



Design, synthesis, in vitro evaluation, molecular docking and ADME properties studies of hybrid bis-coumarin with thiadiazole as a new inhibitor of Urease

Munther Alomari^a, Muhammad Taha^{b,*}, Syahrul Imran^c, Waqas Jamil^d, Manikandan Selvaraj^e, Nizam Uddin^f, Fazal Rahim^g

^a Department of Stem Cell Biology, Institute for Research and Medical Consultations, Imam Abdulrahman Bin Faisal University, P.O. Box. 1982, Dammam 31441, Saudi Arabia

^b Department of Clinical Pharmacy, Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University, P.O. Box 1982, Dammam 31441, Saudi Arabia

^c Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Universiti Teknologi MARA Cawangan Selangor Kampus Puncak Alam, 42300 Bandar Puncak Alam, Selangor D. E., Malaysia

^d Institute of Advance Research Studies in Chemical Sciences, University of Sindh Jamshoro, Hyderabad 76080, Pakistan

^e Monash University School of Chemical Engineering, 47500 Selangor Alam Campus, Bandar Subway 42300, Malaysia

^f Department of Chemistry, University of Karachi, Karachi 75270, Pakistan

^g Department of Chemistry, Hazara University, Mansehra 21300, Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Hybrid bis-coumarin derivatives **1–18** were synthesized and evaluated for their in vitro urease inhibitory potential. All compounds showed outstanding urease inhibitory potential with IC₅₀ value (The half maximal inhibitory concentration) ranging in between 0.12 SD 0.01 and 38.04 SD 0.63 μ M (SD standard deviation). When compared with the standard thiourea (IC₅₀ = 21.40 $\pm$ 0.21 μ M). Among these derivatives, compounds **7** (IC₅₀ = 0.29 $\pm$ 0.01), **9** (IC₅₀ = 2.4 $\pm$ 0.05), **10** (IC₅₀ = 2.25 $\pm$ 0.05) and **16** (IC₅₀ = 0.12 $\pm$ 0.01) are better inhibitors of the urease compared with thiourea (IC₅₀ = 21.40 $\pm$ 0.21 μ M). To find structure–activity relationship molecular docking as well as absorption, distribution, metabolism, and excretion (ADME) studies were also performed. Various spectroscopic techniques like ¹H NMR, ¹³C NMR, and EI-MS were used for characterization of all synthesized analogs. All compounds were tested for cytotoxicity and found non-toxic.

1. Introduction

Urease is an enzyme that catalyzes the hydrolysis of urea to produce ammonia and carbon dioxide, and the most vital role is to protect the bacteria in the acidic environment of the stomach [1]. The urease inhibitors can play a vital role to counter effect the negative function of urease in living organisms. Urease inhibitors are effective against several serious infections caused by the secretion of urease by *Helicobacter pylori* which include gastric tract syndromes and urinary tract infection. Research on urease inhibitions yielded several vital therapeutic drugs [2]. Moreover, it has been demonstrated that *H. pylori* lacking urease activity are incapable of causing infection in animal models. Thus, it is most likely that urease is essential for bacterial colonization and perhaps the pathogenesis of related disease *in vivo*. World Health Organization has categorized *H. pylori* as a class I carcinogen [3]. Inhibition of

urease was extensively studied because of their potential uses like therapy against bacterial urease e.g. *H. pylori* that induced pathogenic conditions i.e. urinary stone formation, peptic ulcer pyelonephritis and hepatic coma. Urease inhibitor dissolves crystals and struvite kidney stones and prevents new crystal formation in urine [4,5].

Coumarin and its derivatives are widely distributed naturally occurring compounds with diverse biological activities. Many of natural products such as, warfarin (**1**), umbelliferone (7-hydroxycoumarin) (**2**), aesculetin (6,7-dihydroxycoumarin) (**3**), herniarin, psoralen (**4**) and imperatorin (**5**) containing coumarin moiety [6]. Coumarin derivatives have been found to have numerous therapeutic applications including anti-HIV [7–10], anti-inflammatory [11–13], analgesic [14], anti-mutagenic [11], anticancer [15–19], antibiotic [20,21], antitumor, [22,23]. Furthermore, coumarins are known to be lipid lowering agents with moderate triglyceride lowering activity [24]. They also found as a

* Corresponding author.

E-mail address: mtaha@uod.edu.sa (M. Taha).

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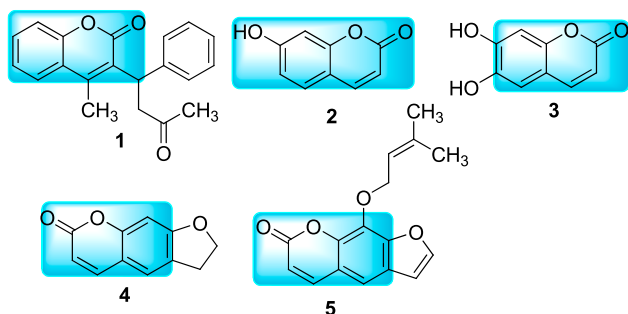


Fig. 1. Structures of some naturally occurring Coumarin.

powerful chain breaking antioxidants [25]. The pattern of substitutions on the basic chemical structure is said to influence both the coumarin's pharmacological and biochemical properties, including the therapeutic applications, and can beneficially affect toxicity. Now the diversity of coumarin derivatives, both natural and synthetic, has grown in search of better therapeutics [26] (see Fig. 1).

1,3,4-Thiadiazoles are well known heterocyclic compounds, having diverse biological properties, such as anticonvulsant [27], anti-inflammatory effects [28], antidiabetic, [29], antibacterial [30,31], antioxidant [32,33], antifungal [34], and antidepressant [35,36], etc. Nowadays single drug therapy concept has been replacing by "hybrid drugs" ideas, due to insufficient therapeutic effect to control the diseases. So, different pharmacotherapeutic profile combination within one drug is now a developing field in medicinal chemistry. The known side effect of one drug may be suppressed by incorporating another drug with it *i.e.* hybrid drug [37].

Furthermore, almost all reported compounds have originated from urea and thiourea, the later one is standard drug used for the treatment of urease inhibition, whereas compound having some other origin (heterocyclic or carbocyclic) was found to be less potent. Therefore, it is a need of time that to synthesize compounds having some unique structural features and can show potential either containing electron withdrawing or electron donating substitutions. The above literature revealed that both coumarin and thiazole are active compounds, Hence the combination (hybridization) of these two may result a better therapeutic lead with pronounced effect on activity as well as helpful to minimize the adverse effect of single drug [38].

2. Result and discussion

2.1. Chemistry

In the demand to synthesize hybrid biscoumarin derivatives started with refluxing 40 mmol of 4-hydroxy coumarin with 20 mmol of 4-Nitrobenzaldehyde in ethanol to form 4-hydroxy-3-((4-hydroxy-2-oxo-2H-chromen-3-yl)(4-nitrophenyl)methyl)-2H-chromen-2-one (a). In the second step 4-hydroxy-3-((4-hydroxy-2-oxo-2H-chromen-3-yl)(4-nitrophenyl)methyl)-2H-chromen-2-one (a) was reduced to 4-hydroxy-3-((4-hydroxy-2-oxo-2H-chromen-3-yl)(4-aminophenyl)methyl)-2H-chromen-2-one (b). 4-hydroxy-3-((4-hydroxy-2-oxo-2H-chromen-3-yl)(4-aminophenyl)methyl)-2H-chromen-2-one (b) was further treated with carbon disulfide to form intermediate which is further reflux with different benzoyl hydrazide to form (1–18) desired compounds. Different spectroscopic techniques such as EI-MS, ^1H NMR and C^{13}NMR were used to determine the structure of all analogs Scheme1 (Table 1).

