

# Synthesis and $\alpha$ -glucosidase inhibition of some novel quinazolin-4(3H)-one derivatives bearing a 2-thioxothiazolidine-4-one heterocycle

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## ABSTRACT

Aimed at finding new compounds capable of inhibiting the enzyme  $\alpha$ -glucosidase, six new *N*-(5-arylidene-4-oxo-2-thioxothiazolidin-3-yl)-2-[[4-oxo-3-(*p*-tolyl)-3,4-dihydroquinazolin-2-yl]thio]acetamides **5a-f** were synthesized by the reaction of *N*-(4-oxo-2-thioxothiazolidin-3-yl)-2-[[4-oxo-3-(*p*-tolyl)-3,4-dihydroquinazolin-2-yl]thio]acetamide **4** and appropriate aromatic aldehydes. The structural features of all synthesized compounds were confirmed based on their spectral data, including HR-MS, IR, <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra. The results of the evaluation of the  $\alpha$ -glucosidase enzyme inhibitory activity of acetamide compounds **5a-f** showed that these compounds possess good inhibitor activity against the  $\alpha$ -glucosidase enzyme, in which compound **5a** containing the 4-methylbenzylidene moiety exhibited the best activity. Furthermore, molecular docking was conducted to better comprehend the binding properties of the compounds. The results indicated that synthesized quinazolinone derivatives are potential antidiabetic compounds.

**Key words:** quinazolinone, 2-thioxothiazolidin-4-one,  $\alpha$ -glucosidase inhibition, acetamide, docking

## INTRODUCTION

According to the International Diabetes Federation, currently, between the ages of 20 and 79, 6.7 million adults will have diabetes, and three-quarters of people with diabetes will come from low-income countries<sup>1</sup>. Diabetes is a common disease and causes severe symptoms. Therefore, new drugs as well as new methods to treat this disease are being studied by many scientists.

The  $\alpha$ -glucosidase enzyme is one of the causes of diabetes. Therefore, compounds with the ability to inhibit the  $\alpha$ -glucosidase enzyme have the potential to become drugs for the treatment of diabetes. In addition to possessing some bioactivities, such as hypoglycemia<sup>2,3</sup>, antimicrobial<sup>4</sup>, anticancer<sup>5</sup>, etc., hybrid compounds including quinazolinone heterocycles incorporating thiazolidine heterocycles show good activity in the inhibition of the  $\alpha$ -glucosidase enzyme, and the procedure to synthesize them is not very complicated<sup>6-9</sup>. This work reported the synthesis and evaluation of the  $\alpha$ -glucosidase enzyme inhibitory activity of *N*-(5-arylidene-4-oxo-2-thioxothiazolidin-3-yl)-2-[[4-oxo-3-(*p*-tolyl)-3,4-dihydroquinazolin-2-yl]thio]acetamide compounds as an extension of our study on the

synthesis and bioactivity of compounds containing both quinazolin-4-one and thiazolidin-4-one heterocycles<sup>10</sup>.

## MATERIALS AND METHODS

All chemicals (from Acros) and solvents (from Xilong) were utilized without any further purification. Melting point determination of solid substances was performed by the capillary method with a Galenkamp apparatus 5A-6797, which was uncorrected. The IR spectra were measured on a Shimadzu FT-IR Affinity – 1S spectrometer using potassium bromide (KBr) pellets.

The <sup>1</sup>H-NMR (500 MHz) and <sup>13</sup>C-NMR (125 MHz) spectra were recorded on a Bruker Advance spectrometer using DMSO-*d*<sub>6</sub> as the solvent and internal standard. The chemical shift ( $\delta$ ) and *spin-spin* coupling constants (*J*) are described in ppm and Hz, respectively. The abbreviations *s* (singlet), *d* (doublet), *dd* (double doublet), *t* (triplet), *q* (quartet), *m* (multiplet), and *br* (broad) represent NMR signal multiplicity.

Mass spectra were acquired from the Bruker micrOTOF-Q 10187.

Compounds containing a 2-thioxothiazolidin-4-one nucleus incorporated into the 3-(*p*-tolyl)quinazolin-

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4(3H)-one heterocycle were produced following the synthetic sequences illustrated in Scheme 1.

The examination of the enzyme  $\alpha$ -glucosidase inhibitor activity was performed as described in <sup>11</sup>. The enzymes, substrates and sample solutions were established in 0.1 M potassium phosphate buffer (pH 7). A reaction mixture of 50  $\mu$ L each of  $\alpha$ -glucosidase solution and serially diluted sample mixed with 100  $\mu$ L buffer reagent was incubated at 37 °C for 20 minutes. Then, 50  $\mu$ L of the substrate (pNPG) was added, and the obtained mixture was kept at 37 °C for another 30 minutes. The absorbance of the final solution was measured at a wavelength of 405 nm.

The formula aGI (%) = (A – B)/A × 100% was utilized to calculate the inhibition of the  $\alpha$ -glucosidase enzyme, where aGI stands for  $\alpha$ -glucosidase inhibitory activity, A is the absorbance of the mixture without a sample (inhibitor), and B is the absorbance of the mixture containing the sample. The inhibitor concentration required for 50% inhibition against  $\alpha$ -glucosidase function under the assay conditions was defined as the IC<sub>50</sub> value and determined by Table Curve AISN software.

**Synthesis of 2-mercapto-3-(p-tolyl)-quinazolin-4(3H)-one 1:** Anthranilic acid (6.85 g, 0.05 mol) and potassium hydroxide (2.8 g, 0.05 mol) were dissolved in 60 mL of methanol. Carbon disulfide (9.5 g, 0.125 mol) and *p*-toluidine (5.35 g, 0.05 mol) were slowly added to the mixture above and then refluxed for 10 hours. After pouring the reaction mixture into ice water, the precipitate was separated, dissolved in 10% potassium hydroxide and filtered to remove other solid impurities. Hydrochloric acid was added to the filtrate until the maximum precipitation. The pinkish precipitate was filtered and recrystallized from the mixture of DMF and H<sub>2</sub>O to produce compound 1.

