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Synthesis of novel inhibitors of α-amylase based on thiazolidine-4-one skeleton containing pyrazole moiety and their configurational studies

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Abstract

Postprandial hyperglycemia can be controlled by delaying the absorption of glucose resulting from carbohydrate digestion. α-amylase is the initiator of the hydrolysis of polysaccharides, therefore developing α-amylase inhibitors can lead to development of new treatments for metabolic disorders like diabetes mellitus. In the present work, we set out to rationally develop α-amylase inhibitors based on thiazolidine-4-one scaffold. The structure of all these newly synthesized hybrids was confirmed by spectroscopic analysis (IR, ^{1}H-NMR, MS). Appearance of two sets of signals for some protons in ^{1}H NMR revealed the existence of a mixture of 2*E*,5*Z* (37.1-42.0%) and 2*Z*,5*Z* isomer (58.4-62.8%) which was further supported by DFT studies. All the newly synthesized compounds have potential inhibitory properties as revealed through *in vitro* α-amylase inhibition activity. Compound **5a** in 100 μg/mL concentration showed remarkable inhibition of 90.04%. *In vitro* α-amylase inhibition was further supported by docking studies of compound **5a** against active site of human pancreatic α-amylase (PDB ID: 2QV4). Docking studies revealed that the bonding interactions found between **5a** and human pancreatic α-amylase are similar to those responsible for α-amylase inhibition by acarbose.

Keywords: Thiazolidin-4-one, pyrazole, α-amylase, configurational isomer, ^{1}H NMR, docking

Introduction

DM (Diabetes mellitus) is a metabolic disorder characterized by chronic hyperglycemia or increased blood glucose levels with disturbances in carbohydrate, fat and protein metabolism resulting from absolute or relative lack of insulin secretion.[1] Diabetes, being one of the most common global diseases, affects approximately 200 million individuals worldwide.[2] The

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management of the blood glucose level as close to normal as possible in patients with diabetes mellitus is the most challenging task. Therefore, enzymes that regulate gluconeogenic or glycogenolytic pathways are key biological targets for therapeutic interventions. Out of several enzymes known α-amylase is an important key enzyme responsible for carbohydrate digestion. So inhibitors of α-amylase can effectively retard the digestion and assimilation at the early steps of starch digestion, and thus succeed in a significant delay of postprandial hyperglycemia and have a beneficial effect on insulin resistance.[3] α-Amylase are considered to be one of the best targets for the development of type II diabetes therapeutic agents due to their ability to catalyze the hydrolysis of α-(1,4)-glycosidic linkages in starch.[4,5] Acarbose and voglibose are two known inhibitors for α-amylase which are in clinical use now days. However, they often cause severe gastrointestinal side effects such as abdominal pain, flatulence, and diarrhea.[6] In recent years α-amylase has been a point of interest for the development of novel anti-obesity and antidiabetic drugs.[7]

Thiazolidine-4-one represents one of the important class of compounds incorporated in several anti-diabetic drugs.[8] This compound exhibit a wide range of pharmacological activities such as anti-microbial,[9] anti-tubercular activity,[10] anti-inflammatory,[11] anti-cancer,[12] anti-oxidant,[13] anti-hyperglycemic agents[14,15] and anti-viral.[16] There is a wide scope for different pharmacological properties associated with this molecule due to presence of active methylene position in this compound. Similarly, pyrazole also represents an important class of compounds not only for their theoretical interest but also for anti-inflammatory, analgesic, anti-tumor, anti-hypertensive, anti-pyretic, sedatives, anti-bacterial and anti-diabetic activities.[17-23]

In the light of these facts and in continuation of our interest in the synthesis of heterocycles containing a multi-structure for biological activity, we, herein report a new class of thiazolidin-4-one linked pyrazole to see the additive effect of these rings towards α-amylase inhibition.

Result and Discussion

Chemistry

The target compounds 5-((3-(aryl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-2-(*p*-tolylimino) thiazolidin-4-one (**5a-g**) were synthesized by a following synthetic protocol. Firstly, a reaction of *p*-tolyl thiourea (**2**) and ethyl bromoacetate was employed to synthesise 2-(*p*-tolylimino) thiazolidin-4-one (**3**) which is a key reactant for the development of novel hybrids. Compound **3** was further subjected to Knoevenagel condensation with 3-(aryl)-1-phenyl-1*H*-pyrazole-4-

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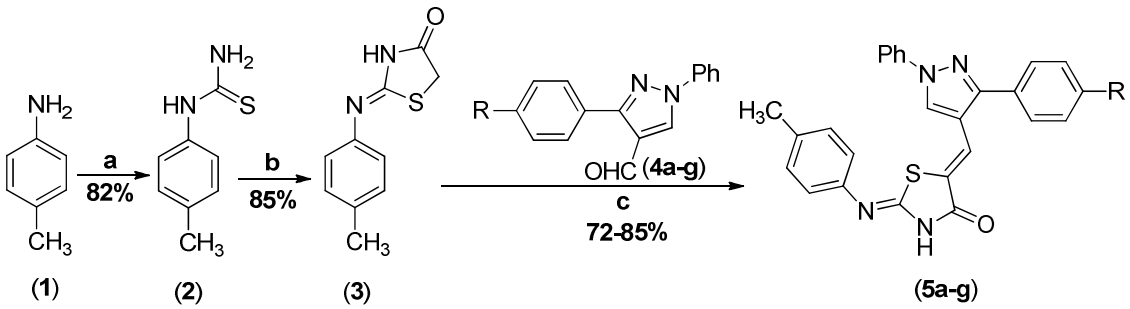
carbaldehyde[24] (**4a-g**) using catalytic amount of piperidine in refluxing ethanol for 10-12 h. The progress of the reaction was monitored by TLC using petroleum ether: ethylacetate (60:40, *v/v*). After completion of the reaction, the reaction mixture was cooled down at room temperature and the solid so obtained was filtered to yield 5-((3-(aryl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-2-(*p*-tolylimino) thiazolidin-4-one (**5a-g**) in 72-85% yield (Scheme 1, Table 1). The formation of the compounds (**5a-g**) was confirmed by using IR, ^{1}H NMR, Mass and elemental analysis.



