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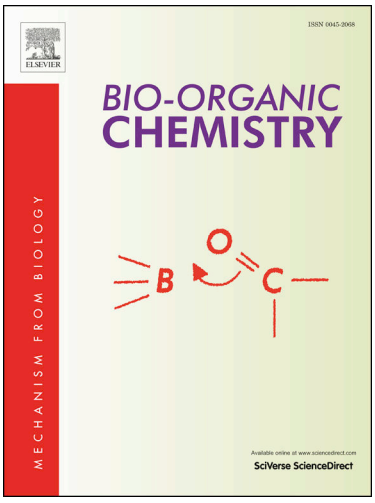

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4-Oxycoumarinyl Linked Acetohydrazide Schiff bases as Potent Urease Inhibitors

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Abstract: Urease enzyme is responsible to catalyze the hydrolysis of urea into carbamate and ammonia. Then carbamate hydrolyzed to ammonia and carbon dioxide. Excess release of ammonia leads to increase pH in stomach that actually encourages the survival of *Helicobacter pylori*. *H. pylori* involves in various disorders most commonly peptic ulcer, pyelonephritis, hepatic coma, kidney stone formation, urolithiasis, and encephalopathy. Apart from many pharmacological properties, coumarin and Schiff bases are known to possess urease inhibitory activity. Therefore, these two pharmacologically important scaffolds are combined into single hybrid molecules to assess their potential as urease inhibitors. For this aim, *N*'-benzylidene-2-((2-oxo-2*H*-chromen-4-yl)oxy)acetohydrazide Schiff base derivatives **3-27** were synthesized by following a three step reaction strategy. Structures of all synthetic molecules were characterized by EI-MS, ^{1}H-, and ^{13}C-NMR spectroscopic techniques. All molecules were assessed for urease inhibitory activity and found to possess a varying degree of inhibitory potential in the range of IC_{50} = 12.3 ± 0.69 to 88.8 ± 0.04 μM. Amongst the active analogs, compounds **7** (IC_{50} = 16.2 ± 0.11 μM), **9** (IC_{50} = 15.2 ± 0.14 μM), **10** (IC_{50} = 12.3 ± 0.69 μM), **12** (IC_{50} = 16.3 ± 0.45 μM), and **15** (IC_{50} = 17.6 ± 0.28 μM) were identified as potent inhibitors compared to standard urea (IC_{50} = 21.5 ± 0.47 μM). It is conferred from structure-activity relationship (SAR) that variation in inhibitory activity is due to

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different substitutions pattern on aryl ring. Moreover, molecular docking studies were carried out to understand the interactions of ligand with the active pocket of urease enzyme.

Keywords: Synthesis; chromene/coumarin; urease inhibitory activity; *In vitro*; structure-activity relationship; *In silico*

Introduction

Coumarin also known as 2*H*-chromen-2one, belongs to the benzopyrone family which consists of a benzene ring fused to a pyrone ring hence also known as benzopyrane-2-one. It is isolated from plants, animals, and microorganisms [1, 2]. The role of coumarin is well established as it is associated with a diverse range of biological activities. It is the privileged scaffold in the domain of medicinal chemistry and it is amongst the natural products those have been extensively used as medicines [3-6]. Over the years, a number of natural and synthetic coumarin based compounds have been reported for their wide range of pharmacological activities such as antimicrobial, antioxidant [7], anticancer [8, 9], antitubercular [10, 11], and antiproliferative [12]. Coumarin scaffold reported to cure fibrotic diseases [13] as well as also revealed enzyme inhibitory activities [14-16]. Apart from various biological applications, coumarin is also used as optical brightening agents, tunable laser dye, cosmetics, agrochemicals, perfumes, and food additive [17,18]. Coumarin derivatives has been identified as potent urease inhibitors [19-23]. Previously, our research group has already been engaged to synthesize coumarin based bioactive molecules and explored their distinct biological activities [19, 21-25].

Schiff bases possess azomethine (-C=N-) group which is very well-known for being active pharmacophore. Schiff bases are also known to exhibit tremendous biological potential [26]. Schiff bases with various heterocyclic rings have been extensively reported to exhibit bioactivities including anticancer, anticonvulsant, antifungal, antiproliferative [27], antiviral, antibacterial, anti-inflammatory, antimalarial [28], and antitumor [29]. Apart from range of enzyme inhibitory activities, Schiff bases are also recognized as urease inhibitors [30].

Urease is a nickel containing metalloenzyme belonging to the superfamily of phosphotriesterases and amidohydrolases. It catalyzes the hydrolysis of urea into carbamate and carbon dioxide which eventually converts to ammonia [31]. Hence, by increasing the pH due to the excess release of ammonia, it promotes the survival of *Helicobacter pylori* by countering the acidic pH of stomach

[32]. *Helicobacter pylori* is harmful for human health as it is involved in various ailments including pyelonephritis, peptic ulcer, hepatic coma, kidney stone formation, urolithiasis, and encephalopathy [33]. Therefore, identification of novel and potent urease inhibitors with high stability and less toxicity is extremely required. During last two decades, our research group is actively involved in the search of new potent urease inhibitors [19, 21-23, 34-38]. We have identified biscoumarins as non-cytotoxic and selective urease inhibitors [21]. Since, urease possess two nickel centers for coordination and Schiff bases are capable to form metal complexes [39, 40] which encourage us to combine both of these moieties within a single scaffold and to explore further biological aspects related to them (Figure-1). The present study is covering the multi-step synthesis of coumarin linked with Schiff base and their evaluation as urease inhibitors.

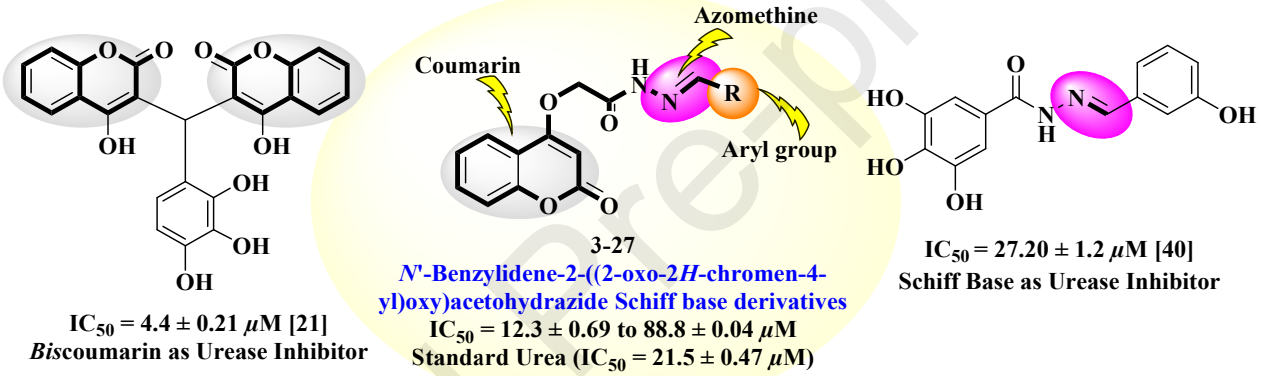


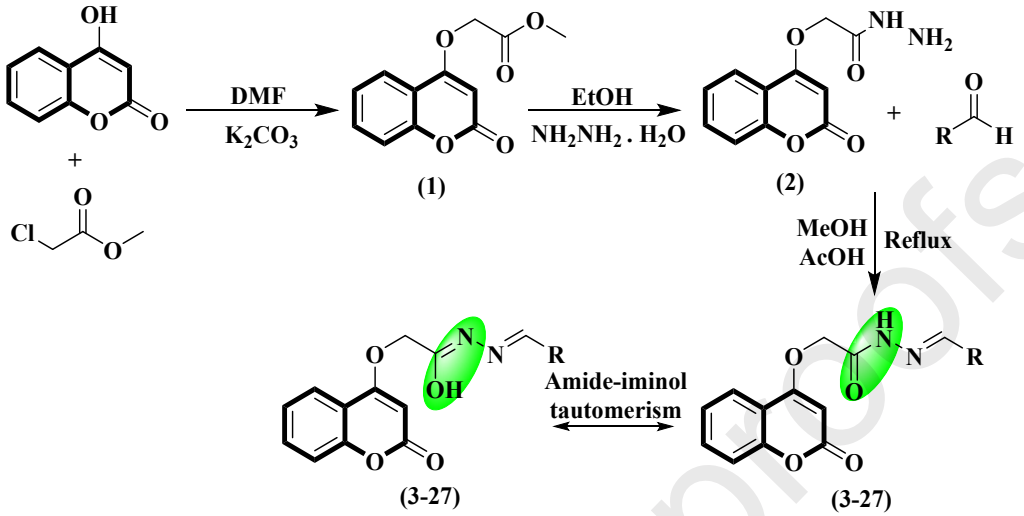
Figure-1: Coumarin and Schiff base as a Potent Urease Inhibitors.

Results and discussion

Chemistry

A mixture of 4-hydroxy coumarin and methyl chloroacetate in DMF was refluxed for two hours in the presence of K_2CO_3. Formation of methyl 4-oxycoumarinyl acetate (**1**) reaction was monitored by thin layer chromatography (TLC). In second step, intervening intermediate (**1**) was treated with hydrazine hydrate (NH_2NH_2. H_2O) in ethanol and stirred at room temperature to get 2-((2-oxo-2*H*-chromen-4-yl)oxy)acetohydrazide (**2**). After that, compound **2** was condensed with different substituted aldehydes in methanol in the presence of glacial acetic acid to obtain a variety of 4-oxycoumarinyl linked acetohydrazide Schiff bases **3-27** (Scheme-1). Second and third steps were also monitored by TLC analysis. As a result of above reaction scheme, two geometrical isomers *E* and *Z* were obtained in the ratio of 75% and 25%, respectively, analyzed by integrations in NMR

spectra. Chemical shifts corresponds to minor isomer "*Z*" were marked by steric (*) symbol. To the best knowledge, all synthesized compounds are new, except compounds **4** and **18** [41].



Scheme-1: Synthesis of 4-oxycoumarinyl linked acetohydrazide Schiff bases **3-27**

Since, amide group may undergo amide-iminol tautomerism and could leads to geometric isomerism around single bond. There are four possible isomers including s-cis and s-trans with *E*/*Z* isomerism (Figure-2). s-Cis isomers are less stable than s-trans, due to more steric crowding . However, there is more chance that molecules attained the s-trans conformation and less chance to get the mixture of s-trans and s-cis. The single chemical shift of iminol OH (refer NMR data of all compounds) confirmed the presence of one isomerism around single bond that is s-trans.

