RSC Advances

PAPER

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Cite this: *RSC Adv.*, 2016, **6**, 114879

Synthesis and bioevaluation of 2-phenyl-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acid/carbohydrazide derivatives as potent xanthine oxidase inhibitors†

Ailong Shi,[ab] Defa Wang,[a] He Wang,[a] Yue Wu,[a] Haiqiu Tian,[a] Qi Guan,*[b] Kai Bao*[a] and Weige Zhang*[a]

Received 3rd October 2016
Accepted 30th November 2016

DOI: 10.1039/c6ra24651f

www.rsc.org/advances

A series of 2-phenyl-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acids/carbohydrazides as analogues of febuxostat were synthesized and evaluated for their *in vitro* xanthine oxidase (XO) inhibitory activity. Among these compounds, the carboxylic acid derivatives **7a–h** and **8a–h** exhibited high potency in the submicromolar/nanomolar range. Steady-state kinetics experiment revealed that **7f** was a mixed-type inhibitor of xanthine oxidase. In addition, a molecular docking study of **7f** was performed to determine its binding mode at the active site of xanthine oxidase.

Introduction

Xanthine oxidase (XO) is a ubiquitous molybdo-flavoenzyme existed in organism which catalyzes the oxidation of hypoxanthine to xanthine and uric acid (UA).[1,2] Normally, UA dissolves in the blood and passes through the kidneys into urine. Excess UA accumulating in tissues leads to a hyperuricemic condition such as gout.[3] In addition, it has been demonstrated that high uric acid level is related to other major diseases including diabetes, cardiovascular disease and metabolic syndrome.[4–6] XO inhibitors have been of excellent prospect as a therapeutic approach of hyperuricemia and its complications by reducing UA levels.

Allopurinol (**1**), a structural isomer of hypoxanthine, is the first XO inhibitor marketed in 1966 for the treatment of gout.[7] However, severe drug-induced hypersensitivity syndrome impedes its widely application.[8] Febuxostat (**2**), the first non-purine XO inhibitor in clinic, is an effective alternative to allopurinol and shows greater urate-lowering efficacy and minor side effects.[9,10]

In view of the attractive biological activity exhibited by febuxostat, numerous analogues of febuxostat have been designed and synthesized in order to have a better understanding of the structure–activity relationships (SAR). Ring bioisosterism is an efficient strategy for the discovery of new non-purine XO inhibitors, such as **3**, **4** and **5** (Fig. 1).[11–13] By following this strategy, we designed and synthesized a series of selenazole derivatives. Among them, **6** displayed potent activity against XO (Fig. 2).[14]

In the light of our ongoing efforts to develop new non-purine XO inhibitors, we herein attempt to replace the thiazole moiety of febuxostat with an 1,2,3-triazole which is one of the significant structural fragments of drugs and bioactive molecules.[15–17] Furthermore, there have been few reports, to the best of our knowledge, concerning the structure modification on the carboxyl group of febuxostat analogous. Hence, a series of 2-phenyl-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acids (**7a–h** and **8a–h**) and the hydrazide derivatives (**9e–f** and **10e–f**) were synthesised and evaluated their biological activities (Fig. 2). The

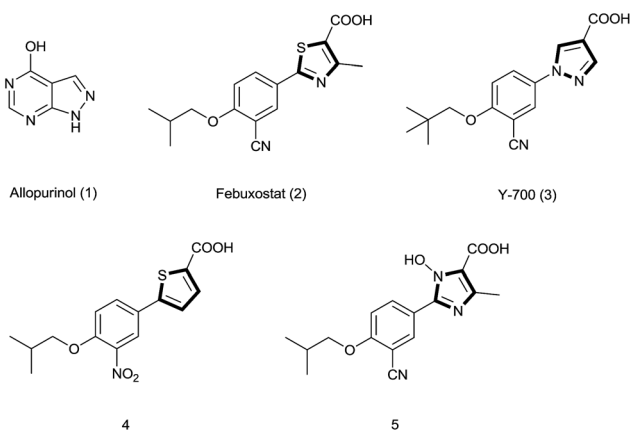


Fig. 1 Chemical structures of allopurinol and non-purine XO inhibitors.