Reaction conditions: (a) KSCN, 6N HCl, 80°C, 8-10 h; (b) $BrCH_2COOEt$, glacial acetic acid, CH_3COONa, reflux 2 h; (c) Piperidene, ethanol, reflux 10-12 h.

Scheme 1: Synthesis of 5-((3-(aryl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-2- (*p*-tolylimino) thiazolidin-4-one **5a-g**.

Table 1: Synthesis of 5-((3-(aryl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-2-(*p*-tolylimino) thiazolidin-4-one **5a-g**

S. No.	Compound	-R	Yield (%)	M.pt. °C
1	**5a**	$-OCH_3$	85	298-300
2	**5b**	$-CH_3$	76	270-275
3	**5c**	-H	83	263-265
4	**5d**	-Cl	78	292-295
5	**5e**	-F	72	288-290
6	**5f**	-Br	74	290-292
7	**5g**	$-NO_2$	85	288-290

Characterization of compounds and their configurational studies

The structure of these hybrids was ascertained by IR, ^{1}H NMR and mass spectral data. The absorption signals corresponding to N–H stretching appeared at 3,050-3,127 cm^{-1} and C=O stretching appeared in the region of 1,700-1,720 cm^{-1} of thiazolidinone ring respectively. The presence of a band in the range 1,646–1,691 cm^{-1} reveals the formation of exocyclic C=C bond at 5th position. It is assumed that the compound **5**, restricted rotation about (C=C) linkage as well as

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the imine (C=N) linkage led to the formation of 4 possible configuration that is *2Z,5E*; *2E,5E*; *2E,5Z* and *2Z,5Z* as shown in the (Fig. 1)(1 to 4)

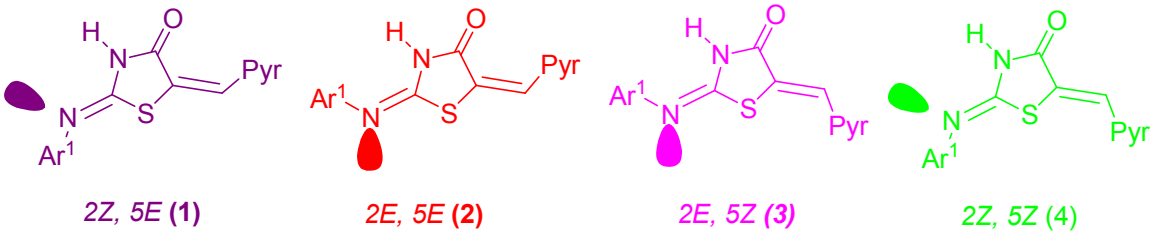

Figure 1: Four possible isomers for **5.**

Literature survey reveals that the 2-arylimino-4-thiazolidinone **3**exists as a mixture of *E* and *Z* isomers of imino form in dimethylsulphoxide-d_6 and predominantly in Z form.[25] In ^{1}H and ^{13}C NMR of compound **3** also contains two set of signals for N-H, -CH_2, and aromatic protons as shown in (Fig. 2).

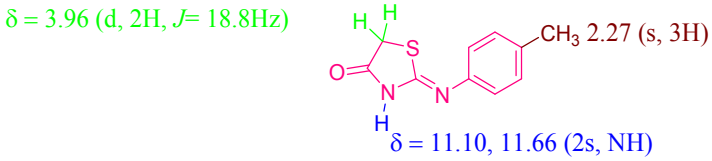

Figure 2: Characteristic peak assigned in^{1}H NMR of compound **3.**

In the ^{1}H NMR spectra of compound (**5a-g**) the signals for the olefinic proton (**H_7**) was detected at δ 7.24-7.57 ppm, deshielded by the adjacent C=O, a higher chemical shift value than expected one for *E* isomer.[26] In *E* isomer, due to lesser deshielding effect of sulphur, such a proton should resonate at lower delta values.[27] Therefore we omit the probability of 2*Z,5E* and 2*E,5E* isomers. The NMR (^{1}H) spectra of compounds **5a-g** also gave two sets of signals which confirmed the existence of two configurational isomers in dimethylsulphoxide-d_6 (2*E,5Z* and 2*Z,5Z*) and according to the literature[25-27] the predominance was assigned to the 2Z,5Z isomer.

Compound **5a** was taken as a test sample to study the configurational isomers by means of IR, ^{1}H- NMR, mass, ^{1}H-^{1}H COSY (Fig. 3 and Fig.4). Disappearance of signal of methylene proton at δ 3.80 and appearance of signal in the range of δ 7.24-7.57 due to C=C-H olefinic hydrogen confirmed the formation of exocyclic C=C bond. The numeration of structure is given specifically for NMR analysis only. The amide proton (O=C-N-**H**) observed at □ 12.00-12.53 ppm showed that substitution is on 2nd position of thiazolidin-4-one which is in agreement with a lactam proton, since an aryl amine proton appears at much higher field.[24] Two singlet at □ 2.30

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(39.94%) and □ 2.31 (60.55%) were observed due to methyl protons of aryl ring of imine. Signals at δ 8.52 (61.72%) and δ 8.61 (39.21%) in form of two sharp singlet were observed for proton of pyr-H. Duplication of signals have also been observed for protons showing resonance in aromatic region associated with the proton of *E* and *Z* geometrical isomers of the compound. Two protons $H_{28/32}$, exhibits two doublets at δ 7.92 (59.31%) and δ 7.96 (40.74%) with a coupling constant 7.6 and 8.0 Hz. Two doublets δ 6.97 (*J*=8.0 Hz) (40.82%) and δ 7.19-7.21 (59.17%) are due to two protons $H_{10/12}$. In ESI-MS mass spectra of compound **5a**, *m/z* value was observed at 466.85. In order to understand the effect of solvent on configurational isomer distribution, the NMR of compound **5a** was taken in TFA. Interestingly the % of 2*Z,5Z* isomer has been increased over 2*E,5Z* isomer in a ratio of 95:5 (supplementary file). This may be due to the solvation and stability of different configuration in different solvent. The chemical shift of olefinic protons increased from δ 7.43 to δ 8.06. Moreover the protons of OCH_3 and CH_3were found downfield. This may be due to the protonation of nitrogen and oxygen.

