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REGULAR ARTICLE

Synthesis, type II diabetes inhibitory activity and docking studies of novel thiazole molecules

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Abstract. A series of novel ethyl (*E*)-2-cyano-3-((4-methyl-5-(arylcarbamoyl)thiazol-2-yl)amino)-3-(methylthio)acrylate have been synthesized starting from various 2-amino-*N*-aryl-4-methylthiazole-5-carboxamide. The reaction of 3-oxo-*N*-arylbutanamide **2a-i** with *N*-bromosuccinimide and cyclization with thiourea under reflux conditions yielded derivatives of 2-amino-*N*-aryl-4-methylthiazole-5-carboxamide **3a-i.** Further reaction of thiazoles **3a-i** with ethyl 2-cyano-3,3-bis(methylthio)acrylate in DMF and K_2CO_3 as a base under room temperature gave new thiazole molecule **4a-i** with excellent yields. The significant features of this reaction procedure are novel, modest, and short time. The spectral characterization of molecules was confirmed by ^{1}H NMR, ^{13}C NMR, FTIR, and MS. Synthesized molecules were evaluated *in vitro* for their α-amylase inhibitory activity and displayed moderate to excellent inhibition with IC_{50} values varying from 12.55 µg/mL to 69.47 µg/mL using acarbose (IC_{50}=23.62 µg/mL) as control. Moreover, a molecular docking study was carried out for synthesized molecules **4a-i** against human pancreatic α-amylase (2QV4) *via* utilizing the Autodock technique. The docking outcomes of molecules **4g** and **4h** showed good cytotoxic activity.

Keywords. Thiazole; α-amylase; molecular docking; ketene dithioacetal.

1. Introduction

Protein enzyme α-amylase aids in converting starch into maltose and glucose. Human beings and other species use sugars and carbohydrates as energy storage.[1] It is extensive between living organisms. Salivary glands produce α-amylase in humans and other mammals; in humans and several other beings, α-amylase production is done by the salivary gland, which is called ptyalin. The optimum pH of α-amylase is 6.7-7.0. It is also found in urine, serum, and human saliva. In recent times, α-amylase has been also found in natural resources.[2] Heterocycles with sulphur atoms like thiazole played a key role in medicinal research (I-V, Figure 1).

The thiazole molecule has fascinated the interest of researchers due to their ready accessibility good chemical and biological activities such as anticancer,[3–5] antidiabetic,[6] antimicrobial,[7] antiviral,[8] antifungal,[9] antioxidant,[10] antitubercular,[11] antihypertensive[12] and antialzheimer's agent.[13] Sravanthi and co-workers screened novel thiazole containing indole and pyrazole moiety and evaluated for antihyperglycemic activity with IC_{50} value 236.1 µg/mL (**A**, Figure 2) was moderate compared to the standard drug acarbose with IC_{50} value 171.8 µg/mL.[14] In 2017, Salar and co-workers developed new hydrazinyl thiazole moieties substituted with chromone and screened them for alpha-amylase inhibitory activity in which the IC_{50} value was observed between 2.186-3.405 µM compared to acarbose (1.9±0.07 µM). They have found that the bromo substitution showed the most potent alpha amylase inhibitory activity based on the structure-activity relationship (SAR) study, and attachment of methyl group on chromone ring also showed improved activity (**B**, Figure 2).[15] Khan and co-workers have synthesized isobutyl substituted thiazole derivatives and performed α-amylase inhibitory activity. The molecule **C** (Figure 2) with methyl ester substitution showed most

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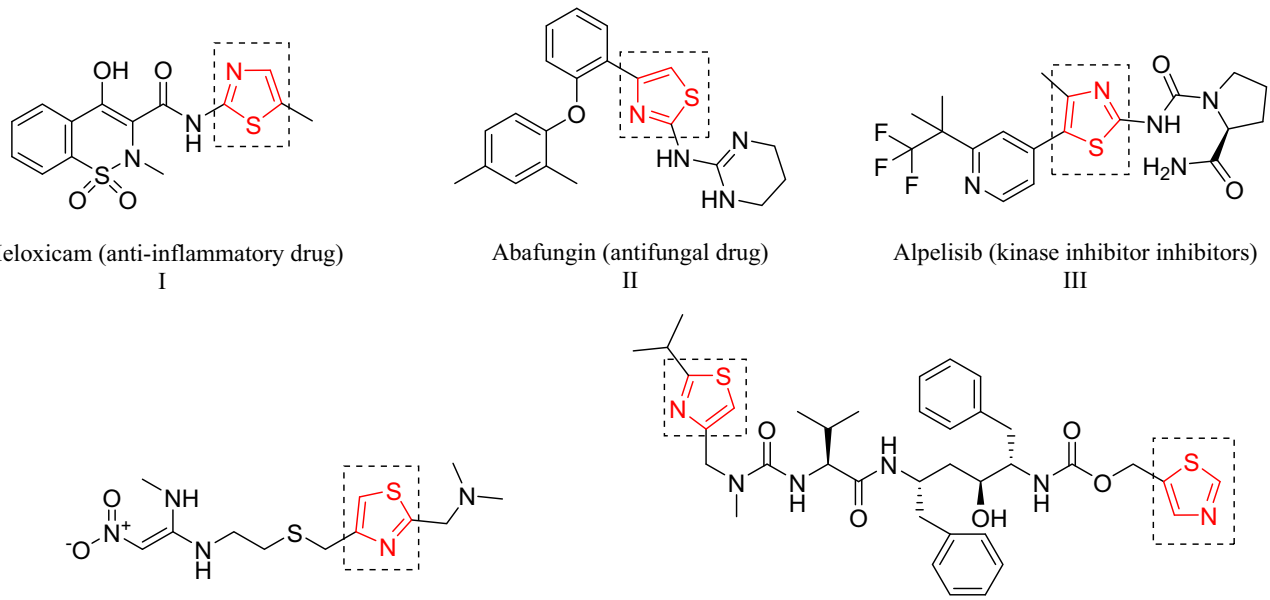