[a]*Key Laboratory of Structure-Based Drug Design and Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, China. E-mail: zhangweige@syphu.edu.cn; baokai@syphu.edu.cn*

[b]*School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, China. E-mail: guanqi@syphu.edu.cn; Fax: +86 2423986393; Tel: +86 24 23986422*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c6ra24651f

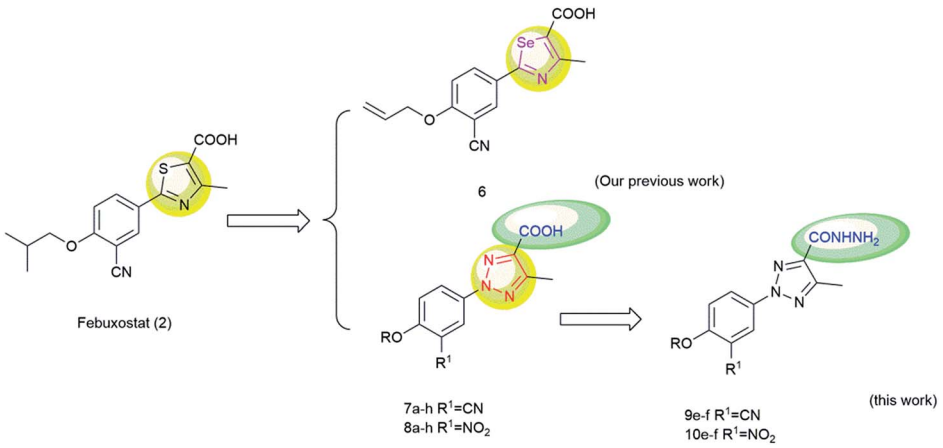


Fig. 2 Design of the target compounds.

inhibition type and the interaction of the most potent inhibitor with the amino acid residues of the enzyme have also been investigated.

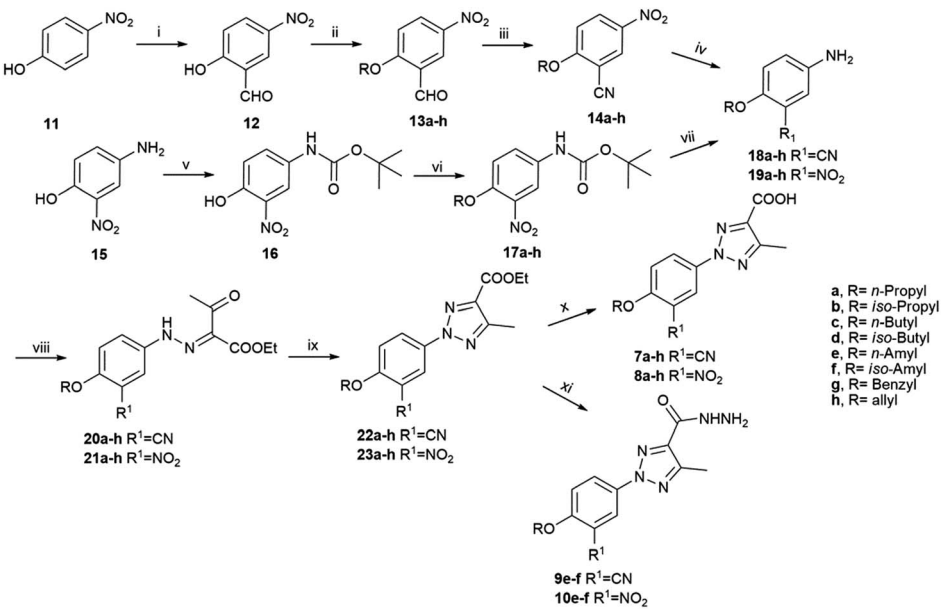
Results and discussion

1. Chemistry

The synthesis of the target compounds was performed as outline in Scheme 1. The commercially available 4-nitrophenol (**11**) was applied as starting material and converted into 2-hydroxy-5-nitrobenzaldehyde (**12**) *via* Duff reaction.[18] The alkylations of **12** with appropriate alkyl halides afforded the corresponding **13a–h**. Compounds **13a–h** were treated with hydrochloride hydroxylamine to give aldoximes and subsequently underwent dehydration in the presence of copper acetate to yield nitriles **14a–h**.[19] The reduction of the nitro group of **14a–h** was performed by using stannous chloride to generate amines **18a–h**. Compounds **19a–h** were obtained from commercially available 4-amino-2-nitrophenol (**15**) *via* *N*-Boc protection, alkylation and deprotection.