In ^{1}H-NMR of 2-(*p*-tolylimino)-5-((3-aryl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)thiazolidin-4-one (**5a-g**), two singlets were observed for -CH_3(□ 2.11-2.31), pyr-H (δ 8.18-8.64) whereas other protons of aromatic rings also showed two sets of signals associated with the protons of 2*E,5Z* and 2*Z,5Z* isomers of the compounds. The ratio of 2*E,5Z* and 2*Z,5Z* isomers was calculated by the NMR integration ratio of respective protons in proton NMR analysis. The integration of the two singlet of methyl (-CH_3) and pyrazole proton were used to calculate the relative abundance of each isomer at 25°C. The percentage of 2*E,5Z* and 2*Z,5Z* isomers was found in the range of 37.1-42.0% and 58.4-62.8%, respectively as shown in (Table S1).

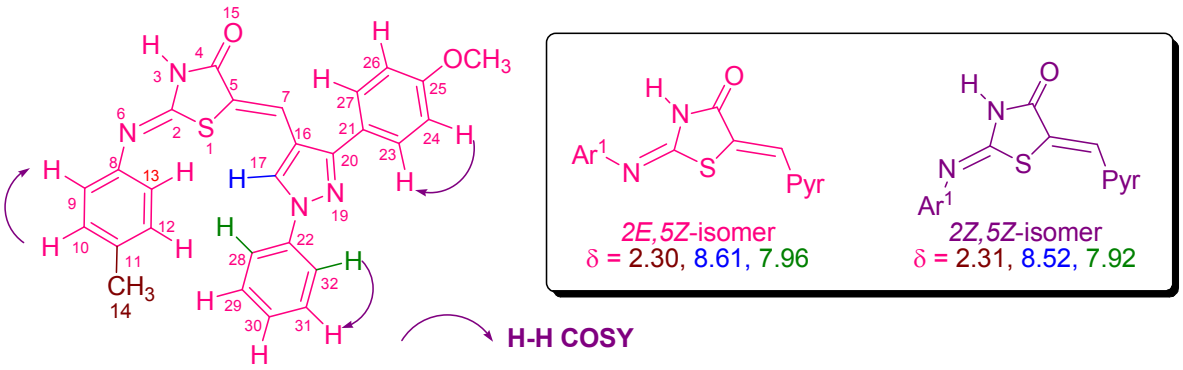

Figure 3: Characteristic peaks for isomers and H-H COSY correlation of **5a.**

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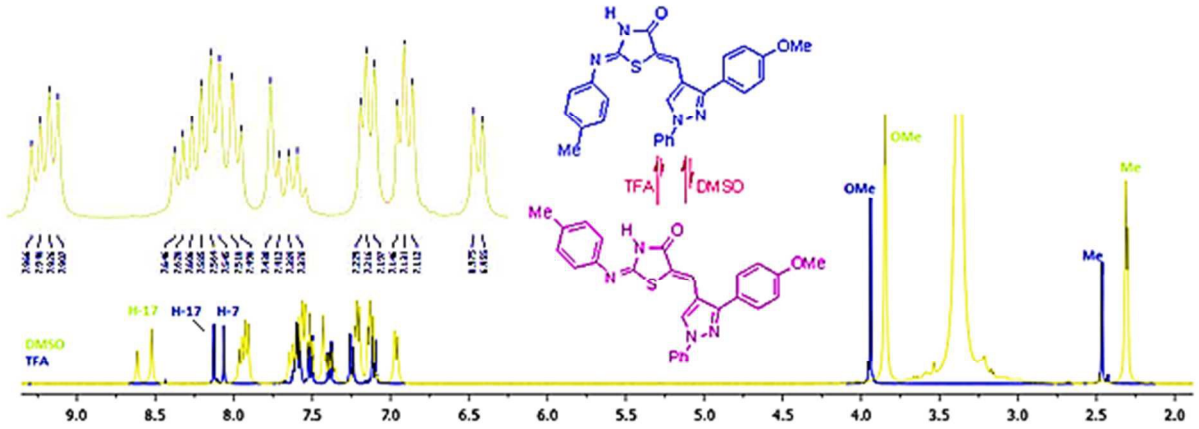

Figure 4: Characteristic peak and comparative ^{1}H NMR of compound **5a**in DMSO and TFA.

Computational studies

The 5-((3-(4-methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-2-(*p*-tolylimino) thiazolidin-4-one (**5a**) can exist in 4 possible configuration that is *2Z,5E*; 2*E,5E*; *2E,5Z* and *2Z,5Z* as shown in the (Fig. 1)(1 to 4). In this work the 3-dimensional structures of 2-(*p*-tolylimino)-5-((3-aryl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)thiazolidin-4-one configurational isomer in their neutral state were obtained by the DFT[28] approach utilizing Becke's three parameter functional[29] with the Vosko *et al.*[30] local and Lee*et al.*[31] non-local correlation, abbreviated as B3LYP1(VWN formula 1 RPA in B3LYP). The rotations about the C2-N6 (Fig. 1) (1nd and 2nd), C5-C7 (Fig. 1) (3rd and 4th) bonds respectively were taken into account. We have calculated the rotational energy barrier by steps of 10° around. All four isomers presented in (Fig. 1) were considered in our study. All the molecules were geometry-optimized until the root-mean square (RMS) gradient value was smaller than 10^{-6} a.u. To structurally characterize the molecule in detail, a systematic investigation of its potential energy surface was undertaken at the DFT (B3LYP1)/6-311G level of approximation.[32] Later, using the surface data generated from firefly software, the distribution of charge in a molecule was calculated. To obtain a 3D plot of the MEP, the electrostatic potential cube file was calculated from total SCF density. The contour maps of the electrostatic potential were then drawn using a distance between grid points of 0.02 Å and the iso value of 0.0004. The firefly software was used to calculate the electrostatic potential maps and surfaces as the distribution of the potential energy of a unit positive charge in a given molecular space, with a resolution controlled by the grid density. The vibrational wave numbers were calculated at the DFT (B3LYP1)/6-311G level of approximation. It is well known in the quantum chemical

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literature that among the available functional, the B3LYP1 functional yields a good description of harmonic vibrational wave numbers for small and medium-sized molecules. All calculations were performed using the fire fly software. The visualizations were prepared by use of the MASK.[33] By considering the calculated relative energy barriers (DFT) for the *2Z,5E*; 2*E,5E*; *2E,5Z* and 2*Z,5Z* configuration, the most stable isomers are 2*Z,5Z* and 2*E,5Z*, due to the highest energy difference (Table S2). However, the minimum difference of energy was observed in 2*Z, 5Z* and 2*E,5Z* isomer i.e. (2.8 kcal/mole) which suggest that these two configurations 2*Z, 5Z* and 2*E, 5Z* may not be experimentally accessible as isolate species.

