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2-Chloroquinolinyl-thiazolidine-2,4-dione Hybrids as Potential α-Amylase Inhibitors: Synthesis, Biological Evaluations, and In Silico Studies

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Recently, molecular hybridization strategy has paved a way to develop novel lead compounds for the α-amylase targeted antidiabetic therapy. In this study, we disclosed a series of new hybrids of thiazolidine-2,4-diones with 2-chloroquinoline-3-yl moiety as potential antidiabetic agents. The molecular structures of all the synthesized hybrids (**15a–n**) were confirmed by spectroscopic studies (FT-IR, ESI-MS, ^{1}H, ^{13}C NMR, and elemental analysis). When in vitro antidiabetic evaluation of these agents was carried out in a dose-dependent manner, it was revealed that several compounds (**15f–h**, **15j,** and **15n**) were endowed with significant antidiabetic activities and exhibited more than 50% α-amylase inhibition at a dose of 50 μg/mL. Particularly, hybrid **15n** was found to be more potent than acarbose with 95% inhibition of α-amylase and IC_{50} of 5.00 ± 0.18 μM under given conditions. Further, it was demonstrated that **15n** bearing 2-chloroquinolinyl and thiazolidin-2,4-dione scaffolds functionalized with 3-OMe and 4-OH groups was not only able to effectively bind with α-amylase receptor site with best docking score (−9.644 kcal/mol) but also possessed drug-likenesses properties with no violations of Lipinski rule. Overall, this study discovered new 2-chloroquinolinyl-thiazolidine-2,4-diones hybrids as novel α-amylase inhibitors and compound **15n** as promising lead for its further development as antidiabetic agent.

1. Introduction

Diabetes mellitus (DM) also known as chronic hyperglycemia, is a metabolic disease that arise due to defects in the secretion process of insulin by salivary glands.[1] Recently, the IDF Diabetes Atlas (2021) has reported DM as a disease of global concern due to its high prevalence (537 million) in the adult population (10.5%).[2] Among these cases, over 90% of people were suffering from type 2 diabetes (DM2).[2] Further, the number of diabetes patients is projected to raise up to 783 million by 2045 which shows the growing global health burdens towards the treatments and prevention of DM.[2]

In the recent past, α-amylase enzyme has been exploited as therapeutic target for the treatment and maintenance of chronic hyperglycemia.[3] The α-amylase is a ubiquitous enzyme produced by many species, including microorganisms, plants, and animals.[4] It is among the major category of amylases produced by salivary glands that are responsible for the carbohydrate metabolism. Structurally, α-amylases proteins are consisting of three domain carrying the three binding sites (catalytic calcium-binding, and chloride-binding) that are vital for digestion of starch.[5] Through its catalytical domain, α-amylase hydrolyses the α-(1,4)-d-glycosidic linkages present in starch and breaks down into maltose, maltotriose, and dextrin by employing aspartate/glutamate mediated double-displacement mechanism.[6] Thus, inhibiting the α-amylase and thereby reducing the postprandial blood glucose level piloted as clinically effective strategy in the treatment of hyperglycemia and obesity.[7] Consequently, Voglibose (**1**) and acarbose (**2**) which are glycoside derivatives (Figure 1) have been developed as α-amylase inhibitors for the treatment of DM2.[8] However, these medications are associated with several side effects which includes abnormal liver functions, abdominal pain, and diarrhea.[8] Therefore, design and discovery of effective and safter α-amylase inhibitors is key areas of research in the antidiabetic drug developments.

The 2,4-thiazolidinedione (TZD) is a crucial skeleton frequently appeared as pharmacophore in a number of bioac-

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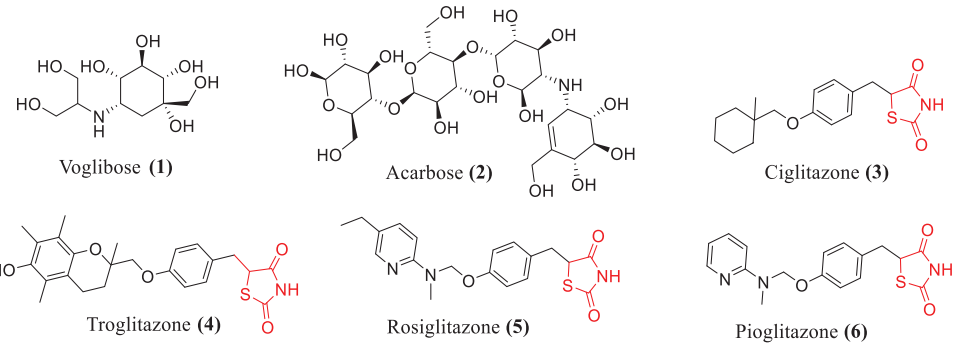


Figure 1. Structures of some clinically used antidiabetic drugs. (a) α-Amylase inhibitors (**1** and **2**); (b) PPARγ receptors inhibitors (**3–6**).

