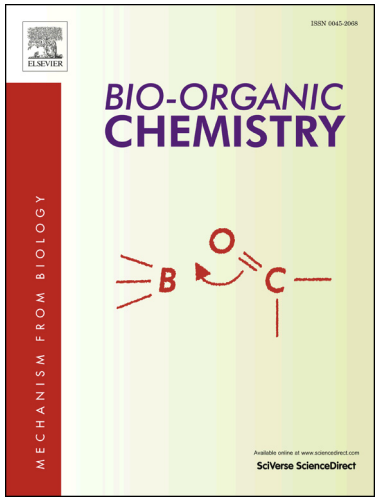

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Synthesis of 2-Acylated and Sulfonated 4-hydroxycoumarins: *In vitro* Urease Inhibition and Molecular Docking Studies

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Abstract:

Sixteen 4-hydroxycoumarins derivatives were synthesized, characterized through EI-MS and ^{1}HNMR and screened for urease inhibitory potential. Three compounds exhibited better urease inhibition than the standard inhibitor thiourea (IC_{50}= 21 ± 0.11 μM and other four compounds exhibited good to moderate inhibition with IC_{50} values between 29.45 ± 1.1 μM and 69.53 ± 0.9 μM. Structure activity relationship was established on the basis of molecular docking studies, which helped to predict the binding interactions of the most active compounds.

Keywords: Synthesis, 2-Acylated and Sulfonated 4-hydroxycoumarin, Urease Inhibition, Molecular Docking, SAR.

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1.0. Introduction

Urease (urea amidohydrolase, E.C.3.5.1.5) is a nickel containing metallo-enzyme found in plants, bacteria, fungi and soil. Ureases derived from different sources have different structural features; however, they share more than 50% amino acids characteristics. Urease catalyzes the hydrolysis of urea into ammonia and carbamate, the later produces ammonia and carbon dioxide on further decomposition [1, 2]. Urease is involved in human and animal pathogenicity of hepatic encephalopathy, hepatic coma urolithiasis, pyelonephritis, gastric and peptic ulcers; while urinary catheter encrustation are also caused by ammonia produced by ureases [3-4]. Urease inhibitors can be considered as tool to control the damaging effects of ureolytic bacterial infections in humans, which occur commonly in the developed countries [5-6].

Ureases are inhibited by different classes of compounds. Urease inhibitors can be broadly classified into two categories; organic compounds and organometallics [7]. The former category includes hydroxamic acid and its derivatives [8], triazoles, coumarins [9], isatins, semicarbazones [10], Schiff bases [11], urea derivatives [12], oxadiazoles [13], piperidines [14]; however, the later includes organophosphorous/phosphinic inhibitors [15]. Coumarin (1,2-benzopyrone) derivatives constitute one of the most common families of green plant secondary metabolites. Several type of coumarins have been reported to display multiple biological properties, e.g. [16-17]. Many products which contain a coumarin subunit exhibit biological activities, such as molluscicidal, anthelmintic, hypnotic, and insecticidal activities [18]. The medicinal properties of coumarins include the inhibition of platelet aggregation, cytochrome P450 and steroid 5α-reductase [19].

4-Hydroxycoumarin is an interesting compound found in many plants and particularly known as the natural precursor of dicoumarol, a powerful anticoagulant that acts as a vitamin K antagonist [20]. In addition, recent studies in a collaborative effort between chemists and biologists have shown that a number of 4-hydroxycoumarins show biological activity as potent non-nucleoside RT inhibitors [21]; HIV integrase [22] or HIV protease [23] inhibitors, promising characteristics have been identified. Khan et al reported a variety of *bis*-coumarins as urease inhibitors with IC_{50} values ranging from m 15.06–91.35 µM [24]. Only compound **1** (**Figure 1a**, R = H) showed good urease inhibition. They concluded that the two hydroxyl groups present on two lactone rings of *bis*-coumarin molecule may be responsible for inhibitory activity while the decline in activity was rationalized due to the steric hindrance of bulky groups (R). In the current study, we

are reporting the synthesis of simple and new derivatives of 4-hydroxycoumarins **(Figure 1b)** with better urease inhibitory potentials than the reported *bis*-coumarins. The molecular docking studies were performed to determine the binding interactions of these compounds.

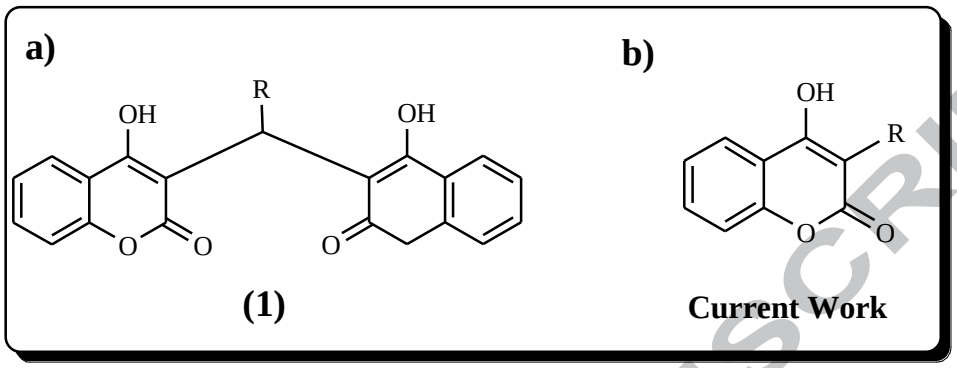
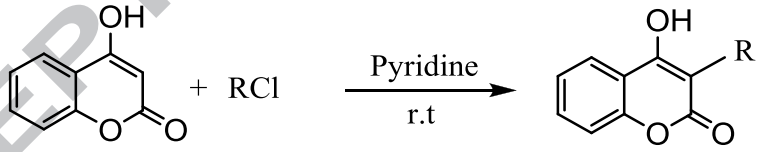


Figure 1: (a) Structure of the previously identified *bis*-coumarin (1) as urease inhibitor and; (b) the structural framework of the current study.