The key intermediates **18a–h** and **19a–h** were diazotized, respectively, in ice bath followed by condensation with ethyl acetylacetate in the presence of sodium acetate to afford **20a–h** and **21a–h**.[20] Treatment of **20a–h** and **21a–h** with $CuBr_2$ in acetic acid/*N*,*N*-dimethyl formamide mixture afforded esters **22a–h** and **23a–h**.[21]

Carboxylic acids **7a–h** and **8a–h** were obtained through hydrolysis of **22a–h** and **23a–h** by sodium hydroxide followed by acidification. On the other hand, hydrazinolysis of esters **22f–g** and **23f–g** in ethanol provided carbohydrazides **9f–g** and **10f–g**, respectively.

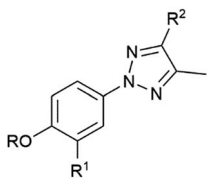


Scheme 1 Reagents and conditions: (i) $C_6H_{12}N_4$, TFA, 80 °C; (ii) alkyl halide, K_2CO_3, DMF; (iii) (a) $NH_2OH \cdot HCl$, MeOH, 50 °C; (b) $Cu(OAc)_2$, MeCN, 60 °C; (iv) $SnCl_2$, HCl aq., EtOH, 55 °C; (v) $(Boc)_2O$, THF, 50 °C; (vi) alkyl halide, K_2CO_3, DMF; (vii) TEA, DCM, rt; (viii) (a) $NaNO_2$, HCl aq., 0 °C; (b) ethyl acetylacetate, NaOAc, EtOH, H_2O; (ix) $CuBr_2$, NH_4OAc, DMF, HOAc, 120 °C; (x) NaOH, aq., THF/EtOH; (xi) $NH_2NH_2 \cdot H_2O$, EtOH, 50 °C.

2. Biological activity

The *in vitro* bovine XO inhibitory activities of compounds **7a–h**, **8a–h** and **9e–f**, **10e–f** were evaluated and compared with the positive control, febuxostat (Table 1).[22,23] As shown in Table 1, most of the target compounds exhibited moderate XO inhibitory activity. Among them, compounds **7f** and **8f** bearing *iso*-amyl substituent were more potent than the other compounds (IC_{50} 0.084 and 0.109 μM, respectively). **7a–h** carrying the cyano group at the 3-position of phenyl moiety showed similar or slightly improved activity with respect to **8a–h** carrying the nitro group. In addition, carboxyl group at the 5-position of the triazole ring was found to be essential for XO inhibition with respect to carbohydrazides (**7e** *vs.* **9e**; **7f** *vs.* **9f**; **8e** *vs.* **10e**; **8f** *vs.* **10f**). Increasing the size of the substituent at the 4′-position of the phenyl moiety from *n*-propyl to *n*-amyl groups improved XO inhibitory activity (**7e** *vs.* **7a**, **7c**; **8e** *vs.* **8a**, **8c**), with the exception of benzyloxy substituent (**7g**, **8g**). Branched alkoxy substituents showed more potent inhibitory activity than corresponding linear chain alkoxy groups (**7b** *vs.* **7a**; **7d** *vs.* **7c**; **7f** *vs.* **7e**; **8b** *vs.* **8a**; **8d** *vs.* **8c**; **8f** *vs.* **8e**). Consequently, compound **7f** represented the most potent molecule among the series. This finding indicated that the combination of a cyano group and an *i*-amyl group is favourable for the XO inhibitory activity of 2-phenyl-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acids/carbohydrazides derivatives.

Table 1 *In vitro* xanthine oxidase inhibitory activity of 2-(3-cyano/nitro-4-phenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acid/carbohydrazide derivatives