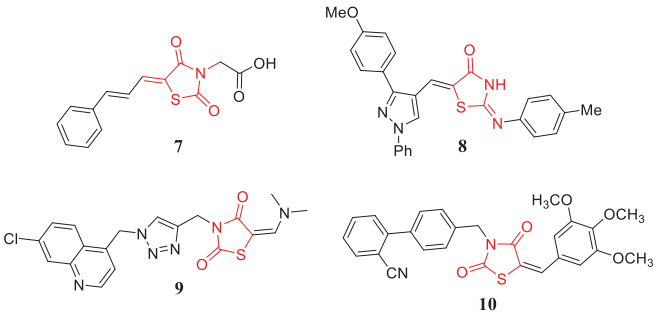


Figure 2. Examples of thiazolidinone hybrids (**7–10**) as α-amylase inhibitors.

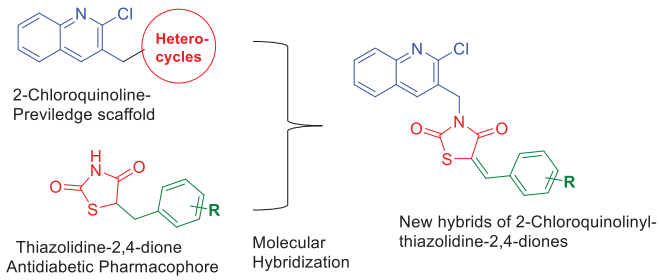


Figure 3. Molecular hybridization approach for the design of new 2-chloroquinolinyl-thiazolidine-2,4-dione hybrids.

tive compounds developed for the broad range of pharmacological applications.[9–11] This includes antitubercular activity,[12] antimicrobial,[13] antimicrobial,[14] anti-inflammatory,[15] antiviral activity and so forth.[16] Particularly, in the late 1990s several antidiabetic drugs based on thiazolidinediones (TZDs) scaffolds (Figure 1) such as ciglitazone (**3**), troglitazone (**4**), rosiglitazone (**5**), and pioglitazone (**6**) have been approved by FDA for clinical uses.[17,18] These, agents exerted their antidiabetic effect via PPARγ receptors which regulate the glucose metabolism.[17,18] These reports demonstrated the α-amylase enzyme as a druggable target. Currently, several thiazolidinedione conjugates have also been reported as effective α-amylase inhibitors.[19] Most of these non-glycosidic α-amylase inhibitors were prepared by employing scaffold combination strategies in which various bioactive heterocyclic systems were appended with thiazolidine-2,4-diones.[19] For instance, chalcone-thiazolidinone conjugates (**7**),[20] pyrazole-thiazolidinone hybrids (**8**),[21] and thiazolidine-2,4-dione tethered 1,2,3-triazoles (**9**)[22] are being investigated and exhibited good antidiabetic activity as α-amylase inhibitors (Figure 2). Recently, we have reported biphenylcarbonitrile-thiazolidinedione hybrids (**10**) having significant α-amylase inhibition activities.[23]

On the other hand, quinoline heterocycle regarded as crucial pharmacophore in antimalarial and antimicrobial drug discovery.[24–30] Particularly, 2-chloroquinoline skeleton containing hybrid compounds displayed wide range of bioactivities including diuretics,[31] apoptotic,[32] EGFR inhibitors,[33] as well as dual inhibitors of SARS-CoV-2.[34]

In our continued research efforts towards design and development of new antidiabetic agents,[35,36] we pursued to employ molecular hybridization approach and prepare 2-chloroquinoline-thiazolidine-2,4-dione hybrids to explore their α-amylase inhibition activities which are unexplored to date (Figure 3).

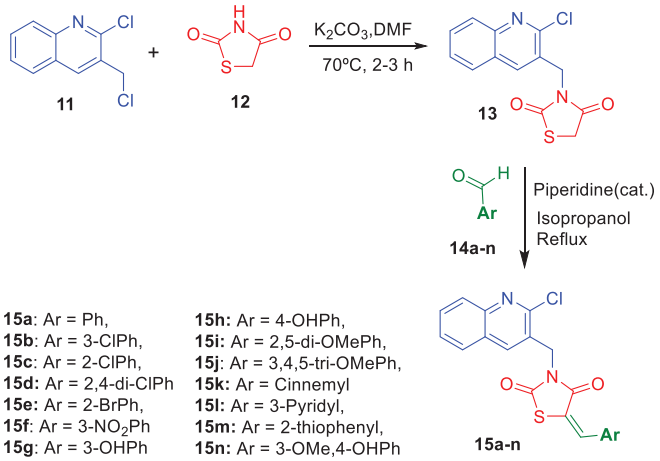
It was envisaged that 2-chloroquinolin-3-yl tethered on thiazolidine-2-,4-dione motif would facilitate the binding of these conjugates with α-amylase and might lead to an effective antidiabetic agent (Figure 3). Herein we reported, synthesis, α-amylase inhibition activities, and in silico profiling of new 2-chloroquinolinyl-thiazolidine-2,4-dione conjugates.

