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New Thiazolidine-4-Carboxylic Acid Derivatives Act as Promising α-Amylase and α-Glucosidase Inhibitors; Synthesis, *In Vitro* Pharmacological Evaluation and *In Silico* Molecular Docking Study

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A B S T R A C T

The management of diabetes presents difficulties to scientists as this deadly condition continues to affect people worldwide. Diabetes control depends on enzyme inhibition which blocks both α-amylase and α-glucosidase play key roles in the breakdown of carbohydrates after meals.The presented study details potent Thiazolidine-4-Carboxylic Acid Derivatives 5(a-l) which exhibit strong inhibition against α-amylase and α-glucosidase. The bio-assays performed in test tubes showed that these compounds could prevent biological effects. The phenyl ring substituents served as the main focus of a structure-activity relationship analysis performed on all synthesized molecules. Molecular docking studies served to determine how the compounds would bind when placed inside the enzyme active pocket.Compound 5e proved its outstanding potential as a α-amylase inhibitor because its IC50 value reached 24.13µg/ml which surpassed the standard acarbose (IC50=32.27µg/ml). The inhibitory properties of compounds 5f and 5g towards α-glucosidase surpassed acarbose reference levels (IC50=30.45µg/ml) with 22.76 and 25.68 µg/ml IC50 values. The reference inhibitor matched suppression levels of the tested compounds 5a, 5b, 5c, and 5d as these compounds displayed strong inhibitory potential against α-amylase as well as against α-glucosidase enzymes.These designed derivatives show effective potential characteristics which can serve as starting compounds to develop forthcoming therapeutic intervention solutions.

Keywords: Thiazolidine-4-Carboxylic Acid; Synthesis; SAR; Alpha-amylase; Docking study; Alpha-glucosidase.

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INTRODUCTION

Heterocyclic compounds form an essential category of organic substances that display varied biological plus pharmacological activities (Ansari and Lal, 2009, Akhtar *et al.,* 2017, Kakkar and Narasimhan, 2019, Shiro *et al.,* 2015). The natural diversity of thiazolidines as well as other heterocyclic compounds makes them exceptional choices for drug creation (Zhang *et al.,* 2020, Lu *et al.,* 2009). The pharmaceutical drugs pioglitazone and benzylpenicillin serve along with other heterocyclic compounds including thiazolidines in treating diabetes and epilepsy and diuresis along with infection through drugs such as etozoline and rosiglitazone and teneligliptin and ralitoline (Sahiba *et al.,* 2020, Makwana and Malani, 2017). Science supports that thiazolidines demonstrate antiviral alongside anti-inflammatory properties as well as acetyl/butyrylcholinesterase inhibition and anticancer effects and neuroprotective and antimicrobial and immunostimulant and antinociceptive and hepatoprotective activities (Joshi *et al.,* 2005, Abdellatif *et al.,* 2016, Zhang *et al.,* 2010, Jagtap *et al.,* 2018, das Neves *et al.,* 2020).

Diabetes exists as an essential management issue in modern society (Kharroubi and Darwish, 2015, Bellary *et al.,* 2021) because hyperglycemia develops due to endocrine disorders (Kharroubi and Darwish, 2015, Bellary *et al.,* 2021). The existence of high blood glucose (hyperglycemia) triggers multiple severe health conditions such as thrombosis (Vazzana *et al.,* 2012), retinopathy (Mensah and Kohner, 2002), nephropathy (Mora-Fernández *et al.,* 2014), encephalopathy (Gispen and Biessels, 2000), Alzheimer's disease (Kong *et al.,* 2020) and cardiovascular disease (O'Gara *et al.,* 2013, Kobayashi and Liang, 2015, Zhao *et al.,* 2021) which contribute to millions of deaths across the world yearly. Dietary carbohydrate inhibitor treatment plays a fundamental role in treating postprandial hyperglycemia for patients with diabetes (Tucci *et al.,* 2010).

Pancreatic α-amylase breaks down complex carbohydrates into glucose while α-glucosidase hydrolyzes both starch and disaccharides into glucose (Joshi *et al.,* 2015). Enzyme blocking enables glucose uptake delay along with decreased blood sugar levels. The drugs acarbose, voglibose, and miglitol prevent α-glucosidase and α-amylase activity yet raise discomforting side effects like stomach problems and diarrhoea and flatulence and bloating (Tundis *et al.,* 2010, Gong *et al.,* 2020). Researchers must investigate different and improved methods to block α-glucosidase and α-amylase functions. The research study investigates new derivatives of thiazolidine-4-carboxylic acid through synthesis and structural investigation followed by enzyme activity evaluation against α-amylase and α-glucosidase. The results from molecular docking exams demonstrated how these compounds binds with binding sites of the protein while confirming the possible use of thiazolidine derivatives to treat hyperglycemia.

MATERIAL AND METHODS

Chemicals

Research chemicals were procured from distinguished suppliers namely Daejung and Sigma-Aldrich and AlfaAesar. The FTIR spectrophotometer (Alpha Bruker) provided analysis of functional groups in synthesized products which produced wavenumbers (νmax) in cm−1. The 1H NMR spectra and 13C NMR spectra were collected by a Bruker AM300 spectrophotometer through DMSO-d6 solution at 300 MHz and 75 MHz frequencies, together with Tetramethyl silane (TMS) reference as the internal standard. Analysis of elements took place through the LECO-183 CHN analyzer. Thin-layer chromatography (TLC) served as the method to monitor all reactions' progress. The final products were purified through column chromatography utilizing as solvent chloroform against silica gel HF-254 (Merck). Research chemicals within this study reached HPLC purity standards of 99% (to define their high quality grade).

Synthesis of Thiazolidine-4-Carboxylic Acid Derivatives 5(a-l)

The synthesis solution involved mixing 0.0035 mol p-Hydroxybenzaldehyde together with 0.035 mol 1,2-dichloroethane within 70 mL dimethylformamide (DMF). The reaction needed potassium carbonate (0.035 mol) as an added component to stir the mixture at room temperature for 5 days in dark conditions. Thin-layer chromatography (TLC) served as the reaction monitoring method for this synthesis while potassium carbonate (K2CO3) solid was filtered out before proceeding. The residue from the vacuum removal process was dissolved in chloroform after the removal of DMF and excess dichloroethane. The researchers eliminated remaining solid impurities before they evaporated the chloroform solution. The researcher obtained the crude liquid product and purified it through silica-gel column chromatography which employed chloroform as its eluting agent. The segmentation method yielded a light yellow pure solid substance (1) as the final product (Laudien *et al.,* 2002).

General Procedure for the Synthesis of Ester Derivatives 3(a-l)

A solution formation required placing compound (1) at a 0.01 mol concentration within 25 mL DMF solution. The reaction solution received 0.01 mol of substituted benzoic acids 2(a-l) together with 0.01 mol triethylamine and 0.01 mol potassium iodide afterward which was stirred at room temperature for an entire night. The reaction was determined complete through the use of thin-layer chromatography (TLC). The mixture received four extractions with ethyl acetate (4×25 mL) to extract product while being stirred over finely crushed ice. The mixture received two separate liquid washes that included 5% potassium carbonate solution followed by an aqueous sodium chloride solution. Following filtration of the dried organic layer the crude products 3(a-l) (Abbas *et al.,* 2017) were obtained through

vacuum distillation of solvent. Silica gel column chromatography method was used for purifying the crude compounds 3(a-l).

