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Some old 2-(4-(Aryl)- thiazole-2-yl)-3a,4,7,7a-tetrahydro-1H-4,7-tethanoisoindole-1,3(2H)-dione derivatives: Synthesis, inhibition effects and molecular docking studies on Aldose reductase and α-Glycosidase

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Abstract

Utilizing the simple chromatic techniques, Aldose reductase (AR) was derived from sheep liver. In addition, α-glycosidase from Saccharomyces cerevisiae was used as the enzyme. It was determined the interactions between compounds and the enzymes. Molecular docking method used to compare biological activity values of molecules against enzymes.
In the current study, the inhibition effect of synthetic isoindol-substitute thiazole derivatives **(3a-f)** on AR, and α-glycosidase enzymes was studied. In the thiazole series, compound **3b** (Ki: 9.70±4.72 μM) showed a maximum inhibitory impact towards AR while compound 3f (Ki: 44.40±17.18 μM) showed a lowest inhibitory impact towards AR. It was investigated potent inhibition profiles with K_i values in the range of 24.54±6.92–44.25±10.34 μM against α-glycosidase. Theoretical results were found consistent with experimental results.

Acting as antidiabetic agents, these compounds have the potential to be the selective inhibitor of α-glycosidase and AR enzymes. The biological activities of the studied molecules against AR and α-glycosidase enzymes will be compared with molecular docking method. ADME analysis of the molecules will be done.

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1. Introduction

In general, naturally occurring, isoindole-1,3-dione is important for sustaining a desired life quality. Extracts of isoindole-1,3-dione have long been the focus for their antifungal, antibacterial, antimicrobial [1], hypoglycemic [2], anti-tumor [3], anti-inflammatory [4], anticovulsants [5], and anti-inflammatory [6]. High blood sugar resulting from the complete or partial deficiency in the secretion of insulin is one of the characteristics of diabetes mellitus. Long-term complications of diabetes are found to be tightly linked with the chronic hyperglycemia of diabetes. Cataracts of both eyes, cardiovascular complications, retinopathy, nephropathy and neuropathy are among the major complications [7].

Under hyperglycemic conditions, various biochemical pathways are activated. The polyol pathway comprises the most promising and the most widely studied one. The polyol pathway is functional in the metabolization of excessive glucose. Through polyol pathway, glucose is transformed to sorbitol by aldose reductase (AR) [8].

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Some studies have displayed the connection between glucose metabolism and long-term complications of diabetes via the polyol pathway. Within the polyol pathway, AR is the crucial enzyme that gives rise to diabetic complications [9]. In the prevention and attenuation of diabetic complications, AR inhibition plays a crucial factor. As a result, the synthesis of new and useful AR inhibitors (ARIs) having antioxidant properties is of great importance [10].

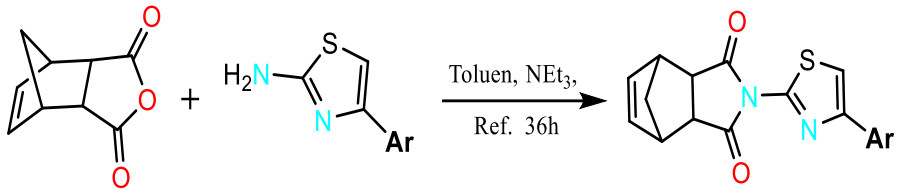
In the small intestine, α-glycosidase hydrolyzes polysaccharide and oligosaccharides to such monosaccharide units as fructose and glucose [8]. α-glycosidase inhibitors (α-GIs) possess key role in keeping human hyperglycemia and type-2 diabetes mellitus (T2DM) under control. α-GIs can repress T2DM and postprandial hyperglycemia and reduce the absorption of carbohydrates through diet. Hence, these types of α-GIs have a sugar molecule that competes with oligosaccharides in binding with the active site of the enzyme, thus efficiently decreasing the amounts of postprandial glucose in T2DM [11].

There are many experimental and theoretical methods to calculate the numerical values of the activities of molecules in studies conducted today. Because of both time and money, theoretical methods have developed too much. Molecular docking is also one of these methods. It should be well known that molecular docking is a common method used to evaluate the molecular level biological activity against certain enzymes [12-13]. In this study, thiazole derivatives (3a-f) against enzymes, which are AR whose ID is 3V36, and α-glycosidase whose ID is 1XSI, were compared for their biological activities. In the present paper, the in vitro inhibition impacts of isoindole-1,3(2H)-dione derivatives (3a-f) on AR and α-glycosidase as metabolic enzymes were studied, and the molecular docking properties were determined.

