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Design, synthesis and biological evaluation of 5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitrile derivatives as xanthine oxidase inhibitors

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Abstract

A series of 5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitrile derivatives (**1a-p**) was designed, synthesized and identified as xanthine oxidase inhibitors with micromolar level potencies. Among them, the most promising compounds **1j** and **1k** were obtained with IC_{50} values of 8.1 μM and 6.7 μM, respectively. The Lineweaver-Burk plot revealed that compound **1k** acted as a mixed-type xanthine oxidase inhibitor. SARs analysis revealed that a carbon atom occupying the X^3 position is not as effective as a nitrogen atom; and an *iso*-pentyloxy or a cyclopentyloxy at the 2-position of benzonitrile moiety will benefit the inhibitory potency. The basis of xanthine oxidase inhibition by **1k** was rationalized by molecular modeling studies.

Keywords: 1,2,3-Triazole; Topiroxostat; Xanthine oxidase inhibitor.

Introduction

Xanthine oxidase (XO) is a critical, rate-limiting enzyme in purine metabolism. It catalyzes the last two steps of purine catabolism in humans, *i.e.*, hydroxylation of hypoxanthine to xanthine and xanthine to uric acid.[1] In parallel with the hydroxylation process, two kinds of reactive oxygen species (ROS), superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), are produced.[2] XO is therefore a critical source of uric acid and ROS. Over-production of uric acid can lead to hyperuricemia, which is the key cause of gout. Thus, XO is considered the most promising target for treating hyperuricemia and gout.[3, 4] Meanwhile, an excess of ROS could cause various pathological states including inflammation, metabolic disorders, atherosclerosis, cancer and chronic obstructive pulmonary disease.[5, 6] Thus, inhibition of XO could reduce the formation of ROS and benefit the treatment of these diseases.[7, 8] Allopurinol is a prototypical XO inhibitor and has been widely used in the treatment of hyperuricemia and gout for several decades. However, in some cases, it has been reported that allopurinol and its analogues, which possess a similar backbone of purine, can cause severe life-threatening side effects.[9] Therefore, searching for novel non-purine XO inhibitors with more potent XO inhibitory potency but fewer side effects has always been a hotspot.

In the past two decades, numerous classical non-purine XO inhibitors characterized by a five-membered ring linking a carboxyl group (febuxostat analogues) or a pyridine (topiroxostat analogues) to an aryl nitrile moiety have been reported (**Fig. 1**). Febuxostat[10] is a classical non-purine XO inhibitor, that bears a thiazole moiety as the five-membered ring linker, has an outstanding inhibitory potency as well as acceptable side effects and was approved by the US Food and Drug Administration (FDA) in 2009. Thereafter, a lot of febuxostat analogues have been reported, such as Y-700,[11] selenazoles,[12] 2-(indol-5-yl)thiazoles,[13] isoxazoles,[14] imidazoles,[15] and 1,2,3-triazoles.[16] Topiroxostat (also known as FYX-051) is another typical non-purine XO inhibitor, which bears a 1,2,4-triazole as five-membered ring linker,[17] and was approved in Japan in 2013. The action mechanism of topiroxostat is not only occupying the active pocket but also covalently bonding with the receptor,[18] which makes it a powerful enzymatic inhibitor. In general, topiroxostat analogues were not as abundant as febuxostat analogues, a typical representative

is the pyridyl-1,2,4-triazoles reported by Takahiro Sato *et al*[19] which possess excellent potency (**Fig. 1**). Furthermore, other XO inhibitors with various structural classes have also been recently published, including isocytosines,[5, 20-22] *N*-(1,3-diaryl-3-oxo-propyl)amides,[23] *N*-acetyl pyrazolines,[24] hydroxylated chalcones,[25] 9-deazaguanines,[26] benzimidazoles,[27] flavonoids,[28, 29] fraxamosides,[30] pyrano[3,2-d]pyrimidines,[31] 2-arylbenzo[b]furans[32] and benzaldehydes.[33]

