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Design, synthesis and molecular docking studies of *N*-(9,10-anthraquinone-2-carbonyl)amino acid derivatives as xanthine oxidase inhibitors

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Abstract

A series of *N*-(9,10-anthraquinone-2-carbonyl)amino acid derivatives (**1a-j**) was designed and synthesized as novel XO inhibitors. Among them, the *L*/*D*-phenylalanine derivatives (**1d** and **1i**) and the *L*/*D*-tryptophan derivatives (**1e** and **1j**) were effective with micromolar level potency. In particular, the *L*-phenylalanine derivative **1d** (IC_{50} = 3.0 μM) and the *D*-phenylalanine derivative **1i** (IC_{50} = 2.9 μM) presented the highest potency, and were both more potent than the positive control

allopurinol (IC_{50} = 8.1 μM). Preliminary SAR analysis pointed that an aromatic amino acid fragment, *e.g.*, phenylalanine or tryptophan, was essential for the inhibition; the *D*-amino acid derivative presented equal or greater potency compared to its *L*-enantiomer; and the 9,10-anthraquinone moiety was welcome for the inhibition. Molecular simulations provided rational binding models for compounds **1d** and **1i** in the XO active pocket. As a result, compounds **1d** and **1i** could be promising lead compounds for further investigation.

Keywords: Anthraquinone; Synthesis; Xanthine oxidase inhibitor; Hyperuricemia

Introduction

Xanthine oxidase (XO) is a key rate-limited enzyme in uric acid production in humans which oxidizes hypoxanthine from nucleic acid metabolites into xanthine, and xanthine into uric acid.[1, 2] The over-production of uric acid can lead to hyperuricemia, which is a major cause of gout. And hyperuricemia and gout are associated with chronic diseases such as metabolic syndrome, renal and cardiovascular disorders.[3, 4] Besides, reactive oxygen species (ROS) overproduction may be also caused by the enhancement of XO expression and activity. This hypothesis is supported by clear evidence showing that XO is involved in various forms of ischemic and vascular injuries, inflammatory diseases, and chronic heart failure.[5] Therefore, inhibition of XO not only is one of the most promising methods for the treatment for hyperuricemia and gout, but also has widely therapeutic potential for series XO-associated diseases.

Allopurinol (**Fig. 1**) is the first XO inhibitor approved in the 1960s and has been the main therapy for the management of gout and conditions associated with hyperuricemia for several decades. However, in some cases, severe life-threatening side effects have been reported such as fulminant hepatitis, renal failure, and Stevense Johnson syndrome.[6] Febuxostat[7] and topiroxostat[8] (**Fig. 1**) are both non-purine XO inhibitors which possess excellent XO inhibitory activities and have been introduced into market in 2009 and 2013, respectively. Their approval greatly promoted the research

of non-purine XO inhibitors and a number of compounds based on the scaffolds of febuxostat and topiroxostat have been recently reported such as Y-700,[9] isoxazoles,[10] imidazoles,[11] 1,2,3-triazoles,[12] selenazoles,[13] 2-(indol-5-yl)thiazoles,[14] isocytosines,[15-18] phenyl-1,2,4-triazoles,[19] 4-(pyridin-4-yl)-1,2,3-triazoles[20] and isonicotinamides.[21]

9,10-Anthraquinone is a class of functionally diverse aromatic compound which exists widely in traditional Chinese medicines such as aloe-emodin, rhein, emodin, chrysophanol and physcion.[22] Up to now, various medicinal values of 9,10-anthraquinone were discovered, including anticancer, antibacterial, anti-inflammatory, antioxidant, antidiabetic, antiviral, hepatoprotection and purgation.[23] Shi *et al* [24] reported a series of aloe-enodin derivatives which presented well XO inhibitory potency. Our previous studies discovered a series of XO inhibitors based on a (1*H*-1,2,3-triazol-4-yl)methoxybenzaldehyde scaffold and contained an 9,10-anthraquinone moiety.[25] Structure-activity relationship (SAR) analysis and molecular modeling studies demonstrated that the 9,10-anthraquinone moiety located at the outer region of XO active pocket and performed a role of the lipophilic fragment as in the case of the isobutoxy group of febuxostat.[25]