2.2. Structure-activity relationship (SAR) for urease inhibitory activity

Compound 1–18 were synthesized in the continuation of your research on enzyme inhibition [39]. All these compounds were evaluated for their urease inhibition activity. The *in-vitro* screening results showed that the combination of the two coumarin skeleton in the cage like

morphology as well as hybridization with substituted thiazole ring structure exhibited comparable urease inhibition potential to that of the reference. Furthermore, the substitution on thiazole ring may play influential role for urease inhibition activity. All the synthesized compounds 1–18 showed excellent urease inhibition activity and most of the compounds found to be better active than the standard thiourea ($\text{IC}_{50} = 21.40 \pm 0.21 \mu\text{M}$) (IC_{50} = The half maximal inhibitory concentration) as shown in Table 1. Mostly halogen (F, Cl, Br) containing derivatives shown better activity but some other substitutions such as -OMe or -Me groups also showed excellent potential towards urease inhibition. Among halogens Fluoro substituted derivatives showed remarkable activity. Compound 16 ($\text{IC}_{50} = 0.12 \pm 0.01 \mu\text{M}$) was found to be most active compound of this series and bears 4-trifluoro methyl substitution at phenyl ring attached with thiadiazole ring Compound 7 ($\text{IC}_{50} = 0.29 \pm 0.01 \mu\text{M}$) having 2-fluoro substitution also showed excellent inhibition activity, both compounds were found to be many folds better active than the standard. The elevated potential of compound 16 may be due to trifluoro substitution which have very high capacity to interact with the enzyme's active sites via hydrogen bonding, while according to structural features of compound 7 the fluoro group is near to -OH group and may have some interaction with -OH group, which made this molecule better fit for the enzyme. Compound 8 ($\text{IC}_{50} = 5.12 \pm 0.06 \mu\text{M}$) and 9 ($\text{IC}_{50} = 2.4 \pm 0.05 \mu\text{M}$) with 3 and 4 fluoro substitution also showed good activity but in lesser extent than the compounds 7 and 16 but these compounds still better active than the standard. Compounds 1 ($\text{IC}_{50} = 7.41 \pm 0.08 \mu\text{M}$), 2 ($\text{IC}_{50} = 15.85 \pm 0.18 \mu\text{M}$), and 3 ($\text{IC}_{50} = 13.97 \pm 0.16 \mu\text{M}$) with bromo substitution at 2nd, 3rd, and 4th position also showed good inhibition potential although chloro substituted compounds at 2nd, 3rd, and 4th position in compound 10 ($\text{IC}_{50} = 2.25 \pm 0.05 \mu\text{M}$), 11 ($\text{IC}_{50} = 14.06 \pm 0.14 \mu\text{M}$), and 12 ($\text{IC}_{50} = 6.8 \pm 0.08 \mu\text{M}$) respectively showed admirable activity. Among chloro and bromo group containing derivatives activity pattern it was revealed that size of the substituent may also effect on activity with its position. The bromo substituted compounds were found to be more potent than chloro substituted compounds (see Fig. 2).

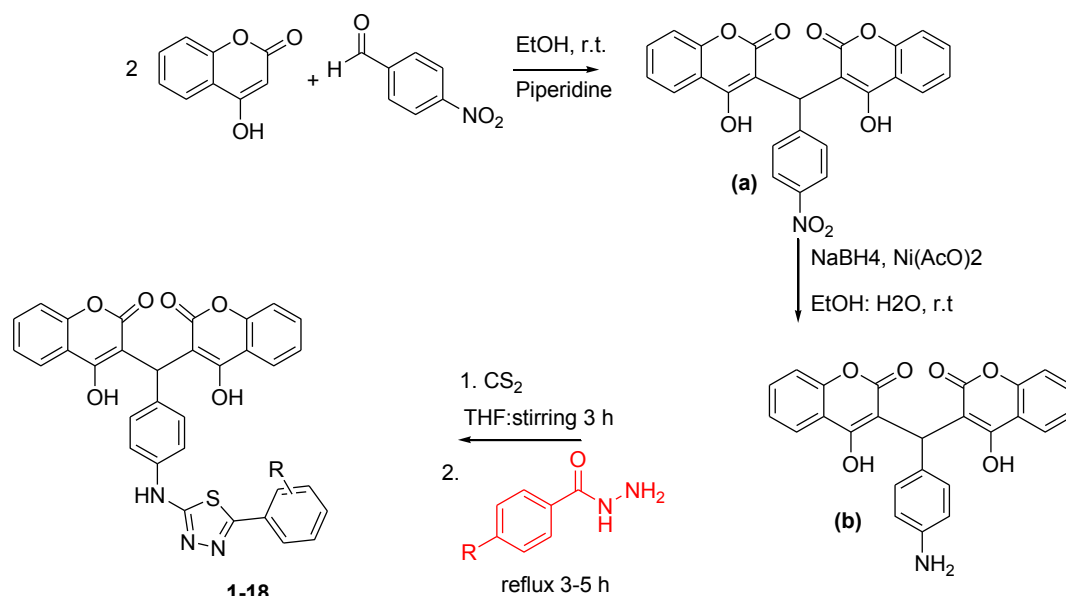
The -OMe substitution at various positions *i.e.* 2-OMe, 3-OMe and 4-OMe in compounds 4 ($\text{IC}_{50} = 5.60 \pm 0.15 \mu\text{M}$), 5 ($\text{IC}_{50} = 15.30 \pm 0.15 \mu\text{M}$) and 6 ($\text{IC}_{50} = 10.51 \pm 0.11 \mu\text{M}$) were found to be favorable for urease inhibition. A decline in activity was observed by replacing -OMe with -Me group as in case of compounds 13 ($\text{IC}_{50} = 15.22 \pm 0.26 \mu\text{M}$), 14 ($\text{IC}_{50} = 38.04 \pm 0.63 \mu\text{M}$), and 15 ($\text{IC}_{50} = 23.36 \pm 0.49 \mu\text{M}$). In case of these compounds the oxygen in -OMe group has some effective role for enzyme inhibition although the position of OMe also matter as compound 4 was found to be more potent than its similar analogues. Compound 18 ($\text{IC}_{50} = 14.69 \pm 0.18 \mu\text{M}$) having NO_2 at 4th position showed better activity than the standard but its look like compound 17 ($\text{IC}_{50} = 31.02 \pm 0.71 \mu\text{M}$) with - NO_2 substitution at 3rd position was not found as active as compound 18. Over all, it was concluded that substitution at 2nd position at phenyl ring may affect the activity but might be the size of substituted group also have important role for enzyme inhibition. So, in order to find better SAR, the compounds were docked.