We have focused on the structural modifications of five-membered ring and the structure activity relationship (SAR) investigations of classical XO inhibitors.[14-16] Our previous studies disclosed that an oxime-like fragment fixing at the X^1 position will be beneficial[15] and a polar N atom occupying the X^2 position will be disadvantage for the potency of febuxostat analogues.[16] Since 1,2,3-triazole is a strictly isostere of 1,2,4-triazole as well as a welcome molecular building block in drug design.[34, 35] in this work, we used a 1,2,3-triazole to replace the 1,2,4-triazole of topiroxostat, and designed a series of 5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitrile derivatives (**1a-p**) as topiroxostat analogues (**Fig. 1**) to investigate the structural modification of the X^3 position. The target compounds were prepared *via* a Huisgen 1,3-dipolar cycloaddition reaction under microwave conditions to build the crucial 1,2,3-triazole ring. The in vitro activity was evaluated by enzymatic inhibition assays. The synthesized compounds have been patented by our team.[36] In addition, molecular modeling and the steady-state kinetic analysis were both carried out to explore the inhibitory behaviors of these compounds.

Results and discussions

Chemistry

The synthesis of target compounds **1a-p** was performed as outlined in **Scheme 1**. Compounds **5** were considered as the key intermediates, of which synthetic method has been reported in our previous studies.[16] In brief, commercially available 2-hydroxybenzonitrile was nitrated by conc. HNO_3 in an AcOH solution to provide 2-hydroxy-5-nitrobenzonitrile (**2**). Compound **2** was alkylated with corresponding alkyl bromides or alkyl chlorides to produced 2-alkoxy-5-nitrobenzonitriles (**3**), which followed by the nitro reduction to yield 2-alkoxy-5-aminobenzonitriles (**4**). The diazotization of **4** followed by the treatment with

sodium azide yielded the desired intermediates 2-alkoxy-5-azidobenzonitriles (**5**). Cyclization of **5** with 4-ethynylpyridine hydrochloride in the presence of copper sulfate and vitamin C under microwave conditions produced 5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitriles **1a-p**. The structures of the synthesized compounds were elucidated by ^{1}H NMR, ^{13}C NMR and MS. All the spectral data were in accordance with the assumed structures.

Biological activity

Bovine XO *in vitro* inhibitory potencies by target compounds **1a**–**p** were determined by spectrophotometrically measuring uric acid levels at 294 nm.[17, 37] Allopurinol and topiroxostat were included as reference compounds. In general, half of the synthesized compounds possessed the micromolar level potencies, some of which were comparable to allopurinol but much lower than the leading compound topiroxostat. Nevertheless, we were interested in summarizing the SARs and investigating their inhibitory behaviors, and expected to provide some insights for the further investigations.

Table 1. *In vitro* XO inhibitory potencies of compounds **1a**-**p**.

Compound	R group	IC_{50} (μM)
1a	Methyl	n.a. [a]
1b	*n*-Propyl	n.a.
1c	*n*-Butyl	20.8± 0.94 [#]
1d	*n*-Pentyl	16.3± 1.96 [#]
1e	Allyl	n.a.
1f	Methoxyethyl	n.a.
1g	*iso*-Propyl	25.4± 1.11 [#]
1h	*iso*-Butyl	n.a.
1i	*sec*-Butyl	18.0± 2.12 [#]

1j	iso-Pentyl	8.1± 1.16
1k	Cyclopentyl	6.7± 0.70 #
1l	Cyclohexyl	n.a.
1m	Benzyl	45.0± 4.43 #
1n	*para*-Methoxybenzyl	23.7± 2.12 #
1o	*para*-Chlorobenzyl	n.a.
1p	*para*-Cyanobenzyl	n.a.
Allopurinol	/	8.5± 0.67
Topiroxostat	/	0.016± 0.003

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

# $P < 0.05$, versus allopurinol.

In the linear alkyl subseries of **1a-e** as shown in **Table 1**, *n*-butyl derivative (**1c**) and *n*-pentyl derivative (**1d**) were effective; especially, **1d** exhibited a relatively high potency with an IC_{50} value of 16.3 μM. The transition of the *n*-butyl (**1c**, IC_{50} =20.8 μM) to the methoxyethyl (**1h**) accompanied by a loss of the potency, meaning that the inserted oxygen atom could damage the potency and that a polar atom fusing in the R group may be not welcome. This point was also true in the 1,2,3-triazole series.[16]

