalence remains constant, the people who are infected by diabetic in the world are expected to approximately double between 2000 and 2030, based solely on demographic changes. In 2000, the number of people with diabetes is 171 million people. The latest estimates showed a global prevalence of 366 million people with diabetes in 2030[2] and expected to rise to 592 million on 2035.[3]

Pyrazolines have played a crucial role in the drug discovery researches and they have been used widely as

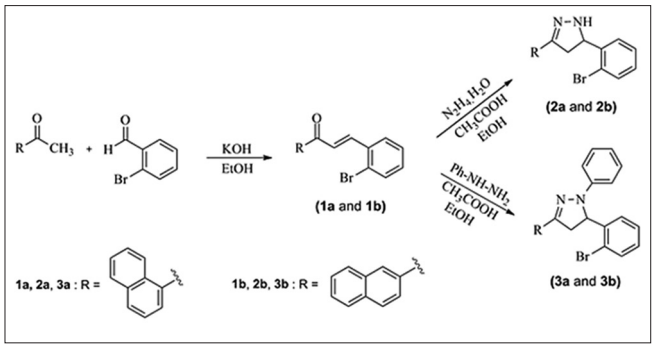

Figure 1: Scheme of the synthesis new brominated pyrazoline analogs

important pharmacophores or intermediate for the synthesis of bioactive compounds.[4] Scheme of the synthesis new brominated pyrazoline analogs is presented in Figure 1. Pyrazolines have numerous prominent effects, such as antimicrobial, antiamoebic, anti-inflammatory, anticancer, antidepressant, antiandrogenic, and antiviral activities.[5] Some patented pyrazolines have been used for the treatment of food disorders, including obesity or metabolic syndrome in patients with developed diabetes[6] and treatment of diabetes and also related diseases, especially Type II diabetes.[7] In addition, the presence of atom bromine in an organic compound has been reported can enhance the hypoglycemic activity. Introducing atom bromine to coumarin sulfonylurea compound has been reported can enhance their hypoglycemic activity.[8] Another work reported that some brominated pyrazoles are also shown the higher hypoglycemic activity than antidiabetic drug.[9] In the view of the importance pharmacological of pyrazolines and bromo substituent, it was thought worthwhile to construct some new pyrazoline analogs containing with bromo substituent.

There are some methods that have been used to synthesize the pyrazolines analogs, such as grinding, stirring, and reflux. However, the microwave-assisted synthesis is a more attractive method for researchers.[10] This method has been widely applied to synthesis some various heterocyclic compound[11] and showed some advantages, such as reduce in reaction time, increase the yield by increasing the selectivity of reaction and it is a very easy and simple method when combined with supporting reagents[12] and appropriate irradiation power.[13] Thus, so far, computational approaches can aid in drug discovery studies in various ways. In this study, we are interested to explore the potential of some brominated pyrazoline analogs as antidiabetic, considering that the prevalence of diabetes mellitus patients is increasing every year. The pyrazoline analogs have been synthesized under microwave irradiation for then can be used as an antidiabetic agent with the aid of *in silico* way (i.e., molecular docking and molecular dynamic [MD] simulation). In addition, *in vivo* evaluation has been also conducted to evaluate the results from *in silico* studies and to confirm the effect of oral administration of compound 3a on the percentage of change in the blood glucose level and also weight loss prevention, decreasing the drinking and urine volume and to obtain the effect of the oral administration on the change of relative weight of liver, kidney, and heart of treated diabetic mice.

MATERIALS AND METHODS

General Information's

The materials used to synthesize the brominated pyrazoline analogs included 1-acetyl naphthalene, 2-acetyl naphthalene, 2-bromobenzaldehyde, potassium hydroxide, hydrochloric acid, hydrazine hydrate, phenylhydrazine, glacial acetic acid, and some organic solvents, such as ethanol, *n*-hexane, and ethyl acetate were produced by Merck and or Sigma-Aldrich. The materials used to evaluate the antidiabetic activity included alloxan (Aldrich), 10% glucose solution (Otsuka), sodium carboxymethyl cellulose (NaCMC) (Brataco), sodium chloride solution (Otsuka), and glibenclamide (IndoFarma).

