Synthesis, α-glucosidase and α-amylase inhibitory activities, acute toxicity and molecular docking studies of thiazolidine-2,4-diones derivatives

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1. Introduction

Over 90% of diabetic patients suffer from type 2 diabetes mellitus (T2DM), which is a multifactorial metabolic disorder, characterized by an uncontrolled chronic hyperglycemia, related to failure of insulin function (insulin resistance) over extended periods in peripheral tissues (Hameed et al., 2015). This chronic high blood glucose level leads to severe complications such as cardiovascular diseases, neuropathy, kidney failure and obesity (Abbas et al., 2019; Hanefeld et al., 1996). This makes it necessary to maintain a normal blood glucose level, especially postprandial hyperglycemia, as a solution for the control of this pathology and prevent their complications. One of the approaches to manage diabetes is to enhance the functionality of pancreatic α-amylase and intestinal α-glucosidase (Joshi et al., 2015; Rafique et al., 2020), which are two enzymes positioned in the digestive system. These enzymes are responsible for the degradation process of starch and oligosaccharides respectively into monosaccharides such as glucose and fructose (Joshi et al., 2015). In this regard, inhibition of α-amylase and α-glucosidase play a critical role in modulating the absorption of carbohydrates and preventing postprandial hyperglycemia, making inhibitors a useful solution in the management of type 2 diabetes (Leroux-Stewart et al., 2014). Thiazolidine-2,4-diones (Glitazones) are an important class of oral antidiabetic agents that have attracted the community attention for their excellent control of glucose increasing effect without causing hypoglycemia (Bloomgarden et al., 2018). Due to their flexible and diverse nature, thiazolidinediones (TZDs) show also a wide range of pharmacological activities which include anti-hyperglycemic (Naim et al., 2017), anti-proliferative (Patil et al., 2010), anti-obesity (Bhattarai et al., 2010), anti-microbial (Avupati et al., 2012; Sun et al., 2019) and anti-inflammatory effects (Prabhakar et al., 1998). Furthermore, TZDs derivatives also reported as α-glucosidase inhibition
(Chinthala et al., 2013). Despite their diverse therapeutic profile and excellent antidiabetic properties, thiazolidinediones possess some severe side effects, such as weight gain (Fonseca, 2003), plasma-volume expansion, bone related disorders and edema (Berlie et al., 2007; Forman et al., 2000; Okazaki et al., 1999; Willson et al., 2000). One of the marketed glitazone drugs (Figure 1), Troglitazone, has been removed due to its hepatotoxicity (Smith, 2003). In this context, extensive research on TZD has been going around the globe to develop novel or improved derivatives better and safer without compromising the diabetic potential. In a recent publication, our research team has reported work on the design and synthesis of new thiazolidine-2,4-dione derivatives, in search of potential lead compounds, which can demonstrate promising results. The aim of this study consists of the evaluation of the in vitro antidiabetic profile of a library of newly synthesized thiazolidine-2,4-dione derivatives (3a–3e) and (4a–4e) by showing their potential to reduce postprandial blood hyperglycemia by the inhibition of digestive enzymes (α-glucosidase and α-amylase), the molecular docking studies were performed on the two enzymes to predict the mechanism of inhibition and the orientation within a targeted binding site.

2. Results and discussion

2.1. Chemistry

First, we examined the survey of the Knoevenagel condensation reaction by using TZD 1 and benzaldehyde 2a as models adsorbed on mineral support under solvent-free MW conditions. All minerals solid supports used are commercially available. These conditions, which are collected in Table 1, included nature of solid support, reaction time and temperature. Therefore, we found that the reaction carried out in basic alumina irradiated in a microwave digestion system in open vessel afforded the desired product 3a as a single (Z)-diastereoisomer in high yield 95% yield at 108°C for short

Table 1. Optimization of different supports for the synthesis of 3a model product.
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that basic Al₂O₃ catalyzes the synthesis of 5-arylidenethiazolidine-2,4-diones (Syassi et al., 1997).

The involvement of surface hydroxyl groups on no calcined supports increases remarkably in the presence of hydroxylated supports, and 6), and the reaction requires a few minutes with improved yields using MW. It is interesting to note that the reactivity has been improved also on acidic alumina and silica (entries 3–5). The condensation reaction realized without support or by using of calcined alumina or K10 bentonite clay, did not work under microwave irradiation even at same temperature and longer reaction time (entries 2, 4 and 8). The condensation reaction has been improved also on acidic alumina and silica (entries 3 and 6), and the reaction requires a few minutes with improved yields using MW. It is interesting to note that the reactivity increases remarkably in the presence of hydroxylated supports, we observed a significant effect leading to an increase of yield of 3a in a short reaction time (5–10 min) (Table 2, entries 5–7). The involvement of surface hydroxyl groups on no calcined alumina, and silica supports plays a determinant role in the chemical activation of methylene group condensation with aromatic aldehydes (Khalfinezhad et al., 2001; Kwon et al., 1997; Syassi et al., 1997).