Figure 1. Several bioactive thiazoles I-V.

potent IC_{50} value 12.00±0.244 µg/mL compared to acarbose (16.59±0.135 µg/mL).[16]

It has already been established that the thiazole acetamide molecule has the ability to inhibit both α-amylase and α-glycosidase. In this context, Wu, Lino, and co-workers presented thiazole moiety bearing amide linkage (**D** and **E**, Figure 2) showed excellent antidiabetic activity,[17,18] Taha and co-workers synthesized methyl substituted thiazole molecules having an amide bridge and screened them as alpha amylase inhibitors. The synthesized molecules showed IC_{50} values ranging from 1.709±0.12 to 3.049±0.25 µM (**F**, Figure 2) compared to acarbose, which showed IC_{50} value 1.637±0.153 µM. The binding relationship of compounds with protein ligands' active sites was evaluated through molecular docking research.[19] The aryl thiazole substituent is another intriguing heterocycle for developing effective α-glycosidase inhibitors. Molecule **G** (Figure 2) (IC_{50}=0.0004±1.097 µM) with thiazole in its core structure with ethyl ester substitution showed promising antiglycating activity compared to aminoguanidine (IC_{50}=25.50±0.337 µM).[20] Also, the ethyl ester group (**H**, Figure 2) attached with nitrile (**I**, Figure 2) showed increased α-amylase inhibitory activity.[21] Very little research work has been performed on the presence of thiomethyl group as an important attachment for antidiabetic activity. Kanwal and co-workers reported indole bearing an amide linkage and thiomethyl (**J**, Figure 2) showed potent alpha-amylase inhibitory activity with IC_{50}=2.15±0.09 µM compared to acarbose (IC_{50}=0.92±0.4 µM).[22] Our continuous research in the field of synthesis of various bioactive heterocyclic compounds[23–27] motivated us to develop some novel thiazoles for medicinal interest.

2. Experimental

2.1 *Chemistry*

Melting points were determined on an electrothermal device using open capillaries and were uncorrected. Thin-layer chromatography was performed on precoated silica-gel 60 F254 (Merck), compounds were visualized with UV light at 254 nm and 365 nm or with iodine vapor. The IR spectra were recorded on a Shimadzu FT-IR spectrometer using the ATR technique. NMR spectra were recorded on a Bruker AVANCE III (400 MHz) spectrometer in DMSO-d_6. Chemical shifts are expressed in δ ppm downfield from Tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded using a direct inlet probe on Shimadzu GCMS QP2010 Ultra mass spectrometer. All reactions were carried out under an ambient atmosphere. All reagents were purchased from Sigma-Aldrich, Loba, Molychem, SRL, and CDH and used without further purification.

