Supplementary Information

Contents:

1. Experimental details

1.1. Materials and reagents

MLN injections (No. 200611032) were generously provided by Jinling Pharmaceutical (Jiangsu, China). Xanthine oxidase (E.C. 1.1.3.22) from bovine milk was purchased from Sigma (St. Louis, MO). Centrifugal ultrafilters (Omega Nanosep, 10K) were obtained from Pall (Ann Arbor, MI). The reference standards of caffeoylquinic acids (CQA) including 5-CQA, 1,3-diCQA, 1,5-diCQA, 3,4-diCQA, 3,5-diCQA and 4,5-diCQA were purchased from the National Institute for the Control of Pharmaceutical and Biological Product (98%). Caffeic acid, 3-CQA, 4-CQA, 3,4-dicaffeoylquinic acid methyl ester (3,4-diCQA-ME) and 3,5-dicaffeoylquinic acid methyl ester (3,4-diCQA-ME) were previously isolated from several species of *Lonicera* in the authors' laboratory. Their structures were identified by MS, ^{1}HNMR and ^{13}CNMR with a purity of more than 95% for each compound. Quinic acid was purchased from Aladdin Industrial Corporation (Shanghai, China) with a purity of more than 98%. Acetonitrile and formic acid (HPLC grade) were purchased from Merck (Darmstadt, Germany). Distilled-deionized water was provided by a Milli-Q water purification system from Millipore (Bedford, MA, USA).

1.2. Screening of XOD binders from MLN injection by UF-LC-MS

In the experimental group, 20 μL of MLN injection, 1 μL of 10 mM febuxostat and 1 μL of 10 mM loganin were incubated with 50 μL of XOD solution (at a final

concentration of 0.9 μM) in 128 μL of phosphate buffer solution (75 mM, pH 7.4) for 1 h at room temperature. After incubation, the mixture was filtered through a 10 kDa molecular weight cutoff ultrafiltration membrane filter by centrifugation at 16,200 × g for 20 min. The XOD-ligand complexes were trapped in the ultrafiltration cell and washed three times with 200 μL aliquots of phosphate buffer solution, followed by another centrifugation at 16,200 × g for 20 min to remove the unbound compounds. The ligands were dissociated from XOD by adding 200 μL 70% methanol aqueous solution for a 15 min ultrasonication. After centrifugation, the ultrafiltrates containing the ligands released from the XOD-ligand complexes were further analyzed via HPLC. The control experiments were carried out in a similar manner without XOD. All assays were performed in triplicate.

1.3. Identification of XOD binders by LC-ESI-MS and NMR experiments

A 20 μL aliquot of ultrafiltrate was analyzed on an Agilent 1200 SL rapid resolution LC system equipped with a binary pump, an online degasser, an auto sampler and a thermostatically controlled column compartment. Chromatographic separation was performed at room temperature using a Zorbax SB-C18 column (250 mm × 250 mm, 5 μm; Agilent) and a C18 guard column. The mobile phase consisted of 0.1% formic acid aqueous solution (A) and acetonitrile (B) was delivered at a flow rate of 0.5 mL/min. The following gradient program was used: 0–10 min, 5–14% B; 10–18 min, 14–19% B; 18–26 min, 19–19% B; 26–34 min, 19–31% B; 34–50 min, 31–54% B; 50–70 min, 54–100% B. The detection wavelength was 254 nm.

QTOF–MS/MS analysis was carried out using a 6520 LC-QTOF mass system (Agilent Technologies, Santa Clara, CA, USA; nominal mass resolution 20,000 at a scan of 5 s^{-1}) equipped with an electrospray ionization (ESI) interface. The ESI source worked in negative mode, and the operating parameters were as follows : drying gas (N_2) flow rate, 10.0 L/min; drying gas temperature, 325 °C; nebulizer, 35 psig; capillary, 3500 V; OCT RF V, 250 V; fragmentor voltage, 120 V; skimmer voltage, 65 V; collision energy, 25 V. The mass range was set at m/z 100–1000. All the operations, acquisition and analysis of data were performed under Masshunter Workstation Software (version B.02.00). NMR experiments were conducted with Instrum AV300 NMR spectrometer operating at 300 MHz for recording ^{1}H NMR spectra and 75 MHz for ^{13}C NMR spectra in CD3OD solvent using TMS as an internal standard.

