

Synthesis of Fluorinated Hydrazinylthiazole Derivatives: A Virtual and Experimental Approach to Diabetes Management

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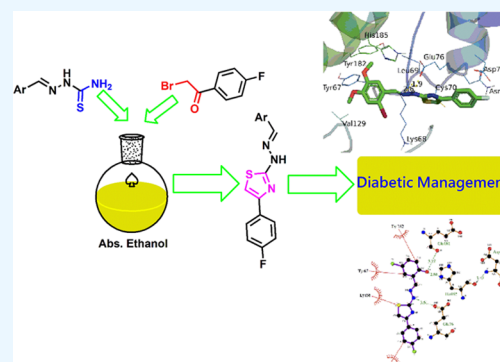


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ABSTRACT: A novel series of fluorophenyl-based thiazoles was synthesized following the Hantzsch method. All of the compounds were initially verified with physical parameters (color, melting point, retardation factor (R_f)), which were further confirmed by several spectroscopic methods, including ultraviolet–visible (UV–visible), Fourier-transform infrared (FTIR), ^1H , ^{13}C , ^{19}F NMR, and high-resolution mass spectrometry (HRMS). The binding interactions of all compounds were studied using a molecular docking simulation approach. Furthermore, each compound was evaluated for its alpha(α)-amylase, antiglycation, and antioxidant potentials. The biocompatibility of all compounds was checked with an *in vitro* hemolytic assay. All synthesized scaffolds were found biocompatible with minimal lysis of human erythrocytes as compared to the standard Triton X-100. Among the tested compounds, the analogue **3h** ($\text{IC}_{50} = 5.14 \pm 0.03 \mu\text{M}$) was found to be a highly potent candidate against α -amylase as compared to the standard (acarbose, $\text{IC}_{50} = 5.55 \pm 0.06 \mu\text{M}$). The compounds **3d**, **3f**, **3i**, and **3k** exhibited excellent antiglycation inhibition potential with their IC_{50} values far less than the standard amino guanidine ($\text{IC}_{50} = 0.403 \pm 0.001 \text{ mg/mL}$). The antidiabetic potential was further supported by docking studies. Docking studies revealed that all synthesized compounds exhibited various interactions along enzyme active sites (π – π , H-bonding, van der Waals) with varied binding energies.



INTRODUCTION

Halogen tagged compounds have diverse distributions in nature and have a wide range of biological activities.^{1–4} Besides natural distribution, synthetic halogen-substituted compounds hold a special place in medicinal chemistry. Their significance and regular use in medicinal chemistry is reinforced by the fact that, despite the COVID-19 pandemic, more than 20 drugs bearing halogen substituents have been approved by FDA for alternative clinical use (Figure 1).^{5,6}

Due to the distinctive stability characteristics of halogens, lead optimization for drug development, frequently incorporates halogens within their core structures. Fluorine and chlorine are the most common substituents in this regard, as indicated in Figure 1. Thus, halo-organic variations are key frontiers of medicinal chemistry.⁷ Their characteristic “halogen bonding”⁸ renders their multitude of organic, inorganic, and biological systems.^{9–12} The directional and strength comparability of halogen bonding with the hydrogen bond has attracted the widespread attention of medicinal chemists in medicinal chemistry.^{13,14}

Fluorinated compounds do not show similar electrostatic potential trends to the other halogens due to small size of the fluorine atom.^{12,15} Fluorine has weak ability to form halogen bonds, but its strong C–F bond facilitates tuning of the physicochemical properties and molecular conformations of

the drug leads.¹⁶ These properties include change in pK_a of neighboring functionalities,¹⁷ lipophilicity,¹⁸ enhanced membrane permeability,¹⁹ and reduced steric effect.²⁰ The importance of fluorine in medicinal chemistry is further supported by the fact that 13 new drugs containing fluorine were approved by the FDA in 2022.²¹ Some representative examples of these are presented in Figure 2.

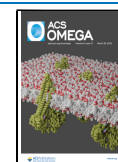
The biological applications of halogenated compounds are also enriched when thiazole and hydrazinyl functionalities are also incorporated into their structures. Halogens in combination with hydrazinyl and thiazole moieties possess versatile biological activities.^{22–28} Some important biological applications of halogenated hydrazinylthiazole compounds are shown in Figure 3.

Recently, Shahzadi et al. reported the antiglycation potential of alkyl-based hydrazinylthiazoles.²⁹ Compounds **I** and **II** (Figure 4) were highlighted as excellent antiglycating agents with IC_{50} values of 1.848 ± 0.646 and $0.0004 \pm 1.097 \mu\text{M}$,

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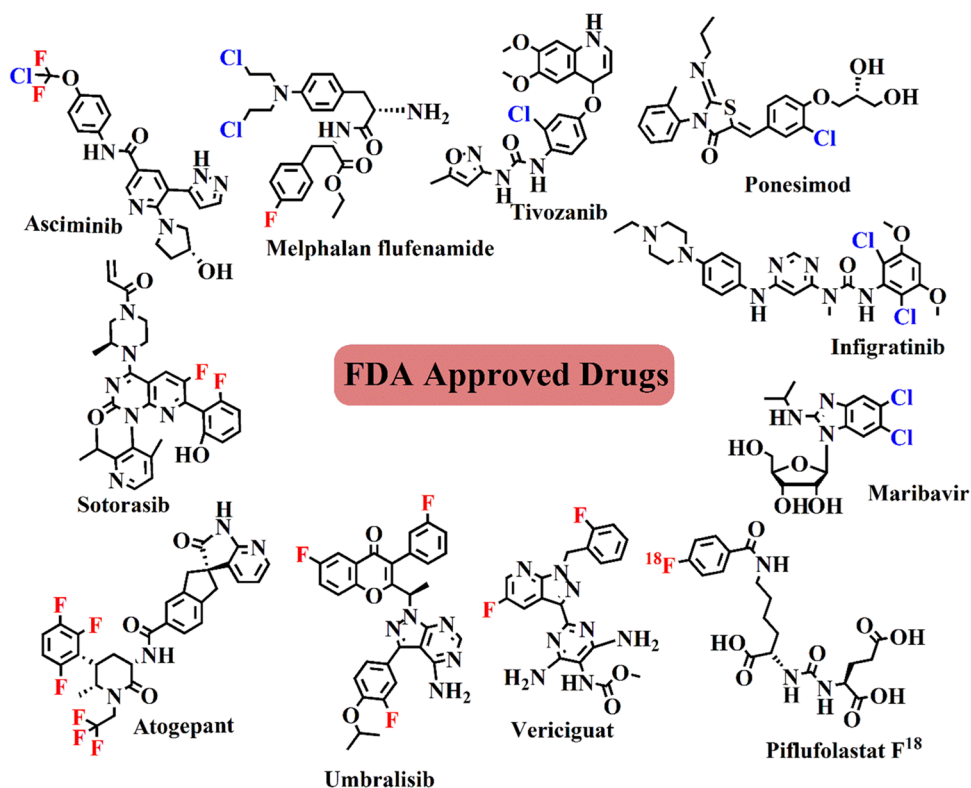


Figure 1. FDA-approved halogen-based drugs.

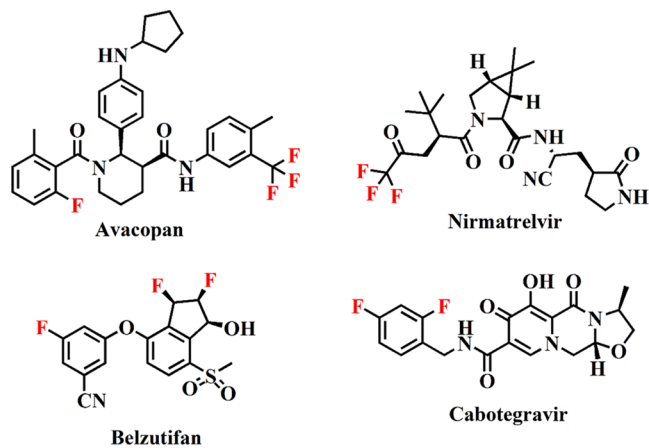


Figure 2. FDA-approved fluorine-containing drugs.

respectively (amino guanidine, $IC_{50} = 25.50 \pm 0.337 \mu M$). In addition, Hasnain et al. reported the syntheses of two series of hydrazinylthiazoles and evaluated their antiglycation and α -amylase inhibition potential.^{30,31} Compounds III and IV (Figure 4) were found to be potential α -amylase inhibitors with IC_{50} values of 4.80 ± 0.07 and $4.79 \pm 0.08 \mu M$, respectively (acarbose, $IC_{50} = 5.62 \pm 0.04 \mu M$). They also reported compound V as an excellent antiglycating agent with IC_{50} value of 0.383 ± 0.001 mg/mL as compared to the standard amino guanidine ($IC_{50} = 0.394 \pm 0.001$ mg/mL). The nonclassical bioisosterism, antidiabetic properties of halogenated compounds,^{26,32–36} and in continuation of our research to explore thiazoles in search of an excellent antidiabetic agent guided us in structural planning of the proposed study (Figure 4).

RESULTS AND DISCUSSION

To synthesize 2-(2-arylidenehydrazinyl)-4-(4-fluorophenyl)-thiazoles (3a–o), an equimolar mixture of the respective thiosemicarbazones^{30,31} (1) and 2-bromo-4-fluoroacetophenone (2) was condensed together under reflux in ethanol for 4–5 h, as shown in Scheme 1. Cyclized products were achieved in moderate to good yields (61–80%).

The formation of new compounds was clearly indicated by the change of physical parameters including color, melting points, and retardation factor (R_f) values. The structures of synthesized compounds were confirmed by spectroscopic studies (ultraviolet–visible (UV–visible), Fourier-transform infrared (FTIR), 1H , ^{13}C and ^{19}F NMR) and mass spectral data. In the IR spectra of the compounds, characteristic absorption bands in the region 3278 – 3138 cm^{-1} were seen due to N–H stretching. The absorption stretchings in the range 3151 – 2933 cm^{-1} were attributed to aliphatic C–H functionality. Azomethine (–CH=N–) linkage was indicated by the characteristic of C=N stretching in the range of 1699 – 1600 cm^{-1} . Aromatic C=C stretching vibrations were observed in the region 1571 – 1436 cm^{-1} . Characteristic thiazole vibrations were interpreted for the absorption bands in the region 1068 – 692 cm^{-1} .³⁷

In the 1H NMR spectra of the synthesized compounds, the N–H group appears in the range 11.26 – 12.50 ppm and one proton singlet at 7.85 – 8.43 ppm was ascribed to the azomethine protons. The thiazole proton present at position 5 of the ring was observed at 6.22 – 7.50 ppm. The signals of all other aromatic and aliphatic protons were observed in their expected regions with multiplicities corresponding to the required substitution pattern of the ring.

In the ^{19}F NMR (proton-decoupled), one or two peaks corresponding to respective number of fluorine atoms present

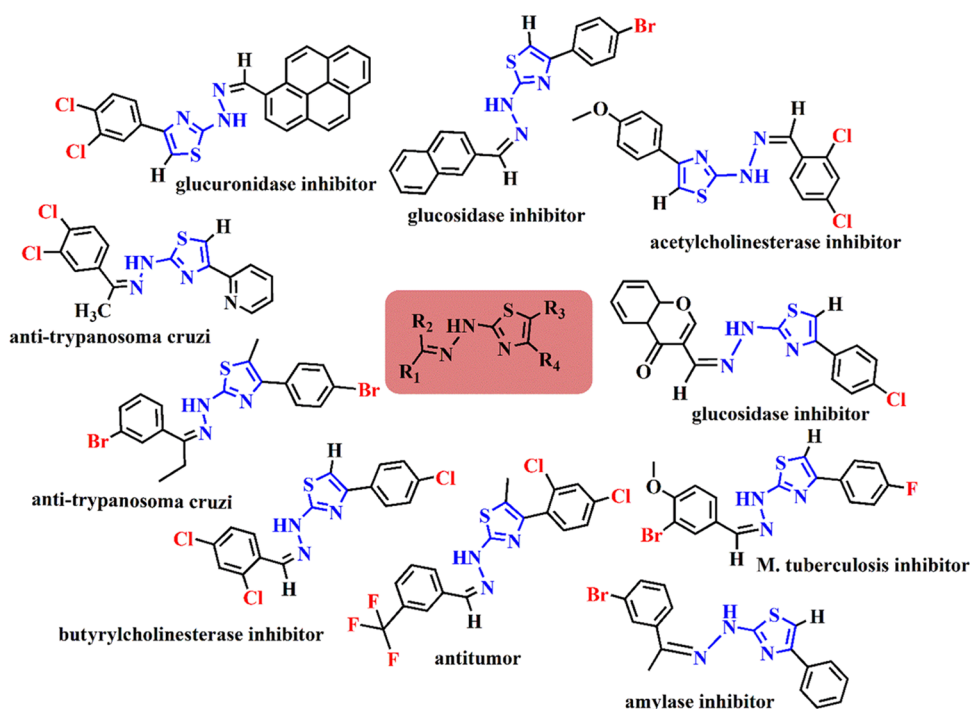


Figure 3. Biologically active halogenated hydrazinylthiazole-based scaffolds.