Quality of this work is it reports new compounds and demonstrated excellent inhibition of urease. Confinements of this work is its should be taken for further examinations like kinetics and *in vivo* investigations.

2.3. Docking study

Analysis of the docking study in the urease binding site were found to be stable for this class of synthesized hybrid bis-coumarin derivatives and the binding mode of the most active compound 16 and the moderate active compound 13 are discussed in detail.

Fig. 3 show the surface model of the urease protein and the binding mode orientation of the reference compound thiourea and co-crystallized compound Acetohydroxamic acid that are found deep bound in the



Scheme 1. Synthetic route of hybrid bis-coumarin derivatives (1-18).

Table 1

Synthesized Hybrid bis-coumarin derivatives (1-18) and their Urease inhibitory potential.

No.	R	IC ₅₀ (μM)	No.	R	IC ₅₀ (μM)
1		7.41 ± 0.08	10		2.25 ± 0.05
2		15.85 ± 0.18	11		14.06 ± 0.14
3		13.97 ± 0.16	12		6.80 ± 0.08
4		5.60 ± 0.07	13		15.22 ± 0.26
5		15.30 ± 0.15	14		38.04 ± 0.63
6		10.5 ± 0.11	15		23.36 ± 0.49
7		0.29 ± 0.01	16		0.12 ± 0.01
8		5.12 ± 0.06	17		31.02 ± 0.71
9		2.4 ± 0.05	18		14.69 ± 0.18
Thiourea					21.40 ± 0.21

active site due to their smaller size, while the compound 16 and 13 are taking feasible fit on the surface of the urease active site.

Fig. 4a show the binding mode of the most active compound 16 in this series. Residue Cys321 forms non-bonded pi-sulfur contact with phenyl ring and coumarin ring of the compound 16 and forms pi-alkyl contact with the same residue. Similar pi-alkyl hydrophobic contact is also established between tri-fluorophenyl group with His322 and Leu252, which seems to be the key interaction feature for the activity. In addition, the His322 imidazole ring forms pi-pi stacking with the tri-fluorophenyl ring and pi-donor hydrogen bond with His322. Moreover, the coumarin ring also forms pi-sulfur phenyl contact with sulfur of

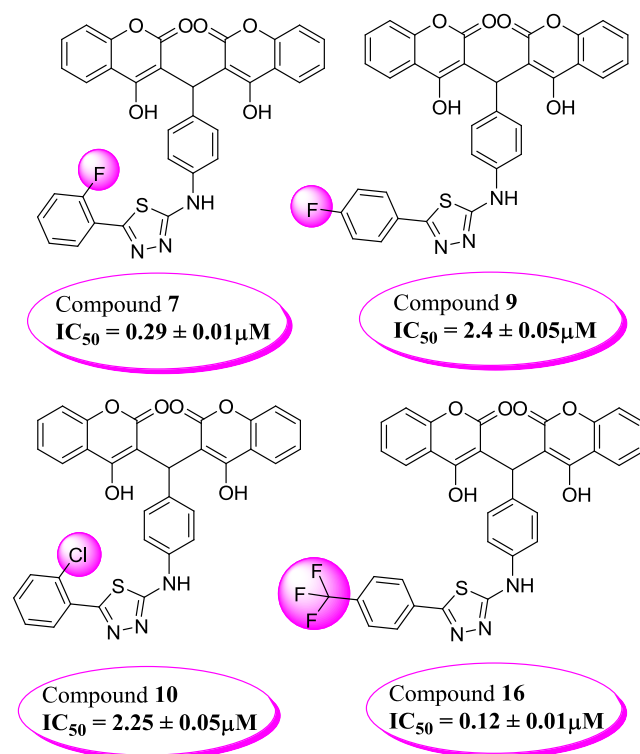


Fig. 2. Comparison of Structure Activity Relationship between Compounds 7, 9, 10 and 16.

Met317.

Fig. 4b shows the binding mode of the moderate active compound 13, here the binding orientation of the compound is slightly different from the compound 16, where the methylphenyl thiadiazol phenyl part of the compound is oriented in a different direction establishing pi-alkyl hydrophobic contact with Trp224 indole ring and similar interaction between Ala169 and toluene ring of the compound 13. The Cys321 residue forms non-bonded pi-sulfur contact with phenyl ring and coumarin ring of the compound and pi-alkyl contact with the same residue. In addition, the coumarin ring also forms pi-sulfur phenyl contact with sulfur of Met317 alike compound 16. The key point to note is that the

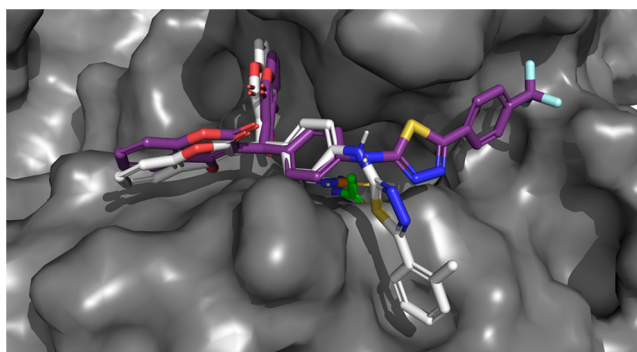


Fig. 3. Shows the binding mode of the reference compound thiourea (green color stick) and co-crystallized compound Acetohydroxamic acid (brown color stick), compound **16** (violet color stick) and **13** (white color stick) and the urease protein is shown in gray color surface model.

methyl in the toluene does not involve in any contact making this molecule moderate active in comparison with compound **16**.

Furthermore, the druggable properties of the compounds were computed considering the Lipinski's rule of 5 (RO5). Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria: No more than 5 hydrogen bond donors (the total number of nitrogen–hydrogen and oxygen–hydrogen bonds), No more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms), A molecular mass less than 500 Daltons and An octanol–water partition coefficient log P not greater than 5. The druggable properties computed as shown in Table 2, for the synthesized hybrid bis-coumarin derivatives are in the range having agreeable ADME properties for oral bio-availability.

3. Experimental

3.1. General

All nuclear magnetic resonance experiments had been carried out using on Advance Bruker 500 MHz. Elemental analysis was performed on Carlo Erba Strumentazione-Mod-1106, Italy. Electron impact mass spectra (EI-MS) were recorded on a Finnigan MAT-311A, Germany. Thin layer chromatography (TLC) was performed on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by UV at 254 and 365 nm.

3.1.1. Synthesis of 3,3'-((4-nitrophenyl)methylene)bis(4-hydroxy-2H-chromen-2-one) (a)

As we have reported in our previous article [40].

Table 2

The druggable properties computed for the synthesized hybrid bis-coumarin derivatives (**1–18**).

Compound	H-bond donor	H-bond acceptor	Mol.wt	logP
1	1	6	664.482	5.364
2	1	6	664.482	5.364
3	1	6	664.482	5.364
4	1	7	615.611	4.599
5	1	7	615.611	4.599
6	1	7	615.611	4.599
7	1	6	603.576	4.821
8	1	6	603.576	4.821
9	1	6	603.576	4.821
10	1	6	620.031	5.28
11	1	6	620.031	5.28
12	1	6	620.031	5.28
13	1	6	599.612	5.102
14	1	6	599.612	5.102
15	1	6	599.612	5.102
16	1	6	653.583	5.558
17	1	6	630.583	4.51
18	1	6	630.583	4.51

3.1.2. Synthesis of 3,3'-((4-aminophenyl)methylene)bis(4-hydroxy-2H-chromen-2-one) (b)

As we have reported in our previous article [40].