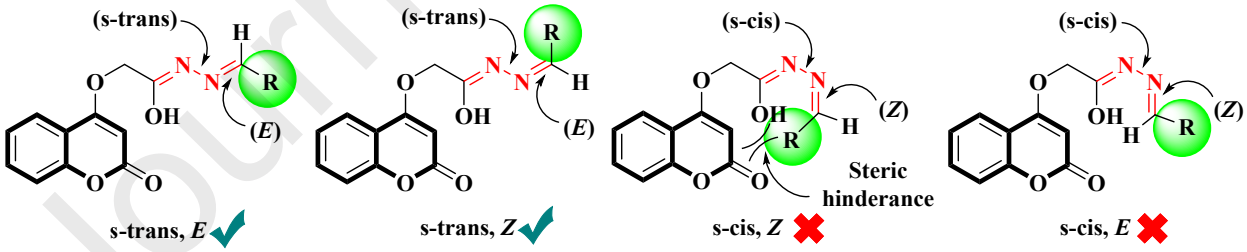


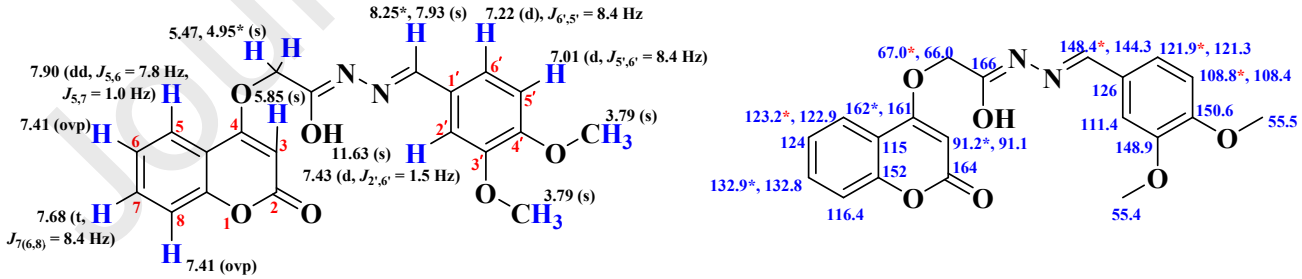
Figure-2: Four possible geometrical isomers

Detailed Spectral Study of Compound 14

^{1}H-NMR spectrum was recorded in DMSO-d_6 on a 300 MHz Bruker AM instrument. The NH proton was resonated at δ_H 11.63 as most downfield singlet (s). Imine proton was appeared at δ_H 8.25* (s-trans, *Z* isomer) and δ_H 7.93 (s-trans, *E* isomer). H-5 of the basic coumarin skeleton was

appeared as doublet of doublet (dd) at δ_H 7.90 and showed *ortho* and *meta* coupling with H-6 (J = 7.8 Hz) and H-7 (J = 1.0 Hz). H-7 appeared as triplet (t) at δ_H 7.68, presenting *ortho* coupling with H-6 and H-8 (J = 8.4 Hz). H-6 and H-8 were appeared as overlapping multiplet (ovp) at δ_H 7.43. H-2' of aryl ring was resonated at δ_H 7.43 as doublet (d) with J = 1.5 Hz, due to *meta* coupling with H-6'. H-6' and H-5' were resonated at δ_H 7.22 and δ_H 7.01, respectively. Both protons appeared as doublet (d), showing *ortho* coupling with each other (J = 8.4 Hz). H-3 was appeared as singlet at δ_H 5.85. Two protons belongs to -OCH_2- were appeared at δ_H 5.47 (*E* isomer) and 4.95* (*Z* isomer) as singlet. Six protons of two OMe groups were appeared at δ_H 3.79 as singlet (Figure-3A).

Broad-band (BB) decoupled ^{13}C-NMR spectrum (in DMSO-d_6 at 100 MHz) showed a total twenty eight carbon signals, including two methoxy, one methylene, eight methine, and eight quaternary carbons. Eight signals corresponds to minor isomer (*Z*). Carbonyl carbons of hydrazide and lactone moieties were appeared as most downfield signals at δ_C 166 and δ_C 164, respectively. C-4 was also appeared downfield at δ_C 162* (*Z* isomer), 161 (*E* isomer) being part of *α*-*β*-unsaturated system. Quaternary C-9, C-4', C-3', and C-10 were appeared at δ_C 152.7, 150.9, 148.9, and 115.1, respectively. Iminic carbon was appeared at δ_C 148.4* (*Z* isomer), 144.3 (*E* isomer). Other carbons belong to coumarin ring such as C-3, C-5, C-6, C-7, and C-8, and aryl ring C-1', C-2', C-5', and C-6' were appeared in the usual aromatic region. Methylene carbon appeared at δ_C 67.0* (*Z* isomer), 66.0 (*E* isomer). Two methoxy carbons were appeared at δ_C 55.5 and δ_C 55.4, respectively (Figure-3B).



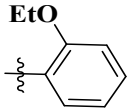
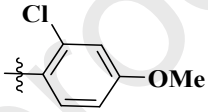
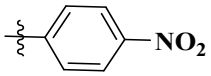
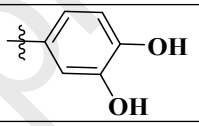
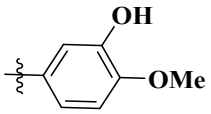
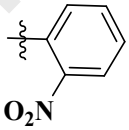
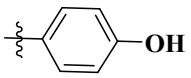
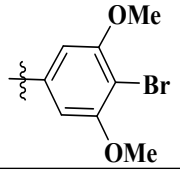
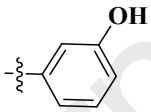
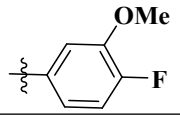
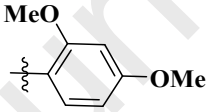
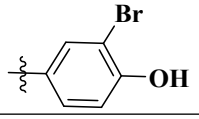
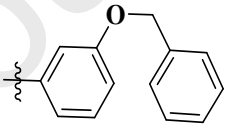
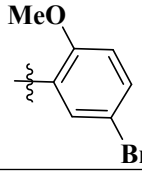
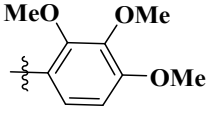
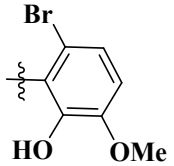
(A) **(B)**

Figure-3: ^{1}H- **(A)** and ^{13}C-NMR **(B)** of symbolic compound **14**

***In vitro* urease inhibitory activity**

All 4-oxycoumarinyl linked acetohydrazide Schiff bases **3-27** were screened to check their inhibitory potential against urease enzyme. Results in terms of IC_{50} values were compared with standard thiourea (IC_{50} = 21.5 ± 0.47 μM). Based on the results listed in Table-1, a number of Schiff bases including **7**, **9-12**, and **15-18** exhibited potent urease inhibitory activity with IC_{50} values less than standard thiourea 21.5 ± 0.47 μM. While rest of the compounds showed good to moderate inhibitory potential.

Table-1: *In vitro* urease inhibition of 4-oxycoumarinyl linked acetohydrazide Schiff bases **3-27**

Comp.	R	IC_{50} ± SEM[a] (μM)	Comp.	R	IC_{50} ± SEM[a] (μM)
3		68.9 ± 0.28	**16**		21.5 ± 0.39
4		64.5 ± 0.47	**17**		18.9 ± 0.10
5		67.8 ± 0.46	**18**		19.6 ± 0.44
6		65.6 ± 0.64	**19**		22.6 ± 0.16
7		16.2 ± 0.11	**20**		27.5 ± 0.07
8		25.6 ± 0.74	**21**		25.8 ± 0.48
9		15.2 ± 0.14	**22**		88.8 ± 0.04
10		12.3 ± 0.69	**23**		64.8 ± 0.09

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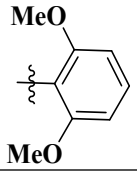
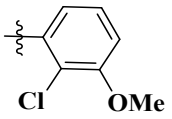
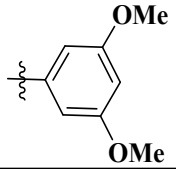
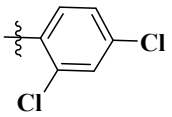
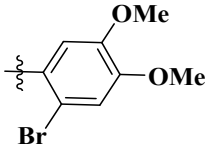
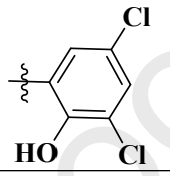
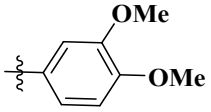
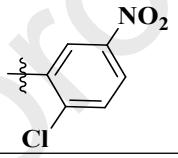
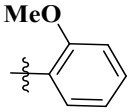
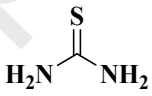
11		19.6 ± 0.36	**24**		87.7 ± 0.48
12		16.3 ± 0.45	**25**		63.6 ± 0.29
13		61.1 ± 0.08	**26**		86.7 ± 0.11
14		66.6 ± 0.16	**27**		69.8 ± 0.17
15		17.6 ± 0.28	Standard = Thiourea[b]		21.5 ± 0.47

SEM[a] (Standard error mean); Thiourea[b] (Standard for urease inhibitory activity)

Structure-activity relationship (SAR)

Worth mentioning that all compounds were found to be active which shows that each structural feature is taking part in the inhibitory activity. Since 4-oxycoumarin ring, and acetohydrazide moiety are exactly similar in all compounds thus limited structure-activity relationship (SAR) is rationalized on the basis of varying features *i.e.* different substitutions pattern in aryl ring.

Amongst the synthetic twenty-five derivatives, tri-methoxy substituted compound **10** (IC_{50} = 12.3 ± 0.69 μM) was found to be the most potent member of this library. Its potent activity might be due to the presence of three electron donating groups which make the aryl ring electron rich. However, di-methoxy substituted analogs **8**, **11**, **12** and **14** exhibited interesting inhibitory activity pattern, based on the positions of the methoxy groups. Activity comparison of compound **10** with compound **8** (IC_{50} = 25.6 ± 0.74 μM) revealed that lacking a methoxy group at *meta* position brought two-fold decreased activity. While absence of methoxy at *ortho* position as in case of compound **14** (IC_{50} = 66.6 ± 0.16 μM) displayed more than six-fold decreased activity. Furthermore, compounds **11** (IC_{50} = 19.6 ± 0.36 μM) and **12** (IC_{50} = 16.3 ± 0.45 μM) with symmetrical dimethoxy substitutions at *ortho* and *meta* positions, respectively, demonstrated

potent urease inhibition which suggests that symmetrical substitutions might play role during interactions with the active site of enzyme. In addition to that *ortho*-methoxy substituted compound **15** (IC_{50} = 17.6 ± 0.28 μM) was also found to be potent which suggests that *ortho* position is favorable for methoxy group to take part in urease inhibition. Activity of compound **15** may compared with *ortho* ethoxy substituted analog **3** (IC_{50} = 68.9 ± 0.28 μM), displayed moderate inhibitory activity might be due to elongation of alkyl chain length. This activity comparison further confirms the active participation of *ortho* methoxy group in case of compound **15** (Figure-4).