2. Results and Discussion

2.1. Chemistry

The synthetic approach for the targeted 2-chloroquinolinyl-thiazolidine-2,4-dione conjugates is shown in Scheme 1.

First, the 2-chloro-3-(chloromethyl)quinoline (**11**) was synthesized from 3-chloro-*N*-phenylpropanamide by reacting with DMF and $POCl_3$ as reported earlier.[37] Similarly, thiourea and chloroacetic acid was reacted in the presence of concentrated HCl at reflux temperature for 10–12 h to produce thiazolidine-2,4-dione (**12**).[38] Further, *N*-alkylation of thiazolidine-2,4-dione (**12**) with 2-chloro-3-(chloromethyl)quinoline (**11**) was carried out using K_2CO_3 in DMF at 70 °C to generate requisite intermediate 3-((2-chloroquinolin-3-yl)methyl)thiazolidine-2,4-dione (**13**) in high yield (86%). Finally, the Knoevenagel condensation reaction of intermediate **13** with various aromatic aldehydes (**14a–n**) in the presence of catalytic piperidine in refluxing isopropanol gave



Scheme 1. Synthesis of 2-chloroquinolin-3-yl tethered on thiazolidine-2-,4-diones (15a–n).

desired 2-chloroquinolinyl-thiazolidine-2,4-dione hybrids (**15a–n**) in excellent yields (78%–98%).

The molecular structures of all the newly synthesized compounds (**13** and **15a–n**) were established by FT-IR, ESI-Ms, NMR (^{1}H and ^{13}C) and elemental analysis. For instance, FT-IR spectrum of **15a** showed two characteristic absorption bands at 1740 and 1681 cm^{-1} corresponds to presence of two carbonyl groups (C═O) in the molecule. The sp^2 stretching (C═C—H) of aromatic ring appeared at 2852, 2925, and 3056 cm^{-1}. Further, in the ^{1}H NMR spectrum of **15a**, the linker group (—CH_2—) exhibited singlet at 5.05 δ ppm. The two signals at 8.45 and 8.02 δ ppm was attributed to methine (—CH) of quinoline ring bearing N-atom and arylidene proton (—C═C—H) respectively. Another five signals of nine protons within aromatic region (8.07 (d), 7.98 (d), 7.83 (t), 7.68 (m), and 7.56 (m) δ ppm) confirmed the presence of quinoline and phenyl ring. Furthermore, ^{13}C NMR spectrum of **15a** confirmed the compound having two carbonyls by two distinct C═O signals (S—CO—N and N—CO—C) at 167.38 and 165.47 δ ppm. The presence of linker —CH_2— was further confirmed from signal at 42.58 δ ppm. The exocyclic sp^2(C) showed signal at 121.36 δ ppm while remaining 14 distinct carbons showed their signals within aromatic region (126–148 δ ppm) which confirmed the carbon skeleton of molecule. The 2D NMR (HSQC) of **15a** further confirmed the structure showing cross peaks of all desired C—H interactions. Moreover, the mass spectrum of compound **15a** revealed a peak having m/z 381.3 (M + 1) consistent to its molecular formula $C_{20}H_{13}ClN_2O_2S$ which was further established by elemental analysis.

2.2. In Vitro α-Amylase Inhibition

Having targeted 2-chloroquinolinyl-thiazolidine-2,4-dione hybrids (**15a–n**) in hand, we next evaluated their in vitro α-amylase inhibitory activities. A five dose-dependent α-amylase inhibition assay was carried out for all newly synthesized hybrids (**15a–n**) using acarbose as reference standard. The results of the in vitro α-amylase inhibition studies are represented in Table 1 with their respective IC_{50} in μM range. As evident, several compounds (**15a**, **15f–h**, **15j**, **15l,** and **15n**) have exhibited significant α-amylase inhibition with $IC_{50} < 10$ μM. Further, the inhibition of α-amylase by **15a–n** was found to be dose-dependent and linearly elevated with respect to the drug concentration (Figure 4). In all of these, **15j** and **15n** were most active compounds having IC_{50} value 5.30 ± 0.11 and 5.00 ± 0.18 μM, respectively, whereas standard drug acarbose exhibited IC_{50} of 6.16 ± 0.08 μM under given conditions. The hybrids **15f**, **15g**, and **15h** have also exhibited substantial α-amylase inhibition and were equipotent with their IC_{50} around 5.50 μM.