All the synthesis reactions were performed in a Samsung ME109F domestic microwave oven. The melting point was determined on a SMP 11 apparatus (Stuart®) (uncorr). Thin-layer chromatography analysis was performed using GF254 (Merck Millipore) under UV Lamp 254/366 nm (Camag™). High-performance liquid chromatography (HPLC) chromatograms were recorded on Shimadzu LC solution. UV spectrum was recorded on Genesys™ 10S UV visible spectrophotometer (Thermo Scientific™), Fourier transform-infrared (FT-IR) spectra were recorded in KBr powder on a Shimadzu® FT-IR Prestige-21 spectrophotometer (Shimadzu Corporation), and ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectral data were recorded on a JEOL JNM ECA at 500 and 125 MHz, respectively. Mass spectral data were recorded on a LC-mass spectrometry (MS) (Mariner Biospectrometry). The blood glucose levels were measured on GlucoDr™ Blood Glucose Test Meter (All Medicus).

General Procedures

Synthesis of brominated pyrazoline analogs

Preparation of compounds 1a and 1b

Acetylnaphthalene (5 mmol) and 2-bromobenzaldehyde (5 mmol) were dissolved in absolute ethanol (10 mL) in an Erlenmeyer and potassium hydroxide 1N (5 mL) was added. Then, the mixture was irradiated using a domestic microwave (180 W) for 3 min. After the reaction was completed, cold distilled water (10 mL) was added into the mixture and was neutralized with hydrochloric acid 1N to afford the precipitate. The precipitate was filtered in vacuo, washed using cold *n*-hexane, dried in a desiccator, and re-crystallized with mixture of ethyl acetate and *n*-hexane to get pure 1a and 1b.

Preparation of compounds 2a, 2b, 3a, and 3b

Compounds 1a and 2b (1 mmol) were dissolved in absolute ethanol (15 mL) in a closed reaction vessel using an ultrasonicator. Then, hydrazine compound (5 mmol) and five drops of glacial acetic acid were added. The mixture was irradiated using a domestic microwave (180 W) for 2 min. After the reaction was completed, the mixture was cooled in the ice bath to afford the precipitate. The precipitate was filtered in vacuo, washed using cold *n*-hexane, dried in a desiccator, and re-crystallized in a mixture of ethyl acetate and *n*-hexane to get pure 2a, 2b, 3a, and 3b.

In silico studies for antidiabetic activity

Preparation of protein

Molecular docking was conducted using AutoDock 1.5.4 software packages. Docking was performed after finishing the preparation of protein and ligand. Protein was prepared with retrieving the three-dimensional of the crystal structure. This crystal structure was downloaded from PDB database with PDB ID 2QMJ (α-glucosidase) for then it can be used as the macromolecule for molecular docking. This protein must be kept rigid. For then, all hydrogen atoms were added, merging non-polar hydrogen atoms, checking and repairing missing atoms, adding Gasteiger charges, checking and fixing total charges on residues, and assigning atom types to the protein structure. Autogrid 4 software was used to generate a grid box of the protein structure with default atom types (carbon, hydrogen, oxygen, and nitrogen), grid spacing of 0.35 Å, with the dimensions of 122 × 116 × 120 points along the X-, Y-, and Z-axes and centered on the protein for the docking.

Preparation of ligands

These six compounds were used as the ligands and drawn using ChemDraw as depicted in Figure 2. In this case, these ligands must be kept flexible. All the ligands were required to minimize the energies for then the minimized structures were subsequently prepared with the detected root of torsion and number of torsions for flexible-ligand docking using AutoDock Tools 1.5.4 software.

Analyzing and output visualization

The docking poses were ranked based on the docking scores. The binding affinity of one ligand to the receptor molecule can be predicted using the scoring function in AutoDock. The lowest binding affinity was selected as the best docking conformation for then it can be used for analysis after the docking process. The molecular visualization of the docked complexes was performed using the Discovery Studio Visualizer (Biovia).

MD simulation

Preliminary study for MD was performed using NAMD (Nanoscale MD Program; v 2.9), it was used for modeled protein for then all the files were generated using VMD (visual MD).[14] The protein was solvated with a TIP3P water box with a 2.0 Å layer of water for each direction of the coordinate structure, and a Chemistry at Harvard Macromolecular Mechanics was used as a force field.