**Synthesis of ethyl 2-[[4-oxo-3-(p-tolyl)-3,4-dihydroquinazolin-2-yl]thio]acetate 2:** After stirring a mixture of 2-mercapto-3-(p-tolyl)-quinazolin-4(3H)-one (1) (2.68 g, 0.01 mol) and potassium carbonate (1.38 g, 0.01 mol) in DMF (50 mL) at room temperature for 30 minutes, ethyl chloroacetate (1.225 g, 0.01 mol) was added. The reaction mixture above was refluxed for 5 hours, cooled to room temperature and poured into ice water. Filtering of the white precipitate and recrystallization from ethanol afforded pure product 2.

**Synthesis of 2-[[4-oxo-3-(p-tolyl)-3,4-dihydroquinazolin-2-yl]thio]acetohydrazide 3:** Hydrazine hydrate (N<sub>2</sub>H<sub>4</sub> · H<sub>2</sub>O) (0.75 g, 0.015 mol) was slowly added dropwise to a solution of ester 2 (3.54 g, 0.01 mol) dissolved in 50 mL of ethanol. After refluxing for 5 hours, the reaction mixture

was kept overnight at room temperature. The solid formed was filtered and recrystallized from ethanol to yield compound 3.

**Synthesis of N-(4-oxo-2-thioxothiazolidin-3-yl)-2-[[4-oxo-3-(p-tolyl)-3,4-dihydroquinazolin-2-yl]thio]acetamide 4:** Thiocarbonyl-bis-thioglycolic acid (1.469 g, 6.5 mmol) was added to a solution of compound 3 (2.04 g, 6 mmol) dissolved in 15 mL of absolute ethanol. The mixture was refluxed for 8 hours and then cooled down. The precipitate was filtered off and recrystallized from acetic acid to give the yellowish powder compound 4.

**General procedure for the synthesis of N-(5-arylidene-4-oxo-2-thioxothiazolidin-3-yl)-2-[[4-oxo-3-(p-tolyl)-3,4-dihydroquinazolin-2-yl]thio]acetamide 5a-f:** Compound 4 (0.456 g, 1 mmol) was dissolved in 15 mL of glacial acetic acid, and then the appropriate aldehyde (1 mmol) and CH<sub>3</sub>COONa (0.082 g, 1 mmol) were added. After refluxing for 5 hours, the reaction mixture was cooled down, poured into ice water and filtered for solid. Recrystallization from a proper solvent produced pure substances 5a-f.

The physical properties and IR spectral data of compounds 5a-f are shown in Table 1; <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and HR-MS spectral data are illustrated in Table 2, Table 3 and Table 4, respectively.

## Docking study

The ligand–protein interactions were investigated by utilizing AutoDock Vina software (version 1.1.2) from the Scripps Research Institute, San Diego, California, USA. The target protein data,  $\alpha$ -glucosidase [PDB: 5KWZ], originated from the RCSB Protein Data Bank. Both the  $\alpha$ -glucosidase and the studied compounds were prepared properly before performing the docking method. Based on the native ligand,  $\alpha$ -glucosidase was prepared, and its active site was also identified via Autodock Tool software version 1.5.6. The geometrical center was determined and illustrated in a three-dimensional grid with a size of 40 Å × 40 Å × 40 Å, in which X = 2.5, Y = -19.2 and Z = 25.2. Subsequently, all the compounds were energy minimized by Avogadro Version 1.2.0. Visualization and evaluation of the outcomes for postdocking analysis were performed via Discovery Studio 2021 Client.

## RESULTS

### Synthesis and structural study

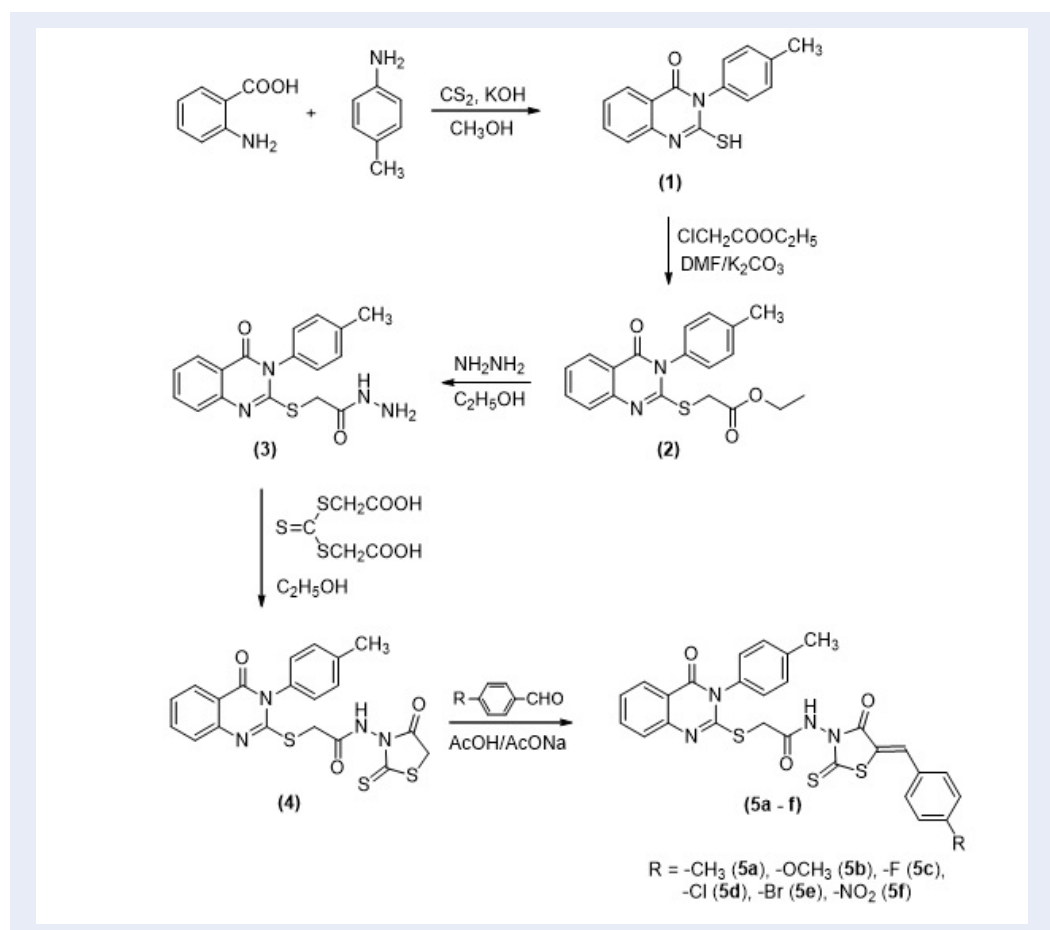
**2-Mercapto-3-(p-tolyl)-quinazolin-4(3H)-one 1:** light-pink crystal (10.05 g), yield 75%, mp: 170 °C (<sup>7</sup>:

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Scheme 1: Pathway for preparation of 2-thioxothiazolidin-4-ones derived from 2-mercapto-3-(p-tolyl)quinazolin-4(3H)-one heterocycles

**Table 1: The physical properties and IR spectral data of the compounds 5a-f (structure was shown in Scheme 2)**

Comp	Solvent re-crystallized	Mp. (°C)	Yield (%)	$\nu$ (cm <sup>-1</sup> )				
				N-H	C-H aromatic	C-H aliphatic	C=O	C=N, C=C
5a	AcOH : H <sub>2</sub> O	215	75	3192	3011	2916	1744, 1690	1593, 1549, 1466
5b	AcOH : H <sub>2</sub> O	223	78	3198	3009	2924	1744, 1688	1580, 1551, 1468
5c	AcOH : H <sub>2</sub> O	233	70	3381	3089	2980	1739, 1688	1651, 1579, 1468
5d	AcOH : H <sub>2</sub> O	238	73	3200	3013	2918	1740, 1688	1603, 1579, 1466
5e	AcOH : H <sub>2</sub> O	253	71	3190	3010	2956	1743, 1688	1594, 1550, 1468
5f	AcOH : H <sub>2</sub> O	235	77	3185	3005	2938	1742, 1686	1599, 1552, 1468

169 – 171 °C). IR ( $\nu$ ,  $\text{cm}^{-1}$ ): 3242, 3122 (N-H, S-H), 3028 (C-H aromatic), 1659 (C=O), 1620, 1522, 1485 (C=N, C=C aromatic);  $^1\text{H-NMR}$  ( $\delta$ , ppm): 13.01 (1H, s, SH), 7.96 (1H, *d*,  $J = 8.0$  Hz, Ar-H), 7.79 (1H, *dd*,  $J_1 = J_2 = 8.0$  Hz, Ar-H), 7.45 (1H, *d*,  $J = 8.5$  Hz, Ar-H), 7.35 (1H, *dd*,  $J_1 = J_2 = 7.5$  Hz, Ar-H), 7.28 (2H, *d*,  $J_1 = J_2 = 8.0$  Hz, Ar-H), 7.15 (2H, *d*,  $J_1 = J_2 = 8.0$  Hz, Ar-H), 2.38 (3H, s, -CH<sub>3</sub>);  $^{13}\text{C-NMR}$  ( $\delta$ , ppm): 176.7 (C=O), 160.3, 140.1, 137.9, 137.2, 136.0, 129.9, 129.2, 127.9, 124.8, 116.7, 116.2 ( $C_{Ar}$ ), 21.3 (CH<sub>3</sub>).

**Ethyl 2-[[4-oxo-3-(*p*-tolyl)-3,4-dihydroquinazolin-2-yl]thio]acetate 2:** white crystal (2.478 g), yield 70%, mp: 101 – 102 °C ( $^{12}$ : 102 °C). IR ( $\nu$ ,  $\text{cm}^{-1}$ ): 3061 (C-H aromatic), 2986, 2914 (C-H aliphatic), 1728 (C=O ester), 1694 (C=O ketone), 1606, 1547, 1466 (C=N, C=C aromatic);  $^1\text{H-NMR}$  ( $\delta$ , ppm): 8.08 (1H, *d*,  $J = 8.0$  Hz, Ar-H), 7.82 (1H, *dd*,  $J_1 = J_2 = 8.0$  Hz, Ar-H), 7.49 (2H, *m*, Ar-H), 7.40 (2H, *d*,  $J = 8.0$  Hz, Ar-H), 7.34 (2H, *d*,  $J = 8.0$  Hz, Ar-H), 4.15 (2H, *q*,  $J = 7.0$  Hz, -CH<sub>2</sub>CH<sub>3</sub>), 3.98 (2H, s, -SCH<sub>2</sub>-), 2.43 (3H, s, -CH<sub>3</sub>), 1.22 (3H, *t*,  $J = 7.0$  Hz, -CH<sub>2</sub>CH<sub>3</sub>);  $^{13}\text{C-NMR}$  ( $\delta$ , ppm): 168.8, 161.1 (C=O), 157.2, 147.5, 140.3, 135.4, 133.5, 130.6, 129.5, 127.1, 126.6, 126.3, 119.9 ( $C_{Ar}$ ), 61.5 (-OCH<sub>2</sub>CH<sub>3</sub>), 34.9 (-SCH<sub>2</sub>), 21.3 (-CH<sub>3</sub>), 14.6 (-OCH<sub>2</sub>CH<sub>3</sub>).