2.0. Results and Discussion

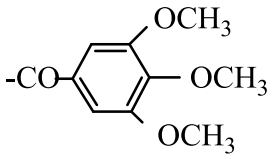
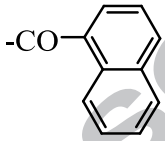
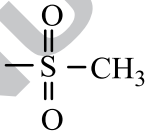
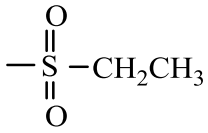
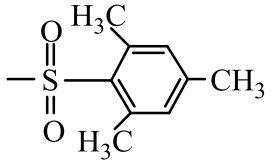
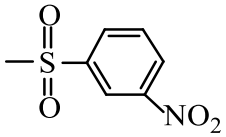
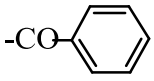
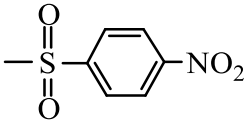
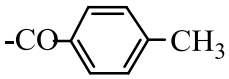
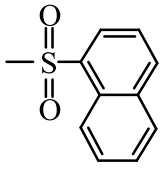
2.1. Chemistry

A mixture of acyl chloride (1 mmol) and 4-hydroxycoumarin (1 mmol) was dissolved in dry pyridine (5 mL), and stirred at room temperature for 10 minutes. Water was added to precipitate out the product, which was filtered on suction and washed with excess of water followed by EtOH. The products were finally recrystallized to obtain pure compounds (**1-16**).



Scheme-1: Synthesis of coumarin analogs **(1-16)**

Table-1: Synthesis of various derivatives of coumarins (1-16)

S. No.	R	Yield (%)	S. No.	R	Yield (%)
1	-CO-CH_3	90	**9**		73
2	-CO-$CHCl_2$	70	**10**		83
3	-CO-$C(CH_3)_3$	78	**11**		82
4	-CO-$(CH_2)_7$ CH_3	84	**12**		88
5	-CO-(CH_2)8 CH3	86	**13**		80
6	-CO-(CH_2)10 CH3	89	**14**		81
7		85	**15**		74
8		82	**16**		74

2.2 Urease Inhibitory Activity

Compounds **1-16** were screened for urease inhibitory potential. Some compounds among the series showed potent urease inhibitory potential (**Table-2**). Compounds **5**, **6**, and **9** exhibited excellent urease inhibition with IC_{50} values 13.01 ± 0.8, 17.09 ± 1.1, and 11.30 ± 1.7 μM, respectively, which is better than the standard inhibitor thiourea (IC_{50} = 21 ± 0.11 μM). Compound **4**, **11**, **14** and **16** exhibited good to moderate potential for urease inhibition with IC_{50} values of 30.87 ± 1.3, 29.45 ± 1.1, 56.2 ± 1.2 and 69.53 ± 0.9 μM, respectively.

Structure-activity relationship predicted that the urease inhibitory potential of molecules was basically governed by the substitution pattern on main skeleton. It is obvious from **Table 2**, with a few exceptions, that incorporation of alkyl chains was well tolerated. The 3, 4, 5-trimethoxy phenyl substituted analog **9** (IC_{50} = 11.30 ±1.7 μM) displayed good potency even better than the standard drug thiourea. At the same time, fusion of another phenyl ring (naphthalene) in compound **10** furnished a substantial loss in the inhibitory potential. 1-decanone substituted analog **5** (IC_{50} = 13.01 ± 0.8 μM) and 1-dodecanone substituted analog **6** (IC_{50} = 17.09 ± 1.1 μM) exhibited outstanding inhibitory potential among the whole series. The greater potential of the most active analog **9** might be due to having electron donating groups on the phenyl ring. Similarly analogs **5** and **6** contain alkyl moieties, which are weakly, electron donor might be responsible for these greater potentials. If we compare these more active compounds with compounds **1**, **2** and **3** which also contain alkyl moiety but show no inhibitions. The reason behind this seems to be the nature of the alkyl group. We observed here that by increasing the length of the alkyl chain, inhibitory potential is increased. This was confirmed from compound **4** which contains nonane-1-one moiety and show weaker potential for the inhibition of urease as compared to the compounds **5** and **6**, both of which contain longer alkyl groups. In case of the sulfonated analogs, the methanesulfonyl substituted analog **11**, 3-nitrophenyl sulfonyl analog **14** and naphthyl sulfonyl analog **16,** having IC_{50} values 29.45±1.1, 56.2±1.2 and 69.53±0.9 μM, respectively, showed good potential for urease inhibition. It is concluded that the replacement of phenyl to naphthyl group, *i.e.* introduction of additional ring resulted in the substantial decrease or loss in activity and this is the reason of the higher inhibitory potential of simple coumarins when compared with the previously studied *bis*-coumarins by Khan *et al.* Our results are further

supported by molecular docking studies and the mean binding energies (**Figure S-1,** Supporting Information).