In this context, we used a *N*-acylamino acid to mimic an open-ring fragment of the thiazole-5-carboxylic acid moiety of febuxostat and adopted an 9,10-anthraquinone moiety to play a combined role of benzonitrile moiety and the lipophilic tail to design a series of *N*-(9,10-anthraquinone-2-carbonyl)amino acid derivatives (**Fig. 2**), expecting to identify a new class of novel XO inhibitors based on the endogenous amino acids and 9,10-anthraquinone moiety. Additionally, molecular modeling simulations were carried out to explore the action mode of the representative compounds.

Materials and methods

Chemistry

Unless otherwise indicated, reagents and solvents were purchased from commercial sources and used without further purification. All reactions were monitored by TLC using silica gel aluminum cards (0.2 mm thickness) with a fluorescent indicator at 254 nm. The column chromatography was

performed using silica gel (200-300 mesh) from Qingdao Ocean Chemicals (Qingdao, Shandong, China). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 600 MHz spectrometer. Chemical shifts were expressed in parts per million using DMSO-d_6 as the solvent.

General procedure for the preparation of *N*-(9,10-anthraquinone-2-formyl)amino acids (1a-j)

A mixture of **7** (10 mmol), 10 M NaOH (5 mL), methanol (1 mL), and tetrahydrofuran (1 mL) was stirred at 50 °C for 4 h. Then the reaction mixture was diluted with water and adjusted pH to 3 with dilute sulfuric acid. The formed precipitate was collected and purified by recrystallization to provide pure **1a-j**.

N-(9,10-anthraquinone-2-formyl)glycine (**1a**): A yellow solid, yield 85%; MS: m/z 308.1 $[M-H]^{-1}$; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 12.71 (s, 1H, COOH), 9.36 (t, 1H, J = 5.7 Hz, Ar-H), 8.66 (d, 1H, J = 1.4 Hz, Ar-H), 8.28 (m, 4H, Ar-H), 7.94 (d, 2H, J = 5.7 Hz, Ar-H), 4.00 (d, 2H, J = 5.8 Hz, CH_2); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 182.54, 182.50, 171.42, 165.41, 139.01, 135.13, 135.06, 135.00, 133.54, 133.47, 133.43, 133.20, 127.57, 127.24, 127.20, 125.91, 41.76, 40.29, 40.15, 40.01, 39.88, 39.74, 39.60, 39.46.

N-(9,10-anthraquinone-2-formyl)-L-alanine (**1b**): A yellow solid, yield 76%; MS: m/z 322.1 $[M-H]^{-1}$; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 12.67 (s, 1H, COOH), 9.21 (s, 1H, NH), 8.67 (s, 1H, Ar-H), 8.34 (d, 1H, J = 7.8 Hz, Ar-H), 8.22 (d, 3H, J = 5.7 Hz, Ar-H), 7.93 (s, 2H, Ar-H), 4.47 (q, 1H, J = 7.5 Hz, CH), 1.45 (d, 3H, J = 7.2 Hz, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 182.57, 182.51, 174.32, 165.03, 139.14, 139.11, 135.12, 135.04, 133.46, 133.41, 127.43, 127.39, 127.22, 127.19, 126.14, 126.10, 48.82, 40.29, 40.15, 40.01, 39.87, 39.73, 39.60, 39.46, 17.16**.**

N-(9,10-anthraquinone-2-formyl)-L-valine (**1c**): A yellow solid, yield 83%; MS: m/z 350.2 $[M-H]^{-1}$; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 12.73 (s, 1H, COOH), 9.04 (d, 1H, J = 8 Hz, Ar-H), 8.68 (s, 1H, NH), 8.36 (d, 1H, J = 8 Hz, Ar-H), 8.26 (m, 3H, Ar-H), 7.96 (m, 2H, Ar-H), 4.35 (t, 1H, J = 4.5 Hz,

CH), 2.24 (dd, 1H, J = 13.2 Hz, J = 6.9 Hz, CH), 1.01 (t, 6H, J = 5.7 Hz, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 182.54, 182.53, 173.25, 165.96, 139.47, 135.06, 135.04, 133.64, 133.50, 133.45, 133.36, 127.33, 127.23, 127.21, 126.47, 126.44, 59.03, 29.86, 19.69, 19.23.