Compound	R	R^1	R^2	IC_{50}[a] (μM)
7a	*n*-Propyl	CN	COOH	0.195
7b	*i*-Propyl	CN	COOH	0.177
7c	*n*-Butyl	CN	COOH	0.135
7d	*i*-Butyl	CN	COOH	0.105
7e	*n*-Amyl	CN	COOH	0.112
7f	*i*-Amyl	CN	COOH	0.084
7g	Benzyl	CN	COOH	0.152
7h	Allyl	CN	COOH	0.225
8a	*n*-Propyl	NO_2	COOH	0.162
8b	*i*-Propyl	NO_2	COOH	0.145
8c	*n*-Butyl	NO_2	COOH	0.151
8d	*i*-Butyl	NO_2	COOH	0.123
8e	*n*-Amyl	NO_2	COOH	0.125
8f	*i*-Amyl	NO_2	COOH	0.109
8g	Benzyl	NO_2	COOH	0.180
8h	Allyl	NO_2	COOH	0.254
9e	*n*-Amyl	CN	$CONHNH_2$	NA[b]
9f	*i*-Amyl	CN	$CONHNH_2$	NA[b]
10e	*n*-Amyl	NO_2	$CONHNH_2$	NA[b]
10f	*i*-Amyl	NO_2	$CONHNH_2$	NA[b]
Febuxostat				0.012

[a] Values are means of the three experiment. [b] NA = not active <50% inhibition@10.0 μM.

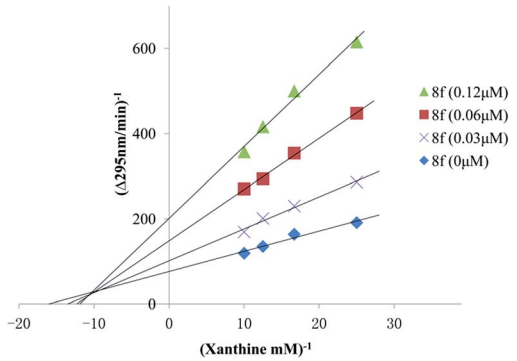


Fig. 3 Lineweaver–Burk plot of the inhibition of xanthine oxidase by compound **7f**.

3. Steady-state kinetic analysis

A Lineweaver–Burk plot for inhibition of XO by compound **7f** was obtained in the presence or absence of compound **7f** with varying concentration of xanthine as the substrate (20–100 μM). The interaction of lines indicated that compound **7f** was a mixed-type inhibitor of XO (Fig. 3). Similar findings have been reported with febuxostat (**2**).[24]

4. Molecular modeling

To elucidate the interactions of the newly synthesized compounds with XO and further understand the SARs of the target compounds, the potential binding mode of compound **7f** at molybdenum-pterin sites was investigated using Discovery Studio 3.0 (Accelrys, San Diego, CA, USA) software with the crystal structure of the xanthine dehydrogenase/febuxostat complex (PDB: 1N5X).[25]

The docking study clearly indicated that **7f** (yellow) was perfectly superimposed with febuxostat (green) in the narrow tunnel towards the molybdenum-pterin center (Fig. 4A). Hydrogen bonds were found between the carboxylate group of **7f** and Thr1010, Arg880, the nitrogen atom at 3-position of triazole and Glu802, the nitrile group and Asn768 (Fig. 4B).[25] The hydrogen bond distances were 2.3, 1.9, 2.0 and 2.3 Å, respectively. Moreover, the triazole ring of **7f** fostered π–π interaction with Phe1009 and the interaction distance is 4.2 Å. The CDOCKER interaction energy of **7f** was −43.06 kcal mol^{-1}, while that of febuxostat was −47.25 kcal mol^{-1}. These computational modeling analyses may help to explain the reason that compound **7f** exhibited potent inhibitory activity, but lower than that of febuxostat.

Conclusions

In summary, a series of novel XO inhibitors containing 1,2,3-triazole segments were designed and synthesized. *In vitro* activity assay indicated that compounds **7a–h** and **8a–h** had