Table-2: Urease inhibitory potential of compounds **1-16**

S. No	IC_{50} ± SEM[a](μ/M)	S. No	IC_{50} ± SEM[a](μ/M)
1	NA	**9**	11.30±1.7
2	NA	**10**	NA
3	NA	**11**	29.45±1.1
4	30.87 ± 1.3	**12**	NA
5	13.01±0.8	**13**	NA
6	17.09±1.1	**14**	56.2±1.2
7	NA	**15**	NA
8	NA	**16**	69.53±0.9
		Thiourea[b]	21 ± 0.11

SEM[a] is the standard error of the mean, Thiourea[b] standard inhibitor for anti-urease activity

NA stands for "Not Active"

2.3. Molecular Docking Studies

To investigate the plausible orientation of the synthesized compounds and the compound-enzyme interactions, molecular docking studies were performed by using crystal structure of *Bacillus pasteurii* (BP) in complex with acetohydroxamic acid (HAE, PDB ID 4UBP). AutoDock 4.2 and a Lamarckian genetic algorithm (LGA) were used for protein-fixed ligand-flexible docking calculations. In order to check the reliability of the docking method, acetohydroxamic acid was docked into the active site of 4UBP. The root mean square deviation (RMSD) between co-crystallized and re-docked conformation was found 0.981 Å suggesting high docking reliability.

The active site of urease is located within the cavity or the crevice in its internal territory in which HAE molecule chelates with two nickel ions (Ni798 and Ni799) *via* hydroxyl oxygen. The key amino acid residues in the catalytic site of BPU are Ala170, His137, His139, Lys220, His249, His275, Gly280, Cys322, His323, His324, Arg339, Ala363 and Asp363. His137, His139 and KCX220 residues are liganded to Ni799. Whereas, His249, His275, Asp363 residues are

liganded to Ni798. Carbamylated Lys490 (KCX220, a non-standard residue), acts as bridging residue between two Ni ions.

We analyzed the computer generated molecular model of compounds and our analysis identified that all active compounds interact well with Ni ions of the urease enzyme. The scoring functions, i.e., the distance of interaction, coordination pattern, nature of interaction and the residues involved in the interaction were carefully examined. The coordination pattern of the most active compound **9** (IC_{50} = 11.30±1.7 µM) is shown in **Figure 2**. From the structure of the docking conformation, it appeared that the greater activity of compound **9** is due to the formation of a stable complex of two nickel atoms with oxygen of the methoxy group at a binding distance of 2.9 Å and HBA with Arg339 at a distance of 2.87 Å. The observed negative fitness values of binding interactions (mean binding energy=−12.08 kcal/mol) revealed that compound **9** is tightly fitted into the active site of 4UBP.

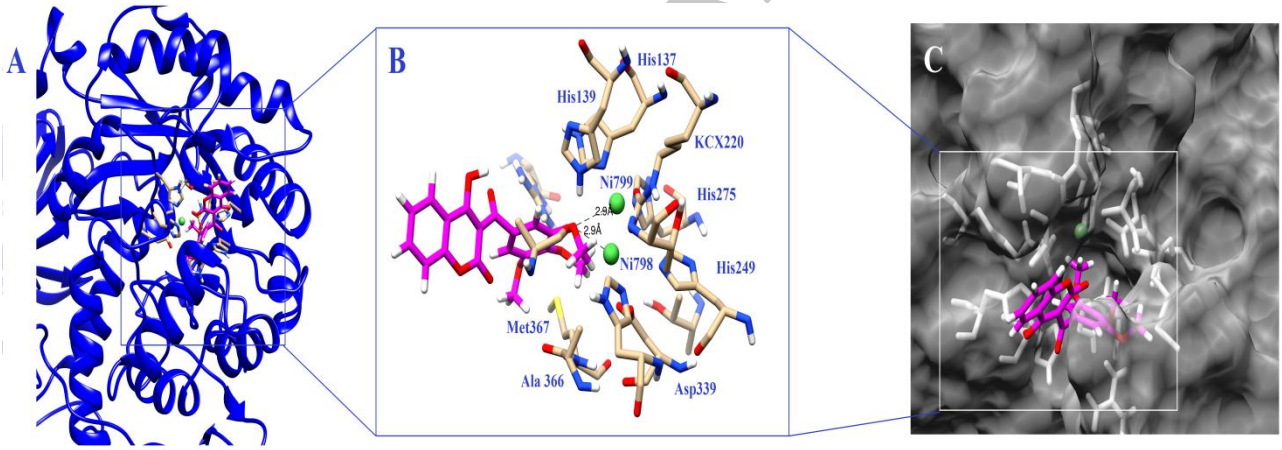


Figure 2: Modeled mode of binding of most active compound **9** (Magenta) into the active site of urease from *Bacillus pasteurii* **(A)** Ribbon form **(B)** Docked conformation showing interactions with Ni bi-center **(C)** Surface form

