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Synthesis, structure–activity relationship analysis and kinetics study of reductive derivatives of flavonoids as *Helicobacter pylori* urease inhibitors

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ABSTRACT

In a continuing study for discovering urease inhibitors based on flavonoids, nineteen reductive derivatives of flavonoids were synthesized and evaluated against *Helicobacter pylori* urease. Analysis of structure–activity relationship disclosed that 4-deoxy analogues are more potent than other reductive products. Out of them, 4′,7,8-trihydroxyl-2-isoflavene (**13**) was found to be the most active with IC_{50} of 0.85 μM, being over 20-fold more potent than the commercial available urease inhibitor, acetohydroxamic acid (AHA). Kinetics study revealed that **13** is a competitive inhibitor of *H. pylori* urease with a K_i value of 0.641 μM, which is well matched with the results of molecular docking. Biological evaluation and mechanism study of **13** suggest that it is a good candidate for discovering novel anti-gastritis and anti-gastric ulcer agent.

1. Introduction

Urease (E.C 3.5.1.5) is found in various living organisms, including bacteria, fungi, higher plants, and some invertebrates [1,2], which catalyzes the hydrolysis of urea to ammonia and carbon dioxide, and is responsible for many microorganisms to use urea as nitrogen source for growth. High concentration of ammonia arising from these reactions causes an increase in pH, which permits *Helicobacter pylori* to endure acidic pH of the stomach during colonization. Urease therefore plays an important role in the pathogenesis of gastric and peptic ulcer induced by *H. pylori* and is known to be a major virulence factor [3]. Urease is also directly involved in the development of infection stones, pyelonephritis, hepatic coma and other disease states [4–6]. Due to diverse functions of this enzyme, its inhibition by potent and specific compounds could provide an invaluable addition for treatment of infections, and secondary complexes such as gastritis and gastric ulcer caused by *H. pylori* [3,7].

Several classes of compounds show significant inhibitory activity against urease with hydroxamic acids being the best recognized inhibitors [8,9] and with phosphoramidates being the most active [10,11]. However, degradation of phosphoramidates at low pH and teratogenicity of hydroxamic acids in rats prevent them from using *in vivo* [12,13]. It is well known that structural diversity and complexity within natural products are unique and the functional complexity found in natural products will never be invented *de novo* in a chemistry laboratory [14]. Over 75% of new chemical entities submitted (1981–2004) were based on natural product lead structures, indicating that the reliance on natural products is so far the most-successful route for new drug discovering [15,16]. In the past decades, exploration of urease inhibitors from natural products has attracted much attention [3,17,18]. In 2001, Bae et al. found daidzein having weak inhibitory activity against *H. pylori* urease, which opened the door for urease inhibitor discovering from flavonoids, a large group of polyphenolic compounds

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naturally occurred in fruit, vegetables, nuts, seeds, flowers, and bark [19–21]. Subsequently, (+)-gallocatechin [22], quercetin [23], datisdirin [24] and other flavonoids [25] were reported as potent urease inhibitors. On the basis of these researches, our current efforts are therefore focused on seeking novel urease inhibitors based on flavonoids [13,26–29]. We have previously demonstrated that substitution of a methylene group for the carbonyl group of a deoxybenzoin (Scheme 1) resulted in a significant increase in activity [26], which is also observed by comparison of the IC_{50} value of (+)-gallocatechin [22] with that of dihydromyricetin (Scheme 1) [28]. These findings lead to a conception that reduction of flavonoid would produce more potent urease inhibitor. In this paper, we discuss our efforts to improve upon the enzyme inhibition of the 4-ol and 4-deoxy series of flavonoids.

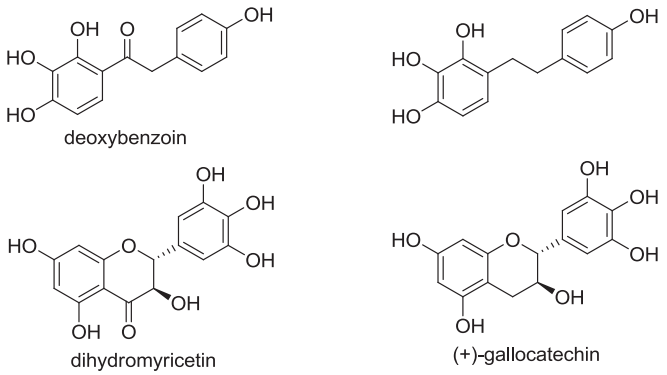
2. Results and discussion

2.1. Chemistry

Nineteen compounds (**1**–**19**) derived from flavonoids were obtained *via* chemical reduction or catalytic hydrogenation (Table 1), and eight of them were synthesized for the first time. All synthetic compounds have been characterized by elemental analyses, MS, and ^{1}H NMR spectra. Out of them, compound **7** was further confirmed by single crystal X-ray Crystallography (Fig. 1).

Reduction of an isoflavone to a ring-opened product was achieved in dry THF by using $LiAlH_4$ as reducing agent [30]. The α-methyldeoxybenzoins **6** and **7** were therefore obtained from formononetin and its methylated product, respectively (Scheme 2). However, treatment of the flavone analogue with $LiAlH_4$ is quite different. The ring-opened products **16**, **17** and **18** were given in a one-pot reaction from 5,7-dimethylated chrysin (Scheme 2), which is a reaction reported for the first time. The presence of chalcone **16** indicates that dihydrochalcone **17** may be the reduction product of **16**. As for compound **18**, we putatively attributed to the demethylation of **16** followed by hydrogenation or/and direct demethylation of **17** with the help of $Al(OAr)_3$, a Lewis acid yielded *in situ* from $LiAlH_4$.

We subsequently turned to hydrogenate flavones and isoflavones in the presence of Pd/C for preparing 4-ol analogues. Tangeretin and methylated formononetin gave the hydrogenated products **3** and **8** respectively, with the reduction of both carbon–carbon double bond and carbonyl group. However, when this system was extended to apigenin and quercetin, flavonoids bearing free hydroxyl groups, only carbon–carbon double bond was reduced [31], forming compounds **1** and **2** respectively (Scheme 3). This clearly indicated that the hydrogenation degree of a flavonoid is strongly dependent on the number of the hydroxyl groups that it



Scheme 1. Molecular structures of some derivatives of flavonoid.

Table 1
In vitro inhibitory activity data of the synthesized compounds against *H. pylori* urease.

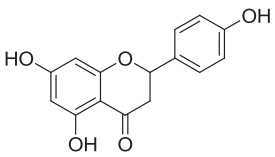
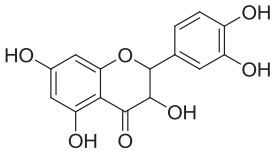
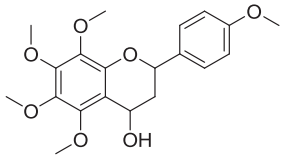
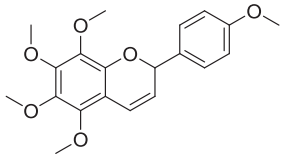
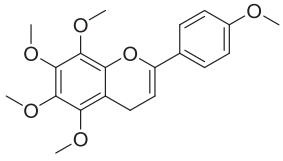
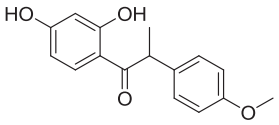
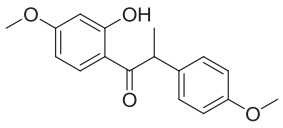
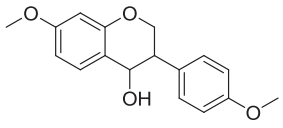
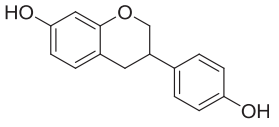
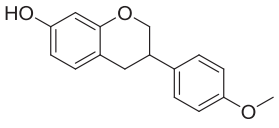
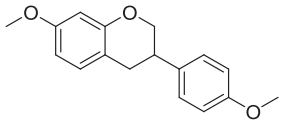
Entry	Structure	Yield	IC_{50}, μM
1		91	328 ± 35
2		64	43.7 ± 3.8
3		44	4853 ± 451
4		37	4172 ± 367
5		62	3791 ± 207
6		46	1050 ± 94
7		62	2178 ± 112
8		40	3867 ± 237
9		63	92.9 ± 8.7
10		79	358 ± 31
11		37	1783 ± 119

Table 1 (*continued*)

