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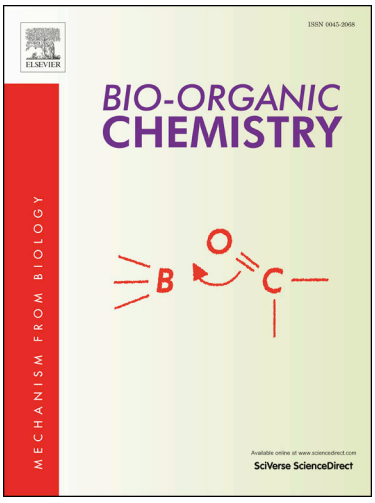

Flavonoid analogues as urease inhibitors: Synthesis, biological evaluation, molecular docking studies and in-silico ADME evaluation

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PII: S0045-2068(20)31668-0
DOI: https://doi.org/10.1016/j.bioorg.2020.104370
Reference: YBIOO 104370

To appear in: *Bioorganic Chemistry*

Received Date: 4 August 2020
Revised Date: 22 September 2020
Accepted Date: 8 October 2020

Please cite this article as: H. Liu, Y. Wang, M. Lv, Y. Luo, B-M. Liu, Y. Huang, M. Wang, J. Wang, Flavonoid analogues as urease inhibitors: Synthesis, biological evaluation, molecular docking studies and in-silico ADME evaluation, *Bioorganic Chemistry* (2020), doi: https://doi.org/10.1016/j.bioorg.2020.104370

Flavonoid analogues as urease inhibitors: Synthesis, biological evaluation, molecular docking studies and in-silico ADME evaluation

Honghui Liu[1,3], Yan Wang[2], Mingxia Lv[1], Yi Luo[4], Bu-Ming Liu[5],·Yan Huang[5], Mian Wang[2]*, Jianyi Wang[1,3]*

1. *Medical College, Guangxi University, Nanning 530004, China.*
   **E-mail: jianyiwang@gxu.edu.cn*
2. *College of Life Science and Technology, Guangxi University, Nanning, 530004, China,*
   **E-mail: mianwang@gxu.edu.cn*
3. *School of Chemistry and Chemical Engineering, Guangxi University, Nanning 530004, China*
4. *Guangxi Institute for Food and Drug Control, Nanning 530021, China*
5. *Guangxi Key Laboratory of Traditional Chinese Medicine, Quality Standards, Nanning 530022, China*

Abstract

A series of novel flavonoid analogues were designed and synthesized. The aimed compounds for urease inhibitory activities were clearly superior to the control drug thiourea (more than 10 times). Among these compounds, L2 (IC_{50} = 1.343 μM) and L12 (IC_{50} = 1.207 μM) exhibited the most excellent urease inhibitory activity in vitro. The molecular dockings of L2, L12 and L22 into urease were performed to explore the binding modes and their structure-activity relationship. Furthermore, these aimed compounds showed good druggable properties.

Keywords

Urease inhibitor; Flavonoid analogues; Synthesis; Docking; Druggability

1. Introduction

Urease (urea amidohydrolase EC3.5.1.5) is nickel-dependent enzyme that can specifically catalyze the hydrolysis of urea to release ammonia(NH_3) and carbon dioxide (CO_2)[1]. The produced ammonia catalyzed by urease helps maintain the pH balance of human intestinal microflora, providing *Helicobacter pylori* a suitable environment for their growth and reproduction in the harsh pH conditions of the stomach[2]. Clearly, Urease is closely related to colonization and survival of *Helicobacter pylori* in the stomach[3], and considered as a therapeutic target of gastric lymphoma, gastric carcinoma and peptic ulcer disease caused by *Helicobacter pylori*.

Some existing urease inhibitors were summarized in **Fig. 1.** The acetohydroxamic

acid (Compound 1) is commonly recognized as urease inhibitor via competitively binding the metal center of the active site of the urease [4], and forming strong complex with the metal center [5], [6].To date acetohydroxamic acid was the only urease inhibitor that is approved by the US Food and Drug Administration for marketing. However, acetohydroxamic acid only shows moderate activity, resulting in a relative large dose [7]. Phosphoramidates (Compounds 2,3) exhibit high urease inhibitory activities because phosphoramidates can tightly bind to the active metallocenter, which is closely similar to the transition state of the enzymatic reaction[8]. But they were not approved for marketing as drugs probably because of their rapid hydrolysis in low pH of gastric juice[9]. The barbiturates or thiobarbiturates (Compounds 4,5,6) were analogous compounds to urea or thiourea fragment, which can competitively bind to the nickel (II) center of urease[10][11][12][13], showing some urease activities. However, they appeared to be weak to moderate uncompetitive or mixed inhibitors, and low practical value. The aromatic heterocyclic compounds (Compounds 7,8,9) bound the active site of the urease were very significant urease inhibitors. Their inhibition activity for urease results from interaction of side chain of cysteine or methionine with π electrons of aromatic fragment of the compound [14][15][16][17]. Some metal complexes can also produce good inhibition of urease[18]. However, the serious side effects and toxicity limit further research of the aromatic heterocyclic compounds and metal complexes. Therefore, it is an urgent need to develop novel urease inhibitors with low toxicity and high efficiency.

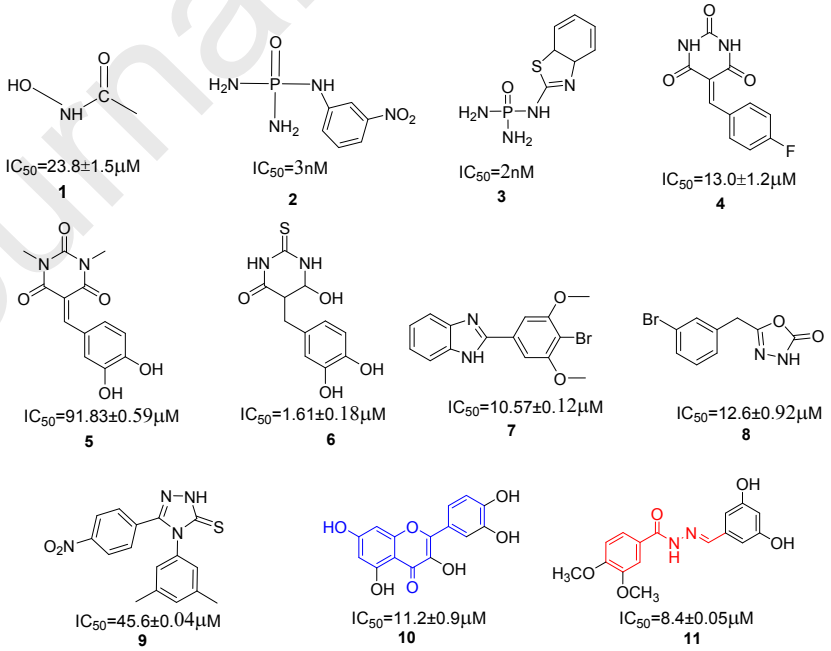


Fig. 1. Some existing urease inhibitors

The natural product flavonoid had been demonstrated significant inhibitory activity on urease[19], beside antioxidant [20], anti-inflammatory[21], antidiabetic [22], anti-bacterial [23], antiviral[24], anti-protozoals [25] and anticancer[26]. A structural resemblance of hydrazide moiety along with thiourea(semicarbazone [27], benzoylhydrazide[28] and isoniazid derivatives[29] make them become good pharmacophore for urease inhibitor.

However, regardless of the flavonoid or urea structure, any single group of urease inhibitory activity is difficult to satisfy. To do so, in this work, we design and synthesize a series of novel urease inhibitor of flavonoid analogues, in which flavonoids could integrate the top of the active chamber, and the hydrazide moiety (semicarbazide, benzoylhydrazide, isoniazid) could bind the active chamber(**Fig. 2**). On this basis, we performed biological evaluation, molecular docking studies and in-silico ADME evaluation to the aimed compounds. It is hopeful to further improve the inhibitory activity of urease and obtain novel candidate compound for urease inhibitors.