The interaction pattern of compounds with moderate and weak activity (Compound **11 and** compound **16**) revealed that Ni metal ligation in the ligand-enzyme complex is essential for stabilizing a ligand-enzyme complex. **Figure 3A** revealed that compound **11** (IC_{50}=29.45±1.1) having methanesulfonyl group formed strong ligation with Ni798 at a binding distance of 1.0 Å. While, Ni799 displaced away from sulfonyl oxygen at the distance of 3.0 Å and this looks to be the apparent reason for the moderate *in vitro* activity of compound **11**. Similarly sulfonyl oxygen

is pointed towards His249 and His275 and acts as a hydrogen bond acceptor. Taking into the consideration of the fitness value, compound **11** have shown mean binding energy= –11.17 kcal/mol. By replacing methanesulfonyl group (compound **11**) to 1-naphthosulfonyl (compound **16)** the urease inhibition activity decreased (IC_{50}=69.53±0.9). In **Figure 3B**, it can be seen that although the compound **16** displaced away from nickel bi-center at the distance of 5.8 Å and 8.0 Å and show no metal ligation,however, it formed hydrogen bond with Asp224 and Arg339. Mean binding energy of –8.29 kcal/mol is the possible explanation for the poor *in vitro* activity of compound **16**.

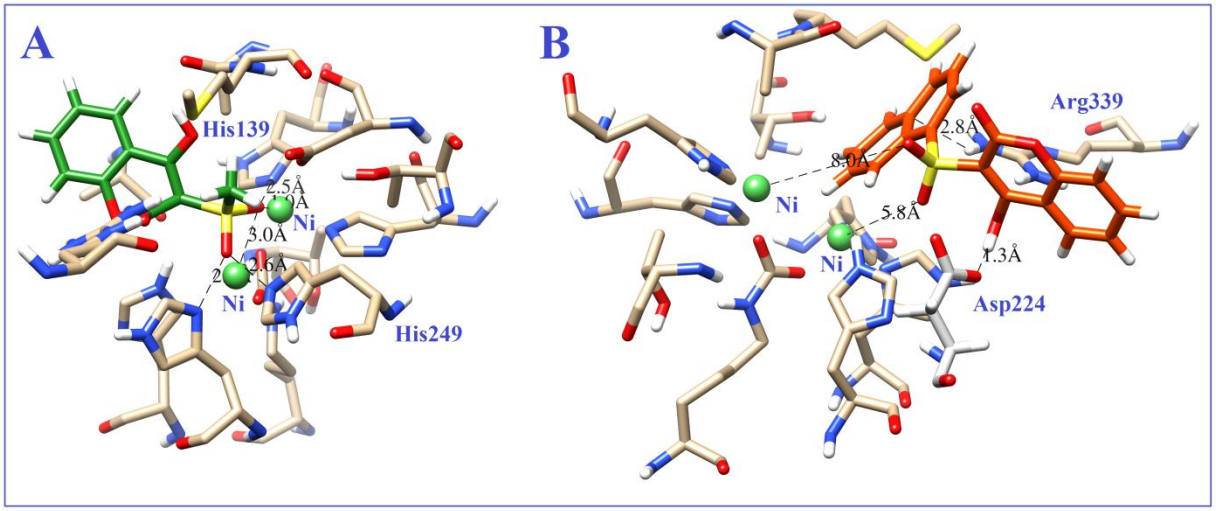


Figure 3: Docked conformation of compounds with moderately and weak urease inhibition activity showing interactions with Ni bi-center and different amino acid residues **(A)** Compound **11** (Green) **(B)** Compound **16** (Orange red).

2.4. Urease Inhibition Assay

Reaction mixtures comprising one unit of urease enzyme (*Bacillus pasteurii*) solution and 55 μL of buffers containing 100 mMol urea were incubated with 5 μL of test compounds (1 mMol concentration) at 30 $^{\circ}$C for 15 min in 96-well plates. Urease activity was determined by measuring ammonia production using the indophenol's method [25]. Momentarily, 45 μL each of phenol reagent and 70 μL of alkali reagent were added to each well. The increasing absorbance at 630 nm was measured after 50 min, using a micro-plate reader (Molecular Devices, USA). All reactions were performed in triplicate in a final volume of 200 μL. The results (change in

absorbance per min.) were processed by using the Soft-Max Pro s4.5.Software (Molecular Devices, USA).

3.0. Conclusion

Synthesis and urease inhibition bioassay of new acylated and sulfonylated 4-hydroxycoumarins has been carried out. Among the newly synthesized derivatives (**1-16**), compound **5**, **6** and **9** were found to be highly potent for urease inhibition. The greater inhibitory potential of these analogs as compared to the standard thiourea was also explained by performing *in silico* molecular docking studies. This work allowed us to obtain a preliminary biological profile of the series indicating which type of compounds might be suitable for further investigation and in which direction the further optimization efforts should be pursued in future.

4.0. Experimental

4.1. General

NMR experiments were performed on Avance Bruker AM 400 MHz machine. CHN Analysis was performed on a Carlo Erba Strumentazion-Mod-1106, Italy. Ultraviolet (UV) spectra were recorded on Perkin–Elmer Lambda-5 UV/vis spectrophotometer in MeOH. Infrared (IR) spectra were recorded on JASCO IR-A-302 spectrometer as KBr (disc). Electron impact mass spectra (EI-MS) were recorded on a Finnigan MAT-311A (Germany) mass spectrometer. Thin layer chromatography (TLC) was performed on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by UV at 254 and 365 nm.