1.4 Lead-based combinatorial compound library design

Three leads, namely, 3,4-diCQA (**a**), 3,5-diCQA (**b**) and 4,5-diCQA (**c**), screened by UF-LC-MS were used as the core molecules for further structure optimization. Lead-based combinatorial compound library design was performed by elimination, dissociation, transposition or introduction of some chemical constitution as shown in Fig. 2 of the main text. In detail, 4-CQA (**d**), 3-CQA (**e**) and 5-CQA (**f**) were obtained by elimination of a caffeoyl group from the three core molecules respectively. Caffeic acid (**g**) and quinic acid (**h**) were the common dissociated products of compounds **d**, **e** and **f**. 1,4-diCQA (**i**), 1,3-diCQA (**j**) and 1,5-diCQA (**k**) were obtained by

transposition of the two caffeoyl groups on the quinic core. 3,4-diCQA-ME (**l**), 3,5-diCQA-ME (**m**) and 4,5-diCQA-ME (**n**) were obtained by introduction of a methyl group into compounds **a**, **b** and **c** respectively.

1.5 Analysis and prediction by in silico molecular docking research

To analyze binding affinities of ligands to XOD and the possible binding sites, an *in silico* protein-ligand docking software AutoDock 4.2 program was applied.[1] The docking steps were performed according to the standard procedures:[2] (1) Crystal structure file of XOD (1FIQ) was downloaded from the RCSB protein data bank; (2) Deletion of unnecessary substructures and water molecules; (3) Hydrogen atoms were added to XOD; (4) Gasteiger charges were calculated for each atom of XOD; (5) Run Autogrid to get grid maps; (6) Run 100 times to generate docked conformations by Lamarckian genetic algorithm (LGA) and obtain former 20 conformations with the lowest binding energies for statistical analysis. The interaction figures of ligands to XOD were generated and the results of docking were recorded with binding orientation ratio and binding energy. The percentage binding orientation ratio of a ligand to XOD was calculated as % binding orientation ratio = EC/TC $\times$ 100, where EC is the number of effective conformations of ligands binding to the function domain of XOD, and TC is the total number of statistical conformations.

1.6 Binding displacement experiment

To verify the docking results of the two diCQA-MEs bound to the function domain of XOD, a binding displacement experiment between them and a known

ligand febuxostat was performed. The mixtures of 50 μM of 3,4-diCQA-ME, 50 μM of 3,5-diCQA-ME and various concentrations of febuxostat (0, 2, 4 μM) were incubated with 0.7 μM XOD in a total volume of 20 μL for 30 min. The following ultrafiltration steps were the same as described in Section 1.2, and the ultrafiltrates were analyzed by HPLC. The detection wavelength was 254 nm.

1.7 Assay of XOD inhibitory activity

The XOD inhibitory activity was measured at 295 nm based on the procedure reported by Filha et al. with modification.[3] The assay mixture consisting of 100 μl of test solution and 50 μl of 0.08 U/mL XOD solution was preincubated at 37 °C for 15 min. After preincubation, the reaction was initiated by adding 50 μL of 0.48 mM xanthine. The reaction mixture was incubated at 37 °C with the absorbance (295 nm) measured every 15 s for 5 min. A blank control contained the same assay mixture except the same volume of phosphate buffer solution (75 mM, pH 7.4) was added instead of a solution of test compounds. Allopurinol and febuxostat, two known XOD inhibitors, were used as the positive controls. Loganin, a non-inhibitor, was used as the negative control. All assays were done in triplicate and inhibition percentages were the means of triplicate observations. XOD inhibitory activity was expressed as the percentage inhibition of XOD in the above assay mixture system, calculated as (%) inhibitory ratio = $(1-\beta/\alpha) \times 100$, where α is the linear change in absorbance per minute of blank solution, and β is the linear change in absorbance per minute of test solution. The IC_{50} values for related compounds were determined on three replicates of several

concentrations. Statistical analyses and the IC_{50} values were calculated using GraphPad Prism version 6.02 (GraphPad Software Inc.).