**2-[[4-Oxo-3-(*p*-tolyl)-3,4-dihydroquinazolin-2-yl]thio]acetohydrazide 3:** white crystal (2.176 g), yield 64%, mp: 146 – 148 °C ( $^{12}$ : 148 °C). IR ( $\nu$ ,  $\text{cm}^{-1}$ ): 3271 (N-H), 3062 (C-H aromatic), 2974 (C-H aliphatic), 1678, 1643 (C=O), 1606, 1548, 1468 (C=N, C=C aromatic);  $^1\text{H-NMR}$  ( $\delta$ , ppm): 9.30 (1H, s, NH), 8.09 (1H, *d*,  $J = 8.0$  Hz, Ar-H), 7.85 (1H, *dd*,  $J_1 = J_2 = 8.0$  Hz, Ar-H), 7.63 (1H, *d*,  $J = 8.0$  Hz, Ar-H), 7.49 (1H, *dd*,  $J_1 = J_2 = 8.0$  Hz, Ar-H), 7.39 (2H, *d*,  $J = 8.0$  Hz, Ar-H), 7.34 (2H, *d*,  $J = 8.0$  Hz, Ar-H), 4.27 (2H, *br*, NH<sub>2</sub>), 3.84 (2H, s, SCH<sub>2</sub>), 2.50 (3H, s, CH<sub>3</sub>).  $^{13}\text{C-NMR}$  ( $\delta$ , ppm): 166.6, 161.2 (C=O), 157.5, 147.6, 140.1, 135.3, 133.6, 130.5, 129.6, 127.0, 126.6, 126.5, 120.0 ( $C_{Ar}$ ), 35.0 (-SCH<sub>2</sub>-), 21.3 (-CH<sub>3</sub>).

2-Mercapto-3-(*p*-tolyl)-quinazolin-4(3H)-one **1**, ethyl 2-[[4-oxo-3-(*p*-tolyl)-3,4-dihydroquinazolin-2-yl]thio]acetate **2**, and 2-[[4-oxo-3-(*p*-tolyl)-3,4-dihydroquinazolin-2-yl]thio]acetohydrazide **3** were synthesized according to <sup>4,6,7,12</sup>. The matching of both melting point and spectral data provides evidence that these compounds were successfully synthesized.

***N*-(4-oxo-2-thioxothiazolidin-3-yl)-2-[[4-oxo-3-(*p*-tolyl)-3,4-dihydroquinazolin-2-yl]thio]acetamide 4:** white crystal (1.97 g), 72% yield, mp: 246 °C. IR ( $\nu$ ,  $\text{cm}^{-1}$ ): 3210 (NH), 3094 (C-H aromatic), 2978

(C-H aliphatic), 1761, 1688 (C=O), 1609, 1549, 1466 (C=N, C=C aromatic);  $^1\text{H-NMR}$  ( $\delta$ , ppm): 11.28 (1H, s, NH), 8.09 (1H, *d*,  $J = 8.0$  Hz, Ar-H), 7.85 (1H, *dd*,  $J_1 = J_2 = 8.0$  Hz, Ar-H), 7.75 (1H, *d*,  $J = 8.0$  Hz, Ar-H), 7.49 (1H, *dd*,  $J_1 = J_2 = 8.0$  Hz, Ar-H), 7.40 (2H, *d*,  $J = 8.0$  Hz, Ar-H), 7.34 (2H, *d*,  $J = 8.0$  Hz, Ar-H), 4.41 (2H, *m*, CH<sub>2</sub> thiazolidine), 4.13 (2H, *m*, -SCH<sub>2</sub>CONH-), 2.43 (3H, s, CH<sub>3</sub>);  $^{13}\text{C-NMR}$  ( $\delta$ , ppm): 200.1 (C=S), 170.5, 166.0, 161.2 (C=O), 156.8, 147.6, 140.3, 135.2, 133.5, 130.5, 129.6, 126.9, 126.5, 120.0 ( $C_{Ar}$ ), 66.8 (C=N), 34.5 (-S-CH<sub>2</sub>-), 33.8 (CH<sub>2</sub> thiazolidine), 21.3 (CH<sub>3</sub>); HR-ESI-MS  $m/z$  479.0275 ( $M + \text{Na}$ )<sup>+</sup>, calc. for C<sub>20</sub>H<sub>16</sub>N<sub>4</sub>NaO<sub>3</sub>S<sub>3</sub> 479.0277.

***N*-(5-arylidene-4-oxo-2-thioxothiazolidin-3-yl)-2-[[4-oxo-3-(*p*-tolyl)-3,4-dihydroquinazolin-2-yl]thio]acetamide 5a-f:** The synthesis results and spectral data of compounds **5a-f** are shown in Table 1, Table 2 and Table 3.