In vivo evaluation for antidiabetic activity

The animal used in this *in vivo* evaluation is male albino mice (*Mus musculus* L.), 20–30 g weight. Before used, the mice were acclimated for a week. The mice were declared as healthy if they did not show 10% changing of the body weight and also did not show any healthy problem symptom, visually. In this study, the animal used is followed by the Ethical Clearance No. 178/UN.19.5.1.1.8/UEPKK/2018. There are 48 mice were used in this study, they were divided into eight mice for the normal control group, eight mice for the negative control group, eight mice for the positive control group, and 24 mice were used in treated groups (i.e. 25, 50, and 100 mg/kg of the body weight).

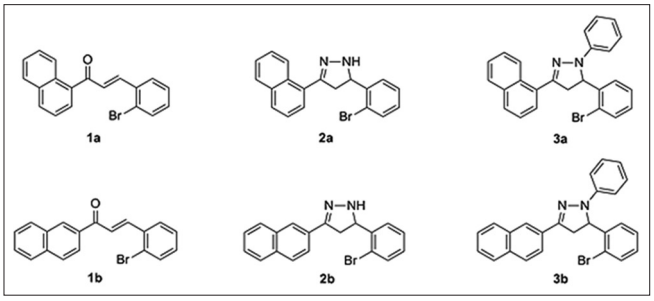

Figure 2: Structure of the six ligands

Preparation of dosage and suspension

Compound 3a was oral administration to the diabetic mice with three dosages (25, 50, and 100 mg/kg of body weight). Volume of oral administration is 1% of mice weight was made in 5 mL of 1% NaCMC suspension. Glibenclamide was oral administration to the diabetic mice with dosage 5 mg/kg of body weight with conversion factor to mice dosage 5 mg/kg of body weight × 0.0026 = 0.013 mg/20 g of body weight = 0.65 mg/kg of body weight. Alloxan was injected in a dosage 175 mg/kg of body weight.

Inducing to diabetic

The albino mice were fasted for 18 h before induced to the diabetic. The mice were injected with alloxan solution, intraperitoneally. The blood glucose level before and after inducing to the diabetic was measured. After alloxan injection, 10% glucose solution was given to the mice for 2 days. The blood glucose level was measured 2 days after alloxan induction.

For 3rd until the 7th day, drinking water was given to the mice and each mouse was moved into a cage, where a cage contained a mice and the blood glucose level of each mouse was also measured. If mice were diabetic (blood glucose level >200 mg/dL), they were treated with suspension of compound 3a, orally. The blood glucose level of treated mice was measured every 2 days (1st, 3rd, 5th, and 7th day).

Treating with compound 3a

Randomly, the albino mice were divided into six groups. Each group contained five mice. The first group is normal mice (did not induced to diabetic and did not treated). The second group is negative control (just treated with 1% NaCMC). The third group is positive control (treated with glibenclamide in a dosage of 0.65 mg/kg of body weight). Group four-six were treated with compound 3a in dosages of 25, 50, and 100 mg/kg of body weight, respectively. The mice were treated with compound 3a, orally, 1 time a day for a week.

Measurement of blood glucose level, weight loss, drinking, and urine volume

Blood was taken from mice tail. The blood was placed on the GlucoDr™ Blood Glucose Test Strips and the blood glucose level on the 1st, 3rd, 5th, and 7th days after diabetic condition was measured with a GlucoDr™ Blood Test Meter. The loss of mice weight was measured on the 1st, 3rd, 5th, and 7th days after diabetic condition with an analytical balance. Drinking volume was measured on the 1st, 3rd, 5th, and 7th days after diabetic condition. Mice were given some water with known initially volume. Then after 24 h, the volume residue of water was measured. The difference of water volume with residue of water volume was calculated as drinking volume. The volume of urine was measured by summing up of urine was collected for 24 h on the 1st, 3rd, 5th, and 7th days after diabetic

condition. Each mouse was transferred to the metabolic cage with equipped using filter on the bottom of cage.

Measurement of relative weight of heart, liver, and kidney

On the 8th day, all mice were sacrificed. The mice were sacrificed by neck dislocation. This process was performed according to good animal test protocol. Then, the heart, liver, and kidney were collected. The organs were dried on the filter paper and weight for each organ was measured using analytical balance.