General Procedure for the Synthesis of Thiazolidine-4-Carboxylic Acid Derivatives 5(a-l)

In a reaction involving a water solution of Hydrated L-Cysteine HCl and NaHCO3 (1.1 equiv), compounds 3(a-l) (1.1 equiv) were added in one addition through 95% ethanol (200 mL) as the solvent medium. The mixture received an alkaline addition of Hydrated L-Cysteine HCl and NaHCO3 and stayed under continuous stirring for 5 hours. The solid product obtained from this reaction was filtered before undergoing ethanol washing then drying which yielded the desired products 5(a-l) (Liu *et al.*, 2011) pictured in Figure 1. Silva gel column chromatography followed where the eluent system consisted of a 3:1 ratio of n-hexane to ethyl acetate.

Spectral Analysis

Compound 5a: White solid; yield: 84%, m.p. 142–144°C; Rf = 0.56 (C_6H_{14}:$C_4H_8O_2$ 3:1); FTIR (νmax cm^{-1}) 1335 (C-N), 1593 (C=C), 1674 (C=O), 1734 (C=O), 2973 (Sp^2 C-H), 3138 (O-H); ^{1}H NMR (DMSO-d6) δ ppm 3.38, 3.35 (m, 4H), 4.30 (dd, J=5.1Hz, 9.3Hz, 1H), 4.44 (dd, J=4.5Hz, 9.0Hz, 1H), 4.61–4.56 (m, 8H), 5.60-5.53 (s, 1H), 8.06–6.91 (m, 16H), 9.88 (s, 2H); ^{13}C NMR (DMSO-d6) δ ppm 40.6, 40.4, 63.7, 63.4, 64.7, 64.8, 66.4, 65.5, 69.6, 69.3, 114.5, 114.1, 115.9, 116.8, 120.7, 120.6, 128.5, 128.1, 129.6, 131.2, 133.4, 134.5, 158.9, 158.0, 162.8, 163.4, 165.6, 166.2, 172.7, 173.3. GC–MS (EI) *m/z* 408 $[M]^+$.

Compound 5b: Off-white solid; yield: 83%, m.p. 163–166°C; Rf = 0.56 (C_6H_{14}:$C_4H_8O_2$ 3:1); FTIR (νmax cm^{-1}) 1309 (C-N), 1595 (C=C), 1634 (C=O), 1739 (C=O), 2959 (Sp^2 C-H), 3161 (O-H); ^{1}H NMR (DMSO-d6) δ ppm 3.37, 3.36 (m, 4H), 4.33 (dd, J=5.1Hz, 9.3Hz, 1H), 4.50 (dd, J=4.5Hz, 9.0Hz, 1H), 4.63–4.60 (m, 8H), 5.59-5.47 (s, 1H), 8.05–6.94 (m, 16H), 9.86 (s, 2H); ^{13}C NMR (DMSO-d6) δ ppm 40.9, 40.3, 63.7, 63.2, 64.3, 63.6, 66.7, 65.4, 71.8, 71.3, 115.3, 114.5, 116.8, 116.2, 120.1, 121.5, 128.6, 129.5, 130.5, 131.2, 133.4, 133.8, 157.4, 158.1, 162.4, 163.4, 165.7, 166.0, 172.4, 173.8. GC–MS (EI) *m/z* 424 $[M]^+$.

Compound 5c: Off-white solid; yield: 83%; m.p. 162–164°C; Rf = 0.66 (C_6H_{14}:$C_4H_8O_2$ 3:1); FTIR (νmax cm^{-1}) 1319 (C-N), 1599 (C=C), 1639 (C=O), 1737 (C=O), 2951 (Sp2 C-H), 3122 (O-H); ^{1}H NMR (DMSO-d6) δ ppm 3.36–3.31 (m, 4H), 4.36 (dd, J=5.3Hz, 9.1Hz, 1H), 4.45 (dd, J=4.2Hz, 9.0Hz, 1H), 4.64–4.61 (m, 8H), 5.59-5.52 (s, 1H), 8.21, 6.79 (m, 16H), 10.35-9.83 (s, 1H); ^{13}C NMR (DMSO-d6) δ ppm 40.4, 40.1, 63.7, 62.5, 65.6, 65.4, 66.3, 66.2, 70.2, 69.5, 114.9, 114.1, 117.6, 117.5, 121.8, 121.1, 128.4, 129.6, 130.7, 132.5, 134.6, 136.1, 157.1, 157.4, 162.5, 163.1, 165.6, 166.3, 172.1, 172.3. GC–MS (EI) *m/z* 446 $[M]^+$.

Compound 5d: Off-white solid; yield: 81%; m.p. 182–187 °C; Rf = 0.67 (C_6H_{14}:$C_4H_8O_2$ 3:1); FTIR (νmax cm^{-1}) 3137 (O-H), 2953 (Sp^2 C-H), 1726 (C=O), 1632 (C=O), 1583 (C=C), 1325 (C-N); ^{1}H NMR (DMSO-d6) δ ppm 10.37, 9.88 (s,1H), 7.94–6.94 (m, 16H),5.66-5.51 (s, 1H), 4.61–4.60 (m, 8H), 4.42 (dd, J=4.5Hz, 8.7Hz), 4.37 (dd, J=5.6Hz, 9.3Hz, 1H), 3.37–3.25(m,4H); ^{13}CNMR(DMSO-d6) δppm173.5,172.6, 167.5,166.4, 163.4,163.2,156.2,155.9, 134.6, 134.0, 131.8, 131.4, 129.3, 128.5, 120.8, 120.3, 117.6, 116.6, 114.7, 114.1, 70.6, 69.8, 67.5, 67.3, 65.5, 65.2, 63.9, 63.2, 40.7, 40.2. GC–MS (EI) *m/z* 458 $[M]^+$.

Compound 5e: Pale yellow solid; yield: 79%; m.p. 180–184 °C; Rf = 0.49 (C_6H_{14}:$C_4H_8O_2$ 3:1); FTIR (νmax cm^{-1}) 3150 (O-H), 2958 (Sp^2 C-H), 1731 (C=O), 1669 (C=O), 1588 (C=C), 1318 (C-N); ^{1}H NMR (DMSO-d6) δ ppm 9.88 (s, 2H), 8.37–6.79 (m, 16H), 5.62, 5.48 (s, 1H), 4.61–4.58 (m, 8H), 4.53 (dd, J=4.2Hz, 9.0Hz, 1H), 4.38 (dd, J=5.6Hz, 9.3Hz, 1H), 3.37–3.30(m,4H); ^{13}CNMR(DMSO-d6) δppm 173.8, 172.5, 166.4, 165.8, 164.7, 163.9, 150.5, 150.5, 135.7, 134.7, 132.4, 132.3, 131.7, 132.2, 130.6, 129.6, 124.6, 124.5, 115.7, 114.6, 71.3, 71.2, 66.7, 66.6, 64.7, 63.7, 63.1, 62.5, 40.6, 40.5. GC–MS (EI) *m/z* 419 $[M]^+$.