### Enzyme $\alpha$ -glucosidase inhibition activity

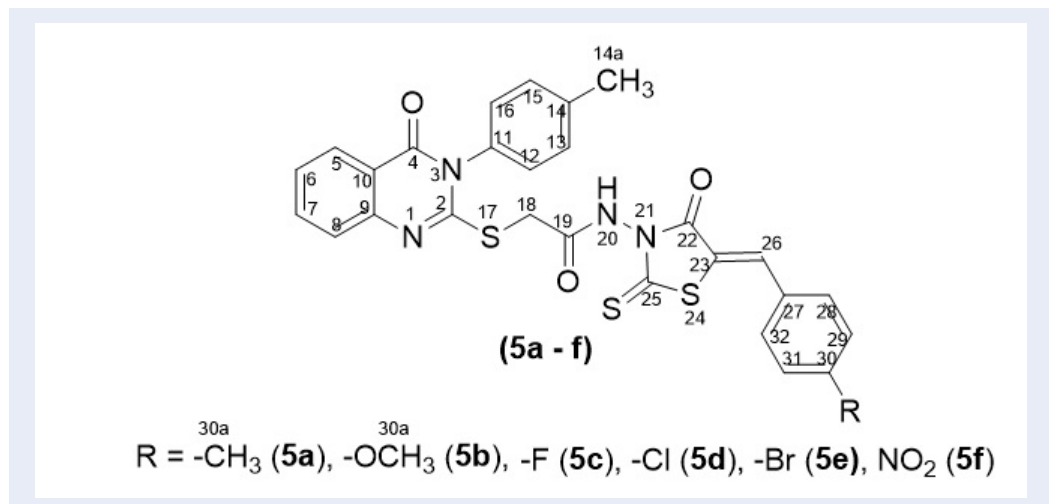
$\alpha$ -Glucosidase is an important enzyme in the hydrolysis of carbohydrates to form glucose. Therefore, the compounds that had  $\alpha$ -glucosidase inhibition can slow the absorption of glucose, which is effective in diabetes type 2 treatment.

Enzyme  $\alpha$ -glucosidase inhibition activity results (see Table 5) of seven compounds **4**, **5a-f** indicated that the substitution of arylidene groups at the 5<sup>th</sup> position in the thiazolidine ring distinctly affected the biological activity of these compounds.

Molecular docking, as an *in silico* approach, is used to study the interaction between receptor (protein) targets and small compounds (ligands)<sup>13</sup>. The docking score, which is also known as the binding energy obtained from docking, is regarded as a function of the binding affinity of the ligand to the protein target<sup>13</sup>. This study modeled synthesized compounds to determine their inhibitory activity toward the enzyme  $\alpha$ -glucosidase through molecular docking. The docking results and IC<sub>50</sub> values of these compounds are shown in Table 5.

## DISCUSSION

The synthesis of **4** starting from anthranilic acid was reported in our previous work<sup>14</sup>. Compound **4** bearing a 2-thioxothiazolidin-4-one moiety in which an active methylene group was reacted with aromatic aldehydes via Knoevenagel condensation to form *N*-(5-arylidene-4-oxo-2-thioxothiazolidin-3-yl)-2-[[4-oxo-3-(*p*-tolyl)-3,4-dihydroquinazolin-2-yl]thio]acetamides **5a-f**, respectively (see the physical properties in Table 1).



Scheme 2: The structure of compounds 5a-f

**Table 2:** <sup>1</sup>H-NMR (δ, ppm and J, Hz) spectral data of compounds 5a-f

Signal	R = -CH <sub>3</sub> (5a)	R = -OCH <sub>3</sub> (5b)	R = -F (5c)	R = -Cl (5d)	R = -Br (5e)	R = -NO <sub>2</sub> (5f)
H <sup>5</sup>	8.09 ( <i>d</i> ) <i>J</i> = 8.0	8.10 ( <i>d</i> ) <i>J</i> = 8.0	8.09 ( <i>d</i> ) <i>J</i> = 7.5	8.09 ( <i>d</i> ) <i>J</i> = 8.5	8.10 ( <i>d</i> ) <i>J</i> = 7.0	8.10 ( <i>d</i> ) <i>J</i> = 9.0
H <sup>6</sup>	7.89 ( <i>dd</i> ) <i>J</i> <sub>1</sub> = <i>J</i> <sub>2</sub> = 7.5	7.89 ( <i>dd</i> ) <i>J</i> <sub>1</sub> = <i>J</i> <sub>2</sub> = 7.5	7.88 ( <i>dd</i> ) <i>J</i> <sub>1</sub> = <i>J</i> <sub>2</sub> = 8.0	7.88 ( <i>dd</i> ) <i>J</i> <sub>1</sub> = <i>J</i> <sub>2</sub> = 8.5	7.88 ( <i>dd</i> ) <i>J</i> <sub>1</sub> = <i>J</i> <sub>2</sub> = 8.0	7.88 ( <i>dd</i> ) <i>J</i> <sub>1</sub> = <i>J</i> <sub>2</sub> = 7.0
H <sup>7</sup>	7.50 ( <i>dd</i> ) <i>J</i> <sub>1</sub> = <i>J</i> <sub>2</sub> = 7.5	7.50 ( <i>dd</i> ) <i>J</i> <sub>1</sub> = <i>J</i> <sub>2</sub> = 8.0	7.50 ( <i>dd</i> ) <i>J</i> <sub>1</sub> = <i>J</i> <sub>2</sub> = 7.0	7.50 ( <i>dd</i> ) <i>J</i> <sub>1</sub> = <i>J</i> <sub>2</sub> = 8.0	7.50 ( <i>dd</i> ) <i>J</i> <sub>1</sub> = <i>J</i> <sub>2</sub> = 8.0	7.51 ( <i>dd</i> ) <i>J</i> <sub>1</sub> = <i>J</i> <sub>2</sub> = 8.0
H <sup>8</sup>	7.78 ( <i>d</i> ) <i>J</i> = 8.0	7.78 ( <i>d</i> ) <i>J</i> = 8.0	7.78 ( <i>d</i> ) <i>J</i> = 8.5	7.78 ( <i>d</i> ) <i>J</i> = 8.0	7.79 ( <i>d</i> ) <i>J</i> = 8.5	7.78 ( <i>d</i> ) <i>J</i> = 7.5
H <sup>12</sup> , H <sup>16</sup>	7.40 ( <i>d</i> ) <i>J</i> = 8.5	7.41 ( <i>d</i> ) <i>J</i> = 8.5	7.41 ( <i>d</i> ) <i>J</i> = 8.0	7.41 ( <i>d</i> ) <i>J</i> = 8.5	7.41 ( <i>d</i> ) <i>J</i> = 8.0	7.41 ( <i>d</i> ) <i>J</i> = 8.5
H <sup>13</sup> , H <sup>15</sup>	7.35 ( <i>d</i> ) <i>J</i> = 7.5	7.35 ( <i>d</i> ) <i>J</i> = 8.5	7.35 ( <i>d</i> ) <i>J</i> = 7.0	7.35 ( <i>d</i> ) <i>J</i> = 8.5	7.35 ( <i>d</i> ) <i>J</i> = 7.5	7.35 ( <i>d</i> ) <i>J</i> = 8.5
H <sup>14a</sup>	2.43 ( <i>s</i> )	2.43 ( <i>s</i> )	2.43 ( <i>s</i> )	2.43 ( <i>s</i> )	2.44 ( <i>s</i> )	2.43 ( <i>s</i> )
H <sup>18</sup>	4.16 ( <i>m</i> )	4.16 ( <i>m</i> )	4.16 ( <i>m</i> )	4.16 ( <i>m</i> )	4.16 ( <i>m</i> )	4.16 ( <i>m</i> )
H <sup>20</sup>	11.51 ( <i>s</i> )	11.50 ( <i>s</i> )	11.54 ( <i>s</i> )	11.55 ( <i>s</i> )	11.54 ( <i>s</i> )	11.59 ( <i>s</i> )
H <sup>26</sup>	7.88 ( <i>s</i> )	7.88 ( <i>s</i> )	7.94 ( <i>s</i> )	7.93 ( <i>s</i> )	7.91 ( <i>s</i> )	8.04 ( <i>s</i> )
H <sup>28</sup> , H <sup>32</sup>	7.58 ( <i>d</i> ) <i>J</i> = 8.0	7.67 ( <i>d</i> ) <i>J</i> = 9.0	7.78 ( <i>m</i> )	7.77 ( <i>d</i> ) <i>J</i> = 9.0	7.79 ( <i>d</i> ) <i>J</i> = 8.5	8.37 ( <i>d</i> ) <i>J</i> = 8.5
H <sup>29</sup> , H <sup>31</sup>	7.39 ( <i>d</i> ) <i>J</i> = 7.5	7.15 ( <i>d</i> ) <i>J</i> = 8.5	7.43 ( <i>m</i> )	7.64 ( <i>d</i> ) <i>J</i> = 8.5	7.64 ( <i>d</i> ) <i>J</i> = 8.5	7.95 ( <i>d</i> ) <i>J</i> = 9.0
H <sup>30a</sup>	2.38 ( <i>s</i> )	3.86 ( <i>s</i> )	-	-	-	-