2. Material and Methods

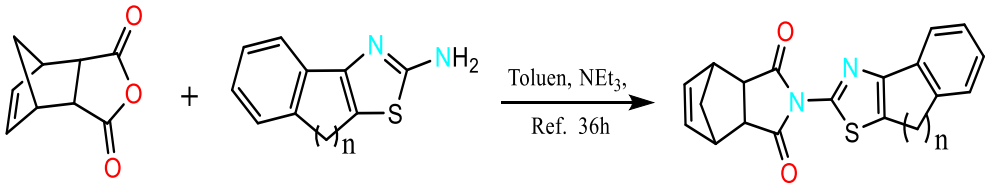
2.1. Chemistry

From the isoindol-substitute thiazole derivatives, a synthesis (3a-f) was carried out in line with to the steps explained in our previous study [14]. From the reaction of thiazole derivatives (2a-f) with dicarboxylic anhydride (1) in the presence of NEt3, the compounds 3a-f were synthesized. The spectral data of all compounds are in agreement with the data reported previously [14].



1 2a-d 3a-d

Ar: a) Ph- b) 4-MePh- c) 4-ClPh- d) 3-OmePh-



1 2e-f 3e-f

2e) n = 0, 2f) n = 1

Scheme 1. Synthesis route of investigated compounds in this study

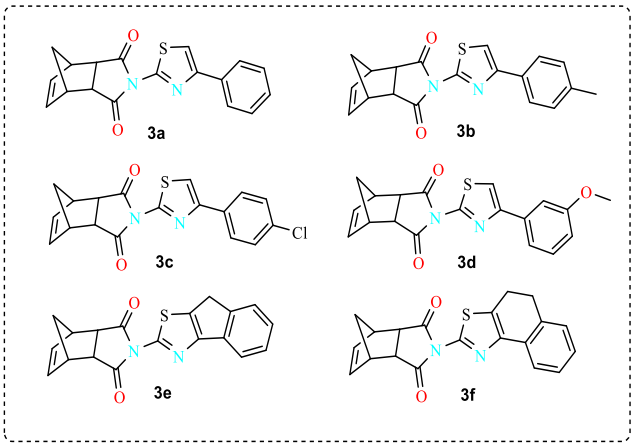


Figure 1. The tested compounds in this study

2.2. Biological studies

2.2.1. α-Glycosidase enzyme assay

α-Glycosidase enzyme was assayed according to previous study [15].

2.2.2. The analysis of AR activity

The analysis of AR activity was conducted the decrease of NADPH as 340 nm spectrophotometrically [16].

2.2.3. Purification of AR

AR enzyme was purified using, DE-52 cellulose, Sephadex G-100 and 2′5′-ADP-Sepharose-4B affinity from sheep liver [17]. Quantitative amounts of AR enzyme were determined in line with the Bradford procedure at 595 nm, spectrophotometrically [18]. The purity level of the enzyme was determined in line with the Laemmli's method [28] as explained previously [19-21].

2.2.4. Enzyme inhibition analysis

In order for determining the effect of isoindol-substitute thiazole derivatives on α-glycosidase, and AR, various concentrations of them were included into the reaction medium. The IC_{50} values were calculated from activity (%) versus some isoindol-substitute thiazole concentration plots. The K_i values were determined by Lineweaver and Burk's curves [22].

2.2.5. Docking studies

Nowadays there are many methods to calculate the molecular activities [23]. One of the most common methods is molecular docking. For molecular docking calculations, optimized structures of molecules are calculated by using the Gaussian Package program [24]. The optimized structures of the molecules were calculated by the Gaussian software program. The structures of the optimized molecules are obtained from *.pdb files.

Molecular docking calculations of the studied molecules were done using Maestro Molecular Modeling platform (version 12.2) by Schrödingder, LLC [25]. Crystal structures of enzyme proteins have been downloaded from the Protein Data Bank (PDB) site. The enzymes studied are AR that is ID: 3V36 [26], and α-glycosidase that is ID: 1XSI [27], respectively. The pH 7.0 ± 2.0 range was used in all calculations for the interaction of isoindol-substitute thiazole derivatives with enzymes. Enzymes that interact with molecules are made up of many proteins. Studied proteins were prepared for calculations using the protein preparation module [28], for calculations.

Later, the proteins in the active regions of the studied proteins were prepared for interactions. The preparation process of the molecules was started. Optimized structures of isoindol-substitute thiazole derivatives were obtained from the Gaussian software program [24]. The drawn structures were prepared for molecular docking calculations using LigPrep module [29]. 3D structures of isoindol-substitute thiazole derivatives were obtained. The Glide ligand docking module [30] was used for the interaction of isoindol-substitute thiazole derivatives with enzymes.