N-(9,10-anthraquinone-2-formyl)-L-phenylalanine (**1d**): A yellow solid , yield 78% ; MS: m/z 398.1 $[M-H]^{-1}$; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 12.85 (s, 1H, COOH), 9.26 (s, 1H, NH), 8.62 (s, 1H, Ar-H), 8.23 (m, 4H, Ar-H), 7.95 (m, 2H, Ar-H), 7.25 (m, 5H, Ar-H), 4.69 (t, 1H, J = 2.4 Hz, CH), 3.22 (m, 2H, CH_2); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 182.54, 182.49, 173.35, 165.20, 139.21, 138.56, 135.07, 135.03, 133.47, 133.42, 133.24, 129.52, 129.45, 129.07, 128.75, 128.57, 128.47, 127.45, 127.22, 127.18, 126.74, 126.02, 54.94, 40.30, 40.16, 40.02, 39.88, 39.74, 39.61, 39.47, 36.65.

N-(9, 10-anthraquinone-2-formyl)-L-tryptophan (**1e**): A purple solid, yield 83%; MS: m/z 437.1 $[M-H]^{-1}$; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 10.86 (s, 1H, NH), 9.27 (s, 1H, NH), 8.66 (s, 1H, Ar-H), 8.25 (m, 4H, Ar-H), 7.95 (m, 2H, Ar-H), 7.63 (d, 1H, J = 7.7 Hz, Ar-H), 7.17 (m, 3H, Ar-H), 4.72 (t, 1H, J = 4.5 Hz, CH), 3.33 (m, 2H, CH_2); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 182.51, 182.47, 173.65, 165.31, 139.18, 136.50, 135.11, 135.08, 135.05, 133.48, 133.46, 133.43, 133.35, 127.51, 127.41, 127.23, 127.19, 126.12, 123.91, 121.35, 118.80, 118.52, 111.82, 110.76, 54.34, 40.30, 40.16, 40.02, 39.88, 39.75, 39.61, 39.47, 26.95.

N-(9,10-anthraquinone-2-formyl)-*L*-aspartic acid (**1f**): A green solid , yield 83%; MS: m/z 366.1 $[M-H]^{-1}$; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 12.73 (s, 2H, COOH), 9.31 (d, 1H, J = 7.7 Hz, Ar-H), 8.66 (d, 1H, J = 1.3 Hz, Ar-H), 8.28 (m, 4H, Ar-H), 7.95 (d, 2H, J = 5.8 Hz, Ar-H), 4.82 (q, 1H, J = 7.6 Hz, CH), 2.85 (m, 2H, CH_2); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 182.51, 182.47, 172.79, 172.23, 164.92, 139.07, 135.12, 135.05, 134.79, 133.49, 133.46, 133.41, 133.36, 127.52, 127.23, 127.19, 125.97, 50.02, 40.28, 40.14, 40.00, 39.86, 39.72, 39.59, 39.45.

N-(9,10-anthraquinone-2-formyl)-L-glutamic acid (**1g**): A yellow solid , yield 81%; MS: m/z 380.1 $[M-H]^{-1}$; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 12.54 (s, 1H, COOH), 9.13 (s, 1H, NH), 8.69 (s, 1H, Ar-H), 8.36 (d, 1H, J = 8.1 Hz, Ar-H), 8.26 (m, 3H, Ar-H), 7.95 (m, 2H, Ar-H), 4.47 (t, 1H, J = 4.5 Hz, CH), 2.40 (t, 2H, J = 7.2 Hz, CH_2), 2.07 (m, 2H, CH_2); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 182.58, 182.53, 174.19, 173.50, 165.51, 139.10, 138.78, 135.22, 135.13, 135.06, 133.48, 133.46, 133.44, 127.46, 127.24, 127.21, 126.14, 52.58, 40.29, 40.15, 40.01, 39.88, 39.74, 39.60, 39.46, 30.78, 26.20.