3.2. Synthetic procedure for 3,3'-((4-(5-(aryl)-1,3,4-thiadiazol-2-ylamino)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one)

The 3,3'-((4-aminophenyl)methylene)bis(4-hydroxy-2H-chromen-2-one) (**b**) 0.5 mmol was taken in 5 ml THF the carbon disulfide was added (1.5 mmol) dropwise and left it for stirring for 3 h. The reaction was monitored by TLC. After completion of reaction the intermediate directly used for next step. The intermediate (4-(bis(4-hydroxy-2-oxo-2H-chromen-3-yl)methyl)phenyl)carbamdithioic acid was further refluxed with 0.5 mmol of arylhydrazide 3–5 h. The reaction completion was monitored by TLC. The solvent from the reaction mixture was removed under reduced pressure and the product partitioned between water and EtOAc. Subsequent work up of the EtOAc phase afforded the crude product which was purified by thin layer chromatography (TLC) using petroleum ether-EtOAc gradient mixture. All synthesized compounds were characterized by different spectroscopic techniques such as EI-MS, ^1H NMR and ^{13}C NMR.

3.2.1. 3,3'-((4-((5-(2-bromophenyl)-1,3,4-thiadiazol-2-yl)amino)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one)

Yield: (0.547 g 82%); ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 9.80 (s, 1H), 9.20 (s, 2H), 7.82 (dd, $J = 8.0$, 2.0 Hz, 2H), 7.58–7.55 (m, 2H), 7.46–7.43 (m, 2H), 7.41–7.38 (m, 1H), 7.27 (d, $J = 8.0$ Hz, 2H),

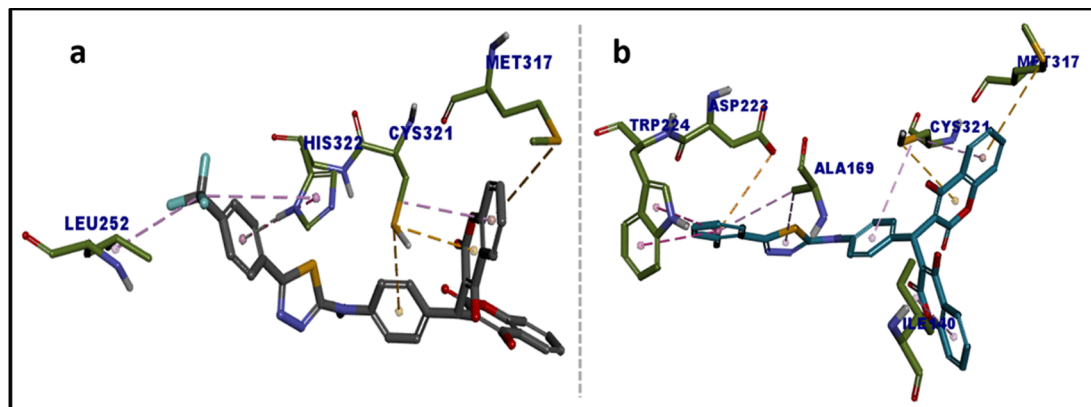


Fig. 4. Shows the binding mode of the compound **16** (gray color stick) and **13** (blue color stick) and the urease key active site residues are shown as green color stick.

7.25–7.24 (m, 4H), 7.19 (dd, $J = 8.0, 2.0$ Hz, 1H), 7.14 (d, $J = 8.0$ Hz, 2H), 6.40 (s, 1H); ^{13}C NMR (150 MHz, DMSO- d_6): δ 164.8 (C), 164.5 (C), 164.5 (C), 160.6 (C), 160.6 (C), 160.4 (C), 152.8 (C), 152.8 (C), 140.9 (C), 134.2 (C), 133.9 (CH), 133.9 (CH), 133.1 (C), 132.2 (CH), 132.2 (CH), 131.3 (CH), 128.6 (CH), 128.2 (CH), 128.2 (CH), 124.3 (CH), 124.3 (CH), 123.6 (CH), 123.6 (CH), 121.9 (C), 121.5 (CH), 121.5 (CH), 116.3 (C), 116.3 (C), 115.7 (CH), 115.7 (CH), 106.1 (C), 106.1 (C), 33.7 (CH); HR-EI-MS: m/z calcd for $\text{C}_{33}\text{H}_{20}\text{BrN}_3\text{O}_6\text{S}$, $[\text{M}]^+ 665.0256$; Found 665.0241.

3.2.2. 3,3'-((4-((5-(3-bromophenyl)-1,3,4-thiadiazol-2-yl)amino)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one)

Yield: (0.560 g 84%); ^1H NMR (500 MHz, DMSO- d_6) δ 9.78 (s, 1H), 9.64 (s, 2H), 7.84–7.81 (m, 3H), 7.70–7.67 (m, 2H), 7.50 (d, $J = 6.5$ Hz, 1H), 7.32–7.27 (m, 8H), 7.12 (d, $J = 8.0$ Hz, 2H), 6.21 (s, 1H); ^{13}C NMR (150 MHz, DMSO- d_6): δ 164.5 (C), 164.5 (C), 163.2 (C), 163.2 (C), 160.6 (C), 160.6 (C), 152.8 (C), 152.8 (C), 140.9 (C), 133.9 (C), 133.8 (C), 132.2 (CH), 132.2 (CH), 132.1 (C), 130.8 (C), 128.2 (CH), 128.2 (CH), 127.9 (CH), 125.3 (CH), 124.3 (CH), 124.3 (CH), 123.6 (CH), 123.6 (CH), 122.7 (C), 121.5 (CH), 121.5 (CH), 116.3 (CH), 116.3 (CH), 115.7 (CH), 115.7 (CH), 106.1 (C), 106.1 (C), 33.7 (CH); HR-EI-MS: m/z calcd for $\text{C}_{33}\text{H}_{20}\text{BrN}_3\text{O}_6\text{S}$, $[\text{M}]^+ 665.0256$; Found 665.0240.

3.2.3. 3,3'-((4-((5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl)amino)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one)

Yield: (0.506 g 76%); ^1H NMR (500 MHz, DMSO- d_6) δ 9.70 (s, 1H), 9.64 (s, 2H), 7.83 (d, $J = 8.0$ Hz, 2H), 7.52 (d, $J = 8.0$ Hz, 2H), 7.47–7.45 (m, 4H), 7.31–7.28 (m, 6H), 7.07 (d, $J = 8.0$ Hz, 2H), 6.22 (s, 1H); ^{13}C NMR (150 MHz, DMSO- d_6): δ 165.2 (C), 164.5 (C), 164.5 (C), 163.5 (C), 160.6 (C), 160.6 (C), 152.8 (C), 152.8 (C), 140.9 (C), 133.9 (C), 133.9 (C), 132.2 (CH), 132.2 (CH), 131.7 (CH), 131.7 (CH), 130.5 (CH), 129.7 (CH), 129.7 (CH), 128.2 (CH), 127.1 (CH), 127.1 (CH), 124.3 (CH), 124.3 (CH), 123.6 (CH), 123.6 (CH), 121.5 (C), 116.3 (C), 116.3 (C), 115.7 (CH), 115.7 (CH), 106.1 (C), 106.1 (C), 33.7 (CH); HR-EI-MS: m/z calcd for $\text{C}_{33}\text{H}_{20}\text{BrN}_3\text{O}_6\text{S}$, $[\text{M}]^+ 665.0256$; Found 665.0238.