Compound 5f: White solid; yield: 77%; m.p. 150–153°C; Rf = 0.58 (C_6H_{14}:$C_4H_8O_2$ 3:1); FTIR (νmax cm^{-1}) 1327 (C-N), 1601 (C=C), 1639 (C=O), 1728 (C=O), 2975 (Sp^2 C-H), 3141 (O-H); ^{1}H NMR (DMSO-d6) δ ppm 3.28, 3.25 (s, 3H), 3.37–3.33 (m, 4H), 4.36 (dd, J=5.1Hz, 9.1Hz, 1H), 4.48 (dd, J=4.5Hz, 9.0Hz, 1H), 4.64–4.62 (m, 8H), 5.59-5.48 (s, 1H), 7.96–6.86 (m, 16H, Ar-H), 9.92- 9.84 (s,1H); ^{13}C NMR (DMSO-d6) δ ppm 41.6, 41.5, 63.7, 63.2, 64.7, 64.3, 66.4, 66.1, 68.3, 69.6, 115.7, 115.3, 117.8, 117.1, 120.7, 120.4, 128.5, 129.8, 131.5, 132.3, 135.5, 136.2, 157.7, 159.3, 162.7, 164.1, 166.4, 167.8, 172.1, 172.7. GC–MS (EI) *m/z* 404 $[M]^+$.

Compound 5g: White solid; yield: 80%; m.p. 167–170°C; Rf = 0.41 (C_6H_{14}:$C_4H_8O_2$ 3:1); FTIR (νmax cm^{-1}) 1339 (C-N), 1586 (C=C), 1656 (C=O), 1735 (C=O), 2972 (Sp^2 C-H), 3125 (O-H), 3265 (O-H); ^{1}H NMR (DMSO-d6) δ ppm 3.39–3.28 (m, 4H), 4.33 (dd, J=5.1Hz, 9.3Hz, 1H), 4.45 (dd, J=4.5Hz, 8.7Hz, 1H), 4.58–4.52 (m, 8H), 5.60-5.45 (s, 1H), 7.89–6.83 (m, 16H), 10.31- 9.88 (s, 1H); ^{13}C NMR (DMSO-d6) δ ppm 40.7, 40.5, 63.2, 63.0, 65.8, 65.2, 66.9, 66.4, 71.9, 71.3, 114.7, 115.0, 115.5, 115.8, 120.5, 120.4, 128.8, 129.1, 131.5, 132.0, 132.3, 133.6, 158.2, 158.7, 162.5, 163.7, 165.9, 165.9, 172.7, 173.5. GC–MS (EI) *m/z* 390 $[M]^+$.

Compound 5h: White solid; yield: 79%; m.p. 144–147°C; Rf = 0.57 (C_6H_{14}:$C_4H_8O_2$ 3:1); FTIR (νmax cm^{-1}) 1342 (C-N), 1593 (C=C), 1678 (C=O), 1736 (C=O), 2981 (Sp^2 C-H), 3140 (O-H); ^{1}H NMR (DMSO-d6) δ ppm 3.37, 3.32 (m, 4H), 4.27 (dd, J=5.1Hz, 9.3Hz, 1H), 4.41 (dd, J=4.5Hz, 9.0Hz, 1H), 4.67–4.60 (m, 8H), 5.64-5.52 (s, 1H), 8.08–6.95 (m, 16H), 9.88 (s, 2H); ^{13}C NMR (DMSO-d6) δ ppm 40.7, 40.4, 63.8, 63.1, 64.5, 64.1, 65.8, 66.6, 69.7, 69.2, 114.0, 114.3, 115.4, 116.9, 120.9, 120.2, 128.4, 128.7, 129.2, 131.5, 133.4, 134.6, 157.8, 158.4, 162.2, 163.7, 165.4, 166.1, 172.4, 173.8. GC–MS (EI) *m/z* 408 $[M]^+$.

Compound 5i: White solid; yield: 91%; m.p. 165–169 °C; Rf = 0.60 (C_6H_{14}:$C_4H_8O_2$ 3:1); FTIR (νmax cm^{-1}) 1308 (C-N), 1598 (C=C), 1634 (C=O), 1735 (C=O), 2958 (Sp^2 C-H),

3165 (O-H); ^{1}H NMR (DMSO-d6) δ ppm 3.37, 3.34 (m,4H), 4.36 (dd, J=5.1Hz, 9.3Hz, 1H), 4.45 (dd, J=4.5Hz, 9.0Hz, 1H), 4.61–4.55 (m, 8H), 5.64-5.47 (s, 1H), 8.12–7.02 (m, 16H), 9.88 (s, 2H); ^{13}C NMR (DMSO-d6) δ ppm 40.6 ,40.1, 62.8, 63.1, 63.5, 64.4, 65.9, 66.7, 71.5, 71.5, 114.7, 115.3, 116.3, 116.7, 120.5, 120.8, 128.6, 129.4, 130.7, 131.2, 133.0, 133.5, 157.6, 158.2, 162.5, 163.8, 165.8, 166.4, 172.4, 173.7. GC–MS (EI) *m/z* 392 $[M]^{+}$.

Compound 5j: Pale yellow solid; yield: 80%; m.p. 183–187 °C; Rf = 0.65 (C_6H_{14}:$C_4H_8O_2$ 3:1); FTIR (νmax cm^{-1}) 1327 (C-N), 1578 (C=C), 1636 (C=O), 1719 (C=O), 2949 (Sp^2 C-H), 3146 (O-H); ^{1}H NMR (DMSO-d6) δ ppm 3.34–3.25 (m,4H), 4.38 (dd, J=5.6Hz, 9.3Hz, 1H), 4.44 (dd, J=4.5Hz, 8.7Hz, 1H), 4.64–4.57 (m, 8H), 5.64-5.47 (s, 1H), 7.93–6.95 (m, 16H), 10.26-9.88 (s,1H); ^{13}C NMR (DMSO-d6) δ ppm 40.7, 40.5, 63.2, 63.6, 65.1, 65.4, 67.0, 67.7, 69.5, 70.6, 114.1, 114.8, 116.9, 117.7, 120.0, 120.4, 128.4, 129.5, 131.2, 131.7, 133.9, 134.1, 155.5, 156.1, 163.0, 163.7, 166.4, 167.5, 172.6, 173.7. GC–MS (EI) *m/z* 500 $[M]^{+}$.