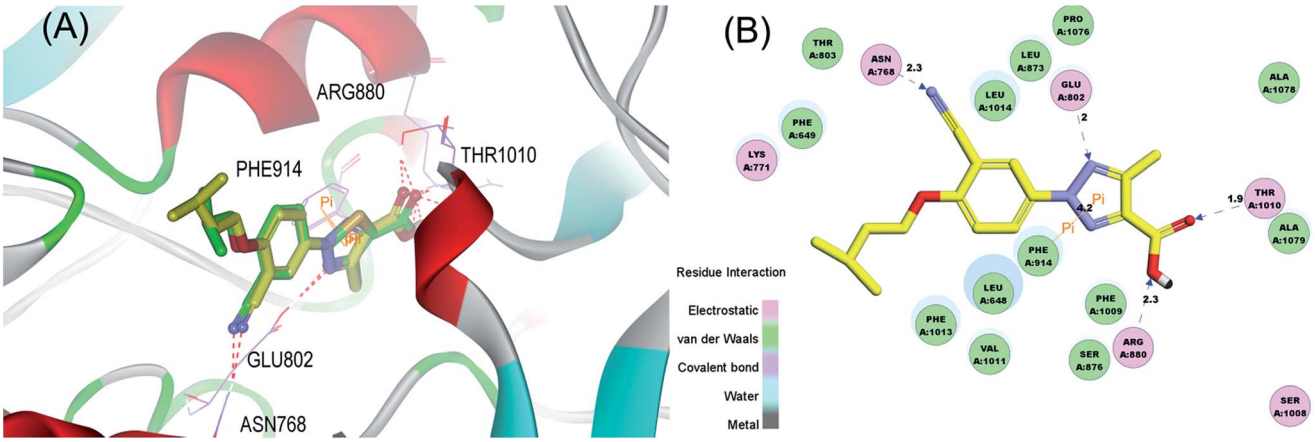


Fig. 4 (A) Overlapping modes for compound **7f** (yellow) and febuxostat (green) binding the active site of xanthine oxidase. (B) The interactions between the active site of xanthine oxidase and compound **7f** on 2D diagram. The surrounding amino acid residues are displayed in ball format and labelled. The dashed blue arrows represent hydrogen bonds, the solid orange line represents π–π interaction and the interaction distances (Å) are shown.

potential inhibitory effects against XO, with IC_{50} values ranging from 0.084 to 0.254 μM. Enzyme inhibition kinetic study revealed that the most promising compound **7f** acted as a mixed-type inhibitor for xanthine oxidase. Furthermore, molecular docking studies were performed to determine the probable binding conformation of compound **7f** in the XO binding site and offered some insights into the further modification of the target compounds. Our results suggested that 2-phenyl-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acids might serve as new molecular scaffold for the development of non-purine XO inhibitors. Further research in this area is in progress in our laboratory.

Experimental section

1. Chemistry

Unless otherwise noted, all materials were commercial available and were used without further purification. Thin-layer chromatography was performed on FLUKA silica gel aluminum cards with fluorescent indicator 254 nm under UV light. The column chromatography was performed using silica gel (200–300 mesh, Qingdao PUKE) with the designated solvents. Melting points were measured by a YRT-3 melting point detector without corrected. ESI-HRMS spectra were performed on Agilent 6530 Accurate-Mass Q-TOF LC/MS (Agilent Technologies, USA). ^{1}H and ^{13}C NMR spectra were obtained on Bruker AVANCE 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) or Bruker AVANCE 600 (^{1}H, 600 MHz; ^{13}C, 150 MHz), in DMSO-d_6 or $CDCl_3$ using TMS as internal standard. Infrared spectra were recorded using KBr plates on a PE Spectrum-100 instrument.

2. Synthesis of 2-hydroxy-5-nitrobenzaldehyde (12)

A mixture of 4-nitrophenol (6.0 g, 43.2 mmol), hexamethylenetetramine (7.9 g, 56.2 mmol) in trifluoroacetic acid (50 mL) was heated at 85 °C for 15 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The mixture was diluted with H_2O (100 mL) and then neutralized with Na_2CO_3 to pH 5. The precipitate formed was collected by filtration, washed with water and dried to afford **12** (6.6 g, 92%) as a yellow solid, mp 146–149 °C.[26]

3. General procedure for the preparation of compounds 13a–h

A mixture of compound **12** (5.0 g, 29.9 mmol), respective alkyl halide (35.8 mmol), anhydrous K_2CO_3 (8.2 g, 59.8 mmol) in DMF (50 mL) was heated at 75 °C for 7 h. The reaction mixture was diluted with H_2O (100 mL) then extracted with EtOAc (100 mL) and the combined organic phases were washed with H_2O (3 × 100 mL) and brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was recrystallized from EtOH.

