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Synthesis, Characterization, Docking and *In-Vitro* evaluation of newly Synthesized Thiazolidinones

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ABSTRACT

This project was designed to measure the enzyme inhibition potential of the synthesized compounds. The following compounds 3-amino-2-thioxothiazolidin-4-one (**1**) and 5-(4-chlorobenzylidene)-3-(methyleneamino)-2-thioxothiazolidin-4-one (**2**) were prepared via three-step method using cheaper reagents. The compounds were characterized by employing different spectroscopic techniques and subjected to biological evaluation. *In vitro* model was used for enzyme inhibition activity through spectrophotometer and suggested that compounds may be helpful in the medicinal field. Compound **2** exhibits more inhibition against BChE (67.7%) while compound **1** showed maximum inhibition of *α*-glucosidase (65.1%). Theoretical study i.e. docking of the prepared compounds was also related to experimental work. *In-Silico* ADMET study of both compounds exhibited various therapeutic properties, which depicted that they are quite similar to the drug-like candidates.
Keywords: Thiazolidinones; ADMET; Docking Study; Enzyme inhibition

INTRODUCTION

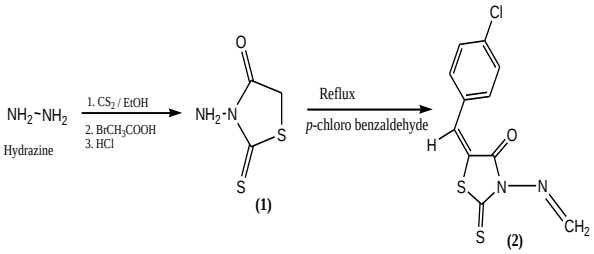
The challenging task for the scientist of this era is to synthesize new drugs, their mechanism, and development (Nueno, 2016). To combine the molecules and to get good results against mortal diseases, the researcher should make newer organic moieties having better medicinal properties (Patel *et al*., 2014). Heterocycles are the basic structure of medicinal chemistry, enzymes, vitamins, natural products, having antioxidants, antiallergic, anti-inflammatory, anticonvulsant, anticancer, herbicidal, anti-HIV, and antidiabetic activities (Al-Mulla, 2017). Different compounds like azo dyes (Grirrane, Corma, & García, 2008), Schiff's bases (Ibrahim & Sharif, 2011), zeolites (Tao, Kanoh, Abrams, & Kaneko, 2006), stationary phase for HPLC (Gocan, 2002), epoxy resins (Gocan, 2002) and plastics (Ismail *et a*l., 2015) are synthesized by aromatic amines. Saturated thiazoles having a carbonyl group on fourth carbon are called thiazolidinones (Belwal & Joshi, 2012). Thiazolidinones are derivatives of thiazolidine and contain 'S' atom at position 1, 'N' at position 3, and at 2, 4, 5 position carbonyl group is attached (Singh, Parmar, Raman, & Stenberg, 1981). US economy costs $1.75 every year and £7-£14 billion in the UK due to AD (Lleo, Greenberg, & Growdon, 2006) (Ernst & Hay, 1994). To handle this multifarious illness researcher put all their struggles to advance the multi-targets drugs which can stop different factors causing the AD-like linked aggregation, protein misfolding, oxidative stress, metal dyshomeostasis, Aβ aggregation, and decreased the ACh levels (Azam, Amer, Abulifa, & Elzwawi, 2014;)Russo, Frustaci, Del Bufalo, Fini, & Cesario, 2013). The biggest universal health problem of this era is diabetes mellitus (DM). Currently, 415 million patients are suffering from diabetes throughout the world shown by the report of the International Diabetes Federation and this number is predicted to be 643 million by 2040. Chronic hyperglycemia is multistage of the metabolic disease called diabetes and classified into type 1 and type 2. Most patients are being suffered from type 2 DM, caused by different behavioral, environmental and genetic hazards (Proença *et al*., 2017). Currently, oral hypoglycemic drugs which are being used in medication of diabetes are biguanides, sulphonylureas and thiazolidene moieties (Aditama, Mujahidin, Syah, & Hertadi, 2015; Aisha *et al*., 2020; Pillai, Subramanian, & Kandaswamy,2013). Throughout the world different researcher are involved in synthesizing new molecules for community. The project was designed to synthesize thiazolidinone compounds and their biological evaluation.

Author Contribution: A., Conducted research; **M. A. R.,** Designed whole project as supervisor; **U. F.,** Did AMET and docking studies.