After optimization of the reaction conditions, this methodology was extended for the synthesis of 5-arylidenethiazolidine-2,4-diene via Knoevenagel condensation reaction between TZD 1 and various aromatic aldehydes. It was found that basic Al₂O₃ catalyzes the synthesis of 5-arylidenethiazolidine-2,4-diones (3a–3e) in an efficient manner and excellent yields (90–95%) of the desired products were obtained (Table 2, entries 1–5). The synthesized products (3a–3e) were characterized by ¹H NMR, ¹³C NMR and ESI-MS, and melting point, which were in accordance with the literature data (Giles et al., 2000; Zhang & Zhou, 2012). Then, the

Table 2. Synthesis of 5-arylidenedithiazolidine-2,4-dione derivatives (3a–3e).

| Entry | Product | R₁ | R₂ | Yield (%) | M.p. (°C) |
|-------|---------|----|----|-----------|-----------|
| 1     | 3a      | H  | H  | 95        | 246–248   |
| 2     | 3b      | CH₃| H  | 92        | 229–231   |
| 3     | 3c      | F  | H  | 97        | 218–220   |
| 4     | 3d      | Br | H  | 93        | 243–245   |
| 5     | 3e      | Cl | Cl | 89        | 215–217   |

*aExperimental conditions: TZD (1 mmol) and aromatic aldehyde (2 mmol) were adsorbed on basic alumina (1 g/mmol of TZD) and irradiated under MW for 5 min at 108°C, temperature measurement in microwave-assisted open teflon reactors.

*bYield of isolated product.

Table 3. Synthesis of N-allyl 5-arylidenedithiazolidine-2,4-dione derivatives (4a–4e).

| Entry | Product | R₁ | R₂ | Yield (%) | M.p. (°C) |
|-------|---------|----|----|-----------|-----------|
| 1     | 4a      | H  | H  | 62        | 102       |
| 2     | 4b      | CH₃| H  | 65        | 116       |
| 3     | 4c      | F  | H  | 58        | 121       |
| 4     | 4d      | Br | H  | 55        | 146       |
| 5     | 4e      | Cl | Cl | 60        | 113       |

*aExperimental conditions: Product 3 (1 mmol) and Allyl bromide (1.2 mmol) in EtOH/H₂O (2:1, 10 mL) at 25°C were added NaOH (1 mmol), and the reaction mixture was stirred and heated at 75°C for 5–6 h.

*bYield of isolated product.

Table 4. α-glucosidase and α-amylase inhibitory activities for the synthesized compounds.

| Compound | R₁ | R₂ | α-glucosidase (IC₅₀ ± SD, [μM]) | α-amylase (IC₅₀ ± SD, [μM]) |
|----------|----|----|--------------------------------|-----------------------------|
| 3a       | H  | H  | 98.88 ± 1.11                  | 126.67 ± 2.10               |
| 3b       | CH₃| H  | 380.10 ± 0.01                 | 286.25 ± 4.30               |
| 3c       | F  | H  | 339.7 ± 1.02                  | 263.73 ± 1.54               |
| 3d       | Br | H  | 156.3 ± 7.93                  | 173.30 ± 4.70               |
| 3e       | Cl | Cl | 84.95 ± 1.01                  | 47.09 ± 0.04                |
| 4a       | H  | H  | 98.45 ± 0.54                  | 108.14 ± 2.05               |
| 4b       | CH₃| H  | 120.8 ± 1.10                  | 167.02 ± 0.97               |
| 4c       | F  | H  | 211.6 ± 3.25                  | 195.77 ± 2.14               |
| 4d       | Br | H  | 101.9 ± 0.43                  | 156.48 ± 3.17               |
| 4e       | Cl | Cl | 43.85 ± 1.06                  | 18.19 ± 0.11                |
| Acarbose | –  | –  | 97.12 ± 0.35                  | 2.975 ± 0.01                |

αSD means Standard Deviation of the Mean.

time (5 min) as compared with reaction carried out under classical synthetic conditions (70%, 5 h) (Table 1, entries 4 and 9). However, the use of the use of closed vessel microwave assisted Knoevenagel reaction has not favored the reaction for 10 min at 110°C. Reversible dehydration condensation under pressure is in principle a difficult transformation because the amount of water removed from the product in a closed system pushes the equilibrium in favor of the hydrated compounds (Brahmachari, 2016; Murase et al., 2012). Moreover, the condensation reaction realized without support or by using of calcined alumina or K10 bentonite clay, did not work under microwave irradiation even at same temperature and longer reaction time (entries 2, 4 and 8). The condensation reaction has been improved also on acidic alumina and silica (entries 3 and 6), and the reaction requires a few minutes with improved yields using MW. It is interesting to note that the reactivity increases remarkably in the presence of hydroxylated supports, we observed a significant effect leading to an increase of yield of 3a in a short reaction time (5–10 min) (Table 2, entries 5–7). The involvement of surface hydroxyl groups on no calcined alumina, and silica supports plays a determinant role in the chemical activation of methylene group condensation with aromatic aldehydes (Khalfinezhad et al., 2001; Kwon et al., 1997; Syassi et al., 1997).