Table S1 Retention time (t_R), MS data and UV spectra for identification of XOD binders in MLN injection by QTOF MS.

Peak No.	t_R (min)	[M-H]$^-$			Elem. Comp.	Fragmentation pathways	UV (λ max)	Identification
		m/z	Cal m/z	Diff. (ppm)				
2	13.76	353.8920	353.0878	-3.79	$C_{16}H_{17}O_9$	[M-H]$^-$	242, 300sh, 325	3-caffeoylquinic acid [a]
		191.0551	191.0561	5.15	$C_7H_{11}O_6$,	[M-H-caffeoyl]$^-$		
12	36.75	515.1241	515.1195	-8.80	$C_{25}H_{23}O_{12}$	[M-H]$^-$	243, 300sh, 327	3,4-dicaffeoylquinic acid [a]
		353.0907	353.0878	-8.10	$C_{16}H_{17}O_9$	[M-H-caffeoyl]$^-$		
		191.0566	191.0561	-2.72	$C_7H_{11}O_6$	[M-H-2caffeoyl]$^-$		
		179.0353	179.0350	-1.61	$C_9H_7O_4$	[caffeoyl]$^-$		
		135.0454	135.0452	-1.62	$C_8H_7O_2$	[caffeoyl-CO_2]$^-$		
13	38.08	515.1226	515.1195	-5.79	$C_{25}H_{23}O_{12}$	[M-H]$^-$	240, 300sh, 327	3,5-dicaffeoylquinic acid [a]
		353.0885	353.0878	-1.87	$C_{16}H_{17}O_9$	[M-H-caffeoyl]$^-$		
		191.0560	191.0561	0.64	$C_7H_{11}O_6$	[M-H-2caffeoyl]$^-$		
		179.0347	179.0350	1.39	$C_9H_7O_4$	[caffeoyl]$^-$		
14	39.00	515.1173	515.1195	4.24	$C_{25}H_{23}O_{12}$	[M-H]$^-$	240, 300sh, 327	4,5-dicaffeoylquinic acid [a]
		353.0869	353.0878	2.67	$C_{16}H_{17}O_9$	[M-H-caffeoyl]$^-$		
		191.0550	191.0561	6.00	$C_7H_{11}O_6$	[M-H-2caffeoyl]$^-$		
		179.0338	179.0350	6.39	$C_9H_7O_4$	[caffeoyl]$^-$		
		135.0446	135.0452	4.17	$C_8H_7O_2$	[caffeoyl-CO_2]$^-$		

[a] Identification was further confirmed with reference standards.

Table S2 Structural identification of XOD binders in MLN injection by NMR data.