MATERIALS AND METHODS

Synthesis of thiazolidinones (1 and 2)

Compound **1** was prepared using hydrazine and triethylamine, CS_2 and bromoacetic acid followed by the reported method. Compound **1** was further condensed with *p*-chloro benzaldehyde and resulting compound **2** as shown in scheme 1 (Aisha *et al*., 2020; Shahwar *et al*., 2011).



Scheme 1: Synthesis of the targeted compounds

ADMET Study

ADMET is a software-based study used to measure various pharmaceutical parameters of synthesized and naturally occurring compounds. ADMET estimation of synthesized thiazolidinones **1** and **2** was done through online software (Dege, Raza, Doğan, Ağar, & Mumtaz, 2021; Raza & Fatima, 2020).

AChE and BChE Inhibitory Activity

The esterase inhibitory potential of synthesized compounds was checked *in vitro* model according to the reported method (Aisha et al., 2020; Ellman, Courtney, Andres Jr, & Featherstone, 1961).

α-Glucosidase Inhibition

The *α*-glucosidase activity of the synthesized compounds was checked *in vitro* model according to the reported method (Aisha et al., 2020).

Docking Studies

The docking studies were conducted viz Molecular Operating Environment software (Aisha et al., 2020; Raza, Fatima, Saqib, Maurin, & Budzianowski, 2019) using PDB file of 1EVE, 1P0I, and 3WEO for AChE, BChE, and α-glucosidase, respectively.

RESULTS AND DISCUSSION

This project is a continuity of our previous work where we used different amine and synthesize their thiazolidinone compounds. The reaction consisted of three steps; in the first step, dithiocarbamate was prepared using an ice bath. The first step product was further reacted with *α*-bromoacetic acid. The cyclization of compound **1** was done by adding the second step product in hot HCl solution. The synthesized compound **1** was further condensed with *p*-chlorobenzaldehyde yielded (*E*)-5-(4-chlorobenzylidene)-2-thioxo-3-(*p*-tolyl) thiazolidin-4-one (**2) (**Aisha *et al*., 2020; Shahwar *et al*., 2011; Shahwar, Tahir, Kashif, Saeed, & Bukhari, 2012; Shahwar, Tahir, Yasmeen, Ahmad, & Khan, 2009). The whole reaction was handled and monitored by TLC and FTIR and NMR confirmed synthesized compounds structures.

Characterization of the Synthesized Compounds

The recognition of the functional group of both compounds was done using FTIR spectroscopy. The appearance of NH_2 peaks around 3400 cm^{-1} in both compounds suggested the formation of the targeted compounds. The peak present at 3011 cm^{-1} revealed the presence of the Ar-H group in the synthesized compound (**1**) while the IR signal of C-N bond was indicated at 1171 cm^{-1}. The C=O signal of thiazolidinone ring of compound **1** comes at 1703 cm^{-1} further confirms the synthesis of the desired ring. The bands appear at 1034 cm^{-1} and 574 cm^{-1} confirms the presence of C=S and C-S bonds in compound **1**. Aromatic stretching vibration (C-H) band of **2** at 3001 cm^{-1} and methyl (C-H) peak at 2583 cm^{-1} shows the presence of the respective group in the compound (**2**). The formation of a new (C=C) band peak in the condensed product comes at 1664 cm^{-1}. The new band at 772 cm^{-1} corresponds to C-Cl is also indicated the synthesis of compound **2**. Furthermore, the IR peaks at 1662, 1038, and 629cm^{-1} are due to C=O, C=S, and C-S respectively. The ^{1}H NMR spectra of the prepared compounds recognized their structure identities. The peaks corresponding to aromatic protons (Ar-N) of compound **1** gave a signal at δ 3.3 in *dd* fashion while in the case of compound **2** the aromatic protons of the aldehydic ring showed signals (6.8-7.0 ppm) at the downfield due to attachment of the chloro group at the *para* position of the ring. Furthermore, a new singlet band at δ 6.2 is due to alkene proton also revealed the synthesis of **2**. The carbon NMR data is also in favor of the

synthesis of compounds. The peak of the carbonyl carbon of thiazolidinone ring in compound **1** indicated at δ 169, which shifted to δ 163 in compound **2**. The peaks present at δ 35 were due to (-CH_2) group of thiazolidinone ring in compound **1** which shifted to δ 138 due to conversion into -C=C group in compound **2**. The signals due to -C=S functionality in the **1** and **2** compounds appeared at δ 196 and δ192, respectively. The C-Cl peak that appeared at δ 134 confirms the synthesis of compound **2**.