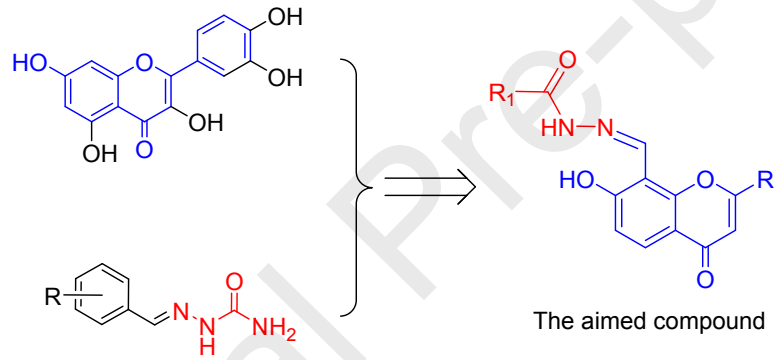
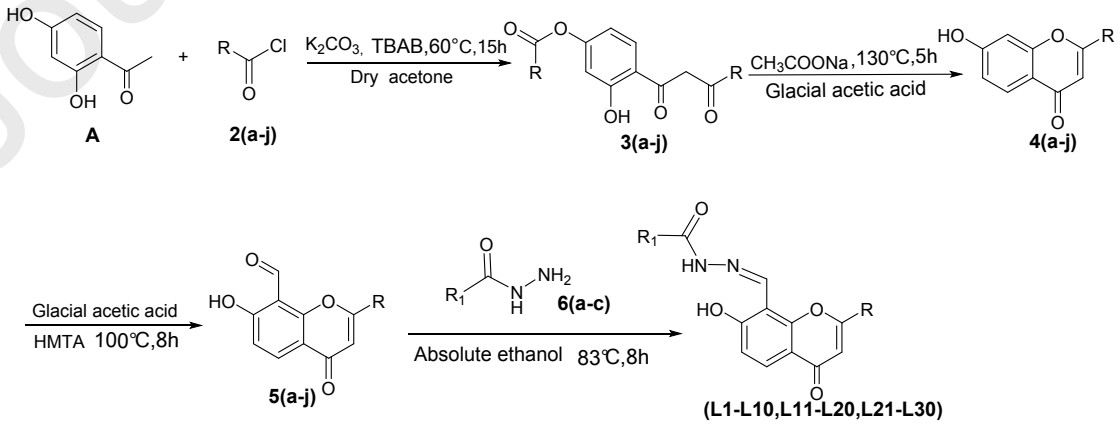


Fig. 2. The designed urease inhibitors

2. Results and discussion

2.1. The synthesis of the aimed compounds

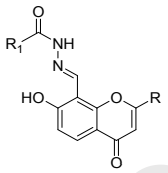


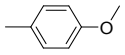
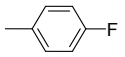
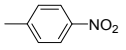
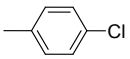
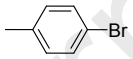
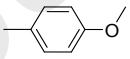
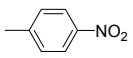
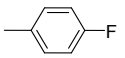
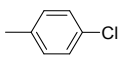
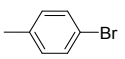
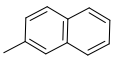
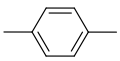
Scheme 1. Synthetic routes of the aimed compounds

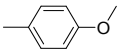
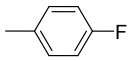
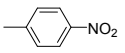
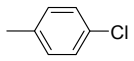
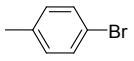
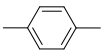
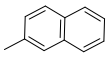
As show in **Scheme 1**, Compound A firstly reacted with compound 2(a-j) to afford an intermediate 3(a-j). Subsequently, the intermediate 3(a-j) was treated with glacial acetic acid and sodium acetate to yield the cyclic intermediate 4(a-j). In the presence of glacial acetic acid, 4(a-j) and hexamethylenetetramine (HMTA) underwent a 8h Duff formylation reaction to provide 5(a-j). In the ethanol, Compound 5 (a-j) and 6 (a-c) further reacted to give the aimed product semicarbazide(L1-L10), benzoyl hydrazide(L11-L20) and isoniazid(L21-L30). The synthesized compounds were characterized by HR-EI-MS, ^{1}H NMR, and ^{13}C NMR spectroscopic techniques.

2.2 The inhibitory activity assays for Urease

Table 1. The inhibitory activities of the aimed compounds for urease.



Species	R	R_1	IC_{50}(μM)[a]	Species	R	R_1	IC_{50}(μM)[a]
L1		NH_2-	1.565±0.102	**L16**			1.663±0.429
L2		NH_2-	1.343±0.288	**L17**			2.002±0.069
L3		NH_2-	1.356±0.381	**L18**			ND
L4		NH_2-	1.907±0.065	**L19**			2.441±0.355
L5		NH_2-	1.965±0.635	**L20**			2.771±0.338
L6		NH_2-	1.833±0.232	**L21**			1.546±0.503
L7		NH_2-	1.936±0.576	**L22**			1.470±0.502
L8		NH_2-	ND	**L23**			2.101±0.842
L9		NH_2-	1.621±0.351	**L24**			1.523±0.922
L10		NH_2-	2.205±1.199	**L25**			1.658±0.621

L11			1.378±0.616	**L26**			1.821±0.846
L12			1.207±0.186	**L27**			1.449±0.247
L13			1.475±0.434	**L28**			ND
L14			1.643±0.415	**L29**			1.645±0.469
L15			1.869±0.734	**L30**			1.566±0.364
thiourea			21.902±0.696				

[a] Values are the mean ± SEM. All experiments were performed at least three times, ND = Not determined.

Next, the synthesized compounds L1–L10, L11–L20 and L21-L30 were performed inhibitory activity assays for urease. As shown in Table 1, thiourea served as a positive control drug (IC_{50} values of 21.902 ± 0.696μM), the inhibitory activities of semicarbazide, benzohydrazide and isoniazid compounds for urease were 10 times more than the thiourea's one.

A comparison of Compounds L2-L4 and L12-L14 show that the H atom in the B ring replaced by an electron-withdrawing halogen atom (-F, -Cl, -Br) with similar size, the inhibitory activities for urease gradually increased with the -Br, -Cl, -F trend. On the other hand, the H atom in the B ring replaced by the electron-withdrawing group -NO_2 (L7, L17) or the electron-donated -CH_3 or –OCH_3 group (L6, L16) with larger size, decrease the inhibitory activity against urease. These show that the polarity (hydrophobicity) of compound and the pocket size of the active site of urease may determine the inhibitory activities of compounds for urease. The H atom in the B ring replaced by electron-withdrawing -F, -Cl, or –Br group was favorable for the formation of the halogen-bond interaction between the B ring and the urease, and the active pocket of urease is not large enough to accommodate the large bulk B ring.

When the B ring of the flavonoid became thiophene, furan, and naphthalene, the urease activity of compounds (L8-L10, L18-L20 and L28-L30) are accordingly decreased compared with compounds L1-L7，L11-L17 and L21- L27, showing that the benzene is the optimal group in B ring. No clear activity difference between L1, L11, and L21, imply that in these compounds the -NH_2 group is not a key group, and can be modified by benzene or pyridine ring.

All aimed compounds (semicarbazide, benzohydrazide and isoniazid) exhibited better inhibitory activities for urease, showing that flavonoid analogues is significant novel lead compounds for urease inhibitors or anti-*Helicobacter pylori* agents. It is worth further structure modification of flavonoid analogues.

2.3. Docking studies

To demonstrate the binding mode and the structure-activity relationship of flavonoid analogues L1-L10，L11-L20 and L21-L30 at the active site of urease, we performed molecular docking studies on representative compounds L2, L12, L22 acting on urease. It should be noticed that, L2, L12 and L22 were surrounded by similar residues CYS322, ALA170, HIS323, Ni798, ASP224 and HIS222 (**Fig. 3-5**), and presented similar interaction with the active site of urease, which may be the reason why L2, L12 and L22 show inhibitory activity of the same order of magnitude toward urease. In the other hand, the binding sites of urease was slightly changed by L2, L12 and L22, which may be the reason why L2, L12 and L22 show different inhibitory activity toward urease.