The structure activity relationship study (SAR) showed remarkable effects of functional groups on bioactivity of these new hybrids. Such as, the electron withdrawing halogen groups (—Cl or —Br) on phenyl ring remained less effective showing $IC_{50} > 10$ μM against α-amylase. However, —NO_2 substitution on phenyl ring (**15f**, $IC_{50} = 5.44 \pm 0.27$ μM) resulted in slightly increased activity compared to unsubstituted derivative (**15a**, $IC_{50} = 5.87 \pm 0.22$ μM). On the other hand, presence of electron releasing —OH, —OMe or its combination groups on phenyl ring significantly increased α-amylase inhibition. For example, compared to **15a** (Ar = Ph, $IC_{50} = 5.87 \pm 0.22$ μM), derivatives bearing 3—OH (**15g**, $IC_{50} = 5.50 \pm 0.18$ μM), 4—OH (**15h** , $IC_{50} = 5.47 \pm 0.20$ μM) and 3,4,5-tri-OMe (**15j**, $IC_{50} = 5.30 \pm 0.11$ μM) were more active. Particularly, 2-chloroquinolinyl-thiazolidine-2,4-dione hybrids (**15n**) bearing 3—OMe and 4—OH functionalities found to be the most active compound of the series with potency of 5.00 ± 0.18 μM. Moreover, replacement of phenyl ring with pyridine heterocycles (**15l**) decreased the activity to some extent ($IC_{50} = 6.02 \pm 0.04$ μM) while hybrid with thiophene ring (**15m**) was found to be the least active candidate of the series ($IC_{50} = 23.93 \pm 1.02$ μM). Overall, the study revealed that molecular hybridization of 2-chloroquinoline-3-yl with electron rich benzylidenethiazolidine-2,4-diones was crucial for effective α-amylase inhibition.

2.3. Molecular Docking Studies

Nowadays, molecular docking studies became an integral parts of modern drug discovery due to its vital role in predicting the binding mode of the inhibitors with receptor site.[39] In addition, it also reveals stability of binding complex with enzyme and types of interactions associated during protein-ligand complex formation.[40] Hence, the synthesized **15a–n** hybrids were also examined for in silico studies to find out their basic interactions and respective energies of binding with α-amylase receptor site. The protein structure was retrieved from PDB (ID:1rpk) and prepared for docking using UCSF Chimera (v.1.17.3).[41,42] The molecular docking of **15a–n** with α-amylase enzyme was performed using AutoDock Vina (v.1.2.5).[43,44] The results of the in silico studies are summarized in Table 2 showing docking scores and protein-ligand binding site interactions are indicated in Table 3. Further, docking poses of most active compounds (**15f**, **15j,** and **15n**) within active site of α-amylase is depicted in Figure 5.

The study revealed that, majority of synthesized 2-chloroquinolinyl-thiazolidine-2,4-dione hybrids could efficiently

Table 1. In vitro α-amylase inhibitory activity of the synthesized compounds (**15a–n**).

Compound	Inhibition of α-Amylase (%)[a] In Vitro Dose (μg/mL)					IC_{50} (μM)
	50	**100**	**150**	**200**	**250**	
15a	54.37 ± 0.14	64.55 ± 1.27	74.97 ± 0.79	81.65 ± 0.94	92.57 ± 0.85	5.87 ± 0.22
15b	28.31 ± 0.98	45.69 ± 1.86	57.42 ± 0.79	65.64 ± 1.06	70.37 ± 0.89	11.18 ± 0.58
15c	22.12 ± 0.98	35.29 ± 1.12	50.22 ± 0.67	60.41 ± 0.58	67.03 ± 0.75	14.57 ± 0.03
15d	32.68 ± 0.29	43.23 ± 0.69	55.67 ± 0.88	64.41 ± 0.38	72.12 ± 1.22	11.35 ± 0.29
15e	14.11 ± 1.90	22.92 ± 0.79	32.45 ± 1.40	40.53 ± 1.65	50.29 ± 0.45	21.01 ± 0.26
15f	51.82 ± 0.49	59.10 ± 0.77	65.42 ± 0.84	71.33 ± 0.48	79.10 ± 0.91	5.44 ± 0.27
15g	53.63 ± 1.33	61.57 ± 0.51	70.16 ± 0.58	78.45 ± 0.67	86.17 ± 0.71	5.50 ± 0.19
15h	56.11 ± 0.87	66.38 ± 0.38	77.37 ± 0.10	88.29 ± 0.56	94.62 ± 0.27	5.47 ± 0.20
15i	34.28 ± 0.63	42.51 ± 0.51	50.22 ± 0.69	57.20 ± 0.80	62.23 ± 0.07	13.37 ± 0.22
15j	53.85 ± 0.77	63.31 ± 1.33	70.66 ± 0.68	79.12 ± 0.69	89.96 ± 0.53	5.30 ± 0.11
15k	24.08 ± 1.78	32.09 ± 0.65	42.13 ± 1.13	53.27 ± 0.35	59.24 ± 0.65	18.07 ± 0.34
15l	56.11 ± 0.43	67.03 ± 0.36	78.31 ± 0.43	88.57 ± 0.38	93.09 ± 0.18	6.02 ± 0.04
15m	23.43 ± 0.47	30.12 ± 1.17	38.78 ± 1.13	46.79 ± 1.15	51.52 ± 0.80	23.93 ± 1.02
15n	56.91 ± 0.76	68.41 ± 0.82	78.67 ± 0.48	89.45 ± 0.05	95.05 ± 0.28	5.00 ± 0.18
Acarbose	47.23 ± 0.95	56.84 ± 1.10	67.90 ± 1.15	79.33 ± 0.58	88.06 ± 0.29	6.16 ± 0.08