3-amino-2-thioxothiazolidin-4-one(1)

Yield: 62%; IR (cm^{-1}) ν_{max}: 3011 (Ar-CH), 1703 (C=O), 1034 (C=S), 1171 (C-N); ^{1}H-NMR (500 MHz, Chloroform) δ 3.3 (d, 2H); ^{13}C-NMR (125 MHz, Chloroform): δ 196, 169, 35.

(*E*)-3-amino-5-(4-chlorobenzylidene)-2-thioxothiazolidin-4-one (2)

Yield: 54%; IR (cm^{-1}) ν_{max}: 3002 (CH), 2583 (CH), 1662 (C=O), 1038 (C=S), 1644 (C=C), 1231(C-N); ^{1}H-NMR (500 MHz, Chloroform) δ 8.0 (s, 1H), 6.8-7.0 (m, 4H), 6.2 (s, 1H); ^{13}C-NMR (125 MHz, Chloroform): δ 192, 163, 138, 134, 132, 130, 129, 127, 119.

***In-Silico* ADMET assessments**

ADMET outcomes of the synthesized Thiazolidinones are tabulated in tables I-VI. It was revealed from results that compounds have physiochemical properties similar to drugs which are as follow: molecular weight (<500), number of rotatable bonds (<10), fractional sp^3 carbon (<0.25), number of hydrogen bond donor (<5), number of hydrogen acceptor (<10), molar refractivity (<130), and topological polar surface area (<90) (Table I). The lipophilicity of compound **1** is less than 5, although it is higher than 5 in some cases of compound **2** (Table II). It was illustrated from ADMET results that **1** has more solubility than **2** (Table III). Compound **1** and **2** have negligible values of Log K_p showing that molecules easily pass through the skin (Table IV). Both Compounds also give zero violation against Lipinski rule and Bio score 0.55 which again confirms properties of the drug (Table V). There is only 1 alert for both against PAINS and synthetic accessibility (SA) is also in a good range (Table VI).

Table I: Physicochemical properties of the synthesized compounds

Code	Formula	Mol. Wt	N.H.A	N.A.H.A	F sp^3	N.R.B	N.H.B.A	N.H.B.D	MR	TPSA(Å^2)
1	$C_{10}H_9NOS_2$	223.31	14	6	0.20	1	1	0	66.82	77.70
2	$C_{17}H_{12}ClNOS_2$	345.87	22	12	0.06	2	1	0	101.45	77.70

Number of heavy atoms (N.H.A), Number of aromatic heavy atoms (N.A.H.A), F(Fraction), Number of rotatable bonds (N.R.B), Number of H bond acceptors (N.H.B.A), Number of H bond donors (N.H.B.D), Molar Refractivity (MR), Topological polar surface area (TPSA)

Table II: Lipophilicity of the proposed compounds

Code	Log $P_{o/w}$ (iLOGP)	Log $P_{o/w}$ (XLOGP3)	Log $P_{o/w}$ (WLOGP)	Log $P_{o/w}$ (MLOGP)	Log $P_{o/w}$ (SILICOS-IT)	Consensus Log $P_{o/w}$
1	2.22	2.78	1.98	1.67	3.55	2.44
2	3.36	5.53	4.56	3.72	5.78	4.59

Table III: Water solubility of the proposed compounds

Code	Log S (ESOL)	Solubility (mg/ml)	Class	Log S (Ali)	Solubility (mg/ml)	Class	Log S (SILICOS-IT)	Solubility (mg/ml)	Class
1	-3.23	$1.32e^{-01}$	Soluble	-4.07	$1.91e^{-02}$	Moderately soluble	-3.05	$1.99e^{-01}$	Soluble
2	-5.74	$6.30e^{-04}$	Moderately soluble	-6.92	$4.15e^{-05}$	Poorly soluble	-6.17	$2.34e^{-04}$	Poorly soluble

Table IV: Pharmacokinetics of the proposed compounds

Code	GI absorption	BBB permeant	*P*-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Log K_p cm/s
1	High	No	No	Yes	Yes	Yes	No	No	-5.69
2	High	No	No	Yes	Yes	Yes	No	Yes	-4.48

Table V: Drug likeness of the proposed compounds

Code	Lipinski	Ghose	Veber	Egan	Muegge	Bio. score
1	Yes; 0 violation	Yes	Yes	Yes	Yes	0.55
2	Yes; 0 violation	Yes	Yes	Yes	No; 1 violation	0.55

Table VI: Medicinal chemistry of the proposed compounds

Code	PAINS	Brenk	Lead likeness	Synthetic accessibility
1	1 alert	1 alert	No	2.23
2	1 alert	2 alerts	No	3.12

Enzyme inhibition Results

Three enzymes i.e., AChE, BChE, and alpha-glucosidase were targeted in a screening of compounds **1** and **2** while using an *in-vitro* model. Compounds **1** and **2** showed normal to good enzyme inhibition activity. Compound **1** showed maximum inhibition against enzyme *α*-glucosidase, while compound **2** showed maximum inhibition for enzyme BChE as shown in table VII.