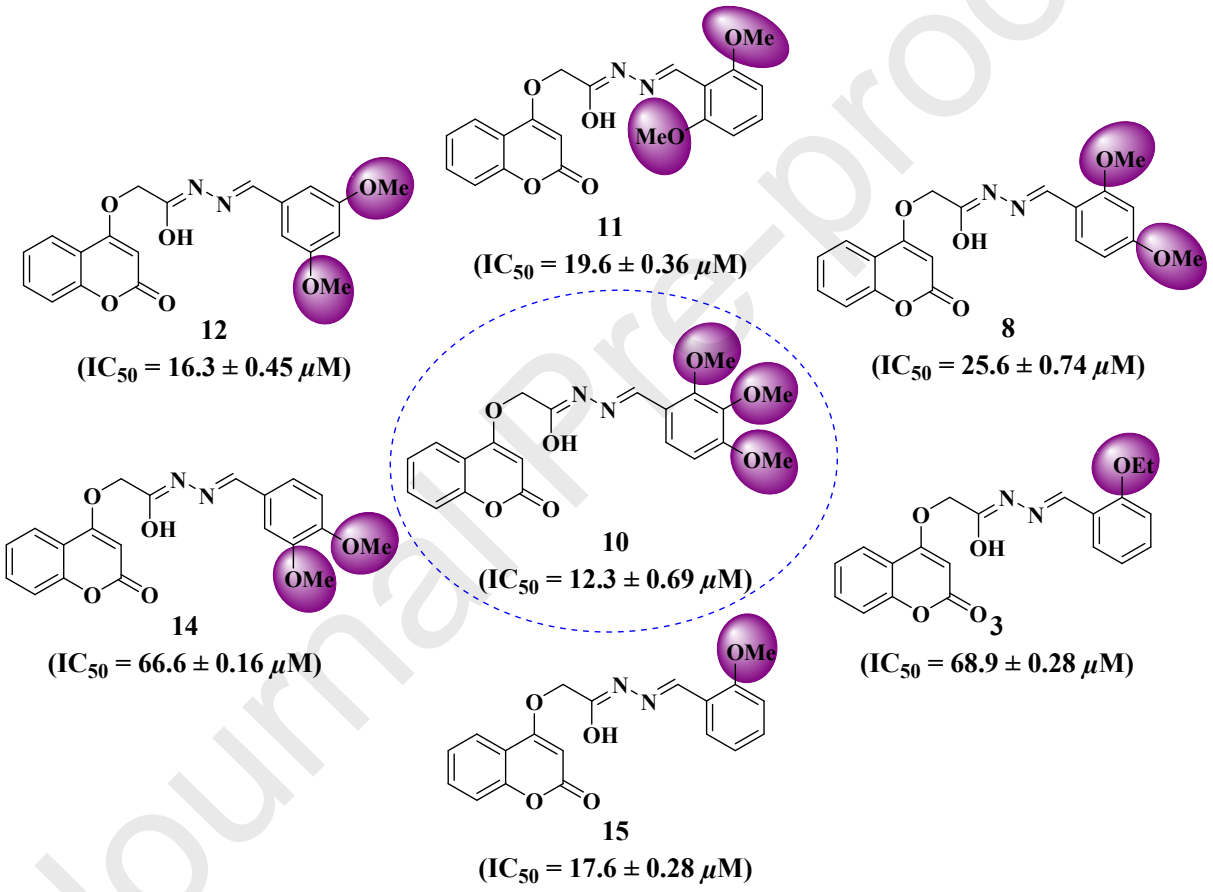


Figure-4: Urease inhibitory activities comparison of analogs **3**, **8**, **10-12**, **14** and **15**, on the basis of their structures

A number of compounds bearing combination of methoxy/s and halogen/s exibited interesting inhibitory potential profile. Amongst them, compound **16** (IC_{50} = 21.5 ± 0.39 μM) which is distinctively similar to compound **8** but have *ortho*-chloro instead of methoxy, showed inhibitory potential better than compound **8** and exactly similar to standard thiourea. Switching the methoxy

group from *para* to *meta* position as in case of compound **24** (IC_{50} = 87.7 ± 0.48 μM), activity sharply decreased. It clearly indicates that chloro and methoxy at adjacent positions is not a good combination for the inhibitory activity as they might create steric hindrance for each other to interact with the active site of urease. Similarly comparing activity of compound **16** with another compound **25** (IC_{50} = 63.6 ± 0.29 μM) revealed that replacing *para*-methoxy with *para*-chloro, exhibited three-fold diminished potential. Moreover, compound **20** (IC_{50} = 27.5 ± 0.07 μM) which has substitution pattern almost similar to compound **14** but *para*-fluoro instead methoxy, revealed much better inhibitory activity which indicates the active participation of fluoro group in inhibition. Compounds **13**, **19**, **22**, and **23** bearing combination of bromo with methoxy showed good to moderate inhibitory strength. Amongst them, compound such as residue **19** (IC_{50} = 22.6 ± 0.16 μM) having *para*-bromo and two methoxy groups at *meta* positions presented inhibitory strength comparable to standard. Changing the positions of bromo and methoxy groups as in case of compound **13** (IC_{50} = 61.1 ± 0.08 μM) showed sharp decline in the activity. It suggested that the symmetrical substitutions pattern in **19** and as observed in previous cases **11** and **12** favors the inhibitory strength of compounds. Besides that, compound **23** (IC_{50} = 64.8 ± 0.09 μM) having hydroxy group along with bromo and methoxy was found to be moderately active. In this case might be positions of the groups are not favorable for the activity. Activity comparison of compound **23** with **22** (IC_{50} = 88.8 ± 0.04 μM) showed the decline in the activity is attributed to the lack of *ortho*-hydroxy group (Figure-5).

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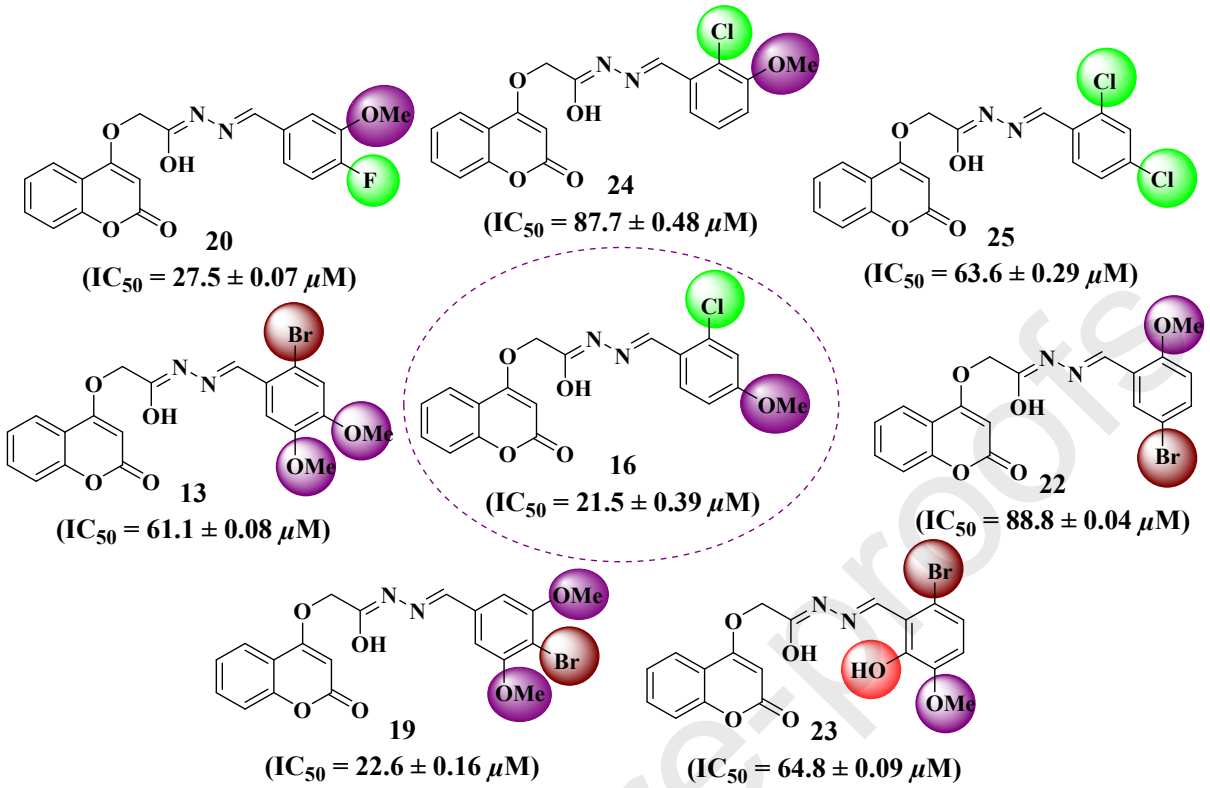

Figure-5: Urease inhibitory activities comparison of analogs **13**, **16**, **19**, **20**, **22** and **25**, on the basis of their structures

Amongst the hydroxy substituted derivatives, *meta* hydroxy substituted analog **7** (IC_{50} = 16.2 ± 0.11 μM) displayed potent activity as compared to standard thiourea (IC_{50} = 21.5 ± 0.47 μM). Repositioning of hydroxy group from *meta* to *para* as in case of compound **6** (IC_{50} = 65.6 ± 0.64 μM), gave four-fold decreased activity. It reveals that the change in position of hydroxy group greatly affects the activity. Di-hydroxy substituted analog **17** (IC_{50} = 18.9 ± 0.10 μM) having hydroxy both at *meta* and *para* position also displayed potent inhibitory activity. Activity of compound **17** can compared with compound **21** (IC_{50} = 25.8 ± 0.48 μM) which showed decreased activity just due to replacement of hydroxy with bromo at *meta* position. Similarly, replacing *para* hydroxy with methoxy as in case of compound **5** (IC_{50} = 67.8 ± 0.46 μM) brought sharp decline in the inhibitory activity (Figure-6).