**Table 3:**  $^{13}\text{C}$ -NMR ( $\delta$ , ppm) spectral data of compounds 5a-f (see structure in Scheme 2)

Comp.	$\delta$ (ppm)					
	R	C=S	C=O	Csp <sup>2</sup>	CH <sub>2</sub>	CH <sub>3</sub>
5a	-CH <sub>3</sub> 21.3	190.5	166.2, 163.6, 161.2	156.8, 147.6, 142.5, 140.3, 135.3, 135.2, 133.5, 131.5, 130.7, 130.6, 130.5, 129.6, 127.0, 126.6, 120.0, 118.5	34.5	21.6
5b	-OCH <sub>3</sub> 56.1	190.5	166.2, 163.6, 162.3	161.2, 156.8, 147.6, 140.3, 135.3, 135.2, 133.7, 133.5, 130.5, 129.6, 127.0, 126.6, 125.8, 120.0, 116.3, 115.7	34.5	21.3
5c	-F	190.4	166.3; 164.9; 163.5	156.8, 147.6, 140.3, 135.2, 134.1, 134.0, 133.9, 133.5, 130.6, 130.0, 129.9, 129.6, 127.0, 126.6, 120.0, 119.5	34.5	21.3
5d	-Cl	190.3	166.3; 163.5; 161.2	156.8, 147.6, 140.3, 136.5, 135.2, 133.8, 133.5, 133.0, 132.1, 130.6, 130.1, 129.6, 127.0, 126.6, 120.5, 120.0	34.5	21.3
5e	-Br	190.2	166.3; 163.5; 161.2	156.8, 147.6, 140.3, 135.2, 133.9, 133.5, 133.1, 132.4, 130.6, 129.6, 128.7, 127.0, 126.6, 125.5, 120.6, 120.0	31.2	21.3
5f	-NO <sub>2</sub>	190.0	166.3; 163.3; 161.2	156.8, 148.4, 147.7, 140.3, 139.2, 135.2, 133.5, 132.3, 132.1, 130.6, 129.6, 127.0, 126.6, 124.9, 124.1, 120.0	34.5	21.3

**Table 4:** HR-ESI-MS spectral data of compounds 5a-f

Comp.	Molecular formula	[M+H] <sup>+</sup> calcd.	[M+H] <sup>+</sup>
5a	C <sub>28</sub> H <sub>22</sub> N <sub>4</sub> O <sub>3</sub> S <sub>3</sub>	C <sub>28</sub> H <sub>22</sub> N <sub>4</sub> O <sub>3</sub> S <sub>3</sub> +H 559.0927	559.0934
5b	C <sub>28</sub> H <sub>22</sub> N <sub>4</sub> O <sub>4</sub> S <sub>3</sub>	C <sub>28</sub> H <sub>22</sub> N <sub>4</sub> O <sub>4</sub> S <sub>3</sub> +H 575.0876	575.0884
5c	C <sub>27</sub> H <sub>19</sub> FN <sub>4</sub> O <sub>3</sub> S <sub>3</sub>	C <sub>27</sub> H <sub>19</sub> FN <sub>4</sub> O <sub>3</sub> S <sub>3</sub> +H 563.0682	563.0695
5d	C <sub>27</sub> H <sub>19</sub> ClN <sub>4</sub> O <sub>3</sub> S <sub>3</sub>	C <sub>27</sub> H <sub>19</sub> ClN <sub>4</sub> O <sub>3</sub> S <sub>3</sub> +H 579.0386	579.0379
5e	C <sub>27</sub> H <sub>19</sub> BrN <sub>4</sub> O <sub>3</sub> S <sub>3</sub>	C <sub>27</sub> H <sub>19</sub> BrN <sub>4</sub> O <sub>3</sub> S <sub>3</sub> +H 622.9875	622.9883
5f	C <sub>27</sub> H <sub>19</sub> N <sub>5</sub> O <sub>5</sub> S <sub>3</sub>	C <sub>27</sub> H <sub>19</sub> N <sub>5</sub> O <sub>5</sub> S <sub>3</sub> +H 590.0621	590.0646