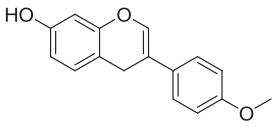
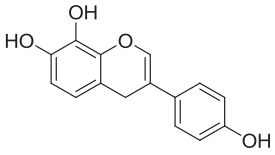
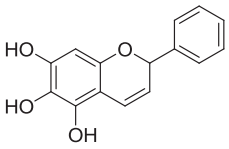
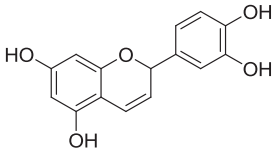
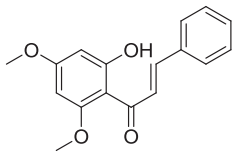
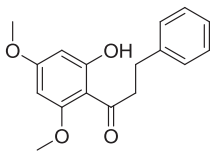
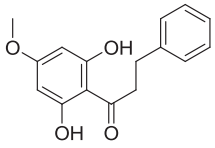
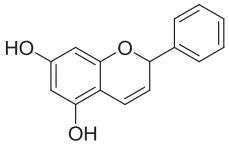
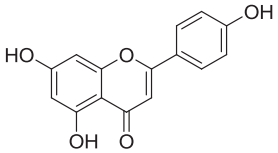
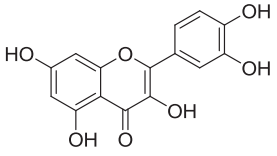
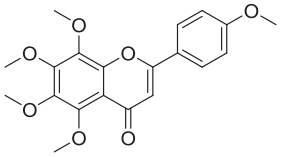
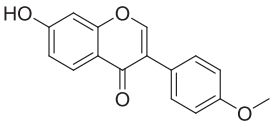
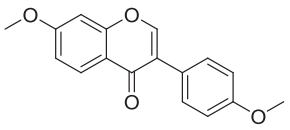
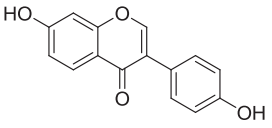
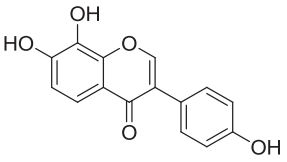
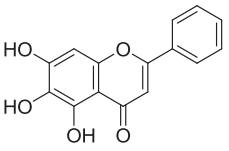
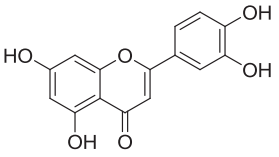
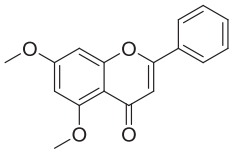
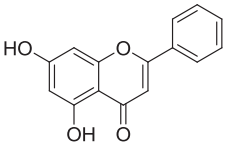
Entry	Structure	Yield	IC_{50}, μM
12		55	86.2 ± 5.9
13		42	0.85 ± 0.06
14		49	22.1 ± 2.0
15		51	4.42 ± 0.31
16		18	1220 ± 105
17		43	1668 ± 132
18		26	978 ± 87
19		91	33.2 ± 1.8
20		–	138 ± 12 [28]
21		–	11 ± 0.9 [28]

Table 1 (*continued*)

Entry	Structure	Yield	IC_{50}, μM
22		–	4628 ± 215 [28]
23		–	2003 ± 115 [28]
24		–	3527 ± 237
25		–	556 ± 31 [28]
26		–	140 ± 3 [26]
27		–	291 ± 29 [28]
28		–	35.4 ± 2.0 [28]
29		–	2185 ± 163
30		–	302 ± 37 [28]
31	AHA	–	18.2 ± 1.6

bears, which may be caused by the adsorption of a flavonoid on the surface of Pd/C being decreased with the increase of the number of hydroxyl groups. It was reported that a flavanone is stable to hydrogenation under neutral conditions but can be further reduced under alkaline conditions and pressure [32]. Triggered by this finding, $Pd(OH)_2/C$ was selected as catalyst to generate

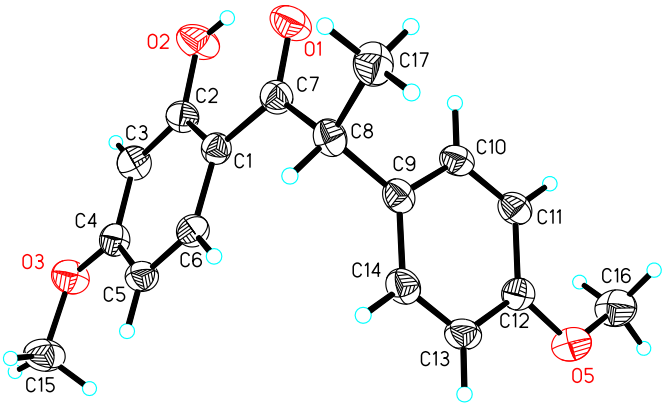


Fig. 1. Molecular structure of **7**.

4-methylene derivatives. As expected, isoflavones daidzein, formononetin, and methylated formononetin were successfully converted to the isoflavanes **9**, **10**, and **11**, respectively (Scheme 4). Contrary to isoflavones, tangeretin, baicalein, luteolin and chrysin failed to furnish the corresponding isoflavanes, but generated 3-flavenes **4**, **14**, **15** and **19** (Scheme 5) irrespective of the number of the bearing hydroxyl groups. To our knowledge, this is the first method for building the 3-flavene structure from flavone, adding the last member into the method spectra for preparing all possible patterns of C-ring.

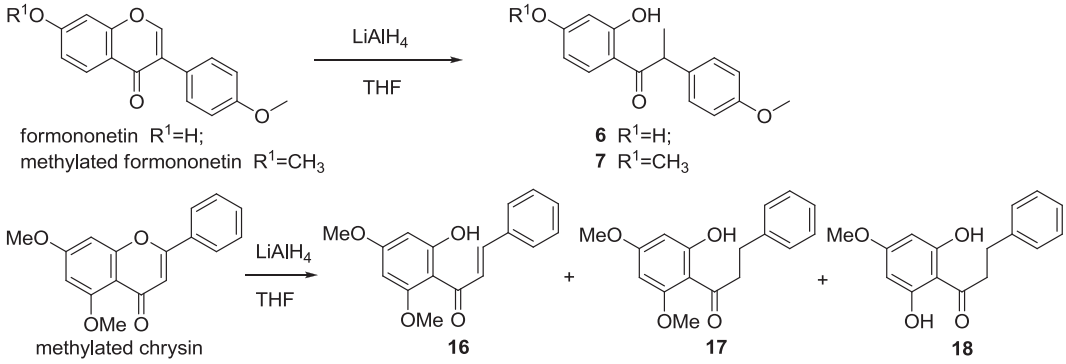
In order to obtaine 2-flavenes and 2-isoflavenes, tangeretin was selected to treat with $LiAlH_4$–$AlCl_3$, and compound **5** was formed in a yield of 52% (Scheme 6). Obviously, this reductive system is not suitable for hydroxylated species. Thus, we developed an expedient route to yield **12** and **13** from formononetin and 7,8,4′-isoflavone (Scheme 7) based on the method for preparing 1,2-diarylethanes [13,33].

2.2. Structure description

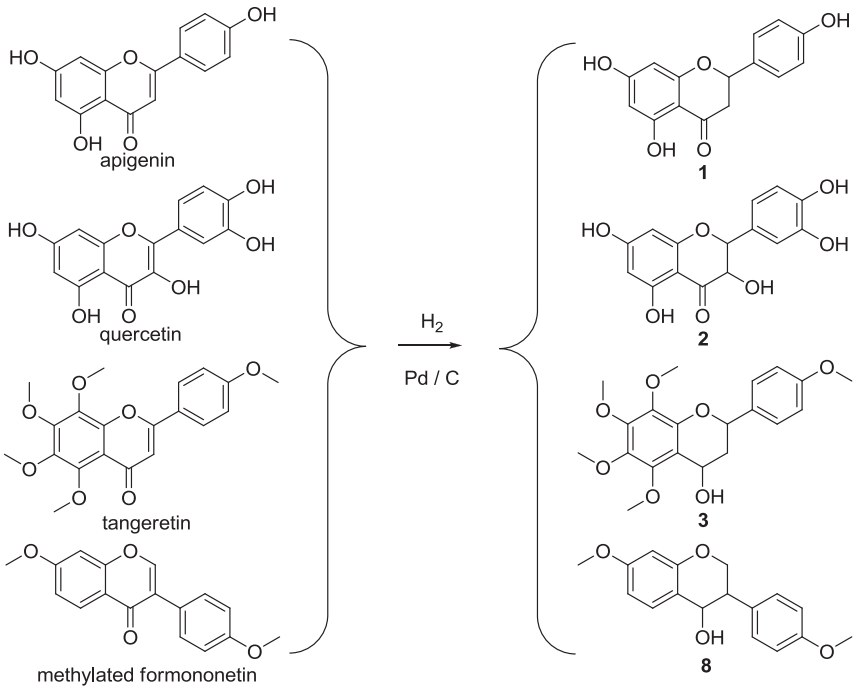
Compounds **4**, **14**, **15** and **19** were reported for the first time and their structures were identified as 3-flavene through ^{1}H NMR spectra. The double doublet signals at about δ 5.16 to 5.59 were assigned to H2 with J about 12.6 and 3.0 Hz, indicating the vicinal coupling of H2–H3 and the long-range coupling of H2–H4. On the other hand, H3 is adjacent to H2 and H4. The double doublet signals at about δ 3.0 with J about 17.0 and 12.7 Hz were therefore attributed to H3. Furthermore, over 14 Hz of coupling constant clearly suggests the *cis*-coupled protons of H3 and H4. The assignments made above were further confirmed from the double doublet signals at about δ 2.7 (H4) with J about 17.0 and 3.0 Hz. The single crystal structure of **7** clearly indicated it is the reductive ring opening product of methylated formononetin. In the ^{1}H NMR spectrum of **7**, the doublet signals at δ 1.49 (J = 6.7 Hz) were assigned to methyl protons and that at δ 4.59 (J = 6.9 Hz) to CH. Based on this assignment, compound **6** was surely determined as the reductive ring opening product of formononetin.