Seen from **Fig.3**, the B ring in L2 surrounded by residues LYS169, GLU166 and ALA170, could form pi-Alkly interaction with ALA170 and Vander waals interaction with LYS169 and GLU166. Clearly, the benzene as B ring is more favorable for these pi-Alkly and Vander waals interaction than the thiophene and the furan as B ring, supporting that in the inhibitory activity for urease, L1, L11 and L21 were superiors to L8-L9, L18-L19 and L28-L29. The benzene H in ring B replaced by a small bulk bioisosteres F group (electron-withdrawing) can increase the halogen bond interaction between B ring and the residues in the active site, and the interaction between B ring and Ni metal center, supporting the inhibitory activities order for urease that the benzene H in ring B replaced by small bulk and electron-withdrawing group are favorable for the inhibitory activity for urease.

The semicarbazone moiety (NNH) in L2 can form hydrogen-bonded interaction with residue ASP363. Beside one conventional hydrogen bonds between the NH group and ASP224, the benzoyl hydrazide moiety in L12 (Fig.4) was observed to form π-π interactions with residue TRP225. This may be the main reason that L12 is superiors to L2 in the inhibitory activities for urease. When the benzene moiety of benzoyl hydrazide in L12 was replaced by the pyridine to give L22(Fig.5), the π-π interactions disappeared between the active site residues and the pyridine moiety of isoniazid,

correspondingly decreasing the inhibitory activity for urease.

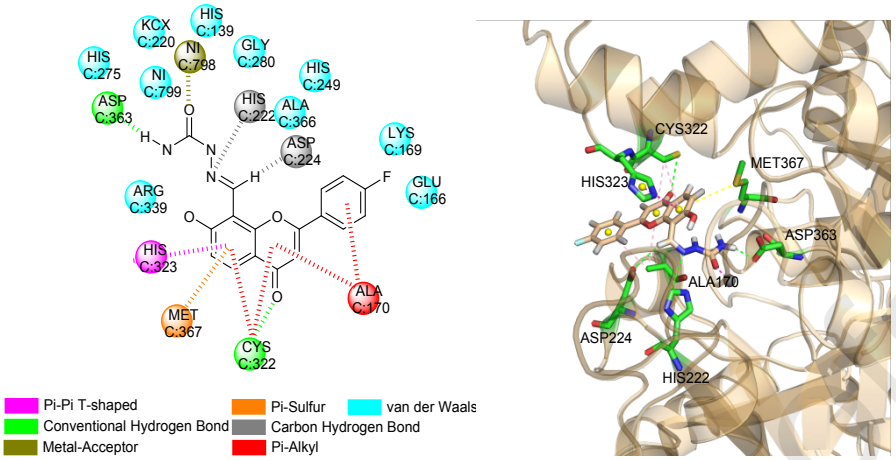


Fig. 3. The interaction map of compound L2 docked into urease.

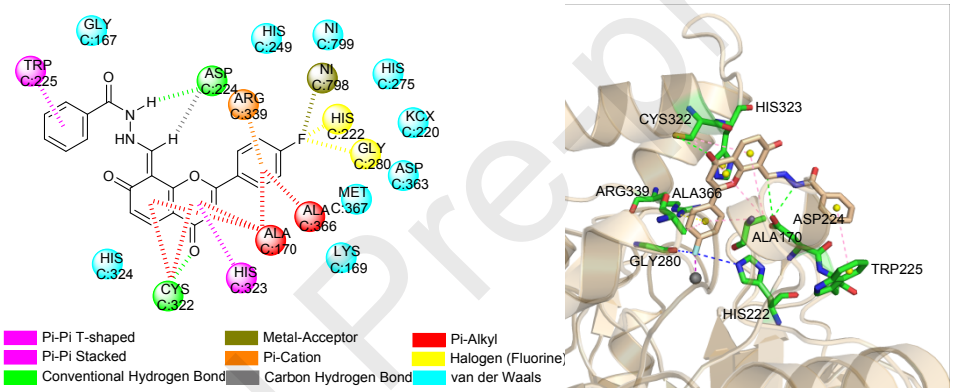


Fig.4. The interaction map of compound L12 docked into urease.

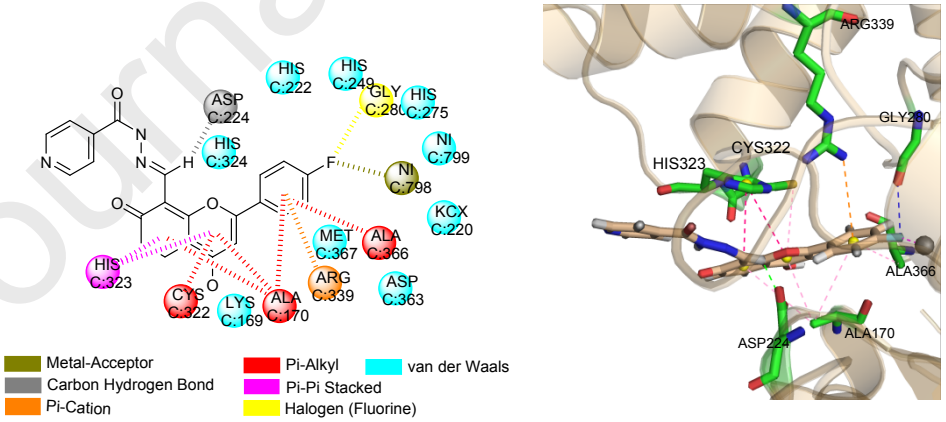


Fig.5. The interaction map of compound L22 docked into urease.

2.4 In-silico ADME evaluation

To further evaluate druggability of these compounds, we performed in-silico

adsorption, distribution, metabolism, excretion and toxicity (ADME) evaluation using Molinspiration and Molsoft online （ http://www.molinspiration.com/ and http://www.molsoft.com/) toolkit[30]. Seen from Table 2, all compounds had 2–4 hydrogen bond donors (HBD) and 5–8 hydrogen bond acceptors (HBA), obeying the Lipinski's rule of 5[31]: The hydrogen bond donors are no more than 5, and the hydrogen bond acceptors are no more than 10. The molecular mass is less than 500 Daltons, and the octanol-water partition coefficient log P is smaller than 5[32]. All compounds had 3-5 rotatable bonds (nROTB) determining the molecular flexibility (should be <10), and 90.90–131.06 $Å^2$ (<140 $Å^2$) topological polar surface area (TPSA) values, showing good permeability of the aimed compounds in the cellular plasma membrane and blood-brain barrier (BBB) [33]. Clearly, most of the aimed compounds possess agreeable ADME properties for oral bio-availability, and good druggability.

Table 2. The druggability of the aimed compounds.

Compound	H-bond donor	H-bond acceptor	nROTB	Lipinski, violation	TPSA($Å^2$)	Mol.wt	logP
L1	4	5	3	Yes, 0	117.92	323.09	3.03
L2	4	5	3	Yes, 0	117.92	341.08	3.20
L3	4	5	3	Yes, 0	117.92	357.05	3.71
L4	4	5	3	Yes, 0	117.92	401.00	3.84
L5	4	5	3	Yes, 0	117.92	337.11	3.48
L6	4	6	4	Yes, 0	127.16	353.10	3.09
L7	4	7	3	Yes, 0	117.92	368.08	2.16
L8	4	6	3	Yes, 0	131.06	313.07	1.98
L9	4	6	3	Yes, 0	117.92	329.05	2.62
L10	4	5	3	Yes, 0	117.92	373.11	4.22
L11	2	5	4	Yes, 0	91.90	384.11	4.79
L12	2	5	4	Yes, 0	91.90	402.10	4.95
L13	2	5	4	Yes, 1	91.90	418.07	5.47
L14	2	5	4	Yes, 1	91.90	462.02	5.60
L15	2	6	4	Yes, 1	91.90	399.12	5.24
L16	2	6	5	Yes, 0	101.14	414.12	4.85
L17	2	7	4	Yes, 0	91.90	429.10	3.92
L18	2	6	4	Yes, 0	105.04	374.09	3.74
L19	2	6	4	Yes, 0	91.90	390.07	4.38

L20	2	5	4	Yes, 1	91.90	434.13	5.97
L21	2	6	4	Yes, 0	104.79	385.11	3.67
L22	2	6	4	Yes, 0	104.79	403.10	3.73
L23	2	6	4	Yes, 0	104.79	419.07	4.18
L24	2	6	4	Yes, 0	104.79	463.02	4.31
L25	2	6	4	Yes, 0	104.79	399.12	3.95
L26	2	7	5	Yes, 0	114.03	415.12	3.56
L27	2	8	4	Yes, 0	104.79	430.09	2.63
L28	2	7	4	Yes, 0	117.93	375.09	2.45
L29	2	7	4	Yes, 0	104.79	391.06	3.09
L30	2	6	4	Yes, 0	104.79	435.12	4.68

