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## New Hybrid Hydrazinyl Thiazole Substituted Chromones: As Potential $\alpha$ -Amylase Inhibitors and Radical (DPPH & ABTS) Scavengers

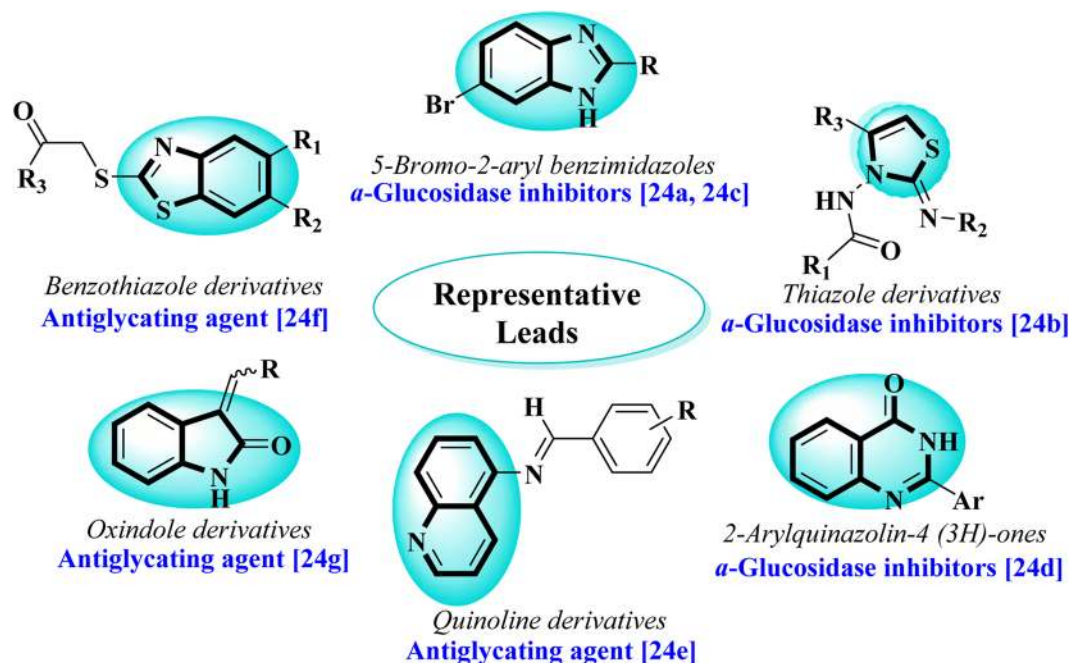
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Current research is based on the identification of novel inhibitors of  $\alpha$ -amylase enzyme. For that purpose, new hybrid molecules of hydrazinyl thiazole substituted chromones 5–27 were synthesized by multi-step reaction and fully characterized by various spectroscopic techniques such as EI-MS, HREI-MS, <sup>1</sup>H-NMR and <sup>13</sup>C-NMR. Stereochemistry of the iminic bond was confirmed by NOESY analysis of a representative molecule. All compounds 5–27 along with their intervening intermediates 1–4, were screened for *in vitro*  $\alpha$ -amylase inhibitory, DPPH and ABTS radical scavenging activities. All compounds showed good inhibition potential in the range of  $IC_{50} = 2.186\text{--}3.405\ \mu\text{M}$  as compared to standard acarbose having  $IC_{50}$  value of  $1.9 \pm 0.07\ \mu\text{M}$ . It is worth mentioning that compounds were also demonstrated good DPPH ( $IC_{50} = 0.09\text{--}2.233\ \mu\text{M}$ ) and ABTS ( $IC_{50} = 0.584\text{--}3.738\ \mu\text{M}$ ) radical scavenging activities as compared to standard ascorbic acid having  $IC_{50} = 0.33 \pm 0.18\ \mu\text{M}$  for DPPH and  $IC_{50} = 0.53 \pm 0.3\ \mu\text{M}$  for ABTS radical scavenging activities. In addition to that cytotoxicity of the compounds were checked on NIH-3T3 mouse fibroblast cell line and found to be non-toxic. *In silico* studies were performed to rationalize the binding mode of compounds (ligands) with the active site of  $\alpha$ -amylase enzyme.

Diabetes mellitus (DM) is a metabolic disorder caused by the insufficient insulin secretion and decreased insulin activity which leads to the disruption of carbohydrate, protein, and fat metabolism<sup>1</sup>. Insulin is a peptide hormone which is responsible to reduce gluconeogenesis, increases the glucose consumption, and drops the blood glucose level<sup>2</sup>. However, failure in insulin secretion or disturbance in insulin sensitivity give rise to uncontrolled blood glucose levels (hyperglycemia) and ultimately results in DM. In addition to that continuous complications of DM further brings out to neuropathy, nephropathy, retinopathy, microangiopathy as well as cardiovascular diseases<sup>1</sup>.

Treatment of type-II DM includes a number of therapeutic approaches such as stimulation of the endogenous insulin secretion, reduction of insulin's demand, and inhibition of carbohydrate degradation<sup>3</sup>. One of therapeutic strategies is to reduce the post-prandial glucose levels by retarding the absorption of glucose. This could possibly be done by the inhibition of enzymes,  $\alpha$ -glucosidase and  $\alpha$ -amylase, those are responsible to hydrolyze oligosaccharides and disaccharides into monosaccharides<sup>4–6</sup>. The  $\alpha$ -amylase ( $\alpha$ -1,4-glucan-4-glucanohydrolases; E.C. 3.2.1.1) is one of the main enzyme secreted by the pancreas (about 5–6%) and salivary glands, and shows a significant role in digestion or breakdown of starch and glycogen and usually found in microbes, plants, and higher organisms<sup>7,8</sup>. Inhibitors of  $\alpha$ -amylase enzyme such as acarbose, function by delaying the carbohydrate digestion and cause a decreased rate of glucose absorption and accordingly diminishing the postprandial plasma glucose level<sup>9,10</sup>. However, adverse effects are also associated such as abdominal discomfort, meteorism, flatulence, and diarrhea which lead to discontinuation of therapy<sup>1</sup>. Some natural products such as flavonoids and phenolic

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**Figure 1.** Identified representative lead candidates.



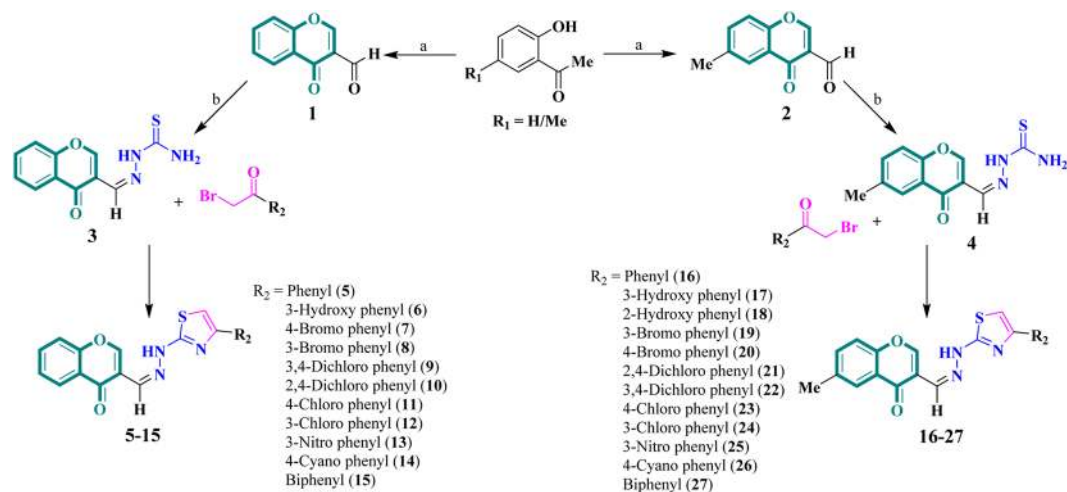
**Figure 2.** Rationale of the current study.

compounds has been identified as  $\alpha$ -amylase inhibitors<sup>1,11–13</sup>. However, the synthetic inhibitors are rarely discovered. There is an urgent need for the discovery of novel therapeutic agents for the management of type-II diabetes mellitus.

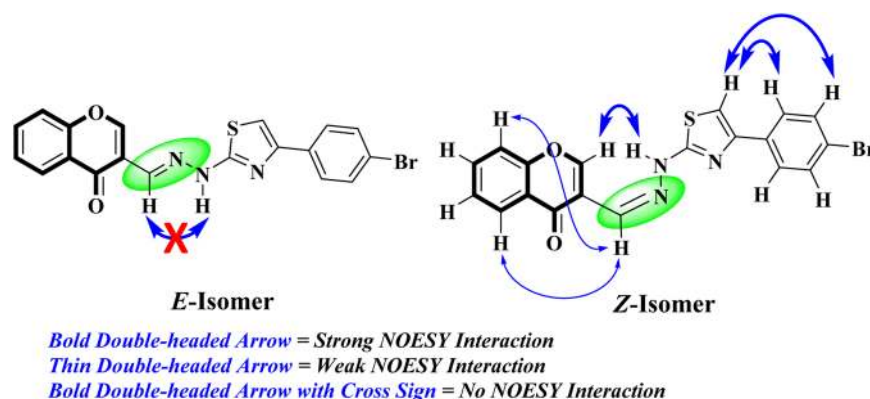
Chromone or 4*H*-chromen-4-one is a naturally occurring heterocycle based on benzopyrone scaffold and widely distributed in nature mainly in plants. It is also the core fragment of several flavonoids *e.g.* flavones and isoflavones. Chromone derived compounds have a wide-range of biological activities such as antioxidant, antihypertensive, antiinflammatory, anticancer, antifungal, antibacterial, antiviral, antimutagenic, and phytotoxic activities<sup>14</sup>. Chromones have also been reported to possess lipoxygenase, thymidine phosphorylase, cyclooxygenase, tyrosine and protein kinase inhibitory activities<sup>15–19</sup>. Similarly, heterocyclic ring thiazole has also reported to be the main part of countless medicinally important molecules due to its notable biological activities<sup>20,21</sup>.

A number of reports available on hybrid scaffolds based on thiazole linked with chromone scaffold with some biological potentials<sup>22–25</sup>. Nevertheless, there is no report available on this hybrid class for their  $\alpha$ -amylase inhibitory activity. Our research group has identified many lead scaffolds having antiglycation and  $\alpha$ -glucosidase inhibitory activities (Fig. 1), as a possible treatment for diabetic management<sup>26–31</sup>.

We have also reported 3-thiazolyl coumarin as potent inhibitors of  $\alpha$ -glucosidase enzyme<sup>32</sup>. It is worth-mentioning that newly synthesized compounds have close structural resemblance with the 3-thiazolyl coumarins (Fig. 2), so that we decided to explore the new hybrid hydrazinyl thiazole substituted chromones 5–27 along with the intervening intermediates for  $\alpha$ -amylase inhibitory activity in order to identify novel inhibitors. Furthermore, by keeping in mind that excess free radical formation is also associated with the diabetic patients, so that synthetic analogs were also evaluated for their radical scavenging activities (DPPH and ABTS). To the best of our knowledge, except compounds 1–5<sup>33–36</sup> all compounds are new.



**Figure 3.** Syntheses of hydrazinyl thiazole substituted chromone derivatives 5–27 *via* intervening intermediates 1–4; **Reaction conditions:** (a)  $\text{POCl}_3$ , DMF; (b) Thiosemicarbazide, AcOH, EtOH, Reflux, 2 h; (c)  $\text{Et}_3\text{N}$ , EtOH, Reflux, 3 h.