RESULTS AND DISCUSSION

Synthesis of Brominated Pyrazoline Analogues

In this work, we have successfully applied the microwave irradiation to synthesize some brominated pyrazolines analogs by two-step reactions. The first step is synthesis of brominated chalcone analogs 1a and 2a through Claisen-Schmidt condensation. The second step is synthesis of brominated pyrazolines analogs through Michael addition and followed by intramolecular cyclization of brominated chalcone with hydrazine hydrate or phenylhydrazine. Both reactions are taken in a short time about 2–3 min of radiation time and we obtained a good yield about 70–97% yield.

Compound 1a was obtained as yellow soli in 73% yield. Mp: 102–103°C. HPLC: t_R = 16.2 min. UV (MeOH) λ_{max} (nm): 212 and 380. FT-IR (KBr) $\bar{\upsilon}$ (cm^{-1}): 3064, 1655, 1597, 1506, 649. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.38 (d, 1H), 8.00 (t, 1H), 7.98 (d, 1H$_\alpha$, J = 15.5 Hz), 7.91 (d, 1H), 7.81 (d, 1H), 7.70 (d, 1H), 7.57 (m, 4H), 7.35 (t, 1H), 7.24 (d, 1H), 7.22 (d, 1H$_\alpha$, J = 15,5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) (ppm): 195.1, 144.3, 136.6, 134.9, 134.0, 133.6, 132.1, 131.5, 130.6, 129.7, 128.6, 128.0, 127.9, 127.7, 127.6, 126.6, 126.0, 125.8, 124.5. HRMS (ES+): m/z $[M+H]^+$ = 337.3219 and $[(M+2)+H]^+$ 339.3165.

Compound 1b was obtained as yellow solid in 92% yield. Mp: 102–104°C. HPLC: t_R = 16.8 min. UV (MeOH) λ_{max} (nm): 261 and 363. FT-IR (KBr) $\bar{\upsilon}$ (cm^{-1}): 3059, 1651, 1600, 1467, 661. ^{1}H NMR ($CDCl_3$) δ (ppm): 8.53 (s, 1H), 8.19 (d, 1H$_\alpha$, J = 15.5 Hz), 8.10 (dd, 1H), 7.98 (d, 1H), 7.93 (d, 1H), 7.89 (d, 1H), 7.79 (dd, 1H), 7.64 (dd, 1H), 7.60 (dt, 1H), 7.57 (d, 1H$_\alpha$, J = 15.5 Hz), 7.56 (dt, 1H), 7.38 (t, 1H), 7.25 (td, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 190.3, 143.2, 135.6, 135.3, 133.7, 132.6, 131.4, 130.3, 129.7, 128.7, 128.6, 128.0, 127.9, 127.8, 126.9, 126.0, 125.2, 124.6. HRMS (ES+): m/z $[M+H]^+$ = 337.2925; m/z $[(M+2)+H]^+$ = 339.2951.

Compound 2a was obtained as white solid in 82% yield. Mp: 102–104°C. HPLC: t_R = 14,5 min. UV (MeOH) λ_{max} (nm): 225 and 317. FT-IR (KBr) $\bar{\upsilon}$ (cm^{-1}): 3328, 3053, 2861, 1592, 1465, 1319, 565. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 9.19 (d, 1H), 7.86 (d, 1H), 7.82 (d, 1H); 7.69 (dd, 1H), 7.58 (t, 2H), 7.51 (t, 2H), 7.43 (t, 1H), 7.33 (t, 1H), 7.15 (td, 1H), 5.33 (t, 1H, J_1 = 10 Hz, J_2 = 9.5 Hz), 3.83 (dd, 1H, J_1 = 16 Hz, J_2 = 10 Hz), 3.11 (dd, 1H, J_1 = 16 Hz, J_2 = 9 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 42.8, 62.7, 123.1, 124.9, 126.1, 127.0, 127.1, 127.4, 128.0, 128,5. 129.1, 129.7, 130.9, 133.0, 134.1, 141.5, 151.9. HRMS (ES-): m/z $[M-H]^+$ = 349.2994; m/z $[(M+2)-H]^+$ = 351.2969.