4.2. Docking Studies

Docking studies were carried out using Autodock 4.2 [26]. Gasteiger charges were added to the ligand and maximum 6 number of active torsions are given to the lead compound using AutoDock Tool. 2D structures of ligands converted to 3D in pdbqt format by Openbabel (ver. 2.3.1) [27]. Kollaman charges and solvation term were added to the protein structure. The Grid for docking calculation was centered to cover the protein binding site residues and accommodate ligand to move freely. Docking parameters were as follows: 30 docking trials, population size of 300, maximum number of energy evaluation ranges of 250000, maximum number of generationsis 27,000, mutation rate of 0.02, cross-over rate of 0.8, Other docking parameters were set to the software's default values. The view of the docking results and analysis of their surface with graphical representations were done using UCSF Chimera package [28].

4.3. General procedure for the synthesis of compounds (1-16)

A mixture of acyl chloride (1 mmol) and 4-hydroxycoumarin (1 mmol) dissolved in dry pyridine (5 mL), was stirred at room temperature for 10 minutes, then water was added till precipitates formed, filtered on suction and washed with excess of water followed by EtOH then recrystallized to obtained pure products.

3-Acetyl-(4-hydroxycoumarin) (1)

Yield: 0.56 g (90%); R_f = 0.5 (Ethyl acetate: Hexane, 9:1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.86 (d, 2H, *J* = 7.8 Hz, H-5), 7.57 (td, 2H, *J* = 7.8, *J* = 1.9 Hz, H-7), 7.32 (d, 2H, *J* = 7.8Hz, H-8), 7.27 (td, 2H, *J* = 7.8, *J* = 2.0 Hz, H-6), 1.44 (s, 3H, -C*H*$_3$); IR (KBr, cm^{-1}) ν_{max} 1553, 1610, 1726, 2928, 3426; UV/Vis. ($CHCl_3$) λ_{max} (log ε) 214.2 (4.35) nm; EI-MS (*m/z*) 204, (M^+, 71), 189 (47), 162 (28), 120 (100), 92 (34), 77 (23), 63 (33), 51 (34); Anal. calcd. for $C_{11}H_8O_4$ C 64.71; H 3.95; Found: C 64.78; H 3.90.

3-Dichloroacetyl-(4-hydroxycoumarin) (2)

Yield: 0.35 g (70%); R_f = 0.64 (Ethyl acetate: Hexane, 9:1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.41 (s, 1H, -C*H*), 7.86 (d, 2H, *J* = 7.8 Hz, H-5), 7.57 (td, 2H, *J* = 7.8, *J* = 1.9 Hz, H-7), 7.32 (d, 2H, *J* = 7.8Hz, H-8), 7.27 (td, 2H, *J* = 7.8, *J* = 2.0 Hz, H-6); IR (KBr) ν_{max} 750, 1560, 1640, 1710, 2970, 3410 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 214.2 (4.42) nm; EI-MS (*m/z*) 442, (M^+, 71), 189 (47), 162 (28), 120 (100), 92 (34), 77 (23), 63 (33), 51 (34); Anal. calcd for $C_{11}H_6O_4Cl_2$ C 48.38; H 2.21; Found: C 48.46; H 2.25.

3-Pivaloyl-(4-hydroxycoumarin) (3)

Yield: 0.41 g (78%); R_f = 0.62 (Ethyl acetate: Hexane, 9:1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.86 (d, 2H, *J* = 7.8 Hz, H-5), 7.57 (td, 2H, *J* = 7.8, *J* = 1.9 Hz, H-7), 7.32 (d, 2H, *J* = 7.8Hz, H-8), 7.27 (td, 2H, *J* = 7.8, *J* = 2.0 Hz, H-6), 1.48 (s, 9H, -C*H*$_3$); IR (KBr) ν_{max} 1550, 1618, 1730, 3010, 3390 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 215.4 (4.71) nm; EI-MS (*m/z*) 442, (M^+, 76), 189 (47), 162 (28), 120 (100), 92 (34), 77 (23), 63 (33), 51 (34); Anal. calcd for $C_{14}H_{14}O_4$C 68.28; H 5.73; Found: C 68.25; H 5.50.

3-Nonanoyl-(4-hydroxycoumarin) (4).

Yield: 0.73 g (84%); R_f = 0.58 (Ethyl acetate: Hexane, 9:1); H^1-NMR (500 MHz, $CDCl_3$): δ 7.89 (d, 1H, *J* = 7.8 Hz, H-5), 7.55 (td, 1H, *J* = 7.55, *J* = 2.0 Hz, H-7), 7.30 (dd, 2H, *J* = 7.8 Hz, H-8), 7.23 (td, 2H, *J* = 7.8, *J* = 2.0 Hz, H-6), 1.59 (2H, t, -C*H*$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_3$), 1.14-1.64 (12H, m, -CH$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-CH$_3$), 0.84 (t, 3H, *J* = 6.6 Hz, -CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-C*H*$_3$); IR (KBr) ν_{max} 1558, 1612, 1730, 2940, 3610 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 214.7 (4.81) nm; EI-MS (*m/z*) 302, (M^+, 53), 189 (34), 162 (58), 120 (100), 92 (67), 77 (6), 63 (27), 51 (18); Anal. calcd for $C_{18}H_{22}O_4$ C 71.50; H 7.33; Found: C 71.60; H 7.5.