2.3. Urease inhibitory activity

It is well known, flavonoids are typically composed of two phenolic rings and a pyrone ring, which are referred to as A, B and C rings (Scheme 8). In this work, we attempted to modify ring C for possible improvements in urease inhibitory activity. All the synthetic compounds were evaluated for their inhibitory activities against cell-free urease from *H. pylori* (Table 1). Reduction of $\Delta^{2,3}$ carbon–carbon double bond yielded compounds with a 2- to 4-fold loss of activity (**1** *vs.* **20**, **2** *vs.* **21**). On the contrary, replacement of the 4-carbonyl group in tangeretin **22** with a methylene group (**5**) produced some improvement in potency for inhibition of *H. pylori* urease. Whereas it was quite surprise that 3-flavenes (**14**, **15** and **19**), another kind of 4-deoxy analogues, show a significant increase in activity when compared to their parent compounds (baicalein **27**, luteolin **28** and chrysin **30**). These results clearly indicated that maintenance of the sp^2 hybridized C3 is very important to improve the urease inhibitory activity. In the series of 3-flavenes, compound **4** must be mentioned. It showed a much less increase in activity than others, proving to be approximately equipotent to the parent compound (**22**), which may be attributed to its extremely high hydrophobicity. To further understand the importance of the sp^2 hybridized C3 in isoflavone series, some isoflavanes and isoflavenes were next synthesized from the reduction of the corresponding isoflavones. Converting isoflavones (**23**, **24** and **25**) to the corresponding isoflavanes (**10**, **12** and **9**) resulted in a 2- to 6-fold increase in potency. However it is quite different to 2-isoflavenes, **11** and **13** get a significant improvement of activity. It is worthy to note that 2-isoflavene **13** with IC_{50} of 0.85 ± 0.06 μM is over 160-fold more potent than its parent compound **26**, being the most active of all tested compounds. This contrasted sharply with that of 2-flavene **5**, which may be also caused by the difference of hydrophobicity. Consistent with our observation in naturally occurred flavonoids, an appropriately hydroxylated flavonoid skeleton is a key structural characteristic for an active compound [28], which gives a reasonable explanation for **13** having much more potent than **11**. The above mentioned results also disclosed that replacement of 4-carbonyl group with CH_2 or CH group is beneficial to both flavone and isoflavone for enhancement of urease inhibitory



Scheme 2. Formation of open-ring products from flavonoids.



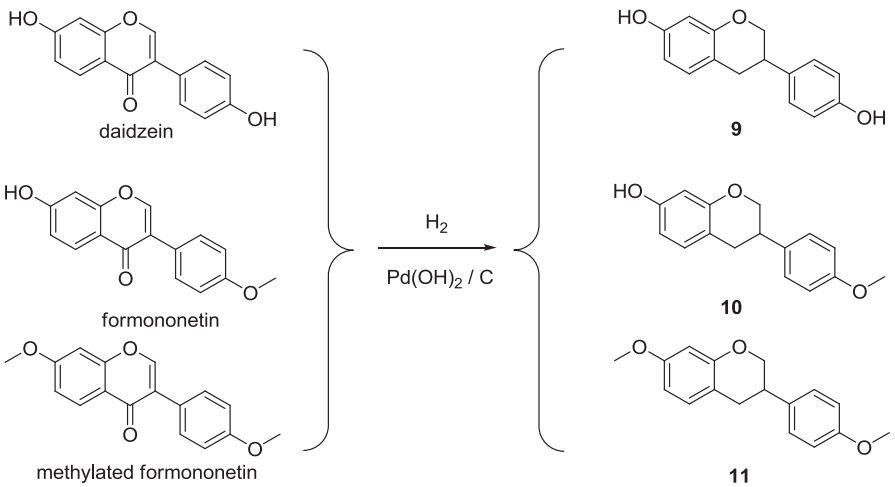
Scheme 3. Hydrogenation of some flavonoids by using Pd/C as catalyst.

activity. This hypothesis was indirectly confirmed by the activities of 4-flavonol **3** and 4-isoflavonol **8** with no improvement as compared to their parent compounds.

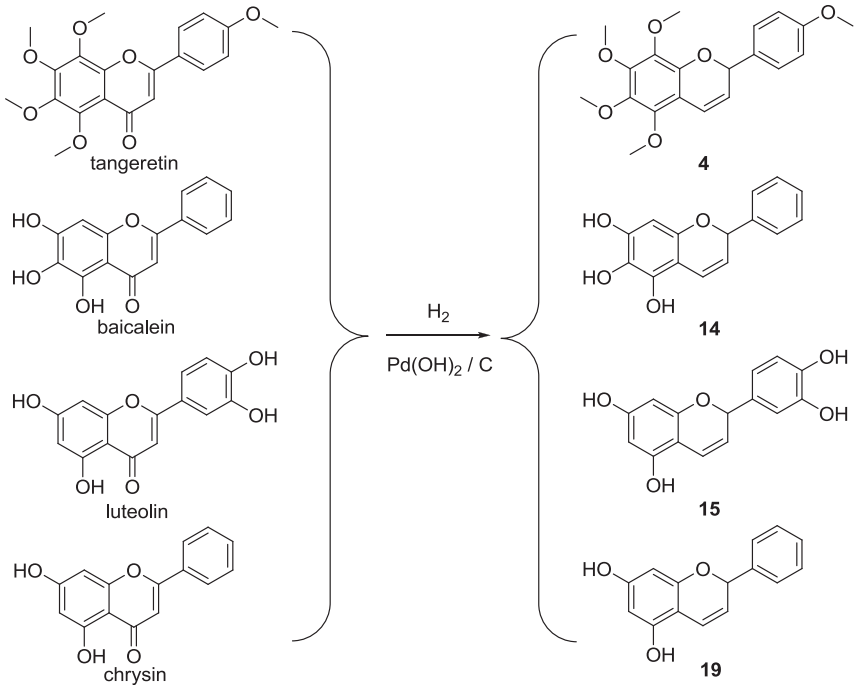
With the respect to the reductive ring opening products of flavonoids, α-methyldeoxybenzoins (**6** and **7**), chalcone (**16**) and dihydrochalcone (**17**) displayed only a slight increase in potency.

2.4. Kinetics of urease inhibition by 2-isoflavene *13*

In the past ten years, several natural flavonoids were documented as potent urease inhibitors, including flavones [24], isoflavones [34], C-glycosylflavonoids [35] and flavonoid glycosides [23]. Although quercetin, a typical flavone, has recently been determined as a reversible inhibitor of *H. pylori* urease with a non-competitive inhibition mechanism by our group [28], the inhibition mechanism of other flavonoids such as isoflavone and C-glycosylflavonoid, especially the synthetic analogues of flavonoids such as catechol derivatives [27], has not been done so far. To gain information on the inhibition mechanism of 4-deoxyflavonoids, the most potent inhibitor **13** was therefore selected for a kinetics study. The type of inhibition was elucidated from the analysis of Lineweaver–Burk plots, in which K_m was decreased in the presence of **13** leaving unaffected V_{max} (Fig. 2). This pattern indicated a pure competitive inhibition, which means that compound **13** competes for the same binding site as the substrate urea. Unexpectively, this is in contrast with the non-competitive inhibition mechanism of quercetin, revealing that removal of 4-oxygen might cause the change of mechanism. The K_i value was calculated directly from Dixon plots (Fig. 3), and was confirmed by a plotting of the slopes of the Lineweaver–Burk plot *vs.* the concentration of **13** (Fig. 4) [36]. The obtained K_i value was 0.146 μg/mL (0.641 μM), demonstrating the strong affinity of **13** towards *H. pylori* urease.