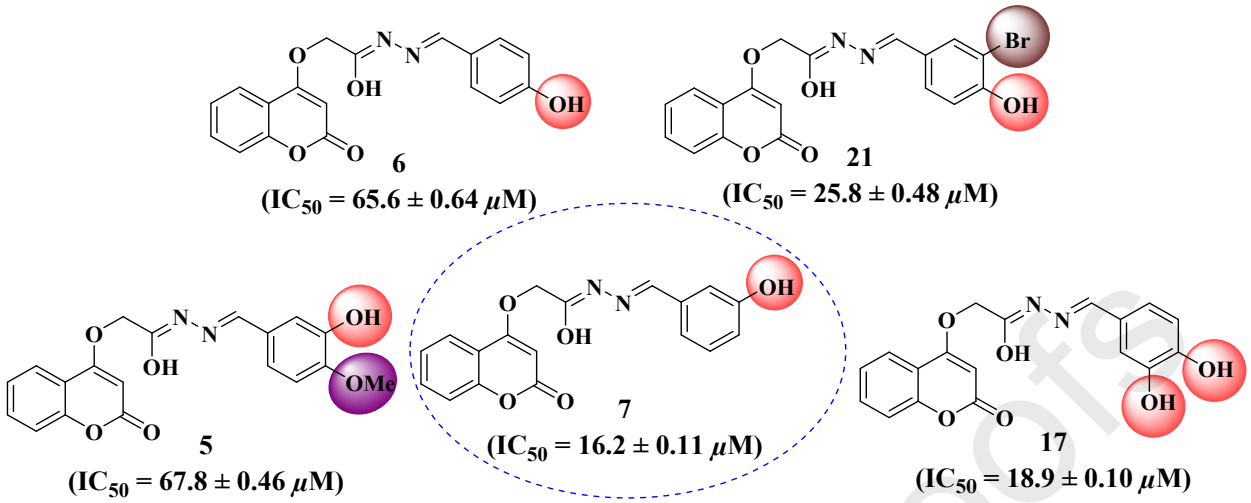

Figure-6: Urease inhibitory activities comparison of analogs **5-7**, **17**, **21** and **23**, on the basis of their structures

Compound **18** (IC_{50} = 19.6 ± 0.44 μM) bearing nitro group at *ortho* position was found to be a potent urease inhibitor as it showed better strength than standard thiourea. Switching of nitro group from *ortho* to *para* as in compound **4** (IC_{50} = 64.3 ± 0.47 μM) leads to more than three-folds reduced inhibitory activity which shows that *ortho* position is favorable for nitro substitution. Another compound **27** (IC_{50} = 69.8 ± 0.17 μM) having nitro and chloro groups *para* to each other showed moderate inhibitory strength (Figure-7).

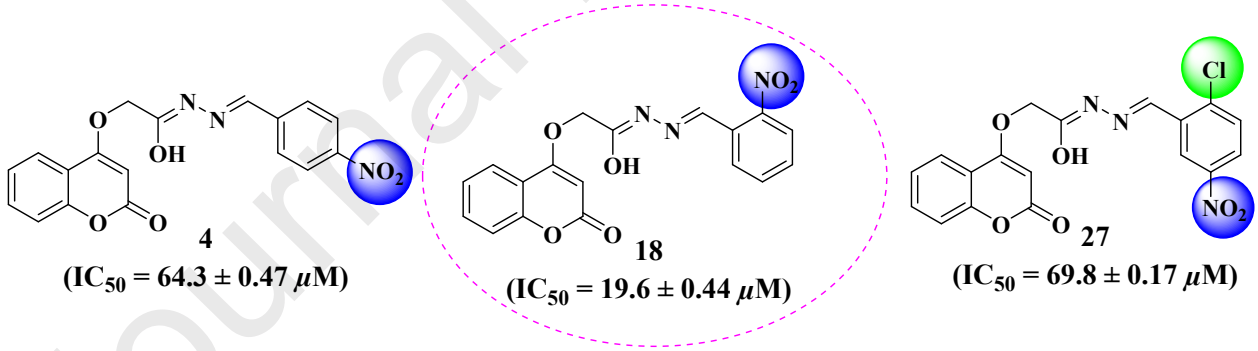

Figure-7: Urease inhibitory activities comparison of analogs **4**, **18**, and **27**, on the basis of their structures

Concisely, limited SAR suggested that almost all substitution/s including methoxy, hydroxy, bromo, chloro, fluoro, and nitro are taking part in the inhibitions but their positions and numbers really alter the inhibitory strength of the molecules.

***In silico* study**

Molecular docking study was carried out to understand the plausible ligand binding mechanism. For that purpose, the crystal structure of Jack bean urease (PDB id: 4GY7, 1.49 Å) was downloaded from the Protein Data Bank (PDB). All steps including standard receptor and ligand preparation, and subsequent docking studies were carried out according to our previously reported protocol [42] by using BioSolveIT's Lead IT software [43]. Discovery Studio Visualizer was used for the visualization of docked conformation.

The docked conformation of compound **10** is shown in Figure-8. The coumarin ring oxygen was making hydrogen bond with Arg609, whereas the carbonyl oxygen was making a bond with Arg609 and His593. The NH group was making a hydrogen bond with carboxymethylated cysteine amino acid Cme592. Several π-alkyl interactions were observed between the coumarin ring and Met637, Ala636. The trimethoxy substituted phenyl ring was also making π-alkyl interaction with Arg439 and Ala436. The pyrone ring of coumarin was also making a π-π T-shaped interaction with His519 and His492, whereas its adjacent ring was making a π-π stacked interaction with His545.

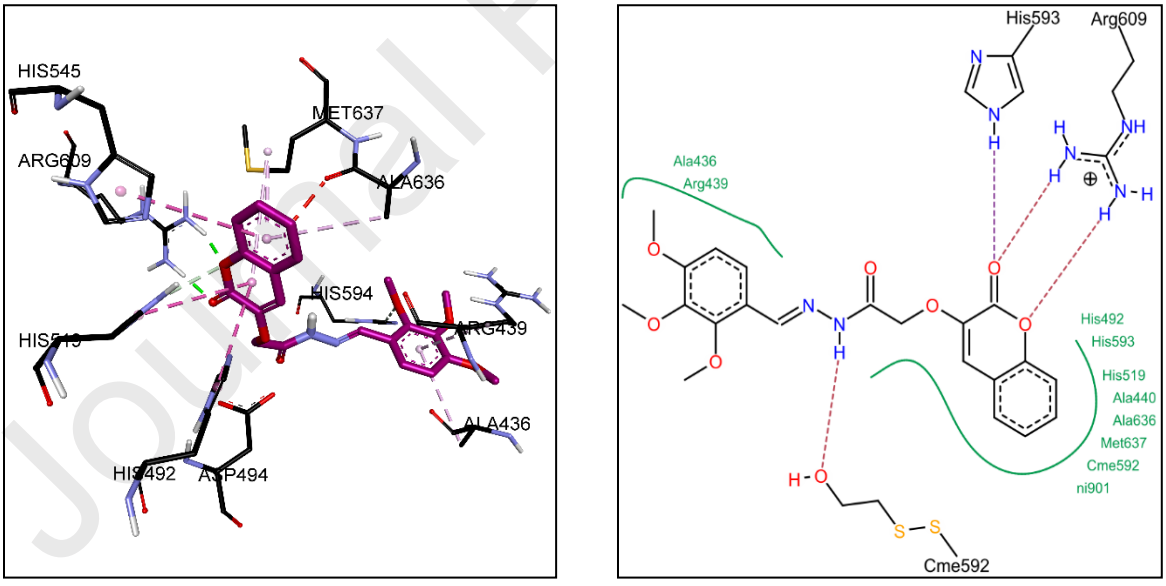

Figure-8: 3D and 2D binding site interactions of docked conformation of compound **10**

Molecular docking studies for compound **14** were also carried out (Figure-9). The carbonyl oxygen of coumarin ring and the oxygen atom of the -OH group were making a hydrogen bond with

Gln635. The two azomethine nitrogen atoms were making hydrogen bonds with Arg439. A π-alkyl interaction was observed between the phenyl ring and Ala436. Another π-alkyl interaction was observed between the coumarin ring and Ala636. Amino acid CMe592 was making π-sulfur interaction with the phenyl ring and the coumarin ring. Another π-sulfur interaction was found between the coumarin ring and Met588.

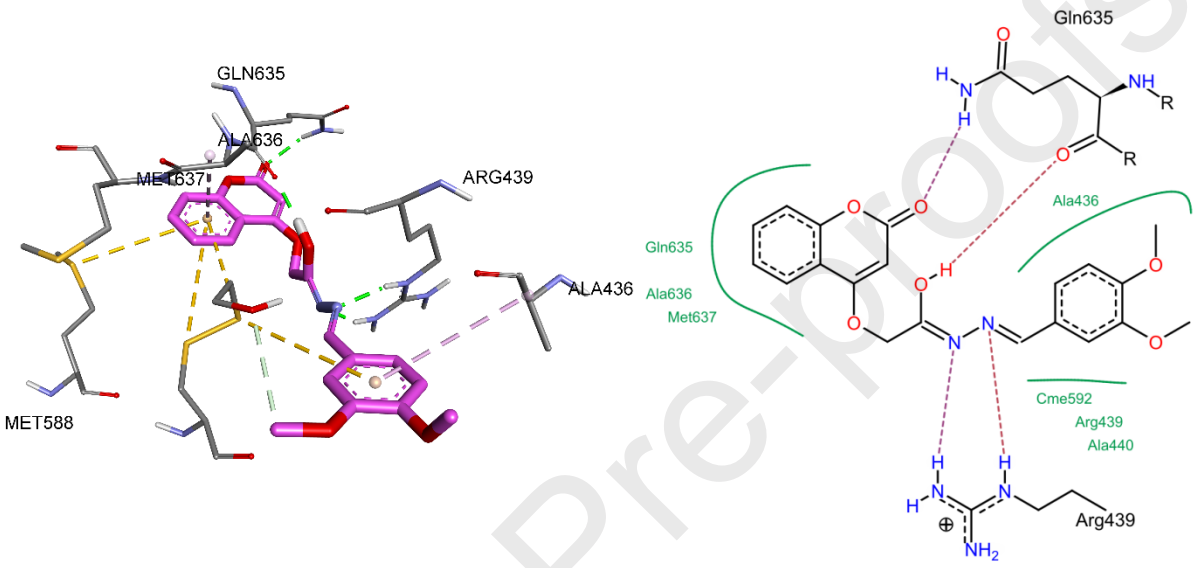

Figure-9: 3D and 2D binding site interactions of docked conformation of compound **14**

Conclusion

A series of 4-oxycoumarinyl linked acetohydrazide Schiff bases **3-27** were synthesized and evaluated for their potential urease inhibitory activity. All compounds were found to be active and it is worth mentioning that many compounds were identified as potent urease inhibitors as compare to standard thiourea. Limited structure activity relationship revealed that all substitutions are taking part in the inhibitory activity but their substitution pattern (number and positions) on aryl ring is responsible for their varying degree of inhibitory strength. Molecular docking studies was also performed on analogs **10** and **14** to understand the binding mechanism of synthetic compounds with active site of enzyme. This study has identified many potent urease inhibitors those may serve as potential leads to identity new drug therapies for gastric and peptic ulcers.

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Experimental

Materials and Methods

All analytical grade reagents were purchased from Sigma-Aldrich, USA. For thin layer chromatography (TLC) silica gel coated aluminum plates GF-254 (Merck, Germany) were used. Spots were visualized under ultraviolet light at 254 and 366 nm or iodine vapors. Electron impact mass spectra were recorded on MAT 113D and MAT 312 mass spectrometers. The ^{1}H- and ^{13}C-NMR spectra were recorded ont 300 and 400 MHz on Bruker AM machines. Chemical shift (δ) values, relative to (TMS) as an internal standard, are presented in ppm, and the coupling constant (J) values are presented in Hz.