Docking studies

The docking of compounds **1** and **2** was performed by using MOE and PDB files of AChE, BChE, and glucosidase were taken from the online way. The docking scores are listed after the process of docking in the given table VII. It was clear that compound **1** showed interactions with Trp84, Gly118, Tyr130, Glu199, Ser200(2.54), Ala201, Phe330, Phe331, and His440 of acetylcholinesterase. Molecule **1** showed H-bonding with residues Gly118, Tyr130, Glu199, Ser200, Ala201, and His440 while with Trp84 showed pi-pi interactions. Amino acid residues i.e., Trp84, Phe330, Phe331, and His440 depicted pi-sulfur interaction as pi system of the following residues and sulfur of the thiazolidone ring. Compound **2** showed interactions through H-bonding with Tyr70, Trp84, Tyr121, Gly123, Tyr334 and with Tyr70, Asp72, Tyr121, Ser122 and Tyr334 molecule **2** showed pi-pi interactions. Pi-sulfur interactions with molecule **2** are shown by Trp84.

In the case of butyrylcholinesterase Gly115, Gly116, Glu197and Ser198 showed H-bonding interactions with molecule **1** while in the case of compound **2** H-bonding interactions are shown by Thr234, Glu238, Arg242, Thr284, Leu286 and Val288. Pi-pi interactions of compound **2** depicted by Arg242 and Thr284. Compound **1** showed interactions with Trp329, Asp568, Trp565, Phe601, and His626 of *α*-glucosidase. Molecule **1** interact through H-bonding with Trp329, Asp568, and His626, while with Trp565 and His626 it showed pi-pi interactions. Compound **1** showed pi-sulfur interactions with Trp329, Trp565, and Phe601 as the pi-system of the following residues and sulfur of thiazolidinone ring. Compound **2** showed interactions with alpha-glucosidase through the following residues i.e., Trp467, Trp432, Met470, Asp469, Arg552, and Asp568. H-bonding interactions are shown by Trp467, Met470, Asp469, Arg552, and Asp568 with compound **2**, while Pi-sulfur interactions are shown by Trp432, Trp467, and MET470.

Table VII: Docking and Enzyme inhibition studies of the synthesized compounds

Compounds	Docking score			Enzyme inhibition (%)		
	AChE	BChE	α-glucosidase	AChE	BChE	*α*-glucosidase
1	-2.8452	-2.5614	-3.4238	66.9	73.9	74.1
2	-6.5485	-5.3348	-4.3028	62.1	67.7	65.1