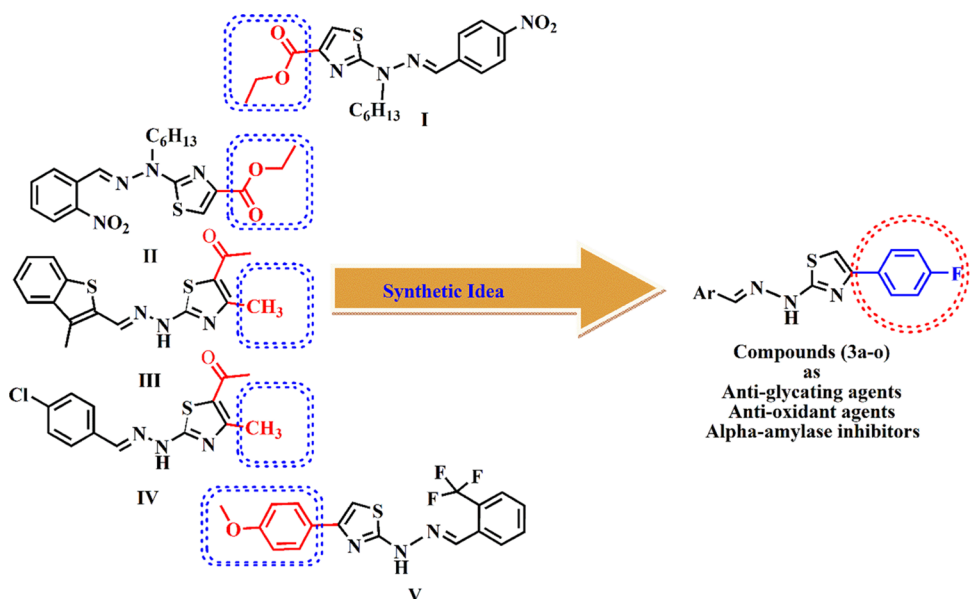


Figure 4. Synthetic inspiration for the targeted antidiabetic agents.

in the compounds were observed. For compounds **3d**, **3g**, **3i**, **3k**, and **3l**, two signals were observed, indicating two fluorine atoms present in the structure, while the remaining monofluorinated compounds gave a single signal in the region -114.49 to -114.77 ppm.

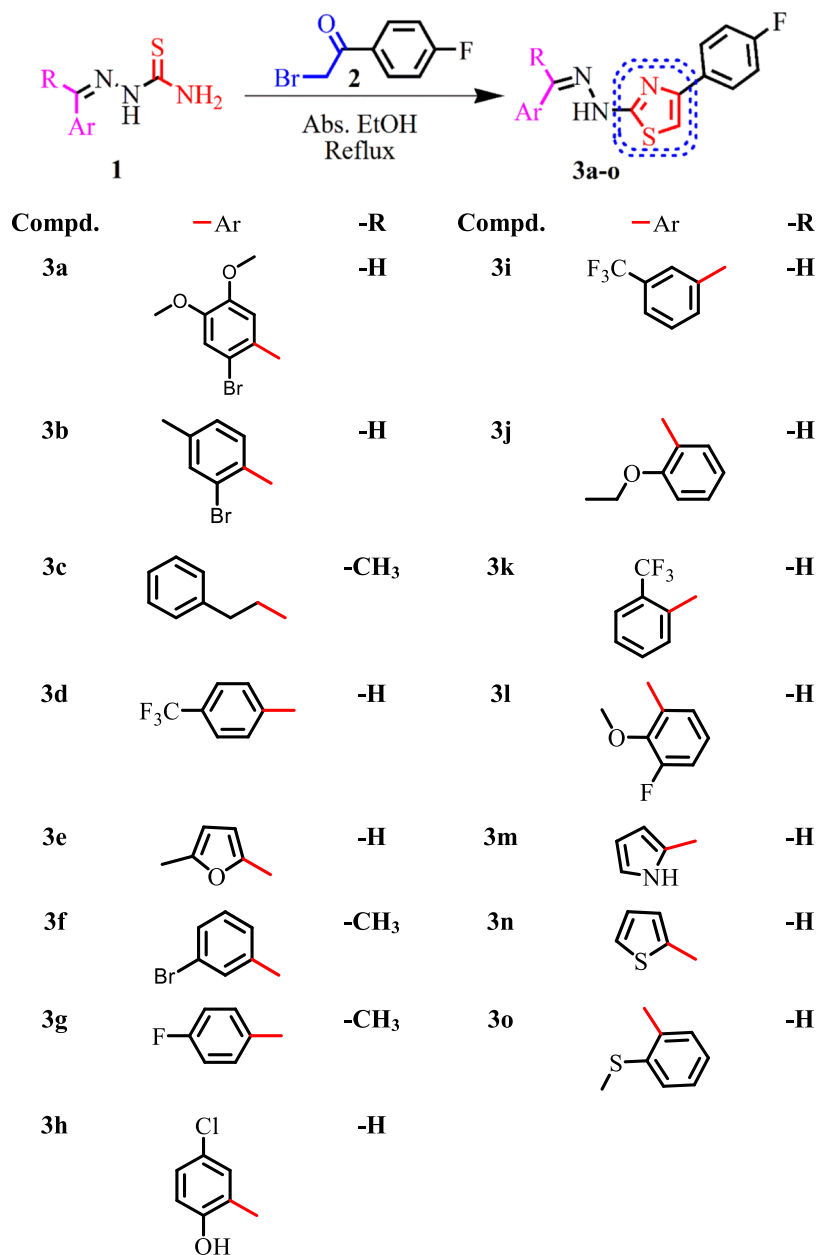
In the ^{13}C NMR spectra, signals of carbons at chemical shift values 168.3 – 170.6 , 148.8 – 160.9 , and 101.8 – 104.5 ppm were assigned to C2, C4, and C5 of the thiazole ring, respectively. The carbon of the azomethine linkage was indicated by a signal in the region 135.5 – 148.3 ppm. A characteristic doublet was observed for ipso carbons bonded with fluorine in the region 162.0 – 164.7 ppm with the coupling constant in the range of 244.3 – 249.4 ($^1J_{\text{CF}}$) Hz. Smaller doublets at 115.9 – 116.1 ppm

were observed with the coupling constant $^2J_{\text{CF}} = 21.6$ Hz. All other aromatic and aliphatic carbons were observed in their respective regions. The syntheses were further corroborated by high-resolution mass spectrometry (HRMS), where the calculated masses of synthesized compounds showed good agreement with the observed masses.

The synthesized compounds were assessed for their biological significance by evaluating the α -amylase, antiglycation, and antioxidant inhibition potentials. The cytotoxicity of all compounds was evaluated by *in vitro* hemolytic activity.

Biological Screening of 2-(2-Arylidenehydrazinyl)-4-(4-fluorophenyl)thiazoles (3a–o) α -Amylase Inhibition Activity. The compounds (**3a–o**) were evaluated for their

Scheme 1. : Synthesis of 2-(2-Arylidenehydrazinyl)-4-(4-fluorophenyl)thiazoles (3a–o)



enzyme inhibition potential against α -amylase, and the results are presented in Table 1. Acarbose was used as the standard (reference inhibitor) with an IC_{50} value of $5.55 \pm 0.06 \mu M$. All tested compounds exhibited enzyme inhibition potency from moderate to high with an effect of concentration. The IC_{50} values revealed that their inhibition potential is dose-dependent. The compound **3h** (5-chloro-2-hydroxy) was found to be a more potent ($IC_{50} = 5.14 \pm 0.03 \mu M$) α -amylase inhibitor when compared with the standard. Besides **3h**, the compound **3n** (thiophen-2-yl, $IC_{50} = 5.77 \pm 0.05 \mu M$) showed good enzyme inhibition compared to the standard.

Similarly, the IC_{50} values of compounds **3b** (2-bromo-4-methyl, $IC_{50} = 6.87 \pm 0.01 \mu M$) and **3f** (3-bromo, $IC_{50} = 5.88 \pm 0.16 \mu M$) indicated that they also have almost comparable enzyme inhibition potential to the standard. The structure–activity relationship (SAR) studies revealed that the compound **3h**, containing the $-OH$ group, enhances the ability of the compound to form strong hydrogen bonding with the enzyme.

The reason for exhibiting the comparable inhibition potential may be because of having the same substituent at different positions, which may be related to compounds **3d**, **3i**, and **3k** showing a similar behavior.

Antiglycation Activity. The synthesized compounds (**3a–o**) were further evaluated for their antiglycation potential using amino guanidine as the reference inhibitor ($IC_{50} = 0.403 \pm 0.001$ mg/mL) as shown in Table 2. All compounds possess good to excellent antiglycation potential, with IC_{50} values ranging from 0.393 ± 0.002 to 0.584 ± 0.006 mg/mL.

An initial SAR was established for targeted compounds, which indicated that the inhibition potential depends upon the nature of the substituent and its position on the aromatic ring. Compound **3i** exhibited the highest inhibition activity ($IC_{50} = 0.393 \pm 0.002$ mg/mL) having the trifluoromethyl group at position 3. Compounds **3d** and **3k** were also found to be more potent than the standard having IC_{50} values of 0.394 ± 0.003 and 0.396 ± 0.002 mg/mL, respectively. The comparable

Table 1. Percentage α -Amylase Inhibition along with IC₅₀ Values of Compounds 3a–o

compd.	% age α -amylase inhibition			IC ₅₀ \pm SEM (μ M)
	1 μ M	5 μ M	10 μ M	
3a	35.96	51.11	69.11	6.41 \pm 0.09
3b	31.95	51.54	62.71	6.87 \pm 0.01
3c	30.03	45.41	58.12	7.52 \pm 0.05
3d	36.97	49.93	62.35	6.92 \pm 0.12
3e	30.06	41.20	57.45	7.77 \pm 0.01
3f	34.92	51.68	62.28	6.88 \pm 0.16
3g	38.00	49.56	62.75	6.90 \pm 0.23
3h	55.89	69.05	82.47	5.14 \pm 0.03
3i	38.90	51.09	64.58	6.70 \pm 0.08
3j	29.58	42.23	54.03	8.07 \pm 0.12
3k	47.84	57.72	70.90	6.03 \pm 0.04
3l	37.06	49.08	61.72	7.00 \pm 0.13
3m	42.76	52.79	63.94	6.66 \pm 0.13
3n	51.64	64.48	71.83	5.77 \pm 0.05
3o	33.07	43.03	57.18	7.69 \pm 0.18
acarbose	53.42	66.81	74.77	5.55 \pm 0.06

antiglycation potential of compounds 3d, 3i, and 3k is ascribed to the presence of the same substituent (trifluoromethyl) at positions 4, 3, and 2, respectively. The compound 3f bearing the bromine atom at position 3 also exhibited more antiglycation potential (IC₅₀ = 0.399 \pm 0.002 mg/mL), compared to the standard. The IC₅₀ values of all other tested compounds indicated that the inhibitory potential depends upon the substituent on the aromatic ring. The results also revealed that the percentage antiglycation is also dose-dependent; with an increase in concentration of the tested sample, the antiglycation activity increases.

SAR studies also revealed that the antiglycation activity is affected by the nature of groups present around the thiazole ring. While comparing our current results with previous studies, it may be concluded that the presence of 4-fluorophenyl functionality around the thiazole ring enhanced the glycation inhibition potential of the compounds. The argument may be strengthened by the current results, where

3k, 3i, and 3d showed enhanced antiglycation activity as compared to the standard (Figure 5).^{30,31}

Free Radical Scavenging Ability (Antioxidant Activity). The compounds 3a–o were screened for their DPPH free radical scavenging ability, and the results are presented in Table 3. Most compounds showed fairly good antioxidant potential but less active than the standard (IC₅₀ = 7.18 \pm 0.01 mM). The compound 3j, bearing the ethoxy group at position 2 of the arylidene ring, was found to be the most active in this series with an IC₅₀ value of 8.33 \pm 0.08 mM, almost comparable to that of the standard. The increased percentage antioxidant potential of the other compounds is as follows: 3o < 3n < 3l < 3a < 3c < 3b < 3d. The percentage antioxidant potential of the other compounds 3e, 3f, 3g, 3h, 3i, 3k, and 3m was less than 50%, so their IC₅₀ values were not calculated. The results revealed that the percentage antioxidant potential is also dose-dependent. Most of the tested compounds at their lower concentration did not show significant activity but were found to be active when the concentration increases.