Synthesis of methyl 4-oxycoumarinyl acetate (1)

4-Hydroxy coumarin (0.163 g) (1 mmol) and K_2CO_3 (0.13 g) were taken in DMF (10 mL) into a 100 mL round-bottommed flask and stirred to completely solubilized. Then, methyl chloro acetate (0.1 mL) (1 mmol) was added in the reaction mixture and refluxed for 2 h. Reaction progress was monitored by TLC analysis. After completion, reaction mixture was poured onto crushed ice. Precipitates were formed which filtered and washed with distilled water. Structure of compound **1** was confirmed by EI-MS and ^{1}H-NMR.

Methyl 2-((2-oxo-2*H*-chromen-4-yl)oxy)acetate (1)

White Solid; Yield: 65%; M.p.: 94-96 ˚C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 7.85 (d, $J_{6(5,4)}$ = 7.8 Hz, 1H, H-7), 7.68 (t, $J_{6(5,7)}$ = 7.2 Hz, 1H, H-6), 7.40 (m, 2H, H-5, H-8), 5.94 (s, 1H, H-3), 5.10 (s, 2H, CH_2), 3.74 (s, 3H, CH_3); EI-MS *m/z* (% rel. abund.): 234 (M^+, 100), 206 (36), 175 (24), 133 (75).

Synthesis of 4-oxycoumarinyl acetohydrazide (2)

Methyl 4-oxycoumarinyl acetate **1** (0.28g) (1 mmol) was taken in ethanol (20 mL) into a 100 mL round-bottommed flask and dissolved by stirring. Then, hydrazine hydrate (1 mL) was added and further stirred at room temperature. Formation of the desired product was monitored by TLC. On completion, precipitates were formed in the reaction which were filtered and washed with cold

ethanol (10 mL). Structure of 4-oxycoumarinyl acetohydrazide **2** was elucidated by EI-MS and ^{1}H-NMR.

2-((2-Oxo-2*H*-chromen-4-yl)oxy)acetohydrazide (2)

White Solid; Yield: 75%; M.p.: 250-252 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.50 (s, 1H, NH), 7.85 (d, $J_{(7,6)}$ = 7.8 Hz, 1H, H-7), 7.68 (t, $J_{6(5,7)}$ = 7.2 Hz, 1H, H-6), 7.40 (m, 2H, H-5, H-8), 5.94 (s, 1H, H-3), 5.10 (s, 2H, CH_2), 4.41 (d, 2H, NH_2); EI-MS *m/z* (% rel. abund.): 234 (M^+, 38), 163 (85), 120 (100).

General procedure for the synthesis of 4-oxycoumarinyl linked acetohydrazide Schiff bases (3-27)

4-Oxycoumarinyl acetohydrazide **2** (0.160 g, 1.0 mmol) and different substituted aldehydes (1.0 mmol) were taken in methanol (20 mL) into a 100 mL round-bottommed flask. Then, two drops of glacial acetic acid was added and refluxed for 2-3 h. Reaction progress was checked by TLC. After completion of the reaction, solid precipitates were appeared in the reaction mixture which were separated out through filtration, washed with distilled water and hexane to afford pure products **3-27**. The crude products were crystallized from ethanol. The structural determination of all compounds was carried out by EI-MS, ^{1}H-, and ^{13}C-NMR spectroscopy.

(*E,Z*)-*N*'-(2-Ethoxybenzylidene)-2-((2-oxo-2*H*-chromen-4-yl) oxy) acetohydrazide (3)

White Solid; Yield: 74%; M.p.: 237-239 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.71 (s, 1H, OH), 8.61*, 8.38 (s, 1H, N=CH), 7.90 (dd, $J_{6',5'}$ = 6 Hz, $J_{6',4'}$ = 3 Hz, 2H, H-6'), 7.68 (t, $J_{7(6,8)}$ = 8.4 Hz, 1H, H-7), 7.43-7.35 (m, 3H, H-6, H-8, H-4'), 7.09 (d, $J_{3',4'}$ = 8.4 Hz, 1H, H-3'), 6.99 (t, $J_{5'(6'',4')}$ = 7.5 Hz, 1H, H-5'), 5.88 (s, 1H, H-3), 5.44, 4.94* (s, 2H, OCH_2), 4.11 (q, 2H, CH_2), 1.39 (t, 3H, CH_3); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 166.9, 164.7, 164.5*, 162.1, 157.1*, 156.9, 152.7, 143.5*, 140.2, 132.9*, 132.8, 131.7*, 131.4, 125.8, 125.5*, 124.2, 123.2*, 122.9, 120.6*, 120.5, 116.4, 115.2, 112,.7, 91.1, 66.9*, 66.0, 63.8, 15.8; EI-MS *m/z* (% rel. abund.): 366 (M^+, 18), 234 (27), 188 (34), 177 (75), 162 (63), 147 (53), 133 (100), 119 (84), 105 (306).

(*E,Z*)-*N*'-(4-Nitrobenzylidene)-2-((2-oxo-2*H*-chromen-4-yl) oxy) acetohydrazide (4)

White Solid; Yield: 75%; M.p.: 320-322 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 12.02 (s, 1H, OH), 8.41*, 8.12 (s, 1H, N=CH), 8.28 (d, $J_{2',3'/6',5'}$ = 6.6 Hz, 2H, H-2', H-6'), 8.04 (d, $J_{3',2'/5',6'}$ = 9 Hz, 2H,

H-3', H-5'), 7.90 (d, $J_{5,6}$ = 6 Hz, 1H, H-5), 7.68 (t, $J_{7(6,8)}$ = 6 Hz, 1H, H-7), 7.43-7.38 (m, 2H, H-6, H-8), 5.95 (s, 1H, H-3), 5.51, 5.01* (s, 2H, OCH_2); EI-MS *m/z* (% rel. abund.): 367 (M^+, 51), 309 (64), 188 (22), 177 (52), 120 (100), 89 (44).

(*E,Z*)-*N*'-(3-Hydroxy-4-methoxybenzylidene)-2-((2-oxo-2*H*-chromen-4-yl)oxy) acetohydrazide (5)

White Solid; Yield: 72%; M.p.: 313-316 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.55 (s, 1H, OH), 9.19 (s, 1H, OH), 8.12*, 7.95 (s, 1H, N=CH), 7.87 (dd, $J_{5,6}$ = 8.4, $J_{5,7}$ = 2.7 Hz, 1H, H-5), 7.68 (m, 1H, H-7), 7.42-7.37 (m, 2H, H-6, H-8), 7.22 (d, $J_{2',6'}$ = 3 Hz, 1H, H-2'), 7.07 (dd, $J_{6',5'}$ = 6.3 Hz, $J_{6',2'}$ = 1.2 Hz, 1H, H-6'), 6.96 (d, $J_{5',6'}$ = 8.4 Hz, 1H, H-5'), 5.84 (s, 1H, H-3), 5.42, 4.93* (s, 2H, OCH_2), 3.79 (s, 3H, OCH_3); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 166.7, 164.7, 164.5*,161.9, 161.4, 161.3*, 152.7, 149.9*,149.7, 148.4*, 146.8*, 146.7, 144.5, 132.9*, 132.8, 126.6, 124.2, 123.3*, 123.0, 120.4*, 120.1, 116.4, 115.2, 114.9*, 112.4, 112.3*, 111.8*, 111.7, 91.2*, 91.1, 67.0*, 65.9, 55.5; EI-MS *m/z* (% rel. abund.): 368 (M^+, 93), 310 (27), 177 (24), 162 (53), 149 (100), 136 (26), 120 (50).

(*E,Z*)-*N*'-(4-Hydroxybenzylidene)-2-((2-oxo-2*H*-chromen-4-yl)oxy) acetohydrazide (6)

White Solid; Yield: 69%; M.p.: 269-272 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.56 (s, 1H, OH), 9.92(s, 1H, OH), 7.92*, 7.90 (s, 1H, N=CH), 7.88 (d, $J_{5,6}$ = 6 Hz,1H, H-5), 7.68 (m, 1H, H-7), 7.57 (d, $J_{2',3'/6',5'}$ = 8.4 Hz, 2H, H-2', H-6'), 7.43-7.37 (m, 2H, H-6, H-8), 6.82 (d, $J_{3',2'/5',6'}$ = 8.7 Hz, 2H, H-3', H-5'), 5.86 (s, 1H, H-3), 5.41, 4.95* (s, 2H, OCH_2); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 166.7, 164.7, 164.5*, 161.9*, 161.5, 161.3*, 159.5*, 159.3, 152.7, 148.5*, 144.5, 132.9*, 132.8, 128.9*, 128.7, 124.8, 124.2, 123.3*, 123.0, 116.4, 115.6*, 115.5, 115.2, 114.9*, 91.2*, 91.1; EI-MS *m/z* (% rel. abund.): 338 (M^+, 51), 280 (33), 177 (52), 162 (47), 120 (100), 106 (37), 92 (41).

(*E,Z*)-*N*'-(3-Hydroxybenzylidene)-2-((2-oxo-2*H*-chromen-4-yl) oxy) acetohydrazide (7)

White Solid; Yield: 80%; M.p.: 312-314 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.68 (s, 1H, OH), 9.61 (s, 1H, OH), 8.38*, 7.93 (s, 1H, N=CH), 7.91 (d, $J_{5,6}$ = 7.8 Hz, 1H, H-5), 7.71 (t, $J_{7(6,8)}$ = 7.8 Hz, 1H, H-7), 7.43 (m, 2H, H-6, H-8), 7.25 (t, $J_{5'(4',6')}$ = 7.5 Hz, 1H, H-5'), 7.17-7.07 (m, 2H, H-2', H-6'), 6.84 (d, $J_{4',5'}$ = 7.2 Hz,1H, H-4'), 5.86 (s, 1H, H-3), 5.44, 4.94* (s, 2H, OCH_2); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 167.0, 164.7, 164.5*, 162.3*, 161.5, 161.3*, 157.6*, 157.5, 152.7,

148.3*, 144.4, 135.1*, 135.0, 132.9*, 132.8, 129.8*, 128.7, 124.2, 123.3*, 123.0, 118.9*,118.4, 117.6*, 117.3, 116.4, 115.2, 112.9, 112.6*, 91.3*, 91.1, 66.9*, 65.9; EI-MS *m/z* (% rel. abund.): 338 (M^+, 85), 280 (39), 177 (96), 120 (100).