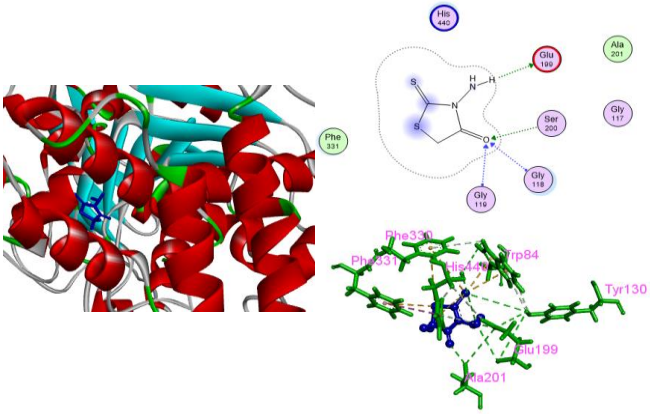


Fig. 1: Docking View of the compound **1** with 1EVE

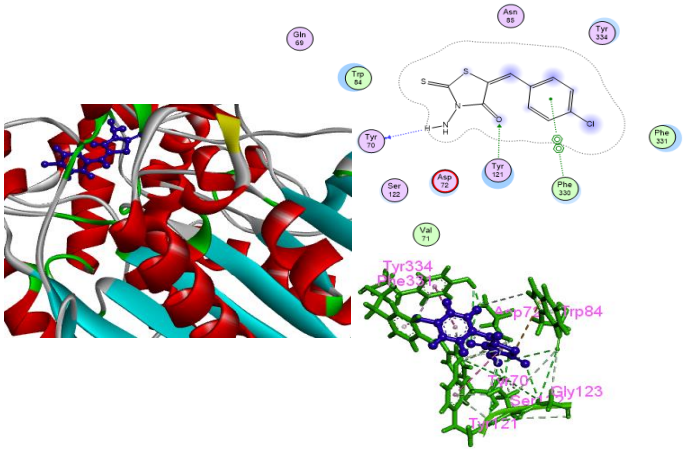


Fig. 2: Docking View of the compound **2** with 1EVE

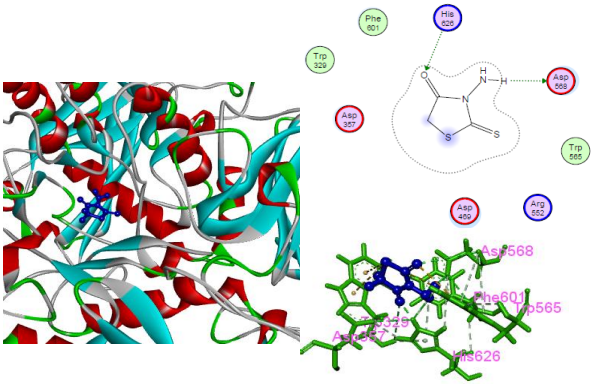


Fig. 3: Docking View of the compound **1** with 1POI

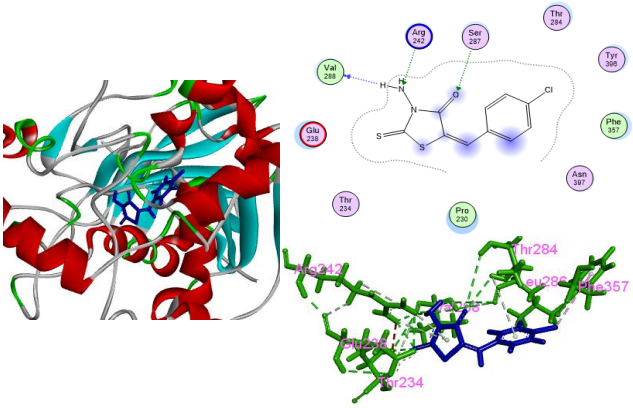


Fig. 4: Docking View of the compound **2** with 1POI

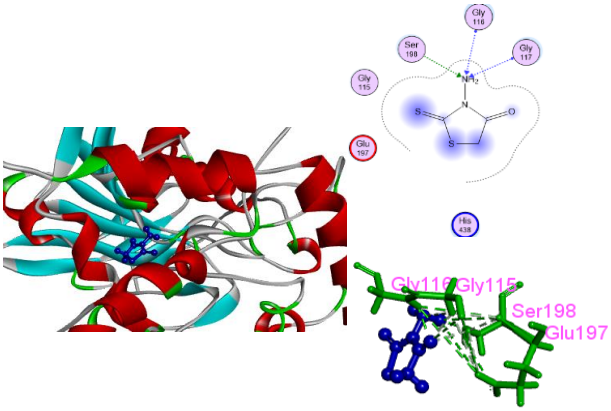


Fig. 5: Docking View of the compound **1** with 3WEO

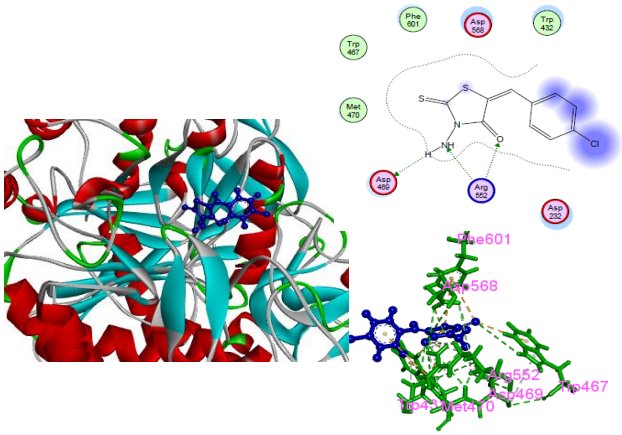


Fig. 6: Docking View of the compound **2** with 3WEO

Conclusion

Compound **1** containing thiazolidinone ring is prepared by using hydrazine by convenient resources and already given method of Aisha. By condensing compound **1** with *p*-chlorobenzaldehyde compound **2** was prepared both compounds **1** and **2** were characterized structurally by using FTIR and NMR techniques and biologically screened against three enzymes by using *in vitro* method. The compounds showed normal to good inhibition activity. The binding energies of the synthesized compounds **1** and **2** were checked through a computational method of docking. All these studies recommend that these types of compounds may be helpful in the treatments of various diseases.

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