N-(9,10-anthraquinone-2-formyl)-L-glutamine (**1h**): A green solid , yield 75%; MS: m/z 379.1 $[M-H]^{-1}$; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 12.54 (s, 1H, COOH), 9.12 (d, 1H, J = 7.6 Hz, Ar-H), 8.68 (d, 1H, J = 1.7 Hz, Ar-H), 8.35 (dd, 1H, J = 8.1 Hz, J = 1.7 Hz, NH), 8.29 (d, 1H, J = 8.0 Hz, Ar-H), 8.23 (m, 2H, Ar-H), 7.94 (m, 2H, Ar-H), 4.47(m, 1H, CH), 2.39 (t, 2H J = 7.6 Hz, CH_2), 2.14 (m, 1H, CH_2), 2.00 (m, 1H, CH_2); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 182.52, 182.47, 174.25, 173.57, 165.45, 139.14, 135.10, 135.05, 134.99, 133.47, 133.44, 133.39, 127.45, 127.23, 127.20, 126.17, 126.13, 52.68, 40.29, 40.15, 40.01, 39.87, 39.73, 39.59, 39.45, 30.86, 26.30.

N-(9,10-anthraquinone-2-formyl)-D-phenylalanine (**1i**): A yellow solid, yield 83%; MS: m/z 398.2 $[M-H]^{-1}$; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 12.85 (s, 1H, COOH), 9.26 (s, 1H, NH), 8.62 (s, 1H, Ar-H), 8.23 (m, 4H, Ar-H), 7.95 (m, 2H, Ar-H), 7.25 (m, 5H, Ar-H), 4.69 (t, 1H, J = 2.4 Hz, CH), 3.22 (m, 2H, CH_2); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 182.57, 182.49, 173.35, 165.20, 139.21, 138.56, 135.07, 135.03, 133.47, 133.42, 133.24, 129.52, 129.45, 129.07, 128.75, 128.57, 127.45, 127.22, 127.18, 126.74, 126.25, 126.02, 54.94, 40.30, 40.16, 40.02, 39.88, 39.74, 39.61, 39.47, 36.65.

N-(9,10-anthraquinone-2-formyl)-D-tryptophan (**1j**): A brown solid, yield 83%; MS: m/z 437.1 $[M-H]^{-1}$; ^{1}H NMR (600 MHz, DMSO-*d6*): δ = 10.86 (s, 1H, NH), 9.28 (s, 1H,NH), 8.66 (s, 1H), 8.25 (m, 4H), 7.95 (m, 2H), 7.63 (d, 1H, J = 7.6 Hz,), 7.16 (m, 3H), 4.86 – 4.65 (m, 1H), 3.32 (dd, 2H, J = 14.3, 7.1 Hz,); ^{13}C NMR (150 MHz, DMSO-d_6) δ = 182.56, 182.51, 173.65, 165.31, 139.18, 136.50,

135.08, 135.05, 134.99, 133.48, 133.46, 133.43, 133.35, 127.51, 127.41, 127.23, 127.19, 126.12, 123.91, 121.35, 118.80, 118.52, 111.82, 110.76, 54.34, 40.30, 40.16, 40.02, 39.88, 39.75, 39.61, 39.47, 26.95.

N-benzoyl-L-phenylalanine (**8a**): A yellow solid, yield 86%; MS: m/z 268.2 $[M-H]^{-1}$; ^{1}H NMR (600 MHz, DMSO-d_6) δ = 12.77 (s, 1H, OH), 8.70 (d, 1H, J = 8.2 Hz, NH), 7.81 – 7.76 (m, 2H, Ar-H), 7.51 (d, 1H, J = 7.4 Hz, Ar-H), 7.45 (d, 2H, J = 7.8 Hz, Ar-H), 7.32 (d, 2H, J = 7.3 Hz, Ar-H), 7.27 (t, 2H, J = 7.6 Hz, Ar-H), 7.18 (t, 1H, J = 7.3 Hz, Ar-H), 4.62 (m, 1H, CH_2), 3.19 (m, 1H, CH_2), 3.07 (m, 1H, CH); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 173.70, 166.82, 138.69, 136.99, 134.38, 131.83, 129.77, 129.53, 129.14, 128.72, 128.65, 127.80, 127.35, 126.82, 54.70, 40.39, 40.25, 40.11, 39.97, 39.84, 39.70, 39.56, 36.71.