Biological studies

In vitro α-amylase inhibition

All the synthesized compounds (**5a-g**) and standard drug were explored for their *in vitro* α-amylase inhibition studies at different concentrations (50-200µg/mL) as shown in the Table 2. All the compounds showed good % inhibition of α-amylase when compared with standard drug acarbose. Compound **5b** and **5e** were found to be more potent among all the synthesized compounds when explored at the concentration of 50µg/mL. Compound **5e** shows 77.12% inhibition followed by **5b** with 79.24% inhibition. There was a significant rise in % inhibition when concentration has been changed to 100µg/ml from 50µg/mL. Among all, **5b** shows 83.72% inhibition followed by **5a** which showed 90.04% inhibition at 100µg/ml. Apart from this **5a** shows a sudden rise in % inhibition from 75.94 to 90.04 % on increasing concentration to 100µg/mL from 50µg/mL. Inspired by the results obtained at 100µg/mL concentration, all the synthesized compounds were further screened for there *in vitro* α-amylase inhibition at 200µg/mL. Compounds **5a, 5b, 5c** and **5f** exhibited a linear rise in % inhibition, but a reduction in % inhibition has been observed for compounds **5d**, **5e** and **5g** owing to their less solubility at concentration of 200µg/mL. Among compounds showing enhanced % inhibition, compound **5a** showed % inhibition of 92.5% followed **5f** with % inhibition of 97.4% at concentration of 200µg/mL. A comparative graph of % inhibition of compounds **5a-g** with standard drug acarbose has been shown in (Fig. 5).

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Table 2: α-amylase inhibition activity of compounds **5a-g**.

Compound	Concentration (µg/mL)	OD at 595 nm	Residual Activity	% Inhibition
5a	50	0.102	24.05	75.94
	100	0.042	9.90	90.04
	200	0.032	7.54	92.15
5b	50	0.088	20.75	79.24
	100	0.069	16.27	83.72
	200	0.068	16.04	83.97
5c	50	0.211	49.76	48.08
	100	0.078	18.39	81.60
	200	0.066	15.73	84.27
5d	50	0.208	49.05	50.94
	100	0.080	18.87	81.13
	200	0.106	24.97	75.03
5e	50	0.097	28.87	77.12
	100	0.078	18.39	81.60
	200	0.093	21.95	78.05
5f	50	0.203	47.87	52.12
	100	0.115	27.12	72.87
	200	0.011	2.59	97.40
5g	50	0.148	11.79	65.09
	100	0.050	34.90	88.20
	200	0.178	41.98	58.00
Acarbose	50	0.218	51.52	48.58
	100	0.196	46.23	53.77
	200	0.154	36.32	63.68

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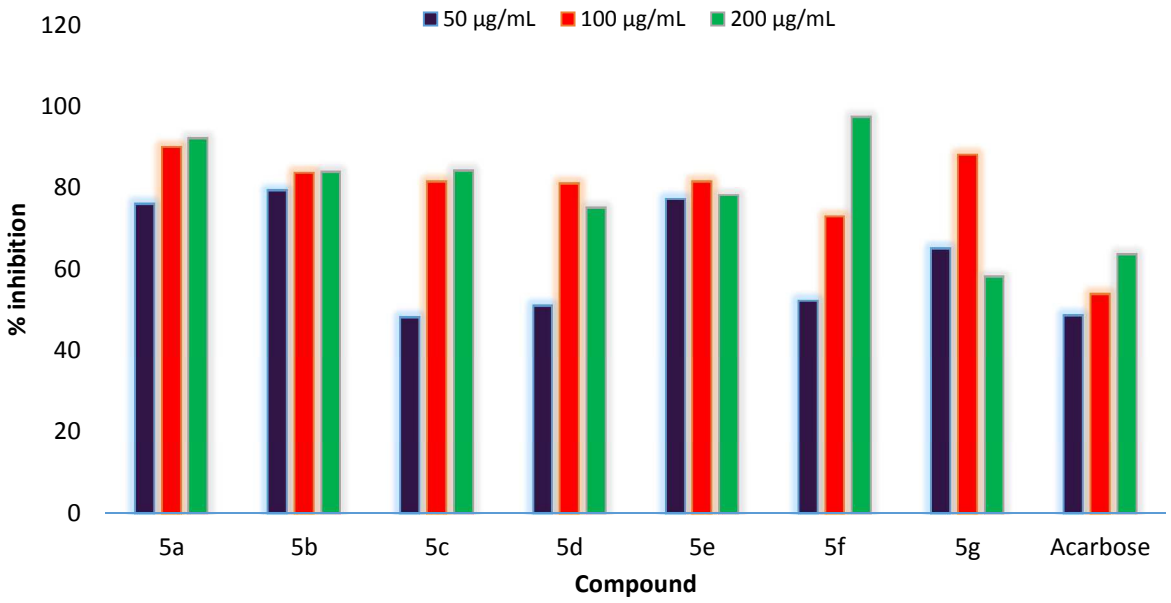

Figure 5: Comparative analysis of % Inhibition of compounds **5a-g**.