In Vitro Hemolytic Activity. The compounds 3a–o were screened for their cytotoxicity behavior through a hemolytic assay, and the results are presented in Table 4. The compounds 3b, 3c, 3f, 3j, and 3l were found safe at their minimum treated concentration (10 μ M), while all other compounds were found to be fatal to red blood cells and caused lysis of RBCs at all tested concentrations. The percentage hemolysis is also found to be dose-dependent; lysis increases when the concentration of the sample increases.

Molecular Docking. The compounds 3a–o were virtually screened against two proteins, *i.e.*, human serum albumin (HSA) and human pancreatic α -amylase (HPA), by molecular docking. HSA is a carrier protein that also serves as a marker for uncontrolled diabetes. This protein becomes heavily glycosylated due to long-term elevated glucose levels.³⁸ The protein glycation may contribute to oxidative stress and inflammation. Here, we have performed antiglycation studies for the synthesized ligands (3a–o) using the HSA three-dimensional structure as a target protein. HAS has multiple glycation sites (F1–9) in its three domains.^{39,40} Sudlow site II is a fatty acid binding site mainly consisting of Leu¹¹⁵, Pro¹¹⁸, Met¹²³, Ala¹²⁶, Phe¹³⁴, Lys¹³⁷, Tyr¹³⁸, Glu¹⁴¹, Ile¹⁴², His¹⁴⁶,

Table 2. Percentage Antiglycation Inhibition along with IC₅₀ Values of Compounds 3a–o

compd.	% age antiglycation inhibition						IC ₅₀ (mg \pm SEM)
	100 ppm	200 ppm	400 ppm	600 ppm	800 ppm	1000 ppm	
3a	80.912	82.097	84.808	85.864	86.983	89.880	0.410 \pm 0.003
3b	81.189	84.602	85.170	86.200	86.768	87.462	0.413 \pm 0.002
3c	67.583	68.855	69.669	75.722	77.932	81.626	0.464 \pm 0.002
3d	87.417	87.923	89.045	89.475	90.859	92.275	0.394 \pm 0.003
3e	68.735	69.659	70.703	71.831	72.794	73.677	0.493 \pm 0.003
3f	84.146	86.734	87.272	87.994	90.418	91.338	0.399 \pm 0.002
3g	24.260	34.557	48.804	54.331	66.643	74.646	0.584 \pm 0.006
3h	50.878	56.497	65.488	68.424	68.964	70.744	0.528 \pm 0.002
3i	87.964	88.535	89.110	89.990	91.164	92.228	0.393 \pm 0.002
3j	52.257	61.537	66.470	70.267	76.184	79.161	0.488 \pm 0.003
3k	87.393	87.943	88.776	89.221	89.978	91.580	0.396 \pm 0.002
3l	79.853	81.769	83.050	84.648	86.160	87.099	0.418 \pm 0.005
3m	76.769	78.789	82.499	83.963	86.080	88.774	0.418 \pm 0.002
3n	79.297	80.987	82.086	83.014	85.776	87.827	0.420 \pm 0.003
3o	83.693	84.223	85.521	86.434	88.18	89.496	0.407 \pm 0.002
amino guanidine	85.190	86.141	87.143	88.011	88.866	89.657	0.403 \pm 0.001

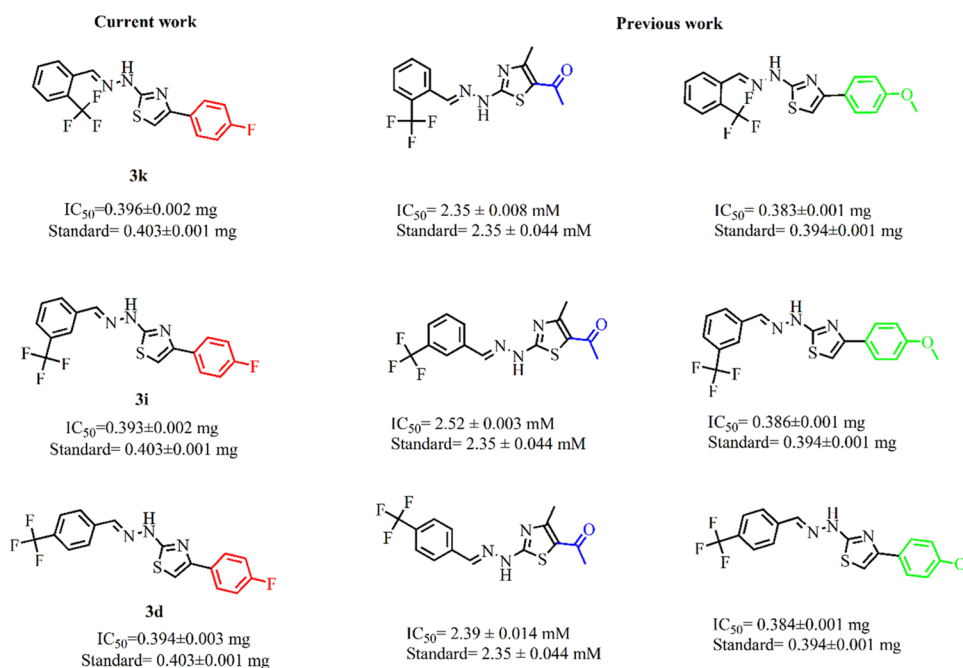


Figure 5. Antiglycation activity: structural comparison of our current work with previously reported work.

Table 3. Percentage Antioxidant Potential along with IC_{50} Values of Compounds 3a–o^a

compd.	% age antioxidant potential						IC_{50} (mM \pm SEM)
	3 mM	6 mM	9 mM	12 mM	15 mM		
3a	0.00	18.82	32.72	47.58	67.95	12.39 \pm 0.10	
3b	8.77	23.69	36.38	53.65	69.82	11.32 \pm 0.14	
3c	0.00	11.13	34.28	57.51	73.34	11.43 \pm 0.12	
3d	23.48	34.45	45.32	56.39	63.78	10.68 \pm 0.08	
3e	0.00	8.26	19.32	44.74	49.04		
3f	0.00	0.00	19.48	28.70	40.73		
3g	0.00	0.00	0.00	0.00	8.45		
3h	0.00	0.00	0.00	8.78	23.25		
3i	0.00	8.64	18.87	32.26	46.53		
3j	18.18	45.90	62.07	75.45	78.51	8.33 \pm 0.08	
3k	0.00	14.08	21.05	30.92	48.28		
3l	0.00	15.62	32.06	46.14	65.18	12.94 \pm 0.01	
3m	0.00	0.00	0.00	15.13	31.05		
3n	0.00	12.72	30.39	45.47	59.36	13.86 \pm 0.04	
3o	0.00	7.68	18.22	30.15	53.73	17.97 \pm 0.27	
ascorbic acid	52.34	61.01	74.41	81.85	84.92	7.18 \pm 0.01	

^a IC_{50} values: Concentration of the sample (mM) at which DPPH is scavenged by 50%.

Phe¹⁴⁹, Leu¹⁵⁴, Phe¹⁵⁷, Tyr¹⁶¹, Leu¹⁸⁵, Arg¹⁸⁶, Gly¹⁸⁹, Lys¹⁹⁰, and Ser¹⁹³ amino acids. The ligand 3a shows binding to the same Sudlow site II with a binding energy of -9.54 kcal/mol and a binding constant of 102 nM (Table 5). In the same site, two lysines and one histidine amino acid, i.e., Lys¹³⁷, His¹⁴⁶, and Lys¹⁹⁰, constitute a major glycation site (FA1) in domain IB.⁴¹ The ligands 3(d, f, i, and k) share the same binding site involving Lys¹³⁷, Leu¹¹⁵, Arg¹¹⁷, Met¹²³, Ala¹²⁶, Phe¹²⁷, Asp¹²⁹, Asn¹³⁰, Thr¹³³, Phe¹³⁴, Lys¹³⁷, Tyr¹³⁸, Glu¹⁴¹, Ile¹⁴², Tyr¹⁶¹, Phe¹⁶⁵, and Leu¹⁸² residues (Figure 6). These ligands form π - π stacking interactions with Tyr¹³⁸ and show interactions with Lys¹³⁷, one of the main residues that undergo glycation.⁴² Tyr¹³⁸ and Tyr¹⁶¹ have previously been reported to interact with macrocycle rings of various drugs by π - π stacking,⁴³ while Tyr¹⁶¹ and His¹⁴⁶ are involved in the heme-Fe atom coordination; any ligand binding to these sites may disrupt

heme-Fe interactions.⁴¹ Among these ligands, 3f shows a binding energy of -9.22 kcal/mol and a dissociation constant of 173 nM (Table 5). Although 3h shows a binding energy of -9.07 kcal/mol, it binds to a shared region of Sudlow sites I and II comprising residues, i.e., Ala¹⁹¹, Lys¹⁹⁵, Leu¹⁹⁸, Lys¹⁹⁹, Ser²⁰², Ala²¹⁰, Phe²¹¹, Trp²¹⁴, Lys⁴³⁶, Pro⁴⁴⁷, Cys⁴⁴⁸, Ala⁴⁴⁹, Asp⁴⁵¹, Tyr⁴⁵², Val⁴⁵⁵, and Leu⁴⁸¹. This binding site includes a part of Sudlow site I, where Lys¹⁹⁹ is responsible for 5% of the total glycation.^{44,45} *In vitro* results also corroborate the best IC_{50} obtained with the same ligands exhibiting the highest binding energy in the *in silico* studies. Previously, triazole-based Schiff bases and thiazole-based thiosemicarbazones had shown antiglycation activities by *in silico* studies.⁴⁶

Human pancreatic α -amylase (HPA) has multiple ligand binding sites in its three-domain structure.^{47,48} Ligands 3a–o

Table 4. Percentage Hemolysis of Compounds 3a–o

compd.	% hemolysis		
	10 μM	50 μM	100 μM
3a	3.06	15.10	32.23
3b	0.00	7.90	31.56
3c	0.00	8.38	32.09
3d	16.05	25.71	40.97
3e	1.20	11.50	40.38
3f	0.00	15.57	40.19
3g	1.98	26.35	52.27
3h	3.11	21.17	52.50
3i	14.70	30.72	44.54
3j	0.00	16.83	32.47
3k	4.43	27.62	45.69
3l	0.00	19.99	36.64
3m	2.58	26.29	46.22
3n	11.42	32.43	61.41
3o	1.99	21.55	49.12
triton X-100	100	100	100

have been found to interact in four of these sites, with the binding energies ranging from -8.78 to -7.22 kcal/mol (Table 6). In HPA, Trp,⁵⁹ Asp¹⁹⁷, and Glu²³³ are the main catalytic site amino acids that interact with α -amylase inhibitors via π - π stacking, van der Waals, and H-bonding interactions, respectively. Many natural and synthetic compounds have been found to interact with active site residues (Trp⁵⁹, Asp¹⁹⁷, and Glu²³³) in HPA.^{49–51} In our study, most of the ligands, i.e., 3(a, c, e, i–o), were found to interact with these active site residues (Figure 7). The ligand 3a was found to be a better α -amylase inhibitor. It interacts with all three domains of HPA while binding at four different sites (Figure 7). The ligand 3a shows the best binding energy (-8.59 kcal/mol) with a dissociation constant of ~ 506 nM when interacting with the C-terminal domain (Domain III) and -7.94 kcal/mol of binding energy with 1.5 μM dissociation constant when binding to the

main active site of the enzyme (Table 6). The active site residues include Trp⁵⁸, Trp⁵⁹, Tyr⁶², Gln⁶³, His¹⁰¹, Gly¹⁰⁴, Asn¹⁰⁵, Ala¹⁰⁶, Val¹⁰⁷, Leu¹⁶², Thr¹⁶³, Gly¹⁶⁴, Leu¹⁶⁵, Arg¹⁹⁵, Asp¹⁹⁷, Ala¹⁹⁸, Glu²³³, His²⁹⁹, and Asp³⁰⁰ (Figure 7). Here, Ala,^{105,106} Gln⁶³, and Arg¹⁹⁵ are found to interact via H-bonding. *In silico* findings for ligand 3n agree with the *in vitro* results. It interacts proximate to the active site residues with a binding energy of -8.31 kcal/mol and a dissociation constant of 8.1 μM (Table 6). The ligand 3h, which has been found to be the best α -amylase inhibitor in experimental IC₅₀, interacts proximate to N- and C-terminal domains and does not directly disrupt the enzyme active site (Figure 7). The ligand 3k interacts with active site residues (Trp⁵⁹, Glu⁶⁰, Tyr⁶², Gln⁶³, His¹⁰¹, Tyr¹⁵¹, Leu¹⁶², Thr¹⁶³, Leu¹⁶⁵, Asp¹⁹⁷, Ala¹⁹⁸, Ser¹⁹⁹, Lys²⁰⁰, His²⁰¹, Glu²³³, Val²³⁴, Ile²³⁵) as well as domain III (C-terminal) of the enzyme with binding energies of -7.76 and -7.71 kcal/mol with dissociation constants of 2.06 and 2.23 μM (Table 6). The ligand 3k also showed an IC₅₀ of 6.03 ± 0.04 μM in *in vitro* amylase inhibition (Table 1). Previously, some α -amylase inhibitors were found to form hydrogen bonds with Ile²³⁵ and Glu²³³ and charge- π interactions with His¹⁵¹. In our findings, His¹⁰¹ and His²⁰¹ have been found to interact with 3a–o ligands via charge- π and π - π interactions.