(*E,Z*)-*N*'-(2,4-Dimethoxybenzylidene)-2-((2-oxo-2*H*-chromen-4-yl) oxy) acetohydrazide (8)

White Solid; Yield: 73%; M.p.: 277-279 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.56 (s, 1H, OH), 8.55*, 8.25 (s, 1H, N=CH), 7.90 (dd, $J_{5,6}$ = 7.5 Hz, $J_{5,7}$ = 0.6 Hz, 1H, H-5), 7.84 (d, $J_{6',5'}$ = 8.1 Hz, 1H, H-6'), 7.70 (t, $J_{7\,(6,8)}$ = 7.2 Hz, 1H, H-7), 7.43 (m, 2H, H-6, H-8), 6.61 (s, 1H, H-3'), 6.59 (d, $J_{5',6'}$ = 8.7 Hz, 1H, H-5'), 5.85 (s, 1H, H-3), 5.41, 4.96* (s, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 166.7, 164.7, 164.5*, 162.6*, 162.3, 161.8*, 161.5, 161.4*, 159.2*, 159.0, 152.7, 143.7*, 140.0, 132.9*, 132.8, 126.9, 126.7*, 124.2, 123.3*, 123.0, 116.4, 115.2, 114.9*, 114.6, 106.4, 98.2*, 98.0, 91.2*, 91.1, 67.0*, 66.0, 55.7, 55.4; EI-MS *m/z* (% rel. abund.): 382 (M^+, 85), 293 (10), 207 (18), 188 (26), 177 (24), 163 (100), 149 (86), 121 (50).

(*E,Z*)-*N*'-(3-(Benzyloxybenzylidene)-2-((2-oxo-2*H*-chromen-4-yl)oxy) acetohydrazide (9)

White Solid; Yield: 73%; M.p.: 280-282 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.7 (s, 1H, OH), 8.35*, 7.97 (s, 1H, N=CH), 7.91 (d, $J_{5,6}$ = 7.8 Hz, 1H, H-5), 7.71 (t, $J_{7,6/7,8}$ = 7.5 Hz, 1H, H-7), 7.47-7.30 (m, 10H, H-6, H-8, H-2', H-5', H-6', H-2'', H-3'', H-4'', H-5'', H-6''), 7.08 (d, $J_{4'/5'}$ = 7.8 Hz, 1H, H-4'), 5.88 (s, 1H, H-3), 5.46 (s, 2H, OCH_2), 5.14, 4.96* (s, 2H, OCH_2); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 167.2, 164.7, 161.5, 158.5, 152.7, 148.0*, 143.9, 136.9, 135.2, 132.8, 129.9, 128.4, 127.8, 127.7, 127.6*, 124.2, 123.3*, 123.0, 120.1, 116.7, 116.4, 112.7*, 112.4, 91.3*, 91.2, 69.2, 66.0; EI-MS *m/z* (% rel. abund.): 428 (M^+, 15), 370 (5), 162 (22), 91 (100).

(*E,Z*)-2-((2-Oxo-2*H*-chromen-4-yl) oxy)-*N*'-(2,3,4-trimethoxybenzylidene) acetohydrazide (10)

White Solid; Yield: 78%; M.p.: 278-280 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.63 (s, 1H, OH), 8.44*, 8.19 (s, 1H, N=CH), 7.90 (d, $J_{5,6}$ =7.5 Hz, 1H, H-5), 7.71-7.62 (m, 2H, H-7), 7.58 (d, $J_{6',5'}$ = 9 Hz, 1H, H-6'), 7.43-7.37 (m, 2H, H-6, H-8), 6.92 (d, $J_{5',6'}$ = 9 Hz, 1H, H-5'), 5.86 (s, 1H, H-3), 5.43, 4.93* (s, 2H, OCH_2), 3.83 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 166.7, 164.7, 164.5, 161.5, 155.1, 152.7, 143.6*, 141.4, 140.1, 132.9*,

132.8, 124.2, 123.3*, 122.9, 120.8, 120.6*, 119.9, 116.4, 115.1, 108.6, 91.2*.91.1, 67.0*, 66.0, 61.7, 60.4, 56.0; EI-MS *m/z* (% rel. abund.): 412 (M^+, 40), 193 (100), 179 (74), 162 (21), 120 (27).

(*E,Z*)-*N*'-(2,6-Dimethoxybenzylidene)-2-((2-oxo-2*H*-chromen-4-yl)oxy) acetohydrazide (11)

White Solid; Yield: 81%; M.p.: 285-287 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.56 (s, 1H, OH), 8.42*, 8.25 (s, 1H, N=CH), 7.90 (d, $J_{5,6}$ = 7.5 Hz, 1H, H-5), 7.68 (t, $J_{7\,(6,8)}$ = 8.1 Hz, 1H, H-7), 7.43-7.31 (m, 2H, H-6, H-8, H-4'), 6.72 (d, $J_{3',4'/5',4'}$ = 8.4 Hz, 2H, H-3', H-5'), 5.71 (s, 1H, H-3), 5.33, 4.92* (s, 2H, OCH_2), 3.81 (s, 6H, OCH_3); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 166.8, 164.8, 161.8*, 161.4, 158.8, 158.7*, 152.7, 143.7*, 139.4, 132.8, 131.4*, 131.3, 124.2, 123.2*, 123.0, 116.4, 115.1, 110.4, 104.4, 104.3*, 91.2*, 90.8, 66.9*, 65.9, 56.0, 55.9; EI-MS *m/z* (% rel. abund.): 382 (M^+, 6), 188 (16), 177 (19), 163 (100), 149 (38), 121 (24).

(*E,Z*)-*N*'-(3,5-Dimethoxybenzylidene)-2-((2-oxo-2*H*-chromen-4-yl)oxy) acetohydrazide (12)

White Solid; Yield: 80%; M.p.: 310-312 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.77 (s, 1H, OH), 8.21*, 7.96 (s, 1H, N=CH), 7.90 (d, $J_{5,6}$ = 9 Hz, 1H, H-5), 7.68 (t, $J_{7\,(6,8)}$ = 8.4 Hz, 1H, H-7), 7.43-7.37 (m, 2H, H-6, H-8), 6.89 (m, 2H, H-2', H-6'), 6.56 (d, $J_{4',2'}$ = 2.1 Hz, 1H, H-4'), 5.87 (s, 1H, H-3), 5.48, 4.96* (s, 2H, OCH_2), 3.77 (s, 6H, OCH_3); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 167.2, 164.7, 162.4*, 161.4, 161.3*, 160.6, 152.7, 148.1*, 144.0, 135.8, 132.9*, 132.8, 124.2, 123.2*, 122.9, 116.4, 115.1, 114.9*, 104.9, 102.4*, 102.0, 91.3*, 91.2, 66.9*, 66.0, 55.3; EI-MS *m/z* (% rel. abund.): 382 (M^+, 80), 219 (12), 177 (42), 163 (100), 149(17), 133 (16), 121 (43).

(*E,Z*)-*N*'-(2-Bromo-4,5-dimethoxybenzylidene)-2-((2-oxo-2*H*-chromen-4-yl)oxy) acetohydrazide (13)

White Solid; Yield: 77%; M.p.: 304-306 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.84 (s, 1H, OH), 8.54*, 8.26 (s, 1H, N=CH), 7.89 (d, $J_{5,6}$ = 6.9 Hz, 1H, H-5), 7.68 (t, $J_{7(6,8)}$ = 8.4 Hz, 1H, H-7), 7.50 (s, 1H, H-3'), 7.43-7.37 (m, 2H, H-6, H-8), 7.19 (s, 1H, H-6'), 5.87 (s, 1H, H-3), 5.52, 4.96* (s, 2H, OCH_2), 3.81 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 167.1, 164.7, 164.5*, 161.4, 152.7, 151.4*, 151.1, 148.5, 146.6*, 142.6, 132.9*, 132.8, 124.7, 124.5*, 124.2, 123.2*,122.9, 116.4, 115.4*, 115.3, 115.1, 114.8, 109.0, 108.4*, 91.3*, 91.2, 66.9*, 66.1, 56.0, 55.6; EI-MS *m/z* (% rel. abund.): 460 (M^+, 17), 462 (M+2, 18), 382 (17), 338 (8), 323 (12), 243 (100), 177 (45), 163 (57).

(*E,Z*)-*N*'-(3,4-Dimethoxybenzylidene)-2-((2-oxo-2*H*-chromen-4-yl) oxy) acetohydrazide (14)

White Solid; Yield: 65%; M.p.: 245-247 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.63 (s, 1H, OH), 8.25*, 7.93 (s, 1H, N=CH), 7.90 (dd, $J_{5,6}$ = 7.8 Hz, $J_{5,7}$ = 1.0 Hz, 1H, H-5), 7.68 (t, $J_{7(6,8)}$ = 8.4 Hz, 1H, H-7), 7.43 (d, $J_{2',6'}$ = 1.5 Hz, 1H, H-2'), 7.41-7.30 (m, 2H, H-6, H-8), 7.22 (d, $J_{6',5'}$ = 8.4 Hz, 1H, H-6'), 7.01 (d, $J_{5',6'}$ = 8.4 Hz, 1H, H-5'), 5.85 (s, 1H, H-3), 5.47, 4.95* (s, 2H, OCH_2), 3.79 (s, 6H, OCH_3); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 166.9, 164.7, 162.0*, 161.4, 152.7, 150.6, 148.9, 148.4*, 144.3, 132.9*, 132.8, 126.5, 124.2, 123.2*, 122.9, 121.9*, 121.3, 116.4, 115.1, 111.4, 108.8, 108.4*, 91.2*, 91.1, 67.0*, 66.0, 55.5, 55.4; EI-MS *m/z* (% rel. abund.): 382 (M^+, 100), 324 (6), 177 (10), 163 (73).

(*E,Z*)-*N*'-(2-Methoxybenzylidene)-2-((2-oxo-2*H*-chromen-4-yl) oxy) acetohydrazide (15)

White Solid; Yield: 72%; M.p.: 275-277 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.70 (s, 1H, OH), 8.64*, 8.35 (s, 1H, N=CH), 7.91-7-88 (m, 2H, H-5, H-6'), 7.67 (m, 1H, H-7), 7.42-7.38 (m, 3H, H-6, H-8, H-4'), 7.10 (d, $J_{3',4'}$ = 7.6 Hz, 1H, H-3'), 7.00 (t, $J_{5'(4',6')}$ = 7.6 Hz, 1H, H-5'), 5.88 (s, 1H, H-3), 5.44, 4.93* (s, 2H, OCH_2), 3.84 (s, 3H, OCH_3); EI-MS *m/z* (% rel. abund.): 352 (M^+, 24), 234 (19), 177 (100), 163 (15), 133 (59), 121 (62), 91 (13).