3.2.4. 3,3'-((4-((5-(2-methoxyphenyl)-1,3,4-thiadiazol-2-yl)amino)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one)

Yield: (0.481 g, 78%); ^1H NMR (500 MHz, DMSO- d_6) δ 9.8 (s, 2H), 9.05 (s, 1H), 8.09 (dd, $J = 8.0, 2.0$ Hz, 1H), 7.87 (d, $J = 8.0$ Hz, 2H), 7.54 (t, $J = 7.0, 1.3$ Hz, 2H), 7.34–7.24 (m, 6H), 7.17–7.12 (m, 3H), 7.08 (d, $J = 8.0$ Hz, 1H), 6.91 (t, $J = 8.0$ Hz, 1H), 6.26 (s, 1H), 3.89 (s, 3H); ^{13}C NMR (150 MHz, DMSO- d_6): δ 163.3 (C), 163.3 (C), 163.1 (C), 159.4 (C), 159.4 (C), 155.6 (C), 154.4 (C), 151.6 (C), 151.6 (C), 139.7 (C), 132.7 (C), 131.0 (CH), 131.0 (CH), 131.0 (CH), 128.1 (CH), 127.0 (CH), 127.0 (CH), 123.1 (CH), 123.1 (CH), 122.5 (CH), 122.5 (CH), 122.1 (C), 120.3 (CH), 120.3 (CH), 118.8 (CH), 115.1 (C), 115.1 (C), 114.5 (CH), 114.5 (CH), 109.9 (CH), 104.9 (C), 104.9 (C), 54.5 (OCH₃), 32.5 (CH); HR-EI-MS: m/z calcd for $\text{C}_{34}\text{H}_{23}\text{N}_3\text{O}_7\text{S}$, $[\text{M}]^+ 617.1257$; Found 617.1239.

3.2.5. 3,3'-((4-((5-(3-methoxyphenyl)-1,3,4-thiadiazol-2-yl)amino)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one)

Yield: 75%. ^1H NMR (500 MHz, DMSO- d_6) δ 9.64 (s, 2H), 9.61 (s, 1H), 7.84 (dd, $J = 7.8, 1.3$ Hz, 2H), 7.51 (dt, $J = 11.9, 2.5$ Hz, 2H), 7.23 (ddd, $J = 18.1, 16.9, 8.3$ Hz, 9H), 7.07 (d, $J = 8.3$ Hz, 1H), 7.03 (d, $J = 7.4$ Hz, 1H), 6.68 (dd, $J = 8.2, 2.1$ Hz, 1H), 6.27 (s, 1H), 3.73 (s, 3H); ^{13}C NMR (150 MHz, DMSO- d_6): δ 165.6 (C), 164.8 (C), 164.8 (C), 163.5 (C), 160.9 (C), 160.9 (C), 160.0 (C), 153.1 (C), 153.1 (C), 141.2 (C), 134.2 (C), 133.0 (CH), 132.5 (CH), 132.5 (CH), 131.0 (CH), 128.5 (CH), 128.5 (CH), 124.6 (CH), 124.6 (CH), 124.0 (CH), 124.0 (CH), 121.8 (CH), 121.8 (CH), 119.3 (C), 117.7 (CH), 116.6 (CH), 116.6 (CH), 116.0 (C), 116.0 (C), 112.5 (CH), 106.4 (C), 106.4 (C), 55.8 (OCH₃), 34.0 (CH); HR-EI-MS: m/z calcd for $\text{C}_{34}\text{H}_{23}\text{N}_3\text{O}_7\text{S}$, $[\text{M}]^+ 617.0210$; Found 617.0212

3.2.6. 3,3'-((4-((5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl)amino)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one)

Yield: 79%. ^1H NMR (500 MHz, DMSO- d_6) δ 9.49 (s, 1H), 9.42 (s, 1H), 7.84 (d, $J = 7.7$ Hz, 2H), 7.54–7.49 (m, 1H), 7.32 (d, $J = 8.6$ Hz, 1H), 7.25 (dd, $J = 16.7, 8.0$ Hz, 2H), 7.06 (d, $J = 8.2$ Hz, 2H), 6.88 (d, $J = 8.9$ Hz, 2H), 6.26 (s, 1H), 3.74 (s, 3H); ^{13}C NMR (150 MHz, DMSO- d_6): δ 164.3 (C), 163.5 (C), 163.5 (C), 162.5 (C), 161.3 (C), 159.6 (C), 159.6 (C), 151.8 (C), 151.8 (C), 139.9 (C), 132.9 (C), 131.2 (CH), 131.2 (CH), 127.7 (CH), 127.7 (CH), 127.2 (CH), 127.2 (CH), 126.4 (C), 123.3 (CH), 123.3 (CH), 122.7 (CH), 122.7 (CH), 120.5 (CH), 120.5 (CH), 115.3 (C), 114.7 (CH), 114.7 (CH), 113.9 (CH), 113.9 (CH), 105.1 (C), 105.1 (C), 54.4 (OCH₃), 32.7 (CH); HR-EI-MS: m/z calcd for $\text{C}_{34}\text{H}_{23}\text{N}_3\text{O}_7\text{S}$, $[\text{M}]^+ 617.0312$; Found 617.0311.

3.2.7. 3,3'-((4-((5-(2-fluorophenyl)-1,3,4-thiadiazol-2-yl)amino)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one)

Yield: 80%. ^1H NMR (500 MHz, DMSO- d_6) δ 9.85 (s, 2H), 9.31 (s, 1H), 7.84 (dd, $J = 7.8, 1.3$ Hz, 2H), 7.64 (t, $J = 7.9$ Hz, 1H), 7.51 (m, 2H), 7.25 (ddd, $J = 19.9, 9.8, 5.7$ Hz, 8H), 7.15 (ddd, $J = 8.5, 5.5, 3.2$ Hz, 1H), 7.08 (d, $J = 8.0$ Hz, 2H), 6.27 (s, 1H); ^{13}C NMR (150 MHz, DMSO- d_6): δ 164.8 (C), 164.5 (C), 164.5 (C), 161.6 (C), 160.6 (C), 160.6 (C), 159.3 (C), 152.8 (C), 152.8 (C), 140.9 (C), 133.9 (C), 133.1 (CH), 132.2 (CH), 132.2 (CH), 128.5 (CH), 128.2 (CH), 128.2 (CH), 124.3 (CH), 124.3 (CH), 123.6 (CH), 123.6 (CH), 123.6 (CH), 121.5 (CH), 121.5 (CH), 119.3 (C), 116.3 (C), 116.3 (C), 115.7 (CH), 115.7 (CH), 115.4 (CH), 106.1 (C), 106.1 (C), 33.7 (CH); HR-EI-MS: m/z calcd for $\text{C}_{33}\text{H}_{20}\text{FN}_3\text{O}_6\text{S}$, $[\text{M}]^+ 605.0142$; Found 605.0140.