MW: Molecular Weight, HBA: a number of H-bond acceptors, HBD: a number of H-bond donors, log P: the octanol-water partition coefficient. nROTB: a number of rotatable bonds. TPSA: the polar surface area

3. Conclusion

In this work, we design and synthesize a series of novel urease inhibitor flavonoid analogues(L1-L10,L11-L20,L21-L30) based on the combination principles. On this basis, we performed inhibitory activity evaluation against urease, molecular docking studies and in-silico ADME evaluation for the aimed compounds. These aimed compounds for urease inhibitory activities were clearly superior to the control drug thiourea (more than 10 times), and L2 and L12 exhibited optimal urease inhibitory activity. The molecular docking studies demonstrated the binding modes of the aimed compounds and their structure-activity relationship. The aimed compounds possess good druggability**.**

4. Experimental part

4.1. Methods and materials

The chemicals and solvents were commercially available and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker 500 MHz at 25 °C, with TMS as the internal standard in deuterium reagent. MS analysis was performed with a Thermo Fisher Scientific LTQ ORBITRAP ELITE (USA).

4.2. General procedure for the preparation of compounds L1-L10,L11-L20 and L21-L30, exemplified with L1

2,4-dihydroxyacetophenone (1.5g, 10mmol) and anhydrous potassium

carbonate(10g, 72mmol),TBAB (1.5g,10mmol) was dissolved in 100 mL Dry acetone at Room temperature. Then benzoyl chloride (2.3ml, 20mmol) , was added dropwise with constant stirring, The resulting solution was stirred for 15 h under reflux condition.and completion of reaction is checked by TLC analysis. Later the solution was cooled to RT and filtered. The filtrate was transferred into 200 mL of 10% glacial acetic acid (GAA) under vigorous stirring and obtained yellow precipitate was filtered and dried. The product was purified by recrystallisation from acetone to give of 3a as a yellow solid.Yellow solid 3a and 3.5g sodium acetate was dissolved in glacial acetic acid(17 mL) and refluxed at 130 °C for 5 h give of 4a. then hexamethylenetetramine (HMTA) (5.6 g, 40mmol) was added and refluxed at 100 °C for 8 h give of 5a. finally, 5a and semicarbazide were dissolved in anhydrous ethanol and refluxed at 83 °C for 8 h.The product was purified by recrystallisation from anhydrous ethanol to give of L1.

4.2. Spectral data of the aimed compounds

(Z)-2-((7-hydroxy-4-oxo-2-phenyl-4H-chromen-8-yl)methylene)hydrazine-1-carboxamide L1

Yield 63.74%; yellow solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.67 (s, 1H, NH), 10.70 (s, 1H, OH), 8.74 (s, 1H, CH=N), 8.18 – 8.12 (m, 2H, Ar), 7.91 (d, J = 8.8 Hz, 1H, Ar), 7.65 – 7.55 (m, 3H, Ar), 7.05 (d, J = 8.8 Hz, 1H, CH), 7.01 (s, 1H, Ar), 6.53 (s, 2H, NH_2). ^{13}C NMR (151 MHz, DMSO-d_6)δ176.68, 162.32, 161.80, 155.99, 154.91, 136.71, 132.24, 131.56, 129.58, 127.03, 126.87, 116.70, 115.74, 107.76, 107.22. HR-EI-MS: m/z calcd for $C_{17}H_{13}N_3O_4$, $[M]^+$324.09998; Found 324.09788;

7(Z)-2-((2-(4-fluorophenyl)-7-hydroxy-4-oxo-4H-chromen-8-yl)methylene)hydrazine-1-carboxamide L2

Yield 64.96%; yellow solid;^{1}H NMR (500 MHz, DMSO-d_6) δ 10.51 (s, 1H, OH), 8.65 (s, 1H, CH=N), 8.29 – 8.21 (m, 2H, Ar), 7.82 (d, J = 8.9 Hz, 1H, Ar), 7.39 (t, J = 8.8 Hz, 2H, Ar), 6.93 (s, 1H, CH), 6.88 (d, J = 8.9 Hz, 1H, Ar), 6.42 (s, 2H, NH_2).^{13}C NMR (151 MHz, DMSO-d_6) δ 176.47, 165.25, 163.59, 160.97, 156.41, 155.51, 137.57, 129.48, 129.42, 128.43, 126.72, 116.62, 116.48, 107.79, 106.95. HR-EI-MS: m/z calcd for $C_{17}H_{12}FN_3O_4$, $[M]^+$Na364.07230; Found 364.07041;

(Z)-2-((2-(4-chlorophenyl)-7-hydroxy-4-oxo-4H-chromen-8-yl)methylene)hydrazine-1-carboxamide L3

Yield 72.28%; yellow solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.53 (s, 1H, OH), 8.64 (s, 1H, CH=N), 8.21 (s, 2H, Ar), 7.83 (d, J = 8.6 Hz, 1H, Ar), 7.61 (d, J = 8.1 Hz, 2H, Ar), 6.95 (d, J = 27.9 Hz, 2H, CH+Ar), 6.45 (s, 2H, NH_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 176.62, 161.82, 161.21, 156.07, 154.83, 136.97, 136.57, 130.49, 129.57, 128.74, 127.02, 116.68, 115.76, 107.86, 107.54.HR-EI-MS: m/z calcd for $C_{17}H_{L26}lN_3O_4$, $[M]^+$Na380.04285; Found 380.04085;

(Z)-2-((2-(4-bromophenyl)-7-hydroxy-4-oxo-4H-chromen-8-yl)methylene)hydrazine-

1-carboxamide L4

Yield 63.50%; yellow solid;^{1}H NMR (500 MHz, DMSO-d_6) δ 10.70 – 10.51 (m, 1H, OH), 8.65 (s, 1H, CH=N), 8.12 (d, J = 8.2 Hz, 2H, Ar), 7.85 (d, J = 8.8 Hz, 1H, Ar), 7.75 (d, J = 8.2 Hz, 2H, Ar), 7.03 – 6.93 (m, 2H, CH+Ar), 6.48 (s, 2H, NH_2).^{13}C NMR (151 MHz, DMSO-d_6) δ 176.54, 161.12, 155.12, 136.99, 132.48, 131.01, 128.88, 126.89, 125.76, 116.47, 107.90, 107.47. HR-EI-MS: m/z calcd for $C_{17}H_{L16}rN_3O_4$, $[M]^+$ 402.01083; Found 402.00839;

(Z)-2-((7-hydroxy-4-oxo-2-(p-tolyl)-4H-chromen-8-yl)methylene)hydrazine-1-carboxamide L5

Yield 72.29%; yellow solid;^{1}H NMR (500 MHz, DMSO-d_6) δ 10.64 (s, 1H, OH), 8.71 (s, 1H, CH=N), 8.05 (d, J = 8.0 Hz, 2H, Ar), 7.88 (d, J = 8.8 Hz, 1H, Ar), 7.39 (d, J = 7.9 Hz, 2H, Ar), 6.99 (d, J = 8.8 Hz, 1H, CH, Ar), 6.93 (s, 1H, Ar), 6.49 (s, 2H, NH_2), 2.42 (s, 3H, CH_3).^{13}C NMR (151 MHz, DMSO-d_6) δ 176.55, 142.28, 130.16, 128.95, 126.88, 126.78, 107.75, 106.50, 21.55.HR-EI-MS: m/z calcd for $C_{18}H_{15}N_3O_4$, $[M]^+$Na 360.09714; Found 360. 09548;

(Z)-2-((7-hydroxy-2-(4-methoxyphenyl)-4-oxo-4H-chromen-8-yl)methylene)hydrazine-1-carboxamide L6