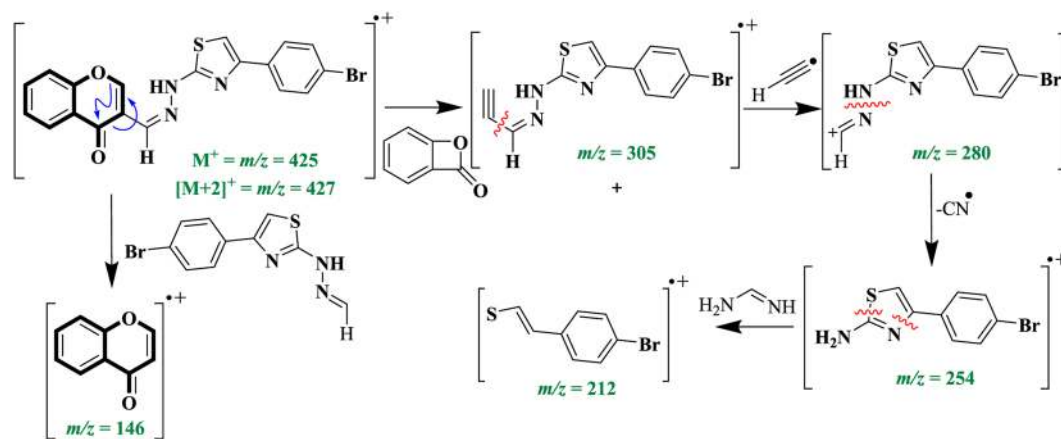
**Figure 4.** Distinctive NOESY interactions.

## Results and Discussion

**Chemistry.** New hybrid hydrazinyl thiazole substituted chromones 5–27 were synthesized by multi-step reaction. First, chromone-3-carbaldehyde **1** and 6-methylchromone-3-carbaldehyde **2** were synthesized by reacting 2-hydroxyacetophenone and 5-methyl-2-hydroxyacetophenone with the dimethyl formamide (DMF) in the presence of phosphoryl chloride ( $\text{POCl}_3$ )<sup>14</sup>. In the next step, chromone-3-carbaldehyde derivatives (**1** and **2**) were condensed with thiosemicarbazide in ethanol to afford their corresponding thiosemicarbazone derivatives (**3** and **4**), in the presence of glacial acetic acid. These thiosemicarbazone derivatives (**3** and **4**) were reacted with different phenacyl bromides which underwent a cyclization reaction in the presence of triethylamine<sup>32</sup> resulting in the formation of desired products (Fig. 3). Reaction progress was checked by periodic thin layer chromatography (TLC). Chemical structures of compounds 1–27 were elucidated by using spectroscopic techniques such as EI-MS, HREI-MS,  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$ .

To confirm the stereochemical assignment of iminic double bond, NOESY (nuclear overhauser enhancement spectroscopy) was performed on a representative derivative **7**. Many interactions were observed in the NOESY spectrum, some of them confirmed the (*Z*) stereochemistry of the iminic double bond. Strong NOESY interaction between the NH proton and CH-2 of chromone ring was observed which can only be observed in case of *Z*-isomer. Similarly, absence of NOESY interaction between the NH and H-C=N protons further confirms the *Z*-stereochemistry of resulting isomer (Fig. 4). Other interactions such as strong interactions of H-5' with H-2''/H-6'' and H-3''/H-5'' as well as weak interactions of iminic proton with H-5 and H-8 were also observed.

**Mass Spectrometry.** Low resolution EI-MS of compound **7** displayed the molecular ion peak  $[\text{M}]^+$  at  $m/z$  425 and  $[\text{M} + 2]^+$  at  $m/z$  427 which confirmed the presence of bromine substitution. High resolution EI-MS displayed  $[\text{M}]^+$  at  $m/z = 424.9806$  with a composition of  $\text{C}_{19}\text{H}_{12}\text{BrN}_3\text{O}_2\text{S}$  (Calcd. 424.9834) which also confirmed the formation of desired compound. Low resolution EI-MS spectrum showed many characteristic fragments. A fragmentation pattern is discussed below.



**Figure 5.** Key EI-MS fragmentation of compound 7.

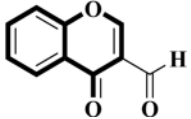
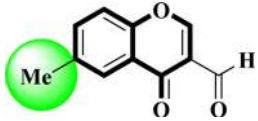
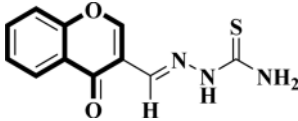
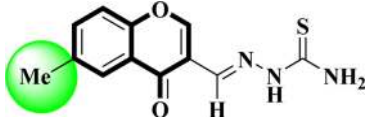
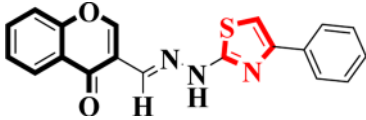
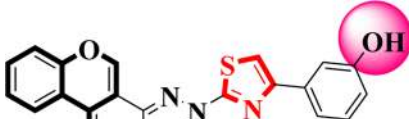
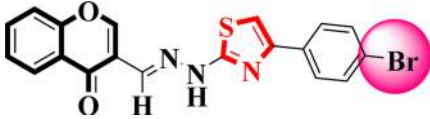
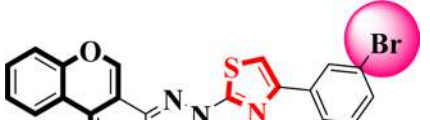
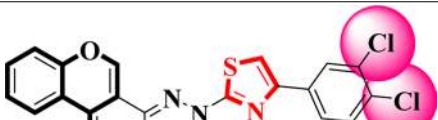
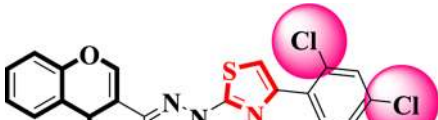
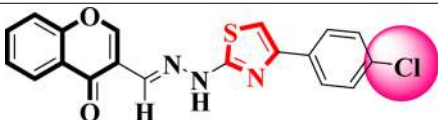
**Structure-fragmentation pattern.** The molecular ion at  $m/z$  425 was fragmented to afford a radical cation at  $m/z$  305 by the neutral loss of 7-oxabicyclo[4.2.0]octa-1,3,5-trien-8-one molecule. The resulting radical cation further cleaved to give a cation at  $m/z$  280 by the loss of hydrogen cyanide radical. Cation obtained at  $m/z$  280 undergo two successive cleavage to afford radical cations at  $m/z$  254 and  $m/z$  212 with the losses of nitrile radical and neutral formimidamide molecule, respectively. Similarly, molecular ion at  $m/z$  425 also fragmented in another fashion to give a chromone radical cation at  $m/z$  146 by the neutral loss of rest of the molecule (Fig. 5).

**In vitro biological activities.** All hybrid hydrazinyl thiazole substituted chromones 5–27 along with the intervening intermediates 1–4 were evaluated to check their  $\alpha$ -amylase inhibitory<sup>37–39</sup>, DPPH<sup>40–42</sup> and ABTS<sup>43</sup> radical scavenging, and cytotoxic<sup>44</sup> activities. Results depicted in Table 1 showed that all compounds displayed comparable  $\alpha$ -amylase inhibitory activities in the range of  $IC_{50} = 2.186 \pm 0.03$ – $3.405 \pm 0.21 \mu M$  as compared to standard acarbose  $IC_{50} = 1.9 \pm 0.07 \mu M$ . All analogs were also showed good DPPH and ABTS radical scavenging activities in the ranges of  $IC_{50} = 0.09 \pm 0.17$ – $2.233 \pm 0.6 \mu M$  and  $IC_{50} = 0.584 \pm 0.07$ – $3.738 \pm 0.6 \mu M$ , respectively, as compared to standard ascorbic acid ( $IC_{50} = 0.33 \pm 0.18 \mu M$  and  $IC_{50} = 0.53 \pm 0.3 \mu M$ , respectively). It is worth-mentioning that all compounds were found to be non-toxic when tested on NIH-3T3 mouse fibroblast cell line by using the standard MTT colorimetric assay<sup>44</sup>.

**Structure-activity relationship (SAR) for  $\alpha$ -amylase inhibitory activity.** Synthetic molecules possess very unique structural features (Fig. 2) and these features or pharmacophores are cordially playing their role in exhibiting  $\alpha$ -amylase inhibition. However, the difference in the inhibitory activity is attributed by the varying features or groups present at aromatic rings *i.e.*  $R_1$  and  $R_2$ . Figure 6 revealed that intervening intermediates 1 and 2 showed similar but two fold less  $\alpha$ -amylase inhibition as compared to standard acarbose. However, thiosemicarbazone intermediate 4 with methyl substitution on chromone found to be better active than intermediate 3 which shows that the methyl substitution is influencing the binding interactions of compound with the active site of enzyme. The influence of methyl group is seemingly persists after the thiazole ring formation. Compound 5 with unsubstituted phenyl ring ( $R_2$ ) showed inhibitory activity comparable to standard. Incorporation of methyl group as  $R_1$  in compound 16 leads to slight decreased  $\alpha$ -amylase inhibition potential. Compounds 6, 17, and 18, having phenol as  $R_2$ , showed decreased  $\alpha$ -amylase inhibition as compared to the unsubstituted analogs 5 and 16. Comparison of inhibitory activity of compound 6 with closely related compounds 17 and 18 revealed that incorporation of methyl group leads to increased activity. Amongst the halogens (Br and Cl) containing compounds, derivatives 7 and 8 with 4''-bromo and 3''-bromo phenyl group as  $R_2$ , respectively, showed good and comparable  $\alpha$ -amylase inhibition. However, analogs 20 and 19 with an additional methyl group as  $R_1$ , showed increased activity. In case of mono-chlorinated derivatives, 6-methyl substituted compounds 23 and 24 having 4''-chloro and 3''-chloro substitutions on phenyl ring ( $R_2$ ), respectively, showed almost similar  $\alpha$ -amylase inhibitory activity. Structurally similar analogs without methyl group as  $R_1$ , *i.e.* 11 and 12 displayed slight decreased activities than 23 and 24. Dichloro substituted derivatives 9, 10, 21, and 22 were found to be more active than mono chloro substituted analogs which confirmed that chloro groups are actively participating in the activity. Amongst the 3''-nitro substituted derivatives, compound 25 with methyl substitution as  $R_1$ , demonstrated better  $\alpha$ -amylase inhibitory activity as compared to compound 13. In case of 4''-cyano substituted analogs 14 and 26, both compounds showed almost similar activities which showed that presence of methyl group in compound 26 didn't really make any difference in the activity. Compounds 15 and 27 having biphenyl ring as  $R_2$ , also showed good activities. Amongst them compound 27 with methyl substitutions as  $R_2$ , showed superior activity as compared to compound 15 which lacks the methyl group (Fig. 6).