3.1. 5-Nitro-2-propoxybenzaldehyde (13a). White solid, (yield: 85%), mp: 64–67 °C.[27]

3.2. 2-Isopropoxy-5-nitrobenzaldehyde (13b). White solid, (yield: 88%), mp: 77–79 °C.[28]

3.3. 5-Nitro-2-propoxybenzaldehyde (13c). White solid, (yield: 90%), mp: 67–69 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.47 (s, 1H), 8.68 (d, J = 2.9 Hz, 1H), 8.41 (dd, J = 9.2, 2.9 Hz, 1H), 7.12 (d, J = 9.2 Hz, 1H), 4.24 (t, J = 6.4 Hz, 2H), 1.95–1.87 (m, 2H), 1.60–1.50 (m, 2H), 1.02 (t, J = 7.4 Hz, 3H).

3.4. 2-Isobutoxy-5-nitrobenzaldehyde (13d). White solid, (yield: 91%), mp: 69–71 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.50 (s, 1H), 8.68 (d, J = 2.9 Hz, 1H), 8.41 (dd, J = 9.2, 2.9 Hz, 1H), 7.11 (d, J = 9.2 Hz, 1H), 4.00 (d, J = 6.4 Hz, 2H), 2.24 (dt, J = 13.3, 6.6 Hz, 1H), 1.11 (d, J = 6.7 Hz, 7H).

3.5. 5-Nitro-2-(amyloxy)benzaldehyde (13e). White solid, (yield: 89%), mp: 62–65 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.48 (s, 1H), 8.68 (d, J = 2.9 Hz, 1H), 8.41 (dd, J = 9.2, 2.9 Hz, 1H), 7.14–7.08 (m, 1H), 4.23 (t, J = 6.5 Hz, 2H), 1.93 (dq, J = 13.3, 6.5 Hz, 2H), 1.53–1.48 (m, 2H), 1.43 (dq, J = 14.4, 7.0 Hz, 2H), 0.96 (t, J = 7.3 Hz, 3H).

3.6. 2-(Isoamyloxy)-5-nitrobenzaldehyde (13f). White solid, (yield: 83%), mp: 67–70 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.47 (s,

1H), 8.68 (d, J = 2.9 Hz, 1H), 8.42 (dd, J = 9.2, 2.9 Hz, 1H), 7.13 (d, J = 9.2 Hz, 1H), 4.26 (t, J = 6.4 Hz, 2H), 1.93–1.85 (m, 1H), 1.82 (q, J = 6.5 Hz, 2H), 1.01 (d, J = 6.4 Hz, 6H).

3.7. 2-(Benzyloxy)-5-nitrobenzaldehyde (13g). White solid, (yield: 86%), mp: 90–92 °C.[29]

3.8. 2-(Allyloxy)-5-nitrobenzaldehyde (13h). White solid, (yield: 78%), mp: 75–78 °C.[30]

4. General procedure for the preparation of compounds 14a–h

A mixture of compound **13** (22.4 mmol), hydroxylamine hydrochloride (7.7 g, 112 mmol) in methanol (50 mL) was heated at 55 °C for 3 h. The reaction was cooled to room temperature, and concentrated under reduced pressure. The mixture was diluted with H_2O (200 mL) then extracted with EtOAc (3 × 150 mL) and the combined organic phases were washed with brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo* to yield aldoximes.

A mixture of respective aldoxime (12.6 mmol), copper acetate (0.18 g, 1 mmol) in acetonitrile (50 mL) was heated at 65 °C for 3 h. The reaction mixture was cooled to room temperature, and concentrated under reduced pressure. The mixture was diluted with H_2O (200 mL) then extracted with EtOAc (3 × 150 mL) and the combined organic phases were washed with brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The collected solid was purified by flash column chromatography (0–25% EtOAc in hexanes).

4.1. 5-Nitro-2-propoxybenzonitrile (14a). White solid, (yield: 74%), mp: 75–77 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.48 (d, J = 2.8 Hz, 1H), 8.42 (dd, J = 9.3, 2.8 Hz, 1H), 7.08 (d, J = 9.3 Hz, 1H), 4.18 (t, J = 6.4 Hz, 2H), 1.99–1.90 (m, 2H), 1.12 (t, J = 7.4 Hz, 3H).

4.2. 2-Isopropoxy-5-nitrobenzonitrile (14b). White solid, (yield: 70%), mp: 82–84 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, J = 2.8 Hz, 1H), 8.43 (dd, J = 9.4, 2.8 Hz, 1H), 7.08 (d, J = 9.4 Hz, 1H), 4.86–4.76 (m, 1H), 1.50 (d, J = 6.1 Hz, 6H).