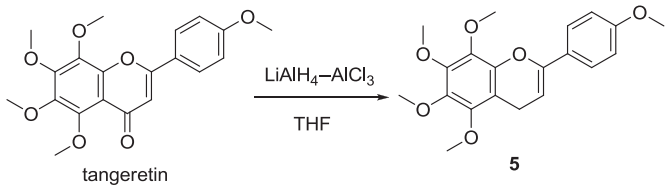
Scheme 4. Hydrogenation of isoflavones by using $Pd(OH)_2/C$ as catalyst.



Scheme 5. Hydrogenation of flavones by using $Pd(OH)_2/C$ as catalyst.

2.5. Molecular docking

In an effort to elucidate the competitive inhibition mechanism revealed by the kinetics study, molecular docking of the most active compound (**13**) into the crystal structure of *H. pylori* urease (entry 1E9Y in the Protein Data Bank) was performed by the AutoDock program, and the best possible binding modes are shown in Fig. 5. As expected by the Lineweaver–Burk plots, 2-isoflavene (**13**) is nicely bound to the region of the urea binding pocket. In detail, the compound is oriented with its benzopyran moiety in proximity to urea binding cavity, letting ring B locate at the mouth of the cavity. The channel to the active site for urea is therefore blocked off (Fig. 6). The 7-hydroxyl group of **13** strongly interacts with the enzyme, providing the main binding affinity for ligand–urease complex. The O atom as acceptor receives two strong hydrogen bonding interactions from the NH_2 groups of Arg338 at distances of 1.915 Å and 1.947 Å, as well as a relative weak interaction from NH group of His322 at a distance of 3.494 Å. This hydroxyl group, on the other hand, is used as a donor, forming O–H···N and O–H···O hydrogen bonding interactions with His248 and Glu222 respectively. In addition, 8-hydroxyl group hydrogen bonds to the O atom of the carboxyl group of Asp223 (distance = 2.220 Å) and to the NH in imidazole ring of His322 (distance = 1.983 Å), which further anchors **13** at the active site. It is worth of note that ring B is also of primary importance for the interactions network of compound **13**. Two strong hydrogen bonds are observed between 4′-hydroxyl group and the NH_2 of Asn168 (distance = 2.121 Å) as well as the O atom of the carboxyl group of Asp165 (distance = 2.184 Å). The binding pose of **13** is therefore stabilized through the attachment of ring B to the protein backbone. The above mentioned hydrogen bond network strongly indicated that a high affinity of **13** to the target enzyme is acquired, and rationalizes its potent activity observed in biological assays.



Scheme 6. Reduction of tangeretin by $LiAlH_4$–$AlCl_3$ system.

3. Conclusion

In summary, we established a structure–activity relationship between nineteen reductive derivatives of flavonoids and their inhibitory activity against *H. pylori* urease. Briefly, 4-deoxy analogues are more potent than other reductive products, with 3-flavenes and 2-isoflavenes being the most active in their own series. Among the assayed compounds, 2-isoflavene **13** with IC_{50} of 0.85 ± 0.06 μM is the most active, showing over 20-fold more potent than the positive control, acetohydroxamic acid (AHA). Kinetics studies revealed that the most active compound **13** is a reversible inhibitor with a pure competitive inhibition mechanism, which is rationalized by the molecular docking study. The high potency and unequivocal inhibition mechanism of **13** against *H. pylori* urease suggests that this compound deserves to be further researched as a good candidate to treat gastritis and gastric ulcer.

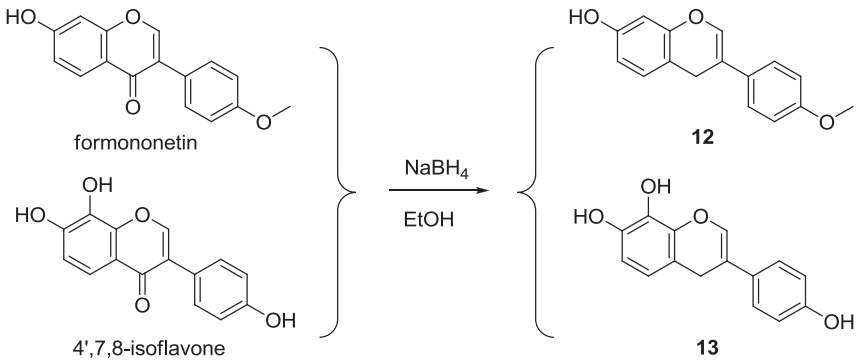
4. Experimental section

4.1. Materials

Protease inhibitors (Complete mini EDTA-free) were purchased from Roche Diagnostics GmbH (Mannheim, Germany) and Brucella broth was from Becton–Dickinson (Cockeysville, MD). Horse serum was from Hyclone (Utah, American).

4.2. Bacteria

H. pylori (ATCC 43504; American Type Culture Collection, Manassas, VA) was grown in Brucella broth supplemented with 10%



Scheme 7. Reduction of formononetin and 4′,7,8-isoflavone by $NaBH_4$.

heat-inactivated horse serum for 24 h at 37 °C under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2), as our previously described [26–28].

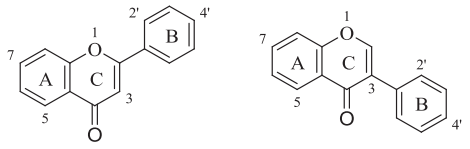
4.3. Preparation of H. pylori urease

For urease inhibition assays, 50 mL broth cultures (2.0×10^8 CFU/mL) were centrifuged (5000 g, 4 °C) to collect the bacteria, and after washing twice with phosphate-buffered saline (pH 7.4), the *H. pylori* precipitation was stored at −80 °C. *H. pylori* was returned to room temperature, and after addition of 3 mL of distilled water and protease inhibitors, sonication was performed for 60 s. Following centrifugation (15,000 g, 4 °C), the supernatant was desalted through Sephadex G-25 column (PD-10 columns, Amersham Pharmacia Biotech, Uppsala, Sweden). The resultant crude urease solution was added to an equal volume of glycerol and stored at 4 °C until use in the experiment.

4.4. Urease activity determination

One unit of urease activity is defined as the amount of enzyme needed to liberate 1.0 μmol of NH_3 from urea per min at pH 7.0 at 25 °C. According to the enzymatic assays of Sigma–Aldrich [37], the activity of the obtained *H. pylori* urease was determined. Briefly, 1 mL of 0.5 M urea with 0.05% (w/v) bovine serum albumin solution was pipetted into a test tube and warmed to 25 °C. Then, 0.20 mL of urease solution containing 0.02 M sodium phosphate buffer (pH 7.0 at 25 °C) was added. The obtained mixture was incubated at 25 °C for exactly 5 min with magnetic stirring, and followed by addition of 0.20 mL of indicator (4.0 mg/mL of *p*-nitrophenol solution), which was titrated immediately with 0.1 M standardized HCl until the colour turns from yellow to colourless. As for blank, only difference is that the urease solution was added after incubation. The urease activity was calculated based on the following formula:

$$\text{U/mL urease} = \frac{0.1\ \text{mmol/mL} \times \Delta V_{\text{HCl}}\text{mL} \times 1000\ \mu\text{mol/mmol}}{5\ \text{min} \times 0.2\ \text{mL}}$$



Scheme 8. The three-ring structure of flavonoid.

4.5. Measurement of urease inhibitory activity

The assay mixture, containing 25 μL (10 U) of *H. pylori* urease and 25 μL of the test compound, was pre-incubated for 1.5 h at room temperature in a 96-well assay plate. Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn [38].

4.6. Kinetics study

Lineweaver–Burk plots of 1/absorbance *vs.* 1/urea were used to determine the type of enzyme inhibition. Urease inhibition was measured by varying the concentration of urea in the presence of different concentrations of **13**. Inhibitory constants (K_i) were determined as the intersection on the *X*-axis of the plots of the slopes *vs.* different concentrations of inhibitor, in which the slopes obtained from the Lineweaver–Burk lines, or directly measured from Dixon plots as the intersection of four lines. All experiments were conducted in triplicate.