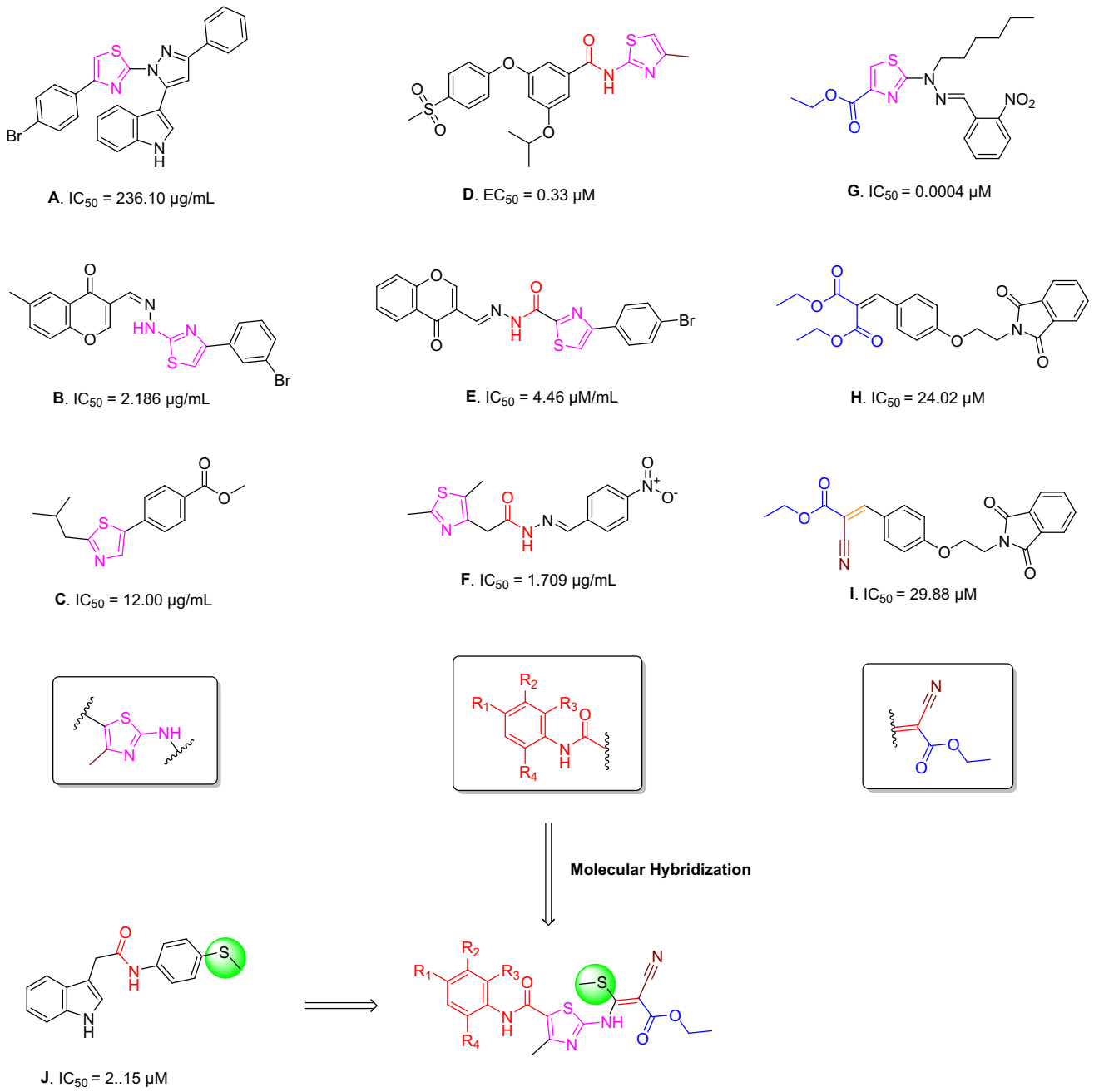


Figure 2. Designing strategy for potent thiazole molecules.

2.1a *General process for the synthesis of Acetoacetanilides (2a-i)*: Various aromatic amines (**1a-i**) (10 mmol) and ethyl acetoacetate containing catalytic amount of Potassium or sodium hydroxide lye (10%) in toluene was refluxed for approximately 24 h. After completion of the reaction, the solvent was evaporated under vacuum and the residue was crystallized from methanol to get pure acetoacetanilide (**2a-i**).[29]

2.1b *General process for the synthesis of thiazoles. (3a-i)*: To a stirred solution of acetoacetanilide (10 mmol) **(2a-i)** in MeOH, *N*-bromosuccinimide (15 mmol) was added and stirred at room temperature for 30 min. To this reaction mixture thiourea (20 mmol) was slowly added and refluxed for 4-5 h. The reaction was being monitored by TLC, after completion of the reaction; mixture was cooled, poured with stirring into ice-water, neutralized with dilute HCl. The reaction mixture was cooled to room temperature, poured into ice-cold water and neutralized with dil. HCl. The separated solid product was filtered, washed with water and dried at room temperature to get analytically pure compound (**3a-i**).[30–33]

2.1c *General procedure for the synthesis of ethyl (E)-2-cyano-3-((5-((arylphenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-3-(methylthio)acrylate (4a-i)*: A mixture of **3a-i** (10 mmol) and ethyl 2-cyano-3,3-bis(methylthio)acrylate (10 mmol) in 10 mL of DMF and anhydrous potassium carbonate (10 mmol) was stirred at room temperature for 1 h. The reaction was being monitored by TLC. After completion of the reaction, the reaction mixture was cooled to room temperature, poured into ice-cold water and neutralized with dil. HCl. The separated solid was filtered, washed with water and purified by recrystallization from DMF to afford crystals (**4a-i**).

Ethyl (E)-2-cyano-3-((5-((4-methoxyphenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-3-(methylthio)acrylate (**4a**): Yellow powder, Yield: 82%, M.p. 129-131 °C; FTIR (ATR, ν_{max}, cm^{-1}): 1453, 1659, 2215, 2971; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.67 (s, 1H, NH-Acetamide), 9.63 (s, 1H, NH-Thiazole), 7.51 (d, J = 9.1 Hz, 2H, Ar-H), 6.90 (d, J = 9.1 Hz, 2H, Ar-H), 4.14 (d, J = 7.3 Hz, 2H, CH_2-Et), 3.74 (s, 3H, OCH_3-Ar), 2.45 (s, 3H, CH_3-Thiazole), 2.32 (s, 3H, CH_3-Thiomethyl), 1.21 (t, J = 7.1 Hz, 3H, CH_3-Et); MS (*m/z*): 432 (M^+); Anal. Calcd. For $C_{19}H_{20}N_4O_4S_2$: C, 52.76; H, 4.66; N, 12.95; Found: C, 52.81; H, 4.65; N, 12.98.

Ethyl (E)-2-cyano-3-((4-methyl-5-(p-tolylcarbamoyl)thiazol-2-yl)amino)-3-(methylthio)acrylate (**4b**): Yellow Powder, Yield: 89%, M.p. 150-152 °C; FTIR (ATR, ν_{max}, cm^{-1}): 1451, 1663, 2217, 2970; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.68 (s, 1H, NH-Acetamide), 9.68 (s, 1H, NH-Thiazole), 7.50 (d, J = 8.1 Hz, 2H, Ar-H), 7.14 (d, J = 8.1 Hz, 2H, Ar-H), 4.15 (d, J = 7.5 Hz, 2H, CH_2-Et), 2.45 (s, 3H, CH_3-Thiazole), 2.32 (s, 3H, CH_3-Thiomethyl), 2.28 (s, 3H, CH_3-Ar), 1.22 (t, J = 7.3 Hz, 3H, CH_3-Et); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.05 (C-3), 163.96 (C-1), 160.17 (C-10), 159.34 (C-14), 158.11 (C-12), 142.40 (C-19), 136.40 (C-17), 133.43 (C-13), 129.69 (C-21), 121.18 (C-20), 117.37 (C-25), 108.19 (C-2), 60.78 (C-27), 20.93 (C-23), 14.70 (C-7), 14.59 (C-24), 14.30 (C-28); MS (*m/z*): 416 (M^+); Anal. Calcd. For $C_{19}H_{20}N_4O_3S_2$: C, 54.79; H, 4.84; N, 13.45; Found: C, 54.68; H, 4.80; N, 13.34.