2.2. Biology

2.2.1. α-Glucosidase inhibition

In an ongoing project which is aimed to develop new enzyme inhibitors, all newly synthesized thiazolidine-2,4-dione derivatives (3a–3e) and (4a–4e) were screened for their in vitro α-glucosidase inhibitory potential. The inhibitory effects were plotted against the log of concentrations using non-linear regression curve approach from which IC₅₀ values were calculated. The all tested compounds exhibited a good to moderate inhibitory activity with IC₅₀ values between 43.85 ± 1.06 and 380.10 ± 1.02 μM, when compared to standard inhibitor acarbose (IC₅₀ = 97.12 ± 0.35 μM) (Table 4). Five compounds 3a, 3e, 4a, 4d and 4e were found to be the most potent inhibitors than the rest of tested compounds, with IC₅₀ values of 98.88 ± 1.11, 84.95 ± 1.01, 98.45 ± 0.54, 101.9 ± 0.43 and 40.67 ± 1.81, respectively, in comparison with the standard drug acarbose. Furthermore, Compounds 3b, 3c, 3d, 4b and 4c showed moderate inhibitory activity with IC₅₀ values of 380.10 ± 1.11, 339.70 ± 1.02, 156.3 ± 7.93, 120.8 ± 1.10, 207.9 ± 4.76 and 211.6 ± 3.25. Based on these results, and to develop the structure-activity relationship (SAR) study, we have divided the synthesized molecules in two groups; Compounds (3a–3e), with different substitutions at variable positions of benzene and (4a–4e) obtained by the N-allylation of compounds (3a–3e). The general structural features of the synthetic molecule comprise of thiazolidine-2,4-dione (TZD) ring, aryl ring and allyl group. All these parts are playing important role in the activity, however, a slight variation in the structure of the synthesized molecules such as the position and the nature of the substituents on the aryl or the N-allylation of TZD could make a great variation in the α-glucosidase inhibitory activity. In fact, within the inhibitory activity of 5-arylidenedithiazolidine-2,4-dione derivatives (3a–3e), compound 3a (IC₅₀ = 98.88 ± 1.11 μM) bearing benzene ring without any substitution displayed good activity among the serie studied. Compound 3d (IC₅₀ = 156.3 ± 7.93 μM) bearing bromo substituent in para position of phenyl ring showed moderate activity. Additionally,
the insertion of a methyl or fluoro groups in para position afforded compounds 3b (IC$_{50}$ = 380.10 ± 1.11 μM) and 3c (IC$_{50}$ = 339.70 ± 1.02 μM), resulted in a sharp decrease in inhibitory activity against α-glucosidase enzyme. However, compound 3d (IC$_{50}$ = 380.10 ± 1.11 μM) substituted with chloro group in ortho and para positions showed the best inhibitory activity compared to compound 3a and acarbose. In general, the presence of electron-releasing (Me) or electron-attracting substituents (Br or F) on phenyl ring reduced the inhibitory activity compared to unsubstituted phenyl (compound 3a), and there was no clear difference between them. In fact, several compounds characterized by the presence of substituents with opposite electronic effects showed the same potency. For example, compound 3b contains the electron-attracting atom Chlorine on benzyl have in ortho and para positions exhibited more inhibition potential, in comparison with their analog non-allylated 3a and acarbose. In fact, the compound 4e obtained by N-allylation of 3e proved to be 2-fold more active than the corresponding analog 3e. Compounds 4b (IC$_{50}$ = 120.8 ± 1.10 μM), 4c (IC$_{50}$ = 211.6 ± 3.25 μM) and 4d (IC$_{50}$ = 101.9 ± 0.43 μM) obtained by N-allylation of 3b, 3c and 3d, showed a considerable increase in inhibitory activity, compared to their non-allylated analogs. For example, compound 4b obtained by N-allylation of 3b proved to be 3-fold more active than the N-allylated analog 3b. Therefore, it can be concluded that the ally group on N-3 of thiazolidine-2,4-dione system once again proved to play a critical role in the binding of compounds (4a–4e) to the active site of α-glucosidase enzyme by resulting in the reduction of IC$_{50}$ values up to one three times, in comparison with N-allylated compounds (3a–3e).

2.2.2. α-Amylase inhibition

In the pursuit of exploration new anti-diabetic compounds with enzymatic inhibition, we have screened our thiazolidine-2,4-dione compounds (3a–3e) and (4a–4e) for human pancreatic α-amylase inhibitory activity. The inhibitory concentrations of the synthesized inhibitors were determined using acarbose as a standard inhibitor with an IC$_{50}$ value of 2.975 ± 0.004 μM. The bioactivity results are presented in Table 4. Among the evaluated molecules, compounds 4e and 3e revealed to be the most active analog in the series with a strong inhibitory effect, providing an IC$_{50}$ value of 18.19 ± 0.103 μM and 47.09 ± 0.037 μM, respectively.