4.7. Protocol of docking study

The automated docking studies were carried out using AutoDock version 4.2. First, AutoGrid component of the program

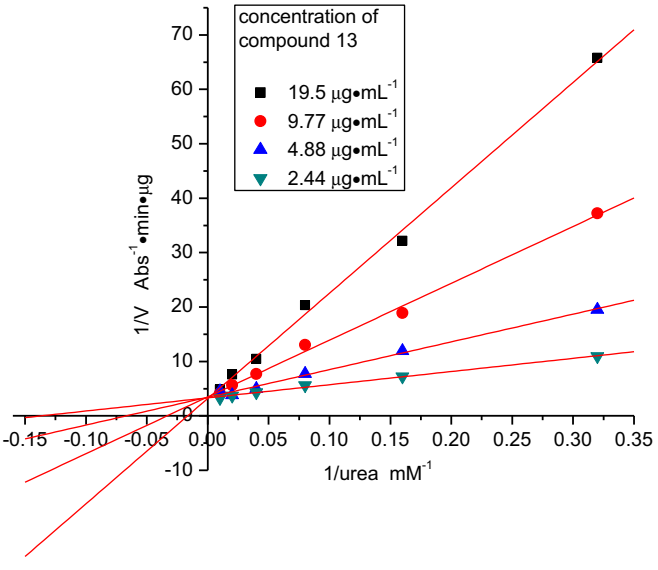


Fig. 2. Double-reciprocal Lineweaver–Burk plot of the inhibition of the *H. pylori* urease activity by compound **13**.

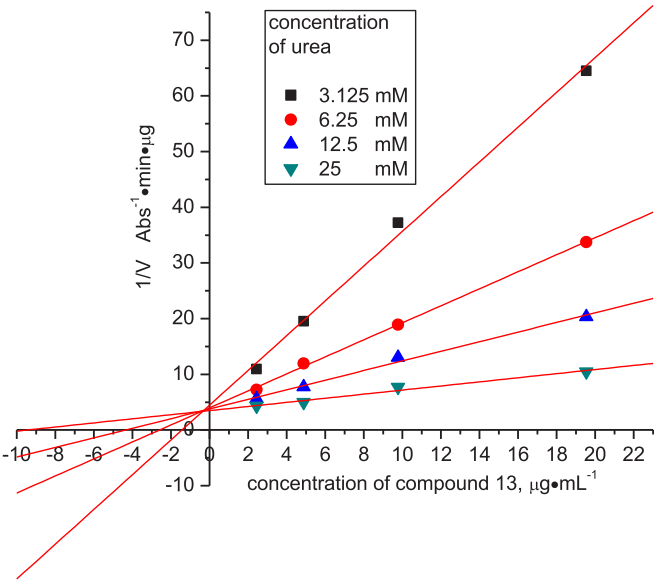


Fig. 3. The Dixon plot of the reciprocal of the initial velocities *vs.* various concentrations of compound **13** at fixed substrate concentrations.

pre-calculates a three-dimensional grid of interaction energies based on the macromolecular target using the AMBER force field. The cubic grid box of 62 Å size (x, y, z) with a spacing of 0.375 Å and grid maps were created representing the catalytic active target site region where the native ligand was embedded. Then automated docking studies were carried out to evaluate the binding free energy of the inhibitor within the macromolecules. The GALS search algorithm (genetic algorithm with local search) was chosen to search for the best conformers. The parameters were set using the software ADT (AutoDockTools package, version 1.5.4) on PC which is associated with AutoDock 4.2. Default settings were used with an initial population of 50 randomly placed individuals, a maximum number of 2.5×10^6 energy evaluations, and a maximum number of 2.7×10^4 generations. A mutation rate of 0.02 and a crossover rate of 0.8 were chosen. Results differing by less than 0.5 Å in positional

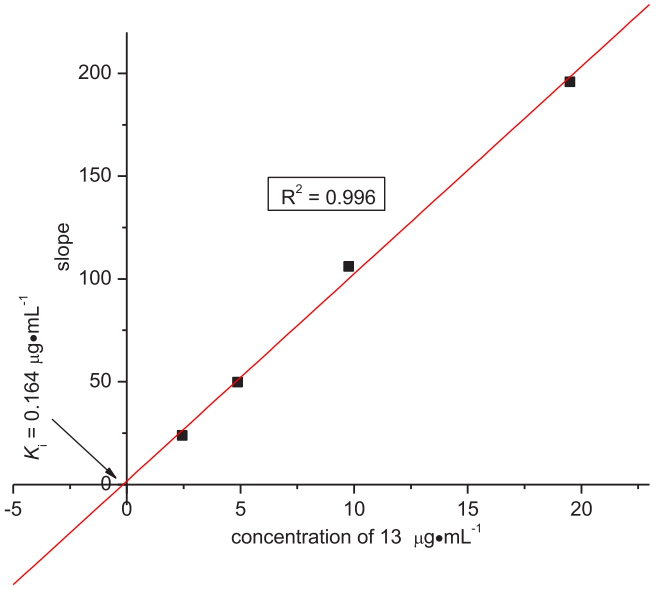


Fig. 4. The secondary replot of the Lineweaver–Burk plot, slopes of the Lineweaver–Burk plot *vs.* various concentrations of compound **13**.

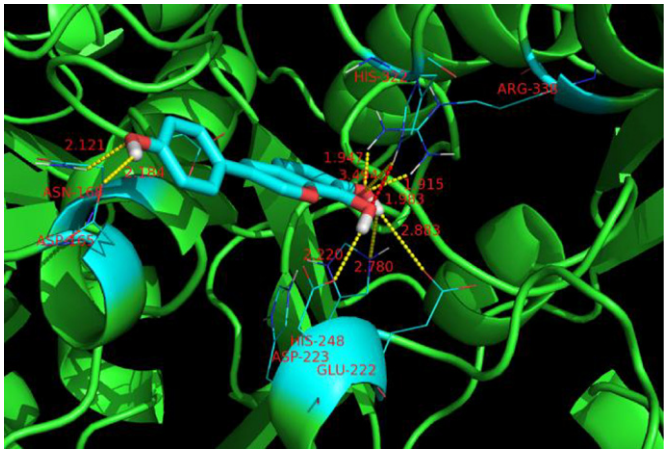


Fig. 5. Binding mode of compound **13** with *H. pylori* urease. For clarity, only interacting residues were labelled. Hydrogen bonding interactions are shown in dash. The figure was made using PyMol.

root-mean-square deviation (RMSD) were clustered together and the results of the most favourable free energy of binding were selected as the resultant complex structures.

4.8. Crystallographic studies

X-ray single-crystal diffraction data for compound **7** was collected on a Bruker SMART APEX CCD diffractometer at 296(2) K using MoKα radiation (λ = 0.71073 Å) by the ω scan mode. The program SAINT was used for integration of the diffraction profiles. All the structures were solved by direct methods using the SHELXS program of the SHELXTL package and refined by full-matrix least-squares methods with SHELXL [39]. All non-hydrogen atoms of compound **7** were refined with anisotropic thermal parameters. All hydrogen atoms were generated theoretically onto the parent atoms and refined isotropically with fixed thermal factors.

4.9. Chemistry

All chemicals (reagent grade) used were purchased from Aldrich (U.S.A) and Sinopharm Chemical Reagent Co., Ltd (China). Melting points (uncorrected) were determined on an XT4 MP apparatus (Taike Corp., Beijing, China). EI mass spectra were obtained on a Waters GCT mass spectrometer, and ^{1}H NMR spectra were recorded

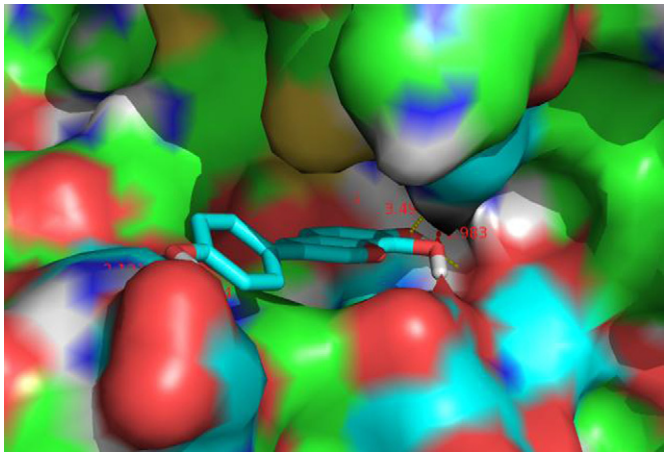


Fig. 6. Binding mode of compound **13** with *H. pylori* urease. The enzyme is shown as surface; while **13** docked structures are shown as sticks.

on a Bruker AV-300 spectrometer at 25 °C with TMS and solvent signals allotted as internal standards. Chemical shifts were reported in ppm (δ). Elemental analyses were performed on a CHN-O-Rapid instrument and were within ±0.4% of the theoretical values.