Compound 5k: Pale yellow solid; yield: 79%; m.p. 180–184 °C; Rf = 0.53 (C_6H_{14}:$C_4H_8O_2$ 3:1); FTIR (νmax cm^{-1}) 1318 (C-N), 1588 (C=C), 1678 (C=O), 1733 (C=O), 2967 (Sp^2 C-H), 3146 (O-H); ^{1}H NMR (DMSO-d6) δ ppm 3.35–3.33(m,4H), 4.38 (dd, J=5.6Hz, 9.3Hz, 1H), 4.51 (dd, J=4.2Hz, 9.0Hz, 1H), 4.67–4.63 (m, 8H), 5.67-5.45 (s, 1H), 8.40–6.79 (m, 16H), 9.88 (s, 2H); ^{13}C NMR (DMSO-d6) δ ppm 40.6, 40.1, 62.7, 63.5, 63.7, 64.3, 66.5, 66.7, 71.4, 71.7, 114.6, 115.8, 124.3, 124.7, 129.6, 130.6, 131.7, 131.8, 132.3, 132.4, 134.6, 135.5, 150.5, 150.9, 163.8, 164.7, 166.3, 166.6, 172.9, 173.7. GC–MS (EI) *m/z* 419 $[M]^{+}$.

Compound 5l: White solid; yield: 82%; m.p. 147–153°C; Rf = 0.50 (C_6H_{14}:$C_4H_8O_2$ 3:1);FTIR (νmax cm^{-1}): 1340 (C-N), 1596 (C=C), 1637 (C=O), 1727 (C=O), 2973 (Sp^2 C-H), 3149 (O-H,).^{1}H NMR (DMSO-d6) δ ppm: 3.29, 3.24 (s, 3H); 3.34–3.31 (m, 4H); 4.36 (dd, J=5.1Hz, 9.1Hz, 1H); 4.46 (dd, J=4.5Hz, 9.0Hz, 1H); 4.66–4.58 (m, 8H); 5.64-5.49 (s, 1H); 7.96–6.84 (m, 16H); 9.92-9.84 (s,1H).^{13}C NMR (DMSO-d6) δ ppm: 41.7, 41.4; 63.7, 63.4; 64.5, 64.4; 66.8, 66.5; 69.4, 68.7; 115.7, 114.8; 117.7, 117.4; 120.6, 120.4; 129.6, 128.7; 132.5, 131.9; 135.8, 135.4; 158.6, 158.0; 163.8, 162.4; 167.8, 166.6; 173.5, 173.1. GC–MS (EI) *m/z* 404 $[M]^{+}$.

In Vitro Pharmacological Evaluation

α-Amylase Activity

The experimental analysis adopted established methods found in literature documentation (Alqahtani, 2021). The testing solution development included the combination of phosphate buffer with alpha amylase and various compound concentrations (31.25, 62.5, 125, 250, and 500 µg/ml). The solution mixtures received starch solution before being incubated for 20 minutes under 37 degrees Celsius conditions. The reaction solution entered a specific time duration at a 100°C water bath after its incubation stage. The color intensity measurements were conducted at 656 nm wavelength through a microplate reader examination. The assessment of percentage inhibition occurred through application of this equation.

$$\text{Percent Inhibition: } \frac{\text{Absorbance (Control)} - \text{Absorbance (Sample)} \times 100}{\text{Absorbance (Control)}}$$

α-Glucosidase Activity

A solution containing glucopyranoside compound enters phosphate buffer solvent while serving as the basis for further studies. Rephrase the following sentence. The synthesized compounds present at concentrations of 31.25, 62.5, 125, 250, and 500 µg/ml make up the sample solutions. Glucosidase enzyme receives addition to the distilled water mixture at 0.5 µg/ml concentration. During the incubation step the mixture needs twenty minutes of time at a temperature of 37°C. The reaction receives an end by adding hydrochloric acid at the termination of the incubation time. The spectrophotometer determines the color intensity by reading 540 nm wavelengths. The percentage inhibition determination follows a certain formula as mentioned below (Huneif *et al.,* 2022)..

$$\text{Percent Inhibition: } \frac{\text{Absorbance (Control)} - \text{Absorbance (Sample)} \times 100}{\text{Absorbance (Control)}}$$

In Silico Docking Studies

A molecular docking analysis predicted the binding affinity together with binding pose and ligand-binding site interactions of potential ligands within target protein areas. For this purpose researchers employed Autodock Vina software version 4.2.6 based at San Diego, CA in the United States. Researchers accessed the target protein structures via RCSB Protein Data Bank found at http://www.rcsb.org/pdb. The DoGSiteScorer software tool identified which sections of the proteins functioned as active sites (Volkamer *et al.*, 2012). Protein structure preparation occurred through Accelrys Discovery Studio Visualizer version 4.1 before the PDBQT file generation with AutoDock Tools version 1.5.6. The removal process of water molecules and co-crystallized ligands produced PDBQT files from the ligand-protein complex. The created structures based on the synthesized compounds and reference ligands originated from ChemSketch yet they maintained Mol. file formats. Open Babel software produced the three-dimensional structures of all ligands (O'Boyle *et al.*, 2011). The AutoDock Tools version 1.5.6 added torsions to PDB structures before saving them as PDBQT files. AutoDock Vina 4.2.6 served for the docking calculations of ligand-protein complexes because it determines the energy values (Trott and Olson, 2010). Accelrys Discovery Studio Visualizer (version 4.1) served to display the protein-ligand binding interactions.

RESULT AND DISCUSSION

Chemistry

The synthesis of twelve newly developed thiazolidine-4-carboxylic acid derivatives occurred through the method depicted in Figure 1. The synthesis of p-(2-chloroethyloxy) benzaldehyde (1) started with 4-hydroxybenzaldehyde during its reaction with 1,2-dichloroethane to produce the

ether derivative. The synthesis of corresponding esters 3(a-l) occurred after running compound (1) through reactions with various substituted carboxylic acids 2(a-l). Evidence for the formation of ester bonds appeared through FTIR spectra as carbonyl stretching signals between 1739 cm^{-1} and 1719 cm^{-1}. The synthesized esters went through reactions with Hydrated L-Cysteine HCl to yield thiazolidine derivatives 5(a-l). Through this condensation reaction new chiral stereocentric centers formed diastereomeric material mixtures (2RS, 4R) in compounds 5(a-l). The 1H-NMR recording at 5.6 ppm indicates the presence of 2R, 4R and 2S, 4R isomers by producing a single characteristic signal. The 70.4 ppm signal in the ^{13}C-NMR spectra for the C-2 carbon atom supported the experimental findings recorded for the obtained results.

In Vitro Pharmacological Evaluation

Alpha-amylase and alpha-glucosidase enzyme inhibitory properties were measured in twelve newly synthesized thiazolidine derivatives (compounds 5(a-l)) compared to acarbose reference inhibitor. The researchers calculated IC50 only for compounds showing more than 50% inhibitory action in the enzymatic test. The selected compounds showed their effects on α-amylase and α-glucosidase by generating data in Table 1.

Table 1: α-Amylase and α-glucosidase inhibition studies of newly synthesized Thiazolidine Derivatives 5(a-l).