Ethyl (E)-3-((5-((3-chlorophenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-2-cyano-3-(methylthio)acrylate (**4c**): Yellow Powder, Yield: 85%, M.p. 152-154 °C; FTIR (ATR, ν_{max}, cm^{-1}): 1481, 1670, 2210, 2973; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.74 (s, 1H, NH-Acetamide), 9.86 (s, 1H, NH-Thiazole), 7.80 (s, 1H, Ar-H), 7.57 (d, J = 8.2 Hz, 1H, Ar-H), 7.36 (t, J = 8.1 Hz, 1H, Ar-H), 7.16 (d, J = 8.0 Hz, 1H, Ar-H), 4.13 (q, J = 7.1 Hz, 2H, CH_2-Et), 2.46 (s, 3H, CH_3-Thiazole), 2.32 (s, 3H, CH_3-Thiomethyl), 1.21 (t, J = 7.1 Hz, 3H, CH_3-Et); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.92 (C-3), 164.04 (C-1), 160.23 (C-12), 159.28 (C-17), 158.07 (C-14), 137.77 (C-20), 133.21 (C-15), 128.12 (C-23), 127.95 (C-21), 121.26 (C-25), 117.41 (C-8), 108.73 (C-2), 60.78 (C-27), 14.71 (C-7), 14.60 (C-16), 14.30 (C-28); MS (*m/z*): 436 (M^+); Anal. Calcd. For $C_{18}H_{17}ClN_4O_3S_2$: C, 49.48; H, 3.92; N, 12.82; Found: C, 49.62; H, 4.02; N, 12.91.

Ethyl (*E*)-2-cyano-3-((4-methyl-5-(phenylcarbamoyl)thiazol-2-yl)amino)-3-(methylthio)acrylate (**4d**): Yellow Powder, Yield: 82%, M.p. 127-129 °C; FTIR (ATR, ν_{max}, cm^{-1}): 1487, 1658, 2217, 2971; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.70 (s, 1H, NH-Acetamide), 9.63 (s, 1H, NH-Thiazole), 7.62 (d, J = 8.3 Hz, 2H, Ar-H), 7.50 (d, J = 8.6 Hz, 1H, Ar-H), 7.32 (t, J = 7.7 Hz, 1H, Ar-H), 7.09 (t, J = 7.4 Hz, 1H, Ar-H), 4.05 (q, J = 7.1 Hz, 2H, CH_2-Et), 2.45 (s, 3H, CH_3-Thiazole), 2.29 (s, 3H, CH_3-Thiomethyl), 1.17 (t, J = 7.0 Hz, 3H, CH_3-Et); MS (*m/z*): 402 (M^+); Anal. Calcd. For $C_{18}H_{18}N_4O_3S_2$: C, 53.72; H, 4.51; N, 13.92; Found: C, 53.82; H, 4.55; N, 13.89.

Ethyl (E)-2-cyano-3-((5-((2,6-dimethylphenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-3-(methylthio)acrylate (**4e**): Yellow Powder, Yield: 93%, M.p. 144-146 °C; FTIR (ATR, ν_{max}, cm^{-1}): 1463, 1665, 2214, 2972; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.68 (s, 1H, NH-Acetamide), 9.20 (s, 1H, NH-Thiazole), 7.11 (s, 3H, Ar-H), 4.14 (q, J = 7.1 Hz, 2H, CH_2-Et), 2.46 (s, 3H, CH_3-Thiazole), 2.33 (s, 3H, CH_3-Thiomethyl), 2.15 (s, 6H, CH_3-Ar), 1.21 (t, J = 7.1 Hz, 3H, CH_3-Et); MS (*m/z*): 430 (M^+); Anal. Calcd. For $C_{20}H_{22}N_4O_3S_2$: C, 54.79; H, 4.84; N, 13.45; Found: C, 54.65; H, 4.84; N, 13.41.

Ethyl (E)-2-cyano-3-((5-((4-fluorophenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-3-(methylthio)acrylate (**4f**): Yellow Powder, Yield: 79%, M.p. 135-137 °C; FTIR (ATR, ν_{max}, cm^{-1}): 1250, 1408, 1676, 2214, 2975; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.71 (s, 1H, NH-Acetamide), 9.79 (s, 1H, NH-Thiazole), 7.63 (dd, J = 8.9, 5.0 Hz, 2H, Ar-H), 7.17 (t, J = 8.9 Hz, 2H, Ar-H), 4.14 (d, J = 7.0 Hz, 2H, CH_2-Et), 2.46 (s, 3H, CH_3-Thiazole), 2.32 (s, 3H, CH_3-Thiomethyl), 1.21 (t, J = 7.1 Hz, 3H, CH_3-Et); MS (*m/z*): 420 (M^+); Anal. Calcd. For $C_{18}H_{17}FN_4O_3S_2$: C, 51.42; H, 4.08; N, 13.32; Found: C, 51.32; H, 3.96; N, 13.35.