3-Decanoyl-(4-hydroxycoumarin) (5).

Yield: 0.86 g (86%); R_f = 0.47 (Ethyl acetate: Hexane, 9:1); ^{1}H-NMR (400 MHz, $CDCl_3$δ 7.86 (d, 2H, *J* = 7.8 Hz, H-5), 7.57 (td, 2H, *J* = 7.8, *J* = 1.9 Hz, H-7), 7.32 (d, 2H, *J* = 7.8Hz, H-8), 7.27 (td, 2H, *J* = 7.8, *J* = 2.0 Hz, H-6), 1.67 (2H, t, -C*H*$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_3$), 1.15-1.51 (14H, m, -C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-CH$_3$), 0.76 (t, 3H, *J* = 6.6 Hz, CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_3$); IR (KBr) ν_{max} 1559, 1608, 1709, 2925, 3398 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 212.8 (4.74) nm; EI-MS (*m/z*) 316, (M^+, 55), 204 (14), 189 (40), 162 (58), 120 (100), 92 (56), 77 (6), 63 (25), 51 (15); Anal. calcd for $C_{19}H_{24}$O4 C 72.13; H 7.65; Found: C 72.20; H 7.70.

3- Lauroyl-(4-hydroxycoumarin) (6)

Yield: 0.46 g (89%); R_f = 0.54 (Ethyl acetate: Hexane, 9:1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.86 (d, 2H, *J* = 7.8 Hz, H-5), 7.57 (td, 2H, *J* = 7.8, *J* = 1.9 Hz, H-7), 7.32 (d, 2H, *J* = 7.8Hz, H-8), 7.27 (td, 2H, *J* = 7.8, *J* = 2.0 Hz, H-6), 1.74 (2H, m, -C*H*$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_3$), 1.15-1.60 (14H, m, -C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-C*H*$_2$-CH$_3$), 0.74 (t, 3H, *J* = 6.6 Hz, -CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-C*H*$_3$); IR (KBr) ν_{max} 1544, 1614, 1705, 2940, 3410 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 209.8 (4.98) nm; EI-MS (*m/z*) 344, (M^+, 70), 204 (5), 189 (3), 162 (71), 120 (100), 92 (37), 77 (5), 63 (15), 51 (33); Anal. calcd for $C_{21}H_{28}O_4$ C 73.23; H 8.19; Found: C 73.30; H 8.20.

3-Benzoyl-(4-hydroxycoumarin) (7)

Yield: 0.70 g (85%); R_f = 0.62 (Ethyl acetate: Hexane, 9:1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.86 (d, 2H, *J* = 7.8 Hz, H-5), 7.57 (td, 2H, *J* = 7.8, *J* = 1.9 Hz, H-7), 7.32 (d, 2H, *J* = 7.8Hz, H-8), 7.27 (td, 2H, *J* = 7.8, *J* = 2.0 Hz, H-6), 7.01 (d, 2H, *J* = 8.3 Hz, H-2′/6′), 6.70 (d, 2H, *J* = 8.3 Hz, H-3′/5′), 5.89 (td, 1H, *J* = 7.8, *J* = 2.0 Hz, H-4); IR (KBr) ν_{max} 1567, 1605, 1637, 1732, 2970,

3419 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 201.6 (5.21) nm; EI-MS (*m/z*) 266, (M^+, 15), 162 (4), 120 (8), 105 (100) 92 (5), 77 (44), 63 (3) 51 (15);

Anal. calcd for $C_{16}H_{10}O_4$ C 72.18; H 3.79; Found: C 72.20; H 43.72.

3-(4-Methylbenzoyl-(4-hydroxycoumarin) (8)

Yield: 0.61 g (82%); R_f = 0.63 (Ethyl acetate: Hexane, 9:1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.86 (d, 2H, *J* = 7.8 Hz, H-5), 7.57 (td, 2H, *J* = 7.8, *J* = 1.9 Hz, H-7), 7.32 (d, 2H, *J* = 7.8Hz, H-8), 7.27 (td, 2H, *J* = 7.8, *J* = 2.0 Hz, H-6), 7.12 (d, 2H, *J* = 8.3 Hz, H-2′/6′), 7.12 (d, 2H, *J* = 8.3 Hz, H-3′/5′), 2.66 (s, 3H, -C*H*$_3$); IR (KBr) ν_{max} 1552, 1615, 1730, 2935, 3389 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 202.7 (5.12) nm; EI-MS (*m/z*) 442, (M^+, 76), 321 (12), 281 (21), 265 (18), 249 (100), 162 (64), 120 (99), 92 (97), 63 (61); Anal. calcd for $C_{17}H_{12}O_4$ C 72.85; H 4.32; Found: C 72.83; H 4.28.

3-(3, 4, 5-Trimethoxybenzoyl-(4-hydroxycoumarin) (9)

Yield: 0.80 g (73%); R_f = 0.61 (Ethyl acetate: Hexane, 9:1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.86 (d, 2H, *J* = 7.8 Hz, H-5/5′), 7.57 (td, 2H, *J* = 7.8, *J* = 1.9 Hz, H-7/7′), 7.32 (d, 2H, *J* = 7.8Hz, H-8/8′), 7.27 (td, 2H, *J* = 7.8, *J* = 2.0 Hz, H-6/6′), 7.01 (d, 2H, *J* = 8.3 Hz, H-2′/6′), 3.78 (s, 3H, OC*H*$_3$), 3.89 (s, 6H, -OC*H*$_3$); IR (KBr) ν_{max} 1594, 1629, 1726, 1750, 2930, 3425 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 201.9 (5.25) nm; EI-MS (*m/z*) 356, (M^+, 30), 195 (100), 167 (7), 120 (3), 109 (9) 92 (3), 63 (2); Anal. calcd for $C_{19}H_{16}O_7$ C 64.04; H 4.53; Found: C 64.10; H 4.58.