Yield 58.70%; yellow solid;1H NMR (500 MHz, DMSO-d6) δ 10.64 (s, 1H, OH), 8.71 (s, 1H, CH=N), 8.12 (d, J = 8.5 Hz, 2H, Ar), 7.88 (d, J = 8.8 Hz, 1H, Ar), 7.11 (d, J = 8.5 Hz, 2H, Ar), 7.00 (d, J = 8.9 Hz, 1H, CH), 6.90 (s, 1H, Ar), 6.51 (s, 2H, NH_2), 3.88 (s, 3H, CH_3).^{13}C NMR (151 MHz, DMSO-d6) δ 176.53, 162.49, 136.95, 128.74, 126.96, 123.82, 114.96, 107.74, 105.68, 55.98. HR-EI-MS: m/z calcd for $C_{18}H_{15}N_3O_5$, $[M]^+$ 354.11069; Found 354.10845;

(Z)-2-((7-hydroxy-2-(4-nitrophenyl)-4-oxo-4H-chromen-8-yl)methylene)hydrazine-1-carboxamide L7

Yield 65.58%; yellow solid;^{1}H NMR (500 MHz, DMSO-d_6) δ 10.52 (s, 1H, OH), 8.62 (s, 1H, CH=N), 8.47 (d, J = 8.4 Hz, 2H, Ar), 8.33 (d, J = 8.5 Hz, 2H, Ar), 7.86 (d, J = 8.9 Hz, 1H, Ar), 7.16 (s, 1H, CH), 6.96 (d, J = 8.9 Hz, 1H, Ar), 6.46 (s, 2H, NH_2).^{13}C NMR (151 MHz, DMSO-d_6) δ 176.44, 159.71, 149.33, 137.83, 136.95, 128.33, 126.88, 124.36, 109.43, 108.07. HR-EI-MS: m/z calcd for $C_{17}H_{12}N_4O_6$, $[M]^+$Na 391.06677; Found 391.06491;

(Z)-2-((2-(furan-2-yl)-7-hydroxy-4-oxo-4H-chromen-8-yl)methylene)hydrazine-1-carboxamide L8

Yield 65.43%; yellow solid;^{1}H NMR (500 MHz, DMSO-d_6) δ 11.42 (s, 1H, NH), 10.55 (s, 1H, OH), 8.66 (s, 1H, CH=N), 8.08 – 8.02 (m, 1H, CH), 7.88 (d, J = 8.8 Hz, 1H, Ar), 7.52 (d, J = 3.5 Hz, 1H, CH), 7.03 (d, J = 8.8 Hz, 1H, CH), 6.87 (dd, J = 3.6, 1.7 Hz, 1H, CH), 6.59 (d, J = 13.5 Hz, 3H, NH_2+Ar).^{13}C NMR (151 MHz, DMSO-d_6) δ 175.87, 161.60, 154.46, 154.39, 147.61, 145.82, 136.41, 127.04, 116.86, 115.61, 114.43, 113.45, 107.60, 104.67. HR-EI-MS: m/z calcd for $C_{15}H_{11}N_3O_5$, $[M]^+$314.07941;Found 314.07715;

(Z)-2-((7-hydroxy-4-oxo-2-(thiophen-2-yl)-4H-chromen-8-yl)methylene)hydrazine-1-carboxamide L9

Yield 62.34%; yellow solid;^{1}H NMR (500 MHz, DMSO-d_6) δ 11.75 (s, 1H, NH), 10.74 (s, 1H, OH), 8.64 (s, 1H, CH=N), 8.06 (dd, J = 3.7, 1.2 Hz, 1H, CH), 8.00 (dd, J = 4.9,

1.2 Hz, 1H, CH), 7.88 (d, *J* = 8.8 Hz, 1H, Ar), 7.32 (dd, *J* = 5.0, 3.8 Hz, 1H, CH), 7.03 (d, *J* = 8.8 Hz, 1H, CH), 6.79 (s, 1H, Ar), 6.51 (s, 2H, NH_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 176.09, 161.79, 158.27, 154.55, 136.41, 134.61, 132.04, 129.97, 129.44, 126.99, 116.59, 115.69, 107.50, 105.66. HR-EI-MS: *m*/*z* calcd for $C_{15}H_{11}N_3O_4S$, $[M]^+$330.05646; Found 330.05430;

(Z)-2-((7-hydroxy-2-(naphthalen-2-yl)-4-oxo-4H-chromen-8-yl)methylene)hydrazine-1-carboxamide L10

Yield 56.59%; yellow solid;^1H NMR (500 MHz, DMSO-d_6) δ 11.71 (s, 1H, NH), 10.73 (s, 1H, OH), 8.84 – 8.72 (m, 2H, CH=N+Ar), 8.23 – 8.16 (m, 2H, Ar), 8.10 (d, *J* = 8.7 Hz, 1H, Ar), 8.06 – 8.02 (m, 1H, Ar), 7.95 (d, *J* = 8.8 Hz, 1H, Ar), 7.70 – 7.63 (m, 2H, Ar), 7.17 (s, 1H, CH), 7.08 (d, *J* = 8.8 Hz, 1H, Ar), 6.58 (s, 2H, NH_2).^{13}C NMR (151 MHz, DMSO-d_6) δ 176.70, 162.30, 161.86, 155.01, 134.67, 133.03, 129.61, 129.26, 128.90, 128.62, 128.22, 127.53, 127.24, 127.07, 123.30, 116.81, 115.73, 107.96, 107.63. HR-EI-MS: *m*/*z* calcd for $C_{15}H_{11}N_3O_4S$, $[M]^+$374.11581;Found 374.11353;

(Z)-N'-((7-hydroxy-4-oxo-2-phenyl-4H-chromen-8-yl)methylene)benzohydrazide L11

Yield 66.50%; yellow solid;^1H NMR (500 MHz, DMSO-d_6) δ 13.10 (s, 1H, NH), 12.51 (s, 1H, OH), 9.27 (s, 1H, CH=N), 8.19 (dd, *J* = 6.7, 2.9 Hz, 2H, Ar), 8.03 – 7.97 (m, 3H, Ar), 7.71 – 7.58 (m, 6H, Ar), 7.11 (d, *J* = 8.8 Hz, 1H, CH), 7.04 (s, 1H, Ar). ^{13}C NMR (151 MHz, DMSO-d_6) δ 176.56, 163.46, 163.33, 162.40, 155.37, 143.70, 132.87, 132.83, 132.29, 131.59, 129.69, 129.19, 128.24, 126.89, 116.58, 116.10, 107.51, 107.04.HR-EI-MS: *m*/*z* calcd for $C_{23}H_{16}N_2O_4$, $[M]^+$385.12039; Found 385.11828; (Z)-N'-((2-(4-fluorophenyl)-7-hydroxy-4-oxo-4H-chromen-8-yl)methylene)benzohydrazide L12

Yield 58.77%; yellow solid;^1H NMR (500 MHz, DMSO-d_6) δ 9.02 (s, 1H, OH), 8.27 (s, 2H, CH=N+Ar), 8.01 (d, *J* = 7.5 Hz, 2H, Ar), 7.70 – 7.50 (m, 3H, Ar), 7.41 (t, *J* = 8.6 Hz, 2H, Ar), 6.75 (s, 1H, CH), 6.46 (s, 1H, Ar).^{13}C NMR (151 MHz, DMSO-d_6) δ 176.42, 165.39, 163.73, 163.46, 161.39, 155.25, 143.57, 132.96, 132.75, 129.62, 129.57, 129.14, 128.23, 128.12, 116.75, 116.61, 116.35, 116.09, 107.31, 107.06.HR-EI-MS: *m*/*z* calcd for $C_{23}H_{15}FN_2O_4$, $[M]^+$403.11133; Found 403.10886;

(Z)-N'-((2-(4-chlorophenyl)-7-hydroxy-4-oxo-4H-chromen-8-yl)methylene)benzohydrazide L13

Yield 57.43%; yellow solid;^1H NMR (500 MHz, DMSO-d_6) δ 12.93 (s, 1H, NH), 12.42 (s, 1H, OH), 9.22 (s, 1H, CH=N), 8.28 (d, *J* = 8.5 Hz, 2H, Ar), 7.99 (t, *J* = 8.2 Hz, 3H, Ar), 7.67 (dd, *J* = 8.2, 3.1 Hz, 3H, Ar), 7.61 (t, *J* = 7.5 Hz, 2H, Ar), 7.11 (d, *J* = 8.9 Hz, 1H, CH), 7.08 (s, 1H, Ar) ^{13}C NMR (151 MHz, DMSO-d_6) δ 176.44, 163.43, 163.29, 161.22, 155.17, 143.48, 137.00, 132.95, 132.76, 130.45, 129.63, 129.14, 128.77, 128.23, 128.14, 116.56, 115.99, 107.71, 107.16.HR-EI-MS: *m*/*z* calcd for $C_{23}H_{L29}lN_2O_4$, $[M]^+$419.08194; Found 419.07931;