CONCLUSIONS

The current study was carried out for the synthesis of fluorophenyl-based hydrazinylthiazole derivatives. 15 compounds were synthesized through a two-step synthetic protocol, and the majority of the compounds were obtained in good yields. The verification of formation and confirmation of the proposed skeleton of all synthesized scaffolds was achieved with spectroscopic techniques (UV-visible, FTIR, ¹H, ¹³C, ¹⁹F NMR, HRMS). Biological screening of all synthesized compounds was carried out against α -amylase, glycation, and oxidation processes. The synthesized compounds showed comparable with standard to excellent activities. The compound 3h was found to be an excellent α -

Table 5. *In Silico* Antiglycation Activity with Binding Energy and Dissociation Constant of All Compounds 3a–o

ligands	binding energy (kcal/mol)	dissociation constant (nM)	active site
3a	-9.54	102.28	Leu ¹¹⁵ , Pro ¹¹⁸ , Met ¹²³ , Ala ¹²⁶ , Phe ¹³⁴ , Lys ¹³⁷ , Tyr ¹³⁸ , Glu ¹⁴¹ , Ile ¹⁴² , His ¹⁴⁶ , Phe ¹⁴⁹ , Leu ¹⁵⁴ , Phe ¹⁵⁷ , Tyr ¹⁶¹ , Leu ¹⁸⁵ , Arg ¹⁸⁶ , Gly ¹⁸⁹ , Lys ¹⁹⁰ , Ser ¹⁹³ .
3b	-8.64	465.05	Leu ¹¹⁵ , Met ¹²³ , Ala ¹²⁶ , Phe ¹²⁷ , Asp ¹²⁹ , Asn ¹³⁰ , Thr ¹³³ , Phe ¹³⁴ , Lys ¹³⁷ , Tyr ¹³⁸ , Glu ¹⁴¹ , Ile ¹⁴² , Tyr ¹⁶¹ .
3c	-9.09	216.92	Leu ¹¹⁵ , Val ¹¹⁶ , Arg ¹¹⁷ , Pro ¹¹⁸ , Met ¹²³ , Phe ¹³⁴ , Lys ¹³⁷ , Tyr ¹³⁸ , Glu ¹⁴¹ , Ile ¹⁴² , His ¹⁴⁶ , Phe ¹⁴⁹ , Phe ¹⁵⁷ , Tyr ¹⁶¹ , Phe ¹⁶⁵ , Leu ¹⁸² , Leu ¹⁸⁵ , Arg ¹⁸⁶ , Gly ¹⁸⁹ , Lys ¹⁹⁰ .
3d	-8.53	557.19	Leu ¹¹⁵ , Met ¹²³ , Ala ¹²⁶ , Phe ¹²⁷ , Asp ¹²⁹ , Asn ¹³⁰ , Glu ¹³¹ , Thr ¹³³ , Phe ¹³⁴ , Lys ¹³⁷ , Tyr ¹³⁸ , Glu ¹⁴¹ , Ile ¹⁴² , Tyr ¹⁶¹ .
3e	-8.64	462.69	Leu ¹¹⁵ , Met ¹²³ , Ala ¹²⁶ , Phe ¹²⁷ , Asp ¹²⁹ , Asn ¹³⁰ , Glu ¹³¹ , Thr ¹³³ , Phe ¹³⁴ , Lys ¹³⁷ , Tyr ¹³⁸ , Glu ¹⁴¹ , Ile ¹⁴² , Tyr ¹⁶¹ .
3f	-9.22	173.72	Leu ¹¹⁵ , Pro ¹¹⁸ , Met ¹²³ , Phe ¹³⁴ , Lys ¹³⁷ , Tyr ¹³⁸ , Glu ¹⁴¹ , Ile ¹⁴² , His ¹⁴⁶ , Tyr ¹⁶¹ , Leu ¹⁸² , Leu ¹⁸⁵ , Arg ¹⁸⁶ , Gly ¹⁸⁹ , Lys ¹⁹⁰ .
3g	-8.7	421.72	Leu ¹¹⁵ , Pro ¹¹⁸ , Met ¹²³ , Phe ¹³⁴ , Lys ¹³⁷ , Tyr ¹³⁸ , Glu ¹⁴¹ , Ile ¹⁴² , His ¹⁴⁶ , Phe ¹⁴⁹ , Tyr ¹⁶¹ , Leu ¹⁸² , Leu ¹⁸⁵ , Arg ¹⁸⁶ , Gly ¹⁸⁹ , Lys ¹⁹⁰ .
3h	-9.07	223.24	Ala ¹⁹¹ , Lys ¹⁹⁵ , Leu ¹⁹⁸ , Lys ¹⁹⁹ , Ser ²⁰² , Ala ²¹⁰ , Phe ²¹¹ , Trp ²¹⁴ , Lys ⁴³⁶ , Pro ⁴⁴⁷ , Cys ⁴⁴⁸ , Ala ⁴⁴⁹ , Asp ⁴⁵¹ , Tyr ⁴⁵² , Val ⁴⁵⁵ , Leu ⁴⁸¹ .
3i	-8.65	457.99	Leu ¹¹⁵ , Arg ¹¹⁷ , Met ¹²³ , Ala ¹²⁶ , Phe ¹²⁷ , Asp ¹²⁹ , Asn ¹³⁰ , Thr ¹³³ , Phe ¹³⁴ , Lys ¹³⁷ , Tyr ¹³⁸ , Glu ¹⁴¹ , Ile ¹⁴² , Tyr ¹⁶¹ , Leu ¹⁸² .
3j	-8.62	484.08	Leu ¹¹⁵ , Arg ¹¹⁷ , Met ¹²³ , Ala ¹²⁶ , Phe ¹²⁷ , Asp ¹²⁹ , Asn ¹³⁰ , Thr ¹³³ , Phe ¹³⁴ , Lys ¹³⁷ , Tyr ¹³⁸ , Glu ¹⁴¹ , Ile ¹⁴² , Tyr ¹⁶¹ , Phe ¹⁶⁵ , Leu ¹⁸² .
3k	-8.53	560.99	Leu ¹¹⁵ , Arg ¹¹⁷ , Met ¹²³ , Ala ¹²⁶ , Phe ¹²⁷ , Asp ¹²⁹ , Asn ¹³⁰ , Thr ¹³³ , Phe ¹³⁴ , Lys ¹³⁷ , Tyr ¹³⁸ , Glu ¹⁴¹ , Ile ¹⁴² , Tyr ¹⁶¹ , Phe ¹⁶⁵ , Leu ¹⁸² .
3l	-9.1	212.44	Leu ¹¹⁵ , Arg ¹¹⁷ , Met ¹²³ , Lys ¹³⁷ , Tyr ¹³⁸ , Glu ¹⁴¹ , Ile ¹⁴² , His ¹⁴⁶ , Phe ¹⁴⁹ , Leu ¹⁵⁴ , Phe ¹⁵⁷ , Tyr ¹⁶¹ , Phe ¹⁶⁵ , Leu ¹⁸² , Leu ¹⁸⁵ , Arg ¹⁸⁶ , Gly ¹⁸⁹ , Lys ¹⁹⁰ , Ser ¹⁹³ .
3m	-8.33	786.45	Leu ¹¹⁵ , Met ¹²³ , Ala ¹²⁶ , Phe ¹²⁷ , Asp ¹²⁹ , Asn ¹³⁰ , Glu ¹³¹ , Thr ¹³³ , Phe ¹³⁴ , Lys ¹³⁷ , Tyr ¹³⁸ , Glu ¹⁴¹ , Ile ¹⁴² , Tyr ¹⁶¹ .
3n	-8.19	1000.00	Leu ¹¹⁵ , Met ¹²³ , Ala ¹²⁶ , Phe ¹²⁷ , Asp ¹²⁹ , Asn ¹³⁰ , Thr ¹³³ , Phe ¹³⁴ , Lys ¹³⁷ , Tyr ¹³⁸ , Glu ¹⁴¹ , Ile ¹⁴² , Tyr ¹⁶¹ .
3o	-8.51	578.33	Leu ¹¹⁵ , Arg ¹¹⁷ , Met ¹²³ , Ala ¹²⁶ , Phe ¹²⁷ , Asp ¹²⁹ , Asn ¹³⁰ , Thr ¹³³ , Phe ¹³⁴ , Lys ¹³⁷ , Tyr ¹³⁸ , Glu ¹⁴¹ , Ile ¹⁴² , Tyr ¹⁶¹ , Phe ¹⁶⁵ , Leu ¹⁸² .

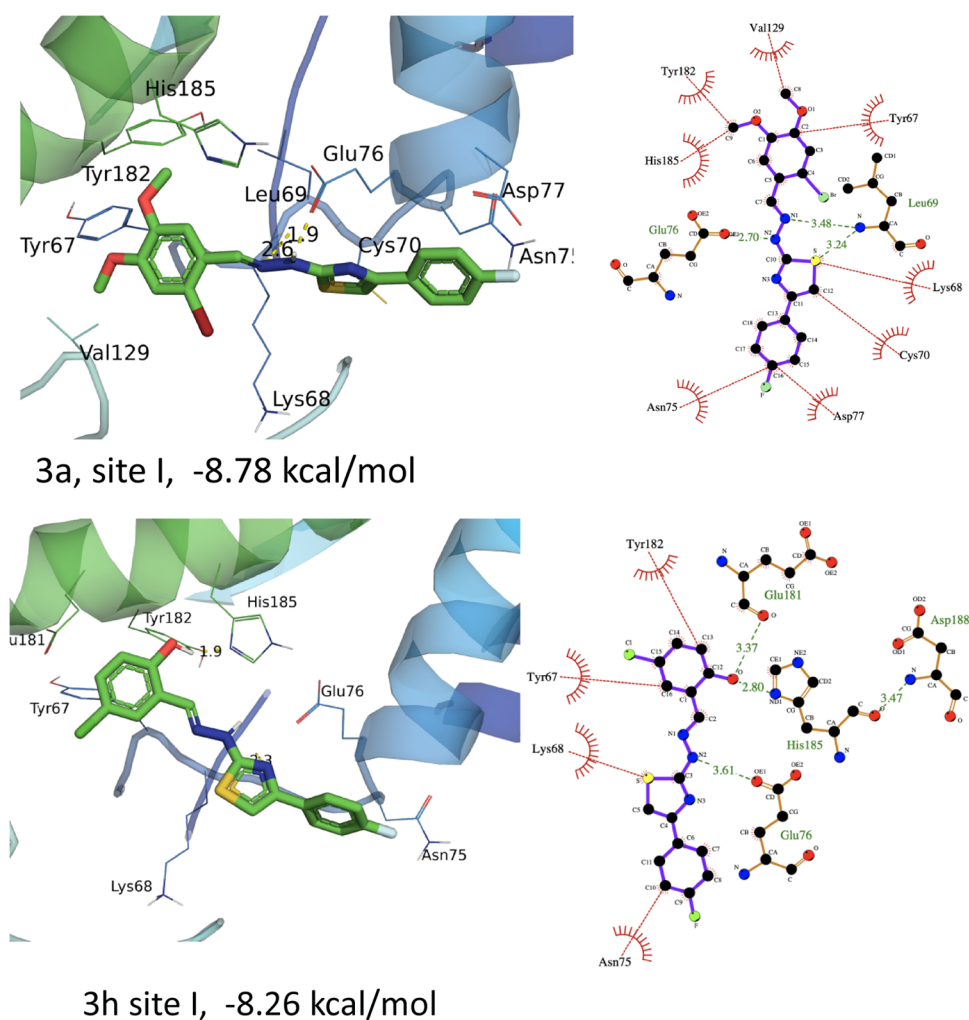


Figure 6. Best docking interactions are shown by ligands **3a** and **3h** with binding energies of -8.78 and -8.26 kJ/mol against human pancreatic α -amylase (HPA).

amylase inhibitor. The compounds **3d**, **3f**, **3i**, and **3k** showed the highest potency for the glycation inhibition process. All synthesized scaffolds were found biocompatible in nature due to minimal lysis of human erythrocytes as compared to the standard Triton X-100. All synthesized compounds exhibited various interactions along enzyme active sites (π - π , H-bonding, van der Waals) with varied binding energies evaluated through a molecular docking study.