**Structure-activity relationship (SAR) for DPPH and ABTS radical scavenging activities.** Variation in the DPPH and ABTS radical scavenging activities are resulted of varying structural features of compounds such as  $R_1$  and  $R_2$ . Figure 7 depicts that the intervening intermediates 1 and 2 showed similar DPPH



Compounds	Structures	$\alpha$ -Amylase inhibitory activity	DPPH radical scavenging activity	ABTS radical scavenging activity
		IC <sub>50</sub> $\pm$ SEM <sup>a</sup>	IC <sub>50</sub> $\pm$ SEM <sup>a</sup>	IC <sub>50</sub> $\pm$ SEM <sup>a</sup>
1		3.405 $\pm$ 0.21	2.23 $\pm$ 0.79	3.738 $\pm$ 0.6
2		3.400 $\pm$ 0.08	2.233 $\pm$ 0.6	2.23 $\pm$ 0.06
3		3.401 $\pm$ 0.18	1.905 $\pm$ 0.06	1.916 $\pm$ 0.09
4		2.842 $\pm$ 0.08	1.145 $\pm$ 0.12	1.258 $\pm$ 0.1
5		2.826 $\pm$ 0.06	1.113 $\pm$ 0.15	1.083 $\pm$ 0.15
6		3.382 $\pm$ 0.23	1.895 $\pm$ 0.03	1.892 $\pm$ 0.01
7		2.706 $\pm$ 0.05	0.892 $\pm$ 0.09	0.83 $\pm$ 0.25
8		2.707 $\pm$ 0.05	0.912 $\pm$ 0.23	0.914 $\pm$ 0.19
9		2.64 $\pm$ 0.05	0.314 $\pm$ 0.12	0.784 $\pm$ 0.12
10		2.335 $\pm$ 0.08	0.09 $\pm$ 0.17	0.677 $\pm$ 0.26
11		2.741 $\pm$ 0.1	0.922 $\pm$ 0.22	0.918 $\pm$ 0.14

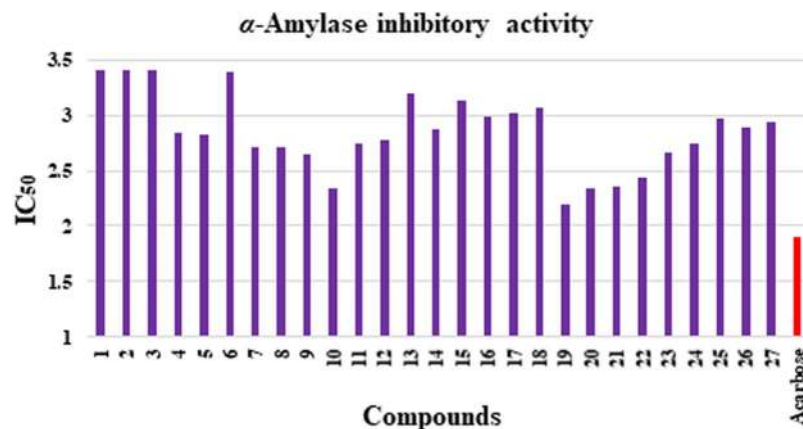
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Compounds	Structures	$\alpha$ -Amylase inhibitory activity	DPPH radical scavenging activity	ABTS radical scavenging activity
		IC <sub>50</sub> ± SEM <sup>a</sup>	IC <sub>50</sub> ± SEM <sup>a</sup>	IC <sub>50</sub> ± SEM <sup>a</sup>
12		2.767 ± 0.07	1.050 ± 0.03	1.083 ± 0.08
13		3.197 ± 0.13	1.745 ± 0.04	1.892 ± 0.05
14		2.877 ± 0.05	1.294 ± 0.1	1.261 ± 0.07
15		3.131 ± 0.28	1.406 ± 0.05	1.643 ± 0.04
16		2.99 ± 0.05	1.368 ± 0.2	1.319 ± 0.2
17		3.024 ± 0.04	1.336 ± 0.09	1.384 ± 0.05
18		3.065 ± 0.02	1.659 ± 0.6	1.734 ± 0.05
19		2.186 ± 0.03	0.781 ± 0.18	0.584 ± 0.07
20		2.335 ± 0.08	0.611 ± 0.29	0.66 ± 0.16
21		2.357 ± 0.1	0.684 ± 0.13	0.71 ± 0.09
22		2.428 ± 0.05	0.688 ± 0.17	0.719 ± 0.1
23		2.669 ± 0.06	0.84 ± 0.1	0.82 ± 0.18

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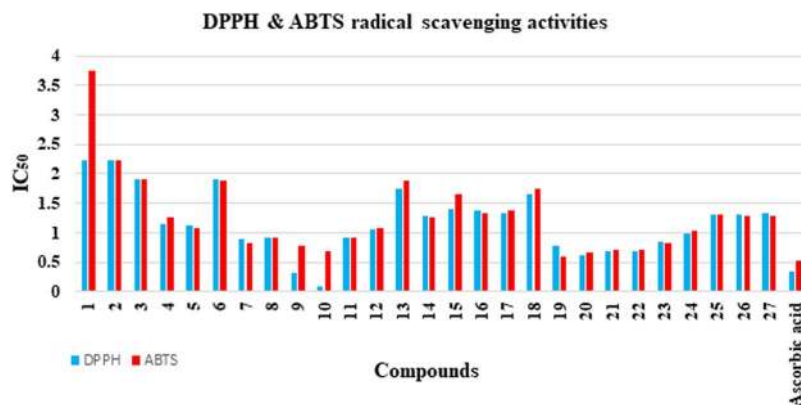
Compounds	Structures	$\alpha$ -Amylase inhibitory activity	DPPH radical scavenging activity	ABTS radical scavenging activity
		IC <sub>50</sub> ± SEM <sup>a</sup>	IC <sub>50</sub> ± SEM <sup>a</sup>	IC <sub>50</sub> ± SEM <sup>a</sup>
24		2.749 ± 0.15	0.99 ± 0.12	1.02 ± 0.04
25		2.97 ± 0.09	1.307 ± 0.06	1.303 ± 0.11
26		2.887 ± 0.08	1.295 ± 0.13	1.277 ± 0.04
27		2.937 ± 0.06	1.336 ± 0.07	1.29 ± 0.07
Standards	Acarbose <sup>b</sup>	1.9 ± 0.07		
	Ascorbic acid <sup>c</sup>		0.33 ± 0.18	0.53 ± 0.3

**Table 1.**  $\alpha$ -Amylase inhibitory, DPPH, and ABTS radical scavenging activities of hydrazinyl thiazole substituted chromones 5–27, and intervening intermediates 1–4. IC<sub>50</sub><sup>a</sup> (Mean ± Standard deviation); Acarbose<sup>b</sup> (Standard Inhibitor for  $\alpha$ -amylase inhibitory activity); Ascorbic acid<sup>c</sup> (Standard for DPPH and ABTS radical scavenging activity).



**Figure 6.** Comparison of  $\alpha$ -amylase inhibitory activity of compounds.

radical scavenging activities, however, compound 2 with methyl group as R<sub>1</sub> showed better ABTS radical scavenging potential than compound 1. Similarly, methyl bearing thiosemicarbazone intermediate 4 showed enhanced DPPH and ABTS radical scavenging activities as compared to non-methylated compound 3. In case of thiazole ring containing compounds 5–27, compound 5 with unsubstituted phenyl ring (R<sub>2</sub>) showed comparable DPPH and ABTS radical scavenging activities to standards. Incorporation of methyl group as R<sub>1</sub> in compound 16 leads to slight decreased DPPH and ABTS radical scavenging activities. Phenol ring (R<sub>2</sub>) containing compounds 6, 17, and 18, demonstrated diminished DPPH and ABTS radical scavenging activities as compared to the unsubstituted analogs 5 and 16. In case of bromo substituted compounds, compound 7 and 8 with 4''-bromo and 3''-bromo phenyl group as R<sub>2</sub>, respectively, showed good and comparable DPPH and ABTS radical scavenging activities. However, their structurally similar analogs 19 and 20 with an additional methyl group as R<sub>1</sub>, showed enhanced activities. In case of mono-chlorinated derivatives, 4-chloro substituted derivative 23 showed better DPPH and ABTS radical scavenging activities as compared to 3-chloro substituted analog 24. Nonetheless, their non-methylated structurally similar analogs *i.e.* 11 and 12 displayed slight decreased activities. Dichloro substituted analogs 9, 10, 21, and 22 were showed superior activities than mono chloro substituted analogs. 4''-Cyano



**Figure 7.** Comparison of DPPH & ABTS radical scavenging activities of compounds.

substituted analogs **14** and **26** showed almost similar activities. Furthermore, 3'-nitro substituted derivative **25** with methyl substitution as R<sub>1</sub>, demonstrated better DPPH and ABTS radical scavenging activities as compared to compound **13**. Compounds **15** and **27** with biphenyl ring as R<sub>2</sub>, also showed good activities (Fig. 7).

Limited structure-activity relationship suggested that all compounds showed almost closed  $\alpha$ -amylase inhibitory, DPPH, and ABTS radical scavenging activities. It indicates that all structural features including R<sub>1</sub> and R<sub>2</sub> are positively contributing in the activities. However, it was noticed that the halogen bearing molecules were found to have better activities than other groups such as OH, CN, NO<sub>2</sub>, and Ph. As well as most of the compounds having methyl group as R<sub>1</sub> were found to be more active than the compounds without methyl substitution. In order to understand the binding interactions of compounds (ligands) with the active site of  $\alpha$ -amylase enzyme, molecular modeling study was carried out.

**In silico studies.** MOE-Dock module implemented in MOE program<sup>45</sup> was utilized to explore the binding conformations of the compounds within the active site of  $\alpha$ -amylase enzyme. The default parameters of MOE-Dock program were used in the docking protocol. At the end of docking experiment, the best conformations on the basis of docking score were analyzed for hydrogen bonding/arene-arene/arene-cation interactions. From the docking calculation study, it was observed that the top-ranked conformations of almost all compounds were well accommodated inside the active site of  $\alpha$ -amylase enzyme and were involved in various type of interactions with the active site residues of  $\alpha$ -amylase enzyme. *i.e.*, Trp58, Trp59, Tyr62, Leu162, Arg195, Asp197, Glu233, Asp300, Asp356 etc. The detail of the docking scores and interactions for all compounds are collected in Table 2. Compound **19** exhibited good inhibitory potential with docking score of  $-9.7919$  against  $\alpha$ -amylase enzyme. Such lower values indicated good fitness of the compound in the binding pocket of the target enzyme and formation of a stable inhibitor protein complex. Compound **20** also showed good but slightly inferior inhibitory potential as compared to compound **19** with docking score of  $-8.9694$  against  $\alpha$ -amylase (Table 2).

Compound **19** has shown good interactions with the active site residues of the receptor protein Asp197, His305 and Asp356 (Fig. 8a). Asp197 formed strong H-donor interaction with the compound and His305 is involved in a strong H-acceptor bond of  $E-0.3$  Kcal/mol (Table 2). Asp356 formed H-donor interaction with the -NH group of the ligand while Trp59 formed arene-arene linkage with the thiazole moiety of the compound. Compound **20** formed two H-donor, one H- $\pi$  and one arene-arene valuable interactions with the enzyme. Asp197 and Asp300 showed H-donor interactions with the compound. Trp59 and His101 formed arene-arene and cation- $\pi$  contact with the thiazole and benzene moiety of compound (Fig. 8b). The good inhibitory potency of the compound **19** is due to the different position of the bromine atom as compared to compound **20**. Presence of electronegative groups like halogens, observed to be actively participated in the activity and among halogens, Br containing compounds were found superior than Cl.

In case of compounds **21** and **22**, it was observed that both compounds have almost similar structure, biological activities and also similar binding interactions with the polar residues. Docking conformation of compound **21** showed that it was making two H-donor, two cation- $\pi$  and one arene-arene contacts with the active residues of the enzyme (Fig. 8c). Compound **22** formed four H-donor and one  $\pi$ -H interactions with the Tyr62, Asp197, Glu233, Asp356 and Ala198 residues of the enzyme, respectively (Fig. 8d). The good inhibitory potential of the compound **21** over compound **22** is due to the diverse positions of the halogen group (-Cl).

The compounds having moderate biological activities such as **17** and **18**, having similar structure demonstrated almost similar binding pattern as shown in Table 2 and Fig. 8e and f. The more effectiveness of the compound **17** as compared to the compound **18** is due to the electronegative OH group at *meta* position. Overall a good correlation was observed between the docking study and biological evaluation of active compounds. The correlation graph and the correlation coefficient values are given in Fig. 9.