Compounds	% Inhibition		IC_{50} (µg/ml)	
	α-Amylase	α-Glucosidase	α-Amylase	α-Glucosidase
5a	59.65	54.89	35.88	35.94
5b	62.78	60.54	36.67	36.76
5c	66.86	61.05	35.33	36.97
5d	71.34	68.58	33.54	33.76
5e	93.45	45.34	24.13	-
5f	41.87	87.32	-	22.76
5g	37.43	84.73	-	25.68
5h	36.66	30.76	-	-
5i	22.67	28.79	-	-
5j	35.93	37.35	-	-
5k	31.67	26.89	-	-
5l	25.65	23.95	-	-

Maximal inhibition of α-amylase was observed in five compounds (5a, 5b, 5c, 5d and 5e) among twelve tested substances with IC50 values of 35.88, 36.67, 35.33, 33.54, and 24.13 µg/ml and the strongest inhibitor 5e exceeded acarbose reference inhibitor (IC50 = 342.27 µg/ml). The seven compounds 5f, 5g, 5h, 5i, 5j, 5k, and 5l failed to provide depression above 50% so IC50 calculations were omitted (Table 1). Structure-activity relationship (SAR) analysis of the thiazolidine-4-carboxylic acid derivatives 5(a-l) indicated that compound 5e, bearing a powerful electron-withdrawing nitro group at position 2 of the phenyl ring, exhibited the most potent inhibition of α-amylase with an IC50 value of 24.13 µg/ml. Compounds 5a, 5b, 5c, and 5d, containing inductively electron-withdrawing halogen substituents at position 2 of the phenyl inhibition strength equal to acarbose in the reference experiments (Table 1). The experimental results indicated that 5f and 5g compounds inhibited the enzyme activity more efficiently than acarbose by showing IC50 values of 22.76 µg/ml and 25.68 µg/ml. None of the other compounds (5e, 5h, 5i, 5j, 5k, or 5l) reached an inhibition of 50% or above which meant they remained inactive. SAR revealed that the bioactivity of the compounds against α-glucosidase was largely affected by the chemical nature along with the positioning of substituents on the phenyl ring. Compounds 5f and 5g containing methoxy and hydroxyl groups positioned at the 2nd carbon of the phenyl ring displayed highly active properties with IC50 values of 22.76 µg/ml and 25.68 µg/ml against α-glucosidase yet acarbose had an IC50 value of 30.45 µg/ml. The α-glucosidase inhibitory capability of compound 5e was non-existent due to its position-2 phenyl ring bearing a strong electron-withdrawing nitro group which produced 45.34% enzyme inhibition. The α-amylase inhibitory response of the compound was superior among all synthesized molecules (Table 2). The present study showed inactivity of 5h 5i 5j 5k and 5l compounds against α-glucosidase inhibition despite their previous inactivity towards α-amylase inhibition (Table 1).

The study establishes electron-withdrawn halogen groups placed at the 2-position on the phenyl ring as critical factors ring, displayed α-amylase inhibition with IC50 values of 35.88, 36.67, 35.33, and 33.54 µg/ml, respectively. The α-amylase inhibitory activities recorded for compounds 5f and 5g that had electron donating methoxy and hydroxy groups at position 2 of the phenyl ring remained under 50% resulting in their categorization as inactive (Table 1). Analysis of compounds 5h, 5i, 5j, 5k and 5l with position 3 phenyl ring substitution showed no α-amylase inhibitory effects since their inhibitory percentages reached 36.66%, 22.67%, 35.93%, 31.67% and 25.65% respectively.

Among the twelve tested compounds six compounds including 5a, 5b, 5c, 5d, 5f and 5g showed effective α-glucosidase

leading to strong inhibitory effects against α-amylase and α-glucosidase. Selective α-amylase or α-glucosidase inhibitors may be designed by changing the position 2 substitution on the phenyl ring according to the study results. The data shows compound 5e (2-nitro substitution) acts as an α-amylase inhibitor whereas compound 5f (2-methoxy substitution) functions as an α-glucosidase inhibitor according to Table 1 observations.

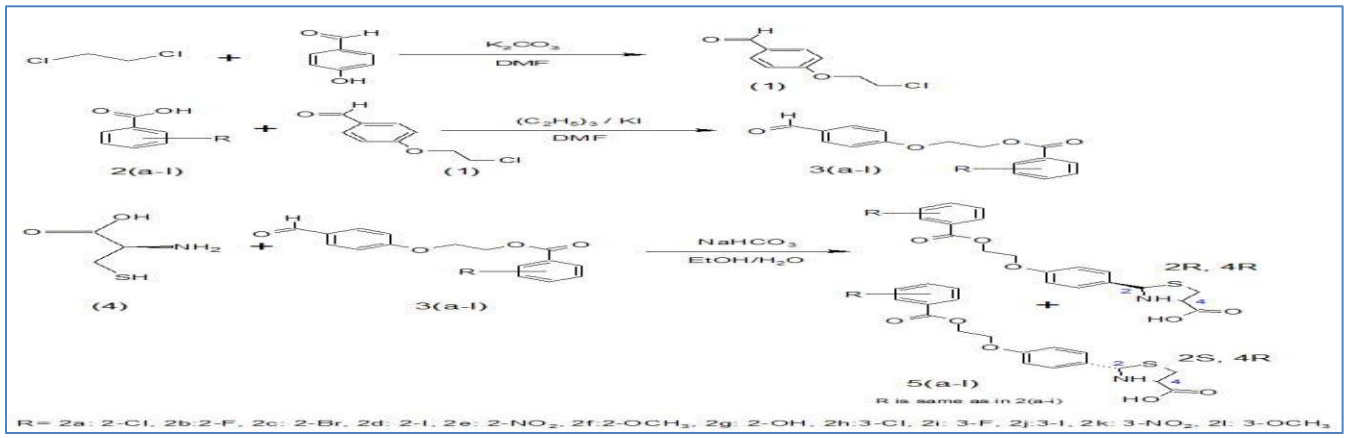

Figure 1: Synthesis of Thiazolidine-4-Carboxylic Acid Derivatives 5(a-l).

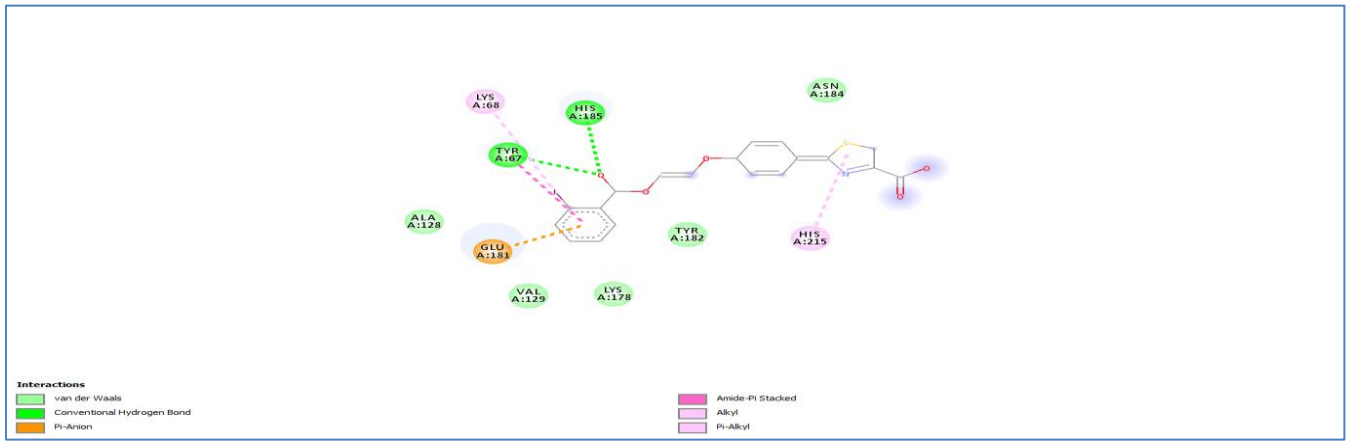

Figure 2: Binding interactions of compound 5d with alpha-amylase (PDB ID 4w93).