4.3. 2-Butoxy-5-nitrobenzonitrile (14c). White solid, (yield: 68%), mp: 87–90 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, J = 2.8 Hz, 1H), 8.44 (dd, J = 9.3, 2.8 Hz, 1H), 7.09 (d, J = 9.3 Hz, 1H), 4.23 (t, J = 6.4 Hz, 2H), 1.95–1.88 (m, 2H), 1.61–1.55 (m, 2H), 1.03 (t, J = 7.4 Hz, 3H).

4.4. 2-Isobutoxy-5-nitrobenzonitrile (14d). White solid, (yield: 73%), mp: 77–80 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, J = 2.8 Hz, 1H), 8.44 (dd, J = 9.3, 2.8 Hz, 1H), 7.08 (d, J = 9.3 Hz, 1H), 3.99 (d, J = 6.5 Hz, 2H), 2.29–2.18 (m, 1H), 1.12 (d, J = 6.7 Hz, 6H).

4.5. 2-Amyloxy-5-nitro-benzonitrile (14e). White solid, (yield: 75%), mp: 69–73 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, J = 2.8 Hz, 1H), 8.44 (dd, J = 9.3, 2.8 Hz, 1H), 7.09 (d, J = 9.3 Hz, 1H), 4.23 (t, J = 6.5 Hz, 2H), 1.98–1.90 (m, 2H), 1.56–1.49 (m, 2H), 1.47–1.38 (m, 2H), 0.97 (t, J = 7.2 Hz, 3H).

4.6. 2-Isoamyloxy-5-nitrobenzonitrile (14f). White solid, (yield: 70%), mp: 87–90 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, J = 2.8 Hz, 1H), 8.45 (dd, J = 9.3, 2.8 Hz, 1H), 7.10 (d, J = 9.3 Hz, 1H), 4.26 (t, J = 6.5 Hz, 2H), 1.96–1.86 (m, 1H), 1.83 (q, J = 6.5 Hz, 2H), 1.02 (d, J = 6.5 Hz, 6H).

4.7. 2-Benzyloxy-5-nitrobenzonitrile (14g). White solid, (yield: 69%), mp: 132–134 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (d, J = 2.8 Hz, 1H), 8.41 (dd, J = 9.3, 2.8 Hz, 1H), 7.40–7.35 (m, 5H), 7.14 (d, J = 9.3 Hz, 1H), 5.37 (s, 2H).

4.8. 2-Allyloxy-5-nitrobenzonitrile (14h). White solid, (yield: 66%), mp: 97–100 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, J = 2.8 Hz, 1H), 8.43 (dd, J = 9.3, 2.8 Hz, 1H), 7.12 (d, J = 9.3 Hz, 1H), 6.07 (ddt, J = 17.2, 10.4, 5.1 Hz, 1H), 5.59–5.49 (m, 1H), 5.43 (m, 1H), 4.82 (dt, J = 5.1 Hz, 2H).

5. Synthesis of *tert*-butyl 4-hydroxy-3-nitrophenylcarbamate (16)

A mixture of 4-amino-2-nitrophenol (6.0 g, 43.2 mmol), di-*tert*-butyl pyrocarbonate (12.0 g, 55.0 mmol) in THF (60 mL) was heated at 55 °C for 10 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was recrystallized from cyclohexane to yield **16** (10.1 g, 91%) as a yellow solid, mp 59–62 °C.[31]

6. General procedure for the preparation of compounds 17a–h

Compounds **17a–h** were synthesized in the same way as compounds **13a–h**.

6.1. *tert*-Butyl 3-nitro-4-propoxyphenylcarbamate (17a). Yellow oil, (yield: 82%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91 (d, J = 2.4 Hz, 1H), 7.49 (s, 1H), 6.99 (d, J = 9.0 Hz, 1H), 6.69 (s, 1H), 4.02 (t, J = 6.4 Hz, 2H), 1.88–1.78 (m, 2H), 1.51 (s, 9H), 1.04 (t, J = 7.4 Hz, 3H).

6.2. *tert*-Butyl 4-isopropoxy-3-nitrophenylcarbamate (17b). Yellow oil, (yield: 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, J = 2.6 Hz, 1H), 7.48 (d, 1H), 7.02 (t, J = 9.5 Hz, 1H), 6.74 (s, 1H), 4.57 (dq, 6.1 Hz, 1H), 1.52 (s, 9H), 1.36 (d, J = 6.1 Hz, 6H).