The selected substituents inserted on the phenyl ring in different positions were found to produce opposite effects on the α-amylase inhibitory activity of the two series of compounds (3a–3e) and (4a–4e). In fact, within the inhibitory activity of 5-aryldienethiazolidine-2,4-dione derivatives (3a–3e), the effects triggered by the introduction of a methyl (3b, IC$_{50}$ = 286.25 ± 4.30 μM), fluoro (3c, IC$_{50}$ = 263.73 ± 1.54 μM) or bromo (3d, IC$_{50}$ = 173.30 ± 4.70 μM) group in the para position of the phenyl ring proved to be indifferent or negligible for the affinity with α-amylase in comparison with the corresponding unsubstituted derivative 3a (IC$_{50}$ = 126.67 ± 2.10 μM). These results confirm clearly that the presence of an electron-attracting substituents (Me) or electron-attracting substituents (Br or F) on phenyl ring reduced the inhibitory activity compared to unsubstituted phenyl, in good agreement with the results found in the α-glucosidase test. On the other hand, the presence of two chloro groups in the ortho and para position (compound 3e) has been found to reduce the IC$_{50}$ value to 47.09 ± 0.04 μM.

Moreover, N-allyl-5-aryldienethiazolidine-2,4-dione derivatives (4a–4e) were even more active than their analogs N-unallylated by inhibiting the enzyme with IC$_{50}$ values ranging from 18.19 ± 0.11 to 208.10 ± 1.80 μM (Table 4).

The allyl group on N-3 of thiazolidine-2,4-dione once again proved to play a critical role in the inhibitory activity of compounds (4a–4e) against α-amylase by resulting in the reduction of IC$_{50}$ values up to two times in comparison with N-unallylated compounds (3a–3e). Among N-allylated inhibitors, compounds 4a (IC$_{50}$ = 108.14 ± 2.05 μM), 4b (IC$_{50}$ = 167.02 ± 0.97 μM), 4c (IC$_{50}$ = 195.77 ± 2.14 μM) and 4d (IC$_{50}$ = 156.48 ± 3.17 μM) showed a considerable increase in inhibitory activity, compared to their non-allylated analogs. For example, compound 4b obtained by N-allylation of 3b proved to be 3-fold more active than the N-allylated analog 3b. Therefore, it can be concluded that the ally group on N-3 of thiazolidine-2,4-dione system once again proved to play a critical role in the binding of compounds (4a–4e) to the active site of α-glucosidase enzyme by resulting in the reduction of IC$_{50}$ values up to one three times, in comparison with N-allylated compounds (3a–3e).

2.3. Molecular docking studies

To understand the observed activities concerning the different molecular structures of inhibitor tested, the molecular docking studies were carried out to shed light on the binding modes between docked ligands and enzyme targets (Hafidi et al., 2021). According to the molecular structures of the ligands (Figure 2), they can be divided into two series with a common carbon skeleton (mentioned in blue color), the change of the organic group (R = CH$_3$, F, Br and Cl) it will be the challenges of the discussion below.

The likeliest docked poses of ligands with the best binding affinity for ligands complexes in the active site of the targeted enzyme α-amylase and α-glucosidase are shown in Figures 3 and 4.

The docking results of the synthesized compounds with the enzyme’s targets have given good information about the nature of the binding mode.

The analysis of the binding site revealed that the synthesized ligands are stabilized by several favorable interactions
Figure 2. Chemical structure designed for the TZD ligand of two series tested against the receptors $\alpha$-amylase (PDB = 1B2Y) and $\alpha$-glucosidase (PDB = 3W37), the common carbon skeleton is mentioned by the blue color.

Figure 3. Ligand-receptor interaction for the best ligand ($a = 3b$, $b = 4a$) which has more hydrogen interactions for the $\alpha$-amylase target enzyme (PDB = 1B2Y).
Figure 4. Ligand-receptor interaction for the best ligand (α = 3b, β = 3d) which has more hydrogen interactions for the α-glucosidase target enzyme (PDB = 3W37).

Table 5. Docking binding energies, number of hydrogen bonds and hydrophobic interaction for the docked ligand TZD and the reference drug within the active binding site of the targeted α-amylase (PDB = 1B2Y) and α-glucosidase (PDB = 3W37).