## Conclusion

New synthetic hybrid molecules of hydrazinyl thiazole substituted chromones **5–27** along with intervening intermediates **1–4** were evaluated for *in vitro*  $\alpha$ -amylase inhibitory, DPPH and ABTS radical scavenging activities. Limited structure-activity relationship revealed that the compounds bearing halogen were found to be more active than the other groups such as OH, CN, NO<sub>2</sub>, and Ph, and compounds with methyl group as R<sub>1</sub> were also



Compounds	Docking scores	Interactions Report				
		Ligand	Receptor	Interaction	Distance	E (kcal/mol)
1	−5.9012	C 13	OD1 ASP 197	H-donor	3.72	−0.6
		6-ring	CD2 LEU 162	$\pi$ -H	4.23	−0.7
2	−5.1034	—				
3	−5.239	N 22	OD1 ASP 197	H-donor	3.26	−1.6
		S 25	NH2 ARG 195	H-acceptor	4	−0.9
		6-ring	5-ring TRP 59	$\pi$ - $\pi$	3.81	−0.0
4	−7.0132	N 22	OD1 ASP 300	H-donor	3.15	−2.9
		N 25	OD2 ASP 300	H-donor	3.16	−2.3
5	−7.1717	6-ring	5-ring TRP 59	$\pi$ - $\pi$	3.9	−0.0
		6-ring	6-ring TRP 59	$\pi$ - $\pi$	3.93	−0.0
6	−6.13	O 38	OD2 ASP 300	H-donor	3.28	−1.6
7	−7.299	BR 38	OE1 GLU 233	H-donor	3.71	−1.9
		6-ring	CG PRO 54	$\pi$ -H	4.08	−0.9
8	−7.2813	S 27	O TYR 62	H-donor	4.35	−0.8
		6-ring	6-ring TRP 59	$\pi$ - $\pi$	3.9	−0.0
9	−7.9712	CL 37	OE1 GLU 233	H-donor	3.58	−1.4
		6-ring	N GLY 306	$\pi$ -H	4.28	−0.6
		6-ring	N GLY 306	$\pi$ -H	3.81	−0.7
10	−9.7828	N 20	6-ring TYR 62	H- $\pi$	4.68	−0.8
		6-ring	6-ring TRP 59	$\pi$ - $\pi$	3.94	−0.0
11	−7.1399	S 27	OD1 ASP 197	H-donor	4.11	−0.7
		6-ring	CD1 LEU 162	$\pi$ -H	4.5	−0.6
		6-ring	5-ring TRP 59	$\pi$ - $\pi$	3.9	−0.0
12	−7.1292	S 27	OD2 ASP 356	H-donor	3.71	−0.9
13	−6.2391	S 27	O THR 163	H-donor	4.07	−0.6
14	−6.9892	5-ring	CB TRP 59	$\pi$ -H	4.48	−0.9
15	−6.1362	N 20	O THR 163	H-donor	3.02	−4.7
		S 27	O TRP 59	H-donor	4.28	−0.5
16	−6.6492	S 26	OD1 ASP 356	H-donor	4.41	−0.4
17	−6.5001	S 26	OD1 ASP 197	H-donor	4.23	−1.5
		6-ring	6-ring TRP 59	$\pi$ - $\pi$	3.51	−0.0
		6-ring	5-ring TRP 59	$\pi$ - $\pi$	3.63	−0.0
		6-ring	5-ring TRP 59	$\pi$ - $\pi$	3	−0.0
18	−6.4289	O 41	OD1 ASP 197	H-donor	1.7	−5.3
		6-ring	6-ring TRP 59	$\pi$ - $\pi$	3.75	−0.0
		6-ring	5-ring TRP 59	$\pi$ - $\pi$	3.78	−0.0
19	−9.7919	C 1	OD1 ASP 197	H-donor	2.2	−0.3
		N 19	OD1 ASP 356	H-donor	3.3	−0.5
		O 15	CD2 HIS 305	H-acceptor	3.8	−0.3
		5-ring	6-ring TRP 59	$\pi$ - $\pi$	3.79	−0.0
20	−8.9694	C 1	OD1 ASP 197	H-donor	2.4	−0.3
		C 4	OD1 ASP 300	H-donor	1.9	−0.0
		C 8	5-ring HIS 101	H- $\pi$	4.87	−0.2
		5-ring	5-ring TRP 59	$\pi$ - $\pi$	3.94	−0.0
21	−8.5183	C 28	OD1 ASP 300	H-donor	2.4	−1
		S 30	OD2 ASP 356	H-donor	3.24	−1.6
		C 16	6-ring TRP 59	H- $\pi$	3.6	−0.3
		N 19	5-ring TRP 59	H- $\pi$	3.47	−1.9
		6-ring	6-ring TRP 58	$\pi$ - $\pi$	4.6	−0.2
22	−8.2417	C 8	OD1 ASP 356	H-donor	2.31	−0.5
		C 24	OD1 ASP 197	H-donor	3.33	−0.3
		S 26	O TYR 62	H-donor	3.58	−0.8
		CL 40	OE1 GLU 233	H-donor	3.31	−1.4
		6-ring	CB ALA 198	$\pi$ -H	3.66	−0.2
23	−7.3154	C 24	OD2 ASP 197	H-donor	2.96	−1.8
24	−7.3456	C 24	OD1 ASP 300	H-donor	3.02	−1.6

Continued

Compounds	Docking scores	Interactions Report				
		Ligand	Receptor	Interaction	Distance	E (kcal/mol)
25	-6.5103	5-ring	6-ring TRP 59	$\pi$ - $\pi$	3.92	-0.0
26	-6.8201	O 15	OG1 THR 163	H-acceptor	3.16	-0.7
27	-6.7643	S 26	OD1 ASP 197	H-donor	2.9	-1.3
		5-ring	CD2 LEU 162	$\pi$ -H	4.34	-0.6
Standard	-11.843	C 19	OD1 ASP 300	H-donor	3.42	-0.7
		C 36	OD1 ASP 300	H-donor	3.09	-1.2
		C 36	OD2 ASP 300	H-donor	2.96	-0.8
		O 61	OD1 ASP 197	H-donor	2.67	-4.2
		O 65	OD1 ASP 356	H-donor	2.83	-3.3
		O 69	OD1 ASP 197	H-donor	2.6	-1.6
		O 79	OG1 THR 163	H-donor	2.81	-1.8
		C 5	6-ring TRP 59	H- $\pi$	3.64	-0.6

**Table 2.** Docking scores and report of predicted interactions of docked conformations.

found better active than the compounds without methyl substitution. All compounds showed good activities as compared to respective standards and also found to be non-toxic. Current study has identified a whole series of lead molecules which can be used in further advance research in order to obtain a powerful inhibitor for  $\alpha$ -amylase enzyme for the development of insulin-independent antidiabetic agents.

## Experimental

**Materials and Methods.** All chemicals were purchased from Sigma-Aldrich, USA. All reagents were of analytical grade and used as received.  $^1\text{H}$  and  $^{13}\text{C}$ -NMR experiments were performed on Avance Bruker AM 300, 400, and 500 MHz instruments. Electron impact mass spectrometric (EI-MS and HREI-MS) experiments were carried out on Finnigan MAT-311A (Germany) mass spectrometer. Thin layer chromatography (TLC) was performed on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). TLC chromatograms were visualized under UV light at 254 and 365 nm or by applying iodine vapors. Melting points of the compounds were determined on a Stuart<sup>®</sup> SMP10 melting point apparatus and are uncorrected.

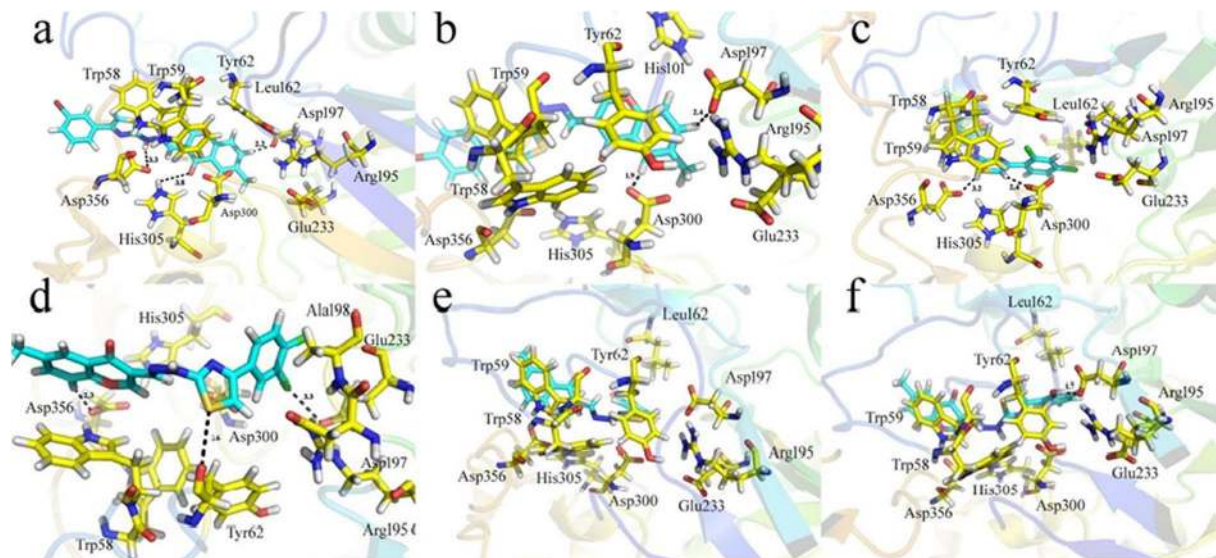
**General procedure for the synthesis of 3-formyl chromone derivatives 1 and 2.** Dry dimethyl formamide (50 mmol) was taken in round-bottomed flask of 250 mL and 50 mmol of  $\text{POCl}_3$  was added drop wise into it with constant stirring at room temperature. After the complete addition of  $\text{POCl}_3$ , reaction mixture was heated for at least 2 h at  $50^\circ\text{C}$ . Then 2-hydroxy acetophenone/2-hydroxy-5-methyl acetophenone was added into it and further heated for 5 h at  $70^\circ\text{C}$ . Reaction completion was monitored by TLC.

**General procedure for the synthesis of thiosemicarbazone derivatives of chromone 3 and 4.** Chromone derivatives 1/2 (1 mmol) and thiosemicarbazide (1 mmol) were taken in 15 mL of ethanol into a 100 mL round-bottomed flask. Then few drops of glacial acetic acid were added into the reaction mixture and refluxed for 2 h. Course of reaction was checked by TLC analysis. Precipitates were appeared in the reaction flask which were collected *via* filtration, washed with distilled water, and dried in air. Solid products were crystallized from ethyl acetate.

**General procedure for the synthesis of hybrid hydrazinyl thiazole chromones 5–27.** Thiosemicarbazone derivative 3/4 (1 mmol) and phenacyl bromide derivative (1 mmol) were taken in 15 mL of ethanol into a 100 mL round-bottomed flask. Triethylamine (1 mmol) was added into the reaction mixture and refluxed for 3 to 4 h. Completion of reaction was checked by TLC analysis. After reaction completion, reaction flask was kept overnight at room temperature. Precipitates were appeared in the reaction flask which were filtered, washed with distilled water, and dried in air. Solid compounds were crystallized from ethyl acetate.