EXPERIMENTAL SECTION

Materials and Methods. All reagents and solvents used in syntheses of compounds **3a–o** were of analytical grade and used as received without further purification. The reagents and solvents were purchased from commercial sources (Sigma-Aldrich Merck, Fischer, and Acros Organics). Precoated thin-layer chromatographic aluminum sheets (Kiesel gel 60, F_{254} , E. Merck, Germany) were used to monitor the progress and purity of synthesized compounds. Melting points (M.p.) were recorded in open capillaries using a DMP-300 A&E Lab, U.K., melting point apparatus and are uncorrected. Ultraviolet (UV) absorption spectra were recorded on a Shimadzu Ultraviolet-1800 spectrophotometer in DMSO. IR spectra were recorded on a Bruker OPUS using attenuated total reflectance (ATR) to identify the functional groups. Proton (^1H), carbon (^{13}C), and fluorine (^{19}F) NMR experiments were performed on Bruker

DPX-400 and 500 MHz spectrometers. Mass spectra were recorded on the Bruker Micro time of flight-electrospray ionization (TOF-ESI) positive targeted mode. Fluorescence of glycated products was measured on a Shimadzu RF-6000 spectrofluorometer.

General Procedure for the Syntheses of 2-(2-Arylidenehydrazinyl)-4-(4-fluorophenyl)thiazoles (3a–o**).** The synthesis of **3a–o** was achieved by the Hantzsch thiazole synthetic protocol. Respective aryl-substituted thiosemicarbazones^{30,31} (0.001 mol) and 2-bromo-4-fluoroacetophenone (0.001 mol) in absolute ethanol were heated under reflux for 4–5 h. Reaction progress and completion were indicated by TLC, measured at regular intervals. Appearance of a single spot on the TLC plate highlighted the completion of reaction. Upon completion, the reaction mixture was allowed to attain room temperature for 30 min. The reaction mixture was then poured on crushed ice, which resulted in precipitation of the solid. The resulting precipitated solid was filtered through suction. The obtained solid was washed with plenty of water to remove the mother liquor. The formed solid was dried under vacuum to obtain the pure product. The NMR spectra (400 MHz) of **3a–o** were recorded in DMSO_d_6 except for **3a**, **3c**, and **3d**, which were run in chloroform CDCl_3 .

2-(2-(2-Bromo-4,5-dimethoxybenzylidene)hydrazinyl)-4-(4-fluorophenyl)thiazole (3a**).** Brick-red solid; yield: 79%;

Table 6. *In Silico* α -Amylase Inhibition Potential with Binding Energy and Dissociation Constant of All Compounds 3a–o

ligands	binding energy (kcal/mol)	dissociation constant (μ M)	active site
3a	-8.78	0.36	Tyr ² , Ser ³ , Asn ⁵ , Thr ⁶ , Gln ⁷ , Gln ⁸ , Arg ⁹² , Leu ²¹⁷ , Trp ²²¹ , Phe ²²² , Pro ²²³ , Gly ²²⁵ , Ser ²²⁶ , Lys ²²⁷ , Pro ²²⁸ , Phe ²²⁹ , Ile ²³⁰ .
3a	-8.59	0.51	Asp ⁴⁵¹ , Ile ⁴⁵³ , Ser ⁴⁵⁴ , Ile ⁴⁶⁵ , Lys ⁴⁶⁶ , Ile ⁴⁶⁷ , Tyr ⁴⁶⁸ , Ala ⁴⁷⁵ , His ⁴⁷⁶ , Phe ⁴⁷⁷ , Ser ⁴⁷⁸ , Ile ⁴⁷⁹ , Ser ⁴⁸⁰ , Ala ⁴⁸³ , Glu ⁴⁸⁴ , Asp ⁴⁸⁵ , Phe ⁴⁸⁷ , Ile ⁴⁸⁸ .
3a	-7.94	1.52	Trp ⁵⁸ , Trp ⁵⁹ , Tyr ⁶² , Gln ⁶³ , His ¹⁰¹ , Gly ¹⁰⁴ , Asn ¹⁰⁵ , Ala ¹⁰⁶ , Val ¹⁰⁷ , Leu ¹⁶² , Thr ¹⁶³ , Gly ¹⁶⁴ , Leu ¹⁶⁵ , Arg ¹⁹⁵ , Asp ¹⁹⁷ , Ala ¹⁹⁸ , Glu ²³³ , His ²⁹⁹ , Asp ³⁰⁰ .
3a	-7.89	1.64	Tyr ⁶⁷ , Lys ⁶⁸ , Leu ⁶⁹ , Cys ⁷⁰ , Asn ⁷⁵ , Glu ⁷⁶ , Asp ⁷⁷ , Cys ¹¹⁵ , Lys ¹⁷⁸ , Glu ¹⁸¹ , Tyr ¹⁸² , His ¹⁸⁵ , Leu ¹⁸⁶ .
3b	-7.91	1.6	Thr ⁴³⁹ , Ile ⁴⁶⁵ , Lys ⁴⁶⁶ , Ile ⁴⁶⁷ , Tyr ⁴⁶⁸ , Ser ⁴⁷⁰ , Asp ⁴⁷² , Lys ⁴⁷⁴ , Ala ⁴⁷⁵ , His ⁴⁷⁶ , Phe ⁴⁷⁷ , Ser ⁴⁷⁸ , Ile ⁴⁸⁸ .
3b	-7.74	2.12	Arg ²⁶⁷ , Gln ³⁰² , Arg ³⁰³ , Gly ³⁰⁴ , His ³⁰⁵ , Ala ³¹⁰ , Ile ³¹² , Thr ³¹⁴ , Trp ³¹⁶ , Asp ³¹⁷ , Arg ³⁴⁶ , Phe ³⁴⁸ , Gly ³⁵¹ , Asn ³⁵² , Asp ³⁵³ , Val ³⁵⁴ , Asp ³⁵⁶ .
3c	-8.1	1.16	Trp ⁵⁹ , Tyr ⁶² , Gln ⁶³ , His ¹⁰¹ , Tyr ¹⁵¹ , Leu ¹⁶² , Thr ¹⁶³ , Leu ¹⁶⁵ , Arg ¹⁹⁵ , Asp ¹⁹⁷ , Ala ¹⁹⁸ , Ser ¹⁹⁹ , Lys ²⁰⁰ , His ²⁰¹ , Glu ²³³ , Val ²³⁴ , Ile ²³⁵ .
3c	-7.62	2.59	Arg ³⁸⁹ , Gln ³⁹⁰ , Asp ⁴⁵¹ , Ile ⁴⁵³ , Ser ⁴⁵⁴ , Ile ⁴⁶⁵ , Lys ⁴⁶⁶ , Ile ⁴⁶⁷ , Tyr ⁴⁶⁸ , His ⁴⁷⁶ , Phe ⁴⁷⁷ , Ser ⁴⁷⁸ , Ile ⁴⁷⁹ , Ser ⁴⁸⁰ , Ala ⁴⁸³ , Glu ⁴⁸⁴ , Asp ⁴⁸⁵ , Pro ⁴⁸⁶ , Phe ⁴⁸⁷ , Ile ⁴⁸⁸ .
3d	-7.61	2.65	Tyr ⁶⁷ , Lys ⁶⁸ , Leu ⁶⁹ , Cys ⁷⁰ , Asn ⁷⁵ , Glu ⁷⁶ , Asp ⁷⁷ , Cys ¹¹⁵ , Ala ¹²⁸ , Val ¹²⁹ , Lys ¹⁷⁸ , Glu ¹⁸¹ , Tyr ¹⁸² , His ¹⁸⁵ .
3e	-7.3	4.44	Tyr 67, Lys 68, Leu 69, Cys 70, Asn 75, Glu 76, Asp 77, Cys 115, Ala 128, Val 129, Lys 178, Glu 181, Tyr 182, His 185
3e	-7.22	5.07	Arg 389, Gln 390, Ser 435, Phe 436, Ser 437, Asp 451, Ile 453, Ser 454, Ile 465, Ser 478, Ile 479, Ser 480, Ala 483, Glu 484, Asp 485, Pro 486, Phe 487, Ile 488
3f	-7.83	1.83	Arg 389, Ser 435, Phe 436, Ser 437, Asp 451, Ile 453, Ser 454, Ile 465, Ser 478, Ile 479, Ser 480, Ala 483, Glu 484, Asp 485, Pro 486, Phe 487, Ile 488
3f	-7.78	2.00	Ser 66, Tyr 67, Lys 68, Leu 69, Cys 70, Asn 75, Glu 76, Asp 77, Cys 115, Ala 128, Val 129, Glu 181, Tyr 182, His 185
3g	-7.42	3.66	Arg 389, Gln 390, Ser 435, Phe 436, Ser 437, Asp 451, Ile 453, Ser 454, Ile 465, Ser 478, Ile 479, Ser 480, Ala 483, Glu 484, Asp 485, Pro 486, Phe 487, Ile 488
3h	-8.26	0.88	Tyr 67, Lys 68, Leu 69, Cys 70, Asn 75, Glu 76, Asp 77, Cys 115, Ala 128, Val 129, Lys 178, Glu 181, Tyr 182, His 185
3h	-8.18	1.01	Thr 439, Ile 465, Lys 466, Ile 467, Tyr 468, Ser 470, Lys 474, Ala 475, His 476, Phe 477, Ser 478, Ile 488
3i	-7.99	1.40	Ser 66, Tyr 67, Lys 68, Leu 69, Cys 70, Asn 75, Glu 76, Asp 77, Cys 115, Ala 128, Val 129, Glu 181, Tyr 182, His 185
3j	-7.74	2.12	Asp 451, Ile 453, Ser 454, Ile 465, Lys 466, Ile 467, Tyr 468, Ala 475, His 476, Phe 477, Ser 478, Ile 479, Ser 480, Ala 483, Glu 484, Asp 485, Pro 486, Phe 487, Ile 488
3j	-7.51	3.11	Trp 59, Glu 60, Tyr 62, Gln 63, His 101, Tyr 151, Leu 162, Thr 163, Leu 165, Asp 197, Ala 198, Ser 199, Lys 200, His 201, Glu 233, Val 234, Ile 235
3k	-7.76	2.06	Trp 59, Glu 60, Tyr 62, Gln 63, His 101, Tyr 151, Leu 162, Thr 163, Leu 165, Asp 197, Ala 198, Ser 199, Lys 200, His 201, Glu 233, Val 234, Ile 235
3k	-7.71	2.23	Thr 439, Ile 465, Lys 466, Ile 467, Tyr 468, Ser 470, Asp 472, Lys 474, Ala 475, His 476, Phe 477, Ser 478, Ile 488
3l	-8.31	0.80	Trp 58, Trp 59, Tyr 62, His 101, Tyr 151, Leu 162, Leu 165, Arg 195, Asp 197, Ala 198, Ser 199, Lys 200, His 201, Glu 233, Val 234, Ile 235, Glu 240, Asn 298, His 299, Asp 300
3m	-7.61	2.65	Arg 267, Gln 302, Arg 303, Gly 304, His 305, Ala 310, Ile 312, Thr 314, Trp 316, Asp 317, Arg 346, Phe 348, Gly 351, Asn 352, Asp 353, Val 354, Asp 356
3m	-7.32	4.31	TRP 59, TYR 62, GLN 63, VAL 98, HIS 101, LEU 162, LEU 165, ASP 197, ALA 198, SER 199, LYS 200, HIS 201, GLU 233, VAL 234, ILE 235
3n	-7.38	3.88	TRP 59, GLU 60, TYR 62, GLN 63, VAL 98, HIS 101, LEU 162, LEU 165, ASP 197, ALA 198, SER 199, LYS 200, HIS 201, GLU 233, VAL 234, ILE 235
3n	-7.1	6.29	TYR 67, LYS 68, LEU 69, CYS 70, ASN 75, GLU 76, ASP 77, CYS 115, VAL 129, LYS 178, GLU 181, TYR 182, HIS 185
3o	-7.67	2.40	ASP 451, ILE 453, SER 454, ILE 465, LYS 466, ILE 467, TYR 468, ALA 475, HIS 476, PHE 477, SER 478, ILE 479, SER 480, ALA 483, GLU 484, ASP 485, PHE 487, ILE 488
3o	-7.65	2.46	TRP 59, GLU 60, TYR 62, GLN 63, HIS 101, TYR 151, LEU 162, THR 163, LEU 165, ASP 197, ALA 198, SER 199, LYS 200, HIS 201, GLU 233, VAL 234, ILE 235
3o	-7.52	3.05	SER 66, TYR 67, LYS 68, LEU 69, CYS 70, ASN 75, GLU 76, ASP 77, CYS 115, ALA 128, VAL 129, LYS 178, GLU 181, TYR 182, HIS 185