[a] Each value is the mean ± S.D. (standard deviation).

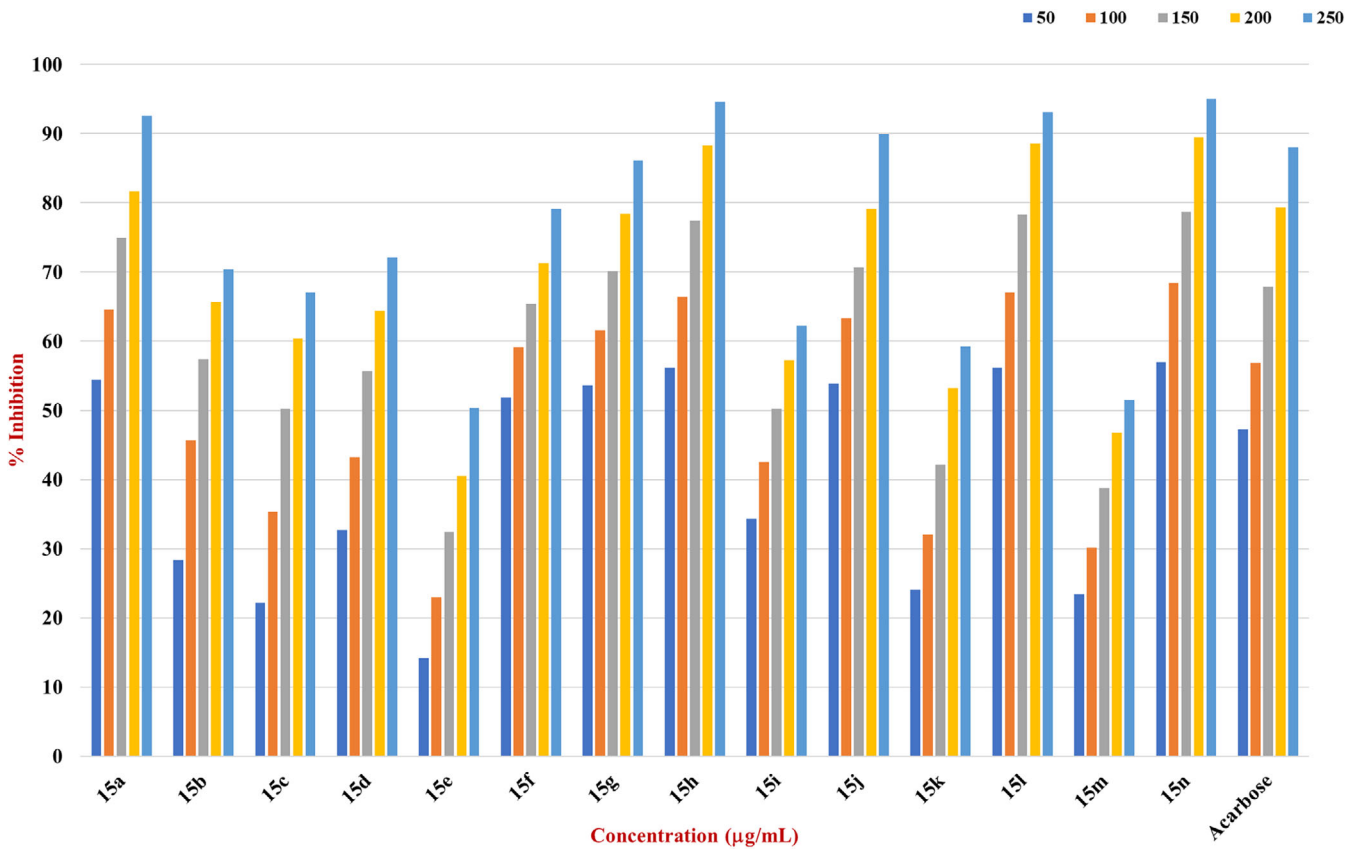


Figure 4. Comparative % inhibitions of α-amylase by hybrids **15a–n**.