Peak No.	^{1}H-NMR	^{13}C-NMR	Identification
2	2.03 (2H, br d, J = 5.67 Hz, H-2), 2.08-2.25 (2H, m, H-6), 3.72 (1H, dd, J = 3.06, 8.46 Hz, H-4), 4.17 (1H, s, H-3), 5.33 (1H, m, H-5), 6.26 (1H, d, J = 15.9 Hz, H-8'), 6.78 (1H, d, J = 8.13 Hz, H-5'), 6.97 (1H, dd, J = 1.65, 8.22Hz, H-6'), 7.05 (1H, d, J = 1.62 Hz, H-2'), 7.56 (1H, d, J = 15.9 Hz, H-7')	76.1 (C-1), 38.2 (C-2), 73.5 (C-3), 72.0 (C-4), 71.3 (C-5), 38.8 (C-6), 127.8 (C-1'), 115.3 (C-2'), 147.1 (C-3'), 149.6 (C-4'), 116.5 (C-5'), 123.0 (C-6'), 146.8 (C-7'), 115.2 (C-8'), 168.7 (C-9'), 177.0 (COOH)	3-caffeoylquinic acid
12	1.99-2.35 (4H, m, 2H-2 and 2H-6), 5.38 (1H, m, H-4), 5.43 (1H, m, H-3), 3.97 (1H, dd, J = 7.32, 3.12 Hz, H-5), 6.27 (1H, d, J = 15.9 Hz, H-8''), 6.35 (1H, d, J = 15.9 Hz, H-8'), 6.78 (2H, d, J = 8.16 Hz, H-5' and H-5''), 6.97 (2H, br d, J = 8.31 Hz, H-6' and H-6''), 7.07 (2H, s, H-2' and H-2''), 7.62 (1H, d, J = 15.6 Hz, H-7''), 7.57 (1H, d, J = 15.6 Hz, H-7')	74.7 (C-1), 37.7 (C-2), 70.6 (C-3), 72.6 (C-4), 72.1 (C-5), 36.0 (C-6), 168.4 (C-1''), 168.9 (C-1'), 115.2 (C-2'), 115.2 (C-2''), 147.0 (C-3'), 147.3 (C-3''), 127.8 (C-4'), 128 (C-4''), 115.3 (C-5''), 115.6 (C-5'), 146.8 (C-6'), 146.8 (C-6''), 149.5 (C-7'), 149.6 (C-7''), 116.5 (C-8''), 116.5 (C-8'), 123.0 (C-9'), 123.0 (C-9''), 177.4 (COOH)	3,4-dicaffeoylquinic acid
13	2.02-2.35 (4H, m, 2H-2 and 2H-6), 3.98 (1H, dd, J = 7.32, 3.06 Hz, H-4), 5.38 (1H, m, H-3), 5.43 (1H, m, H-5), 6.27 (1H, d, J = 15.9 Hz, H-8''), 6.35 (1H, d, J = 15.9 Hz, H-8'), 6.75 (2H, d, J = 8.85 Hz, H-5' and H-5''), 6.97 (2H, br d, J = 7.71 Hz, H-6' and H-6''), 7.06 (2H, s, H-2' and H-2''), 7.58 (1H, d, J = 15.8 Hz, H-7'), 7.62 (1H, d, J = 15.8 Hz, H-7'')	74.7 (C-1), 36.0 (C-2), 72.5 (C-3), 70.6 (C-4), 72.1 (C-5), 37.6 (C-6), 168.4 (C-1''), 168.9 (C-1'), 116.5 (C-2''), 116.5 (C-2'), 146.8 (C-3''), 146.8 (C-3'), 127.8 (C-4''), 128.0 (C-4'), 115.2 (C-5''), 115.2 (C-5'), 147.0 (C-6'), 147.3 (C-6''), 149.5 (C-7''), 149.6 (C-7'), 115.3 (C-8'), 115.6 (C-8''), 123.0 (C-9''), 123.1 (C-9'), 177.3 (COOH)	3,5-dicaffeoylquinic acid
14	2.08-2.32 (4H, m, 2H-2 and 2H-6), 5.11 (1H, dd, J = 8.87, 2.67 Hz, H-4), 4.37 (1H, s, H-3), 5.62 (1H, m, H-5), 6.19 (1H, d, J = 15.9 Hz, H-8''), 6.28 (1H, d, J = 15.9 Hz, H-8'), 6.75 (2H, d, J = 8.07 Hz, H-5' and H-5''), 6.91 (2H, br d, J = 7.89 Hz, H-6' and H-6''), 7.00 (1H, s, H-2''), 7.02 (1H, s, H-2'), 7.52 (1H, d, J = 15.8 Hz, H-7'), 7.60 (1H, d, J = 15.9 Hz, H-7'')	76.0 (C-1), 38.4 (C-2), 69.3 (C-3), 75.7 (C-4), 69.0 (C-5), 39.3 (C-6), 168.2 (C-1'), 168.5 (C-1''), 114.7 (C-2'), 114.8 (C-2''), 147.6 (C-3''), 147.7 (C-3'), 127.7 (C-4'), 127.7 (C-4''), 115.2 (C-5'), 115.2 (C-5''), 146.8 (C-6''), 146.8 (C-6'), 149.7 (C-7''), 149.7 (C-7'), 116.5 (C-8'), 116.5 (C-8''), 123.1 (C-9''), 123.1 (C-9'), 176.7 (COOH)	4,5-dicaffeoylquinic acid