**4-Oxo-4H-chromene-3-carbaldehyde (1).** Yield: 75%; M.p.:  $150$ – $153^\circ\text{C}$ ;  $^1\text{H}$ -NMR (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  10.11 (s, 1H, H-C=O), 8.91 (s, 1H, H-2), 8.15 (dd,  $J_{5,7}=1.2$ ,  $J_{5,6}=8.0$  Hz, 1H, H-5), 7.90 (dt,  $J_{7,5}=1.6$ ,  $J_{7,6}=J_{7,8}=8.4$  Hz, 1H, H-7), 7.76 (d,  $J_{8,7}=8.5$  Hz, 1H, H-8), 7.60 (t,  $J_{6,5}=J_{6,7}=7.6$  Hz, 1H, H-6);  $^{13}\text{C}$ -NMR (300 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  181.3, 174.8, 163.4, 155.5, 135.1, 126.6, 125.2, 124.6, 119.9, 118.8; EI-MS  $m/z$  (% rel. abund.): 174 ( $\text{M}^+$ , 7), 146 (100), 120 (76), 104 (76), 92 (42); HREI-MS Calcd for  $\text{C}_{10}\text{H}_7\text{O}_3$ ;  $m/z=174.0317$ , found 174.0382.

**6-Methyl-4-oxo-4H-chromene-3-carbaldehyde (2).** Yield: 73%; M.p.:  $173$ – $175^\circ\text{C}$ ;  $^1\text{H}$ -NMR (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  10.11 (s, 1H, H-C=O), 8.89 (s, 1H, H-2), 7.93 (bd.s, 1H, H-5), 7.71 (m, 2H, H-7, 8), 2.44 (s, 3H,  $\text{CH}_3$ );  $^{13}\text{C}$ -NMR (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  188.3, 174.8, 163.1, 153.8, 136.4, 136.0, 124.5, 124.3, 119.8, 118.6, 20.3; EI-MS  $m/z$  (% rel. abund.): 188 ( $\text{M}^+$ , 13), 160 (100), 134 (95), 118 (37), 106 (25), 90 (48); HREI-MS Calcd for  $\text{C}_{11}\text{H}_8\text{O}_3$ ;  $m/z=188.0473$ , found 188.0483.



**Figure 8.** Docking conformations of compounds on  $\alpha$ -amylase enzyme. (a) 3D binding mode of compound 19. (b) 3D binding mode of compound 20. (c) 3D binding mode of compound 21. (d) 3D binding mode of compound 22. (e) 3D binding mode of compound 17. (f) 3D binding mode of compound 18 in binding cavity of  $\alpha$ -amylase enzyme. Ligands are shown in cyan color.



**Figure 9.** A correlation graph for predicted docking score and  $IC_{50}$  values.

**(Z)-2-((4-Oxo-4H-chromen-3-yl)methylene)hydrazinecarbothioamide (3).** Yield: 72%; M.p.: 240–242 °C;  $^1H$ -NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  11.52 (s, 1 H, NH), 9.16 (s, 1 H, -N=CH-), 8.24 (s, 1 H, NH), 8.17 (s, 1 H, NH), 8.11 (dd,  $J_{5,6} = 0.8$ ,  $J_{5,7} = 8.0$  Hz, 1 H, H-5), 8.08 (s, 1 H, H-2), 7.85 (dt,  $J_{7,5} = 1.2$ ,  $J_{7,6} = J_{7,8} = 8.4$  Hz, 1 H, H-7), 7.72 (d,  $J_{8,7} = 8.4$  Hz, 1 H, H-8), 7.55 (t,  $J_{6,5} = J_{6,7} = 7.6$  Hz, 1 H, H-6);  $^{13}C$ -NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  178.0, 174.7, 155.7, 155.1, 134.4, 133.9, 125.9, 125.1, 123.3, 118.6, 118.3; EI-MS  $m/z$  (% rel. abund.): 247 ( $M^+$ , 34), 205 (42), 172 (100), 146 (16), 120 (47), 92 (39); HREI-MS Calcd for  $C_{11}H_9N_3O_2S$ :  $m/z = 247.0415$ , found 247.0413.

**(Z)-2-((6-Methyl-4-oxo-4H-chromen-3-yl)methylene)hydrazinecarbothioamide (4).** Yield: 74%; M.p.: 245–247 °C;  $^1H$ -NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  11.51 (s, 1 H, NH), 9.13 (s, 1 H, -N=CH-), 8.23 (s, 1 H, NH), 8.17 (s, 1 H, NH), 8.06 (bd.s, 1 H, H-5), 7.88 (s, 1 H, H-2), 7.66 (m, 2 H, H-7, 8), 2.43 (s, 3 H, CH<sub>3</sub>);  $^{13}C$ -NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  178.0, 174.6, 155.0, 154.0, 135.6, 135.5, 134.1, 124.3, 123.0, 118.4, 118.1, 20.4; EI-MS  $m/z$  (% rel. abund.): 261 ( $M^+$ , 45), 219 (34), 202 (30), 186 (100), 160 (10), 134 (81); HREI-MS Calcd for  $C_{12}H_{11}N_3O_2S$ :  $m/z = 261.0572$ , found 261.0557.

**(Z)-3-((2-(4-Phenylthiazol-2-yl)hydrazono)methyl)-4H-chromen-4-one (5).** Yield: 70%; M.p.: 223–225 °C;  $^1H$ -NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  12.23 (s, 1 H, NH), 8.72 (s, 1 H, -N=CH-), 8.14 (s, 1 H, H-5'),

8.14 (d,  $J_{5,6} = 8.0$  Hz, 1 H, H-5), 7.85 (d,  $J_{2'',3''/6'',5''} = 7.2$  Hz, 3 H, H-7, 2'', 6''), 7.72 (d,  $J_{8,7} = 8.4$  Hz, 1 H, H-8), 7.56 (t,  $J_{6,5} = J_{6,7} = 7.6$  Hz, 1 H, H-6), 7.41 (t,  $J_{3'',2''/5'',6''} = J_{3'',4''/5'',4''} = 7.2$  Hz, 2 H, H-3'', 5''), 7.33 (s, 1 H, H-2), 7.31 (t,  $J_{4'',3''} = J_{4'',5''} = 11.2$  Hz, 1 H, H-4'');  $^{13}\text{C-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  174.5, 171.1, 155.6, 155.2, 150.3, 134.5, 133.8, 133.2, 129.1, 129.1, 128.6, 127.4, 127.4, 125.8, 125.2, 123.4, 118.5, 118.2, 105.1; EI-MS  $m/z$  (% rel. abund.): 347 ( $\text{M}^+$ , 100), 227 (65), 200 (11), 176 (52), 146 (21); HREI-MS Calcd for  $\text{C}_{19}\text{H}_{13}\text{N}_3\text{O}_2\text{S}$ :  $m/z = 347.0728$ , found 347.0731.

**(Z)-3-((2-(4-(3-Hydroxyphenyl)thiazol-2-yl)hydrazono)methyl)-4H-chromen-4-one (6).** Yield: 80%; M.p.: 235–237 °C;  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  11.52 (s, 1 H, NH), 9.16 (s, 1 H, -N=CH-), 8.24 (bd.s, 1 H, H-2''), 8.17 (s, 1 H, H-5'), 8.11 (m, 4 H, H-5, 4'', 5'', 6''), 7.85 (t,  $J_{7,6} = J_{7,8} = 8.0$  Hz, 1 H, H-7), 7.72 (d,  $J_{8,7} = 8.4$  Hz, 2 H, H-2, 8), 7.55 (t,  $J_{6,5} = J_{6,7} = 7.6$  Hz, 1 H, H-6);  $^{13}\text{C-NMR}$  (300 MHz, DMSO- $d_6$ ):  $\delta$  177.8, 171.4, 157.3, 155.8, 155.1, 150.1, 134.7, 134.3, 133.7, 130.5, 125.8, 125.0, 123.2, 120.2, 118.8, 118.2, 116.1, 115.7, 105.2; EI-MS  $m/z$  (% rel. abund.): 247 (28), 213 (8), 205 (45), 188 (34), 172 (85), 146 (16), 120 (100); HREI-MS Calcd for  $\text{C}_{11}\text{H}_9\text{N}_3\text{O}_2\text{S}$ :  $m/z = 247.0415$ , found 247.0404.

**(Z)-3-((2-(4-(4-Bromophenyl)thiazol-2-yl)hydrazono)methyl)-4H-chromen-4-one (7).** Yield: 75%; M.p.: 230–232 °C;  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  12.23 (s, 1 H, NH), 8.72 (s, 1 H, -N=CH-), 8.15 (s, 1 H, H-5'), 8.13 (d,  $J_{5,6} = 8.0$  Hz, 1 H, H-5), 7.86 (t,  $J_{7,6} = J_{7,8} = 7.2$  Hz, 1 H, H-7), 7.80 (d,  $J_{3'',2''/5'',6''} = 8.4$  Hz, 2 H, H-3'', 5''), 7.72 (d,  $J_{8,7} = 8.4$  Hz, 1 H, H-8), 7.60 (d,  $J_{2'',3''/6'',5''} = 8.4$  Hz, 2 H, H-2'', 6''), 7.56 (t,  $J_{6,5} = J_{6,7} = 7.6$  Hz, 1 H, H-6), 7.41 (s, 1 H, H-2);  $^{13}\text{C-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  177.0, 171.3, 155.8, 155.0, 150.4, 134.7, 133.8, 132.2, 131.5, 131.5, 127.5, 127.5, 125.8, 125.0, 123.4, 123.1, 118.7, 118.2, 105.2; EI-MS  $m/z$  (% rel. abund.): 425 ( $\text{M}^+$ , 97), 427 ( $\text{M} + 2$ , 100), 305 (76), 280 (9), 254 (40), 212 (12), 146 (30); HREI-MS Calcd for  $\text{C}_{19}\text{H}_{12}\text{BrN}_3\text{O}_2\text{S}$ :  $m/z = 424.9834$ , found 424.9806.

**(Z)-3-((2-(4-(3-Bromophenyl)thiazol-2-yl)hydrazono)methyl)-4H-chromen-4-one (8).** Yield: 78%; M.p.: 225–227 °C;  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  12.25 (s, 1 H, NH), 8.72 (s, 1 H, -N=CH-), 8.15 (s, 1 H, H-5'), 8.13 (d,  $J_{5,6} = 8.8$  Hz, 1 H, H-5), 8.03 (bd.s, 1 H, H-2''), 7.86 (m, 2 H, H-4'', 5''), 7.72 (d,  $J_{6'',5''} = 8.0$  Hz, 1 H, H-6''), 7.56 (t,  $J_{7,6} = J_{7,8} = 7.2$  Hz, 1 H, H-7), 7.49 (bd.s, 1 H, H-2), 7.49 (d,  $J_{8,7} = 9.2$  Hz, 1 H, H-8), 7.38 (t,  $J_{6,5} = J_{6,7} = 8.0$  Hz, 1 H, H-6);  $^{13}\text{C-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  177.2, 171.3, 155.8, 155.3, 150.1, 134.9, 122.2, 134.3, 133.7, 129.8, 129.4, 128.7, 125.8, 125.5, 125.0, 123.2, 118.7, 118.2, 105.1; EI-MS  $m/z$  (% rel. abund.): 425 ( $\text{M}^+$ , 93), 427 ( $\text{M} + 2$ , 100), 410 (11), 307 (78), 280 (11), 254 (42), 172 (26), 146 (28); HREI-MS Calcd for  $\text{C}_{19}\text{H}_{12}\text{BrN}_3\text{O}_2\text{S}$ :  $m/z = 424.9834$ , found 424.9842.