Compound 2b was obtained as yellow solid in 83% yield. Mp: 87–89°C. HPLC: t_R = 13.9 min. UV (MeOH) λ_{max} (nm): 220 and 309. FT-IR (KBr) $\bar{\upsilon}$ (cm^{-1}): 3323, 3052, 2960, 1601, 1583, 1462, 1030, 683. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.03 (d, 1H), 7.86 (d, 1H), 7.84 (m, 3H), 7.66 (d, 1H), 7.61 (d, 1H), 7.49 (m, 2H), 7.34 (t, 1H), 7.17 (t, 1H), 5.37 (t, 1H, J_1 = J_2 =10 Hz), 3.80 (dd, 1H, J_1 = 16 Hz, J_2 =10.5 Hz), 3,03 (dd, 1H, J_1 = 16 Hz, J_2 = 9.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 151.2, 141.5, 133.5, 133.2, 132.9, 130.3, 129.1, 128.2, 128.2, 128.0, 127.8, 127.4, 126.5, 126.4, 125.7, 123.4, 122.9, 63.4, 40.0. MS (ESI): m/z $[M-H]^+$ = 349.5; m/z $[(M+2)-H]^+$ = 351.2.

Compound 3a was obtained as yellow solid in 97% yield. Mp: 154–155°C. HPLC: t_R = 16.07 min. UV (EtOAc) λ_{max} (nm): 220 and 295. FT-IR (KBr) $\bar{\upsilon}$ (cm^{-1}): 2902, 1595, 1499, 1329, 665. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 9.54 (d, 1H), 7.88 (d, 1H), 7.81 (d, 1H), 7.69 (t, 1H), 7.64 (t, 1H), 7.56 (t,1H), 7.47 (d, 1H), 7.42 (t, 1H), 7.28 (d, 1H), 7.25 (t, 2H), 7.20 (t, 1H), 7.13 (t, 1H), 7.07 (d, 2H), 6.84 (t, 1H), 5.64 (dd, 1H, J_1 = 6.5 Hz, J_2 = 12.5 Hz), 4.19 (dd, 1H, J_1 = 12 Hz, J_2 = 17 Hz), 3.26 (dd, 1H, J_1 = 6.5 Hz, J_2 = 17 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 147.5, 144.3, 140.7, 134.2, 133.2, 130.6, 129.7, 129.2, 128.9, 128.7, 128.4, 127.4, 126.9, 126.2, 124.9, 121.9, 119.4, 113.3, 62.4, 44.5. HRMS (ESI): m/z $[M-H]^+$ = 425.3671; $[(M+2)-H]^+$ = 427.3742.

Compound 3b was obtained as bright yellow solid in 70% yield. Mp: 163–164°C. HPLC: t_R = 16.6 min. UV (EtOAc)λ_{max} (nm): 240 and 307. FT-IR (KBr) $\bar{\upsilon}$ (cm^{-1}): 3037, 1593, 1496, 1335, 689. ^{1}H NMR ($CDCl_3$) δ (ppm): 8.18 (dd, 1H), 7.82 (m, 4H), 7.65 (dd, 1H), 7.47 (m, 2H), 7.23 (m, 4H), 7.14 (td, 1H), 7.05 (d, 2H), 6.83 (t, 1H), 5.68 (dd, 1H, J_1 = 12 Hz, J_2 = 6.5 Hz), 4.11 (dd, 1H, J_1 = 17 Hz, J_2 = 12.5 Hz), 3.17 (dd, 1H, J_1 = 17 Hz, J_2 = 7 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 147.1, 144.3, 140.9, 133.6, 133.4, 133.2, 130.3, 129.2, 128.4, 128.3, 128.2, 127.9, 127.6, 126.6, 126.5, 125.3, 123.5, 121.8, 119.4, 113.3, 63.9, 42.0. HRMS (ESI): m/z $[M-H]^+$ = 425.4034; $[(M+2)-H]^+$ = 427.4004.

In silico Studies for Antidiabetic Activities

In silico docking studies were performed to evaluate the potential of those compounds as antidiabetic. Docking study is a computational technique for the exploration of the possible binding modes of a ligand to a given receptor, enzyme, or other binding site. Docking can be used to predict the preferred and also the best orientation of the molecule (i.e., ligand) to a protein when they bound each other to form a stable and good complex.