Generally speaking, the branched alkyl derivatives (**1g-j**) showed stronger potency than their linear alkyl counterparts (*e.g.*, **1g** versus **1b**; **1i** versus **1c**; **1j** versus **1d**), the exception was **1h**, bearing an *iso*-butyl but being totally inactive. The most potent compound in branched alkyl subseries was **1j** (R = *iso*-pentyl, IC_{50} = 8.1 μM), which showed a comparable potency to allopurinol (IC_{50} = 8.5 μM). Surprisingly, a cyclopentyl derivative (**1k**, IC_{50} = 6.7 μM) presented the highest potency in the series and was slightly more effective than allopurinol. However, expanding the ring to the cyclohexyl (**1l)**, the potency completely lost. In addition, some benzyl derivatives (**1m-p**) were also synthesized. Only **1m** (R = benzyl, IC_{50} = 45.0 μM) and **1n** (R = *para*-methoxybenzyl, IC_{50} = 23.7 μM) exhibited weak potency, but the rest were thoroughly inactive.

To sum up, compounds **1j** and **1k** were identified as the most promising XO inhibitors in the series with comparable inhibitory effects to allopurinol. However, in comparison to topiroxostat (IC_{50} = 0.016 μM) and the pyridyl-1,2,4-triazoles,[19] their potencies were much lower, which may be a direct result of the structural changes at the X^3 and X^4 positions. The X^4 position fusing an N atom would be acceptable (*e.g.*, Y-700); however, a carbon atom occupying the X^3 position may be not as welcome as a nitrogen atom, as nearly all the classical XO inhibitors possessing an N atom at the X^3 position.[9] In order to further investigate their inhibitory behaviors in molecular level, the molecular modeling simulations were performed in the following step.

Molecular modeling

To foresee the possible interactions and to rationalize the activities of the synthesized compounds against XO, molecular modeling simulations of topiroxostat and the representative compound **1k** in the binding pocket of XO were performed with MOE (Molecular Operating Environment, version 2016.08, Chemical Computing Group Inc., Canada) software. The crystal structure of bovine XO in complex with topiroxostat (PDB code: 1V97) was used in the docking calculations.[18] In order to validate the docking protocol, a self-docking of topiroxostat into the binding pocket was firstly performed. As shown in **Fig. 2A**, topiroxostat stacked well with the crystallographic ligand, and the H-bonds predicted by MOE were similar to those found in the crystal structure. Although the covalent bonding, as co-crystal structure emerged,[18] didn't be simulated, this protocol may provide insights into the other interactions besides the covalent bonding.

As shown in **Fig. 2B**, the **1k** docking conformation and the original ligand played a set of similar interactions with XO binding pocket, such as pyridine N atom interacting with Glu1261, the triazole ring linking to Arg880 and Thr1010 residues by a water bridge and the cyano group accepting an H-bond from Asn768. Additionally, the cyclopentyloxy ether tail was surrounded by several lipophilic amino acid residues such as Leu648, Phen649 and Phen1013. The interactions provided a reasonable explanation for the potency of **1k**.

However, some adverse conformation features were also observed. As shown in **Fig. 2B** and **2C**, the topiroxostat X^3-N directly pointed toward the Glu802 terminal carboxyl by a

short distance of 2.84 Å. The transition from the X^3-N of topiroxostat to the X^3-CH of **1k** may induce a hydrophobic repulsion and lead the 1,2,3-triazole moiety twisted to keep away from the Glu802 (**Fig. 2C**). We respectively measured the pyridine-triazole dihedral angles of the **1k** docking pose, the energy minimized **1k** pose and the topiroxostat crystallographic pose by MOE software; the former (20.3°) was much bigger than the latter two (1.2° and 0.6°, respectively), meaning that the stability of **1k** in XO binding pocket was not as well as that of topiroxostat (**Fig. 2C** and 2**D**). This uncomfortable binding mode provided a possibly explanation of the lower potency of **1k**.

Steady-state kinetic analysis

The enzyme kinetic studies were carried out to further investigate the action mode of the representative compound **1k** with XO (**Fig. 3**). The Lineweaver-Burk plots analysis demonstrated that **1k** acted as a mixed-type XO inhibitor. This action mode was distinguished with topiroxostat, but similar with the hydroxy-topiroxosat, a metabolite of the topiroxostat possessing a micromolar level potency.[17] The different inhibition type may be another factor for the lower potency of **1k** than that of topiroxostat.