Molecular docking studies

The aim of specific structural modeling and accurate prediction of activity can be achieved only by molecular docking studies.[34] Interactions between inhibitors and active site of the target protein can be explored using molecular docking studies. The above results showed that all the synthesized molecules were stronger inhibitors of alpha-amylase as compared to acarbose. Although compound **5f** showed maximum inhibition at 200µg/mL but its % inhibition at 100µg/mL was less than compound **5a** which showed a maximum inhibition of 90.04% at concentration of 100µg/mL. After comparison of their molar mass it has been revealed that lesser dose of **5a** was required for maximum inhibition of α-amylase owing to its lower molar mass. Therefore, for ascertaining the binding conformation and interactions responsible for the activity, docking simulation of compound **5a** was performed against active site of human pancreatic alpha-amylase (PDB ID: 2QV4). The docked pose of **5a** with highest binding affinity is shown in (Fig. 6).

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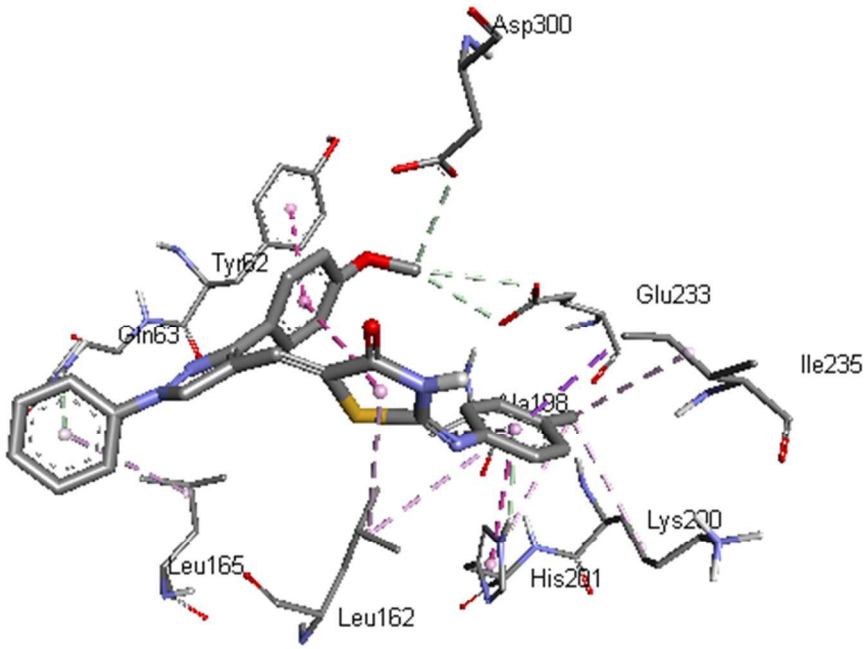

Figure 6: Interactions (dashed lines) of compound **5a** with active site residues of human pancreatic alpha-amylase.

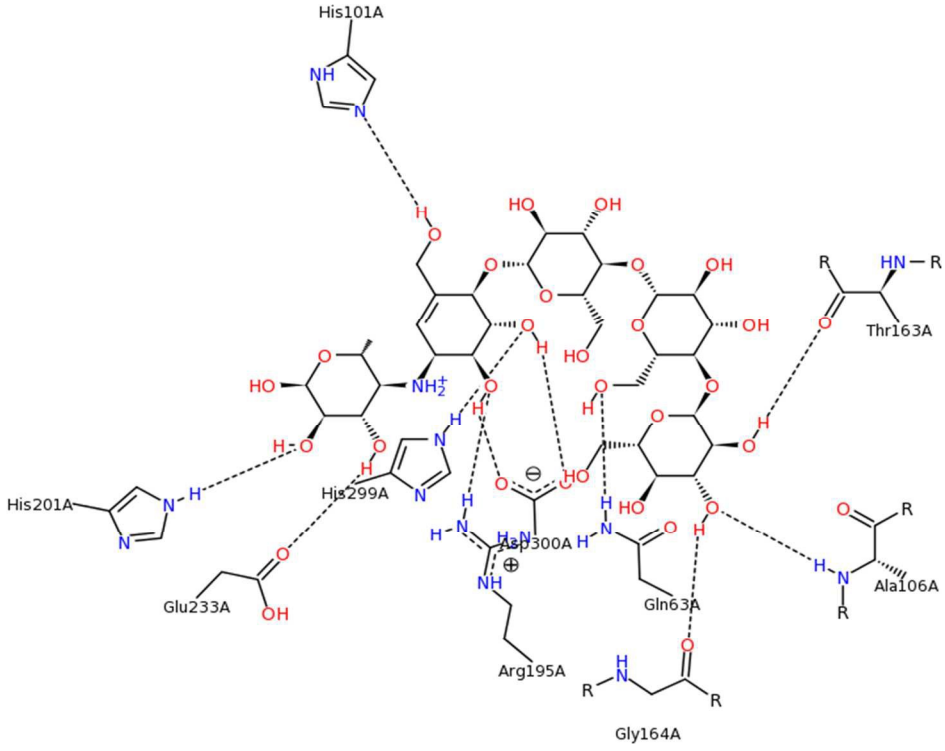

Figure 7: Pose view Image of **QV4** in **2QV4**.

It can be noticed from the Fig. 6 that mainly hydrophobic interactions are responsible for anchoring of the compound **5a**. Methoxy phenyl ring of the compound was stacked against hydroxyl phenyl ring of Tyr^{62} by Pi-Pi interactions. Methyl phenyl ring attached with

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thiazolidinone moiety created T-shaped Pi-Pi interactions with His^{201}. The methyl group was also involved in Pi-alkyl interactions with His^{201}. Thiazolidinone, methyl phenyl and phenyl rings were also involved in Pi-alkyl interactions with Leu^{162}, Ala^{188} and Leu^{165}. Gln^{63} and His^{201} formed Pi-Hydrogen bonds with phenyl and methyl phenyl rings, respectively by acting as hydrogen bond donors. Methoxy group made carbon hydrogen bonds with $Glu^{233}$and Asp^{300}. These two residues (Glu^{233} and Asp^{300}) are reported to act as catalytic residues in hydrolytic reactions of alpha-amylase.[35] Further; Tyr^{62} has been shown as an important residue for binding of some chalcones to active site of alpha-amylase.[36] Also, Tyr^{62}, Gln^{63}, Glu^{233} and Asp^{300} form hydrogen bonding interactions with the acarbose (Fig. 7). All these facts show that binding of compound **5a** to these active site residues might be the cause of alpha-amylase inhibitory activity. In (Fig. 8), compound **5a** is shown along with co-crystalized ligand acarbose in the active site of alpha-amylase.