(Z)-N'-((2-(4-bromophenyl)-7-hydroxy-4-oxo-4H-chromen-8-yl)methylene)benzohydrazide L14

Yield 62.58%; yellow solid;^1H NMR (500 MHz, DMSO-d_6) δ 12.65 (d, *J* = 219.8 Hz, 2H, NH+OH), 9.20 (s, 1H, CH=N), 8.19 (d, *J* = 8.2 Hz, 2H, Ar), 7.98 (dd, *J* = 17.6, 8.2 Hz, 3H, Ar), 7.79 (d, *J* = 8.3 Hz, 2H, Ar), 7.67 (t, *J* = 7.4 Hz, 1H, Ar), 7.60 (t, *J* = 7.5

Hz, 2H, Ar), 7.11 – 7.05 (m, 2H, CH+Ar).^{13}C NMR (151 MHz, DMSO-d_6) δ 176.47, 163.47, 163.35, 161.42, 155.24, 143.58, 132.99, 132.76, 132.59, 130.88, 129.16, 129.01, 128.24, 128.19, 125.95, 116.60, 116.03, 107.77, 107.23.HR-EI-MS: *m*/*z* calcd for $C_{23}H_{L19}rN_2O_4$, $[M]^+$463.03174; Found 463.02880;

(Z)-N'-((7-hydroxy-4-oxo-2-(p-tolyl)-4H-chromen-8-yl)methylene)benzohydrazide L15

Yield 66.13%; yellow solid; ^{1}HNMR (500 MHz, DMSO-d_6) δ 13.06 (s, 1H, NH), 12.48 (s, 1H, OH), 9.23 (s, 1H, CH=N), 8.06 (d, *J* = 8.0 Hz, 2H, Ar), 8.01 – 7.95 (m, 3H, Ar), 7.70 – 7.66 (m, 1H, Ar), 7.61 (dd, *J* = 8.3, 6.7 Hz, 2H, Ar), 7.41 (d, *J* = 8.2 Hz, 2H, Ar), 7.08 (d, *J* = 8.9 Hz, 1H, CH), 6.97 (s, 1H, Ar), 2.42 (s, 3H, CH_3).^{13}C NMR (151 MHz, DMSO-d_6) δ 176.50, 163.45, 163.25, 143.73, 142.53, 132.82, 130.27, 129.20, 128.24, 126.85, 115.99, 106.86, 21.57.HR-EI-MS: *m*/*z* calcd for $C_{24}H_{18}N_2O_4$, $[M]^+$399.13467; Found 399.13393;

(Z)-N'-((7-hydroxy-2-(4-methoxyphenyl)-4-oxo-4H-chromen-8-yl)methylene)benzohydrazide L16

Yield 51.47%; yellow solid;^{1}H NMR (500 MHz, DMSO-d_6) δ 12.73 (d, *J* = 264.6 Hz, 2H, NH+OH), 9.22 (s, 1H, CH=N), 8.12 (d, *J* = 8.4 Hz, 2H, Ar), 8.00 (d, *J* = 7.6 Hz, 2H, Ar), 7.94 (d, *J* = 8.9 Hz, 1H, Ar), 7.67 (t, *J* = 7.4 Hz, 1H, Ar), 7.61 (t, *J* = 7.5 Hz, 2H, Ar), 7.12 (d, *J* = 8.4 Hz, 2H, Ar), 7.06 (d, *J* = 8.8 Hz, 1H, CH), 6.90 (s, 1H, Ar), 3.87 (s, 3H, CH_3).^{13}C NMR (151 MHz, DMSO-d_6) δ 176.35, 163.39, 163.15, 162.52, 162.36, 155.19, 143.66, 132.91, 132.78, 129.16, 128.67, 128.24, 128.14, 123.71, 116.48, 115.82, 115.02, 106.94, 105.92, 55.97.HR-EI-MS: *m*/*z* calcd for $C_{24}H_{18}N_2O_5$, $[M]^+$415.13144; Found 415.12885;

(Z)-N'-((7-hydroxy-2-(4-nitrophenyl)-4-oxo-4H-chromen-8-yl)methylene)benzohydrazide L17

Yield 62.77%; yellow solid;^{1}H NMR (500 MHz, DMSO-d_6) δ 12.74 (s, 1H, NH), 12.34 (s, 1H, OH), 9.20 (s, 1H, CH=N), 8.65 – 8.60 (m, 2H, Ar), 8.40 – 8.38 (m, 2H, Ar), 8.03 – 7.99 (m, 3H, Ar), 7.69 – 7.66 (m, 1H, Ar), 7.61 (dd, *J* = 8.2, 6.7 Hz, 2H, Ar), 7.26 (s, 1H, CH), 7.13 (d, *J* = 8.8 Hz, 1H, Ar).^{13}C NMR (151 MHz, DMSO-d_6) δ 176.46, 163.53, 163.43, 160.12, 155.22, 149.42, 143.38, 137.57, 133.09, 132.70, 129.13, 128.59, 128.24, 124.43, 116.76, 116.09, 109.63, 107.55.HR-EI-MS: *m*/*z* calcd for $C_{23}H_{15}N_3O_6$, $[M]^+$430.10583; Found 430.10336;

(Z)-N'-((2-(furan-2-yl)-7-hydroxy-4-oxo-4H-chromen-8-yl)methylene)benzohydrazide L18

Yield 67.07%; yellow solid;^{1}H NMR (500 MHz, DMSO-d_6) δ 12.89 (s, 1H, NH), 12.37 (s, 1H, OH), 9.13 (s, 1H, CH=N), 8.06 (d, *J* = 1.7 Hz, 1H, CH), 8.00 – 7.96 (m, 2H, Ar), 7.91 (d, *J* = 8.8 Hz, 1H, Ar), 7.67 (t, *J* = 7.2 Hz, 1H, Ar), 7.60 (dd, *J* = 8.3, 6.7 Hz, 2H, Ar), 7.49 (d, *J* = 3.5 Hz, 1H, CH), 7.04 (d, *J* = 8.8 Hz, 1H, CH), 6.87 (dd, *J* = 3.5, 1.7 Hz, 1H, CH), 6.56 (s, 1H, Ar).^{13}C NMR (151 MHz, DMSO-d_6) δ 175.66, 163.36, 163.14, 154.76, 154.22, 147.72, 145.80, 143.45, 132.89, 132.79, 129.18, 128.18, 128.17, 116.61, 115.87 , 114.40, 113.39, 106.70, 104.74.HR-EI-MS: *m*/*z* calcd for $C_{21}H_{14}N_2O_5$, $[M]^+$Na397.08139; Found 397.07949;

(Z)-N'-((7-hydroxy-4-oxo-2-(thiophen-2-yl)-4H-chromen-8-yl)methylene)benzohydrazide L19

Yield 62.07%; yellow solid;^{1}H NMR (500 MHz, DMSO-d_6) δ 13.16 (s, 1H, NH), 12.57 (s, 1H, OH), 9.16 (s, 1H, CH=N), 8.07 (dd, J = 3.7, 1.2 Hz, 1H, Ar), 8.02 (dd, J = 4.9, 1.2 Hz, 1H, CH), 8.00 – 7.98 (m, 2H, Ar), 7.94 (d, J = 8.8 Hz, 1H, CH), 7.70 – 7.66 (m, 1H, CH), 7.61 (dd, J = 8.2, 6.7 Hz, 2H, Ar), 7.34 (dd, J = 5.0, 3.7 Hz, 1H, Ar), 7.07 (d, J = 8.9 Hz, 1H, CH), 6.82 (s, 1H, Ar).^{13}C NMR (151 MHz, DMSO-d_6) δ 175.91, 163.53, 163.28, 158.19, 154.95, 143.32, 134.56, 132.85, 132.82, 132.04, 129.96, 129.51, 129.17, 128.26, 128.17, 116.43, 116.03, 106.66, 105.84. HR-EI-MS: m/z calcd for $C_{21}H_{14}N_2O_4S$, $[M]^+$391.07663; Found 391.107470;