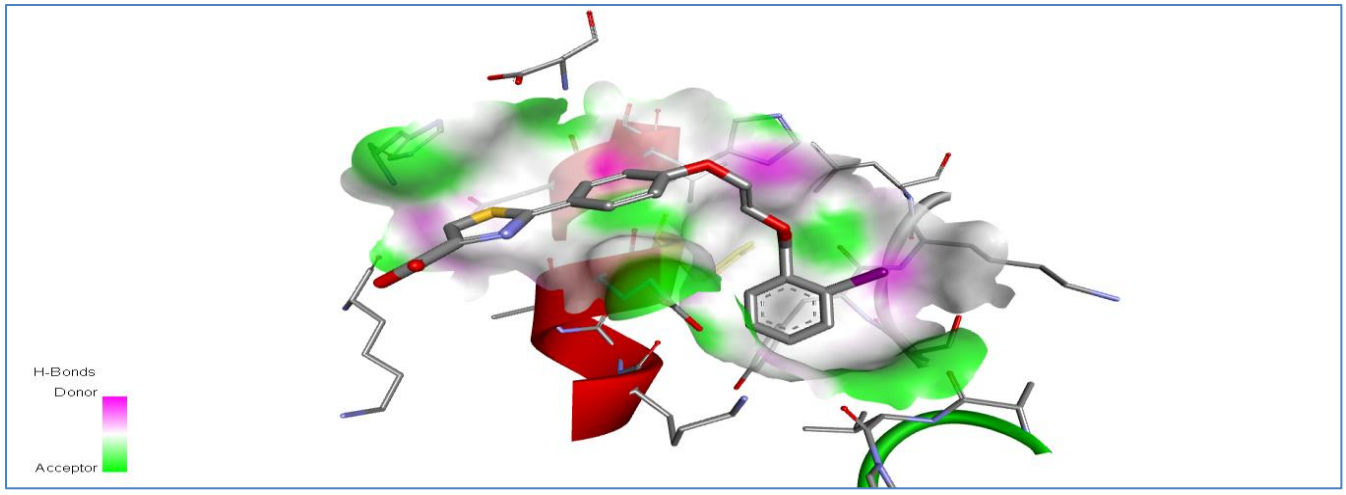

Figure 3: 3D pose of compound 5d docked in alpha-amylase.

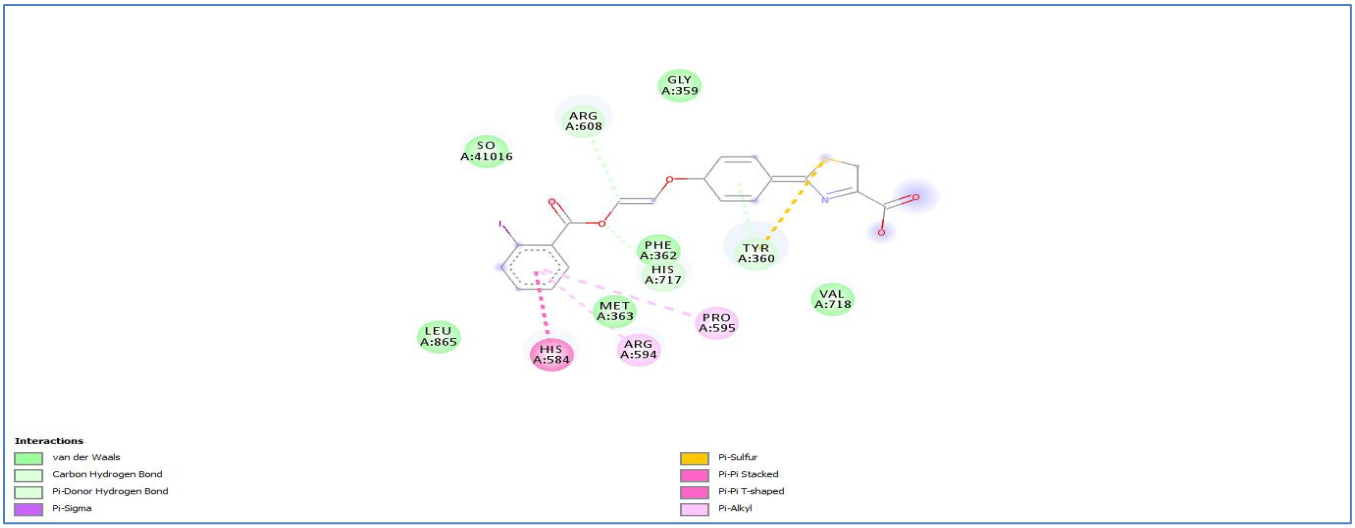

Figure 4: Binding interactions of compound 5d with alpha-glucosidase (PDB ID 5nn3).

In Silico Docking Studies

Results from docking scores show that all synthesized compounds exhibit activities similar to those of acarbose which serves as the standard. All active compounds demonstrate their strongest target protein binding behavior as shown in Table 2. Results validate how hydrophobic interactions namely pi-pi, pi-sulfur and pi-alkyl interactions bind the synthesized compound thiazolidine rings to specific protein residues (Figures 2,3,4, and 5).

The absence of hydrogen bonding occurred together with the particular ring positioning in the hydrophobic pocket that produced the observed hydrophobic interactions. Test outcomes from in vitro α-amylase and α-glucosidase inhibition assays matched the results from thiazolidine derivatives testing because these derivatives demonstrated greater effectiveness than the standard used in the experiments. Compound 5e with a 2-nitro moiety on its phenyl ring displayed the maximum binding capability toward alpha-amylase enzyme (4w93). The binding interaction between the thiazolidine ring and phenyl ring of the ligand occurs through pi-pi and pi-alkyl interactions which allow the ligand to extend toward Tryptophan (A:203) residue and lysine (A:140). The 2-nitro substitution added new Vander Waals bond systems between proline (A:204) (Figure 6 and 7). The docking results concerning glucosidase enzyme examination showed direct correspondence with experimental outcomes. Compound 5f with its phenyl ring substituted by 2-methoxy group engaged in both stacking and pi-pi interaction with tyrosine (A:360). An extra pi-sigma bonding interaction between the 2-methoxy group and molecular receptors was enabled (Figure 8 and 9). The alpha-glucosidase enzyme (PDB ID: 5nn3) formed all the documented pi-pi, pi-alkyl, and pi-anion interactions with various ligands.