Six compounds (1a, 1b, 2a, 2b, 3a, and 3b) were docked into protein (PDB ID: 2QMJ). Based on the docking results, there are three compounds (1a, 3a, and 2b) are find to be active as antidiabetic. Compound 1a showed three hydrophobic interactions with the residues Lys513, Lys534, and Lys776. This molecule also constructed Van der Waals interaction with Glu510 and Asp777. In addition, hydrogen bonding was also observed with the residues Lys513. Based on this, probably the reason the molecule (1a) can be assumed as an active compound.[10]

Another compounds assumed active compounds are compounds 3a and 2b. Based on the docking results,

compound 3a seems to have one hydrogen bonding with the residue Arg547, two hydrophobic interactions with Lys765 and Glu767. There is Van der Waals interaction between the ligand and residue Glu767. Compound 2b has four Van der Waals interactions with the residues Lys513, Arg520, Lys534, and Lys775. In addition, compound 2b also has hydrophobic interaction with amino acid Asp777. The spatial arrangement of compounds 1a, 3a, and 2b is depicted in Figure 3 and the interaction of the ligands with the amino acid is presented in Table 1.

MD simulation was performed with the main purpose is to explore the first stage of docking by assessing the binding pose prediction by MD simulation and also to demonstrate that MD simulation can provide useful information to complement the docking prediction.[15] MD simulation starting from a stable energy minimum, the system fluctuates around the initial conformation and the ligand continues to have initial binding modes.[16] In this study, the MD simulations were performed at temperature 300 K to see the affinity of the ligand to the binding site. In general, the conformation of this active ligand (1a, 3a, and 2b) is maintained to binding with the important residues as presented like a visualization of MD simulation is presented in Figure 4. Based on the estimated free energy of binding and MD simulation, it seems that molecule 3a is the most active compound and furthermore it can be used as new active agents for antidiabetic.

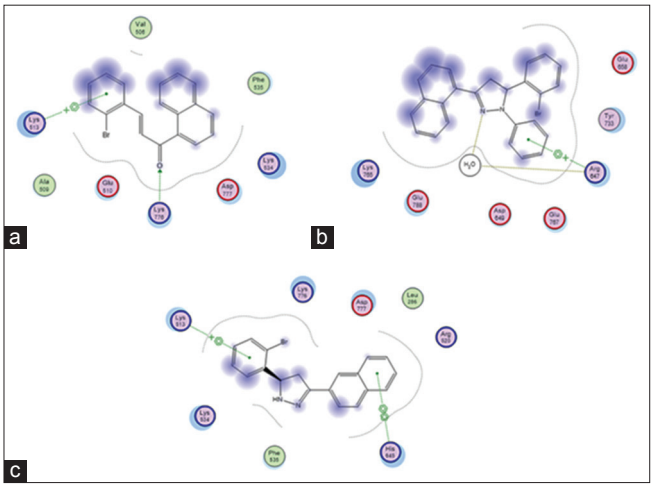

Figure 3: Spatial arrangement of three assumed active compounds (a) compound 1a, (b) compound 3a, (c) compound 2b

In Vivo Evaluation for Antidiabetic Activity of Compound 3a

in vivo evaluation was also conducted to confirm the effect of oral administration of compound 3a with dosages 25, 50, and 100 mg/kg of body weight on percentage of change in blood glucose level and in weight loss prevention, decreasing the drinking, and urine volume and to obtain the effect of the oral administration on the change in relative weight of heart, liver, and kidney of treated diabetic mice.

The % change in blood glucose level was calculated using equation below.

$$\% \text{ Change} = (Li - Lo)/Li \times 100\% \quad \text{(e.q. 1)}$$

Where Li is the initial level of blood glucose of diabetic mice and Lo is observed level of blood glucose (every 2 days after administration of tested compound). The oral administration of compound 3a with dosages 25, 50, and 100 mg/kg of body weight showed that compound 3a has ability to increase the change of percentage in blood glucose level and in loss of weight prevention of treated diabetic mice, significantly ($P < 0.05$) compared than negative control as depicted in Figure 5. These results shown that compound 3a has ability to reduce the blood glucose level. However, the ability is lower than glibenclamide. Figure 5a showed that on the 7th day, compound 3a with dosages of 25, 50, and 100 mg/kg body weight was able to increase the change of percentage in blood glucose level by 26.26%, 37.38%, and 38.13%, respectively, whereas the glibenclamide as the antidiabetic drug was able to increase the change of percentage in blood glucose level by 47.91%. Then, Figure 5b showed that on the 7th day, compound 3a with dosages of 25, 50, and 100 mg/kg body weight was able to increase the change of percentage in loss of weight prevention by 3.38%, 5.66%, and 5.84%, respectively, whereas the glibenclamide was able to increase the change of percentage in loss of weight prevention by 8.60%. Based on the statistical analysis, the oral administration of compound 3a with the dosage of 50 and 100 mg/kg body weight did not show the significant difference ($P > 0.05$) in ability to prevent the weight loss of treated diabetic mice, compared than the glibenclamide.