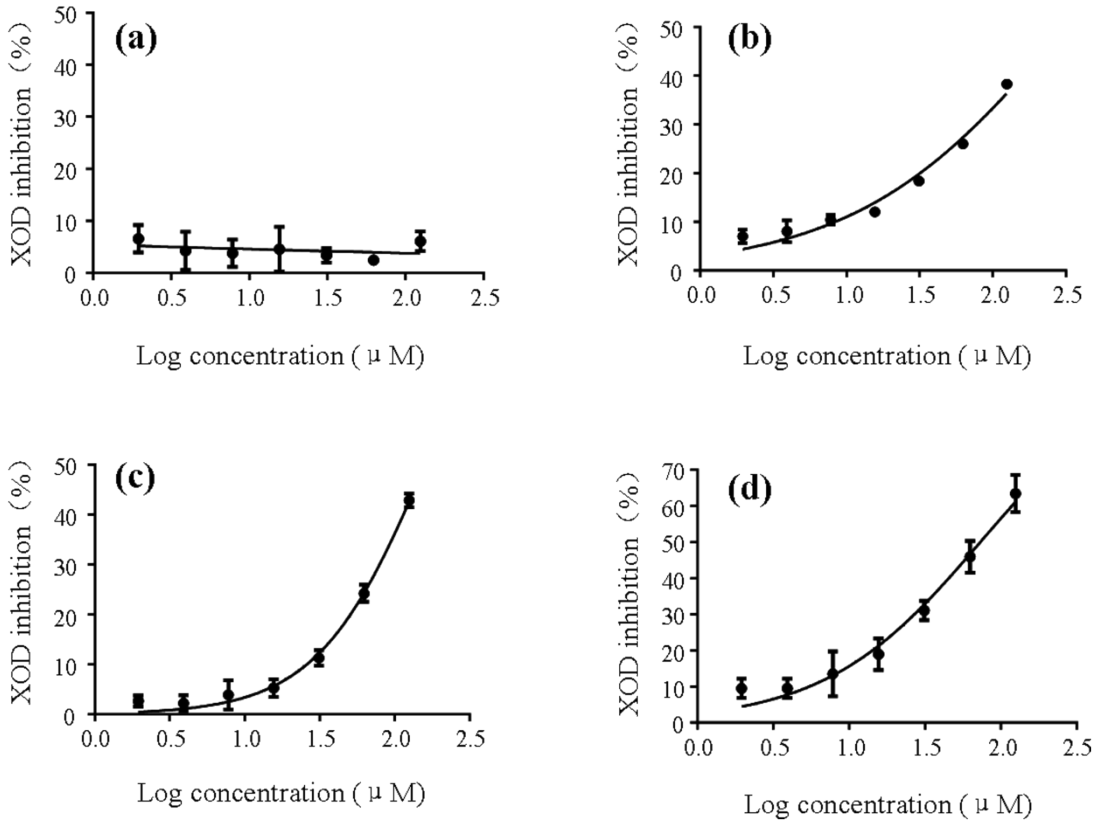

Fig. S1 Dose-response curves of 3-CQA (a), 3,4-diCQA (b), 3,5-diCQA (c) and 4,5-diCQA (d) against XOD.

2. Discussion about docking results

It had been demonstrated previously that a potent XOD inhibitor should satisfy the following requirements: (1) strong binding affinity with XOD and (2) binding located at the active site.[4] Two parameters of binding energy and binding orientation ratio in docking research were chosen to reflect the two characteristics respectively. The detailed information of the two parameters was as follows: (1) Binding energy is applied to evaluate binding affinity. It was calculated as binding energy = A+B+C-D, where A is the sum of final intermolecular energy, Wandervalls energy, hydrogen bonds, desolvation energy and electrostatic energy, B is final total internal energy, C is torsional free energy, and D is unbound system's energy. The complexes of small molecules with XOD possessing lower binding energy were more likely to be stable, and their binding affinities were more likely to be stronger. (2) The parameter binding orientation ratio was used to assess the possibility of the small molecule binding to the function domain of XOD. The molecular structure of XOD could be divided into three major domains as shown in Fig. S2a. The domains are Fe/S-center domain (pink), FAD domain (green) and Mo-pt domain (red), among which Mo-pt domain is the function domain where the oxidation of xanthine takes place.[5] The possibility of a ligand binding to the function domain of XOD was expressed as % binding orientation ratio = EC/TC $\times$ 100, where EC is the number of effective conformations of ligands binding to the Mo-pt domain, and TC is the total number of statistical conformations. A ligand with higher binding orientation ratio indicated that the binding position was more likely at the Mo-pt domain.