N-benzoyl-D-phenylalanine (**8b)**: A brown solid, yield 89%; MS: m/z 268.1 $[M-H]^{-1}$; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 12.76 (s, 1H, OH), 8.70 (d, 1H, J = 8.1 Hz, NH), 7.81 – 7.77 (m, 2H, Ar-H), 7.52 (t, 1H, J = 7.3 Hz, Ar-H), 7.45 (t, 2H, J = 7.8 Hz, Ar-H), 7.32 (d, 2H, J = 7.5 Hz, Ar-H), 7.27 (t, 2H, J = 7.6 Hz, Ar-H), 7.18 (t, 1H, J = 7.3 Hz, Ar-H), 4.62 (m, 1H, CH_2), 3.19 (m, 1H, CH_2), 3.07 (m, 1H, CH); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 173.70, 166.82, 138.69, 136.99, 134.38, 131.83, 129.77, 129.53, 129.14, 128.72, 128.65, 127.80, 127.35, 126.82, 54.70, 40.39, 40.25, 40.11, 39.97, 39.84, 39.70, 39.56, 36.71.

N-benzoyl-L-tryptophan (**8c**): A brown solid, yield 88%; MS: m/z 307.2 $[M-H]^{-1}$; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 12.69 (s, 1H, OH), 10.81 (s, 1H, NH), 8.63 (s, 1H, NH), 7.82 (d, 1H, J = 7.1 Hz, Ar-H), 7.60 (d, 1H, J = 7.9 Hz, Ar-H), 7.52 (t, 1H, J = 7.4 Hz, Ar-H), 7.44 (t, 2H, J = 7.6 Hz, Ar-H), 7.32 (d, 1H, J = 8.1 Hz, Ar-H), 7.21 (d, 1H, J = 2.2 Hz, Ar-H), 7.08 – 7.03 (m, 2H, Ar-H), 6.98 (t, 1H, J = 7.4 Hz, Ar-H), 4.66 (m, 1H, CH_2), 3.30 (d, 1H, J = 4.4 Hz, CH_2), 3.22 (m, 1H, CH); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 173.70, 166.83, 138.68, 136.99, 134.37, 131.84, 129.77, 129.53, 129.14, 128.72, 128.66, 127.81, 127.35, 126.83, 54.69, 40.38, 40.25, 40.11, 39.97, 39.83, 39.69, 39.55, 36.71.

N-benzamido-D-tryptophan (**8d**): A brown solid ,yield 85%; MS: m/z 307.2 $[M-H]^{-1}$; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 12.69 (s, 1H, OH), 10.81 (s, 1H, NH), 8.63 (s, 1H, NH), 7.83 – 7.81 (m, 2H, Ar-H), 7.60 (d, 1H, *J* = 7.9 Hz, Ar-H), 7.52 (t, 1H, *J* = 7.4 Hz, Ar-H), 7.44 (t, 2H, *J* = 7.6 Hz, Ar-H), 7.32 (d, 1H, *J* = 8.1 Hz, Ar-H), 7.21 (d, 1H, *J* = 2.0 Hz, Ar-H), 7.06 (t, 1H, *J* = 7.2 Hz, Ar-H), 6.98 (t, 1H, *J* = 7.2 Hz, Ar-H), 4.66 (m, 1H, CH_2), 3.30 (d, 1H, *J* = 4.4 Hz, CH_2), 3.22 (dd, 1H, *J* = 14.7, 10.0 Hz, CH); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 174.08, 166.82, 136.57, 134.41, 132.00, 131.82, 129.14, 128.69, 127.84, 127.61, 124.04, 121.42, 118.85, 118.61, 111.90, 110.92, 54.15, 40.38, 40.24, 40.10, 39.96, 39.82, 39.68, 39.54, 27.10.