| Name of synthesized compounds | Free binding energy (kcal mol⁻¹) | H-Bonds Numbers | Hydrophobic interaction number | IC₅₀ ± SEM |
|-------------------------------|-----------------|----------------|-----------------------------|---------|
| α-amylase (PDB = 1B2Y)       |                 |                |                             |         |
| 3a                            | −7.1            | 3              | 2                          | 126.67 ± 2.10 |
| 3b                            | −7.5            | 3              | 4                          | 286.25 ± 4.3 |
| 3c                            | −7.3            | 2              | 2                          | 263.73 ± 1.54 |
| 3d                            | −7.2            | 0              | 5                          | 173.30 ± 4.70 |
| 3e                            | −8              | 2              | 5                          | 47.09 ± 0.04  |
| 4a                            | −8              | 4              | 4                          | 108.14 ± 2.05 |
| 4b                            | −7.5            | 2              | 3                          | 167.02 ± 0.97 |
| 4c                            | −8              | 2              | 3                          | 195.77 ± 2.14 |
| 4d                            | −7.3            | 0              | 5                          | 156.48 ± 3.17 |
| 4e                            | −6.8            | 0              | 4                          | 18.19 ± 0.11  |
| Acarbose                      | −10.4           | 7              | 0                          | 2.975 ± 0.01  |
| α-glucosidase (PDB = 3W37)    |                 |                |                             |         |
| 3a                            | −6.3            | 3              | 0                          | 98.88 ± 1.11  |
| 3b                            | −6.3            | 3              | 5                          | 380.10 ± 0.01 |
| 3c                            | −6.3            | 2              | 0                          | 339.7 ± 1.02  |
| 3d                            | −6.2            | 1              | 3                          | 156.3 ± 7.93  |
| 3e                            | −6.1            | 1              | 3                          | 84.95 ± 1.01  |
| 4a                            | −7.5            | 0              | 3                          | 98.45 ± 0.54  |
| 4b                            | −6.8            | 0              | 4                          | 120.8 ± 1.10  |
| 4c                            | −7.7            | 3              | 2                          | 211.6 ± 3.25  |
| 4d                            | −6.6            | 0              | 8                          | 101.9 ± 0.43  |
| 4e                            | −6.8            | 0              | 6                          | 43.85 ± 1.06  |
| Acarbose                      | −11.4           | 15             | 2                          | 97.12 ± 0.35  |
including polar, hydrogen bond, hydrophobic and electrostatic interactions (Tables S1–S4). It was observed that all the compounds exhibited high free energy binding between −6 and −8 kcal/mol. For the reference drug Acarbose, we can see from Table 5 that is obvious from the docking results in that the high activity of the reference drug acarbose compared to the others studied compounds is due mainly to the stability of the complex acarbose with target enzyme and the high number of hydrogen bonds formed between the acarbose and the active residues.

2.3.1. Against α-amylase enzyme target (PDB = 1B2Y)

We can see from Table S1 that the 3a, 3b, 3c, 3d and 3e compounds of the series 1 present the same categories of interaction (hydrogen and hydrophobic interactions) with the same residues: ARG195, HIS299, ASP300, LEU165 and, TYR62. The number of interactions brought into contact between the inhibitors and the residues shows a dependence on the molecular structure. The two additional hydrophobic interactions Pi-Alkyl type have been observed against TRP95 in the molecular structure. The two additional hydrophobic interaction modes Pi-Alkyl type have been observed against TRP95 in the molecular structure which essentially due to the existence in its molecular structure of a methyl group (–CH₃).

The halogen grouping (Fluorine) does not present any modification concerning the number and the type of interaction, in the structure of 3c, the fluorine group affects just the interaction distance against the same residue presented in the 3a interaction mode.

The contribution of the sulfur atom was observed just in the interaction modes of 3d and 3e against residues TRP58, TYR62, HIS299 and ASP197. The only electrostatic interaction has been observed for 3d against ASP300 residue, further, the halogen Br and Cl groups appear in the chemical structure of 3e and 3d participates just in hydrophobic interaction against the following residues TRP59, TYR62, LEU165 and LEU162. The 3d does not present any hydrogen interaction, on the other hand, two Hydrogen interactions have been observed in the mode of interaction of 3d against the residues ASP300 and HIS299.

From Table S2, by comparison with 4a molecular structure, the addition of a methyl group (-CH₃) in the structure of 4b affecting a decrease of the participation of the two groups Ethylene (C=C) and Phenyl (Ring(C6)) in the hydrophobic interaction. Moreover, the molecular structure of 4b promotes the contribution of the sulfur atom in three Pi-sulfur interactions with the residues TRP58, TYR62 and HIS299.

Two hydrogen bonds have been observed in the mode of action of 4c, in which the carbonyl (C=O) and H (CH₃) are in contribution against ARG195 and GLU233. With comparison to 4b, the same Pi-sulfur interaction with the same residue was observed in the interaction of 4c. Moreover, just hydrophobic interactions have been observed in the 4d and 4e interaction modes against residues TRP59, ALA198, LEU162 and LEU165.

2.3.2. Against α-glucosidase target enzyme (PDB = 3W37)

From Table S3, all the ligands of series 1 exhibit, in their interaction mode different interactions, hydrogen, hydrophobic, electrostatic and halogen bonding with different residues.

The presence of -CH₃ in 3b structures hinder the contribution of the two common active site S (sulfur) and the hydrogen of NH, on the other hand, these two sites represent their activity for the ligands 3a and 3c. The absence of the contribution of the NH site has noted in the 3d and 3e mode of interaction. 3d and 3e exhibit only one hydrogen interaction in which the carbonyl group is in contribution against the residue ARG552. Similar to the 1B2Y enzyme, against α-glucosidase, the contribution of Br and Cl for 3d and 3e is limited only in the hydrophobic interaction.

Among series 1, the 3b is the most one that makes more of the hydrogen interaction with different residue ASP232, ASN496 and SER497. Compared to the 3a, the presence of the methyl group in 3b molecular structure increases the number of hydrophobic interactions with different residues PHE476, ALA231 and ILE233. The specificity of the existence of Halogen interactions has only been observed for 3c interaction mode against residues ASP357 and ASP469.