**(Z)-3-((2-(4-(3,4-Dichlorophenyl)thiazol-2-yl)hydrazono)methyl)-4H-chromen-4-one (9).** Yield: 73%; M.p.: 245–247 °C;  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  12.26 (s, 1 H, NH), 8.73 (s, 1 H, -N=CH-), 8.15 (s, 1 H, H-5'), 8.13 (d,  $J_{5,6} = 8.0$  Hz, 1 H, H-5), 8.07 (d,  $J_{2'',6''} = 2.0$  Hz, 1 H, H-2''), 7.85 (m, 2 H, H-7, 5''), 7.72 (d,  $J_{6'',5''} = 8.4$  Hz, 1 H, H-6''), 7.67 (d,  $J_{8,7} = 8.8$  Hz, 1 H, H-8), 7.56 (s, 1 H, H-2), 7.56 (t,  $J_{6,5} = J_{6,7} = 8.0$  Hz, 1 H, H-6);  $^{13}\text{C-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  177.7, 171.4, 155.8, 155.2, 150.4, 134.5, 133.7, 133.1, 132.6, 132.3, 130.8, 128.9, 127.2, 125.7, 125.3, 123.2, 118.7, 118.4, 105.1; EI-MS  $m/z$  (% rel. abund.): 415 ( $\text{M}^+$ , 94), 417 ( $\text{M} + 2$ , 64), 295 (100), 244 (46), 208 (12), 172 (21), 146 (17), 120 (13); HREI-MS Calcd for  $\text{C}_{19}\text{H}_{12}\text{Cl}_2\text{N}_3\text{O}_2\text{S}$ :  $m/z = 414.9949$ , found 414.9956.

**(Z)-3-((2-(4-(2,4-Dichlorophenyl)thiazol-2-yl)hydrazono)methyl)-4H-chromen-4-one (10).** Yield: 77%; M.p.: 243–245 °C;  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  12.24 (s, 1 H, NH), 8.73 (s, 1 H, -N=CH-), 8.15 (s, 1 H, H-5'), 8.13 (d,  $J_{5,6} = 8.0$  Hz, 1 H, H-5), 7.90 (d,  $J_{6'',5''} = 8.8$  Hz, 1 H, H-6''), 7.87 (t,  $J_{7,6} = J_{7,8} = 11.2$  Hz, 1 H, H-7), 7.72 (d,  $J_{8,7} = 8.4$  Hz, 1 H, H-8), 7.68 (d,  $J_{3'',5''} = 2.0$  Hz, 1 H, H-3''), 7.56 (t,  $J_{6,5} = J_{6,7} = 7.6$  Hz, 1 H, H-6), 7.51 (dd,  $J_{5'',3''} = 2.0$ ,  $J_{5'',6''} = 8.4$  Hz, H-5''), 7.42 (s, 1 H, H-2);  $^{13}\text{C-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  177.7, 171.1, 155.5, 155.2, 150.0, 135.8, 134.3, 133.8, 133.4, 130.8, 130.1, 128.2, 127.5, 125.8, 125.0, 123.4, 118.7, 118.2, 105.1; EI-MS  $m/z$  (% rel. abund.): 415 ( $\text{M}^+$ , 61), 417 ( $\text{M} + 2$ , 49), 380 (50), 295 (100), 244 (41), 202 (23), 172 (27); HREI-MS Calcd for  $\text{C}_{19}\text{H}_{12}\text{Cl}_2\text{N}_3\text{O}_2\text{S}$ :  $m/z = 414.9949$ , found 414.9936.

**(Z)-3-((2-(4-(4-Chlorophenyl)thiazol-2-yl)hydrazono)methyl)-4H-chromen-4-one (11).** Yield: 74%; M.p.: 239–241 °C;  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  12.23 (s, 1 H, NH), 8.72 (s, 1 H, -N=CH-), 8.15 (s, 1 H, H-5'), 8.13 (d,  $J_{5,6} = 8.0$  Hz, 1 H, H-5), 7.87 (d,  $J_{2'',3''/6'',5''} = 8.4$  Hz, 2 H, H-3'', 5''), 7.87 (t,  $J_{6,5} = J_{6,7} = 6.8$  Hz, 1 H, H-6), 7.72 (d,  $J_{8,7} = 8.4$  Hz, 1 H, H-8), 7.56 (t,  $J_{7,6} = J_{7,8} = 7.6$  Hz, 1 H, H-7), 7.46 (d,  $J_{3'',2''/5'',6''} = 8.4$  Hz, 2 H, H-2'', 6''), 7.40 (s, 1 H, H-2);  $^{13}\text{C-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  177.8, 171.6, 155.8, 155.0, 150.0, 134.5, 134.2, 133.8, 131.2, 128.5, 128.5, 127.1, 127.1, 125.7, 125.0, 123.4, 118.7, 118.2, 105.1; EI-MS  $m/z$  (% rel. abund.): 381 ( $\text{M}^+$ , 100), 383 ( $\text{M} + 2$ , 38), 261 (95), 234 (7), 210 (37), 168 (19), 146 (13); HREI-MS Calcd for  $\text{C}_{19}\text{H}_{12}\text{ClN}_3\text{O}_2\text{S}$ :  $m/z = 381.0339$ , found 381.0323.

**(Z)-3-((2-(4-(3-Chlorophenyl)thiazol-2-yl)hydrazono)methyl)-4H-chromen-4-one (12).** Yield: 75%; M.p.: 228–230 °C;  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  12.25 (s, 1 H, NH), 8.72 (s, 1 H, -N=CH-), 8.15 (s, 1 H, H-5'), 8.13 (d,  $J_{5,6} = 8.0$  Hz, 1 H, H-5), 7.88 (bd.s, 1 H, H-2''), 7.87 (m, 2 H, H-4'', 5''), 7.72 (d,  $J_{8,7} = 8.4$  Hz, 1 H, H-8), 7.56 (t,  $J_{7,6} = J_{7,8} = 7.6$  Hz, 1 H, H-7), 7.50 (bd.s, 1 H, H-2), 7.45 (t,  $J_{6,5} = J_{6,7} = 7.6$  Hz, 1 H, H-6), 7.36 (d,  $J_{6'',5''} = 8.0$  Hz, 1 H, H-6'');  $^{13}\text{C-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  176.7, 170.9, 155.7, 153.5, 148.9, 136.6, 134.5, 133.7, 133.4, 130.4, 127.2, 125.9, 125.2, 125.1, 124.0, 123.2, 118.6, 118.4, 105.4; EI-MS  $m/z$  (% rel. abund.): 381 ( $\text{M}^+$ , 100), 383 (36), 261 (94), 234 (8), 210 (49), 172 (21), 146 (26); HREI-MS Calcd for  $\text{C}_{19}\text{H}_{12}\text{ClN}_3\text{O}_2\text{S}$ :  $m/z = 381.0339$ , found 381.0307.

**(Z)-3-((2-(4-(3-Nitrophenyl)thiazol-2-yl)hydrazono)methyl)-4H-chromen-4-one (13).** Yield: 71%; M.p.: 235–237 °C;  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  12.35 (s, 1 H, NH), 8.74 (s, 1 H, -N=CH-), 8.66 (s, 1 H,



H-2''), 8.30 (d,  $J_{5,6} = 7.6$  Hz, 1 H, H-5), 8.16 (m, 3 H, H-5', 4'', 5''), 7.87 (t,  $J_{7,6} = J_{7,8} = 6.8$  Hz, 1 H, H-7), 7.72 (d,  $J_{8,7} = J_{6',5''} = 8.4$  Hz, 2 H, H-8, 6''), 7.67 (bd.s, 1 H, H-2), 7.56 (t,  $J_{6,5} = J_{6,7} = 7.6$  Hz, 1 H, H-6);  $^{13}\text{C-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  177.4, 171.4, 155.8, 155.2, 150.3, 148.5, 134.3, 133.8, 133.7, 133.5, 130.5, 125.8, 125.2, 123.8, 123.4, 122.6, 118.7, 118.3, 105.2; EI-MS  $m/z$  (% rel. abund.): 392 ( $\text{M}^+$ , 67), 375 (71), 272 (100), 221 (20), 172 (22), 146 (21), 120 (32); HREI-MS Calcd for  $\text{C}_{19}\text{H}_{12}\text{N}_4\text{O}_4\text{S}$ :  $m/z = 392.0579$ , found 392.0577.

**(Z)-4-(2-(2-((4-Oxo-4H-chromen-3-yl)methylene)hydrazinyl)thiazol-4-yl)benzotrile (14).**

Yield: 72%; M.p.: 225–227 °C;  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  12.30 (s, 1 H, NH), 8.73 (s, 1 H, -N=CH-), 8.16 (s, 1 H, H-5'), 8.13 (d,  $J_{5,6} = 6.8$  Hz, 1 H, H-5), 8.03 (d,  $J_{2'',3''/6'',5''} = 8.4$  Hz, 2 H, H-2'', 6''), 7.87 (d,  $J_{3'',2''/5'',6''} = 8.4$  Hz, 3 H, H-7, 3'', 5''), 7.72 (d,  $J_{8,7} = 8.4$  Hz, 1 H, H-8), 7.65 (s, 1 H, H-2), 7.56 (t,  $J_{6,5} = J_{6,7} = 7.2$  Hz, 1 H, H-6);  $^{13}\text{C-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  177.9, 171.6, 155.8, 155.2, 150.3, 140.9, 140.7, 134.5, 133.8, 131.7, 129.3, 129.3, 128.1, 128.1, 127.9, 127.9, 127.6, 127.1, 127.1, 125.8, 125.2, 123.4, 118.7, 118.2, 105.3; EI-MS  $m/z$  (% rel. abund.): 372 ( $\text{M}^+$ , 92), 252 (100), 225 (8), 201 (43), 172 (17), 159 (23), 146 (14); HREI-MS Calcd for  $\text{C}_{20}\text{H}_{12}\text{N}_4\text{O}_2\text{S}$ :  $m/z = 372.0681$ , found 372.0669.

**(Z)-3-((2-(4-(Biphenyl-4-yl)thiazol-2-yl)hydrazono)methyl)-4H-chromen-4-one (15).**

Yield: 79%; M.p.: 234–236 °C;  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  12.25 (s, 1 H, NH), 8.73 (s, 1 H, -N=CH-), 8.16 (s, 1 H, H-5'), 8.14 (d,  $J_{5,6} = 7.2$  Hz, 1 H, H-5), 7.95 (d,  $J_{2'',3''/6'',5''} = 8.0$  Hz, 2 H, H-2'', 6''), 7.85 (t,  $J_{7,6} = J_{7,8} = 7.2$  Hz, 1 H, H-7), 7.72 (bd.d,  $J_{3'',2''/5'',6''/2''',3'''/6''',5'''} = 8.8$  Hz, 4 H, H-3'', 5'', 2''', 6'''), 7.56 (t,  $J_{6,5} = J_{6,7} = J_{4''',3'''} = J_{4''',5'''} = 7.6$  Hz, 1 H, H-6, 4'''), 7.49 (t,  $J_{3''',2'''/5''',6'''} = 7.6$  Hz, 1 H, H-3''', 5'''), 7.40 (s, 1 H, H-2), 7.38 (d,  $J_{8,7} = 7.2$  Hz, 1 H, H-8);  $^{13}\text{C-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  177.7, 171.4, 155.8, 155.2, 150.3, 135.8, 134.5, 133.9, 133.5, 130.8, 130.2, 128.1, 127.5, 125.8, 125.2, 123.4, 118.7, 118.2, 105.1; EI-MS  $m/z$  (% rel. abund.): 423 ( $\text{M}^+$ , 100), 303 (48), 252 (35), 210 (23), 178 (8), 165 (7), 146 (6); HREI-MS Calcd for  $\text{C}_{25}\text{H}_{17}\text{N}_3\text{O}_2\text{S}$ :  $m/z = 423.0810$ , found 423.0820.