M.p.: 213–215 °C; R_f : 0.44 (acetone/*n*-hexane, 1:3); λ_{\max} = 283, 357 nm; FTIR (ATR) cm^{-1} : 3057 (=C–H stretching), 2937 (C–H aliphatic stretching), 1693 (C=N stretching), 1595 (thiazole skeletal vibrations), 1504, 1436 (C=C aromatic ring stretchings), 1305 (C–H aliphatic bending), 1093 (C–O stretching), 1024–731 (characteristic thiazole vibrations); ^1H NMR (400 MHz): δ (ppm) 8.29 (s, 1H, H–C=N–), 7.80 (dd, 2H, Ar–H, J = 9.0, 5.2 Hz), 7.49 (s, 1H, Ar–H), 7.19–7.13 (m, 2H, Ar–H), 7.03 (s, 1H, thiazole-H), 6.78 (s, 1H, Ar–H), 3.98 (s, 3H, –OCH₃), 3.94 (s, 3H, –OCH₃); ^{19}F NMR (375 MHz): δ (ppm) –114.53; ^{13}C NMR (100 MHz): δ (ppm) 168.7 (thiazole ring C2), 162.9(d, $^1J_{\text{CF}}$ = 248.3 Hz, Ar_{ipso}), 151.3 (Ar–C–O–Me), 148.8 (thiazole ring

C4), 148.2, 142.7 (H–C=N–), 129.3, 127.7 (d, $^3J_{\text{CF}}$ = 8.4 Hz, Ar), 124.9, 115.9 (d, $^2J_{\text{CF}}$ = 21.6 Hz, Ar), 115.3 (d, $^2J_{\text{CF}}$ = 26.0 Hz, Ar), 108.7 (Ar–C), 102.5 (thiazole ring C5), 56.3 (–OCH₃), 56.0 (–OCH₃); HRMS: m/z calculated for C₁₈H₁₅BrFN₃O₂S, [M + H]⁺: calcd: 436.0130, found: 436.0123; [M + Na]⁺: calculated: 457.9950, found: 457.9934; [2M + Na]⁺: calculated: 893.0002, found: 892.9978.

2-(2-(2-Bromo-4-methylbenzylidene)hydrazinyl)-4-(4-fluorophenyl)thiazole (3b). Light-yellow solid; yield: 80%; M.p.: 190–192 °C; R_f : 0.49 (acetone/*n*-hexane, 1:3); λ_{\max} = 270, 357 nm; FTIR (ATR) cm^{-1} : 3192 (N–H stretching), 2974 (C–H aliphatic stretching), 1600 (C=N stretching), 1489 (thiazole skeletal vibrations), 1436 (C=C aromatic ring

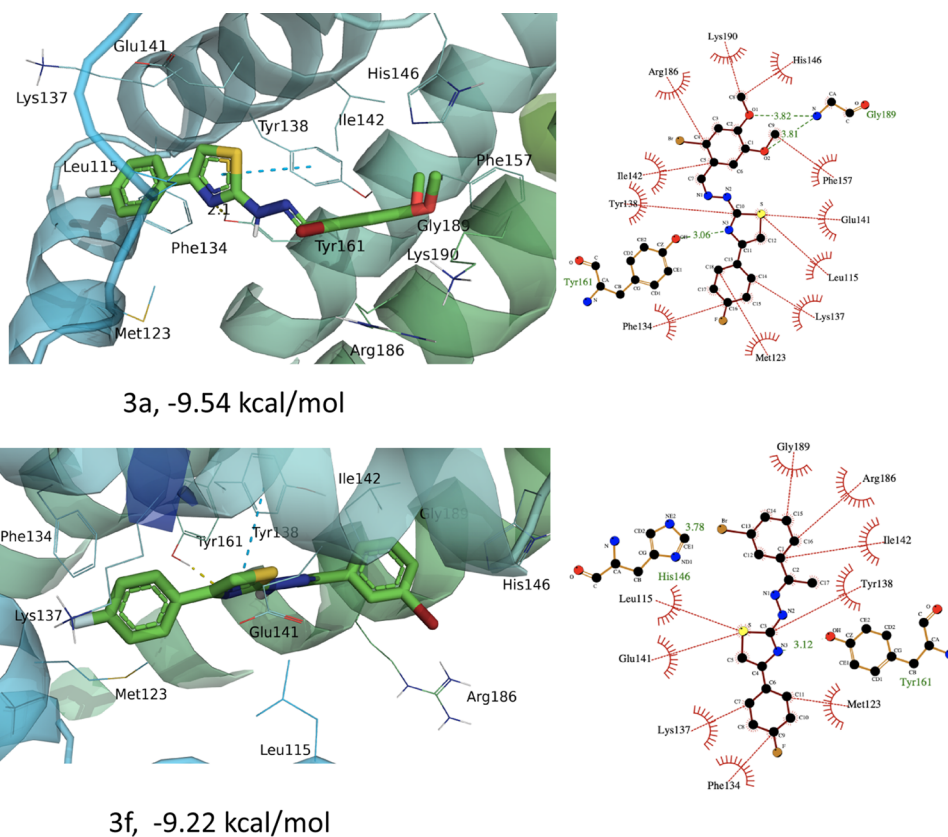


Figure 7. Best docking interactions are shown by ligands **3a** and **3f** with binding energies of -9.54 and -9.22 kJ/mol against human serum albumin (HSA).

stretchings), 1039–837 (characteristic thiazole vibrations); ^1H NMR (400 MHz): δ (ppm) 12.35 (s, 1H, H–N–), 8.34 (s, 1H, H–C=N–), 7.89 (dd, 2H, Ar–H, $J = 9.0, 5.6$ Hz), 7.79 (d, 1H, Ar–H, $J = 8.0$ Hz), 7.50 (s, 1H, thiazole–H), 7.32 (s, 1H, Ar–H), 7.28–7.21 (m, 3H, Ar–H), 2.32 (s, 3H, $-\text{CH}_3$); ^{19}F NMR (375 MHz): δ (ppm) -114.57 ; ^{13}C NMR (100 MHz): δ (ppm) 168.5 (thiazole ring C2), 162.1 (d, $^1J_{\text{CF}} = 248.3$ Hz, Ar_{ipso}), 150.0 (thiazole ring C4), 141.6 (H–C=N–), 140.0 (Ar–C– CH_3), 133.7, 131.7, 130.8, 129.4, 127.9 (d, $^3J_{\text{CF}} = 8.1$ Hz, Ar–C), 126.7, 122.9, 115.9 (d, $^2J_{\text{CF}} = 21.6$ Hz, Ar–C), 104.1 (thiazole ring C5), 20.9 ($-\text{CH}_3$); HRMS: m/z calculated for $\text{C}_{17}\text{H}_{13}\text{BrFN}_3\text{S}$, $[\text{M} + \text{H}]^+$: calcd: 390.0076, found: 390.0068; $[\text{M} + \text{Na}]^+$: calcd: 411.9896, found: 411.9885; $[\text{2M} + \text{Na}]^+$: calcd: 800.9894, found: 800.9878.

4-(4-Fluorophenyl)-2-(2-(4-phenylbutan-2-ylidene)hydrazinyl)thiazole (3c). Beige solid; yield: 78%; M.p.: 181–183 °C; R_f : 0.53 (acetone/*n*-hexane, 1:3); $\lambda_{\text{max}} = 278, 365$ nm; FTIR (ATR) cm^{-1} : 3056 ($=\text{C}-\text{CH}_3$ stretching), 2951 (C–H aliphatic stretching), 1618 (C=N stretching), 1598 (thiazole skeletal vibrations), 1558, 1489 (C=C aromatic ring stretchings), 1448 (C–H aliphatic bending), 1037–700 (characteristic thiazole vibrations); ^1H NMR (400 MHz): δ (ppm) 7.75 (dd, 2H, Ar–H, $J = 8.9, 5.1$ Hz), 7.35–7.29 (m, 2H, Ar–H), 7.27–7.21 (m, 3H, Ar–H), 7.14 (t, 2H, Ar–H, $J = 8.7$ Hz), 6.73 (s, 1H, thiazole–H), 2.96 (t, 2H, $-\text{CH}_2-\text{CH}_2-$, $J = 7.0$ Hz), 2.70 (t, 2H, $-\text{CH}_2-\text{CH}_2-$, $J = 7.0$ Hz), 2.04 (s, 3H, $-\text{CH}_3$); ^{19}F NMR (375 MHz): δ (ppm) -114.63 ; ^{13}C NMR (100 MHz): δ (ppm) 169.7 (thiazole ring C2), 163.1 (d, $^1J_{\text{CF}} = 249.4$ Hz, Ar_{ipso}), 155.7 (thiazole ring C4), 145.3 (CH₃–C=N–), 140.9, 128.5 (d, $^3J_{\text{CF}} = 11.4$ Hz, Ar–C), 127.7 (d, $^3J_{\text{CF}} = 8.1$ Hz, Ar–C), 126.2, 116.1 (d, $^2J_{\text{CF}} = 21.6$

Hz, Ar–C), 101.8 (thiazole ring C5), 40.0 ($-\text{CH}_2-\text{CH}_2-\text{C}=\text{N}-$), 32.2 ($-\text{CH}_2-\text{CH}_2-$), 17.2 (CH_3-CH_2-); HRMS: m/z calcd for $\text{C}_{19}\text{H}_{18}\text{FN}_3\text{S}$, $[\text{M} + \text{H}]^+$: calcd: 340.1283, found: 340.1273; $[\text{M} + \text{Na}]^+$: calculated: 362.1103, found: 362.1092; $[\text{2M} + \text{Na}]^+$: calcd: 701.2308, found: 701.2290.

4-(4-Fluorophenyl)-2-(2-(4-(trifluoromethyl)benzylidene)hydrazinyl)thiazole (3d). Off-white solid; yield: 74%; M.p.: 166–168 °C; R_f : 0.44 (acetone/*n*-hexane, 1:3); $\lambda_{\text{max}} = 268, 350$ nm; FTIR (ATR) cm^{-1} : 1693 (C=N stretching), 1570 (thiazole skeletal vibrations), 1489, 1438 (C=C aromatic ring stretchings), 1409 (C–H aliphatic bending), 1035–729 (characteristic thiazole vibrations); ^1H NMR (400 MHz): δ (ppm) 8.26 (d, 1H, Ar–H, $J = 8.3$ Hz), 7.76 (ddt, 3H, Ar–H, $J = 6.8, 5.4, 2.3$ Hz), 7.69–7.63 (m, 3H, Ar–H), 7.16 (t, 2H, Ar–H, $J = 8.7$ Hz), 6.77 (s, 1H, thiazole–H); ^{19}F NMR (375 MHz): δ (ppm) $-62.72, -112.97$; ^{13}C NMR (100 MHz): δ (ppm) 170.6 (thiazole ring C2), 162.8 (d, $^1J_{\text{CF}} = 248.3$ Hz, Ar_{ipso}), 148.9 (thiazole ring C4), 141.5, 137.3 (H–C=N–), 130.4, 128.1 (d, $^2J_{\text{CF}} = 8.1$ Hz, Ar–C), 126.8 (Ar–C), 125.6 (d, $^4J_{\text{CF}} = 3.9$ Hz, Ar–C), 115.9 (d, $^2J_{\text{CF}} = 21.6$ Hz, Ar–C), 103.2 (thiazole ring C5); HRMS: m/z calculated for $\text{C}_{17}\text{H}_{11}\text{F}_4\text{N}_3\text{S}$, $[\text{M} + \text{H}]^+$: calcd: 366.0688, found: 366.0685; $[\text{M} + \text{Na}]^+$: calcd: 388.0508, found: 388.0508; $[\text{2M} + \text{Na}]^+$: calcd: 753.1118, found: 753.1098.