Table 2. Docking scores (kcal/mol) of the synthesized compounds (**15a–n**) and acarbose with α-amylase (PDB ID:1rpk).

Compound	Score (kcal/mol)	Compound	Score (kcal/mol)
15a	−8.798	**15i**	−8.276
15b	−8.981	**15j**	−9.455
15c	−8.788	**15k**	−9.221
15d	−9.130	**15l**	−8.603
15e	−8.956	**15m**	−8.137
15f	−9.027	**15n**	−9.644
15g	−8.987	**Acarbose**	−7.750
15h	−9.136		

Table 3. Protein-ligand interactions of the inhibitors **15f**, **15j,** and **15n** with the amino acid residues of the acarbose binding site of α-amylase (PDB ID:1rpk).

Compound	Score (kcal/mol)	Binding Site Interactions
15f	−9.027	Hydrophobic: Trp^{207}, Met^{298}, Trp^{299} Hydrogen bond: Arg^{183}
15j	−9.455	Hydrophobic: Trp^{10}, Try^{52}, Trp^{207} Hydrogen bond: Arg^{183} π-Stacking: Trp^{207}
15n	−9.644	Hydrophobic: Try^{52}, Phe^{144}, Phe^{181}, Trp^{207} Hydrogen bonds: Arg^{178}, Arg^{183} π-Stacking: Trp^{207} Halogen bond: Glu^{205}
Acarbose	−7.750	Hydrogen bonds: His^{93}, Arg^{178}, Asp^{180}, Arg^{183}, Glu^{205}, Asn^{209}, His^{290}

bounded with the receptor site of α-amylase with comparable docking scores (7–9 kcal/mol) as of acarbose. Especially, compound **15n** demonstrated best docking score (−9.644 Kcal/mol) indicating favorable binding affinity against targeted protein.

Although, compounds **15f**, **15j,** and **15n** bounded α-amylase through different poses, it showed comparable docking scores (−9.027, −9.455, and −9.644 kcal/mole respectively) and formed some common interactions within active site such as H-bond interactions with Arg^{183} and hydrophobic interactions with Trp^{207}. Notably, compound **15n** was able to form hydrophobic interactions (i.e., Phe^{144}, Try^{52}, and Trp^{207}) utilizing arylidene and quinoline ring systems whereas thiazolidine 2,4-dione moiety was involved in the H-bonding and π-stacking with residues Arg^{178}, Arg^{183}, and Trp^{207} respectively. Furthermore, 2-chloroquinoline ring formed important halogen bond interaction with protein residue Glu^{205}. As shown, compared to acarbose, compound **15n** bounded α-amylase effectively with low docking score along with additional favorable interactions within receptor site. Consequently, the results of molecular docking studies corroborated the in vitro analysis indicating a stable enzyme-ligand complex formation by **15n** which might have resulted in potent α-amylase inhibition.

2.4. Drug-Likeness Properties

To exert a desired therapeutic action upon binding with active site of enzyme, the drug molecule must have favorable pharmacokinetic profile with certain similarities in their physicochemical properties. The characteristic of drug-likeness referred as the "Lipinski rule of five" which predicts that drug molecule should have H-bond donors $\leq$ 5, H—bond acceptors $\leq$ 10, MW (mass) $\leq$ 500, and value of LogP $\leq$ 5 for its high therapeutic effect.[45]