XO inhibitory activity

Bovine in vitro XO inhibitory potency was assayed spectrophotometrically by measuring the production of uric acid at 294 nm at 25°C. The reactive mixture contained 0.1 M sodium pyrophosphate buffer (pH 8.3), 0.3 mM Na_2EDTA, 100 μM xanthine, 25 U/L XO (Sigma, X1875) and the tested compound. The inhibition of XO was evaluated by the reduction of the uric acid formation. The enzyme was pre-incubated for 10 min with the tested compound at 25℃, and the reaction was started by an addition of xanthine. All tests were performed in triplicate. Compounds presenting inhibitory effects over 50% at a concentration of 66 μM were further tested at a wide range of concentrations to calculate their IC_{50} values using SPSS 20.0 software.

Molecular modeling

Molecular modeling studies were performed with MOE (Molecular Operating Environment, version 2016.08, Chemical Computing Group Inc., and Canada) software. The crystal structure of bovine XO in complex with febuxostat (PDB code: 1N5X) was adopted as docking receptor.[7] The structure was protonated, polar hydrogens were added and energy minimization was carried out (RMSD gradient = 0.1 kcal/mol, AMBER10: EHT field).[28] The binding site was designated by the original ligand atoms and other parameters were maintained as the defaults.[29] The standard protocol implemented in MOE was used in docking calculations. The 3-D binding modes were analyzed by *Surfaces and Maps tool* of the MOE software.

Results and discussion

Chemistry

The synthesis of compounds **1a-j** is outlined in **Scheme 1**. Commercially available phthalic anhydride reacted with toluene *via* a Friedel-Craft reaction to yield 2-(4-methylbenzoyl)benzoic acid (**2**), which underwent an intramolecular cyclization in conc. H_2SO_4 conditions, leading to 2-methyl-9.10-anthraquinone (**3**).[25] The oxidation of **3** with chromium trioxide in an acetic acid solution obtained 9,10-anthraquinone-2-carboxylic acid (**4**),[26] which was then treated with thionyl chloride to get 9,10-anthraquinone-2-carbonyl chloride (**5**). A solution of amino acid in methanol was treated with thionyl chloride to provide its methyl ester (**6**).[27] Acylation of **6** with **5** produced methyl *N*-(9,10-anthraquinone-2-carbonyl)amino carboxylates (**7**, as shown in the Supporting Information), which were hydrolyzed with sodium hydroxide to provide target compounds **1a-j**. Compounds **9a-d** were synthesized by a similar procedure for the preparation of compounds **1a-j** as shown in **Scheme 2**. The structures of the synthesized compounds were elucidated by MS, ^{1}H NMR and ^{13}C NMR. All spectral data were in accordance with assumed structures.

Biological activity

In vitro bovine XO inhibitory potency was spectrophotometrically measured by determining the uric acid formation at 294 nm. The testing method has been described in our previous study.[12] Allopurinol was included as a reference compound. Compounds presenting inhibitory effects higher than 50% at the concentration of 60 μM were further tested at a wide range of concentrations to calculate associated IC_{50} values. The results are shown in **Table 1**.

Eight natural amino acid derivatives (**1a-h**) were initially synthesized as shown in **Table 1**. Only two aromatic amino acid derivatives (**1d** and **1e**) were effective, whereas the rest were inactive (*i.e.*, possessing less 50% inhibition at the concentration of 60 μM). Among them, the *L*-tryptophan

derivative **1e** (IC_{50} = 35.6 μM), bearing an (indol-3-yl)methyl for R_1 group, possessed a weak potency; and the *L*-phenylalanine derivative **1d** (IC_{50} = 3.0 μM) with a benzyl for R_1 group, fortunately presented a promising potency and was 2.7-fold more effective than the positive control allopurinol (IC_{50} = 8.1 μM). Removal of the R_1 group produced inactive glycine derivative (**1a**), meaning that the R_1 group is requisite for the potency; moreover, replacement of the aromatic R_1 group with alkyl (**1b** and **1c**) or further introduction of a carboxyl (**1f** and **1g**) or an amide fragment (**1h**) in R_1 group resulted in vanished potency, suggesting that the aromatic R_1 group played a crucial role for the potency.