Ethyl (E)-3-((5-((4-chlorophenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-2-cyano-3-

(methylthio)acrylate (**4g**): Yellow Powder, Yield: 86%, M.p. 152-154 °C; FTIR (ATR, ν_{max}, cm^{-1}): 1590, 1658, 2218, 2972; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.73 (s, 1H, NH-Acetamide), 9.86 (s, 1H, NH-Thiazole), 7.66 (d, J = 8.9 Hz, 2H, Ar-H), 7.39 (d, J = 8.9 Hz, 2H, Ar-H), 4.14 (q, J = 7.1 Hz, 2H, CH_2-Et), 2.45 (s, 3H, CH_3-Thiazole), 2.32 (s, 3H, CH_3-Thiomethyl), 1.21 (t, J = 7.1 Hz, 3H, CH_3-Et); MS (*m/z*): 436 (M^+); Anal. Calcd. For $C_{18}H_{17}ClN_4O_3S_2$: C, 49.48; H, 3.92; N, 12.82; Found: C, 49.18; H, 3.76; N, 12.96.

Ethyl (E)-3-((5-((4-bromophenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-2-cyano-3-(methylthio)acrylate (**4h**): Yellow Powder, Yield: 91%, M.p. 172-174 °C; FTIR (ATR, ν_{max}, cm^{-1}): 1488, 1667, 2214, 2979; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.73 (s, 1H, NH-Acetamide), 9.86 (s, 1H, NH-Thiazole), 7.60 (d, J = 9.0 Hz, 2H, Ar-H), 7.51 (d, J = 8.9 Hz, 2H, Ar-H), 4.14 (d, J = 7.4 Hz, 2H, CH_2-Et), 2.45 (s, 3H, CH_3-Thiazole), 2.31 (s, 3H, CH_3-Thiomethyl), 1.21 (t, J = 7.0 Hz, 3H, CH_3-Et); MS (*m/z*): 481 (M^+); Anal. Calcd. For $C_{18}H_{17}BrN_4O_3S_2$: C, 44.91; H, 3.56; N, 11.64; Found: C, 44.87; H, 3.19; N, 11.43.

Ethyl (E)-2-cyano-3-((4-methyl-5-(o-tolylcarbamoyl)thiazol-2-yl)amino)-3-(methylthio)acrylate (**4i**): Yellow Powder, Yield: 87%, M.p. 131-133 °C; FTIR (ATR, ν_{max}, cm^{-1}): 1451, 1665, 2208, 2974; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.68 (s, 1H, NH-Acetamide), 9.38 (s, 1H, NH-Thiazole), 7.30 - 7.12 (m, 4H, Ar-H), 4.14 (q, J = 7.1 Hz, 3H, CH_2-Et), 2.47 (s, 3H, CH_3-Thiazole), 2.32 (s, 3H, CH_3-Thiomethyl), 2.19 (s, 3H, CH_3-Ar), 1.21 (t, J = 7.0 Hz, 3H, CH_3-Et); MS (*m/z*): 416 (M^+); Anal. Calcd. For $C_{19}H_{20}N_4O_4S_2$: C, 52.76; H, 4.66; N, 12.95; Found: C, 52.79; H, 4.82; N, 13.01.

2.2 *Experimental protocol of molecular docking study*

The ChemSketch 2021.2.0 software was used for the generation of ligand structures. Furthermore, the energy minimization of every molecule was performed using the Dundee PRODRG2 server and Autodock Vina 1.1.2 was used to for the docking studies.[34] Human pancreatic α-amylase was obtained from PDB with ID 2QV4. The grid box size was set to 40, 40 and 40 Å for x, y and z correspondingly. The grid center was set to 17.390, 61.804 and 15.925 for x, y and z individually. Value of exhaustiveness was set to 40. Powerful molecular graphics viewer Discovery Studio Visualizer v21.0 was used to figure out the most probable binding mode.[35]

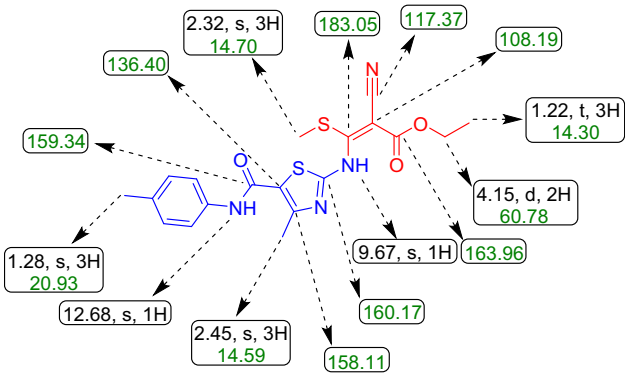
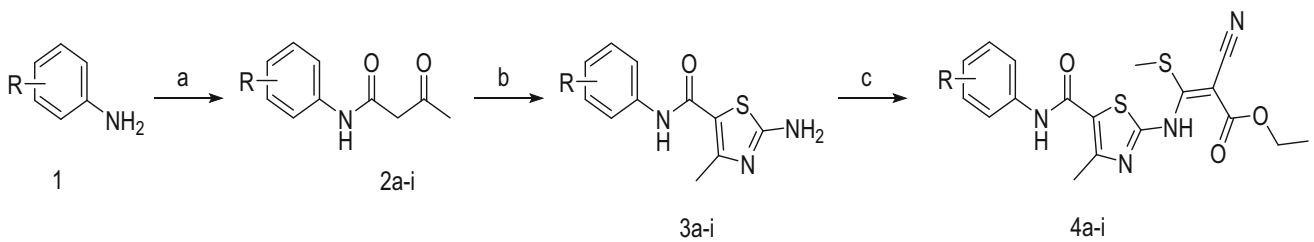


Figure 3. Selected ^{1}H (black) and ^{13}C NMR (green) of **4b**.