3.2.8. 3,3'-((4-((5-(3-fluorophenyl)-1,3,4-thiadiazol-2-yl)amino)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one)

Yield: 83%. ^1H NMR (500 MHz, DMSO- d_6) δ 9.82 (s, 2H), 9.75 (s, 1H), 7.82 (d, $J = 8.0$ Hz, 2H), 7.58–7.53 (m, 3H), 7.42 (t, $J = 6.5$ Hz, 1H), 7.38–7.34 (m, 1H), 7.22 (d, $J = 6.6$ Hz, 7H), 6.92 (t, $J = 8.1$ Hz, 1H), 6.27 (s, 1H); ^{13}C NMR (150 MHz, DMSO- d_6): δ 164.8 (C), 164.5 (C), 164.5 (C), 161.6 (C), 160.6 (C), 160.6 (C), 159.3 (C), 152.8 (C), 152.8 (C), 140.9 (C), 133.9 (C), 133.1 (CH), 132.2 (CH), 132.2 (CH), 128.5 (CH), 128.2 (CH), 128.2 (CH), 124.3 (CH), 124.3 (CH), 123.6 (CH), 123.6 (CH), 123.6 (CH), 121.5 (CH), 121.5 (CH), 119.3 (C), 116.3 (C), 116.3 (C), 115.7 (CH), 115.7 (CH), 115.4 (CH), 106.1 (C), 106.1 (C), 33.7 (CH); HR-EI-MS: m/z calcd for $\text{C}_{33}\text{H}_{20}\text{FN}_3\text{O}_6\text{S}$, $[\text{M}]^+ 605.0232$; Found 605.0230;

3.2.9. 3,3'-((4-((5-(4-fluorophenyl)-1,3,4-thiadiazol-2-yl)amino)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one)

Yield: 78%. ^1H NMR (500 MHz, DMSO- d_6) δ 9.67 (s, 2H), 9.57 (s, 1H), 7.83 (dd, $J = 7.8, 1.0$ Hz, 2H), 7.54–7.48 (m, 2H), 7.46 (dd, $J = 8.8, 5.0$ Hz, 2H), 7.25 (dd, $J = 16.8, 8.2$ Hz, 6H), 7.13 (t, $J = 8.8$ Hz, 2H), 7.06 (d, $J = 8.2$ Hz, 2H), 6.26 (s, 1H); ^{13}C NMR (150 MHz, DMSO- d_6): δ 165.2 (C), 164.6 (C), 164.6 (C), 163.5 (C), 162.7 (C), 160.6 (C), 160.6 (C), 152.8 (C), 152.8 (C), 140.9 (C), 133.9 (C), 132.2 (CH), 132.2 (CH), 129.0 (CH), 129.0 (CH), 128.3 (C), 128.2 (CH), 128.2 (CH), 124.3 (CH), 124.3 (CH), 123.6 (CH), 123.6 (CH), 121.5 (CH), 121.5 (CH), 116.5 (C), 116.5 (C), 116.3 (CH), 116.3 (CH), 115.7 (CH), 115.7 (CH), 106.1 (C), 106.1 (C), 33.7 (CH); HR-EI-MS: m/z calcd for $\text{C}_{33}\text{H}_{20}\text{FN}_3\text{O}_6\text{S}$, $[\text{M}]^+ 605.0243$; Found 605.0241; Anal. Calcd: C, 65.45; H, 3.33; N, 6.94; Found C, 65.43; H, 3.34; N, 6.96.

3.2.10. 3,3'-((4-((5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl)amino)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one)

Yield: 80%. ^1H NMR (500 MHz, DMSO- d_6) δ 9.86 (s, 1H), 9.26 (s, 2H), 7.82 (d, $J = 7.8, 2$ Hz), 7.62 (d, $J = 8.0, 1$ Hz), 7.58–7.54 (m, 3H), 7.43–7.37 (m, 8H), 7.09 (d, $J = 8.0$ Hz, 2H), 6.23 (s, 1H); ^{13}C NMR (150 MHz, DMSO- d_6): δ 164.8 (C), 164.5 (C), 164.5 (C), 161.7 (C), 160.6 (C), 160.6 (C), 152.8 (C), 152.8 (C), 140.9 (C), 133.9 (C), 132.9 (C), 132.2 (CH), 132.2 (CH), 132.2 (CH), 131.8 (CH), 130.9 (CH), 130.2 (CH), 128.2 (CH), 128.2 (CH), 128.0 (C), 124.3 (CH), 124.3 (CH), 123.6

(CH), 123.6 (CH), 121.5 (CH), 121.5 (CH), 116.3 (C), 116.3 (C), 115.7 (CH), 115.7 (CH), 106.1 (C), 106.1 (C), 33.7 (CH); HR-EI-MS: m/z calcd for $C_{33}H_{20}ClN_3O_6S$, $[M]^+$ 622.0480; Found 622.0479;

3.2.11. 3,3'-((4-((5-(3-chlorophenyl)-1,3,4-thiadiazol-2-yl)amino)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one)

Yield: 80%. 1H NMR (500 MHz, DMSO- d_6) δ 9.89 (s, 1H), 9.79 (s, 2H), 7.90 (s, 2H), 7.88 (dd, J = 7.5, Hz, 2H), 7.54–7.48 (m, 3H), 7.44 (d, J = 8.0 Hz, 1H), 7.29–7.24 (m, 6H), 7.09 (d, J = 8.0 Hz, 2H), 6.26 (s, 1H); ^{13}C NMR (150 MHz, DMSO- d_6): δ 165.2 (C), 164.5 (C), 164.5 (C), 163.2 (C), 160.6 (C), 160.6 (C), 152.8 (C), 152.8 (C), 140.9 (C), 136.3 (C), 133.9 (C), 132.4 (CH), 132.4 (CH), 132.2 (CH), 131.3 (CH), 130.1 (CH), 128.2 (CH), 128.2 (CH), 128.2 (CH), 124.6 (C), 124.3 (CH), 124.3 (CH), 123.6 (CH), 123.6 (CH), 121.5 (CH), 121.5 (CH), 116.3 (C), 116.3 (C), 115.7 (CH), 115.7 (CH), 106.1 (C), 106.1 (C), 33.7 (CH); HR-EI-MS: m/z calcd for $C_{33}H_{20}ClN_3O_6S$, $[M]^+$ 622.0480; Found 622.0482.