Following, ADME analysis was performed to examine the use of isoindol-substituted thiazole derivatives as drugs in future experimental studies. The Qik-prop module of Schrödinger software [31] was used to perform this analysis. many parameters are calculated using this module. With these parameters, information is obtained about the properties of the molecules.

3. Results and Discussion

AR has been linked to diabetes complications such as nephropathy, cataractogenesis, retinopathy and neuropathy. Hence, ARIs are used the therapeutic approach in the treatment complications of the diabetic. The development of reliable and novel ARIs is necessary to enhance the quality of life for patients with diabetic [32].

In the present study, synthetic isoindol-substitute thiazole derivatives (3a-f) were studied for their potential to inhibit the AR enzyme. In order for this, AR was the purified from sheep liver. The enzyme was obtained with a specific activity of 1.48 EU/mg protein and 113.85-fold purification (Table 1). SDS–PAGE was done following the purification of the AR enzyme. (Figure 2). The molecular weight of AR was determined as about 38 kDa. There is a growing demand for new and potent ARIs. The primary objective of this study was to recognize extremely useful and potent inhibitors for AR and α-glycosidase.

The inhibitory effect results of studied isoindole-substitute thiazole derivatives are displayed in Table 2.

In the related literature, there are some research about the inhibitory effect of AR. For instance, Stefek et al. [33] investigated 15 different compounds, which display an indole-1-acetic acid moiety inhibit AR. Fatmawati et al. [34] isolated prenylated xanthones from *Garcinia mangostana* Linn. They found that 3-isomangostin showed a better inhibitory effect against AR, with an IC_{50} of 3.48 μM. In another study, Ali et al. [35] synthesized and evaluated iminothiazolidin-4-one acetate derivatives were as AR inhibitors. The highest AR inhibitory potency in the series was evaluated for 2k with IC_{50} values 2.54 mM, respectively. In another study, Taslimi et al. [36] studied the inhibitory impact of bromophenols, diarylmethanes, and diarylmethanons on AR. In that study, it was found that 2d showed the best inhibition effect for AR. Demir et al. [10] displayed the effects of diarylmethanones and bromophenols on α-glycosidase and AR. They reported that 2d showed the best inhibition effect for α-glycosidase and 1f for AR.

The synthetic isoindol-substitute thiazole derivatives (3a–f) displayed a potent inhibition towards AR. K_i values order of compounds exhibiting inhibitory potency was 3b (9.70±4.72 μM) > 3a (10.03±0.51 μM) > 3e (19.82±1.99 μM) > 3d (21.22±3.33 μM) > 3c (26.62±2.63 μM) > 3f (44.40±17.18 μM) against purified AR. In the thiazole series, compound 3b exhibited a maximum inhibitory impact against AR while compound 3f showed a lowest inhibitory effect against AR. When an internal comparison is conducted between the compounds 3a and 3b, the addition of the methyl group in the aromatic ring on 3b has shown better inhibitory activity. Replacement of methyl group in 3b with chloro ion (3c) exhibited lower inhibitory activity. Adding methoxy group to 3a decreased to inhibitory effect. (3d K_i: 21.22±3.33 μM). In this series derivatives, the methyl group may be more a in AR inhibition according to our results.

α-Glycosidase hydrolyzes to polysaccharide and oligosaccharides to such monosaccharides as fructose and glucose. It also hydrolyzes the final stage in the digestive activity of carbohydrates [37,38] a-glycosidase inhibitors have the potential of preventing complications resulting from diabetic conditions [39].

In addition, the α-glycosidase enzyme was studied in the current paper. Studied compounds (3a-f) were determined for their inhibition impacts towards α-glycosidase enzyme, which displayed a significant inhibition commonly. The results of this research can be seen from Table 2. For this enzyme, the compounds had IC_{50} values in 19.75-30.25 range and K_i values in 24.54±6.92-44.25±10.34 μM range (Table 2). The results displayed that all studied compounds had a rather porent α-glycosidase inhibitory effects in comparison to that of acarbose (IC_{50}: 22800 nM) as the standard α-glycosidase inhibitor. The order of compounds with K_i values exhibiting inhibition effect was 3c (24.54±6.92 μM) > 3f (27.94±03.44 μM) > 3e (29.16±4.51 μM) > 3a (30.85±5.82 μM) > 3b (37.03±3.05 μM) > 3d (44.25±10.34 μM) against α-glycosidase. In the thiazole series, compound 3c displayed a maximum inhibitory impact towards α-glycosidase while compound 3d showed a lowest inhibitory impact towards α-glycosidase (Figure 3 and 4). When the 3a and 3b compounds are evaluated among themselves, the addition of the methyl group in the aromatic ring on 3b has shown lower inhibitory activity by contrast with AR enzyme. Replacement of methyl group in 3b with chloro ion (3c) exhibited better inhibitory activity. Adding methoxy group to 3a decreased to inhibitory effect. (3d K_i: 44.25±10.34 μM).