Conclusions

We designed, synthesized and identified a series of 5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitrile derivatives as XO inhibitors (**1a-p**). SARs analysis revealed that a carbon atom occupying the X^3 position is not as potent as a nitrogen atom; and an *iso*-pentyloxy or a cyclopentyloxy at the 2-position of benzonitrile moiety will benefit the inhibitory potency. As a result, the most promising compounds **1j** and **1k** were obtained with IC_{50} values of 8.1 μM and 6.7 μM, respectively. The Lineweaver-Burk plot showed that compound **1k** acted as a mixed-type XO inhibitor and molecular modeling provided a reasonable bonding mode for its inhibitory behaviors. Further investigations based on **1j** and **1k** were in progress.

Experimental section

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). Melting points were recorded on an RD-1 melting apparatus and were uncorrected. ^{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. ESI-HRMS data were gathered using a Bruker microTOF-Q instrument.

General procedure for the synthesis of 2-alkoxy-5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitriles (1a-p)

A solution of **5** (742 μmol), 4-ethynylpyridine hydrochloride (1110 μmol), copper sulfate pentahydrate (89 μmol) and vitamin C (89 μmol) in ethanol (10 mL) and water (10 mL) was heated under microwave conditions at 50°C for 8 min. After the completed reaction, about half of the solution was evaporated in vacuum. The residue was diluted with water. The formed precipitate was collected by filtration and purified by column chromatography (ethyl acetate: petroleum ether = 1: 3 to 1:1) to provide the corresponding 2-alkoxy-5-(4-(pyridin-4-yl)-1H-1,2,3-triazol-1-yl)benzonitriles (**1a-p**).

2-Methoxy-5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitrile (1a)

A pink powder, yield 39.0%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.71 (br s, 2H), 8.36 (d, J = 2.7 Hz, 1H), 8.27 (dd, J = 9.1, 2.7 Hz, 1H), 7.87 (d, J = 4.9 Hz, 2H), 7.53 (d, J = 9.2 Hz, 1H), 4.02 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.91, 150.53, 144.98, 137.25, 129.73, 127.16, 125.43, 122.01, 119.64, 115.36, 113.77, 101.13, 57.03. ESI-HRMS calcd. for $C_{15}H_{12}N_5O$ $[M + H]^+$ 278.1036, found: 278.1050.

2-Propoxy-5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitrile (1b)

A white powder, yield 41.2%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.70 (d, J = 4.6 Hz, 2H), 8.34 (d, J = 2.7 Hz, 1H), 8.23 (dd, J = 9.1, 2.7 Hz, 1H), 7.86 (d, J = 5.9 Hz, 2H), 7.52 (d, J = 9.2 Hz, 1H), 4.21 (t, J = 6.4 Hz, 2H), 1.87 – 1.75 (m, 2H), 1.03 (t, J = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.29, 150.50, 144.95, 137.27, 129.58, 127.03, 125.33, 121.92, 119.56, 115.29, 114.45, 101.35, 70.85, 21.73, 10.15. ESI-HRMS calcd. for $C_{17}H_{16}N_5O$ $[M + H]^+$ 306.1349, found: 306.1352.

2-Butoxy-5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitrile (1c)

A yellow powder, yield 80.4%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.50 (s, 1H), 9.15 (br s, 2H),8.33 (d, J = 2.7 Hz, 1H), 8.22 (dd, J = 9.1, 2.7 Hz, 1H), 8.10 (br s, 2H), 7.52 (d, J = 9.2 Hz, 1H), 4.24 (t, J = 6.4 Hz, 2H), 1.83 – 1.73 (m, 2H), 1.54 – 1.43 (m, 2H), 0.97 (t, J = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.27, 152.25, 145.26, 136.46, 129.56, 126.95, 125.26, 121.76, 116.87, 115.28, 114.41, 101.33, 69.20, 30.32, 18.55, 13.62. ESI-HRMS calcd. for $C_{18}H_{18}N_5O$ $[M + H]^+$ 320.1506, found: 320.1521.