**(Z)-6-Methyl-3-((2-(4-phenylthiazol-2-yl)hydrazono)methyl)-4H-chromen-4-one (16).**

Yield: 79%; M.p.: 235–237 °C;  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  12.21 (s, 1 H, NH), 8.69 (s, 1 H, -N=CH-), 8.14 (s, 1 H, H-5'), 7.91 (bd.s, 1 H, H-5), 7.85 (d,  $J_{2'',3''/6'',5''} = 8.0$  Hz, 2 H, H-2'', 6''), 7.65 (m, 2 H, H-7, 8), 7.41 (t,  $J_{3'',2''/5'',6''/3''',4''',4'''} = 7.6$  Hz, 2 H, H-3'', 5''), 7.33 (s, 1 H, H-2), 7.31 (t,  $J_{4''',3'''/4''',5'''} = 7.2$  Hz, 1 H, H-4''), 2.44 (s, 3 H, CH<sub>3</sub>);  $^{13}\text{C-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  177.8, 172.0, 155.1, 154.2, 150.1, 135.7, 135.4, 134.2, 133.1, 129.3, 129.3, 128.8, 127.6, 127.6, 124.4, 123.1, 118.4, 118.0, 105.2, 20.5; EI-MS  $m/z$  (% rel. abund.): 361 ( $\text{M}^+$ , 100), 227 (100), 200 (7), 176 (32), 160 (16), 134 (57); HREI-MS Calcd for  $\text{C}_{20}\text{H}_{15}\text{N}_3\text{O}_2\text{S}$ :  $m/z = 361.0885$ , found 361.0900.

**(Z)-3-((2-(4-(3-Hydroxyphenyl)thiazol-2-yl)hydrazono)methyl)-6-methyl-4H-chromen-4-one (17).**

Yield: 74%; M.p.: 237–239 °C;  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  11.51 (s, 1 H, NH), 9.13 (s, 1 H, -N=CH-), 8.23 (s, 1 H, H-5'), 8.17 (s, 1 H, H-2), 8.07 (bd.s, 1 H, H-5), 7.88 (s, 1 H, H-2''), 7.66 (m, 5 H, H-7, 8, 4'', 5'', 6''), 2.43 (s, 3 H, CH<sub>3</sub>);  $^{13}\text{C-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  177.9, 172.0, 157.6, 155.1, 154.2, 150.0, 135.7, 135.6, 134.5, 134.2, 130.7, 124.4, 123.2, 120.0, 118.5, 118.0, 115.9, 115.7, 105.1, 20.5; EI-MS  $m/z$  (% rel. abund.): 377 ( $\text{M}^+$ , 2), 261 (27), 219 (28), 202 (34), 186 (80), 134 (100); HREI-MS Calcd for  $\text{C}_{20}\text{H}_{13}\text{N}_3\text{O}_3\text{S}$ :  $m/z = 377.0834$ , found 377.0830.

**(Z)-3-((2-(4-(2-Hydroxyphenyl)thiazol-2-yl)hydrazono)methyl)-6-methyl-4H-chromen-4-one (18).**

Yield: 71%; M.p.: 230–232 °C;  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  11.51 (s, 1 H, NH), 9.13 (s, 1 H, -N=CH-), 8.23 (bd.s, 1 H, H-5), 8.17 (s, 1 H, H-5'), 8.16 (s, 1 H, H-2), 8.06 (bd.s, 1 H, H-8), 7.88 (bd.s, 1 H, H-7), 7.66 (m, 4 H, H-3'', 4'', 5'', 6''), 2.43 (s, 3 H, CH<sub>3</sub>);  $^{13}\text{C-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  178.0, 172.5, 155.4, 155.0, 154.2, 147.7, 135.7, 135.4, 134.2, 131.6, 130.2, 124.4, 123.1, 121.9, 120.6, 118.5, 118.0, 117.7, 105.2, 20.5; EI-MS  $m/z$  (% rel. abund.): 377 ( $\text{M}^+$ , 10), 318 (7), 261 (16), 219 (14), 202 (36), 186 (38), 160 (10), 134 (100); HREI-MS Calcd for  $\text{C}_{20}\text{H}_{15}\text{N}_3\text{O}_3\text{S}$ :  $m/z = 377.0834$ , found 377.0831.

**(Z)-3-((2-(4-(3-Bromophenyl)thiazol-2-yl)hydrazono)methyl)-6-methyl-4H-chromen-4-one (19).**

Yield: 71%; M.p.: 239–241 °C;  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  12.23 (s, 1 H, NH), 8.69 (s, 1 H, -N=CH-), 8.14 (s, 1 H, H-5'), 8.03 (s, 1 H, H-5), 7.91 (s, 1 H, H-2''), 7.85 (d,  $J_{8,7} = 8.0$ , 1 H, H-8), 7.67 (m, 2 H, H-4'', 6''), 7.49 (s, 1 H, H-2), 7.49 (d,  $J_{7,8} = 7.2$  Hz, 1 H, H-7), 7.38 (t,  $J_{5'',4''} = J_{5'',6''} = 8.0$  Hz, 1 H, H-5''), 2.44 (s, 3 H, CH<sub>3</sub>);  $^{13}\text{C-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  177.9, 170.7, 155.3, 154.2, 150.0, 135.7, 135.4, 135.3, 134.2, 131.6, 131.0, 128.2, 126.6, 124.2, 123.1, 122.3, 118.5, 118.0, 105.2, 20.6; EI-MS  $m/z$  (% rel. abund.): 439 ( $\text{M}^+$ , 88), 441 ( $\text{M} + 2$ , 91), 307 (100), 254 (30), 227 (10), 186 (20), 160 (17), 134 (73); HREI-MS Calcd for  $\text{C}_{20}\text{H}_{14}\text{BrN}_3\text{O}_2\text{S}$ :  $m/z = 438.9990$ , found 438.9993.

**(Z)-3-((2-(4-(4-Bromophenyl)thiazol-2-yl)hydrazono)methyl)-6-methyl-4H-chromen-4-one (20).**

Yield: 73%; M.p.: 235–237 °C;  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  12.22 (s, 1 H, NH), 8.69 (s, 1 H, -N=CH-), 8.14 (s, 1 H, H-5'), 7.91 (s, 1 H, H-5), 7.80 (d,  $J_{2'',3''/6'',5''} = 8.4$  Hz, 2 H, H-2'', 6''), 7.67 (m, 4 H, H-7, 8, 3'', 5''), 7.41 (s, 1 H, H-2), 2.44 (s, 3 H, CH<sub>3</sub>);  $^{13}\text{C-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  177.9, 172.0, 155.2, 154.1, 150.3, 135.7, 135.6, 134.2, 132.2, 132.2, 132.0, 128.4, 128.4, 124.4, 123.2, 123.0, 118.5, 118.2, 105.1, 20.5; EI-MS  $m/z$  (% rel. abund.): 439 ( $\text{M}^+$ , 88), 441 ( $\text{M} + 2$ , 90), 305 (100), 280 (5), 254 (3), 212 (9), 186 (13), 160 (15), 134 (37); HREI-MS Calcd for  $\text{C}_{20}\text{H}_{14}\text{BrN}_3\text{O}_2\text{S}$ :  $m/z = 438.9990$ , found 438.9992.

**(Z)-3-((2-(4-(2,4-Dichlorophenyl)thiazol-2-yl)hydrazono)methyl)-6-methyl-4H-chromen-4-one (21).**

Yield: 78%; M.p.: 245–247 °C;  $^1\text{H-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  12.22 (s, 1 H, NH), 8.70 (s, 1 H, -N=CH-), 8.14 (s, 1 H, H-5'), 7.91 (s, 1 H, H-5), 7.89 (d,  $J_{8,7} = 8.4$ , 1 H, H-8), 7.68 (m, 3 H, H-7, 3'', 6''), 7.51 (d,  $J_{5'',6''} = 8.4$  Hz, 1 H, H-5''), 7.42 (s, 1 H, H-2), 2.44 (s, 3 H, CH<sub>3</sub>);  $^{13}\text{C-NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  177.9, 172.1, 155.3, 154.2, 150.4, 135.6, 135.4, 134.0, 133.5, 132.8, 132.4, 130.8, 128.9, 127.1, 124.4, 123.1, 118.5, 118.0, 105.2,

20.3; EI-MS  $m/z$  (% rel. abund.): 429 ( $M^+$ , 64), 431 ( $M + 2$ , 44), 394 (29), 295 (100), 244 (32), 202 (14), 186 (24), 160 (14), 144 (6), 134 (64); HREI-MS Calcd for  $C_{20}H_{13}Cl_2N_3O_2S$ :  $m/z = 429.0106$ , found 429.0091.

**(Z)-3-((2-(4-(3,4-Dichlorophenyl)thiazol-2-yl)hydrazono)methyl)-6-methyl-4H-chromen-4-one (22).**  
Yield: 72%; M.p.: 246–248 °C;  $^1H$ -NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  12.25 (s, 1 H, NH), 8.70 (s, 1 H, -N=CH-), 8.17 (s, 1 H, H-5'), 8.07 (s,  $J_{5,7} = 4.0$  Hz, 1 H, H-5), 7.91 (s, 1 H, H-2''), 7.84 (dd,  $J_{7,5} = 1.6$ ,  $J_{7,8} = 8.8$  Hz, 1 H, H-7), 7.67 (d,  $J_{5'',6''} = J_{6'',5''} = 8.4$  Hz, 2 H, H-5'', 6''), 7.62 (d,  $J_{8,7} = 8.0$ , 1 H, H-8), 7.55 (s, 1 H, H-2), 2.43 (s, 3 H, CH<sub>3</sub>);  $^{13}C$ -NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  177.8, 172.2, 155.3, 154.1, 150.0, 135.7, 135.5, 134.2, 133.5, 132.8, 132.4, 130.8, 128.9, 127.1, 124.4, 123.2, 118.5, 118.0, 105.2, 20.5; EI-MS  $m/z$  (% rel. abund.): 429 ( $M^+$ , 86), 431 ( $M + 2$ , 61), 295 (100), 244 (27), 204 (12), 186 (15), 134 (54); HREI-MS Calcd for  $C_{20}H_{14}Cl_2N_3O_2S$ :  $m/z = 429.0106$ , found 429.0098.

**(Z)-3-((2-(4-(4-Chlorophenyl)thiazol-2-yl)hydrazono)methyl)-6-methyl-4H-chromen-4-one (23).**  
Yield: 71%; M.p.: 245–247 °C;  $^1H$ -NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  12.22 (s, 1 H, NH), 8.69 (s, 1 H, -N=CH-), 8.14 (s, 1 H, H-5'), 7.91 (bd.s, 1 H, H-5), 7.86 (d,  $J_{2'',3''/6'',5''} = 8.8$  Hz, 2 H, H-2'', 6''), 7.65 (m, 2 H, H-7, 8), 7.46 (d,  $J_{3'',2''/5'',6''} = 8.4$  Hz, 2 H, H-3'', 5''), 7.40 (s, 1 H, H-2), 2.44 (s, 3 H, CH<sub>3</sub>);  $^{13}C$ -NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  177.8, 172.5, 155.3, 154.1, 150.0, 135.7, 135.6, 134.2, 133.6, 131.7, 128.5, 128.5, 127.1, 127.1, 124.2, 123.1, 118.5, 118.0, 105.1, 20.5; EI-MS  $m/z$  (% rel. abund.): 395 ( $M^+$ , 60), 397 ( $M + 2$ , 23), 261 (100), 210 (27), 186 (14), 168 (18), 134 (24); HREI-MS Calcd for  $C_{20}H_{14}ClN_3O_2S$ :  $m/z = 395.0495$ , found 395.0490.