Oral administration of compound 3a also showed that compound 3a has an ability to decrease the drinking volume in 24 h for a week and it is also has ability to decrease the urine volume in 24 h for a week, significantly ($P < 0.05$) compared than negative control as depicted in Figure 6. Figure 6a showed

Table 1: Docking output

Ligands	Estimated free energy of binding (kcal/mol)	Interactions		
		Van der Waals	Hydrophobic	H-bonding
1a	−6.58	Glu510, Asp777	Lys513, Lys534, Lys776	Lys513
2a	−4.36	Glu114, Asp777	His645, Lys534, Lys778	-
3a	−7.87	Glu767	Lys765 and Glu767	Arg547
1b	−4.23	Glu11	His50	Lys45
2b	−5.08	Lys513, Arg520, Lys534 and Lys775	Asp777	-
3b	−3.45	Glu276	Arg283, Arg647	-

that on the 7th day, compound 3a with dosages of 25, 50, and 100 mg/kg body weight was able to reduce the drinking volume from 5.98 mL to 4.40 mL, 4.24 mL, and 4.12 mL, respectively, whereas the glibenclamide was able to reduce the drinking volume to 3.92 mL. Then, Figure 6b showed that on the 7th day, compound 3a with dosages of 25, 50, and 100 mg/kg body weight was able to reduce the urine volume from 1.16 mL to 0.72 mL, 0.60 mL, and 0.56 mL, respectively, whereas the glibenclamide was able to reduce the urine volume to 0.60 mL. Based on the statistical analysis, the oral administration of compound 3a with the dosage of 25, 50, and 100 mg/kg body weight did not show the significant difference ($P > 0.05$) in ability to reduce the drinking and urine volume of treated diabetic mice compared than the glibenclamide.

Figure 7 showed that the oral administration of compound 3a with dosages of 25, 50, and 100 mg/kg of body weight did not show the significant effect ($P > 0.05$) on the change in relative weight of heart, liver, and kidney of treated diabetic mice compared to the normal control. The result shown that the oral administration of the compound 3a to the diabetic mice had no effect on damage that interfered the functional process of heart, liver, and kidney of the treated diabetic mice. It was done by determination of the relative weight of organs and is performed as a preliminary illustration to observe whether the tested compound gives the potential toxic effects on the organs or not.

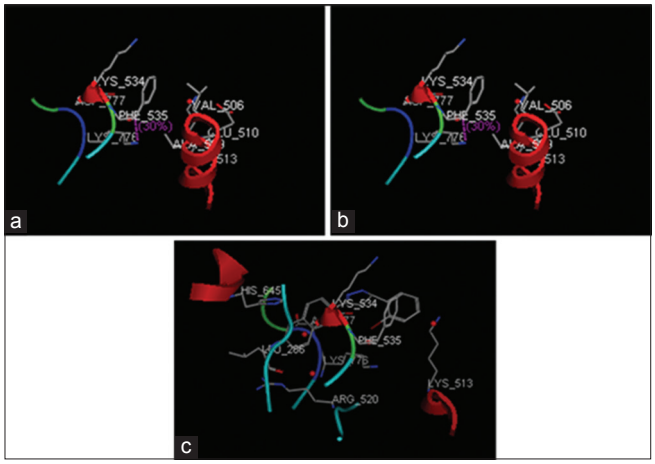

Figure 4: Visualization of binding mode of active compounds (a) compound 1a, (b) compound 3a, and (c) compound 2b