2-Pentyloxy-5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitrile (1d)

A white powder, yield 55.0%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.76 (br s, 2H), 8.34 (d, J = 2.7 Hz, 1H), 8.23 (dd, J = 9.1, 2.7 Hz, 1H), 7.88 (s, 2H), 7.52 (d, J = 9.2 Hz, 1H), 4.24 (t, J = 6.5 Hz, 2H), 1.84 – 1.75 (m, 2H), 1.50 – 1.41 (m, 2H), 1.38 (m, 2H), 0.92 (t, J = 7.2 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.29, 150.50, 145.01, 137.10, 129.57, 127.01, 125.32, 121.89, 120.01, 115.29, 114.44, 101.34, 69.48, 27.94, 27.45, 21.77, 13.89. ESI-HRMS calcd. for $C_{19}H_{20}N_5O$ $[M + H]^+$ 334.1662, found: 334.1674.

2-Allyloxy-5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitrile（1e）

An off-white powder, yield 65.0%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.70 (d, J = 3.4 Hz, 2H), 8.37 (d, J = 2.7 Hz, 1H), 8.24 (dd, J = 9.1, 2.7 Hz, 1H), 7.86 (d, J = 5.8 Hz, 2H), 7.53 (d, J = 9.2 Hz, 1H), 6.10 (ddd, J = 22.3, 10.4, 5.1 Hz, 1H), 5.50 (m, 1H), 5.36 (dd, J = 10.6, 1.3 Hz, 1H), 4.86 (d, J = 5.1 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.76, 150.51, 144.95, 137.26, 132.30, 129.79, 126.99, 125.42, 121.97, 119.55, 118.41, 115.29, 114.78, 101.50, 69.70. ESI-HRMS calcd. for $C_{17}H_{14}N_5O$ $[M + H]^+$ 304.1193, found:

304.1185.

2-(2-Methoxyethoxy)-5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitrile（1f）

A white powder, yield 64.8%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.70 (s, 2H), 8.35 (d, J = 2.7 Hz, 1H), 8.23 (dd, J = 9.1, 2.7 Hz, 1H), 7.86 (d, J = 5.7 Hz, 2H), 7.54 (d, J = 9.2 Hz, 1H), 4.44 – 4.34 (m, 2H), 3.80 – 3.70 (m, 2H), 3.36 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.19, 150.51, 144.96, 137.26, 129.74, 127.00, 125.42, 121.96, 119.57, 115.33, 114.59, 101.41, 69.99, 69.09, 58.43. ESI-HRMS calcd. for $C_{17}H_{16}N_5O_2$ $[M + H]^+$ 322.1299, found: 322.1306.

2-Isopropoxy-5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitrile (1g)

An off-white powder, yield 57.5%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.73 (br s, 2H), 8.33 (d, J = 2.2 Hz, 1H), 8.21 (dd, J = 9.1, 2.2 Hz, 1H), 7.87 (s, 2H), 7.56 (d, J = 9.2 Hz, 1H), 4.91 (m, 1H), 1.37 (d, J = 5.9 Hz, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.40, 150.53, 144.98, 137.18, 129.45, 127.04, 125.56, 121.97, 119.97, 115.51, 115.47, 102.12, 72.27, 21.54. ESI-HRMS calcd. for $C_{17}H_{16}N_5O$ $[M + H]^+$ 306.1349, found: 306.1355.

2-Isobutoxy-5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitrile (1h)

An off-white powder, yield 49.7%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.70 (d, J = 4.6 Hz, 2H), 8.35 (d, J = 2.7 Hz, 1H), 8.28 – 8.14 (m, 1H), 7.86 (d, J = 6.0 Hz, 2H), 7.52 (d, J = 9.2 Hz, 1H), 4.03 (d, J = 6.5 Hz, 2H), 2.11 (m, 1H), 1.04 (d, J = 6.7 Hz, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.36, 150.51, 144.95, 137.28, 129.60, 127.02, 125.28, 121.93, 119.55, 115.23, 114.49, 101.37, 75.27, 27.59, 18.72. ESI-HRMS calcd. for $C_{18}H_{18}N_5O$ $[M + H]^+$ 320.1506, found: 320.1509.