2.3 *In vitro α-amylase inhibition*

Mor's technique[28] was used to assess the inhibition activity of human pancreatic α-amylase using acarbose as reference compound. The molecule **4a-i** were added to 5 mL DMSO and dissolved at ambient temperature to give concentrations ranging from 12.5, 25, 50 and 100 µg/mL. In 25 mL 0.4 M NaOH solution, 500 mg starch was dissolved at 100 °C for 5 min and used as substrate solution, following cooling to room temperature, pH 7 was attained by addition of 2 M HCl solution and 100 mL water was added to make the volume. In microplates, samples (20L) and substrate (40L) were mixed and incubated at 37 °C for



Scheme 1. Reagents and conditions: (**a**) Ethyl acetoacetate, KOH, Reflux, 24 h (**b**) NBS, Thiourea, MeOH, Reflux, 4 h (**c**) ethyl 2-cyano-3,3-bis(methylthio)acrylate, K_2CO_3, DMF, Rt, 1 h.

Table 1. Optimization of the reaction conditions.

Entry	Solvent	Base [a]	Temp. (°C) [b]	Yield (%) [c]	Purification Necessary/By-product formation
1	No Solvent	-	80	-	-
2	H_2O	-	rt	-	-
3	H_2O	K_2CO_3	rt	-	-
4	Acetone	Et_3N	rt	16	Yes
5	Acetone	K_2CO_3	rt	19	Yes
6	MeOH	Et_3N	rt	19	Yes
7	MeOH	K_2CO_3	rt	25	Yes
8	EtOH	Et_3N	rt	32	Yes
9	EtOH	K_2CO_3	rt	48	Yes
10	THF	Et_3N	rt	29	Yes
11	THF	K_2CO_3	rt	35	Yes
12	MeCN	Et_3N	rt	40	Yes
13	MeCN	K_2CO_3	rt	57	Yes
14	DMF	Et_3N	rt	72	Yes
15	DMF	K_2CO_3	rt	94	No

[a]Amount of base was 1 equivalent

[b]Reaction time was 1 h

[c]Yield is given for isolated product without purification

three minutes. Afterward, 20 µL α-amylase solution (50 µg/mL) was added to each well followed by incubation for 15 min. To stop the reaction, 0.1 M HCl (80 µL) was added. 1 mM iodine solution (200 µL) was added to reaction mass and absorbance was measured at 650 nm using Elisa microplate reader. The α-amylase inhibitory activity was demonstrated as percentage inhibition.

$$\%\text{inhibition} = \{1 - (Abs2 - Abs1)/(Abs4 - Abs3) \times 100\}$$

3. Results and Discussion

3.1 *Chemistry*

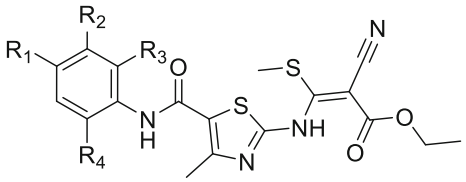
To find novel anti-diabetic molecules and synthesis of different heterocyclic molecules, here, we report newly synthesized thiazole molecules having arylamide, nitrile, ester, and methylthio substitution. The compounds **4a-i** were elucidated by inspecting their spectroscopic data like ^{1}H-NMR, FTIR, and Mass spectroscopy. In the first step, 3-oxo-*N*-arylbutanamide **2a-i** and *N*-bromosuccinimide reacted at ambient temperature to get 2-bromo-3-oxo-*N*-arylbutanamide. Then, thiourea was added for ring closure to obtain 2-amino-*N*-aryl-4-methylthiazole-5-carboxamide **3a-i.**

Then compound **3a-i** was reacted with ethyl 2-cyano-3,3-bis(methylthio)acrylate with potassium carbonate in DMF to obtain novel and highly functionalized derivatives of thiazole **4a-i** as shown in Scheme 1. The ^{1}H-NMR graph of molecules revealed that methyl proton of ester seen at t 1.15-1.23 ppm (CH_3), which were triplet peaks, at s 2.28-2.32 ppm (SCH_3) for thiomethyl protons as a singlet peak. Thiazole methyl protons were detected at s 2.44-2.46

ppm (CH_3) as a singlet, ester methylene protons were seen at t 4.02 to 4.16 ppm (CH_3), which were triplet peaks. The aromatic region was seen between 6.89-7.80 ppm. A sharp singlet peak seen at s 9.02-9.86 ppm (NH) indicated the thiazole amine proton. Broad acetamide protons were observed as a singlet at s 12.67-12.74 ppm (NH). The specific value based on ^{1}H and ^{13}C NMR shift of molecule **4b** has been illustrated (Figure 3).

To improve the experimental conditions for the preparation of molecule **4a**, several bases, such as anhydrous potassium carbonate and triethylamine were used in different solvents such as, methanol, ethanol, tetrahydrofuran, and acetonitrile. As a result, we found that the reaction of **3a** with ethyl 2-cyano-3,3-bis(methylthio)acrylate was faster and gave thiazole derivatives **4a** an excellent yield when potassium carbonate was used with DMF. The optimized reaction condition was used for the synthesis of all novel thiazoles **4a**. Moreover, the one-pot reaction of acetoacetanilide, *N*-bromosuccinimide, and thiourea followed by the addition of ethyl 2-cyano-3,3-bis(methylthio)acrylate was not successful, and the reaction did not yield the desired product.

Table 2. Physicochemical characteristics of the novel thiazole derivatives **4a-i**.