Furthermore, a *D*-phenylalanine derivative (**1i**, IC_{50} = 2.9 μM) and a *D*-tryptophan derivative (**1j**, IC_{50} = 11.8 μM) were synthesized to explore the influence of three-dimensional configuration. Interestingly, compound **1i** showed comparable potency with its *L*-enantiomer **1d** and compound **1j** exhibited 3-fold higher effects compared with its *L*-counterpart **1e**. This result demonstrated that the potency of *D*-amino acid derivative was equal to its *L*-enantiomer or better.

In addition, to investigate the role of the 9,10-anthraquinone moiety, four structure simplified derivatives (**9a-d**) were involved by replacing the 9,10-anthraquinone moiety of **1d**, **1e**, **1i** and **1j** by a phenyl group, respectively. Expectedly, only compound **9c** (IC_{50} = 50.5 μM) exhibited a weak potency, whereas the rest were totally useless (**Table 2**)**,** showing that the 9,10-anthraquinone moiety obviously benefits the potency of compounds **1d**, **1e**, **1i** and **1j**.

Molecular modeling

Molecular modeling simulations were performed to further understand the action modes of compounds **1d** and **1i** in the XO binding pocket with MOE (Molecular Operating Environment, version 2016.08, Chemical Computing Group Inc., and Canada) software. The crystal structure of bovine XO/febuxostat complex (PDB code 1N5X) was adopted as the protein template.[7] The small molecules were built and minimized with MOE software. The carboxyl group of **1d** and **1i** was calculated in its dissociated form. The docking modes are illustrated in **Fig. 3**.

As presented in **Fig. 3**, compound **1d** appeared as a flat conformation in the active pocket and partially overlaid with the original ligand febuxostat.[7] The benzyl moiety inserted into the long-narrow active pocket at a position of 2.75 Å from Glu1261 and 3.50 Å from molybdenum atom and the phenyl ring formed a strong Pi-Pi stack interaction with Phe914. The presence of the benzyl moiety made the ligand draw back from the cavity and therefore led the carboxyl group far away from Arg880. However, the carboxyl group was till a powerful pharmacophore which strongly interacted with Thr1010 and Val1011 by H-bonds. Additionally, the flat 9,10-anthraquinone ring occupied the outer region of the active pocket and stacked with isobutoxyphenyl moiety of febuxostat and a carbonyl group of 9,10-anthraquinone formed an H-bond with Lys771, meaning that the 9,10-anthraquinone played a well combined role of the benzonitrile moietyand the lipophilic tail of febuxostat as expected. The binding model of **1i** in XO binding pocket was as similar as that of **1d**. The differences are that **1i** was more close to Glu1261 (2.57 Å) and absented the H-bond interaction with Lys771. However, the H-bond interactions between carboxylate and the Thr1010 were stronger than that of **1d**. As a result, compounds **1d** and **1i** exhibited the equal potency. The above interactions provided rational action modes of **1d** and **1i** in XO binding pocket and well interpreted their XO inhibitory potency.

Conclusion

Herein, we report a series of *N*-(9,10-anthraquinone-2-carbonyl)amino acid derivatives (**1a-j**) as novel XO inhibitors. Among them, the *L/D*-phenylalanine derivatives (**1d** and **1i**) and *L/D*-tryptophan derivatives (**1e** and **1j**) were effective with micromolar level potency. Particularly, **1d** and **1i** presented the highest potency, which were better than that of allopurinol (IC_{50} = 8.1 μM). Preliminary SAR analysis exhibited that an aromatic amino acid fragment, *e.g.*, phenylalanine or tryptophan, was essential for the inhibition; the *D*-amino acid derivative presented equal or greater effects compared with its *L*-enantiomer; and the 9,10-anthraquinone moiety was welcome for the inhibitory potency. Furthermore, molecular modeling simulations of **1d** and **1i** indicated that the phenyl moiety could insert into the long-narrow pocket and form a Pi-Pi stack interaction with Phe914; and the carboxyl group strongly interacted with Thr1010 and Val1011 by H-bonds. In a word two promising lead compounds, **1d** and **1i,** were successfully discovered in this work and further investigation based on **1d** and **1i** is in progress.