(*E,Z*)-*N*'-(2-Chloro-3-methoxybenzylidene)-2-((2-oxo-2*H*-chromen-4-yl)oxy) acetohydrazide (16)

White Solid; Yield: 73%; M.p.: 319-321 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.94 (s, 1H, OH), 8.75*, 8.42 (s, 1H, N=CH), 7.90 (d, $J_{5,6}$ = 7.8 Hz, 1H, H-5), 7.71-7.65 (m, 2H, H-7, H-6'), 7.44-7.34 (m, 3H, H-6, H-8, H-3'), 7.21 (d, $J_{4',5'}$ = 9 Hz, 1H, H-5'), 5.92 (s, 1H, H-3), 5.47, 4.92* (s, 2H, OCH_2), 3.87 (s, 3H, OCH_3); EI-MS *m/z* (% rel. abund.): 386 (M^+, 4), 388 (M+2, 2), 293 (31), 182 (28), 177 (100), 162 (62), 155 (31), 120 (59).

(*E,Z*)-*N*'-(3,4-Dihydroxybenzylidene)-2-((2-oxo-2*H*-chromen-4-yl)oxy) acetohydrazide (17)

White Solid; Yield: 71%; M.p.: 325-327 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.52 (s, 1H, OH), 9.30 (s, 2H, OH), 8.15*, 7.8 (s, 1H, N=CH), 7.90 (d, $J_{5,6}$ =7.5 Hz, 1H, H-5), 7.71 (t, $J_{7,6/7,8}$ = 8.1 Hz, 1H, H-7), 7.43 (ovp, 2H, H-6, H-8), 7.16 (s, 1H, H-2'), 6.95 (ovp, 1H, H-6'), 6.77 (d, $J_{5',6'}$ = 8.1 Hz, 1H, H-5'), 5.83 (s, 1H, H-3), 5.40, 4.95* (s, 2H, OCH_2); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 166.6, 164.7, 164.5*, 161.8*, 161.4, 161.3*, 152.7, 148.7, 148.2*, 147.9, 145.7*, 145.6, 144.9, 132.9*, 132.8, 125.2, 124.2, 123.3*, 123.0, 120.7*, 120.2, 116.4, 115.5, 115.2, 114.9, 112.9,

112.6*, 91.2*, 91.1, 67.0*, 65.9; EI-MS *m/z* (% rel. abund.): 354 (M^+, 15), 294 (15), 176 (37), 120 (100).

(*E,Z*)-*N*'-(2-Nitrobenzylidene)-2-((2-oxo-2*H*-chromen-4-yl)oxy) acetohydrazide (18)

White Solid; Yield: 76%; M.p.: 312-314 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 12.04 (s, 1H, OH), 8.69*, 8.39 (s, 1H, N=CH), 8.19 (d, $J_{6',5'}$ = 7.7 Hz, 1H, H-6'), 8.07 (d, $J_{3',4'}$ = 9 Hz, 1H, H-3'), 7.90 (d, $J_{5,6}$ = 6.9 Hz, 1H, H-5), 7.82 (t, $J_{7(6,8)}$ = 7.5 Hz, 1H, H-7), 7.71-7.64 (m, 2H, H-6, H-8), 7.43-7.37 (m, 2H, H-4', H-5'), 5.93 (s, 1H, H-3), 5.46, 5.00* (s, 2H, OCH_2); EI-MS *m/z* (% rel. abund.): 367 (M^+, 19), 337 (9), 203 (20), 177 (100), 162 (36).

(*E,Z*)-*N*'-(4-Bromo-3,5-dimethoxybenzylidene)-2-((2-oxo-2*H*-chromen-4-yl)oxy) acetohydrazide (19)

White Solid; Yield: 77%; M.p.: 331-334 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.89 (s, 1H, OH), 8.35*, 7.98 (s, 1H, N=CH), 7.90 (d, $J_{5,6}$ = 7.5 Hz, 1H, H-5), 7.68 (t, $J_{7(6,8)}$ = 8.1 Hz, 1H, H-7), 7.43-7.37 (m, 2H, H-6, H-8), 7.09 (s, 1H, H-2'), 7.06 (s, 1H, H-6'), 5.87 (s, 1H, H-3), 5.52, 4.98* (s, 2H, OCH_2), 3.88 (s, 6H, OCH_3); EI-MS *m/z* (% rel. abund.): 460 (M^+, 2), 462 (M+2, 2), 279 (3), 243 (11), 178 (100), 103 (05), 44 (14).

(*E,Z*)-*N*'-(4-Fluoro-3-methoxybenzylidene)-2-((2-oxo-2*H*-chromen-4-yl)oxy) acetohydrazide (20)

White Solid; Yield: 76%; M.p.: 308-310 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.77 (s, 1H, OH), 8.25*, 7.95 (s, 1H, N=CH), 7.88 (d, $J_{5,6}$ = 9 Hz, 1H, H-5), 7.66 (t, $J_{7(6,8)}$ = 8.4 Hz, 1H, H-7), 7.51 (d, $J_{6',5'}$ = 9 Hz, 1H, H-6'), 7.41-7.35 (m, 2H, H-6, H-8), 7.28 (s, 1H, H-2'), 7.24 (d, $J_{5',6'}$ = 9 Hz, 1H, H-5'), 5.86 (s, 1H, H-3), 5.47, 4.95* (s, 2H, OCH_2), 3.86 (s, 3H, OCH_3); EI-MS *m/z* (% rel. abund.): 370 (M^+, 51), 312 (8), 188 (15), 177 (100), 152 (50), 138 (30), 121 (49).

(*E,Z*)-*N*'-(3-Bromo-4-hydroxybenzylidene)-2-((2-oxo-2*H*-chromen-4-yl) oxy) acetohydrazide (21)

White Solid; Yield: 79%; M.p.: 320-324 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.64 (s, 1H, OH), 10.75 (s, OH), 8.15*, 7.89 (s, 1H, N=CH), 7.89 (m, 2H, H-5, H-2') 7.68 (t, $J_{7(6,8)}$ = 8.4 Hz, 1H, H-7), 7.56 (dd, $J_{6',2'}$ = 3 Hz, $J_{6',5'}$ = 8.4 Hz, 1H, H-6'), 7.43-7.38 (m, 2H, H-6, H-8),7.00 (d, $J_{5',6'}$ = 8.4 Hz, 1H, H-5'), 5.88 (s, 1H, H-3), 5.44, 4.93* (s, 2H, OCH_2); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 166.9, 164.7, 161.5, 155.7, 152.7, 164.9*, 143.0, 132.9*, 132.8, 131.6*, 131.2, 128.0, 127.8*,

126.5, 124.2, 123.2*, 122.9, 116.4, 116.3*, 115.2, 109.8, 91.2, 66.9*, 66.0; EI-MS *m/z* (% rel. abund.): 416 (M^+, 51), 418 (M+2, 49), 358 (13), 199 (37), 177 (100), 134 (35), 121 (52), 105 (58).

(*E,Z*)-*N*'-(5-Bromo-2-methoxybenzylidene)-2-((2-oxo-2*H*-chromen-4-yl)oxy) acetohydrazide (22)

White Solid; Yield: 75%; M.p.: 274-276 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.79 (s, 1H, OH), 8.56*, 8.26 (s, 1H, N=CH), 8.01 (d, $J_{6',4'}$ = 2.7 Hz, 1H, H-6'), 7.90 (d, $J_{5,6}$ = 9 Hz, 1H, H-5), 7.68 (t, $J_{7(6,8)}$ = 8.1 Hz, 1H, H-7), 7.57 (dd, $J_{4',6'}$ = 2.4 Hz, $J_{4',3'}$ = 9 Hz, 1H, H-4'), 7.43-7.37 (m, 2H, H-6, H-8), 7.11 (d, $J_{3',4'}$ = 9 Hz, 1H, H-3'), 5.90 (s, 1H, H-3), 5.50, 4.98* (s, 2H, OCH_2), 3.84 (s, 3H, OCH_3); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 167.2, 164.7, 162.4, 161.5, 161.3*, 156.7, 152.7, 142.0*, 138.3, 133.9*, 133.8, 132.9*, 132.7, 127.7, 127.4*, 124.2, 124.0*, 123.3*, 122.9, 116.4, 115.2, 114.4*, 114.2, 112.5, 91.2, 67.0*, 66.1, 56.1; EI-MS *m/z* (% rel. abund.): 430 (M^+, 25), 432 (M+2, 21), 341 (8), 234 (20), 211 (46), 188 (30), 177 (100), 121 (32), 91 (26).

(*E,Z*)-*N*'-(6-Bromo-2-hydroxy-3-methoxybenzylidene)-2-((2-oxo-2*H*-chromen-4-yl)oxy) acetohydrazide (23)

White Solid; Yield: 84%; M.p.: 322-324 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 12.43 (s, 1H, OH), 12.32 (bs, 1H, OH), 8.84, 8.49* (s, 1H, N=CH), 8.00 (dd, $J_{5,6}$ = 7.2 Hz, $J_{5,7}$ = 1.6 Hz, 1H, H-5), 7.71 (ovp, 1H, H-7), 7.44 (ovp, 2H, H-6, H-8), 7.15 (d, $J_{5',4'}$ = 8.8 Hz, 1H, H-5'), 6.99 (d, $J_{4',5'}$ = 8.8 Hz, 1H, H-4'), 5.95 (s, 1H, H-3), 5.44*, 5.03 (s, 2H, OCH_2), 3.84 (s, 3H, OCH_3); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 166.5*, 164.6*, 164.3, 162.6, 161.4*, 161.3, 152.7, 149.9, 149.6, 148.1*, 147.9, 145.2*, 132.9, 132.8*, 124.2, 123.4, 122.9*, 122.7, 116.7*, 116.4, 115.4, 115.1, 114.8, 114.7, 113.7, 113.1*, 91.5*, 91.3, 66.8, 65.9*, 56.0*, 55.9; EI-MS *m/z* (% rel. abund.): 446 (M^+, 100), 448 (M+2, 98), 309 (29), 177 (45), 163 (74), 135 (30).

(*E,Z*)-*N*'-(2-Chloro-3-methoxybenzylidene)-2-((2-oxo-2*H*-chromen-4-yl)oxy) acetohydrazide (24)

White Solid; Yield: 83%; M.p.: 323-325 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 11.92 (s, 1H, OH), 8.71*, 8.42 (s, 1H, N=CH), 7.90 (dd, $J_{5,6}$ = 6.8 Hz, $J_{5,7}$ = 1.2, 1H, H-5), 7.70-7.65 (m, 2H, H-7, H-6'), 7.43-7.34 (m, 3H, H-6, H-8, H-5'), 7.22 (d, $J_{4',5'}$ = 8.8 Hz, 1H, H-4'), 5.92 (s, 1H, H-3), 5.47,

4.95* (s, 2H, OCH_2), 3.87 (s, 3H, OCH_3); EI-MS *m/z* (% rel. abund.):386 (M^+, 10), 388 (M+2, 5), 293 (33), 182 (26), 177 (100), 167 (45), 155 (28), 121 (32).