3.2.12. 3,3'-((4-((5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)amino)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one)

Yield: 83%. 1H NMR (500 MHz, DMSO- d_6) δ 9.74 (s, 1H), 9.62 (s, 1H), 7.85 (d, J = 8.0 Hz, 2H), 7.55–7.52 (m, 4H), 7.35 (d, J = 8.0 Hz, 2H), 7.29–7.25 (m, 6H), 7.06 (d, J = 8.2 Hz, 2H), 6.28 (s, 1H); ^{13}C NMR (150 MHz, DMSO- d_6): δ 165.2 (C), 164.5 (C), 163.5 (C), 160.6 (C), 160.6 (C), 152.8 (C), 152.8 (C), 140.9 (C), 136.4 (C), 136.4 (C), 133.9 (C), 132.2 (CH), 132.2 (CH), 130.5 (C), 129.4 (CH), 129.4 (CH), 128.2 (CH), 128.2 (CH), 128.0 (CH), 128.0 (CH), 124.3 (CH), 124.3 (CH), 123.6 (CH), 123.6 (CH), 121.5 (CH), 121.5 (CH), 116.3 (C), 116.3 (C), 115.7 (CH), 115.7 (CH), 106.1 (C), 106.1 (C), 33.7 (CH); HR-EI-MS: m/z calcd for $C_{33}H_{20}ClN_3O_6S$, $[M]^+$ 622.0360; Found 622.0361;

3.2.13. 3,3'-((4-((5-(*o*-tolyl)-1,3,4-thiadiazol-2-yl)amino)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one)

Yield: 71%. 1H NMR (500 MHz, DMSO- d_6) δ 9.59 (s, 1H), 9.13 (s, 2H), 7.89 (d, J = 7.0 Hz, 2H), 7.58 (t, J = 7.0 Hz, 2H), 7.43–7.38 (m, 8H), 7.18–7.15 (m, 2H), 7.06 (d, J = 8.0 Hz, 2H), 6.29 (s, 1H), 2.24 (s, 3H); ^{13}C NMR (150 MHz, DMSO- d_6): δ 164.8 (C), 164.8 (C), 164.5 (C), 160.6 (C), 160.6 (C), 158.9 (C), 152.8 (C), 152.8 (C), 140.9 (C), 136.4 (C), 133.9 (C), 132.2 (CH), 132.2 (CH), 131.9 (CH), 131.4 (CH), 129.4 (CH), 128.2 (CH), 128.2 (CH), 128.2 (CH), 127.2 (C), 124.3 (CH), 124.3 (CH), 123.6 (CH), 123.6 (CH), 121.5 (CH), 121.5 (CH), 116.3 (C), 116.3 (C), 115.7 (CH), 115.7 (CH), 106.1 (C), 106.1 (C), 33.7 (CH), 21.2 (CH₃); HR-EI-MS: m/z calcd for $C_{34}H_{23}N_3O_6S$, $[M]^+$ 601.0330; Found 601.0334;

3.2.14. 3,3'-((4-((5-(*m*-tolyl)-1,3,4-thiadiazol-2-yl)amino)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one)

Yield: 75%. 1H NMR (500 MHz, DMSO- d_6) δ 9.66 (s, 1H), 9.57 (s, 2H), 7.85 (dd, J = 7.8 Hz, 2H), 7.59–7.55 (m, 2H), 7.31–7.24 (m, 9H), 7.08 (d, J = 8.0 Hz, 2H), 6.94 (d, J = 8.0 Hz, 1H), 6.29 (s, 1H), 2.25 (s, 3H); ^{13}C NMR (150 MHz, DMSO- d_6): δ 166.2 (C), 164.2 (C), 164.2 (C), 163.2 (C), 160.6 (C), 160.6 (C), 152.8 (C), 152.8 (C), 142.8 (C), 140.9 (C), 133.9 (C), 132.8 (CH), 132.4 (CH), 132.2 (CH), 132.2 (CH), 131.5 (CH), 131.2 (CH), 128.2 (CH), 128.2 (CH), 124.3 (C), 124.3 (CH), 124.3 (CH), 123.6 (CH), 123.6 (CH), 121.5 (CH), 121.5 (CH), 116.3 (C), 116.3 (C), 115.7 (CH), 115.7 (CH), 106.1 (C), 106.1 (C), 33.7 (CH), 22.0 (CH₃); HR-EI-MS: m/z calcd for $C_{34}H_{23}N_3O_6S$, $[M]^+$ 601.0230; Found 601.0231.

3.2.15. 3,3'-((4-((5-(*p*-tolyl)-1,3,4-thiadiazol-2-yl)amino)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one)

Yield: 79%. 1H NMR (500 MHz, DMSO- d_6) δ 9.56 (s, 1H), 9.52 (s, 2H), 7.86 (d, J = 7.5 Hz, 2H), 7.50 (t, J = 7.0 Hz, 2H), 7.37 (d,

J = 8.0 Hz, 2H), 7.28–7.23 (m, 6H), 7.13 (d, J = 8.0 Hz, 2H), 7.04 (d, J = 8.0 Hz, 2H), 6.28 (s, 1H), 2.27 (s, 3H); ^{13}C NMR (150 MHz, DMSO- d_6): δ 165.4 (C), 164.6 (C), 164.6 (C), 163.6 (C), 160.7 (C), 160.7 (C), 152.9 (C), 152.9 (C), 141.0 (C), 138.6 (C), 134.0 (C), 132.3 (CH), 132.3 (CH), 130.8 (CH), 130.8 (CH), 130.8 (CH), 130.8 (CH), 129.3 (C), 128.3 (CH), 128.3 (CH), 124.4 (CH), 124.4 (CH), 123.7 (CH), 123.7 (CH), 121.6 (CH), 121.6 (CH), 116.4 (C), 116.4 (C), 115.8 (CH), 115.8 (CH), 106.2 (C), 106.2 (C), 33.8 (CH), 21.5 (CH₃); HR-EI-MS: m/z calcd for $C_{34}H_{23}N_3O_6S$, $[M]^+$ 601.0210; Found 601.0209.

3.2.16. 3,3'-((4-((5-(4-(trifluoromethyl)phenyl)-1,3,4-thiadiazol-2-yl)amino)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one)

Yield: 74%. 1H NMR (500 MHz, DMSO- d_6) δ 9.95 (s, 1H), 9.93 (s, 2H), 7.86 (d, J = 7.5 Hz, 2H), 7.79 (d, J = 8.0 Hz, 2H), 7.66 (d, J = 8.0 Hz, 2H), 7.54 (d, J = 8.0 Hz, 2H), 7.39–7.34 (m, 6H), 7.08 (d, J = 8.0 Hz, 2H), 6.27 (s, 1H); ^{13}C NMR (150 MHz, DMSO- d_6): δ 166.5 (C), 166.5 (C), 165.7 (C), 164.7 (C), 161.8 (C), 161.8 (C), 154.0 (C), 154.0 (C), 142.1 (C), 135.3 (C), 135.3 (C), 135.1 (C), 134.2 (CH), 133.4 (CH), 133.4 (CH), 130.9 (CH), 129.4 (CH), 129.4 (CH), 128.2 (CH), 128.2 (CH), 127.7 (CH), 127.7 (CH), 125.5 (CH), 125.5 (CH), 123.6 (CH), 123.6 (CH), 122.7 (C), 117.5 (C), 117.5 (C), 116.9 (CH), 116.9 (CH), 107.3 (C), 107.3 (C), 34.9 (CH); HR-EI-MS: m/z calcd for $C_{34}H_{20}F_3N_3O_6S$, $[M]^+$ 655.0421; Found 655.0420.