(Z)-N'-((7-hydroxy-2-(naphthalen-2-yl)-4-oxo-4H-chromen-8-yl)methylene)benzohydrazide L20

Yield 56.79%; yellow solid;^{1}H NMR (500 MHz, DMSO-d_6) δ 13.08 (s, 1H, NH), 12.54 (s, 1H, OH), 9.37 (s, 1H, CH=N), 8.82 (s, 1H, Ar), 8.32 – 7.96 (m, 7H, Ar), 7.67 (dt, J = 21.9, 7.3 Hz, 5H, Ar), 7.25 – 7.10 (m, 2H, CH+Ar).^{13}C NMR (151 MHz, DMSO-d_6) δ 176.47, 163.40, 163.32, 162.30, 155.38, 143.75, 134.68, 133.01, 132.90, 132.81, 129.61, 129.31, 129.20, 128.89, 128.60, 128.26, 128.22, 128.20, 127.53, 127.29, 123.23, 116.65, 116.02, 107.87, 107.12.HR-EI-MS: m/z calcd for $C_{27}H_{18}N_2O_4$, $[M]^+$435.13483; Found 435.13393;

(Z)-N'-((7-hydroxy-4-oxo-2-phenyl-4H-chromen-8-yl)methylene)isonicotinohydrazide L21

Yield 59.42%; yellow solid;^{1}H NMR (500 MHz, DMSO-d_6) δ 13.03 – 12.56 (m, 2H, NH+OH), 9.24 (s, 1H, CH=N), 8.86 (s, 2H, pyridine), 8.26 – 8.18 (m, 2H, pyridine), 8.00 (d, J = 8.7 Hz, 1H, Ar), 7.90 (d, J = 4.9 Hz, 2H, Ar), 7.63 (d, J = 5.4 Hz, 3H, Ar), 7.10 (d, J = 8.7 Hz, 1H, CH), 7.04 (s, 1H, Ar). ^{13}C NMR (151 MHz, DMSO-d_6) δ 176.50, 163.37, 162.44, 162.01, 155.41, 151.00, 144.79, 140.07, 132.27, 131.55, 129.67, 128.56, 126.96, 122.06, 116.66, 116.01, 107.49, 106.99.HR-EI-MS: m/z calcd for $C_{22}H_{15}N_3O_4$, $[M]^+$386.11597; Found 386.11353;

(Z)-N'-((2-(4-fluorophenyl)-7-hydroxy-4-oxo-4H-chromen-8-yl)methylene)isonicotinohydrazide L22

Yield 52.15%; yellow solid;^{1}H NMR (500 MHz, DMSO-d_6) δ 9.06 (s, 1H, CH=N), 8.77 (s, 2H, pyridine), 8.22 (s, 2H, pyridine), 7.93 (d, J = 5.0 Hz, 2H, Ar), 7.73 (d, J = 9.2 Hz, 1H, Ar), 7.43 (t, J = 8.7 Hz, 2H, Ar), 6.81 (s, 1H, CH), 6.57 (d, J = 9.1 Hz, 1H, Ar). ^{13}C NMR (151 MHz, DMSO-d_6) δ 176.40, 163.33, 161.97, 161.32, 155.21, 150.94, 144.55, 140.16, 136.98, 130.40, 129.60, 128.93, 128.48, 122.06, 116.69, 115.85, 107.65, 107.23. HR-EI-MS: m/z calcd for $C_{22}H_{14}FN_3O_4$, $[M]^+$404.10638; Found 404.10411;

(Z)-N'-((2-(4-chlorophenyl)-7-hydroxy-4-oxo-4H-chromen-8-yl)methylene)isonicotinohydrazide L23

Yield 56.73%; yellow solid;^{1}H NMR (500 MHz, DMSO-d_6) δ 12.59 (d, J = 52.2 Hz, 2H, NH+OH), 9.14 (s, 1H, CH=N), 8.85 (d, J = 5.0 Hz, 2H, pyridine), 8.29 (d, J = 8.3 Hz, 2H, pyridine), 7.96 (d, J = 8.8 Hz, 1H, Ar), 7.92 – 7.88 (m, 2H, Ar), 7.63 (d, J = 8.3 Hz, 2H, Ar), 7.08 – 7.03 (m, 2H, CH+Ar).^{13}C NMR (151 MHz, DMSO-d_6) δ 176.33, 165.37, 163.71, 150.93, 129.69, 128.39, 128.17, 122.06, 116.72, 116.57, 107.29. HR-EI-MS: m/z calcd for $C_{22}H_{L28}lN_3O_4$, $[M]^+$420.07709; Found 420.07456;

(Z)-N'-((2-(4-bromophenyl)-7-hydroxy-4-oxo-4H-chromen-8-

yl)methylene)isonicotinohydrazide L24

Yield 47.58%; yellow solid;^{1}H NMR (500 MHz, DMSO-d_6) δ 12.66 – 12.49 (m, 2H, NH+OH), 9.13 (d, J = 2.2 Hz, 1H, CH=N), 8.86 (s, 2H, pyridine), 8.27 – 8.18 (m, 2H, pyridine), 7.96 (dd, J = 8.8, 2.1 Hz, 1H, Ar), 7.91 (d, J = 4.3 Hz, 2H, Ar), 7.77 (dd, J = 8.5, 2.1 Hz,2H, , Ar), 7.09 – 7.02 (m, 2H, CH+Ar).^{13}C NMR (126 MHz, DMSO-d_6) δ 176.42, 163.33, 162.00, 161.46, 155.22, 150.95, 144.57, 140.19, 132.55, 130.79, 129.12, 128.50, 125.94, 116.73, 115.85, 107.66, 107.27.HR-EI-MS: *m*/*z* calcd for $C_{22}H_{L18}rN_3O_4$, $[M]^+$464.02502; Found 464.02405;

(Z)-N'-((7-hydroxy-4-oxo-2-(p-tolyl)-4H-chromen-8-yl)methylene)isonicotinohydrazide L25

Yield 63.86%; yellow solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.73 (d, J = 76.9 Hz, 1H, NH+OH), 9.20 (s, 1H, CH=N), 8.92 – 8.82 (m, 2H, pyridine), 8.08 (d, J = 8.1 Hz, 2H, pyridine), 7.97 (d, J = 8.8 Hz,2H, Ar), 7.91 – 7.89 (m, 1H, Ar), 7.40 (d, J = 8.0 Hz, 1H, Ar), 7.08 (d, J = 8.8 Hz, 1H, CH), 6.96 (s, 1H, Ar), 2.41 (s, 3H, CH_3). ^{13}C NMR (151 MHz, DMSO-d_6) δ 176.45, 163.29, 162.59, 161.99, 155.33, 150.99, 144.78, 142.48, 140.09, 130.23, 130.17, 128.72, 128.52, 126.87, 122.07, 116.65, 115.89, 106.96, 106.78, 21.56. HR-EI-MS: *m*/*z* calcd for $C_{23}H_{17}N_3O_4$, $[M]^+$400.13181; Found 400.12918;

(Z)-N'-((7-hydroxy-2-(4-methoxyphenyl)-4-oxo-4H-chromen-8-yl)methylene)isonicotinohydrazide L26

Yield 55.63%; yellow solid;^{1}H NMR (500 MHz, DMSO-d_6) δ 12.70 (d, J = 73.5 Hz, 2H, NH+OH), 9.19 (s, 1H, CH=N), 8.91 – 8.82 (m, 2H, pyridine), 8.14 (d, J = 8.4 Hz, 2H, pyridine), 7.95 (d, J = 8.8 Hz, 1H, Ar), 7.89 (d, J = 5.0 Hz, 2H, Ar), 7.11 (d, J = 8.4 Hz, 2H, Ar), 7.06 (d, J = 8.8 Hz, 1H, CH), 6.91 (s, 1H, Ar), 3.86 (s, 3H, CH_3).^{13}C NMR (151 MHz, DMSO-d_6) δ 176.33, 163.20, 162.53, 162.46, 161.96, 155.26, 150.98, 144.80, 140.08, 128.78, 128.50, 123.67, 122.04, 116.58, 115.75, 115.01, 106.91, 105.90, 55.97. HR-EI-MS: *m*/*z* calcd for $C_{23}H_{17}N_3O_5$, $[M]^+$416.12659; Found 416.12410;