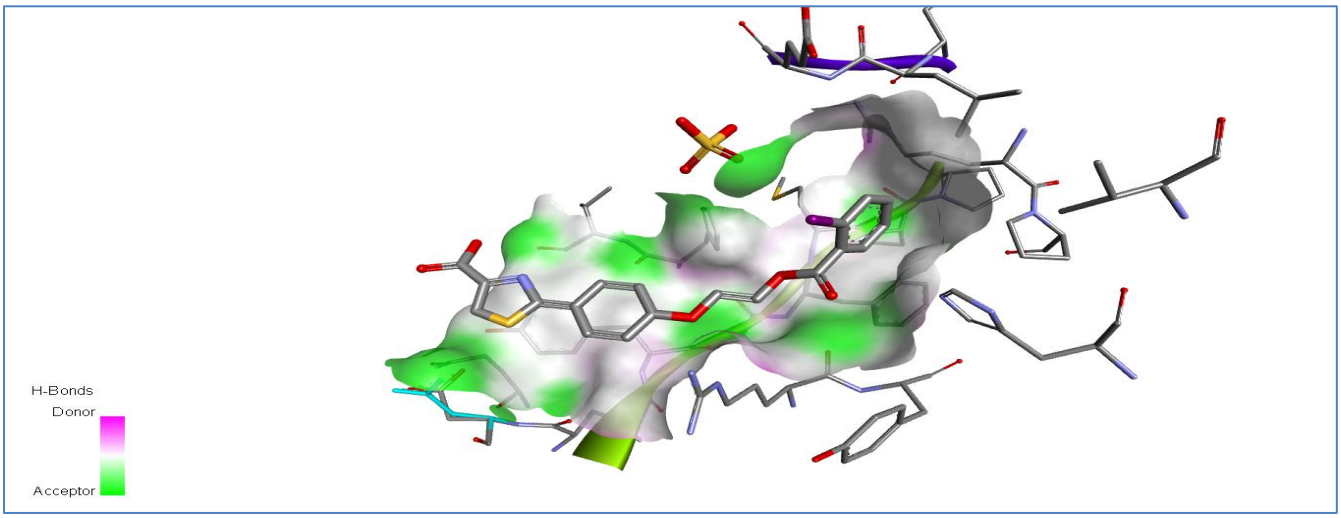

Figure 5: 3D pose of compound 5d docked in alpha-glucosidase.

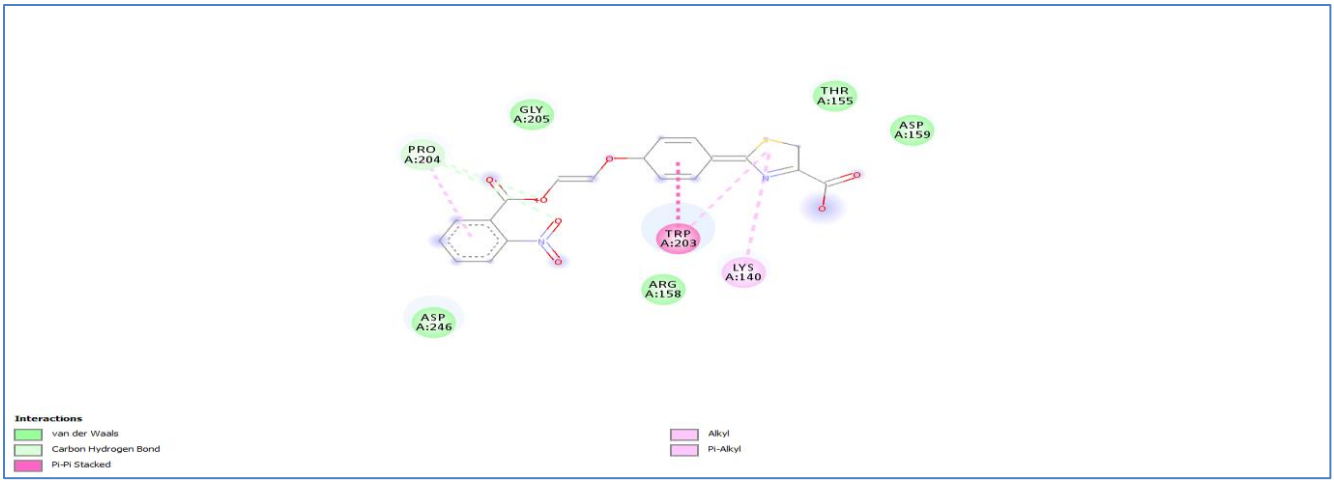

Figure 6: Binding interactions of compound 5e with alpha-amylase (PDB ID 4w93).

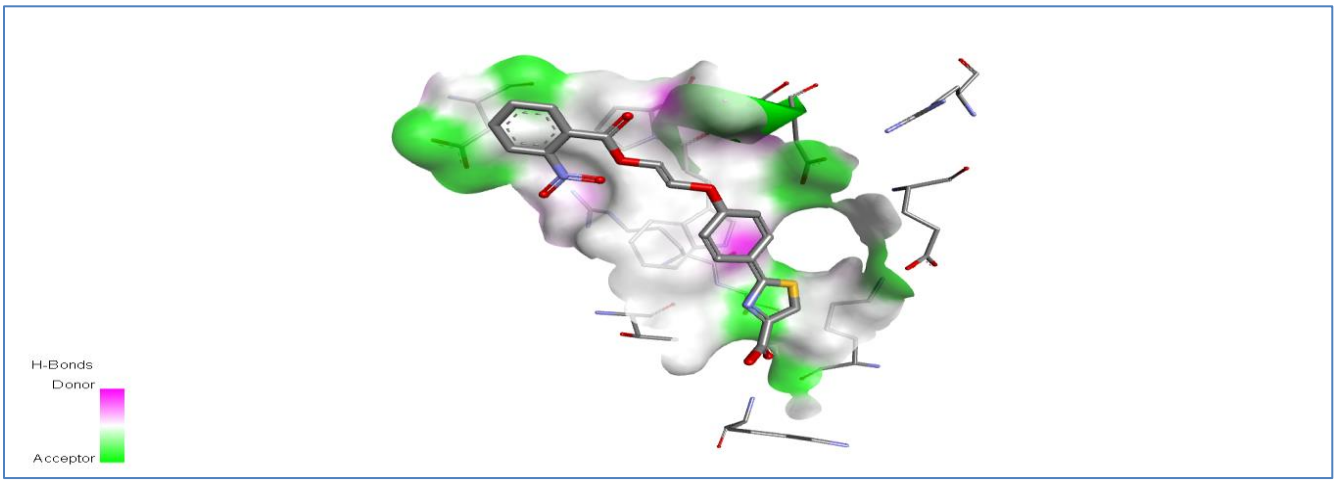

Figure 7: 3D pose of compound 5e docked in alpha-amylase.