Different types of interaction have been observed in the interaction modes of the ligands of series 2. According to Table S4, among the ligands of this series, the 4c with a Fluorine group is the only one that contributed to three hydrogen bonding against the residues ARG552 and ASP469. The contribution of the C=C group in hydrophobic interactions was observed in the interaction modes of all ligands. For 4d and 4e, their interaction mode exhibits no hydrogen interaction, the presence of Br and Cl in the two structures of 4d and 4e precisely increases their hydrophobic interaction character. The only halogen interaction was observed of 4c ligand against the residues ASP357 and ASP469.

According to the behavior of the ligands tested against the two enzymes, we can build a general idea concerning the contribution by which each fragment participated in the interaction modes.

From the results presented in Tables S1–S4 of docking results, we note that at the level of the common carbon skeleton between all the ligands (mentioned in blue color Scheme 1), the two reactive rings sites with 6 and 5 carbons atoms participate in the majority of cases in the hydrophobic interactions types, sometimes we can see their contribution in the interaction of Pi-anion types.

Between the two series 1 and 2, the substitution of the hydrogen atom of nitrogen by a propene group leads to a decrease of participation of the common carbon skeleton in the hydrogen interactions type, but this replacing group has a very important role to increase the number of hydrophobic interactions against the numerous residues in the two enzyme target.

Among the halogen groups (Br, Cl and F) only the fluorine which exhibits the halogen interaction types precisely against the 3W37 enzyme target for the ligands 3c and 4c, the other halogens such as bromide and chloride their main contribution are hydrophobic interactions in the two proteinic pockets 3W37 and 1B2Y.

It is important to mention here that the residues Asp197, Glu233 and Asp300 have been observed in the interaction
modes for certain ligands against the target of the enzyme 1B2Y, each residue among those motioned having a very important role, according to the work of Rydberg and Zhang (Rydberg et al., 2002; Zhang et al., 2009). For the residue Asp197, it was previously reported as a nucleophilic catalytic in the hydrolysis reactions of polymeric substrates as food starch, besides (Rydberg et al., 2002; Zhang et al., 2009), the Asp300 residue has been identified as the player for optimizing the orientation of the substrate molecule using the hydrogen bonding interaction and as a steric conflict regulator for better binding conformation of the substrate (Williams et al., 2012). The last residue Glu233 is known to act as an acid-base catalyst during substrate hydrolysis reactions (Li et al., 2005; Williams et al., 2012).

2.4. Acute oral toxicity

The maximal dose (2000 mg/kg B.W) of each thiazolidinde-2,4-diones derivatives do not shows any related signs of mortality or toxicity tested animals of each group, during the 14 days of test. No weight loss or abnormal changes in the behavioral pattern or any undesired pathologic changes of the animals have been observed during the treatment. Therefore, the oral lethal doses of these products are greater than 2000 mg/kg (Table 6).

Table 6. Effects of 3e and 4e products on body weight variation of swiss mice with a dose of 2000 mg/kg.

| Groups  | Dose (mg/kg) | Initial weight (1st day)* | Final weight (14th day)* | Difference |
|---------|-------------|---------------------------|--------------------------|------------|
| 3e      | 2000        | 29.99 ± 1.88              | 32.67 ± 0.95             | + 2.48     |
| 4e      | 2000        | 28.82 ± 1.82              | 31.23 ± 0.86             | + 2.41     |
| Control | D.W         | 27.68 ± 3.53              | 29.63 ± 3.08             | + 1.95     |

*Data are expressed as mean ± SD (n = 6).

3. Conclusion

A variety of synthesized thiazolidine-2,4-dione derivatives were screened for their in vitro α-glucosidase and α-amylase inhibitory activities and acute oral toxicity on Swiss mice in order to get more potent and non-toxic molecules for treatment of type 2 diabetes mellitus. In the present investigation, we identified lead compounds that have shown to be dual inhibitors of α-glucosidase and α-amylase. The enzymatic screening revealed compounds 3e, 4a and 4e were significantly highly potent against two enzymes α-glucosidase (IC50: 84.95 ± 1.01; 98.45 ± 0.54; 43.85 ± 1.06 μM, respectively) and α-amylase (IC50: 47.09 ± 0.04; 108.14 ± 2.05; 18.19 ± 0.11 μM, respectively) as well as standard acarbose (IC50glucosidase = 97.12 ± 0.35 μM; IC50amylase = 2.975 ± 0.01 μM). The most potent compounds were also found to be non-toxic at concentration of 2000 mg/kg. Additionally, the molecular docking studies were also carried out on all the molecules, in order to obtain an insight into the binding interactions with the active sites. Some molecules showed high binding affinity with enzymes due to their high interaction with some sensible amino acid residues, thus indicating their high stability along with activity potentials similar to control Acarbose.