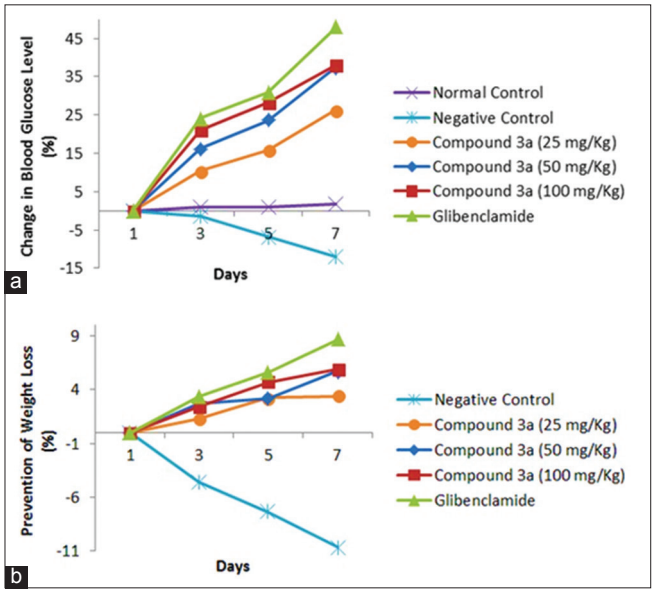

Figure 5: The effect of oral administration on (a) the percentage of change in blood glucose level and (b) in weight loss prevention for a week

CONCLUSION

Some new analogs of brominated pyrazolines has been synthesized under microwave irradiation. The *in silico* studies showed that three compounds (1a, 3a, and 2b) have a good potency as antidiabetic agent and based on the *in vivo* evaluation, compound 3a was able to increase the percentage of decreasing in blood glucose level and in weight loss prevention, decreasing the drinking, and urine volume, significantly ($P < 0.05$) in dosages 25, 50, and 100 mg/kg of body weight compared to the negative control. Then, the oral administrations of compound 3a with dosages 50 and 100 mg/kg of body weight did not show the significant difference ($P > 0.05$) in ability to increase the percentage of change in loss of weight prevention compared

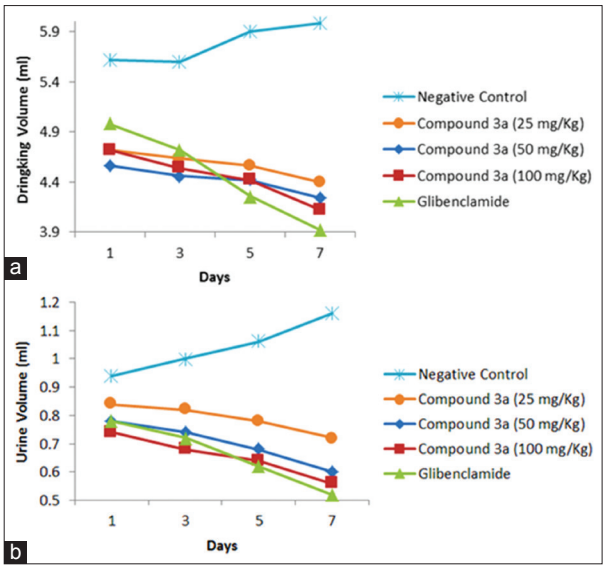

Figure 6: The effect of oral administration on (a) drinking volume and (b) urine volume, in 24 h for a week after oral administration

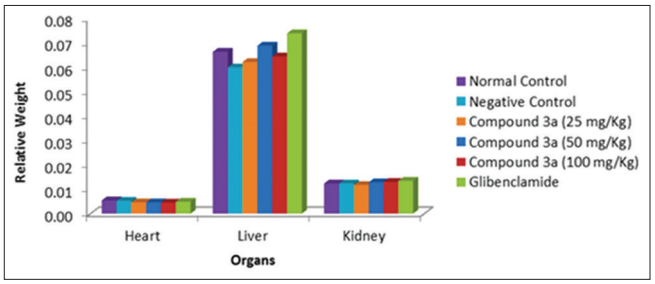

Figure 7: The effect of oral administration for a week on the change of relative weight of mice organs.

than glibenclamide. The oral administration of compound 3a with all given dosages did not show the significant difference ($P > 0.05$) in ability to reduce the drinking and urine volume of diabetic mice compared to the glibenclamide. In addition, based on the measurement of relative weight of organs, the oral administrations of compound 3a for a week had no effect on damage that interfered the functional process of heart, liver, and kidney of treated diabetic mice. This strategy reflects a logical progression for early-stage drug discovery that can be used to successfully identify drug candidates for antidiabetic agents.

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