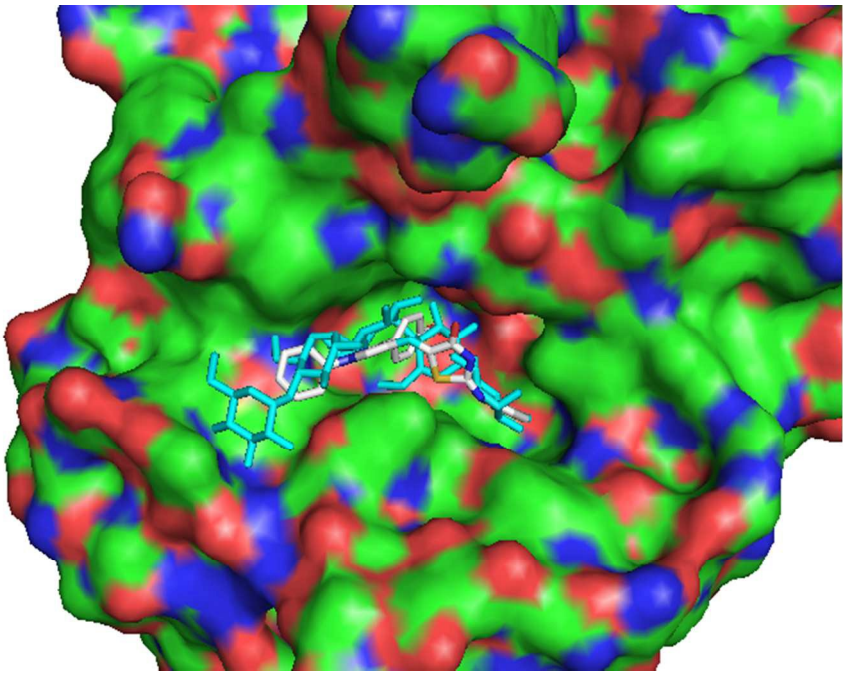

Figure 8: Compound **5a** along with co-crystalized ligand Acarbose in the active site of human pancreatic alpha-amylase (shown as surface)

Experimental

Structures of all the compounds were identified by their spectral data. Silica gel 60 F_{254} (Precoated aluminium plates) from Merck were used to monitor reaction progress. All the melting points were determined in open glass capillary tubes and are uncorrected. IR spectra were taken on a Cary 660 Agilent IR spectrophotometer and the values are expressed as ν_{max} cm^{-}

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[1]. The ^{1}H and ^{13}C spectra were recorded on Bruker (Avance-II) at 400 MHz and 100 MHz in DMSO-d_6 and trifluoroacetic acid using tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) and coupling constants (*J*) are expressed in ppm and Hz, respectively. All chemicals used were supplied by Sigma Aldrich and Merck chemical companies. Mass spectrum was recorded using Waters Micromass Q-Tof Micro. 1-Phenyl-3-aryl-1*H*-pyrazole-4-carbaldehydes and 1-p-tolylthiourea have been synthesized using reported procedure.[37,38]

Synthesis of 2-(*p*-tolylimino)thiazolidin-4-one (3)[38]

It was prepared by a three component reaction of 1-*p*-tolylthiourea (1) (1.0 mmol), ethyl bromoacetate (1.0 mmol) and sodium acetate (2.0 mmol) in glacial acetic acid under the refluxing condition for 2 h. After completion of reaction, reaction was quenched using ice and the solid so formed was filtered under suction and recrystallized from ethanol. Yield 80%; M.pt:167-170°C; ^{1}H NMR (400 MHz, DMSO,d_6 ppm): δ 2.27 (s, 3H), 3.96 (d, 2H, *J* = 18.8 Hz), 6.92 (d, *J* =7.2, 2H, Ar), 7.18 (d, *J* = 7.2 Hz, 2H, Ar), 7.59 (d, *J* =7.6, 2H, Ar), 11.10 & 11.66 (2s, NH); ^{13}C NMR (400 MHz, DMSO, d_6, ppm): 20.9, 21.4, 35.6, 38.8, 120.6, 122.1, 129.8, 130.2, 134.3, 134.5, 136.8, 143.2, 177.6, 178.2, 188.5.

Synthesis of 5-((3-(aryl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-2-(*p*-tolylimino)thiazoli din-4-one(5a-g)[38]

2-Arylimino-thiazolidin-4-ones **1** (0.5 mmol) and 1-phenyl-3-(p-substituted phenyl)-1*H*-pyrazole-4-carbaldehydes **2** (0.6 mmol) were dissolved in absolute ethanol. Piperidine (0.5 mmol) was added to the reaction mixture and the reaction mixture was stirred for 8 h at 60°C until precipitate formed. Then the mixture was cooled to room temperature, and the precipitates formed were filtered and washed with absolute ethanol to yield the final compound **5a-g** in good to excellent yield.

5-((3-(4-Methoxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-2-(*p*-tolylimino) thiazolidin-4-one (5a)

Yield 85%, M.pt. 298-300°C; IR (ν_{max} cm^{-1}, KBr): 3070 (N-H lactam), 3032 (C-H str. of aromatic), 1720 (C=O lactam), 1655 (C=C), 1574 (C=N); ^{1}H NMR (400 MHz, DMSO,d_6, ppm): δ 2.30 & 2.31 (2s, 3H, $-CH_3$), 3.84 (s, 3H, $-OCH_3$), 6.97 (d, *J*= 8.0 Hz, 2H, H_{10}/H_{12}), 7.11-7.14 (m, 2H, H_{24}/H_{26}), 7.19-7.22 (m, 4H, H_9/H_{13}, H_{10}/H_{12}), 7.35-7.43 (m, 2H, H_7, H_{30}), 7.49-7.64 (m,

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6H, H_9/H_{13}, H_{23}/H_{27}, H_{29}/H_{31}), 7.92 (d, *J*=7.6 Hz, 2H, H_{28}/H_{32}), 7.96 (d, *J*=8.0s Hz, 2H, H_{28}/H_{32}), 8.52 & 8.61 (2s, 2H, H_{17}), 11.95 (broad s, 1H, NH); ESI-MS (m/z):466.85;Anal. Calc. $C_{27}H_{22}N_4O_2S$:C, 69.51; H, 4.75; N, 12.01; O, 6.86; S, 6.87; Found: C, 69.45; H, 4.71; N, 11.97; O, 6.84; S, 6.85.