2-(*sec*-Butoxy)-5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitrile (1i)

A yellow powder, yield 38.5%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.77 (br s, 2H), 8.33 (d, J = 2.8 Hz, 1H), 8.20 (dd, J = 9.2, 2.8 Hz, 1H), 7.89 (s, 2H), 7.55 (d, J = 9.3 Hz, 1H), 4.79 – 4.63 (m, 1H), 1.72 (m, 2H), 1.33 (d, J = 6.1 Hz, 3H), 0.97 (d, J = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.66, 150.44, 144.98, 137.13, 129.42, 126.95, 125.45, 121.87, 120.15, 115.44, 115.40, 102.10, 76.89, 28.33, 18.74, 9.17. ESI-HRMS calcd. for

$C_{18}H_{18}N_5O$ $[M + H]^+$ 320.1506, found: 320.1519.

2-Isopentyloxy-5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitrile (1j)

A white powder, yield 29.7%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.69 (d, J = 4.4 Hz, 2H), 8.33 (d, J = 2.6 Hz, 1H), 8.22 (dd, J = 9.1, 2.6 Hz, 1H), 7.85 (d, J = 5.7 Hz, 2H), 7.53 (d, J = 9.2 Hz, 1H), 4.34 – 4.22 (m, 2H), 1.83 (m, 1H), 1.70 (q, J = 6.6 Hz, 2H), 0.97 (d, J = 6.6 Hz, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.30, 129.60, 127.07, 125.37, 121.54, 115.29, 114.47, 101.34, 68.07, 36.95, 24.62, 22.38. ESI-HRMS calcd. for $C_{19}H_{20}N_5O$ $[M + H]^+$ 334.1662, found: 334.1669.

2-Cyclopentyloxy-5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitrile（1k）

A white powder, yield 55.0%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.70 (s, 2H), 8.33 (d, J = 2.7 Hz, 1H), 8.22 (dd, J = 9.1, 2.8 Hz, 1H), 7.86 (d, J = 5.4 Hz, 2H), 7.52 (d, J = 9.2 Hz, 1H), 5.25 – 4.97 (m, 1H), 2.16 – 1.91 (m, 2H), 1.91 – 1.70 (m, 4H), 1.65 (dd, J = 7.7, 5.5 Hz, 2H). ESI-HRMS calcd. for $C_{19}H_{18}N_5O$ $[M + H]^+$ 332.1506, found: 332.1512.

2-Cyclohexyloxy-5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitrile（1l）

An off-white powder, yield 50.8%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.82 (br s, 2H), 8.33 (d, J = 2.7 Hz, 1H), 8.20 (dd, J = 9.2, 2.7 Hz, 1H), 7.91 (br s, 2H), 7.58 (d, J = 9.3 Hz, 1H), 4.72 (m, 1H), 1.94 (m, 2H), 1.80 – 1.69 (m, 2H), 1.60 (m, 2H), 1.55 – 1.34 (m, 4H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.24, 150.54, 144.99, 137.13, 129.48, 126.99, 125.52, 121.97, 119.82, 115.76, 115.43, 102.25, 76.37, 30.65, 24.88, 22.60. ESI-HRMS calcd. for $C_{20}H_{20}N_5O$ $[M + H]^+$ 346.1662, found: 346.1677.

2-Benzyloxy-5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitrile（1m）

An off-white powder, yield 37.8%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.74 (br s, 2H), 8.38 (d, J = 2.7 Hz, 1H), 8.25 (dd, J = 9.1, 2.7 Hz, 1H), 7.87 (s, 2H), 7.63 (d, J = 9.2 Hz, 1H), 7.52 (d, J = 7.3 Hz, 2H), 7.45 (t, J = 7.6 Hz, 2H), 7.39 (t, J = 7.3 Hz, 1H), 5.41 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.92, 150.54, 144.98, 137.23, 135.65, 129.90,

128.65, 128.33, 127.71, 127.09, 125.54, 122.05, 119.44, 115.32, 115.01, 101.71, 70.77. ESI-HRMS calcd. for $C_{21}H_{16}N_5O$ $[M + H]^+$ 354.1349, found: 354.1338.

2-((4-Methoxybenzyl)oxy)-5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitrile（1n）

An off-white powder, yield 58.2%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.51 (s, 1H), 9.10 (br s, 2H), 8.36 (d, J = 2.7 Hz, 1H), 8.24 (dd, J = 9.1, 2.7 Hz, 1H), 8.04 (br s, 2H), 7.63 (d, J = 9.2 Hz, 1H), 7.45 (d, J = 8.6 Hz, 2H), 7.00 (d, J = 8.6 Hz, 2H), 5.31 (s, 2H), 3.77 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.00, 159.37, 150.18, 145.17, 129.79, 129.73, 127.43, 127.02, 125.49, 121.96, 120.15, 115.35, 115.06, 114.03, 109.52, 101.69, 70.69, 55.13. ESI-HRMS calcd. for $C_{22}H_{18}N_5O_2$ $[M + H]^+$ 384.1455, found: 384.1462.