**Table 5: Enzyme  $\alpha$ -glucosidase inhibitor activity and docking results of compounds 4 and 5a-f**

Compounds	IC <sub>50</sub> ( $\mu\text{g mL}^{-1}$ )	Docking score (kcal mol <sup>-1</sup> )	H-bonds
Acarbose	235.36 $\pm$ 3.08	-	-
4	-	-7.8	TRP126, ALA93
5a	15.07 $\pm$ 0.97	-8.8	GLY123, TRP126, ALA93
5b	15.11 $\pm$ 1.94	-8.6	TRP126, ALA93
5c	71.17 $\pm$ 2.22	-8.8	TRP126, ALA93
5d	15.15 $\pm$ 0.15	-8.5	TRP126, ALA93
5e	95.42 $\pm$ 1.88	-8.9	TRP126, ALA93
5f	20.44 $\pm$ 2.64	-8.6	TRP126, ALA93

In compounds **5a-f**, the carbonyl group of the thioxothiazolidin-4-one ring joins the conjugation system with the benzylidene moiety therefore, its signal in the IR spectra (see Table 1) appears at a lower frequency than that of compound **4**. HR-MS spectral data of compounds **5a-f** (see Table 4) showed molecular ion peaks highly matched to their expected structures.

In comparison with the <sup>1</sup>H-NMR spectrum of compound **4**, the signal of the methylene protons in the thiazolidine heterocycle at  $\delta$  4.41 ppm did not appear in compounds **5a-f**, whereas a new singlet signal attributed to the methine proton (<sup>26</sup>H) was found at approximately 7.88-8.04 ppm in the spectra. Additionally, the methylene group peak outside the thiazolidine ring (-SCH<sub>2</sub>CONH-) and the aryl proton peak also appeared as predicted. All signals in <sup>13</sup>C-NMR (see Table 3) were attached to all carbons in the expected structure. These results are evidence that compounds **5a-f** were successfully synthesized.

Compound **4**, with an unsubstituted 5<sup>th</sup> position of the thiazolidine ring, did not show bioactivity against  $\alpha$ -glucosidase. Six compounds, **5a-f** (Table 5), exhibited good interactions with different amino acids found in the binding site of the enzyme  $\alpha$ -glucosidase. Several hydrogen bonds were found to involve many noncovalent interactions, such as Pi-sigma bonds, Pi-cation, Pi-Pi stacking, and alkyl interactions (Figure 1). The compounds in which the electron-donating group existed in the benzylidene moiety revealed better bioactivity than the electron-withdrawing group. Mainly, compound **5a** with the methyl group in the benzylidene moiety exhibited the best  $\alpha$ -glucosidase inhibition activity.

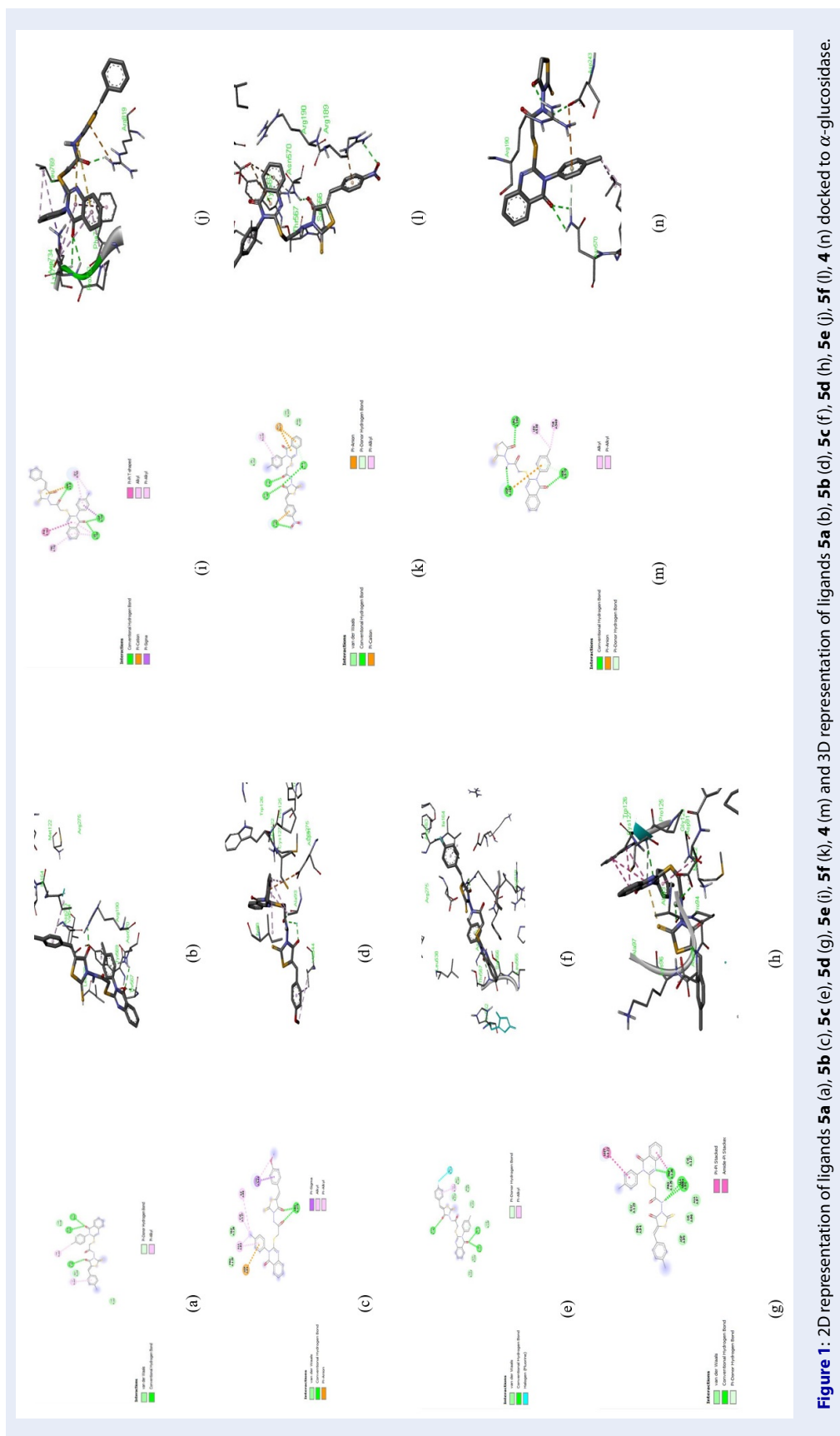
Despite the excellent outcome with IC<sub>50</sub>, the docking score of **5a** is the second top scorer. This compound has a hydrogen bond acceptor at N1 of the quinazoline ring with TRP126, a van der Waals interaction with