4-(4-Fluorophenyl)-2-(2-((5-methylfuran-2-yl)methylene)hydrazinyl)thiazole (3e). Light-yellow solid; yield: 77%; M.p.: 142–143 °C; R_f : 0.50 (acetone/*n*-hexane, 1:3); $\lambda_{\text{max}} = 278, 353$ nm; FTIR (ATR) cm^{-1} : 2922 (C–H aliphatic stretching), 1610 (C=N stretching), 1490 (thiazole skeletal vibrations), 1450 (C=C aromatic ring stretchings), 1053–729 (characteristic thiazole vibrations); ^1H NMR (400 MHz): δ (ppm) 7.89

(d, 1H, furan-H, $J = 5.5$ Hz), 7.85 (s, 1H, H-C=N-), 7.30–7.18 (m, 3H, Ar-H), 7.13–7.03 (m, 1H, Ar-H), 6.69 (d, 1H, furan-H, $J = 3.3$ Hz), 6.22 (s, 1H, thiazole-H), 2.35 (s, 3H, CH₃-); ¹⁹F NMR (375 MHz): δ (ppm) –114.63; ¹³C NMR (100 MHz): δ (ppm) 168.5 (thiazole ring C2), 162.1 (d, ¹J_{CF} = 244.3 Hz, Ar_{ipso}), 154.4 (thiazole ring C4), 149.8 (furan C2), 148.3 (H-C=N- azomethine), 132.3 (furan C5), 130.4, 127.9 (d, ³J_{CF} = 8.4 Hz, Ar-C), 115.9 (d, ²J_{CF} = 21.6 Hz, Ar-C), 114.4 (furan C4), 108.9 (furan C3), 103.7 (thiazole ring C5), 14.0 (CH₃-); HRMS: m/z calcd for C₁₅H₁₂FN₃OS, [M + H]⁺: 302.0763, found: 302.0749; [M + Na]⁺: calcd: 324.0583, found: 324.0569; [2M + Na]⁺: calcd: 625.1268, found: 625.1242.

2-(2-(1-(3-Bromophenyl)ethylidene)hydrazinyl)-4-(4-fluorophenyl)thiazole (3f). Orange solid; yield: 72%; M.p.: 120–122 °C; R_f: 0.52 (acetone/*n*-hexane, 1:3); $\lambda_{\max} = 267$, 355 nm; FTIR (ATR) cm⁻¹: 1670 (C=N stretching), 1598 (thiazole skeletal vibrations), 1571, 1508, 1442 (C=C aromatic ring stretchings), 1029–696 (characteristic thiazole vibrations); ¹H NMR (400 MHz): δ (ppm) 11.39 (s, 1H, H-N-), 7.94 (s, 1H, Ar-H), 7.93–7.88 (m, 2H, Ar-H), 7.77 (d, 1H, Ar-H, $J = 8.2$ Hz), 7.57 (d, 1H, Ar-H, $J = 8.9$ Hz), 7.39 (t, 1H, Ar-H, $J = 7.9$ Hz), 7.32 (s, 1H, thiazole-H), 7.24 (t, 2H, Ar-H-C-F, $J = 9.0$ Hz), 2.32 (s, 3H, H₃C-C=N-); ¹⁹F NMR (375 MHz): δ (ppm) –114.60; ¹³C NMR (100 MHz): δ (ppm) 170.2 (thiazole ring C2), 162.1 (d, ¹J_{CF} = 244.3 Hz, Ar_{ipso}), 160.9 (thiazole ring C4), 145.3 (CH₃-C=N-), 140.8, 131.7, 131.1, 128.6, 127.9 (d, ³J_{CF} = 8.1 Hz, Ar-C), 125.2 (Ar-C), 122.4 (Ar-C-Br), 115.9 (d, ²J_{CF} = 21.6 Hz, Ar-C), 104.5 (thiazole ring C5), 14.4 (CH₃-C=N-); HRMS: m/z calculated for C₁₇H₁₃BrFN₃S, [M + H]⁺: calculated: 390.0076, found: 390.0071; [M + Na]⁺: calculated: 411.9896, found: 411.9889; [2M + Na]⁺: calculated: 800.9894, found: 800.9874.

4-(4-Fluorophenyl)-2-(2-(1-(4-fluorophenyl)ethylidene)hydrazinyl)thiazole (3g). Lemon-yellow solid; yield: 70%; M.p.: 133–134 °C; R_f: 0.58 (acetone/*n*-hexane, 1:3); $\lambda_{\max} = 266$, 350 nm; FTIR (ATR) cm⁻¹: 3057 (C-H stretching), 2926 (C-H aliphatic stretching), 1605 (C=N stretching), 1485 (thiazole skeletal vibrations), 1442 (C=C aromatic ring stretchings), 1068–692 (characteristic thiazole vibrations); ¹H NMR (400 MHz): δ (ppm) 11.26 (s, 1H, H-N-), 7.92 (dd, 2H, Ar-H, $J = 8.9$, 5.5 Hz), 7.83 (dd, 2H, Ar-H, $J = 9.0$, 5.5 Hz), 7.31 (s, 1H, thiazole-H), 7.25 (td, 4H, Ar-H, $J = 8.8$, 6.3 Hz), 2.33 (s, 3H, -CH₃); ¹⁹F NMR (375 MHz): δ (ppm) –113.23, –114.67; ¹³C NMR (100 MHz): δ (ppm) 170.4 (thiazole ring C2), 163.7 (d, ¹J_{CF} = 244.3 Hz, Ar_{ipso}), 161.3 (d, ¹J_{CF} = 244.3 Hz, Ar_{ipso}), 150.1 (thiazole ring C4), 146.1 (H-C=N-), 134.9 (d, ⁴J_{CF} = 3.3 Hz, Ar-C), 131.9 (Ar-C), 128.3 (d, ³J_{CF} = 8.3 Hz, Ar-C), 127.9 (d, ³J_{CF} = 8.3 Hz, Ar-C), 115.9 (d, ³J_{CF} = 13.9 Hz, Ar-C-C-F), 115.7 (d, ³J_{CF} = 13.9 Hz, Ar-C), 104.3 (thiazole ring C5), 14.5 (CH₃-C=N-); HRMS: m/z calculated for C₁₇H₁₃F₂N₃S, [M + H]⁺: calcd: 330.0876, found: 330.0865; [M + Na]⁺: calcd: 352.0696, found: 352.0682; [2M + Na]⁺: calcd: 681.1494, found: 681.1464.

4-Chloro-2-((2-(4-(4-fluorophenyl)thiazol-2-yl)-hydrazinylidene)methyl)phenol (3h). Orange solid; yield: 66%; M.p.: 192–193 °C; R_f: 0.27 (acetone/*n*-hexane, 1:3); $\lambda_{\max} = 265$, 358 nm; FTIR (ATR) cm⁻¹: 3138 (O-H stretching), 3101 (C-H stretching), 2924 (C-H aliphatic stretching), 1610 (C=N stretching), 1562 (thiazole skeletal vibrations), 1477 (C=C aromatic ring stretchings), 1031–734 (characteristic thiazole vibrations); ¹H NMR (400 MHz): δ

(ppm) 12.25 (s, 1H, H-N-), 10.36 (s, 1H, H-O), 8.28 (s, 1H, H-C=N-), 7.89 (dd, 2H, Ar-H, $J = 8.8$, 5.6 Hz), 7.63 (d, 2H, Ar-H, $J = 2.8$ Hz), 7.30 (s, 1H, thiazole-H), 7.28–7.17 (m, 3H, Ar-H), 6.93 (d, 1H, Ar-H, $J = 8.8$ Hz); ¹⁹F NMR (375 MHz): δ (ppm) –114.49; ¹³C NMR (100 MHz): δ (ppm) 168.5 (thiazole ring C2), 162.1 (d, ¹J_{CF} = 244.7 Hz, Ar_{ipso}), 155.1 (thiazole ring C4), 149.1 (Ar-C-OH), 137.8 (H-C=N-), 131.7, 130.3 (Ar-C), 127.9 (d, ³J_{CF} = 8.1 Hz, Ar-C), 125.2 (Ar-C), 123.7 (Ar-C-Cl), 122.6, 118.4 (Ar-C), 115.9 (d, ³J_{CF} = 21.3 Hz, Ar-C), 103.8 (thiazole ring C5); HRMS: m/z calcd for C₁₆H₁₁ClFN₃OS, [M + H]⁺: calcd: 348.0373, found: 348.0365; [M + Na]⁺: calcd: 370.0193, found: 370.0190; [2M + Na]⁺: calcd: 717.0488, found: 717.0472.

4-(4-Fluorophenyl)-2-(2-(3-(trifluoromethyl)benzylidene)hydrazinyl)thiazole (3i). Gray solid; yield: 72%; M.p.: 157–159 °C; R_f: 0.38 (acetone/*n*-hexane, 1:3); $\lambda_{\max} = 264$, 358 nm; FTIR (ATR) cm⁻¹: 3061 (C-H stretching), 2929 (C-H aliphatic stretching), 1695 (C=N stretching), 1570 (thiazole skeletal vibrations), 1500, 1448 (C=C aromatic ring stretchings), 1068–736 (characteristic thiazole vibrations); ¹H NMR (400 MHz): δ (ppm) 8.13 (s, 1H, H-C=N-), 7.97 (d, 2H, Ar-H, $J = 8.7$ Hz), 7.90 (dd, Ar-H, $J = 8.9$, 5.5 Hz), 7.73–7.65 (m, 2H, Ar-H), 7.34 (s, 1H, thiazole-H), 7.24 (t, 2H, Ar-H, $J = 8.8$ Hz); ¹⁹F NMR (375 MHz): δ (ppm) –61.39, –114.54; ¹³C NMR (100 MHz): δ (ppm) 168.6 (thiazole ring C2), 162.1 (d, ¹J_{CF} = 244.7 Hz, Ar_{ipso}), 150.0 (thiazole ring C4), 139.9 (H-C=N-), 136.1, 131.7 (d, ⁴J_{CF} = 3.3 Hz, Ar-C), 130.4 (d, ³J_{CF} = 11.7 Hz, Ar-C), 129.9, 127.9 (d, ³J_{CF} = 8.1 Hz, Ar-C), 125.9, 123.2, 122.9 (d, ⁴J_{CF} = 4.0 Hz, Ar-C), 115.9 (d, ²J_{CF} = 21.3 Hz, Ar-C), 104.3 (thiazole ring C5); HRMS: m/z calcd for C₁₇H₁₁F₄N₃S, [M + H]⁺: calcd: 366.0688, found: 366.0680; [2M + Na]⁺: calcd: 753.1118, found: 753.1086.

2-(2-(2-Ethoxybenzylidene)hydrazinyl)-4-(4-fluorophenyl)thiazole (3j). Light-yellow solid; yield: 78%; M.p.: 127–128 °C; R_f: 0.40 (acetone/*n*-hexane, 1:3); $\lambda_{\max} = 265$, 370 nm; FTIR (ATR) cm⁻¹: 2930 (C-H aliphatic stretching), 1607 (C=N stretching), 1500 (thiazole skeletal vibrations), 1495, 1457 (C=C aromatic ring stretchings), 1018–703 (characteristic thiazole vibrations); ¹H NMR (400 MHz): δ (ppm) 8.41 (s, 1H, H-C=N-), 7.89 (dd, Ar-H, $J = 8.9$, 5.5 Hz), 7.79 (d, 1H, Ar-H, $J = 7.8$ Hz), 7.37–7.20 (m, 4H, Ar-H), 7.08–6.95 (m, 3H, Ar-H), 4.14–4.07 (m, 2H, -CH₂-CH₃), 1.38 (t, 3H, CH₃-CH₂-, $J = 7.0$ Hz); ¹⁹F NMR (375 MHz): δ (ppm) –114.63; ¹³C NMR (100 MHz): δ (ppm) 168.8 (thiazole ring C2), 164.7 (d, ¹J_{CF} = 285.4 Hz, Ar_{ipso}), 160.9 (Ar-C-O) 156.9, 149.9 (thiazole ring C4), 137.7 (H-C=N-), 131.8 (d, ⁴J_{CF} = 2.9 Hz, Ar-C), 131.1 (Ar-C), 127.9 (d, $J = 8.1$ Hz, Ar-C), 125.4, 123.1, 121.2 (Ar-C), 115.9 (d, ²J_{CF} = 21.3 Hz), 113.3 (Ar-C), 103.8 (thiazole ring C5), 64.3 (CH₃-CH₂-O-), 15.1 (CH₃-CH₂-); HRMS: m/z calculated for C₁₈H₁₆FN₃OS, [M + H]⁺: calcd: 342.1076, found: 342.1058; [M + Na]⁺: calcd: 364.0896, found: 364.0882; [2M + Na]⁺: calcd: 705.1894, found: 705.1853.