Entry	R^1	R^2	R^3	R^4	Molecular weight	Molecular formula	Yield (%)	Melting point (°C)
4a	OCH_3	H	H	H	432.51	$C_{19}H_{20}N_4O_4S_2$	82	129-131
4b	CH_3	H	H	H	416.51	$C_{19}H_{20}N_4O_3S_2$	89	150-152
4c	H	Cl	H	H	436.93	$C_{18}H_{17}ClN_4O_3S_2$	85	152-154
4d	H	H	H	H	402.49	$C_{18}H_{18}N_4O_3S_2$	82	127-129
4e	H	H	CH_3	CH_3	430.54	$C_{20}H_{22}N_4O_3S_2$	93	144-146
4f	F	H	H	H	420.48	$C_{18}H_{17}FN_4O_3S_2$	79	135-137
4g	Cl	H	H	H	436.93	$C_{18}H_{17}ClN_4O_3S_2$	86	152-154
4h	Br	H	H	H	481.38	$C_{18}H_{17}BrN_4O_3S_2$	91	172-174
4i	H	H	OCH_3	H	416.51	$C_{19}H_{20}N_4O_4S_2$	87	131-133

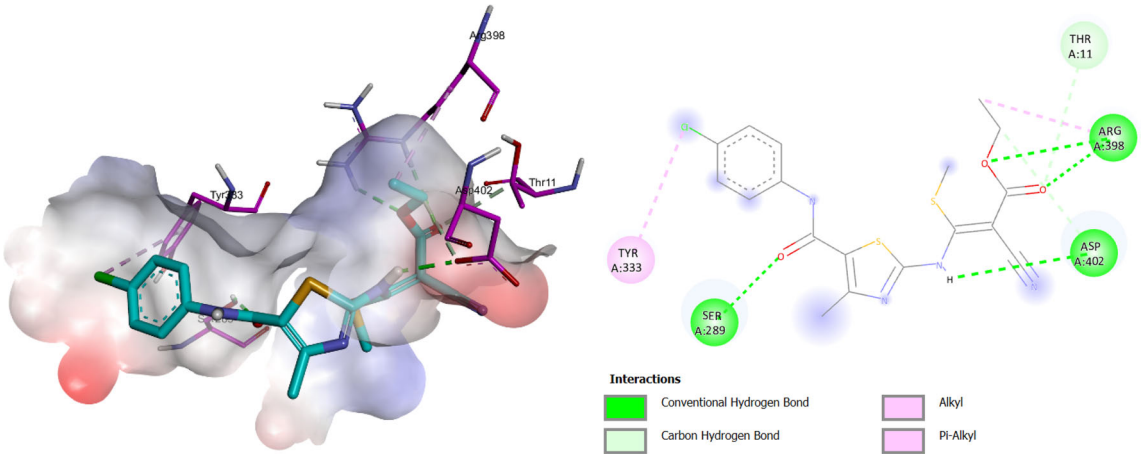


Figure 4. Docking pose of **4g** with human pancreatic α-amylase.

Firstly, the reaction conditions were kept neat with no use of solvent or catalyst at 80 °C, but no product formation was seen (Table 1, entry 1). Therefore, the following trial was carried out, taking water as a solvent and at room temperature without a base for 1 hour. Once more, product formation was none (entry 2), so with the addition of potassium carbonate, it was stirred for 1 h, but the result was again no product formation was seen (entry 3). Then, the reaction was carried out using triethylamine as the base and acetone as a solvent and stirred at rt for 1 h, resulting in the formation of 16% of the product (entry 4) and the formation of 19% of the product when potassium carbonate as a base (entry 5). The reaction condition was then modified with respect to the solvent using methanol and triethylamine as a base. This resulted in the product being obtained with a yield of 19% (Entry 6), and as a base, when potassium carbonate was used, the yield was 25% (entry 7). Further optimization of the reaction was done by using ethyl alcohol as a solvent and triethylamine as a base, the reaction mass was stirred at rt for 1 h, the desired product was obtained in 32% yield (entry 8), and 48% yield was obtained when potassium carbonate was used as a base (entry 9). Subsequently, the use of tetrahydrofuran as solvent and triethylamine as a base yielded a product of 29% (entry 10) while using potassium carbonate yielded a 35% yield was obtained (entry 11). Surprisingly, when using acetonitrile as a solvent and triethylamine as a base, the yield was 40% (entry 12)

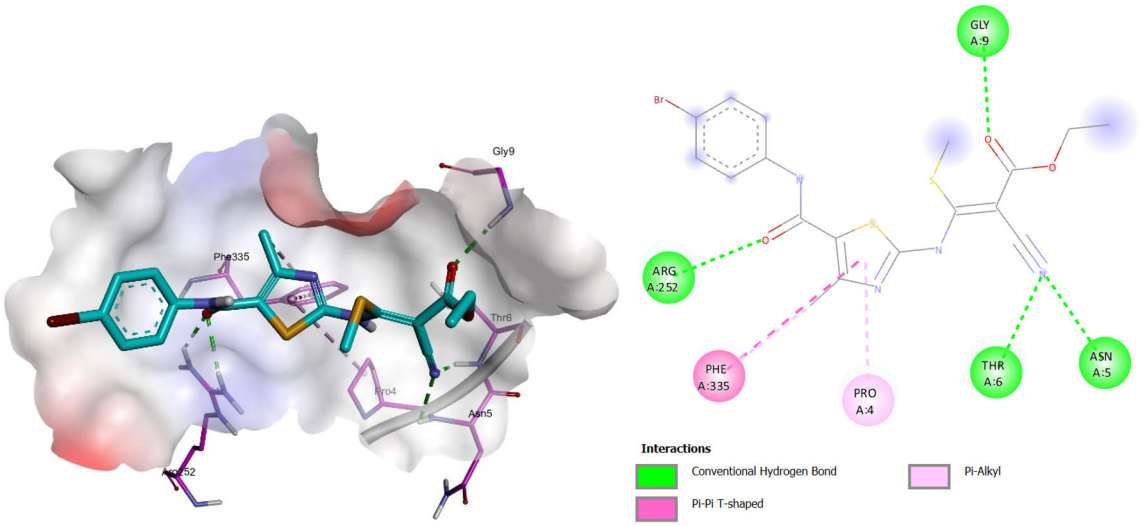


Figure 5. Docking pose of **4h** with human pancreatic α-amylase.