Table S3 showed the binding energies of related compounds based on their rank. Compared with the three lead compounds of 3,4-diCQA, 3,5-diCQA and 4,5-diCQA,

4-CQA, 3-CQA, 5-CQA, caffeic acid, 1,4-diCQA, 1,5-diCQA, 3,4-diCQA-ME and 3,5-diCQA-ME exhibited obviously lower binding energies. The results indicated that their ligand-enzyme complexes were more likely to be stable and they might have stronger binding affinities to XOD than the leads. The binding orientation ratios were closely related with their chemical scaffolds, which could be ranked as: 3,4-diCQA, 3,5-diCQA and 4,5-diCQA (50-65%) ≈ 3,4-diCQA-ME, 3,5-diCQA-ME and 4,5-diCQA-ME, (50-65%) > 1,4-diCQA, 1,3-diCQA and 1,5-diCQA (40-50%) > 4-CQA, 3-CQA and 5-CQA (20-40%) > caffeoyl acid (15%) = quinic acid (15%). These data were summarized in Fig. S2b. Among the above CQA derivatives except the lead compounds of 3,4-diCQA, 3,5-diCQA and 4,5-diCQA, diCQA-MEs showed the highest ratios which were more than 50%, indicating that 3,4-diCQA-ME, 3,5-diCQA-ME and 4,5-diCQA-ME had the greatest possibilities of binding to the function domain of XOD.

By comprehensive analysis of the two parameters above, 3,4-diCQA-ME and 3,5-diCQA-ME were the common candidate high-quality XOD inhibitors which possessed low binding energies and high binding orientation ratios based on the strategy described in Scheme 1, and the other CQA derivatives were weak inhibitors or non-inhibitors. To evaluate the reliability of Autodock 4.2 program, allopurinol and febuxostat, two known XOD inhibitors, were used as the positive controls, and loganin, a non-inhibitor, was used as the negative control. Noteworthy, allopurinol showed the highest binding orientation ratio of 95%, suggesting that its binding position was at Mo-pt domain, which was in agreement with the result of crystal structure analysis.[6] The other positive febuxostat exhibited the lowest binding energy ranging from -6.79 and -6.10 kcal/mol, indicating its high binding affinity with XOD, which was consistent with the reported literature.[7] The binding orientation ratio of

loganin was merely 30%, showing that its binding position was possibly not at the function domain of XOD and it might be a non-inhibitor. The prediction was confirmed by the related data in Table S4.

Table S3 Binding energies of the compounds based on their rank (the top 10).