**(Z)-3-((2-(4-(3-Chlorophenyl)thiazol-2-yl)hydrazono)methyl)-6-methyl-4H-chromen-4-one (24).**  
Yield: 75%; M.p.: 230–232 °C;  $^1H$ -NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  12.24 (s, 1 H, NH), 8.69 (s, 1 H, -N=CH-CH-), 8.15 (s, 1 H, H-5'), 7.91 (s, 1 H, H-5), 7.88 (s, 1 H, H-2''), 7.82 (d,  $J_{8,7} = 7.6$ , 1 H, H-8), 7.67 (m, 2 H, H-4'', 6''), 7.49 (s, 1 H, H-2), 7.45 (t,  $J_{5'',4''} = J_{5'',6''} = 8.0$  Hz, 1 H, H-5''), 7.36 (d,  $J_{7,8} = 8.0$  Hz, 1 H, H-7), 2.44 (s, 3 H, CH<sub>3</sub>);  $^{13}C$ -NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  177.8, 172.4, 155.2, 154.1, 150.0, 135.7, 135.4, 134.9, 134.5, 134.2, 129.7, 129.4, 128.9, 125.7, 124.4, 123.1, 118.5, 118.2, 105.2, 20.5; EI-MS  $m/z$  (% rel. abund.): 395 ( $M^+$ , 99), 397 ( $M + 2$ , 54), 261 (100), 234 (9), 210 (47), 186 (17), 168 (28), 160 (17), 134 (54); HREI-MS Calcd for  $C_{20}H_{14}ClN_3O_2S$ :  $m/z = 395.0495$ , found 395.0482.

**(Z)-6-Methyl-3-((2-(4-(3-nitrophenyl)thiazol-2-yl)hydrazono)methyl)-4H-chromen-4-one (25).**  
Yield: 74%; M.p.: 240–242 °C;  $^1H$ -NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  12.23 (s, 1 H, NH), 8.69 (s, 1 H, -N=CH-), 8.17 (s, 1 H, H-5'), 8.03 (s, 1 H, H-5), 7.91 (s, 1 H, H-2''), 7.85 (d,  $J_{8,7} = 8.0$ , 1 H, H-8), 7.67 (m, 2 H, H-4'', 6''), 7.49 (s, 1 H, H-2), 7.49 (d,  $J_{7,8} = 7.2$  Hz, 1 H, H-7), 7.38 (t,  $J_{5'',4''} = J_{5'',6''} = 7.6$  Hz, 1 H, H-5''), 2.44 (s, 3 H, CH<sub>3</sub>);  $^{13}C$ -NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  177.9, 172.3, 155.3, 154.1, 150.3, 148.5, 135.7, 135.4, 134.2, 133.9, 133.5, 130.5, 124.4, 123.8, 123.2, 122.6, 118.5, 118.0, 105.1, 20.6; EI-MS  $m/z$  (% rel. abund.): 406 ( $M^+$ , 59), 389 (25), 272 (34), 221 (18), 186 (15), 134 (100); HREI-MS Calcd for  $C_{20}H_{14}N_4O_4S$ :  $m/z = 406.0736$ , found 406.0730.

**(Z)-4-(2-(2-((6-Methyl-4-oxo-4H-chromen-3-yl)methylene)hydrazinyl)thiazol-4-yl)benzoxazole (26).** Yield: 68%; M.p.: 238–240 °C;  $^1H$ -NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  12.28 (s, 1 H, NH), 8.70 (s, 1 H, -N=CH-), 8.16 (s, 1 H, H-5'), 8.03 (d,  $J_{2'',3''/6'',5''} = 8.0$  Hz, 2 H, H-2'', 6''), 7.91 (bd.s, 1 H, H-5), 7.86 (d,  $J_{3'',2''/5'',6''} = 8.4$  Hz, 2 H, H-3'', 5''), 7.67 (m, 3 H, H-2, 7, 8), 2.44 (s, 3 H, CH<sub>3</sub>);  $^{13}C$ -NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  177.8, 171.8, 155.2, 154.1, 150.3, 137.4, 135.7, 135.5, 134.2, 132.8, 132.8, 126.0, 126.0, 124.4, 123.1, 118.7, 118.3, 118.0, 112.7, 105.1, 20.4; EI-MS  $m/z$  (% rel. abund.): 386 ( $M^+$ , 100), 252 (37), 201 (28), 186 (28), 159 (47), 134 (82); HREI-MS Calcd for  $C_{21}H_{14}N_4O_2S$ :  $m/z = 386.0837$ , found 386.0824.

**(Z)-3-((2-(4-(Biphenyl-4-yl)thiazol-2-yl)hydrazono)methyl)-6-methyl-4H-chromen-4-one (27).**  
Yield: 76%; M.p.: 235–237 °C;  $^1H$ -NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  12.24 (s, 1 H, NH), 8.70 (s, 1 H, -N=CH-), 8.15 (s, 1 H, H-5'), 7.95 (d,  $J_{2'',3''/6'',5''} = 8.4$  Hz, 2 H, H-2'', 6''), 7.92 (bd.s, 1 H, H-5), 7.72 (bd.d,  $J_{3'',2''/5'',6''/2'',3''/6'',5''} = 8.4$  Hz, 4 H, H-3'', 5'', 2''', 6'''), 7.68 (m, 2 H, H-7, 8), 7.49 (t,  $J_{3'',2''/5'',6''} = J_{3'',4''/5'',4''} = 7.6$  Hz, 2 H, H-3'', 5''), 7.40 (s, 1 H, H-2), 7.38 (t,  $J_{4'',3''/4'',5''} = 7.6$  Hz, 1 H, H-4'''), 2.44 (s, 3 H, CH<sub>3</sub>);  $^{13}C$ -NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  177.9, 172.0, 155.1, 154.2, 150.0, 140.9, 140.6, 135.7, 135.5, 134.2, 131.8, 129.2, 129.2, 128.1, 128.1, 127.8, 127.8, 127.5, 127.0, 127.0, 124.2, 123.1, 118.5, 118.0, 105.1, 20.6; EI-MS  $m/z$  (% rel. abund.): 437 ( $M^+$ , 100), 303 (80), 276 (4), 252 (25), 210 (15); HREI-MS Calcd for  $C_{26}H_{19}N_3O_2S$ :  $m/z = 437.1198$ , found 437.1205.

**$\alpha$ -Amylase inhibition assay.** The  $\alpha$ -amylase inhibitory activity was determined by an assay modified from Kwon, Apostolidis & Shetty<sup>37,38</sup>. A volume of 500  $\mu$ L of test sample (100  $\mu$ g/mL, 200  $\mu$ g/mL, 400  $\mu$ g/mL, 800  $\mu$ g/mL, 1000  $\mu$ g/mL) and 500  $\mu$ L of  $\alpha$ -amylase solution (0.5 mg/mL) in 0.2 mM phosphate buffer (pH 6.9) were incubated at 25 °C for 10 min. After pre-incubation, 500  $\mu$ L of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube and incubated at 25 °C for 10 minutes. The reaction was arrested with 1 mL of dinitrosalicylic acid colour reagent. The tubes were then incubated in boiling water for 5 min and cooled to room temperature. The solutions were diluted after adding 10 mL distilled water and the absorbance was measured at 540 nm<sup>39</sup>.

The percentage of inhibition was calculated as illustrated,

$$\% \text{Inhibition} = (\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}}) / \text{Absorbance}_{\text{Control}} \times 100$$

The IC<sub>50</sub> values, concentration required to inhibit the  $\alpha$ -amylase activity by 50% were calculated by a non-linear regression graph plotted between percentage inhibition (x axis) versus concentrations (y axis), using a Graph Pad Prism Software (Version 5).

**DPPH Free radical scavenging assay.** The ability of the sample to scavenge, 2-diphenyl-1-picrylhydrazyl (DPPH) free radicals was evaluated by standard method<sup>40</sup>. The sample solutions were prepared in absolute alcohol, ranging from 0.01 mg/mL to 1 mg/mL. A total of 500  $\mu$ L of sample was added with 500  $\mu$ L of 2  $\mu$ mol DPPH solution. After 20 min of incubation, the samples were placed in the dark at room temperature, the absorbance was taken at 517 nm. 500  $\mu$ L of prepared DPPH solution and 500  $\mu$ L of absolute alcohol were used as control. The similar procedure was repeated for ascorbic acid as standard<sup>41,42</sup>.

The percentage inhibition of radical scavenging activity was calculated as illustrated,

$$\% \text{Inhibition} = (\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}}) / \text{Absorbance}_{\text{Control}} \times 100$$

**ABTS Free radical cation scavenging assay.** The (ABTS+) 2,2'-azino-bis(3-ethylbenzothiazolin e-6-sulphonic acid) free radical cation scavenging ability of the compounds was determined by standard method<sup>43</sup>. 7 mM ABTS was dissolved in distilled water and 2.45 mM potassium persulfate was added. The solution was kept in the dark for 12–16 h at room temperature. The sample solutions were prepared in absolute alcohol ranging from 0.01 mg/mL to 1 mg/mL. The samples were added with ABTS solution and incubated for 30 min. The absorbance was taken at 734 nm and the procedure was repeated for ascorbic acid as standard.

The percentage inhibition of radical scavenging activity was calculated as illustrated,

$$\% \text{Inhibition} = (\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}}) / \text{Absorbance}_{\text{Control}} \times 100$$

**MTT Cytotoxicity assay.** Cytotoxicity of the newly synthesized compounds on NIH-3T3 fibroblast cells (ATCC, Manassas, USA) was checked by the standard MTT colorimetric assay<sup>44</sup>. Briefly, 100  $\mu$ L of  $5 \times 10^4$  cells/mL in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% FBS were plated into 96-wells flat bottom plate and incubated overnight at 37 °C in 5% CO<sub>2</sub>. Three different concentrations of test compound (1, 10 and 100  $\mu$ g/mL) were added to the plate in triplicates and incubated for 48 hrs. 50  $\mu$ L of 0.5 mg/mL MTT was added to each well and plate was then further incubated for 4 hours. MTT was aspirated and 100  $\mu$ L of DMSO was then added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 540 nm, using spectrophotometer (Spectra Max plus, Molecular Devices, CA, USA). The cytotoxic activity was recorded as concentration causing 50% growth inhibition (IC<sub>50</sub>) for 3T3 cells.

**Methodology of *in silico* study.** The 3D structure of  $\alpha$ -amylase (PDB ID: 1HNY) was obtained from Protein Data Bank. Water molecules were removed and the 3D protonation of the protein molecule was carried out. Energy of the protein molecule was minimized with the help of energy minimization algorithm implemented in MOE (Molecular Operating Environment) software and the minimized structure was used for docking. The 3D structures of ligands were built using builder tool in MOE ([www.chemcomp.com](http://www.chemcomp.com)). All the built structures were 3D protonated and were energy minimized. The 3D structure were saved in mdb file format as input file for docking.

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## Author Contributions

U.S. contributed to the study design, performed all the chemical reactions and spectral analysis of the synthesized compounds, and the preparation of manuscript. K.M.K. provided conceptual and technical guidance, along with the laboratory equipment and expertise to conduct the chemical synthesis, and involved in critical reviewing of the manuscript. S.C. and M.T. provided the laboratory equipment, technical guidance and expertise to conduct the bioassay on the synthetic compounds, as well as contributed in reviewing the manuscript. A.W. provided the laboratory equipment and technical guidance for the *in silico* study and reviewing of manuscript. S.V. performed the bioassay work on the synthesized compounds and statistical analysis. M.G. contributed in performing the molecular modelling study and writing of the manuscript. S.P. also contributed in reviewing the manuscript.

## Additional Information

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