Table 1. Purification steps of AR from sheep liver

Purification Steps	Activity (EU/mL)	Total volume (mL)	Protein (mg/mL)	Total protein (mg)	Total activity (EU)	Specific activity (EU/mg)	Yield (%)	Purification fold
Homogenate	0.30	20	23.43	468.60	6.00	0.013	100	1
Ammonium sulfate precipitation and dialysis	0.33	16	19.15	306.40	5.28	0.017	88.00	1.31
DE-52 Cellulose anion exchange chromatography	0.17	10	5.43	54.30	1.70	0.031	28.33	2.38
Sephadex G-100 gel filtration chromatography	0.095	6	0.84	5.04	0.57	0.113	9.50	8.69
Affinity chromatography	0.013	3	0.009	0.027	0.040	1.48	0.67	113.85

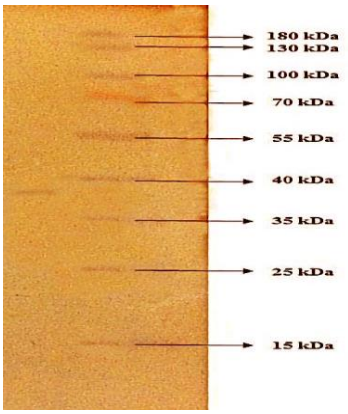
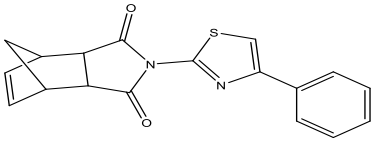
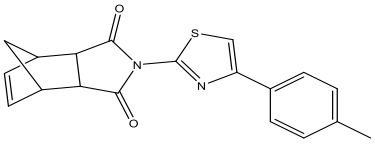
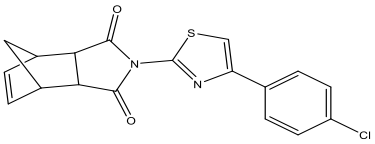
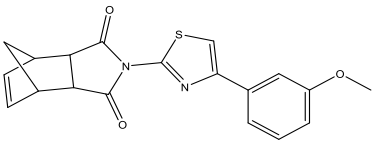
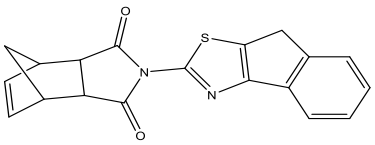
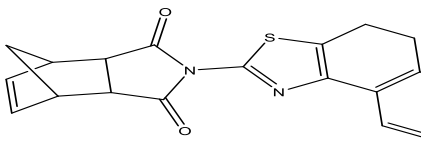
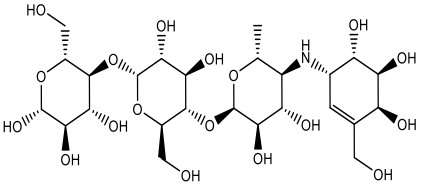
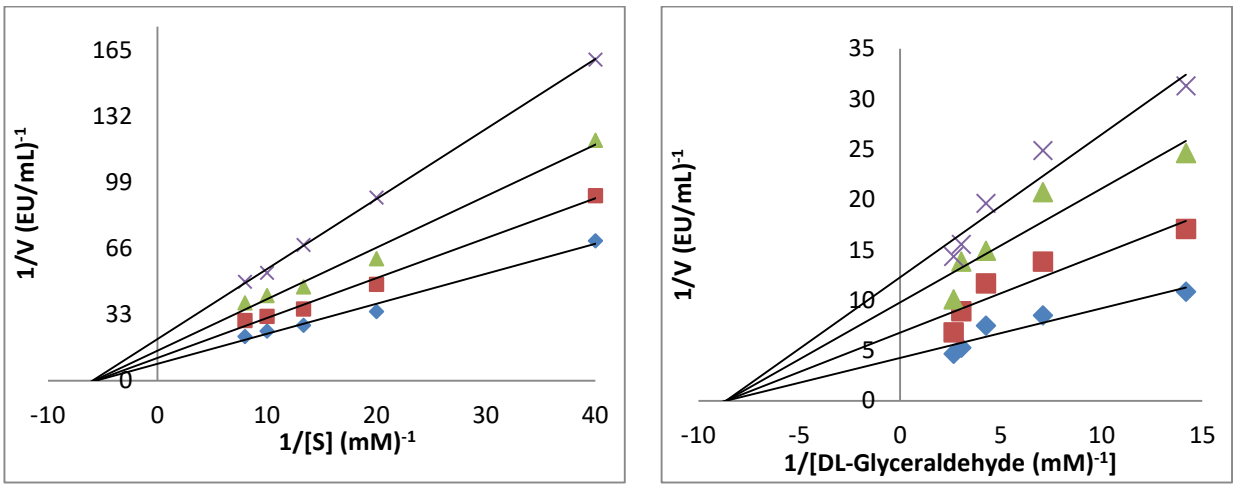