*4.9.1. General procedure for the preparation of compounds **1**–**3** and **8***

To a solution of flavonoid (4 mmol) in dry tetrahydrofuran (15 mL) or *N*,*N*-dimethylformamide (15 mL) was added 20–400 mg of 10% Pd/C in a hydrogen atmosphere at room temperature. The resulted mixture was stirred for 3–6 h, and the catalyst was removed by filtration. Concentration under reduced pressure furnished a residue, which was purified over a silica gel column eluting with EtOAc–petroleum ether.

*4.9.1.1. 4′,5,7-Trihydroxydihydroflavanone (**1**).* White powder, yield 91%, mp 222–224 °C, ^{1}H NMR (DMSO-d_6): 4.01 (t, J = 6.6 Hz, 1H); 4.53 (d, J = 6.6 Hz, 2H); 5.89 (d, J = 1.5 Hz, 2H); 6.72 (d, J = 8.6 Hz, 2H); 7.07 (d, J = 8.4 Hz, 2H); 9.41 (s, 1H); 10.84 (s, 1H); 12.19 (s, 1H); EIMS m/z 272 (M^+). Anal. Calcd for $C_{15}H_{12}O_5$: C, 66.17; H, 4.44; Found: C, 66.22; H, 4.43.

*4.9.1.2. 3,3′,4′,5,7-Pentahydroxyflavanone (**2**).* White powder, yield 64%, mp over 300 °C, ^{1}H NMR ($CDCl_3$): 4.26 (d, J = 11.6 Hz, 1H); 4.71 (d, J = 11.0 Hz, 1H); 5.86 (d, J = 1.8 Hz, 1H); 6.02 (d, J = 2.0 Hz, 1H); 6.56 (d, J = 8.6 Hz, 1H); 7.25 (dd, J = 8.4 Hz, J = 2.0 Hz, 1H); 7.39 (d, J = 2.0 Hz, 1H); 8.15 (s, 1H); 9.79 (s, 1H); 11.84 (s, 1H); EIMS m/z 304 (M^+). Anal. Calcd for $C_{15}H_{12}O_7$: C, 59.21; H, 3.98; Found: C, 59.35; H, 3.97.

*4.9.1.3. 4′,5,6,7,8-Pentamethoxy-4-flavanol (**3**).* White powder, yield 44%, mp 132–133 °C, ^{1}H NMR ($CDCl_3$): 1.94–2.04 (m, 1H); 2.74–2.83 (m, 2H); 3.77 (s, 3H); 3.84 (s, 6H); 3.86 (s, 3H); 3.93 (s, 3H); 4.96 (d, J = 8.0 Hz, 1H); 6.91 (d, J = 8.6 Hz, 2H); 7.34 (d, J = 8.6 Hz, 2H); EIMS m/z 376 (M^+). Anal. Calcd for $C_{20}H_{24}O_7$: C, 63.82; H, 6.43; Found: C, 63.71; H, 6.44.

*4.9.1.4. 4′,7-Dimethoxy-4-flavanol (**8**).* White powder, yield 40%, mp 125–127 °C, ^{1}H NMR ($CDCl_3$): 2.34–2.38 (m, 1H); 3.09 (d, J = 8.3 Hz, 2H); 3.79 (d, J = 8.0 Hz, 1H); 5.92 (d, J = 1.8 Hz, 1H); 6.65 (d, J = 8.4 Hz, 1H); 6.88 (d, J = 8.6 Hz, 2H); 7.16 (dd, J = 8.4 Hz, J = 2.0 Hz, 1H); 7.30 (d, J = 8.6 Hz, 2H); EIMS m/z 286 (M^+). Anal. Calcd for $C_{17}H_{18}O_4$: C, 71.31; H, 6.34; Found: C, 71.39; H, 6.34.

*4.9.2. General procedure for the preparation of compounds **4**, **14**, **15**, **19**, **9**, **10** and **11***

To a solution of flavonoid (4 mmol) in dry tetrahydrofuran (15 mL) or *N*,*N*-dimethylformamide (15 mL) was added 100 mg of 20% $Pd(OH)_2$/C in a hydrogen atmosphere at room temperature. The resulted mixture was stirred for 4–8 h, and the catalyst was removed by filtration. Concentration under reduced pressure furnished a residue, which was purified over a silica gel column eluting with EtOAc–petroleum ether.

*4.9.2.1. 4′,5,6,7,8-Pentamethoxy-3-flavene (**4**).* White powder, yield 37%, mp 109–110 °C, ^{1}H NMR ($CDCl_3$): 2.83 (dd, J = 16.7 Hz, J = 2.9 Hz, 1H); 3.03 (dd, J = 16.7 Hz, J = 12.8 Hz, 1H); 3.83 (s, 6H); 3.85 (s, 3H); 3.90 (s, 3H); 4.05 (s, 3H); 5.38 (dd, J = 12.8 Hz, J = 2.7 Hz, 1H); 6.94 (d, J = 8.6 Hz, 2H); 7.39 (d, J = 8.4 Hz, 2H); EIMS m/z 358 (M^+). Anal. Calcd for $C_{20}H_{22}O_6$: C, 67.03; H, 6.19; Found: C, 67.16; H, 6.18.

*4.9.2.2. 4′,5,6,7,8-Pentamethoxy-3-flavene (**14**).* Light yellow powder, yield 49%, mp 149–151 °C, ^{1}H NMR ($CDCl_3$): 2.55 (dd, J = 17.0 Hz, J = 3.1 Hz, 1H); 2.83 (dd, J = 17.2 Hz, J = 12.8 Hz, 1H); 5.15 (dd, J = 12.8 Hz, J = 2.9 Hz, 1H); 5.87 (s, 2H); 7.14–7.25 (m, 5H); EIMS m/z 256 (M^+). Anal. Calcd for $C_{15}H_{12}O_4$: C, 70.31; H, 4.72; Found: C, 70.25; H, 4.73.

*4.9.2.3. 3′,4′,5,7-Tetrahydroxy-3-flavene (**15**).* Light yellow powder, yield 51%, mp 224–226 °C, ^{1}H NMR ($CDCl_3$): 2.62 (dd, J = 17.0 Hz, J = 3.0 Hz, 1H); 2.76 (dd, J = 17.0 Hz, J = 12.6 Hz, 1H); 5.19 (dd, J = 12.8 Hz, J = 2.8 Hz, 1H); 5.82 (d, J = 1.8 Hz, 1H); 5.99 (d, J = 2.0 Hz, 1H); 6.04 (s, 1H); 6.53 (d, J = 8.4 Hz, 1H); 6.88 (dd, J = 8.6 Hz, J = 2.2 Hz, 1H); 6.96 (d, J = 1.8 Hz, 1H); 7.58 (s, 1H); EIMS m/z 272 (M^+). Anal. Calcd for $C_{15}H_{12}O_5$: C, 66.17; H, 4.44; Found: C, 66.08; H, 4.45.

*4.9.2.4. 5,7-Dihydroxy-3-flavene (**19**).* White powder, yield 91%, mp 199–200 °C, ^{1}H NMR (DMSO-d_6): 2.78 (dd, J = 17.2 Hz, J = 3.2 Hz, 1H); 3.26 (dd, J = 17.0 Hz, J = 12.6 Hz, 1H); 5.59 (dd, J = 12.6 Hz, J = 2.7 Hz, 1H); 5.90 (d, J = 1.8 Hz, 1H); 5.93 (d, J = 2.0 Hz, 1H); 7.36–7.47 (m, 3H); 7.52 (d, J = 6.6 Hz, 2H); 10.83 (s, 1H); 12.13 (s, 1H); EIMS m/z 240 (M^+). Anal. Calcd for $C_{15}H_{12}O_3$: C, 74.99; H, 5.03; Found: C, 74.92; H, 5.03.

*4.9.2.5. 4′,7-Dihydroxyflavane (**9**).* White powder, yield 63%, mp 157–159 °C, ^{1}H NMR ($CDCl_3$): 2.62 (d, J = 9.3 Hz, 2H); 2.77–2.89 (m, 1H); 3.65 (t, J = 10.4 Hz, 1H); 5.90 (dd, J = 10.4 Hz, J = 2.9 Hz, 1H); 6.07 (d, J = 2.4 Hz, 1H); 6.12 (dd, J = 8.2 Hz, J = 2.4 Hz, 1H); 6.55 (d, J = 8.4 Hz, 2H); 6.61 (d, J = 8.0 Hz, 1H); 6.79 (d, J = 8.4 Hz, 2H); EIMS m/z 242 (M^+). Anal. Calcd for $C_{15}H_{14}O_3$: C, 74.36; H, 5.82; Found: C, 74.43; H, 5.81.