GLY123, and a hydrogen bond donor at the amide nitrogen with ALA93 (Figure 1a).

Three compounds, **5b**, **5d** and **5f**, show similarities between IC<sub>50</sub> and docking score. Compound **5b** has a hydrogen bond acceptor (Figure 1c) at N1 of quinazoline with TRP126 and a hydrogen bond donor at the amide nitrogen with ALA93 and a pi-cation interaction between benzene, which has a methoxy group, and ARG275; moreover, there is an alkyl interaction of the -CH<sub>3</sub> group with VAL544. Compound **5d** has a hydrogen bond acceptor at N1 of quinazoline with TRP126 and a hydrogen bond donor with ALA93 at N of the amide functional group (Figure 1g). Furthermore, this compound has a hydrogen bond with ASP91 and many van der Waals interactions. Compound **5f** has a hydrogen bond acceptor at N1 of quinazoline with TRP126 and a hydrogen bond donor with ALA93 at N of the amide functional group (Figure 1k).

Compounds **5e** (Figure 1i) and **5c** (Figure 1e) with relatively high IC<sub>50</sub> values (95.42  $\pm$  1.88  $\mu\text{g mL}^{-1}$  and 71.17  $\pm$  2.22  $\mu\text{g mL}^{-1}$ , respectively) do not show good activity, but they have the best docking score (Table 5) instead (-8.9 kcal mol<sup>-1</sup> with compound **5e** and -8.8 kcal mol<sup>-1</sup> with compound **5c**). These compounds have a hydrogen bond acceptor at N1 of quinazoline with TRP126 and a hydrogen bond donor with ALA93 at N in the amide group.

Hydrogen bond interactions with the binding site and the catalytic residues of an enzyme significantly affect the activity of the target proteins and result in inhibitory modulation of their training<sup>15</sup>. The findings in this study show several hydrogen bonds interacting with the residues of the receptor binding site. The residues residing out of the binding site ranged from two to four conventional hydrogen bond interactions. Hydrogen bond interactions of residues outside an





enzyme's active site area can affect the target receptor's activity by disordering the polypeptides that constitute the active site, as mentioned by William *et al.*, 2012<sup>16</sup> in their study with myricetin and ethyl caffeine.

Noncovalent binding interactions such as Pi-pi stacking, Pi-sigma stacking, and alkyl stacking are crucial for exacting necessary biological activities during ligand-protein interactions<sup>15,17</sup>. Compound **5a**, having the best IC<sub>50</sub> activity, has shown noncovalent interactions (Figure 1a) with the receptor under study, which might support its *in vitro* activity.

## CONCLUSIONS

By a simple and convenient method, compound **4** and six 5-arylidene derivatives were successfully obtained. Their chemical structures were elucidated by the typical analysis of IR, NMR and HR-MS spectral data. Compounds **4** and **5a-f** were evaluated for  $\alpha$ -glucosidase inhibition activity, and compounds with an electron donating group in the benzylidene moiety showed good activity, especially compound **5a** containing the 4-methylbenzylidene moiety, which exhibited the strongest activity among them. The results of molecular docking, including docking score and interactions, prove that quinazoline derivatives are potential antidiabetic compounds.

## LIST OF ABBREVIATIONS

<sup>13</sup>C-NMR: Carbon-13 nuclear magnetic resonance

DMSO: dimethyl sulfoxide

<sup>1</sup>H-NMR: Proton nuclear magnetic resonance

HR-MS: High-resolution mass spectrometry

IR: Infrared spectroscopy

IC<sub>50</sub>: Half-maximal inhibitory concentration

pNPG: 4-Nitrophenyl- $\alpha$ -D-glucopyranoside

## COMPETING INTERESTS

The authors declare that there are no competing interests or any personal problems that could affect this work reported.

## AUTHORS' CONTRIBUTIONS

**Bui Thi Thuy Linh**: conceptualization, methodology, investigation, writing – original draft preparation; **Nguyen Nguyen Cuong Phat**, **Nguyen Tuan Kiet**: investigation; **Nguyen Thi Kim Yen**: reviewing and editing; **Nguyen Tien Cong**: conceptualization, supervision; **Le Trong Duc**: analysis, writing – reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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