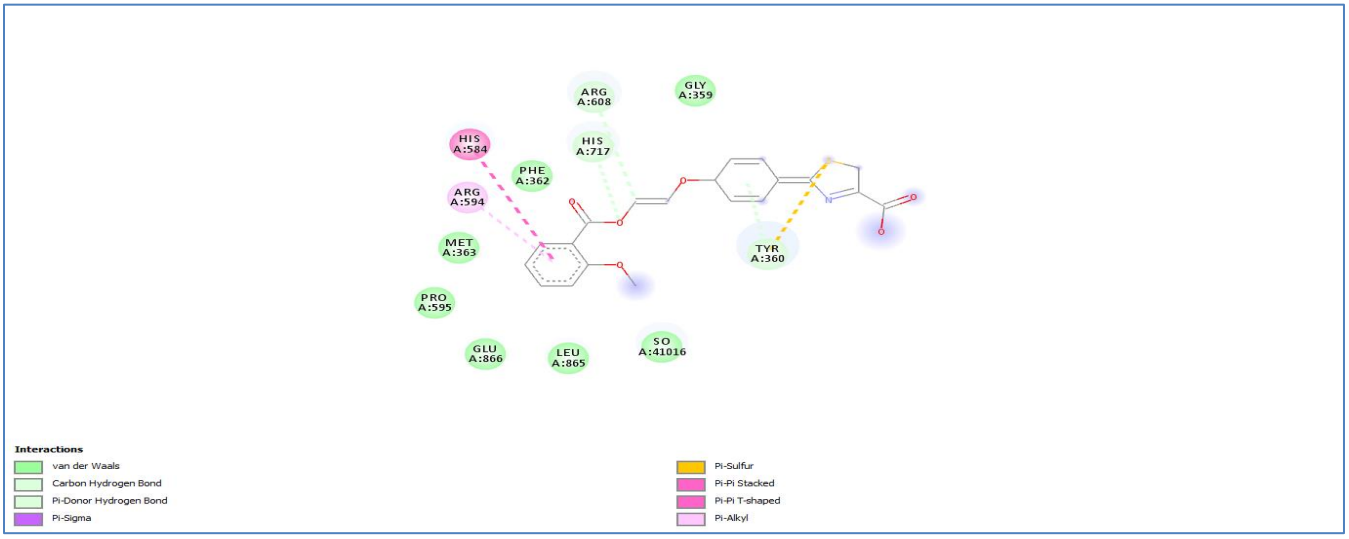

Figure 8: Binding interactions of compound 5f with alpha-glucosidase (PDB ID 5nn3).

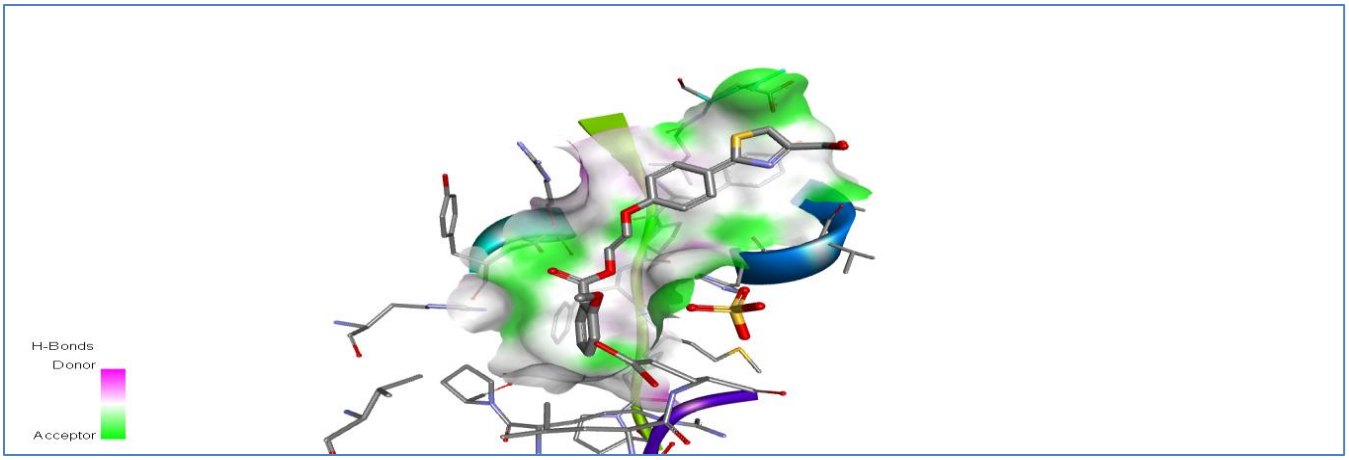

Figure 9: 3D pose of compound 5f docked in alpha-glucosidase.

Table 2 Docking scores of ligands with alpha amylase (4w93) and alpha glucosidase (5nn3).

`	Alpha-Amylase (PDBID:4w93) (Affinity in Kcal/mol)	Alpha Glucosidase (PDBID:5nn3) (Affinity in Kcal/mol)
5a	-7.8	-7.7
5b	-7.8	-7.6
5c	-7.7	-7.5
5d	-7.9	-7.8
5e	-8.9	-7.0
5f	-7.1	-8.8
5g	-7.2	-9.0
5h	-6.8	-6.3
5i	-6.7	-6.4
5j	-6.8	-6.6
5k	-6.6	-6.7
5l	-6.3	-6.1
Acarbose	-7.6	-7.4

CONCLUSIONS

Through our study we synthesized compounds 5(a-l) which presented potential inhibition activities for α-amylase and α-glucosidase enzymes. Academy results showed that compounds 5a, 5b, 5c, 5d and 5e demonstrated strong α-amylase inhibitory effects along with α-glucosidase inhibition by compounds 5a, 5b, 5c, 5d and 5f and 5g. The investigated compounds proved equivalent to the extent of effects as the reference drug acarbose. Compound 5a together with 5b and 5c and 5d showed strong inhibitory effects on the α-amylase and α-glucosidase enzyme activity. Research on structure-activity relationship (SAR) demonstrated that halogen substitutions at position 2 of the phenyl ring enabled the best inhibition outcomes against both enzymes. The selective inhibition properties of compounds 5e 5f and 5g against either α-amylase or α-glucosidase enzymes arose from position 2 phenotype ring substituents modifications. Molecular docking techniques revealed the interaction mechanisms between the active compounds and α-amylase (PDB ID: 4w93) as well as α-glucosidase (PDB ID: 5nn3) enzymes. The strong inhibitory activities of these structurally simple and readily accessible molecules against α-amylase and α-glucosidase offer promising prospects for the development of cost-effective therapeutic options against diabetes a widespread disease.

DECLARATIONS

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Conflict of Interest/Competing Interests: There are no conflicts of interest, whether of a financial or non-financial nature, that could influence the impartiality of the research.

Ethics Approval: It is noted that this investigation did not entail the involvement of either animal subjects or human participants, thereby rendering ethics approval unnecessary.

Consent to Participate: The concept of obtaining consent for participation does not apply to the scope of this study.

Consent for Publication: All the authors have diligently examined and provided their approval for the final version of the manuscript, endorsing its readiness for publication.

AUTHORS' CONTRIBUTIONS

All the authors have significantly contributed to the

conceptualization and design of the research.

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