Figure 2. SDS–PAGE analysis of purified AR which obtained single band

Table 2. Inhibition effects of some isoindole- 1,3(2H)-dione derivatives on α-glycosidase and purified AR from sheep liver

Chemical names	STRUCTURES of MOLECULES	AR		α-Glycosidase	
		K_i (μM)	IC_{50}	K_i (μM)	IC_{50}
(3aR,4S,7R,7aS)-2-(4-phenylthiazol-2-yl)-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindole-1,3(2H)-dione (3a)		10.03±0.51	12.16	30.85±5.82	24.54
(3aR,4S,7R,7aS)-2-(4-(p-tolyl)thiazol-2-yl)-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindole-1,3(2H)-dione (3b)		9.70±4.72	10.83	37.03±3.05	28.17
(3aR,4S,7R,7aS)-2-(4-(4-chlorophenyl)thiazol-2-yl)-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindole-1,3(2H)-dione (3c)		26.62±2.63	28.88	24.54±6.92	19.75
(3aR,4S,7R,7aS)-2-(4-(3-methoxyphenyl)thiazol-2-yl)-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindole-1,3(2H)-dione (3d)		21.22±3.33	17.77	44.25±10.34	30.25
(3aR,4S,7R,7aS)-2-(8H-indeno[1,2-d]thiazol-2-yl)-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindole-1,3(2H)-dione (3e)		19.82±1.99	16.50	29.16±4.51	23.11
(3aR,4S,7R,7aS)-2-(4,5-dihydronaphtho[1,2-d]thiazol-2-yl)-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindole-1,3(2H)-dione (3f)		44.40±17.19	36.48	27.94±3.44	20.83
ACR*		-	-	12600±780	2800

ü*Acarbose (ACR) was used as positive control for α-glycosidase enzyme



A B

Figure 3. Determination of Lineweaver-Burk graphs for excellent inhibitors of α-Gly (3c) compounds (A) and AR (3b) (B)