3-(1-Nephthoyl-(4-hydroxycoumarin) (10)

Yield: 0.82 g (83%); R_f = 0.48 (Ethyl acetate: Hexane, 9:1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.25 (d,1H, *J* = 7.8 Hz, H-4′), 8.25 (d,1H, *J* = 7.8 Hz, H-4″), 7.86 (d, 2H, *J* = 7.8 Hz, H-2′/8′), 7.57 (td, 2H, *J* = 7.8, *J* = 1.9 Hz, H-7/7′), 7.29-7.31 (m, 3H, H-3′/5′-7′), 7.32 (d, 2H, *J* = 7.8 Hz, H-8), 7.27 (td, 2H, *J* = 7.8, *J* = 2.0 Hz, H-6); IR (KBr) ν_{max} 1555, 1608, 1725, 2915, 3425 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 202.4 (5.20) nm; EI-MS (*m/z*) 442, (M^+, 76), 321 (12), 281 (21), 265 (18), 249 (100), 162 (64), 120 (99), 92 (97), 63 (61); Anal. calcd for $C_{20}H_{12}O_4$ C 75.94; H 3.82; Found: C 75.88; H 3.90.

3-(Methanesulfonoyl-(4-hydroxycoumarin) (11)

Yield: 0.61 g (82%); R_f = 0.63 (Ethyl acetate: Hexane, 9:1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.86 (d, 2H, *J* = 7.8 Hz, H-5), 7.57 (td, 2H, *J* = 7.8, *J* = 1.9 Hz, H-7), 7.32 (d, 2H, *J* = 7.8Hz, H-8/8′), 7.27 (td, 2H, *J* = 7.8, *J* = 2.0 Hz, H-6), 3.46 (s, 3H, -C*H*$_3$); IR (KBr) ν_{max} 1050, 1550, 1614,

1720, 2923, 3468 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 206.2 (4.87) nm; EI-MS (*m/z*) 442, (M^+, 76), 321 (12), 281 (21), 265 (18), 249 (100), 162 (64), 120 (99), 92 (97), 63 (61); Anal. calcd for $C_{10}H_8O_5S$ C 50.0; H 3.36; Found: C 50.03; H 3.39.

3-(Ethanesulfonoyl-(4-hydroxycoumarin) (12)

Yield: 0.49 g (88%); R_f = 0.56 (Ethyl acetate: Hexane, 9:1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.86 (d, 2H, *J* = 7.8 Hz, H-5), 7.57 (td, 2H, *J* = 7.8, *J* = 1.9 Hz, H-7), 7.32 (d, 2H, *J* = 7.8Hz, H-8), 7.27 (td, 2H, *J* = 7.8, *J* = 2.0 Hz, H-6), 7.03 (s, 2H, H-3′/5′), 2.66 (s, 6H,C$\underline{H}_3$), 2.33 (s, 3H, -C$\underline{H}_3$);IR (KBr) ν_{max} 1046, 1550, 1610, 1729, 2938, 3460 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 207 (4.84) nm; EI-MS (*m/z*) 442, (M^+, 76), 321 (12), 281 (21), 265 (18), 249 (100), 162 (64), 120 (99), 92 (97), 63 (61); Anal. calcd for $C_{11}H_{10}O_5S$ C 51.96; H 3.96; Found: C 51.94; H 3.99.

3-(Mesitylenebenzosulfonoyl-(4-hydroxycoumarin) (13)

Yield: 0.74 g (80%); R_f = 0.68 (Ethyl acetate: Hexane, 9:1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.86 (d, 2H, *J* = 7.8 Hz, H-5), 7.57 (td, 2H, *J* = 7.8, *J* = 1.9 Hz, H-7), 7.32 (d, 2H, *J* = 7.8 Hz, H-8), 7.27 (td, 2H, *J* = 7.8, *J* = 2.0 Hz, H-6), 7.03 (s, 2H, *J* = 7.8 Hz, H-3′/5′), 2.66 (s, 6H, *J* = 8.3 Hz, C$\underline{H}_3$), 2.33 (s, 3H, -C$\underline{H}_3$); IR (KBr) ν_{max} 1064, 1568, 1605, 1736, 2946, 3446 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 205.6 (4.80) nm; EI-MS (*m/z*) 442, (M^+, 76), 321 (12), 281 (21), 265 (18), 249 (100), 162 (64), 120 (99), 92 (97), 63 (61); Anal. calcd for $C_{18}H_{16}O_5$ C 62.78; H 4.68; Found: C 62.79; H 4.65.