4. Experimental

4.1. General methods

Different reagents and solvents were of analytical reagent (AR) grade and Sigma Aldrich. p-Nitrophenyl-α-D-glucopyranoside (pNPG), α-glucosidase from Saccharomyces cerevisiae, soluble starch, α-amylase from Human Pancreas, acarbose were also purchased from Sigma-Aldrich (France). TLC has been performed on pre-coated silica gel plates (Kieselgel 60 F254, Merck, Germany). NMR spectroscopies were recorded
in dry deuterated DMSO on a Bruker AC spectrometer at 200 MHz for $^1$H NMR and 50 MHz for $^{13}$C NMR. Mass spectra (ESI-MS) were recorded on a Bruker Daltonics Esquire 3000+ and the samples were diluted in methanol.

4.2. Chemistry

4.2.1. General procedure for the synthesis of (Z)-5-arylidene-thiazolidine-2,4-dione 3a–3e

A mixture of TZD (1 mmol) and aromatic aldehyde (1 mmol) was dissolved in CH$_2$Cl$_2$ (5 mL) then mineral spirit (1 g) was added and stirred. After 5 min the solvent was removed under vacuum and the dry-powder was irradiated in a microwave for appropriate time. After the completion of reaction, CH$_2$Cl$_2$ is added the chilled reaction mixture, then stirred for 5 min. The reaction mixture is filtered under vacuum and washed with of CH$_2$Cl$_2$ (3 × 5 mL). The combined organics were dried over MgSO$_4$ and the solvent was evaporated under reduced pressure, the product was crystalized in ethanol to give pure product.

4.2.1.1. (Z)-5-benzylidenethiazolidine-2,4-dione (3a).

White solid, Yield: 95% (144 mg), mp = 247 °C (EtOH), Lit (Giles et al., 2000).

4.2.1.2. (Z)-5-(4-methylbenzylidene) thiazolidine-2,4-dione (3b).

White solid, Yield: 92%, mp = 230 °C (EtOH), Lit (Zhang & Zhou, 2012).

4.2.1.3. 5-(4-Fluorobenzylidene) thiazolidine-2,4-dione (3c).

Yellow solid, Yield: 97%, mp = 219 °C (EtOH), Lit (Giles et al., 2000).

4.2.1.4. 5-(4-Bromobenzylidene) thiazolidine-2,4-dione (3d).

White solid, Yield: 93%, mp = 246 °C (EtOH), Lit (Giles et al., 2000).

4.2.1.5. (Z)-5-(2,4-dichlorobenzylidene) thiazolidine-2,4-dione (3e).

White solid, Yield: 89%, mp = 216 °C (EtOH), Lit (Zhang & Zhou, 2012).

4.2.2. General procedure for the synthesis of 3-allyl-5-arylidene-thiazolidine-2,4-dione 4a–4e

The thiazolidine-2,4-dione derivatives (4a–4e) were synthesized according to the previously described procedures (Thari et al., 2020). A mixture of 5-arylidene-thiazolidine-2,4-dione (3a–3e) (1 mmol) and allyl bromide (1.2 mmol) in EtOH/H$_2$O (v/v: 1: 2) (10 mL) was treated with sodium hydroxide (1 mmol), were added. The resulting mixture was stirred and heated at 75 °C for 5–6 h. The completion of the reaction was monitored by TLC. The reaction mixture was cooled and acidified with diluted HCl (4 N). The precipitated solid was filtered and purified by recrystallization from ethanol to give pure compounds 4a–4e.

4.2.2.1. 3-Allyl-5-(benzylidene) thiazolidine-2,4-dione (4a).

White solid, Yield: 62%, mp = 102 °C (EtOH). FTIR (ATR, cm$^{-1}$): 3210 (NH), 3048 (Ar–CH), 1731 (C = O), 1674 (C = O).

4.2.2.2. 3-Allyl-5-(4-methylbenzylidene) thiazolidine-2,4-dione (4b).

White solid, Yield: 65%, mp = 117 °C (EtOH). FTIR (ATR, cm$^{-1}$): 3320 (NH), 3049 (Ar–CH), 1728 (C = O), 1668 (C = O).

4.2.2.3. 3-Allyl-5-(4-fluorobenzylidene) thiazolidine-2,4-dione (4c).

White solid, Yield: 58%, mp = 121 °C (EtOH). FTIR (ATR, cm$^{-1}$): 3225 (NH), 3042 (Ar–CH), 1735 (C = O), 1684 (C = O).

4.2.2.4. 3-Allyl-5-(4-bromobenzylidene) thiazolidine-2,4-dione (4d).

White solid, Yield: 90%, mp = 112 °C (EtOH). FTIR (ATR, cm$^{-1}$): 3118 (NH), 3040 (Ar–CH), 1728 (C = O), 1674 (C = O).
4.2.2.4. 3-Allyl-5-(4-bromobenzylidene)thiazolidine-2,4-dione (4d). White crystals, Yield: 58%, mp = 113 °C (EtOH). FTIR (ATR, cm⁻¹): 3290 (NH), 3033 (ArC-H), 1735 (C=O). ¹H-NMR (200 MHz, DMSO-d₆, ppm): 7.76 (1H, s, ArCH=C); 7.54 (2H, d, J = 8.5 Hz, H Ar); 7.29 (2H, d, J = 8.6 Hz, H Ar); 5.92–5.66 (1H, m, CH=CH₂); 5.24 (1H, dd, J = 12.0, 2.0 Hz, CH=CH₂); 5.17 (1H, dd, J = 5.0, 2.0 Hz, CH=CH₂); 4.28 (2H, d, J = 6.0 Hz, NCH₂). ¹³C-NMR (50 MHz, DMSO-d₆, ppm): 166.8, 165.2, 132.5, 132.4, 132.0, 131.4, 130.0, 125.0, 122.1, 119.1, 43.9. MS (ESI⁺): m/z = 348.4 [M + Na]⁺.