*4.9.2.6. 7-Hydroxy-4′-methoxyflavane (**10**).* White powder, yield 79%, mp 158–160 °C, ^{1}H NMR ($CDCl_3$): 2.93 (d, J = 8.6 Hz, 2H); 3.11–3.23 (m, 1H); 3.81 (s, 3H); 3.96 (t, J = 10.4 Hz, 1H); 4.29 (dd, J = 10.4 Hz, J = 2.9 Hz, 1H); 5.04 (s, 1H); 6.36 (d, J = 2.2 Hz, 1H); 6.39 (dd, J = 8.0 Hz, J = 2.4 Hz, 1H); 6.89 (d, J = 8.4 Hz, 2H); 6.93 (d, J = 8.0 Hz, 1H); 7.16 (d, J = 8.6 Hz, 2H); EIMS m/z 256 (M^+). Anal. Calcd for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29; Found: C, 74.87; H, 6.30.

*4.9.2.7. 4′,7-Dimethoxyflavane (**11**).* White powder, yield 37%, mp 116–118 °C, ^{1}H NMR ($CDCl_3$): 2.75 (d, J = 8.4 Hz, 2H); 3.06–3.18 (m, 1H); 3.69 (s, 3H); 3.73 (s, 3H); 3.91 (t, J = 10.4 Hz, 1H); 4.31 (dd, J = 10.6 Hz, J = 2.8 Hz, 1H); 6.25 (d, J = 2.0 Hz, 1H); 6.30 (dd, J = 8.2 Hz, J = 2.2 Hz, 1H); 6.84 (d, J = 8.4 Hz, 2H); 6.93 (d, J = 8.0 Hz, 1H); 7.19 (d, J = 8.4 Hz, 2H); EIMS m/z 270 (M^+). Anal. Calcd for $C_{17}H_{18}O_3$: C, 75.53; H, 6.71; Found: C, 75.46; H, 6.72.

*4.9.3. Preparation of 4′,5,6,7,8-pentamethoxy-2-flavene (**5**)*

A solution of tangeretin (1.49 g, 4 mmol) in dry tetrahydrofuran (20 mL) was dropped during 20 min into a stirred solution of aluminium chloride (1.6 g, 12 mmol) and lithium aluminium with hydride (0.23 g, 6 mmol) in dry tetrahydrofuran (25 mL) at 0 °C. Tetrahydrofuran was removed under reduced pressure, then the obtained residue was decomposed with wet EtOAc followed by dropwise addition of water on an ice-water bath. The mixture was extracted with EtOAc. After removal of the solvent, the resulting residue was purified over a silica gel column eluting with EtOAc–petroleum ether (V:V = 1:12), and gave **5** as colourless crystal in a yield of 62%. Mp 137–138 °C, ^{1}H NMR (DMSO-d_6): 3.38 (d, J = 3.7 Hz, 2H); 3.76 (s, 3H); 3.78 (s, 6H); 3.83 (s, 6H); 5.60 (t, J = 3.5 Hz, 1H); 6.99 (d, J = 8.8 Hz, 2H); 7.63 (d, J = 8.8 Hz, 2H); EIMS m/z 358 (M^+). Anal. Calcd for $C_{20}H_{22}O_6$: C, 67.03; H, 6.19; Found: C, 66.94; H, 6.20.

*4.9.4. General procedure for the preparation of compounds **6**, **7**, **16**, **17** and **18***

A solution of flavonoid (5 mmol) in dry tetrahydrofuran (20 mL) was dropped into a stirred solution of lithium aluminium

hydride (10 mmol) in dry tetrahydrofuran (20 mL) at room temperature. Concentration under reduced pressure furnished a residue, which was decomposed with wet EtOAc followed by dropwise addition of water on an ice-water bath. The mixture was extracted with EtOAc. After removal of the solvent, the resulting residue was purified over a silica gel column eluting with EtOAc–petroleum ether.

*4.9.4.1. 2,4-Dihydroxyl-4′-methoxyl-α-methyldeoxybenzoin (**6**).* White powder, yield 46%, mp 86–88 °C, ^{1}H NMR ($CDCl_3$): 1.49 (d, J = 6.8 Hz, 3H); 3.76 (s, 3H); 4.59 (q, J = 6.6 Hz, 1H); 6.30 (dd, J = 9.0 Hz, J = 2.0 Hz, 1H); 6.34 (d, J = 2.0 Hz, 1H); 6.84 (d, J = 8.6 Hz, 2H); 7.21 (d, J = 8.4 Hz, 2H); 7.69 (d, J = 8.8 Hz, 1H); 12.89 (s, 1H); EIMS m/z 272 (M^+). Anal. Calcd for $C_{16}H_{16}O_4$: C, 70.57; H, 5.92; Found: C, 70.53; H, 5.92.

*4.9.4.2. 2-Hydroxyl-4,4′-dimethoxyl-α-methyldeoxybenzoin (**7**).* Colourless crystal, yield 62%, mp 74–75 °C, ^{1}H NMR ($CDCl_3$): 1.50 (d, J = 6.8 Hz, 3H); 3.76 (s, 3H); 3.79 (s, 3H); 4.59 (q, J = 6.8 Hz, 1H); 6.35 (dd, J = 8.8 Hz, J = 2.0 Hz, 1H); 6.38 (d, J = 2.0 Hz, 1H); 6.84 (d, J = 8.4 Hz, 2H); 7.21 (d, J = 8.4 Hz, 2H); 7.69 (d, J = 9.0 Hz, 1H); 12.92 (s, 1H); EIMS m/z 286 (M^+). Anal. Calcd for $C_{17}H_{18}O_4$: C, 71.31; H, 6.34; Found: C, 71.38; H, 6.33.

*4.9.4.3. 2-Hydroxyl-4,6-dimethoxylchalcone (**16**).* Light yellow powder, yield 18%, mp 85–88 °C, ^{1}H NMR ($CDCl_3$): 3.84 (s, 3H); 3.92 (s, 3H); 5.97 (d, J = 2.2 Hz, 1H); 6.12 (d, J = 2.4 Hz, 1H); 7.38–7.41 (m, 3H); 7.59–7.62 (m, 2H); 7.78 (d, J = 15.6 Hz, 1H); 7.91 (d, J = 15.8 Hz, 1H); 14.29 (s, 1H); EIMS m/z 284 (M^+). Anal. Calcd for $C_{17}H_{16}O_4$: C, 71.82; H, 5.67; Found: C, 71.76; H, 5.68.

*4.9.4.4. 2-Hydroxyl-4,6-dimethoxyldihydrochalcone (**17**).* White powder, yield 43%, mp 108–110 °C, ^{1}H NMR ($CDCl_3$): 2.99 (t, J = 7.3 Hz, 2H); 3.32 (t, J = 7.3 Hz, 2H); 3.82 (s, 3H); 3.83 (s, 3H); 5.92 (d, J = 2.2 Hz, 1H); 6.07 (d, J = 2.2 Hz, 1H); 7.18–7.33 (m, 5H); 14.02 (s, 1H); EIMS m/z 286 (M^+). Anal. Calcd for $C_{17}H_{18}O_4$: C, 71.31; H, 6.34; Found: C, 71.37; H, 6.33.

*4.9.4.5. 2,6-Dihydroxyl-4-methoxyldihydrochalcone (**18**).* White powder, yield 26%, mp 172–173 °C, ^{1}H NMR (DMSO-d_6): 2.89 (t, J = 7.9 Hz, 2H); 3.29 (t, J = 7.9 Hz, 2H); 3.74 (s, 3H); 5.96 (s, 2H); 7.16–7.31 (m, 5H); 12.33 (s, 1H); EIMS m/z 272 (M^+). Anal. Calcd for $C_{16}H_{16}O_4$: C, 70.57; H, 5.92; Found: C, 70.49; H, 5.93.

*4.9.5. General procedure for the preparation of compounds **12** and **13***

To a stirred solution of flavonoid (5 mmol) in ethanol (30 mL) was added sodium borohydride (10 mmol) at 0 °C in a nitrogen atmosphere. After decomposition with dropwise addition of water, the mixture was extracted with EtOAc. After removal of the solvent, the resulting residue was purified over a silica gel column eluting with EtOAc–petroleum ether.