3.2.17. 3,3'-((4-((5-(3-nitrophenyl)-1,3,4-thiadiazol-2-yl)amino)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one)

Yield: 82%. 1H NMR (500 MHz, DMSO- d_6) δ 10.00 (s, 2H), 8.57 (s, 1H), 7.93 (dd, J = 8.0, 1.5 Hz, 2H), 7.80 (d, J = 8.0, Hz, 2H), 7.58 (t, J = 8.0 Hz, 1H), 7.56–7.53 (m, 2H), 7.31–7.25 (m, 6H), 7.11 (d, J = 8.0 Hz, 2H), 6.27 (s, 1H); ^{13}C NMR (150 MHz, DMSO- d_6): δ 164.4 (C), 163.6 (C), 163.6 (C), 162.4 (C), 159.7 (C), 159.7 (C), 151.9 (C), 151.9 (C), 147.3 (C), 140.1 (C), 133.1 (C), 132.7 (CH), 131.4 (CH), 131.4 (CH), 128.6 (CH), 127.3 (CH), 127.3 (CH), 127.1 (C), 123.9 (CH), 123.4 (CH), 123.4 (CH), 122.8 (CH), 122.8 (CH), 122.1 (CH), 120.6 (CH), 120.6 (CH), 115.4 (C), 115.4 (C), 114.8 (CH), 114.8 (CH), 105.2 (C), 105.2 (C), 32.8 (CH); HR-EI-MS: m/z calcd for $C_{33}H_{20}N_4O_8S$, $[M]^+$ 632.0300; Found 632.0301.

3.2.18. 3,3'-((4-((5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)amino)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one)

Yield: 82%. 1H NMR (500 MHz, DMSO- d_6) δ 10.28 (s, 1H), 10.17 (s, 2H), 8.16 (d, J = 8.0 Hz, 2H), 7.88–7.84 (m, 4H), 7.50 (t, J = 7.5 Hz, 2H), 7.39–7.23 (m, 6H), 7.07 (d, J = 8.0 Hz, 2H), 6.25 (s, 1H); ^{13}C NMR (150 MHz, DMSO- d_6): δ 166.2 (C), 166.2 (C), 165.2 (C), 162.3 (C), 162.3 (C), 154.5 (C), 154.5 (C), 150.2 (C), 142.6 (C), 135.6 (C), 135.1 (C), 135.1 (C), 133.9 (CH), 133.9 (CH), 129.9 (CH), 129.9 (CH), 128.3 (CH), 128.3 (CH), 127.4 (CH), 127.4 (CH), 126.0 (CH), 126.0 (CH), 125.4 (CH), 125.4 (CH), 123.2 (CH), 123.2 (CH), 118.0 (C), 118.0 (C), 117.4 (CH), 117.4 (CH), 107.8 (C), 107.8 (C), 35.4 (CH); HR-EI-MS: m/z calcd for $C_{33}H_{20}N_4O_8S$, $[M]^+$ 632.0402; Found 632.0400.

3.3. Bioassay

3.3.1. Urease

Spectrophotometrically urease inhibition assay was performed. For urease inhibition assay 5 μ L of synthetic compound was incubated with 25 μ L of urease solution (1 U/well) (250 μ L) at 30 °C for 15 min. After that, 55 μ L substrate urea with 100 mM concentration was added and the plate was again incubated at 30 °C. After incubation 70 μ L of basic reagent (0.5% w/v NaOH and 0.1% NaOCl) and 45 μ L of carbolic acid (1% w/v carbolic acid and 0.005% w/v Na₂[Fe(CN)₅NO]) were added at each well. Again, plate was incubated for 50 min at 30 °C. Rate of

production ammonia was used for determining urease inhibitory activity by following Weather burn method and change in absorbance was monitored at 630 nm on a ELISA plate reader (Spectra Max M2, Molecular Devices, CA, USA) [41]. Acetohydroxamic acid was used as a standard compound [42].

3.3.2. Molecular docking studies with urease

Docking simulation was performed targeting the crystal structure of Urease (PDB ID: 1E9Y) [43] in order to reveal the binding modes of synthesized hybrid bis-coumarin derivatives (1–18). For the purpose of docking studies, the crystal structure of the Urease was optimized using protein preparation module in Discovery Studio 2018 (Dassault Systemes BIOVIA, USA) [44].

The crystal structure was retrieved from the protein data bank (PDB) and further, the structure was optimized by removing the water molecules, hetero atoms, and co-factors. Hydrogen bonds, missing atoms, and charges were computed. The synthesized hybrid bis-coumarin derivatives (1–18) used in these docking studies was prepared and optimized using built and Ligand Preparation module implemented in Discovery Studio 2018 (DassaultSystemes BIOVIA, USA).

To study the protein drug interaction docking program Gold was used, Ligand preparation includes generating various tautomer's, assigning bond orders and stereochemistry. Following it, receptor grid was generated targeting around the Urease active site by choosing centroid of complexed ligand (Acetohydroxamic acid). The active site was defined with a radius of 15 Å around the Acetohydroxamic acid binding site. Docking calculations were accomplished using ChemPLP scoring function. The docking results were further analyzed, and each derivative binding mode were visually inspected using Discover studio visualizer. In addition, ADME properties/Bioavailability for the compounds were also computed.

3.3.3. Cytotoxicity assays using 3T3-L1 and CC-1 cell-lines and MTT

In vitro cytotoxicity assays were performed as described by Scholz et al.56, using the 3T3-L1 mouse embryo fibroblast cell line (American Type Culture Collection 'ATCC', Manassas, VA 20108, USA), and CC-1 cells, a rat Wistar hepatocyte cell line (European Collection of Cell Cultures, Salisbury, UK). The CC-1 cells were suspended in Minimum Essential Medium Eagle (MEM) supplemented with 10% FBS, 2 mM glutamine, 1% non-essential amino acids and, 20 mM HEPES. While the 3T3-L1 cells were suspended in Dulbecco's Modified Eagle's Medium (DMEM) formulated with 10% FBS. Using flat bottomed plates, both cell-lines were plated at a concentration of 6×10^4 cells/mL and incubated for 24 h at 37 °C and 5% CO₂ environment. After removal of media, cells were challenged with three different concentrations (1.0, 5.0, and 20 µg/mL) of compounds in triplicates and were then further incubated for 48 h at 37 °C in CO₂ incubator. Following exposure to each compound, cells viability was assessed by using 0.5 mg/mL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) for 4 h followed by removal of supernatant and addition of DMSO to solubilize the formazan complex. Plates were read at 540 nm after one minute shaking and readings were processed using MS Excel software. Results were expressed as means $\pm$ SD of triplicate readings.

4. Conclusion

It is concluded that, we have synthesized eighteen hybrid bis-coumarin derivatives (1–18) and evaluated against urease inhibitory potential. All these compounds showed remarkable urease inhibition activity and found to be better active than the standard. This study unveiled the bis coumarin and thiazole ring hybrid drugs for urease inhibition and further open the doors to synthesize these types of

compounds for urease inhibition. The interactions of the active compounds and enzyme active site with the help of docking studies were established.

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