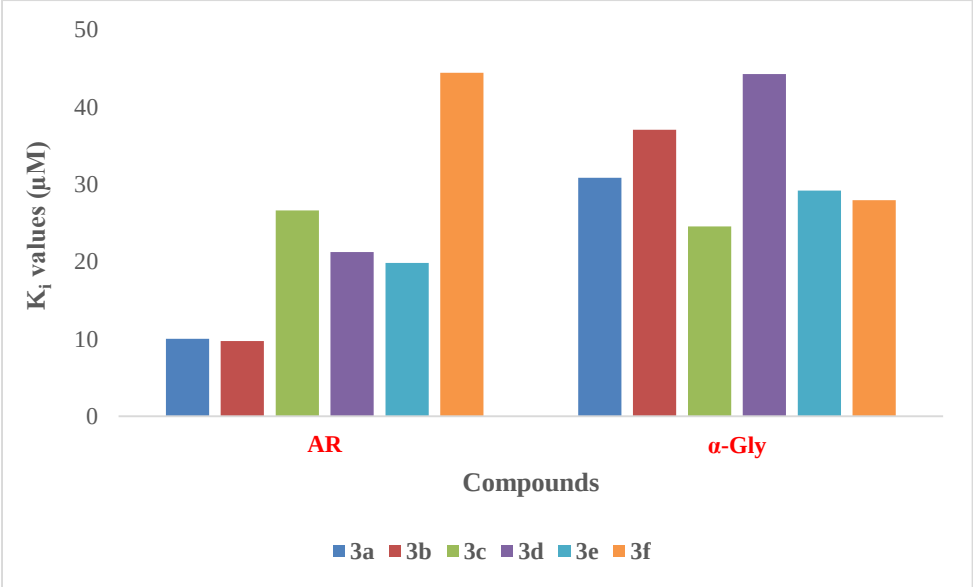


Figure 4. K_i values of studied enzymes

The isoindol-substituted thiazole derivatives were interacted with enzymes and their biological activities were compared. Molecular docking calculations were made with enzymes of isoindol-substituted thiazole derivatives. Biological activity comparison of isoindol-substituted thiazole derivatives can be made by using the numerical value of these parameters. It is thought that the biological activity value of the molecule with the lowest numerical value of this parameter is high [40]. As a result, the biological activities of compounds are listed according to the numerical value of this parameter. Other parameters obtained are used to explain the interactions of molecules with enzymes. As a result of docking studies, the interactions of isoindol-substituted thiazole derivatives with enzymes are given in Figure 5 and 6. The numerical value of the parameters obtained as a result of these interactions are given in Table 3.

Table 3. Numerical values of the parameters obtained from interaction of enzymes studied with enzymes

		3a	3b	3c	3d	3e	3f
α-Gly	Docking Score	-3.6	-3.3	-3.8	-3.3	-3.7	-3.6
	Glide hbond	-0.15	0.00	0.00	0.00	-0.16	-0.15
	Glide emodel	-31.6	-34.8	-38.2	-35.8	-33.4	-33.4
	Glide ligand efficiency	-0.15	-0.14	-0.14	-0.13	-0.15	-0.14
Ald. red.	Docking Score	-3.5	-3.9	-3.1	-3.7	-3.8	-3.0
	Glide hbond	0.00	0.00	0.00	0.00	0.00	0.00
	Glide emodel	-34.9	-34.2	-37.6	-36.9	36.7	-34.0
	Glide ligand efficiency	-0.15	-0.14	-0.13	-0.15	-0.15	-0.12

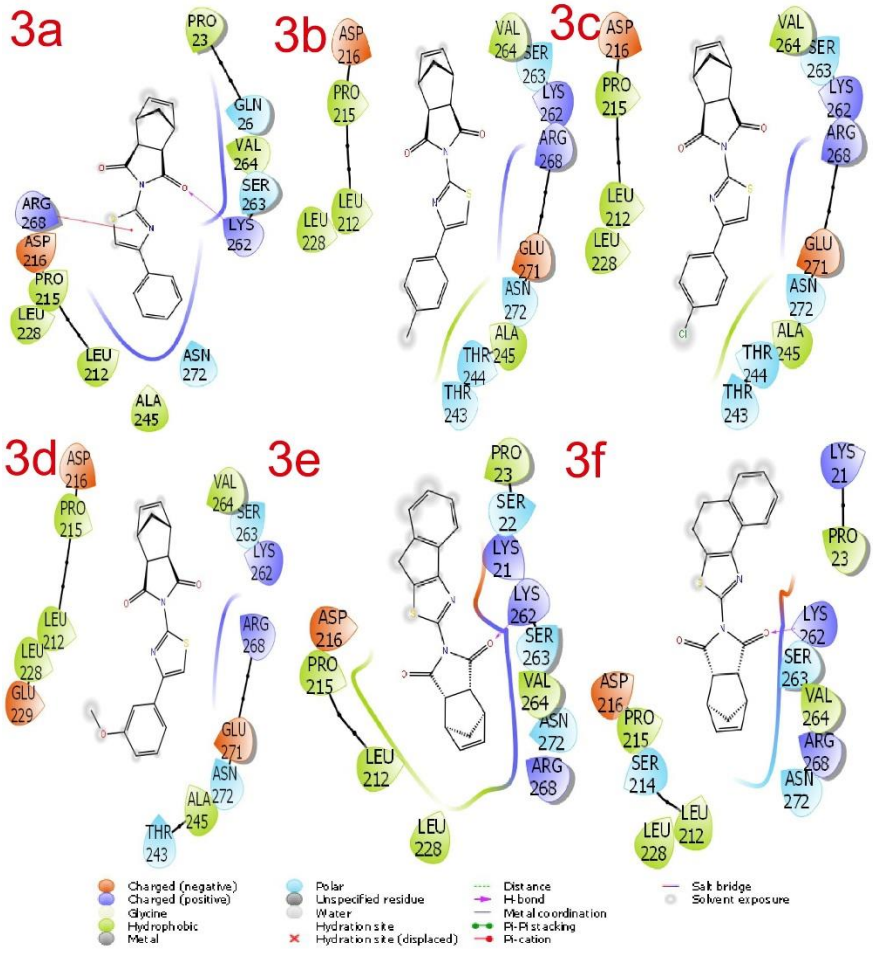


Figure 5. Interaction of molecules against AR enzyme

Docking calculations have shown that as the interactions of isoindol-substituted thiazole derivatives with enzymes increase, the biological activity values of isoindol-substituted thiazole derivatives increase. Interactions between molecules and enzymes are very significant and showed in Figure 7.