4.2.2.5. 3-Allyl-5-(2,4-dichlorobenzylidene)thiazolidine-2,4-dione (4e). Yellow solid, Yield 58%, mp = 113 °C (EtOH). FTIR (ATR, cm⁻¹): 3290 (NH), 3033 (ArC-H), 1735 (C=O). ¹H-NMR spectrum (200 MHz, DMSO-d₆, ppm): 8.09 (1H, s, ArCH=C); 7.44 (1H, d, J = 2.0 Hz, H Ar); 7.39 (1H, d, J = 8.5 Hz, H Ar); 7.29 (1H, dd, J = 8.5, 2.0 Hz, H Ar); 5.88–5.69 (1H, m, CH=CH₂); 5.24 (1H, dd, J = 13.0, 1.2 Hz, CH=CH₂); 5.18 (1H, dd, J = 5.8, 1.1 Hz, CH=CH₂); 4.30 (2H, d, J = 6.0 Hz, NCH₂). ¹³C-NMR (50 MHz, DMSO-d₆, ppm): 166.8, 165.2, 136.6, 136.6, 130.4, 130.3, 129.9, 129.5, 128.8, 127.7, 124.9, 119.3, 44.1. MS (ESI⁻): m/z = 313.1 [M + H]⁺.

4.3. Biological activity

4.3.1. In vitro α-glucosidase and α-amylase inhibition assay

The α-glucosidase and α-amylase inhibitory activities of synthesized compounds were determined according to the method described in our previous work (Fettach et al., 2019; Pillai et al., 2019).

4.4. Molecular docking studies

In order to reveal the binding modes of synthesized 5-arylidene-2,4-thiazolidindione (3a–3e) and (4a–4e), molecular docking simulation was carried out as a significant tool for computer-aided drug design and molecular structural biology using AutodockVina (Trott & Olson, 2010). It comprised a series of steps that includes a selection of protein and its preparation, receptor grid generation, preparation of ligands, and then docking to the receptors. The 3D Crystal Structure of human pancreatic α-amylase (PDB ID: 1B2Y) (Nahoum et al., 2000) and α-glucosidase (PDB ID: 3W37) (Tagami et al., 2013) complexed with the carbohydrate inhibitor (acarbose) was used as target enzyme. The pretreatment of the target crystal structure (1B2Y and 3W37) was done using PYMOL (http://www.pymol.org) (Chen et al., 2017), for the removal of water molecules, heteroatom, ions, and ligands of origin in the crystalline structure (Ladbury, 1996; Lu et al., 2007). All the ligands are drawn using Chemdraw12.0 software (Li et al., 2004). To select the most stable conformation, the geometry of these ligands was subsequently optimized using Molecular Force Field (MMFF94) as implemented in the same Software. The Discovery Studio Client (version 17.2.0) was used for graphical visualization for the best interactions of complex protein-ligand conformations. To evaluate and validate the binding prediction of docking protocol, the crystallized ligand Acarbose was re-docked into the active site of 1B2Y and 3W37 targets. The precision evaluation for protocol docking was based on the Root-Mean-Square Deviation (RMSD) parameter, the prediction is acceptable if its value does not exceed 2 angstroms (Kadukova & Grudinin, 2018). The superimposition (Figure 5) of the re-docked and the cocrystallized acarbose show the low RMSD value of 0.50 Å and 0.40 Å for 1B2Y and 3W37, respectively.

4.5. Acute oral toxicity

The thiazolidine-2,4-diones derivatives that showed the best inhibitory effect of the two enzymes have been assessed for their acute oral toxicity, according to the guideline of the Organization for Economic Testing of Chemicals no 423, the assay was tested on swiss females mice (Fettach et al., 2019). Six mice for each group were received a maximal single oral dose of 2000 mg/kg B.W, after fasting overnight. A follow-up of the mortality rate, macroscopic parameters (neurological, autonomic behaviors) and toxic sings for 5 h after product administration and daily for 2 weeks was carried out. Body weight was recorded daily for 14 days. The control group (n = 6) received distilled water as a vehicle.

4.6. Statistical analysis

All the experiments were carried out in triplicate (n = 3). The data were described as the mean ± standard deviation (SD) of mean and expressed by one-way analysis of variance (ANOVA), followed by Duncan’s new analysis to identify significant differences between means using the Multiple Range Test (p < 0.05). All statistical analysis was determined using GraphPad Prism 8.0.2.

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Disclosure statement

The authors declare that they have no competing financial interests that could have appeared to influence the work reported in this paper.
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