Author contributions

F. Meng and T. Zhang conceived and designed the experiments; S. Li and W. Yuan synthesized the compounds and evaluated the activity; Y. Zhang collected the spectral data; T. Zhang performed the molecular modeling and wrote the manuscript; F. Meng reviewed the manuscript. All authors have read and approved the final manuscript.

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Conflict of interest

The authors have declared no conflict of interest.

Legends for Fig.1, Fig. 2, Fig. 3, Scheme 1, Scheme 2, Table 1 and Table 2.

Fig. 1 Chemical structures of allopurinol, febuxostat and topiroxostat.

Fig. 2 Design of the target compounds **1a-j**.

Fig. 3 (**A**) Binding mode of compoud **1d** (blue) overlaid with the original ligand febuxostat (orange) in XO binding pocket; (**B**) binding mode of compoud **1i** (cyan) overlaid with the original ligand febuxostat (orange) in XO binding pocket; (**C**) interaction of compound **1d** in XO binding pocket; (**D**) interaction of compound **1i** in XO binding pocket.

Scheme 1. Reagents and conditions: (i) toluene, $AlCl_3$, 50 °C, 4 h; (ii) H_2SO_4, 100 °C, 1 h; (iii) CrO_3, AcOH, reflux, 1 h; (iv) $SOCl_2$, DMF, DCM, 40 °C, overnight; (v) $SOCl_2$, MeOH, 0 °C then rt, overnight; (vi) Et_3N, DCM, 0 °C then rt, overnight; (vii) NaOH, MeOH, THF, 50 °C, 4 h.

Scheme 2. Reagents and conditions: (i) $SOCl_2$, DMF, DCM, 40 °C, overnight; (ii) $SOCl_2$, MeOH, 0 °C then rt, overnight; (iii) Et_3N, DCM, 0 °C then rt, overnight; (iv) NaOH, MeOH, THF, 50 °C, 4 h.

Table.1 in vitro XO inhibitory potency of designed compounds **1a-j**.

Table.2 in vitro XO inhibitory potency of compounds **9a-d.**

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Table.1 in vitro XO inhibitory potency of designed compounds **1a-j**.

Compound	R_1	R_2	(*L/D*)	Inhibition rate at 60 μM (%)	IC_{50} (μM)
1a	H	glycine	/	29.3	n.a. [a]
1b	CH_3	alanine	*L*	20.8	n.a.
1c	$(CH_3)_2CH_2$	valine	*L*	24.0	n.a.
1d	benzyl	phenylalanine	*L*	97.5	3.0± 0.32 [#]
1e	(indol-3-yl)methyl	tryptophan	*L*	70.4	35.6± 1.23 [#]
1f	$HOOCCH_2$	aspartic acid	*L*	28.2	n.a.
1g	$HOOCCH_2CH_2$	glutamic acid	*L*	10.6	n.a.
1h	$H_2NCOCH_2CH_2$	glutamine	*L*	20.8	n.a.
1i	benzyl	phenylalanine	*D*	99.1	2.9± 0.26 [#]
1j	(indol-3-yl)methyl	tryptophan	*D*	90.1	11.8± 0.84 [#]
Allopurinol	/	/	/	99.6	8.1± 1.02

[a] n.a.: not active (<50% inhibition at 60 μM).

[#] $P < 0.05$, versus allopurinol.

Table.2 in vitro XO inhibitory potency of compounds **9a-d.**

Compound	R_1	R_2	(*L/D*)	Inhibition rate at 60 μM (%)	IC_{50} (μM)
9a	benzyl	phenylalanine	*L*	17.9	n.a. [a]
9b	benzyl	phenylalanine	*D*	60.3	50.5± 2.12
9c	(indol-3-yl)methyl	tryptophan	*L*	18.9	n.a.
9d	(indol-3-yl)methyl	tryptophan	*D*	21.5	n.a.

[a] n.a.: not active (<50% inhibition at 60 μM).