(*E,Z*)-*N*'-(2,4-Dichlorobenzylidene)-2-((2-oxo-2*H*-chromen-4-yl)oxy) acetohydrazide (25)

White Solid; Yield: 76%; M.p.: 338-340 ˚C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ11.97 (s, 1H, OH), 8.63*, 8.34 (s, 1H, N=CH), 8.12 (d, $J_{6',5'}$ = 8.7 Hz, 1H, H-6'), 7.90 (d, $J_{5,6}$ = 6.9 Hz, 1H, H-5), 7.72 (d, $J_{6',5'}$ = 3 Hz, 1H, H-3'), 7.66 (t, $J_{7(6,5)}$ = 6 Hz, 1H, H-7), 7.52 (dd, $J_{5',6'}$ = 6.9, $J_{5',3'}$ = 1.8, Hz, 1H, H-5'), 7.43-7.37 (m, 2H, H-6, H-8), 5.94 (s, 1H, H-3), 5.48, 4.98* (s, 2H, OCH_2): ^{13}C-NMR (400 MHz, DMSO-d_6): δ167.3, 164.6, 162.7*, 161.5, 152.7, 143.0*, 139.1, 135.0, 133.6, 132.9*, 132.8, 130.2, 129.3, 128.4, 128.1*, 128.0*, 127.8, 124.2, 123.3*, 122.9, 116.4, 115.1, 91.3, 66.9*, 66.0; EI-MS *m/z* (% rel. abund.): 390 (M^+, 11), 392 (M+2, 8), 394 (M+4, 3), 297 (10), 219 (13), 188 (19), 177 (100), 162 (14), 121 (37).

(*E,Z*)-*N*'-(3,5-Dichloro-2-hydroxybenzylidene)-2-((2-oxo-2*H*-chromen-4-yl)oxy) acetohydrazide (26)

White Solid; Yield: 75%; M.p.: 309-311 ˚C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ11.90 (s, 1H, OH), 10.33 (s, 1H, OH), 8.48*, 8.26 (s, 1H, N=CH), 7.89 (d, $J_{5,6}$ = 7.2 Hz, 1H, H-5), 7.78 (d, $J_{6',4'}$ = 2.4 Hz, 1H, H-4'), 7.68-7.62 (m, 2H, H-6, H-7), 7.59 (d, $J_{6',4'}$ = 2.4 Hz, 1H, H-6'), 7.44 (d, $J_{8,7}$ = 7.5 Hz, 1H, H-8'), 5.94 (s, 1H, H-3), 5.51, 5.04* (s, 2H, OCH_2); EI-MS *m/z* (% rel. abund.): 406 (M^+, 73), 408 (M+2, 52), 410 (M+4, 10), 348 (15), 219 (34), 187 (65), 177 (37), 163 (100), 120 (78), 92 (37).

(*E,Z*)-*N*'-(2-Chloro-5-nitrobenzylidene)-2-((2-oxo-2*H*-chromen-4-yl)oxy) acetohydrazide (27)

White Solid; Yield: 76%; M.p.: 298-300 ˚C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ12.11 (s, 1H, OH), 8.74 (d, $J_{6',4'}$ = 2.7 Hz, 1H, H-6'), 8.66*, 8.41 (s, 1H, N=CH), 8.24 (dd, $J_{4',3'}$ = 9 Hz, $J_{4',6'}$ = 2.7 Hz, 1H, H-4'), 7.90 (d, $J_{5,6}$ = 7.5 Hz, 1H, H-5), 7.85 (d, J = 8.7 Hz, 1H, H-3'), 7.68 (t, 1H, $J_{7(6,8)}$ = 8.4 Hz, H-7), 7.44-7.37 (m, 2H, H-6, H-8), 5.93 (s, 1H, H-3), 5.57, 5.02* (s, 2H, OCH_2); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 167.4, 164.6, 164.4*, 162.8*, 161.5, 161.3*, 152.7, 148.1*, 148.0, 143.7*, 139.7, 133.8*, 133.4, 132.9*, 132.8, 130.8,*, 130.6, 128.6, 128.4*, 128.1*, 128.0, 124.6*, 124.4, 124.5, 123.2*, 122.9, 116.4, 115.1, 114.9*, 91.3*, 91.2, 66.8*, 66.0; EI-MS *m/z* (% rel. abund.): 401 (M^+, 80), 403 (M+2, 27), 365 (69), 308 (47), 203 (50), 176 (100).

Urease inhibitory assay

Urease inhibitory activity was performed by following the method of Weatherburn (1967). Test compound (250 μM, 5 μL) was incubated with urease solution (1 U/well) 25 μL for 15 min at 30 $^{\circ}$C. After that, urea (substrate) (100 mM, 55 μL) was added and then plate was incubated for 10 min at 30 $^{\circ}$C. Then, 70 μL of alkali reagents (0.5% w/v sodium hydroxide and 0.1% sodium hypochlorite, and 45 μL of phenol (1% w/v phenol and 0.005% w/v sodium nitroprusside)) was added. Plate was again incubated for 50 min at 30 $^{\circ}$C. Urease activity was calculated with the rate of production of ammonia and alteration in absorbance was examined at 630 nm on ELISA plate reader (Spectra Max M2, Molecular Devices, CA, USA). Thiourea was used as a standard control [44].

Docking methodology

MOE (Molecular Operating Environment) [45] was used to perform molecular docking study in order to predict the binding mode of the synthetic compounds in the active site of urease enzyme. Using the builder tool implemented in MOE software, the three dimensional structures of the synthetic derivatives were generated. The generated compounds were 3D protonated and energy minimized using the default parameters of the MOE (gradient: 0.05, Force Field: MMFF94X). For further evaluation of compounds in molecular docking study, all the compounds were then saved in mdb (Molecular Data Base) file format.

3D structure of the target protein was retrieved from the protein databank (PDB ID 4ubp). The retrieved protein was opened in MOE, water molecules were removed and 3D protonation was carried. After 3D protonation, the protein was energy minimized to get a stable conformation of the protein using the default parameters of MOE. For docking studies, the default parameters of MOE were used *i.e.* Placement: Triangle Matcher, rescoring 1: London dG, Refinement: Force field, Rescoring 2: GBVI/WSA. For each ligand ten conformations were allowed to be formed and the top ranked conformations on the basis of docking score were selected for further analysis.

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4-Oxycoumarinyl Linked Acetohydrazide Schiff bases as Potent Urease Inhibitors

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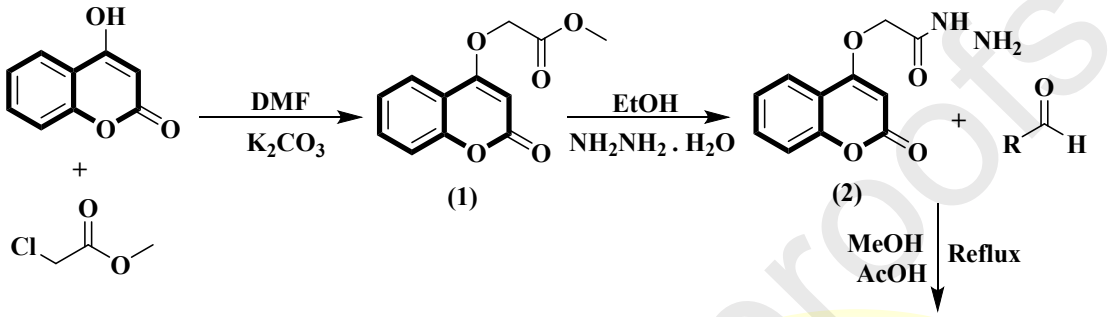
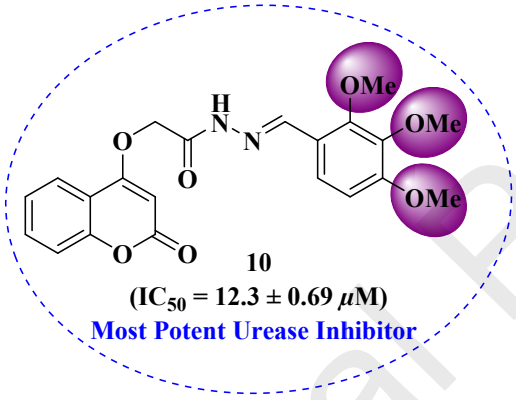
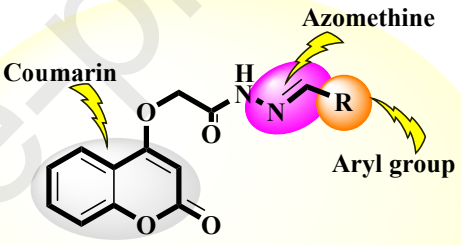




10

(IC_{50} = 12.3 ± 0.69 μM)

Most Potent Urease Inhibitor

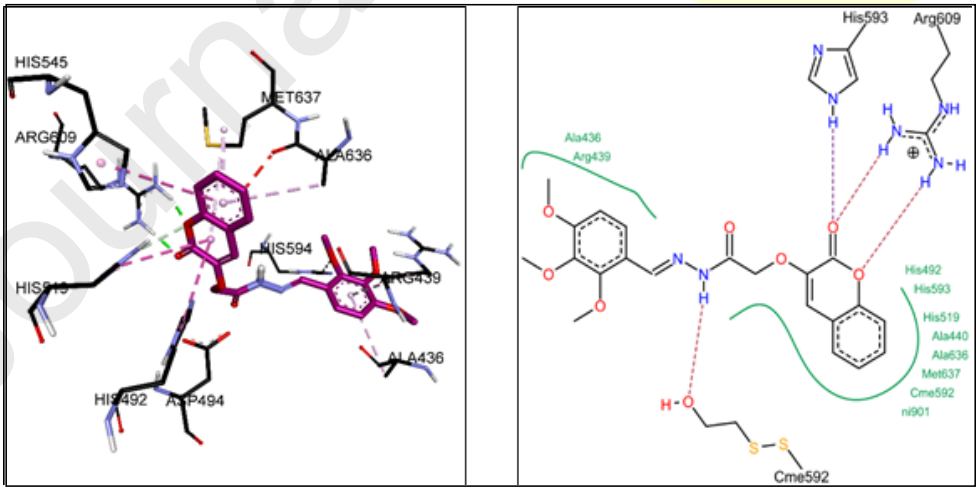


3-27

***N*'-Benzylidene-2-((2-oxo-2*H*-chromen-4-yl)oxy)acetohydrazide Schiff base derivatives**

IC_{50} = 12.3 ± 0.69 to 88.8 ± 0.04 μM

Standard Urea (IC_{50} = 21.5 ± 0.47 μM)



3D and 2D binding site interactions of docked conformation of compound 10

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Highlights:

- Synthesis of 4-oxycoumarinyl linked acetohydrazide schiff bases **3-27**
- Characterization of all analogs by various spectroscopic techniques
- Urease inhibitory activity *in vitro*
- Structure-activity relationship (SAR)
- Molecular docking studies to rationalize the binding interactions

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