Table 4. ADME properties of molecules

	3a	3b	3c	3d	3e	3f	Referance Range
mol MW	322	336	357	352	334	348	130-725
dipole	2.1	1.6	4.4	3.1	2.0	1.9	1.0-12.5
SASA	564	597	588	596	575	592	300-1000
FOSA	139	227	139	226	192	235	0-750
FISA	89	89	89	85	97	91	7-330
PISA	299	243	251	241	250	234	0-450
WPSA	37	37	109	44	35	32	0-175
volume	984	1045	1028	1060	1005	1048	500-2000
donorHB	0	0	0	0	0	0	0.0-6.0
accptHB	4.5	4.5	4.5	5.25	4.5	4.5	2.0-20.0
glob	0.8	0.8	0.8	0.8	0.8	0.8	0.75-0.95
QPpolrz	36.1	38.0	37.4	37.9	36.4	38.0	13.0-70.0
QPlogPC16	10.3	10.5	10.9	10.7	10.2	10.5	4.0-18.0
QPlogPoct	14.5	15.1	15.5	15.5	14.6	15.1	8.0-35.0
QPlogPw	8.2	7.9	7.9	8.3	7.9	7.8	4.0-45.0
QPlogPo/w	3.4	3.7	3.9	3.5	3.4	3.7	-2.0-6.5
QPlogS	-4.7	-5.3	-5.5	-4.8	-4.9	-5.2	-6.5-0.5
CIQPlogS	-4.8	-5.1	-5.5	-5.1	-5.0	-5.3	-6.5-0.5
QPlogHERG	-5.4	-5.4	-5.4	-5.2	-5.2	-5.2	*
QPPCaco	1412	1412	1412	1554	1180	1353	**
QPlogBB	-0.2	-0.2	0.0	-0.2	-0.3	-0.2	-3.0-1.2
QPPMDCK	1149	1150	2833	1380	917	1022	**
QPlogKp	-2.1	-2.3	-2.3	-2.1	-2.4	-2.4	Kp in cm/hr
IP(eV)	9.3	9.1	9.2	9.1	9.2	9.1	7.9-10.5
EA(eV)	1.2	1.2	1.3	1.2	1.1	1.1	-0.9-1.7
#metab	5	6	5	6	6	7	1-8
QPlogKhsa	0.3	0.4	0.4	0.2	0.3	0.5	-1.5-1.5
HumanOralAbsorption	3	3	3	3	3	3	-
PercentHumanOralAbsorption	100	100	100	100	100	100	***
PSA	67	67	67	75	69	68	7-200
RuleOfFive	0	0	0	0	0	0	Maximum is 4
RuleOfThree	0	0	0	0	0	1	Maximum is 3
Jm	0.0	0.0	0.0	0.0	0.0	0.0	-

* corcern below -5, **<25 is poor and >500 is great.
*** <25% is poor and >80% is high.