2-((4-Chlorobenzyl)oxy)-5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitrile（1o）

An off-white powder, yield 49.2%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.70 (br s, 2H), 8.38 (d, J = 2.7 Hz, 1H), 8.26 (dd, J = 9.1, 2.7 Hz, 1H), 7.86 (d, J = 5.0 Hz, 2H), 7.61 (d, J = 9.2 Hz, 1H), 7.53 (m, 4H), 5.40 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.72, 150.53, 144.97, 137.25, 134.69, 132.96, 129.98, 129.56, 128.68, 127.05, 125.51, 122.02, 119.61, 115.25, 114.98, 101.73, 69.94. ESI-HRMS calcd. for $C_{21}H_{15}ClN_5O$ $[M + H]^+$ 388.0960, found: 388.0968.

2-((4-Cyanobenzyl)oxy)-5-(4-(pyridin-4-yl)-1*H*-1,2,3-triazol-1-yl)benzonitrile（1p）

An off-white powder, yield 31.0%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.52 (s, 1H), 8.74 (br s, 2H), 8.41 (d, J = 2.7 Hz, 1H), 8.27 (dd, J = 9.1, 2.7 Hz, 1H), 7.94 (d, J = 8.2 Hz, 2H), 7.88 (br s, 2H), 7.71 (d, J = 8.2 Hz, 2H), 7.60 (d, J = 9.2 Hz, 1H), 5.53 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.52, 150.54, 145.04, 141.38, 132.64, 130.13, 128.00, 127.11, 125.56, 122.02, 121.28, 118.65, 115.20, 114.93, 110.95, 101.77, 69.73. ESI-HRMS calcd. for $C_{22}H_{15}N_6O$ $[M + H]^+$ 379.1302, found: 379.1312.

XO inhibitory activity

Bovine XO inhibitory potency *in vitro* was assayed spectrophotometrically by measuring the production of uric acid at 294 nm using a UV-2100 spectrophotometer (UNICO). The testing method was based on the procedure reported by Matsumoto *et al.*,[17] with

modification. 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, X4875) 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 50 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 software.[38] The crystal structure of bovine XO in complex with topiroxostat (PDB code: 1V97) was adopted in docking calculations.[39] The structure was protonated, polar hydrogens were added and energy minimization was carried out (RMSD gradient = 0.1 kcal/mol, AMBER10: EHT field).[40] Ligand structures were built and minimized with MOE software. The docking procedure adopted the standard protocol implemented in MOE. The binding site was designated by the original ligand atoms and other parameters were maintained as the defaults.[41] The 3-D binding modes were analyzed by *Surfaces and Maps tool* of the MOE software.

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Conflict of interest

The authors have declared no conflict of interest.

Legend for Fig.1, Fig. 2, Fig. 3 and Scheme 1.

Fig. 1 The chemical structures of some XO inhibitors and designed compounds **1a-p**.

Fig. 2 (A) docking conformation of topiroxostat (green) overlaid with the crystal conformation of topiroxostat (orange); **(B)** and **(C)** docking conformation of **1k** (cyan) overlaid with the crystal conformation of topiroxostat (orange); **(D)** docking conformation of **1k** (cyan) overlaid with its energy minimized conformation (pink) calculated by MOE software.

Fig. 3 Lineweaver-Burk plot analysis of XO inhibition by compound **1k**.

Scheme 1. Reagents and conditions: (i) HNO_3, AcOH, 50°C, 4 h; (ii) RCl or RBr, K_2CO_3, KI, DMF, 60°C, overnight; (iii) Fe, NH_4Cl, EtOH, H_2O, reflux, 3 h; (iv) 1) $NaNO_2$, AcOH, H_2O, -10°C, 30 min; 2) NaN_3, 0°C, 3 h; (v) 4-ethynylpyridine hydrochloride, $CuSO_4$, vitamin C, 50°C, microwave for 8 min.

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