Table 3. *In vitro* α-amylase assay of thiazoles **4a-i**.

Compounds	R^1	R^2	R^3	R^4	% Inhibition				IC_{50} (μg/mL)
					12.5 (μg/mL)	25 (μg/mL)	50 (μg/mL)	100 (μg/mL)	
4a	OCH_3	H	H	H	27.43	36.62	60.56	86.13	32.97
4b	CH_3	H	H	H	35.12	44.87	85.21	88.61	22.20
4c	H	Cl	H	H	23.14	51.61	70.14	86.38	26.30
4d	H	H	H	H	19.40	53.36	59.19	75.44	31.94
4e	H	H	CH_3	CH_3	14.74	30.13	39.42	59.57	69.47
4f	F	H	H	H	28.47	40.28	51.31	80.42	36.11
4g	Cl	H	H	H	58.85	66.11	74.82	92.57	11.73
4h	Br	H	H	H	53.38	68.71	78.63	82.61	12.55
4i	H	H	OCH_3	H	25.21	41.10	56.80	82.55	33.99
Acarbose	-	-	-	-	28.43	49.22	79.33	90.42	23.62

and when potassium carbonate was used as a base, 57% yield (entry 13) was archived. When N, N-Dimethylformamide was used with triethylamine, this yielded a yield of 72% (entry 14), but using potassium carbonate as the base reaction mixture was stirred at rt for 30 min, and the resultant yield was 94% (entry 15). This variation led to no by-product formation and gave a high-purity yield. It was clearly observed that while using triethylamine yield was low compared to potassium carbonate. Solvents such as methanol and acetone reduced the yield of the product, respectively (Entry 2 and 3); keeping water as a solvent, the reaction did not proceed further, probably because of the minor solubility of reactants in water.

So, with the optimized reaction environments, the technique was used to produce novel ten thiazole **4a-i** molecules (Table 2).

Newly prepared thiazole molecules were also subjected to molecular docking using Autodock to discover the various binding poses with affinity.

3.2 *Molecular docking with α-amylase*

The *in vitro* outcomes revealed that prepared molecules **4a-i** displayed moderate to good inhibition towards α-amylase compared to acarbose, which showed binding energy of -6.2. The validation of the docking study involved redocking and superimposing the co-crystallized ligand with the extracted ligand from the crystal structure. The root mean square deviation (RMSD) was determined to be less than 1.00 Å, indicating the validity and reliability of the molecular docking procedure. Molecule **4g** and **4h**

were exposed to higher inhibition than other prepared molecules. So, molecule **4g** and **4h** were utilized to determine the binding position and interactions accountable for α-amylase (2QV4) activity. Molecule **4g,** with binding energy of -6.9 showed hydrogen bond interaction with Arg-398, Asp-402, and Ser-289 and pi-alkyl interactions with Tyr-333 and Arg-398, as displayed in Figure 4. Molecule **4h** with a binding energy of -6.6 showed four hydrogen bond with Arg-252, Thr-6, Gly-9, and Asn-5, one pi-pi interaction with Phe-335 and pi-alkyl contacts with Pro-4 as shown in Figure 5.

3.3 *In vitro α-amylase assay*

The synthesized Thiazole molecules **4a-i** were assessed for *in vitro* activity study against human pancreatic α-amylase enzyme by utilizing acarbose as a control following Mor's technique.[28] The outcomes of the α-amylase inhibitory study shown in Table 3 exposed that tested molecule **4a-i** showed moderate to high % inhibition. Molecule **4b**, **4f**, **4g** and **4h** at 12.5 μg/mL, **4c**, **4d**, **4g** and **4h** at 25 μg/mL, **4b** at a concentration 50 μg/mL, and **4g** at a concentration 100 μg/mL displayed more inhibition than the control drug acarbose at a concentration 100 μg/mL. Furthermore, the other molecules showed lesser inhibition compared to the control at various concentrations. Between the prepared molecules, **4b** (IC_{50}=22.20 μg/mL), **4g** (IC_{50}=11.73 μg/mL), and **4h** (IC_{50}=12.55 μg/mL) showed excellent inhibition as compared to the acarbose (IC_{50}=23.62 μg/mL) as a control (Table 3).

4. Conclusions

A novel series of thiazole **4a-i** derivatives bearing amide, nitrile, alkyl, and methylthio groups have been designed, synthesized, characterized, and evaluated for antidiabetic activity.

Among the synthesized compounds, few compounds exhibited notable α-amylase inhibitory activity. This study furthermore marks that molecule **4g** (IC_{50}=11.73 μg/mL) and **4h** (IC_{50}=12.55 μg/mL), having a halogen group on the aromatic rings feature a more powerful inhibitory activity against human pancreatic α-amylase compared to reference compound acarbose (IC_{50}=23.62 μg/mL). Molecular docking studies have shown insight into the binding manners of prepared molecules with the target enzyme. Hence, these thiazole molecules are esteemed nominees deserving of additional investigation for the upcoming development of potential antidiabetic molecules.

Supplementary Information (SI)

Figures S1-S20 are available at www.ias.ac.in/chemsci.

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