4-(4-Fluorophenyl)-2-(2-(2-(trifluoromethyl)benzylidene)hydrazinyl)thiazole (3k). Off-white solid; yield: 79%; M.p.: 168–169 °C; R_f: 0.49 (acetone/*n*-hexane, 1:3); $\lambda_{\max} = 265$, 364 nm; FTIR (ATR) cm⁻¹: 3057 (C-H stretching), 2933 (C-H aliphatic stretching), 1699 (C=N stretching), 1572 (thiazole skeletal vibrations), 1499, 1446 (C=C aromatic ring stretchings), 1060–734 (characteristic thiazole vibrations); ¹H

NMR (400 MHz): δ (ppm) 12.50 (s, 1H, H–N–), 8.37 (s, 1H, H–C=N–), 8.14 (d, 1H, Ar–H, $J = 7.8$ Hz), 7.90 (dd, 2H, Ar–H, $J = 8.8, 5.6$ Hz), 7.81–7.72 (m, 2H, Ar–H), 7.58 (t, 1H, Ar–H, $J = 7.6$ Hz), 7.37 (s, 1H, thiazole–H), 7.25 (t, 2H, Ar–H, $J = 8.9$ Hz); ^{19}F NMR (375 MHz): δ (ppm) –57.06, –114.48; ^{13}C NMR (100 MHz): δ (ppm) 168.3 (thiazole ring C2), 162.1 (d, $^1J_{\text{CF}} = 244.7$ Hz, Ar_{ipso}), 150.1 (thiazole ring C4), 136.6 (H–C=N–), 133.3, 132.7, 131.6, 129.7, 128.1 (d, $^3J_{\text{CF}} = 8.1$ Hz, Ar–C), 127.9, 126.6–126.1 (m, Ar–C), 115.9 (d, $^2J_{\text{CF}} = 21.6$ Hz, Ar–C), 104.5 (thiazole ring C5); HRMS: m/z calculated for $\text{C}_{17}\text{H}_{11}\text{F}_4\text{N}_3\text{S}$, $[\text{M} + \text{H}]^+$: calcd: 366.0688, found: 366.0680; $[\text{2M} + \text{Na}]^+$: calcd: 753.1118, found: 753.1086.

2-(2-(3-Fluoro-2-methoxybenzylidene)hydrazinyl)-4-(4-fluorophenyl)thiazole (3l). Light-yellow solid; yield: 61%; M.p.: 115–117 °C; R_f : 0.39 (acetone/*n*-hexane, 1:3); $\lambda_{\text{max}} = 276, 368$ nm; FTIR (ATR) cm^{-1} : 3055 (C–H stretching), 1604 (C=N stretching), 1575 (thiazole skeletal vibrations), 1489, 1444 (C=C aromatic ring stretching), 1006–696 (characteristic thiazole vibrations); ^1H NMR (400 MHz): δ (ppm) 8.29 (s, 1H, H–C=N–), 7.90 (dd, 2H, Ar–H, $J = 8.9, 5.5$ Hz), 7.63 (d, 1H, Ar–H, $J = 8.0$ Hz), 7.33 (s, 1H, thiazole–H), 7.31–7.21 (m, 3H, Ar–H), 7.18 (td, 1H, Ar–H, $J = 8.2, 5.1$ Hz), 3.91 (s, 3H, –CH₃); ^{19}F NMR (375 MHz): δ (ppm) –114.55, –131.04; ^{13}C NMR (100 MHz): δ (ppm) 168.6 (thiazole ring C2), 162.1 (d, $^1J_{\text{CF}} = 244.3$ Hz, Ar_{ipso}), 155.7 (d, $^1J_{\text{CF}} = 245.0$ Hz, Ar_{ipso}), 150.0 (thiazole C4), 145.8 (Ar–C–O), 136.1 (H–C=N–), 131.7 (d, $^4J_{\text{CF}} = 2.9$ Hz, Ar–C), 129.5 (d, $^4J_{\text{CF}} = 2.9$ Hz, Ar–C), 127.9 (d, $^3J_{\text{CF}} = 8.4$ Hz, Ar–C), 124.9 (d, $^3J_{\text{CF}} = 8.1$ Hz, Ar–C), 121.1 (d, $^4J_{\text{CF}} = 3.3$ Hz, Ar–C), 117.8 (d, $^2J_{\text{CF}} = 19.1$ Hz, Ar–C), 115.9 (d, $^2J_{\text{CF}} = 21.6$ Hz, Ar–C), 104.2 (thiazole ring C5), 62.5 (d, $^5J_{\text{HF}} = 5.1$ Hz, –OCH₃); HRMS: m/z calculated for $\text{C}_{17}\text{H}_{13}\text{F}_2\text{N}_3\text{OS}$, $[\text{M} + \text{H}]^+$: calcd: 346.0825, found: 346.0823; $[\text{M} + \text{Na}]^+$: calcd: 368.0645, found: 368.0642; $[\text{2M} + \text{Na}]^+$: calcd: 713.1392, found: 713.1378.

2-(2-((1*H*-Pyrrol-2-yl)methylene)hydrazinyl)-4-(4-fluorophenyl)thiazole (3m). Dark-gray solid; yield: 77%; M.p.: 130–132 °C; R_f : 0.45 (acetone/*n*-hexane, 1:3); $\lambda_{\text{max}} = 282, 358$ nm; FTIR (ATR) cm^{-1} : 3278 (N–H stretching), 1626 (C=N stretching), 1593 (thiazole skeletal vibrations), 1500 (C=C aromatic ring stretchings), 1029–730 (characteristic thiazole vibrations); ^1H NMR (400 MHz): δ (ppm) 11.77 (s, 1H, H–N–), 11.21 (s, 1H, H–N, pyrrole), 7.91 (s, 1H, H–C=N–), 7.88 (dd, 2H, Ar–H, $J = 8.9, 5.6$ Hz), 7.30–7.18 (m, 3H, Ar–H), 6.90 (d, 1H, pyrrole–H, $J = 1.5$ Hz), 6.40 (s, 1H, thiazole), 6.12 (d, 1H, pyrrole–H, $J = 3.6$ Hz); ^{19}F NMR (375 MHz): δ (ppm) –114.77; ^{13}C NMR (100 MHz): δ (ppm) 168.8 (thiazole ring C2), 162.0 (d, $^1J_{\text{CF}} = 244.3$ Hz, Ar_{ipso}), 154.7 (thiazole ring C4), 149.7, 135.5 (H–C=N–), 131.9 (pyrrole C2), 127.9 (d, $^3J_{\text{CF}} = 8.1$ Hz, Ar–C), 127.6 (Ar–C), 122.1 (pyrrole C3), 115.9 d, $^2J_{\text{CF}} = 21.6$ Hz, 111.8 (Ar–C), 109.6 (pyrrole–C5), 103.3 (thiazole ring C5).

4-(4-Fluorophenyl)-2-(2-(thiophen-2-ylmethylene)hydrazinyl)thiazole (3n). Yellow solid; yield: 67%; M.p.: 143–145 °C; R_f : 0.54 (acetone/*n*-hexane, 1:3); $\lambda_{\text{max}} = 279, 358$ nm; FTIR (ATR) cm^{-1} : 1602 (C=N stretching), 1479 (C=C aromatic ring stretching), 1004–777 (characteristic thiazole vibrations); ^1H NMR (400 MHz): δ (ppm) 8.23 (s, 1H, H–C=N–), 7.89 (dd, 2H, Ar–C, $J = 8.9, 5.6$ Hz), 7.58 (d, 1H, thiophene C5–H, $J = 5.0$ Hz), 7.37 (d, 1H, thiophene C3–H, $J = 2.5$ Hz), 7.29 (s, 1H, thiazole–H), 7.24 (t, 2H, Ar–H, $J = 8.9$ Hz), 7.10 (dd, 1H, Ar–H, $J = 5.1, 3.5$ Hz); ^{19}F NMR (375

MHz): δ (ppm) –114.57; ^{13}C NMR (100 MHz): δ (ppm) 168.4 (thiazole ring C2), 162.1 (d, $^1J_{\text{CF}} = 244.3$ Hz, Ar_{ipso}), 149.9 (thiophene C2), 139.6 (thiophene C3), 137.2 (H–C=N–), 131.7, 129.6, 128.2 (d, $^2J_{\text{CF}} = 18.0$ Hz, Ar–C), 127.9 (d, $^3J_{\text{CF}} = 8.1$ Hz, Ar–C), 115.9 (d, $^2J_{\text{CF}} = 21.3$ Hz, Ar–C), 103.9 (thiazole ring C5); HRMS: m/z calcd for $\text{C}_{14}\text{H}_{10}\text{FN}_3\text{S}_2$, $[\text{M} + \text{H}]^+$: calcd: 304.0378, found: 304.0375; $[\text{M} + \text{Na}]^+$: calcd: 326.0198, found: 326.0193; $[\text{2M} + \text{Na}]^+$: calcd: 629.0498, found: 629.0487.

4-(4-Fluorophenyl)-2-(2-(2-(methylthio)benzylidene)hydrazinyl)thiazole (3o). Pink solid; yield: 80%; M.p.: 152–154 °C; R_f : 0.48 (acetone/*n*-hexane, 1:3); $\lambda_{\text{max}} = 287, 357$ nm; FTIR (ATR) cm^{-1} : 3138 (N–H stretching), 3053 (C–H stretching), 1601 (C=N stretching), 1489, 1446 (C=C aromatic ring stretchings), 1411 (C–H aliphatic bending), 1006–731 (characteristic thiazole vibrations); ^1H NMR (400 MHz): δ (ppm) 8.43 (s, 1H, H–C=N–), 7.90 (dd, 2H, Ar–C, $J = 8.9, 5.5$ Hz), 7.74 (d, 1H, Ar–H, $J = 8.5$ Hz), 7.40–7.33 (m, 2H, Ar–H), 7.31 (s, 1H, thiazole–H), 7.24 (td, 3H, Ar–H, $J = 8.5, 3.7$ Hz), 2.50 (s, 3H, CH₃–S); ^{19}F NMR (375 MHz): δ (ppm) –114.53; ^{13}C NMR (100 MHz): δ (ppm) 168.7 (thiazole ring C2), 162.1 (d, $^1J_{\text{CF}} = 244.3$ Hz, Ar_{ipso}), 149.9 (thiazole C4), 139.6 (H–C=N–), 137.8 (Ar–C–S), 132.2, 131.7 (d, $^4J_{\text{CF}} = 3.3$ Hz, Ar–C), 130.0, 127.9 (d, $^3J_{\text{CF}} = 8.1$ Hz, Ar–C), 127.0 (d, $^2J_{\text{CF}} = 30.1$ Hz, Ar–C), 125.7, 115.9 (d, $^2J_{\text{CF}} = 21.6$ Hz, Ar–C), 104.1 (thiazole ring C5), 16.2 (CH₃–S); HRMS: m/z calculated for $\text{C}_{17}\text{H}_{14}\text{FN}_3\text{S}_2$, $[\text{M} + \text{H}]^+$: calcd: 344.0691, found: 344.0688; $[\text{M} + \text{Na}]^+$: calcd: 366.0511, found: 366.0507; $[\text{2M} + \text{Na}]^+$: calcd: 709.1124, found: 709.1124.

(Please see the Supporting Information for protocols of α -amylase, antiglycation inhibition, antioxidant, *in vitro* hemolysis, and molecular docking studies).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.3c00265>.

UV–vis, FTIR, ^1H , ^{13}C , ^{19}F NMR, and HRMS spectra of all compounds, and protocols of all biological assays along with molecular docking experiment (PDF)

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Dr. Muhammad Khalid, confirm that all authors played their role in this work and is briefed as follows: H.M.: performed all experimental work, characterization, and write-up; M.H.: data compilation, assisting in syntheses, and write-up; T.A.: supervisor of the first author, designed the project, refined the paper, and also corresponding author; S.W.: assisted first author (characterization) in his lab, paper writing, and improvement; M.K., M.A.A., R.B., R.O., S.P.: molecular docking and writing of the part.

Notes

The authors declare no competing financial interest.

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