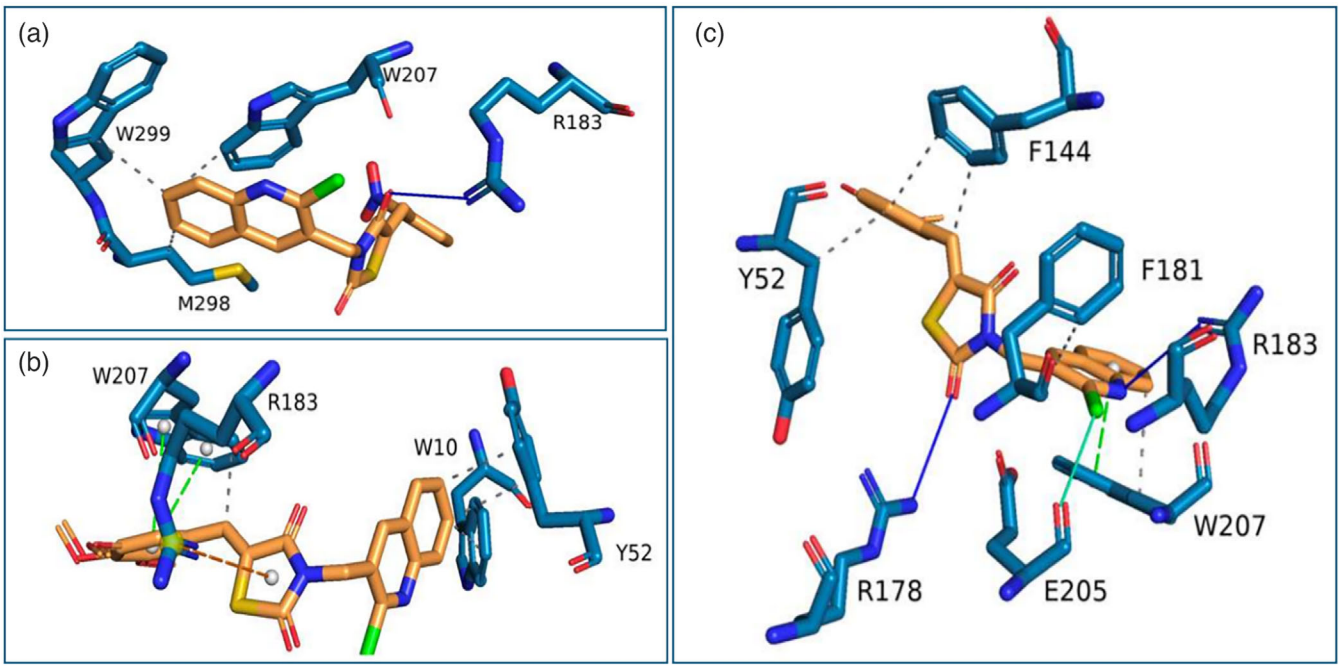


Figure 5. Binding poses and interactions of the compounds **15f** (a), **15j** (b), and **15n** (c) with α-amylase binding site (PDB ID:1rpk).

Table 4. The calculated drug-likeness properties for the synthesized compounds **15a–n**.

Compound	Molecular Weight	LogP	Rotatable Bonds	H-Bond Acceptors	H-Bond Donors	Surface Area	Lipinski Violation
15a	380.85	5.12	3	4	0	159.02	Yes
15b	415.29	5.77	3	4	0	169.33	Yes
15c	415.29	5.77	3	4	0	169.33	Yes
15d	449.73	6.43	3	4	0	179.63	Yes
15e	459.74	5.88	3	4	0	172.89	Yes
15f	425.84	5.03	4	6	0	173.68	Yes
15g	396.85	4.83	3	5	1	163.82	No
15h	396.85	4.83	3	5	1	163.82	No
15i	440.90	5.14	5	6	0	181.98	Yes
15j	470.92	5.15	6	7	0	193.46	Yes
15k	406.88	5.68	4	4	0	171.06	Yes
15l	381.83	4.51	3	5	0	158.24	No
15m	386.87	5.18	3	5	0	156.66	Yes
15n	426.87	4.83	4	6	1	175.30	No
Acarbose	645.61	−8.56	9	19	14.00	250.23	Yes

Therefore, an in silico study was carried out employing web server pkCSM[46] to evaluate the drug-likeness properties of the synthesized hybrids **15a–n**. As revealed in Table 4, most of the compounds showed compliance with at least four criteria of Lipinski rule of five except compounds **15b–e** and **15k** which have LogP > 5. Remarkably, compound **15n** demonstrated strong correlation with drug-likeness properties without any violations of Lipinski rule whereas control acarbose exhibited three violations. Thus, favorable drug-likeness attributes of **15b** such as flexible structure, typical LogP, higher polar surface area, low molecular weight along with aptly substituted ring systems for H-acceptor/donor bonds might be responsible for its significant inhibitory activity.

3. Conclusion

In the present study, a series of new 2-chloroquinolinyl-thiazolidin-2,4-dione hybrids (**15a–n**) has been prepared and evaluated for their antidiabetic activity as α-amylase inhibitors. It was revealed that several of the synthesized hybrids (**15f–h**, **15j,** and **15n**) exhibited significant in vitro α-amylase inhibition at low doses (5.00–5.50 μM) as compared to standard acarbose (6.16 μM). The in vitro results were well corroborated with their in silico studies showing good affinity towards α-amylase enzyme with low docking scores. Particularly compound **15n** emerged as potential candidates against α-amylase exhibiting potency of 5.00 ± 0.18 μM. Furthermore, in silico profiling of **15n** revealed that 2-chloroquinolinyl and thiazolidin-2,4-dione scaffolds were engaged to interact with protein residues and efficiently bind within pocket of α-amylase receptor site. Moreover, **15n** also possessed drug-likenesses properties and regarded it as novel lead for further development as antidiabetic agents.