	Compounds	Binding energies of compounds based on their rank (kcal/mol)									
		1	2	3	4	5	6	7	8	9	10
A	3,4-diCQA	-2.94	-2.92	-2.91	-2.83	-2.81	-2.68	-2.63	-2.63	-2.58	-2.41
B	3,5-diCQA	-3.97	-3.77	-3.76	-3.25	-3.23	-3.05	-2.95	-2.93	-2.72	-2.65
C	4,5-diCQA	-4.18	-3.57	-3.23	-3.20	-3.17	-3.12	-3.05	-2.38	-2.12	-2.10
D	4-CQA	-4.35	-4.34	-3.79	-3.78	-3.62	-3.53	-3.51	-3.45	-3.35	-3.32
E	3-CQA	-5.22	-4.62	-4.34	-4.22	-4.03	-4.00	-3.85	-3.81	-3.78	-3.73
F	5-CQA	-4.78	-4.67	-4.30	-4.26	-4.10	-3.91	-3.90	-3.90	-3.85	-3.82
G	Caffeic acid	-4.73	-4.71	-4.61	-4.58	-4.50	-4.46	-4.42	-4.32	-4.31	-4.31
H	Quinic acid	-3.66	-3.51	-3.44	-3.10	-2.87	-2.79	-2.68	-2.68	-2.67	-2.66
I	1,4-diCQA	-4.77	-4.05	-3.77	-3.74	-3.64	-3.33	-3.24	-3.11	-3.05	-2.95
J	1,3-diCQA	-3.38	-3.35	-3.11	-2.75	-2.56	-2.56	-2.25	-2.15	-2.09	-2.08
K	1,5-diCQA	-5.32	-4.77	-3.56	-3.49	-3.29	-3.23	-3.16	-3.01	-2.81	-2.66
L	3,4-diCQA-ME	-4.43	-3.76	-3.46	-2.98	-2.89	-2.86	-2.80	-2.80	-2.78	-2.54
M	3,5-diCQA-ME	-5.16	-4.94	-4.74	-4.02	-3.94	-3.37	-3.22	-3.10	-2.98	-2.41
N	4,5-diCQA-ME	-3.70	-3.55	-3.34	-3.29	-3.07	-2.79	-2.26	-2.22	-2.19	-2.14
Negative control	Loganin	-4.55	-3.92	-3.85	-3.82	-3.77	-3.64	-3.58	-3.55	-3.42	-3.27
Positive control 1	Allopurinol	-4.93	-4.80	-4.55	-4.43	-4.24	-4.19	-4.13	-4.10	-4.03	-4.01
Positive control 2	Febuxostat	-6.79	-6.45	-6.43	-6.36	-6.22	-6.21	-6.17	-6.17	-6.16	-6.10

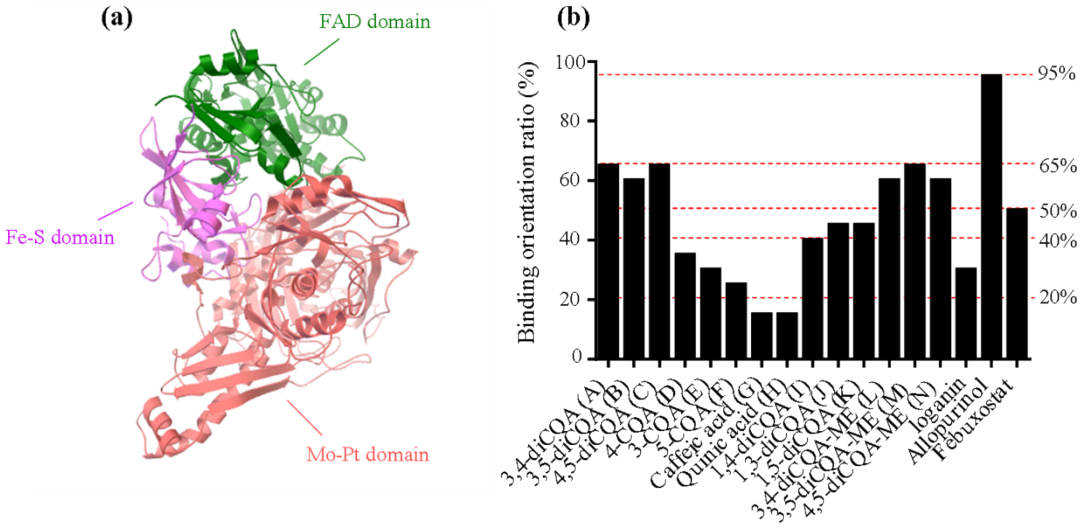

Fig. S2 (a) The molecular structure of the XOD divided into three major domains. The domains are Fe/S-center domain (pink), FAD domain (green) and Mo-pt domain (red). Mo-pt domain is the function domain where the oxidation of xanthine takes place. (b) Histogram shows the probabilities of the related compounds binding to Mo-pt domain represented by binding orientation ratios.

3. Discussion about structure-activity relationship of the XOD inhibitors

Table S4 XOD inhibition activities of the related compounds.