3-(3-Nitrobenzosulfonoyl-(4-hydroxycoumarin) (14)

Yield: 0.86 g (81%); R_f = 0.58 (Ethyl acetate: Hexane, 9:1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.70 (s, 1H, H-2), 8.45 (d, 2H, *J* = 7.8 Hz, H-), 8.37 (d, 1H, *J* = 7.8 Hz, H-), 8.24 (td, 2H, *J* = 7.8, *J* = 1.9 Hz, H-),7.86 (d, 2H, *J* = 7.8 Hz, H-5), 7.57 (td, 2H, *J* = 7.8, *J* = 1.9 Hz, H-7), 7.32 (d, 2H, *J* = 7.8Hz, H-8), 7.86 (d, 2H, *J* = 7.8 Hz, H-5);IR (KBr) ν_{max} 1045, 1349, 1545, 1550, 1610, 1730, 2940, 3428 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 205 (4.90) nm; EI-MS (*m/z*) 442, (M^+, 76), 321 (12), 281 (21), 265 (18), 249 (100), 162 (64), 120 (99), 92 (97), 63 (61); Anal. calcd for $C_{15}H_9NO_7S$ C 51.87; H 2.61; Found: C 51.90; H 2.60.

3-(4-Nitrobenzosulfonoyl-(4-hydroxycoumarin) (15)

Yield: 0.80 g (74%); R_f = 0.56 (Ethyl acetate: Hexane, 9:1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.45 (d, 2H, *J* = 7.8 Hz, H-2′/6′), 8.37 (d, 1H, *J* = 7.8 Hz, H-3′/5′), 7.86 (d, 2H, *J* = 7.8 Hz, H-5), 7.57 (td, 2H, *J* = 7.8, *J* = 1.9 Hz, H-7), 7.32 (d, 2H, *J* = 7.8Hz, H-8), 7.27 (td, 2H, *J* = 7.8, *J* = 2.0 Hz,

H-6); IR (KBr) ν_{max} 1061, 1348, 1544, 1548, 1612, 1732, 2930, 3448 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 205.6 (4.94) nm; EI-MS (*m/z*) 442, (M^+, 76), 321 (12), 281 (21), 265 (18), 249 (100), 162 (64), 120 (99), 92 (97), 63 (61); Anal. calcd for $C_{15}H_9NO_7S$ C 51.87; H 2.61; Found: C 51.85; H 2.65.

3-(1-Nephthosulfonoyl-(4-hydroxycoumarin) (16)

Yield: 0.80 g (74%); R_f = 0.56 (Ethyl acetate: Hexane, 9:1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.27 (d, 1H, *J* = 7.8 Hz, H-4′), 7.92 (d, 2H, *J* = 7.8 Hz, H-2′/8′), 7.86 (d, 2H, *J* = 7.8 Hz, H-5), 7.57 (td, 2H, *J* = 7.8, *J* = 1.9 Hz, H-7), 7.43-7.55 (m, 3H, H-3′/5′-7′), 7.32 (d, 2H, *J* = 7.8 Hz, H-8), 7.27 (td, 2H, *J* = 7.8, *J* = 2.0 Hz, H-6); IR (KBr) ν_{max} 1025, 1555, 1602, 1748, 2974, 3375 cm^{-1}; UV ($CHCl_3$) λ_{max} (log ε) 204.9 (4.96) nm; EI-MS (*m/z*) 442, (M^+, 76), 321 (12), 281 (21), 265 (18), 249 (100), 162 (64), 120 (99), 92 (97), 63 (61); Anal. calcd for $C_{19}H_{12}O_5S$ C 64.76; H 3.43; Found: C 64.78; H 3.40.

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Graphical Abstract

Synthesis of 2-Acylated and Sulfonated 4-hydroxycoumarins:

***In vitro* Urease Inhibition and Molecular Docking Studies**

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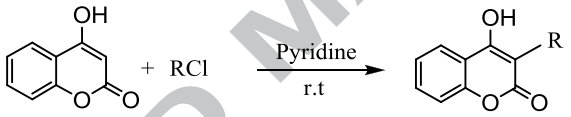
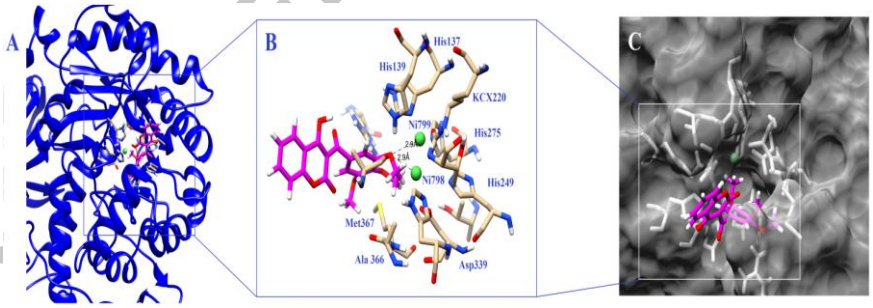


Synthesis of coumarin analogs **(1-16)**



Modeled mode of binding of most active compound **9** (Magenta) into the active site of urease from *Bacillus pasteurii* **(A)** Ribbon form **(B)** Docked conformation showing interactions with Ni bi-center **(C)** Surface form

Highlights

- ✓ Acyl and Sulfonated coumarin derivatives are synthesized
- ✓ Compounds are evaluated for their urease inhibitory potential
- ✓ SAR is established on the basis of Molecular Modeling studies
- ✓ A comparison with previously synthesized biscoumarins is also provided
- ✓ New coumarin derivatives proved better inhibitors of urease as compared to previous biscoumarin derivatives