5-((3-(4-Methylphenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-2-(*p*-tolylimino)thiazolidin-4-one (5b)

Yield 76%; M.pt. 270-275°C; IR (ν_{max} cm^{-1}, KBr): 3114 (N-H lactam), 3006 (C-H str. of aromatic), 1700 (C=O lactam), 1646 (C=C), 1596 (C=N); ^{1}H NMR (400 MHz, DMSO,d_6, ppm): δ 2.30 & 2.31 (2s, 3H, $-CH_3$), 2.40 (s, 1H, $-CH_3$), 6.96 (d, 2H, *J*= 7.6 Hz, H_{10}/H_{12}), 7.19-7.23 (m, 4H, H_9/H_{13}, H_{10}/H_{12}), 7.36-7.43 (m, 4H, H_{24}/H_{26}, H_7, H_{30}), 7.50-7.64 (m, 6H, H_9/H_{13}, H_{23}/H_{27}, H_{29}/H_{31}), 7.93 (d, *J*=8 Hz, 2H, H_{28}/H_{32}), 7.97 (d, 2H, *J*=7.6 Hz, H_{28}/H_{32},), 8.54 & 8.64 (2s, 1H, H_{17}), 12.08 (broad s, 1H, NH); Calc. For $C_{27}H_{22}N_4OS$: C, 71.98; H, 4.92; N, 12.44; O, 3.55; S, 7.12. Found: C, 71.89; H, 4.82; N, 12.39; O, 3.45; S, 7.09.

5-((1-phenyl-3-*p*-tolyl-1*H*-pyrazol-4-yl)methylene)-2-(*p*-phenylimino)thiazolidin-4-one (5c)

Yield 83%; M.pt: 263-265°C; IR (ν_{max} cm^{-1}, KBr): 3050 (N-H lactam), 3031 (C-H str. of aromatic), 1716 (C=O lactam), 1650 (C=C), 1535 (C=N). ^{1}H NMR (400 MHz, DMSO,d_6, ppm): δ 2.11& 2.13 (2s, 3H, $-CH_3$), 6.74 (d, 2H Hz, *J*= 8.4 Hz, H_{10}/H_{12}), 6.93-6.98 (m, 4H, H_9/H_{13}, H_{10}/H_{12}), 7.02-7.19 (m, 2H, H_{25},H_{30}), 7.24-7.35 (m, 5H, H_{29}/H_{31}, H_{24}/H_{26}, H_7), 7.38-7.48 (m, 4H, H_9/H_{13}, H_{23}/H_{27}), 7.66 (d, *J*=8.0 Hz, 2H, H_{28}/H_{32}), 7.72(d, 2H, *J*=8.4 Hz, H_{28}/H_{32}), 8.180 & 8.309 (2s, 1H, H_{17}); Anal. Calc. For $C_{26}H_{20}N_4OS$:C, 71.54; H, 4.62; N, 12.83; O, 3.67; S, 7.35. Found: C, 71.45; H, 4.60; N, 12.79; O, 3.60; S, 7.29.

5-((3-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-2-(*p*-tolylimino)thiazolidine-4-one (5d)

Yield 78%; M.pt: 295-298°C; IR (ν_{max} cm^{-1}, KBr): 3072 (N-H lactam), 3029 (C-H str. of aromatic), 1718 (C=O lactam), 1656 (C=C), 1598 (C=N); ^{1}H NMR (400 MHz, DMSO, d_6 ppm): δ 2.30 & 2.31 (2s, 3H, $-CH_3$), 6.93-7.66 (m, 12H, H_{10}/H_{12} , H_9/H_{13} , H_{24}/H_{26} , H_7, H_{30} , H_{23}/H_{27}, H_{29}/H_{31}), 7.88 (d, *J*=8.0 Hz, 2H, H_{28}/H_{32}), 7.93 (d, 2H, *J*=7.6 Hz, H_{28}/H_{32}), 8.47 & 8.58 (2s, 1H, H_{17}); Anal. Calc. For $C_{26}H_{19}ClN_4OS$:C, 66.30; H, 4.07; Cl, 7.53; N, 11.90; O, 3.40; S, 6.81. Found: C, 66.21; H, 4.01; Cl, 7.45; N, 11.85; O, 3.35; S, 6.75.

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5-((3-(4-Fluorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-2-(*p*-tolylimino)thiazolidine-4-one (5e)

Yield 72%; M.pt: 288-290°C; IR (ν_{max} cm^{-1}, KBr): 3127 (N-H lactam), 3006 (C-H str. of aromatic), 1706 (C=O lactam), 1664 (C=C), 1598 (C=N); ^{1}H NMR (400 MHz, DMSO,d_6, ppm): δ 2.30 & 2.31 (2s, 3H, -CH_3), 6.93-7.88 (m, 14H, H_{10}/H_{12} , H_9/H_{13} , H_{24}/H_{26} , H_7, H_{30} , H_{28}/H_{32}, H_{23}/H_{27}, H_{29}/H_{31}), 8.42 & 8.54 (2s, 1H, H_{17}); Anal. Calcd. For $C_{26}H_{19}FN_4OS$: C, 68.71; H, 4.21; F, 4.18; N, 12.33; O, 3.52; S, 7.05. Found: C, 68.69; H, 4.14; F, 4.08; N, 12.30; O, 3.45; S, 6.98.