4. Experimental Section

4.1. Synthesis of 3-((2-Chloroquinolin-3-yl) methyl) Thiazolidine-2,4-dione (13)

To a solution of 2-chloro-3-(chloromethyl) quinoline[38] (**11**) (2.332 g, 11 mmol) and thiazolidine-2,4-dione (**12**) (0.129 g, 11 mmol) in DMF (2 mL) was added K_2CO_3 (0.38 g, 27.6 mmol) and the resultant reaction mixture was stirred at 70 °C for 3 h. The reaction was monitored by TLC, after completion of the reaction, the reaction mixture was poured into ice cold water. The solid product separated out was filtered, washed thoroughly with water, and dried to give intermediate **13** which was directly used for next step. Yield: 2.76 gm (86%); Pale yellow; m.p. 172 °C; 1H NMR (DMSO d6, 400 MHz, δ ppm): 8.30 (s, 1H, Ar-H), 8.04 (d, 1H, J = 8.4 Hz, Ar-H), 7.97 (d, 1H, J = 8.4 Hz, Ar-H), 7.83 (t, 1H, J = 7.6 Hz, Ar-H), 7.69 (t, 1H, J = 7.6 Hz, Ar-H), 4.89 (s, 2H, CH2), 4.34 (s, 2H, CH2); MS m/z (ES+) 292.

4.2. General Procedure for the Synthesis of 2-Chloroquinolin-3-yl-thiazolidine-2,4-diones Hybrids (15a–n)

To a solution of 3-((2-chloroquinolin-3-yl)methyl)thiazolidine-2,4-dione (**13**, 2.0 mmol) and appropriate aromatic aldehyde (**14a–n**, 2.0 mmol) in isopropyl alcohol (5 mL), catalytical amount of piperidine was added and the resulting mixture was stirred and refluxed for 1–3 h. The progress of the reaction was monitored by TLC, after completion of the reaction, the separated product was filtered and washed with isopropyl alcohol to get a crude product which was triturated with *n*-hexane and filtered to afford analytically pure products **15a–n**. (Refer Supporting Information for detailed experimental and characterizations data of **15a–n**).

4.3. In Vitro α-Amylase Inhibitory Studies

The in vitro α-amylase inhibitory studies of compounds **15a–n** were carried by our previously reported method.[23–35] Briefly, stock

solutions (1 mg/mL) of synthesized hybrids (**15a–n**) and α-amylase (barley malt procured from HIMEDIA) were prepared in DMSO. A mixture of sodium phosphate buffer (20 mM, pH 6.9), α-amylase (200 µL), and 200 µL of test solutions (50, 100, 150, 200, and 250 µg/mL) were incubated at 30 °C for 10 min. Further, the substrate starch solution (1% w/v, 200 µL) prepared in deionized water was added and mixtures were incubated for further 10 min at 30 °C. Reagent 3,5-dinitrosalicylic acid (200 µL) was added to the reaction and incubated for a further five min at 85–87 °C. The mixture was cooled to room temperature and the intensities resulting colored solutions were measured in the form of absorbance at 540 nm using a UV–visible spectrophotometer and compared with blank and control solutions. The % inhibition of α-amylase was calculated by following formula:

$$\%\ \alpha - \text{ Amylase inhibition} = 100 \times \frac{A_{\text{Blank}} - A_{\text{Sample}}}{A_{\text{Blank}}} \quad (1)$$

The IC_{50} values were determined as mean ± SD in triplicates from a non-linear regression graph using Graph Pad Prism software.

4.4. Computational Studies

The synthesized 2-chloroquinolinyl-thiazolidin-2,4-dione hybrids (**15a–n**) were docked into the active site of the crystal structure of barley (malt) α-amylase (PDB:1RPK)[47] obtained from the Protein Data Bank[41] and were studied by molecular docking using AutoDock Vina (v.1.2.5).[43,44] The protein was prepared for docking using UCSF Chimera (v.1.17.3),[42] where all solvent and ions were deleted, the highest occupancy conformations were retained, incomplete side chains were replaced using Dunbrack 2010 rotamer library,[48] hydrogens were added to complete the valencies and Kollman charges were added to the protein. The docking grid with 40 Å extents in x, y, and z directions was defined around the co-crystallized ligand acarbose. The structures of 2-chloroquinolinyl-thiazolidin-2,4-dione derivatives were prepared for docking using Openbabel (v.3.1.1)[49] where the 3D conformations were generated, hydrogens were added to complete the valencies at pH 7.4 and necessary ionization states, subsequently adding the EEM partial charges[50] to the structures. The interactions of the inhibits protein complexes were mapped using Protein Ligand Interaction Profiler Web Server.[51,52] The drug-likeness profiling of the targeted compounds **15a–n** including different parameters was studied on a web-based server viz. pkCSM.[46]

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

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