(Z)-N'-((7-hydroxy-2-(4-nitrophenyl)-4-oxo-4H-chromen-8-yl)methylene)isonicotinohydrazide L27

Yield 57.13%; yellow solid;^{1}H NMR (500 MHz, DMSO-d_6) δ 12.49 (s, 2H, NH+OH), 9.14 (s, 1H, CH=N), 8.85 (s, 2H, pyridine), 8.68 (d, J = 8.3 Hz, 2H, pyridine), 8.37 (d, J = 8.4 Hz, 2H, Ar), 8.00 (d, J = 8.8 Hz, 1H, Ar), 7.92 (d, J = 4.9 Hz, 2H, Ar), 7.25 (s, 1H, CH), 7.11 (d, J = 8.8 Hz, 1H, Ar). ^{13}C NMR (151 MHz, DMSO-d_6) δ 149.14, 144.18, 136.13, 129.67, 128.97, 124.28, 107.48 (d, J = 73.0 Hz).HR-EI-MS: *m*/*z* calcd for $C_{22}H_{14}N_4O_6$, $[M]^+$431.10129; Found 431.09861;

(Z)-N'-((2-(furan-2-yl)-7-hydroxy-4-oxo-4H-chromen-8-yl)methylene)isonicotinohydrazide L28

Yield 66.62%; yellow solid;^{1}H NMR (500 MHz, DMSO-d_6) δ 12.72 (s, 1H, NH), 12.61 (s, 1H, OH), 9.21 (s, 1H, CH=N), 8.90 – 8.82 (m, 2H, pyridine), 8.09 (d, J = 1.7 Hz, 1H, pyridine), 7.99 (d, J = 8.8 Hz, 1H, pyridine), 7.93 – 7.89 (m, 2H, CH), 7.63 (d, J = 3.5 Hz, 1H, Ar), 7.11 (d, J = 8.9 Hz, 1H, CH), 6.90 (dd, J = 3.5, 1.8 Hz, 1H, CH), 6.63 (s, 1H, Ar).^{13}C NMR (151 MHz, DMSO-d_6) δ 175.71, 163.22, 162.03, 154.94, 154.41, 151.02, 147.78, 145.82, 144.76, 140.10, 128.62, 116.79, 115.88, 114.67, 113.48,

106.77, 104.83. HR-EI-MS: *m/z* calcd for $C_{20}H_{13}N_3O_5$, $[M]^+$376.09503; Found 376.09280;

(Z)-N'-((7-hydroxy-4-oxo-2-(thiophen-2-yl)-4H-chromen-8-yl)methylene)isonicotinohydrazide L29

Yield 60.24%; yellow solid;^{1}H NMR (500 MHz, DMSO-d_6) δ 13.06 – 12.68 (m, 2H, NH+OH), 9.19 – 9.12 (m, 1H, CH=N), 8.85 (d, *J* = 4.9 Hz, 2H, pyridine), 8.10 – 7.87 (m, 5H, pyridine+CH), 7.34 (d, *J* = 4.4 Hz, 1H, Ar), 7.07 (d, *J* = 11.0 Hz, 1H, CH), 6.82 (t, *J* = 4.4 Hz, 1H, Ar) ^{13}C NMR (151 MHz, DMSO-d_6) δ 175.89, 163.33, 162.13, 158.25, 155.07, 151.00, 144.54, 140.03, 134.52, 132.08, 130.11, 129.54, 128.55, 122.08, 116.53, 116.02, 106.58, 105.88.HR-EI-MS: *m/z* calcd for $C_{20}H_{13}N_3O_4S$, $[M]^+$392.07230; Found 392.06995;

(Z)-N'-((7-hydroxy-2-(naphthalen-2-yl)-4-oxo-4H-chromen-8-yl)methylene)isonicotinohydrazide L30

Yield 56.37%; yellow solid;^{1}H NMR (500 MHz, DMSO-d_6) δ 12.77 (d, *J* = 43.9 Hz, 2H, NH+OH), 9.32 (s, 1H, CH=N), 8.85 (d, *J* = 32.8 Hz, 3H, Ar), 8.28 – 8.19 (m, 2H, Ar), 8.12 (d, *J* = 8.7 Hz, 1H, Ar), 8.02 (t, *J* = 7.7 Hz, 2H, Ar), 7.94 (d, *J* = 4.8 Hz, 2H, Ar), 7.70 – 7.63 (m, 2H, Ar), 7.19 (s, 1H, CH), 7.12 (d, *J* = 8.8 Hz, 1H, Ar). ^{13}C NMR (151 MHz, DMSO-d_6) δ 176.51, 163.38, 162.50, 162.02, 155.50, 151.04, 145.01, 134.71, 133.04, 129.64, 129.35, 128.88, 128.64, 128.24, 127.56, 127.38, 123.36, 122.06, 116.78, 115.99, 107.90, 107.15.HR-EI-MS: *m/z* calcd for $C_{26}H_{17}N_3O_4$, $[M]^+$436.13184; Found 436.12918;

4.3.Urease inhibitory activity

The reaction mixtures, comprising 25 µL of enzyme (jack bean urease, 5 U/mL) solution and 55 µL of buffers containing 100 mM urea, were incubated with 5 µL of the test compounds (0.5 mM concentration) at 30 °C for 15 min in 96-well plates[34].Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn[35]. Briefly, 45 µL of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprussside) and, 70 µL of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50 min, using a microplate reader (M200 PQO NanoQuant). All reactions were performed in triplicate in a final volume of 200 µL. The entire assays were performed at pH 6.8.Thiourea was used as the standard inhibitor for urease.

4.4. Molecular docking study

Docking study was performed based on the crystal structure of urease in order to reveal the binding modes of synthesized Flavonoid analogues. 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)[36]. The three-dimensional (3D) protein structure of Bacillus Pasteurii Urease (PDB ID: 4UBP, resolution 1.55 Å), determined by X-ray crystallography, was retrieved from the RCSB Protein Databank (http://www.rcsb.org/pdb/home/home.do.). All the water molecules, hetero atoms, and co-factors were removed from the protein structure. Then hydrogen bonds, missing atoms, and charges were computed, and the energy optimization was carried out using default force field. The synthesized flavonoid analogues used in these docking studies was prepared and optimized using built and Ligand Preparation module implemented in Discovery Studio 2018 (Dassault Systemes BIOVIA, USA). The active site was defined with a radius of 26 Å around nickel atoms in the urease enzyme. Lastly, the conformations with the lowest predicted binding free energy were selected for the each test compound. Graphic manipulations were visualized using Accelrys discovery studio visualizer software.

Notes. The authors declare no competing financial interest.

Acknowledgement: This work was supported by the National Natural Science Foundation of China (No. 21967003), the National Natural Science Foundation of China (No. 81960649), Guangxi Natural Science Foundation, China (2018GXNSFAA281030).

Appendix A. Supplementary data.

Supplementary data to this article can be found online at.

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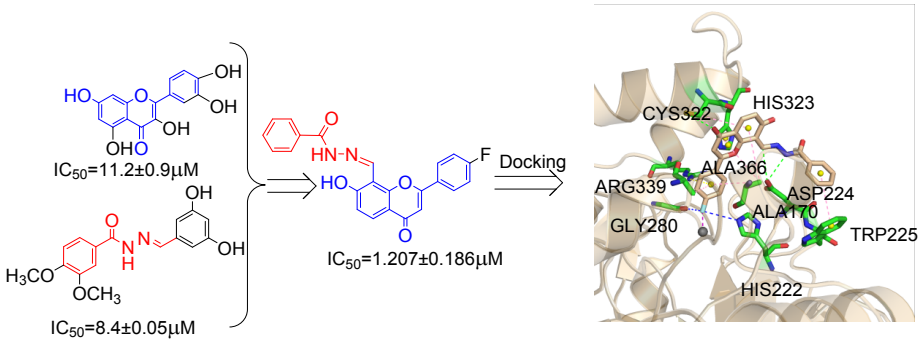
Graphical abstract



Highlights

1. A series of flavonoid analogues were designed and synthesized.
2. The aimed compounds for urease inhibitory activities were superior to thiourea.
3. Compound L2 and L12 show the most potent inhibitory activities
4. The action mode and druggability were explored.