5-((3-(4-Bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-2-(*p*-tolylimino)thiazolidine-4-one (5f)

Yield 74%; M.pt: 290-292°C; IR (ν_{max} cm^{-1}, KBr): 3064 (N-H lactam), 3035 (C-H str. of aromatic), 1720 (C=O lactam), 1654 (C=C), 1596 (C=N); ^{1}H NMR (400 MHz, DMSO,d_6, ppm): δ 2.30 & 2.31 (2s, 3H, -CH_3), 6.93 (d, *J*= 8.0 Hz, 2H, H_{10}/H_{12}), 7.11-7.18 (m, 4H, H_9/H_{13}, H_{10}/H_{12}), 7.32-7.43 (m, 2H, H_{30}, H_7), 7.46-7.72 (m, 8H, H_{24}/H_{26},H_9/H_{13}, H_{23}/H_{27}, H_{29}/H_{31}), 7.87 (d, *J*=8.0 Hz, 2H, H_{28}/H_{32}), 7.92 (d, *J*=8.0 Hz, 2H, H_{28}/H_{32}), 8.44 & 8.56 (2s, 1H, H_{17}); Anal. Calc. For $C_{26}H_{19}BrN_4OS$:C, 60.59; H, 3.72; Br, 15.50; N, 10.87; O, 3.10; S, 6.22. Found: C, 60.55; H, 3.69; Br, 15.45; N, 10.81; O, 3.01; S, 6.19.

5-((3-(4-Nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-2-(*p*-tolylimino)thiazolidine-4-one (5g)

Yield 85%; M.pt: 288-290°C; IR (ν_{max} cm^{-1}, KBr): 3072 (N-H lactam), 3037 (C-H str. of aromatic), 1716 (C=O lactam), 1691 (C=C), 1600 (C=N); ^{1}H NMR (400 MHz, DMSO, d_6, ppm): δ 2.30 & 2.31 (s, 3H, -CH_3), 6.91 (d, *J*= 8.0 Hz, 2H, H_{10}/H_{12}), 7.16 (d, *J*= 8.0 Hz, 2H, H_9/H_{13}, H_{10}/H_{12}), 7.34-7.41 (m, 1H, H_{30}), 7.48-7.57 (m, 3H, H_{29}/H_{31}, H_7), 7.62-7.64 (d, 2H, H_9/H_{13}, *J*=8.4 Hz), 7.87-7.91 (m, 2H, H_{27}/H_{31}), 7.93-7.96 (m, 2H, H_{28}/H_{32}), 8.35-8.39 (m, 2H, H_{24}/H_{26}); 8.49 & 8.61 (2s, 1H, H_{17}); Anal. Calc. For $C_{26}H_{19}N_5O_3S$:C, 64.85; H, 3.98; N, 14.54; O, 9.97; S, 6.66. Found: C, 64.81; H, 3.87; N, 14.43; O, 9.91; S, 6.60.

Inhibition assay for α-Amylase activity

A stock solution of 10 mg/10 mL concentration was prepared by using DMSO solvent. Activity of amylase was assayed with different concentrations (50, 100, 200 µg/mL) of sample with control and reagent solution without test sample was used as control. Starch solution (1% *w/v*) or (0.5% *w/v*) was prepared by stirring and boiling 0.5 g of soluble potato starch in 50 mL of

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deionized water for 15 minutes. The enzyme solution (1 unit/mL) was prepared by mixing 100 mg in 100 mL of 20 mM sodium phosphate buffer (pH 6.9).The color reagent was a solution containing 96 mM 3,5-dinitrosalicylic acid (DNSA) (20 mL), 5.31 M sodium potassium tartrate in 2 M NaOH (8 mL) and deionized water (12 mL). Acarbose was used as a standard at the concentration of 1mg/mL. 100 µl of test solution and 100 µL of enzyme solution were mixed in viols and incubated at 25°C for 30 min. To this mixture 100 µL of color reagent was added and the mixture was heated on water bath at 85°C for 15 min. After then, the reaction mixture was removed from water bath, cooled and absorbance value determined at 595 nm. Individual blanks were prepared for correcting the background absorbance. Control experiment was conducted in the same manner by replacing the drug sample with 1 mL DMSO. Inhibition percentage of α-amylase was calculated by the formula.[39] Enzyme activity was calculated and percentage of inhibition shown in the table.

$$\%\text{Inhibition} = \frac{\text{Control}-\text{Test}}{\text{Control}} \times 100$$

Docking simulations

The 3D structure of the compound **5a** was made and optimized with Marvinsketch.[40] The enzyme alpha-amylase (PDB ID:2QV4) was downloaded from Brookhaven Protein Data Bank (http://www.rcsb.org/pdb). The preparation of protein was performed with UCSF Chimera[41] and Auto Dock Tools[42] using reported procedure.[43] The docking simulation was performed using well established AutoDock Vina program.[44] The validation of the docking protocols was done following the reported technique[45] and these protocols were adopted for carrying out docking studies. The search space for docking was centered at x = 11.0976587428, y = 47.4125222511, and z = 26.0545925088 with size x = 25.0, y = 25.0 and z = 25.0. The exhaustiveness was set to be 100. The results were visualized with Discovery Studio[46] and PyMol[47].

Conclusion

In conclusion, the present study describe the synthesis of seven novel 5-((3-(aryl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)-2-(*p*-tolylimino)thiazolidin-4-one (**5a-g**) compounds by condensation of 1-phenyl-3-(aryl)-1*H*-pyrazole-4-carbaldehyde (**4a-g**) with 2-(*p*-tolylimino)thiazolidin-4-one (**3**) and evaluated for their α-amylase inhibition. The structures of all newly synthesized compounds were confirmed by elemental and spectroscopic analysis (IR, ^{1}H-NMR, MS).^{1}H NMR shows that *2Z,5Z*-isomer is present in 58.4-62.8% which was further supported by DFT studies. The

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biological potential of newly synthesized compounds was investigated through *in vitro* α-amylase inhibition activity. The results showed that some of the synthesized compounds exhibited significant inhibitory activities. Compound **5a** in 100 µg/mL concentration showed remarkable inhibition of 90.04%. Docking studies of compound **5a** was performed against active site of human pancreatic alpha-amylase (PDB ID: 2QV4). It has been revealed from docking studies that the bonding interactions found between **5a** and human pancreatic α-amylase are similar to those responsible for α-amylase inhibition by acarbose.

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Conflict of Interest

The authors declare no competing interests.

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