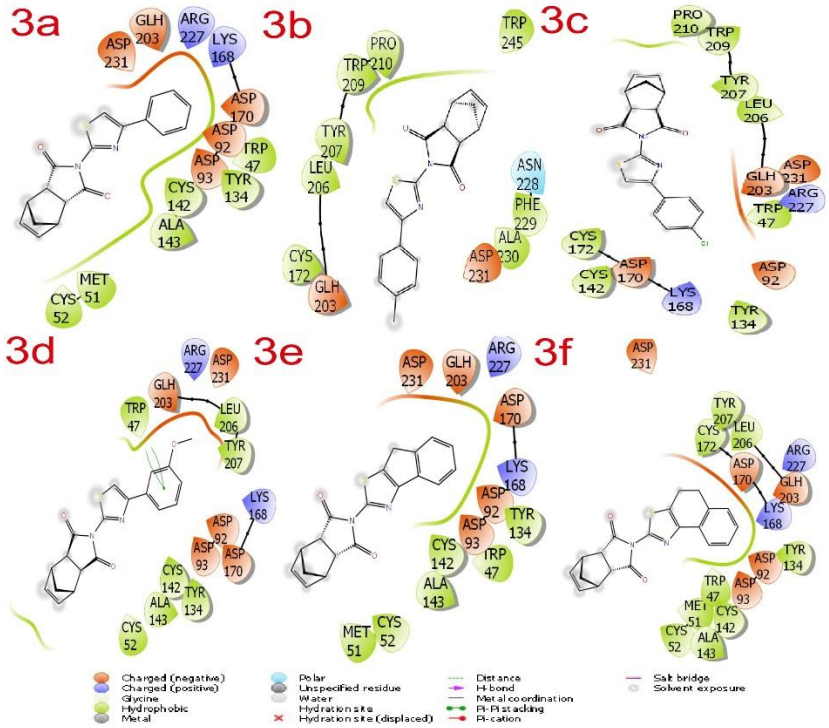


Figure 6. Interaction of molecules against α-Glycosidase enzyme

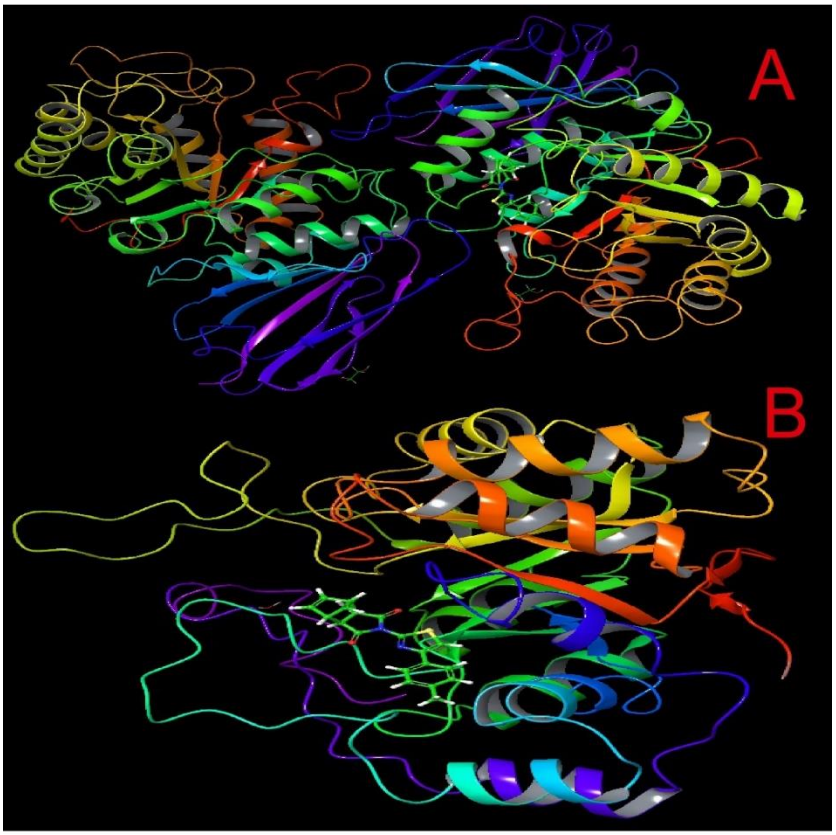


Figure 7. A. Interaction of molecule 3b with proteins of AR enzyme B. Interaction of molecule 3c with proteins of α-Glycosidase enzyme

After molecular docking calculations, it provides us to comment on whether the molecules can be used as medicines in the future with ADME analysis of isoindol-substituted thiazole derivatives. Many parameters were obtained by using Qik-prop module for ADME analysis of molecules. If the numerical values of these parameters are in a certain range, it is thought to be used as a medicine in the future.

The parameters obtained for the ADME properties of the molecules are given in table 4. Many parameters for molecules are calculated in this table. Considering the results given table, Solute as Donor-Hyrogen Bonds is number of hydrogen bond donors [12], Solute as Acceptor-Hyrogen Bonds is number of hydrogen bond acceptors, QP log p for octanol/water is octanol/water partitiopn coefficient, Apparent MDCK Permeability is cell permeability in nm/s, QP log BB for brain/blood is Predicted brain/blood partition coefficient for orally delivered drugs, etc [11]. The numerical values of these parameters give researchers a lot of information [13]. At the end of ADME analysis of molecules, it can be seen that the molecules can be used as a drug in the future.

Conclusions

In the current paper, studied compounds showed strong inhibition profiles towards α-glycosidase and AR enzymes. Micromolar levels of IC_{50} values were obtained for all derivatives on AR and α-glycosidase enzymes, these compounds can be a selective inhibitor of α-glycosidase and AR enzymes as antidiabetic. Molecular docking scores were in agreement with the experimental results. The biological activity of molecule 3b was found to be highest against AR enzyme. however, the biological activity of molecule 3c is highest against α-Glycosidase.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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