*4.9.5.1. 7-Hydroxyl-4′-methoxyl-2-isoflavene (**12**).* White powder, yield 55%, mp 185–187 °C, ^{1}H NMR (DMSO-d_6): 3.77 (s, 3H); 4.92 (s, 2H); 6.11 (d, J = 2.4 Hz, 1H); 6.27 (dd, J = 8.2 Hz, J = 2.4 Hz, 1H); 6.41 (d, J = 8.0 Hz, 1H); 6.73 (s, 1H); 6.81 (d, J = 8.6 Hz, 2H); 7.35 (d, J = 8.6 Hz, 2H); 8.46 (s, 1H); EIMS m/z 254 (M^+). Anal. Calcd for $C_{16}H_{14}O_3$: C, 75.57; H, 5.55; Found: C, 75.63; H, 5.54.

*4.9.5.2. 4′,7,8-Trihydroxyl-2-isoflavene (**13**).* White powder, yield 42%, mp 202–205 °C, ^{1}H NMR (DMSO-d_6): 4.99 (s, 2H); 6.32 (d, J = 8.0 Hz, 1H); 6.44 (d, J = 8.0 Hz, 1H); 6.71 (s, 1H); 6.76 (d, J = 8.4 Hz, 2H); 7.32 (d, J = 8.8 Hz, 2H); 8.37 (s, 1H); 9.01 (s, 1H); 9.55 (s, 1H); EIMS m/z 256 (M^+). Anal. Calcd for $C_{15}H_{12}O_4$: C, 70.31; H, 4.72; Found: C, 70.26; H, 4.73.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.03.016.

References

[1] M.A.S. Aslama, S. Mahmood, M. Shahid, A. Saeed, J. Iqbal, Eur. J. Med. Chem. 46 (2011) 5473–5479.
[2] M. Arfan, H. Amin, I. Khan, M.R. Shah, H. Shah, A.Z. Khan, S.M.A. Halimi, N. Khan, W.A. Kaleem, M. Qayum, A. Shahidullah, M.A. Khan, Med. Chem. Res. 21 (2012) 2454–2457.
[3] K.S.T. Ramsay, P. Wafo, Z. Ali, A. Khan, O.O. Oluyemisi, B.P. Marasini, I.A. Khan, N.T. Bonaventure, M.I. Choudhary, Atta-ur-Rahman, Fitoterapia 83 (2012) 204–208.
[4] B. Krajewska, R. van Eldik, M. Brindell, J. Biol. Inorg. Chem. 17 (2012) 1123–1134.
[5] Ł. Berlicki, M. Bochno, A. Grabowiecka, A. Białas, P. Kosikowska, P. Kafarski, Amino Acids 42 (2012) 1937–1945.
[6] Z.-L. You, D.-H. Shi, J.-C. Zhang, Y.-P. Ma, C. Wang, K. Li, Inorg. Chim. Acta 384 (2012) 54–61.
[7] D.C. Menezes, E. Borges, M.F. Torres, J.P. Braga, Chem. Phys. Lett. 548 (2012) 85–89.
[8] K. Kobashi, J. Hase, K. Uehare, Biochim. Biophys. Acta 65 (1962) 380–383.
[9] B. Krajewska, J. Mol. Catal. B Enzymatic 59 (2009) 9–21.
[10] Z. Amtul, Atta-ur-Rahman, R.A. Siddiqui, M.I. Choudhary, Curr. Med. Chem. 9 (2002) 1323–1348.
[11] M. Kot, W. Zaborska, K. Orlinska, J. Enzym. Inhib. Med. Chem. 16 (2001) 507–516.
[12] M.J. Dominguez, C. Sanmartin, M. Font, J.A. Palop, S. San Francisco, O. Urrutia, F. Houdusse, J. Garcia-Mina, J. Agric. Food Chem. 56 (2008) 3721–3731.
[13] Z.-P. Xiao, T.-W. Ma, W.-C. Fu, X.-C. Peng, A.-H. Zhang, H.-L. Zhu, Eur. J. Med. Chem. 45 (2010) 5064–5070.
[14] F. von Nussbaum, M. Brands, B. Hinzen, S. Weigand, D. Häbich, Angew. Chem. Int. Ed. 45 (2006) 5072–5129.
[15] F.E. Koehn, G.T. Carter, Nat. Rev. Drug Discov. 4 (2005) 206–220.
[16] D.J. Newman, G.M. Cragg, K.M. Snader, J. Nat. Prod. 66 (2003) 1022–1037.
[17] S. Firdous, N.H. Ansari, I. Fatima, A. Malik, N. Afza, L. Iqbal, M. Lateef, Arch. Pharm. Res. 35 (2012) 1133–1137.
[18] N. Brigitte, T.S. Valerie, V.J. Catherine, S.A. Muhammad, L. Mehreen, I. Lubna, A. Nigaht, N.A. Ephrem, J. Enzyme Inhib. Med. Chem. (2012), http://dx.doi.org/10.3109/14756366.2012.719025.
[19] Z.-P. Xiao, Z.-Y. Peng, M.-J. Peng, W.-B. Yan, Y.-Z. Ouyang, H.-L. Zhu, Mini-rev. Med. Chem. 11 (2011) 169–177.
[20] N.C. Cook, S. Samman, J. Nutr. Biochem. 7 (1996) 66–76.
[21] R. Slimestad, T. Fossen, I.M. Vågen, J. Agric. Food Chem. 55 (2007) 10067–10080.
[22] S. Matsubara, H. Shibata, F. Ishikawa, T. Yokokura, M. Takahashi, T. Sugimura, Biochem. Biophys. Res. Commun. 310 (2003) 715–719.
[23] S. Shabana, A. Kawai, K. Kai, K. Akiyama, H. Hayashi, Biosci. Biotechnol. Biochem. 74 (2010) 878–880.
[24] M. Ahmad, N. Muhammad, M. Ahmad, M. Arif Lodhi, N. Jehan, Z. Khan, R. Ranjit, F. Shaheen, M.I. Choudhary, J. Enzym. Inhib. Med. Chem. 23 (2008) 386–390.
[25] A. Muhammad, I. Anis, A. Khan, B.P. Marasini, M.I. Choudhary, M.R. Shah, Arch. Pharm. Res. 35 (2012) 431–436.
[26] Z.-P. Xiao, D.-H. Shi, H.-Q. Li, L.-N. Zhang, C. Xu, H.-L. Zhu, Bioorg. Med. Chem. 15 (2007) 3703–3710.
[27] H.-Q. Li, Z.-P. Xiao, Y. Luo, T. Yan, P.-C. Lv, H.-L. Zhu, Eur. J. Med. Chem. 44 (2009) 2246–2251.
[28] Z.-P. Xiao, X.-D. Wang, Z.-Y. Peng, S. Huang, P. Yang, Q.-S. Li, L.-H. Zhou, X.-J. Hu, L.-J. Wu, Y. Zhou, H.-L. Zhu, J. Agric. Food Chem. 60 (2012) 10572–10577.
[29] Z.-P. Xiao, F. Zhang, Y.-C. Wang, S.-F. Yi, X.-C. Peng, H.-L. Zhu, Chin. J. Struct. Chem. 30 (2011) 330–335.
[30] A. Salakka, K. Wahala, J. Chem. Soc. Perkin Trans. I (Org. Biomol. Chem.) 18 (1999) 2601–2604.
[31] K. Pihlaja, P. Tähtinen, K.D. Klika, T. Jokela, A. Salakka, K. Wähälä, J. Org. Chem. 68 (2003) 6864–6869.
[32] H.G. Krishnamurty, S. Sathyanarayana, Synth. Commun. 19 (1989) 119–123.
[33] C.K. Lau, C. Dufresne, P.C. Bélanger, S. Piétré, J. Scheigetz, J. Org. Chem. 51 (1986) 3038–3043.

[34] E.-A. Bae, M.J. Han, D.-H. Kim, Planta Med. 67 (2001) 161–163.
[35] S. Perveen, A.M. El-Shafae, A. Al-Taweel, G.A. Fawzy, A. Malik, N. Afza, M. Latif, L. Iqbal, J. Asian Nat. Prod. Res. 13 (2011) 799–804.
[36] K.-F. Chan, I.L.K. Wong, J.W.Y. Kan, C.S.W. Yan, L.M.C. Chow, T.H. Chan, J. Med. Chem. 55 (2012) 1999–2014.
[37] Sigma. https://www.safcglobal.com/technical-documents/protocols/biology/enzymatic-assay-of-urease-from-jack-beans.html, 1995.
[38] M.W. Weatherburn, Anal. Chem. 39 (1967) 971–974.
[39] G.M. Sheldrick, SHELXTL-97, Program for Crystal Structure Solution and Refinement, University of Göttingen, Göttingen, Germany, 1997.