	Compounds	Inhibition rate (%)[a]	IC_{50} (μM)
A	3,4-diCQA	38.32 ± 0.80	310.00 ± 9.05
B	3,5-diCQA	42.86 ± 1.37	159.03 ± 3.44
C	4,5-diCQA	63.51 ± 5.16	68.47 ± 0.79
D	4-CQA	11.95 ± 2.38	NI [b]
E	3-CQA	6.06 ± 1.86	NI [b]
F	5-CQA	9.08 ± 0.96	NI [b]
G	Caffeic acid	5.74 ± 2.64	NI [b]
H	Quinic acid	1.71 ± 1.64	NI [b]
I	1,4-diCQA	- [c]	- [c]
J	1,3-diCQA	22.12 ± 0.97	259.43 ± 8.86
K	1,5-diCQA	71.38 ± 0.86	47.26 ± 1.08
L	3,4-diCQA-ME	97.70 ± 0.81	3.16 ± 0.04
M	3,5-diCQA-ME	79.25 ± 2.65	7.54 ± 0.20
N	4,5-diCQA-ME	- [c]	- [c]
Negative control	Loganin	1.66 ± 0.97	NI [b]
Positive control 1	Allopurinol	97.57 ± 0.15	8.36 ± 0.07
Positive control 2	Febuxostat	99.55 ± 0.78	32.26 ± 0.72[d]

[a] Inhibition by 125 μM reference standards.
[b] Less than 15% inhibition at concentration of 125 μM.
[c] Not tested due to the absence of the reference standard.
[d] The unit is nanomole.

As shown in Table S4, the XOD inhibition activities of the related compounds can be ordered as 3,4-diCQA-ME > 3,5-diCQA-ME > 1,5-diCQA > 4,5-diCQA > 3,5-diCQA > 3,4-diCQA > 1,3-diCQA > 4-CQA > 5-CQA > 3-CQA > caffeic acid > quinic acid based on their inhibition rates at concentration of 125 μM and IC_{50} values. Their structures were shown in Fig. 2 of the main text. The structure-activity relationship could be evaluated and summarized as follows:

1. All the CQA derivatives discussed here were esters formed between quinic acid and caffeic acid, where quinic acid was used as the core molecule and one or two

caffeic acids were conjugated to the core. Based on the above activity order, quinic acid and caffeic acid showed the weakest inhibition effect. Thus, the CQA derivatives cannot simply own their XOD inhibitory ability to either the caffeoyl groups or the quinic cores. The conjunction manner of them may be a key factor influencing the activity.

2. The five diCQAs of 1,5-diCQA, 4,5-diCQA, 3,5-diCQA, 3,4-diCQA and 1,3-diCQA exhibited more enhanced XOD inhibitory activity compared with the three monoCQAs of 4-CQA, 5-CQA and 3-CQA, indicating that caffeoyl group played an important role in the inhibitory process. Based on the knowledge that caffeoyl group contains a conjugated ring and two hydroxyl groups, it is reasonable to assume that these structural characteristics are closely related with XOD inhibition.

3. Among the five diCQAs, XOD inhibition activities of 1,5-diCQA and 4,5-diCQA were more enhanced than those of 3,4-diCQA and 1,3-diCQA. Because these five isomers of diCQAs are discriminated by the positions of caffeoyl moieties, the difference in the XOD inhibitory activity could be due to the linkage positions of caffeoyl groups on the quinic core. The results indicate that the caffeoyl moiety at position 5 contributes to increase of inhibitory activity, whereas the caffeoyl moiety at position 3 results in decrease of inhibitory activity. This conclusion could be supported by 3,5-diCQA whose activity order was in the middle of the five diCQAs because of the compromise of the caffeoyl moieties substituted at position 3 and position 5.

4. The two diCQA-MEs of 3,4-diCQA-ME and 3,5-diCQA-ME exhibited the strongest XOD inhibitory effect with IC_{50} values of 3.16 and 7.54 μM respectively. Their IC_{50} values were even lower than that of a positive control allopurinol (IC_{50} =

8.36 μM). By comparing the XOD inhibition of 3,4-diCQA-ME and 3,5-diCQA-ME with that of 3,4-diCQA and 3,5-diCQA, it was easy to see that the methyl group could enhance the activity of diCQAs at a high level. A possible explanation is that the introduction of a hydrophobic methyl group into diCQA contributes to improve its binding affinity with the active site of XOD which is also a hydrophobic region surrounding with several hydrophobic amino acid residues.[8]

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