6.3. *tert*-Butyl 4-butoxy-3-nitrophenylcarbamate (17c). Yellow oil, (yield: 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, 1H), 7.51 (t, 1H), 6.99 (d, 1H), 6.79 (s, 1H), 4.05 (t, J = 6.4 Hz, 2H), 1.78 (m, 2H), 1.51 (s, 9H), 1.47 (m, 2H), 0.96 (t, J = 7.4 Hz, 3H).

6.4. *tert*-Butyl 4-isobutoxy-3-nitrophenylcarbamate (17d). Yellow oil, (yield: 83%), ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, 1H), 7.54 (d, 1H), 7.00 (d, 1H), 6.53 (s, 1H), 3.84 (d, J = 6.5 Hz, 2H), 2.14 (dt, J = 13.3, 6.5 Hz, 1H), 1.53 (s, 9H), 1.05 (d, J = 6.7 Hz, 6H).

6.5. *tert*-Butyl 4-amyloxy-3-nitrophenylcarbamate (17e). Yellow oil, (yield: 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, 1H), 7.52 (t, 1H), 7.01 (d, 1H), 6.61 (s, 1H), 4.07 (t, J = 6.5 Hz, 2H), 1.86–1.78 (m, 2H), 1.53 (s, 9H), 1.49–1.44 (m, 2H), 1.42–1.34 (m, 2H), 0.94 (t, J = 7.1 Hz, 3H).

6.6. *tert*-Butyl 4-(isoamyloxy)-3-nitrophenylcarbamate (17f). Yellow oil, (yield: 90%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.89 (d, J = 2.6 Hz, 1H), 7.51 (s, 1H), 7.00 (d, J = 9.1 Hz, 1H), 6.59 (s, 1H), 4.08 (t, J = 6.6 Hz, 2H), 1.85 (m, 1H), 1.70 (q, J = 6.7 Hz, 2H), 1.51 (s, 9H), 0.95 (d, J = 6.7 Hz, 6H).

6.7. *tert*-Butyl 4-(benzyloxy)-3-nitrophenylcarbamate (17g). Yellow solid, (yield: 89%), mp: 77–79 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.92 (d, J = 2.7 Hz, 1H), 7.47 (s, 1H), 7.43 (d, J = 7.3 Hz,

2H), 7.37 (dd, 2H), 7.31 (t, J = 7.3 Hz, 1H), 7.03 (d, J = 9.1 Hz, 1H), 6.51 (s, 1H).

6.8. *tert*-Butyl 4-(allyloxy)-3-nitrophenylcarbamate (17h). Yellow oil, (yield: 87%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.94 (d, J = 2.4 Hz, 1H), 7.52 (s, 1H), 7.02 (d, J = 9.0 Hz, 1H), 6.65 (s, 1H), 6.11–5.98 (m, 1H), 5.49–5.42 (m, 1H), 5.34–5.27 (m, 1H), 4.65 (d, J = 5.0 Hz, 2H), 1.53 (s, 9H).

7. General procedure for the preparation of compounds 18a–h

A mixture of compound **14** (9.1 mmol), tin(II) chloride dehydrate (2.3 g, 12 mmol), 2 mL concentrated hydrochloric acid in EtOH (40 mL) was heated at reflux for 5 h. The reaction mixture was cooled down to room temperature and concentrated under reduced pressure. The mixture was diluted with H_2O (50 mL) and basified with a 1 M NaOH solution to pH 10. Then the mixture was extracted with EtOAc (3 × 100 mL) and the combined organic phases were washed with brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The collected solid was used directly in the next step without further purification.

8. General procedure for the preparation of compounds 19a–h

A mixture of compound **17** (1.61 mmol), trifluoroacetic acid (2 mL), in DCM (20 mL) was stirred at room temperature for 6 h. The mixture was quenched with water (20 mL) and neutralized with sodium bicarbonate to pH 7. The mixture was extracted with DCM (2 × 20 mL) and the combined organic phases were washed with brine (20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The collected solid was used directly in the next step without further purification.

9. General procedure for the preparation of compounds 20a–h and 21a–h

To a mixture of substituted phenylamine (2.6 mmol) and 15% HCl (5 mL) was added drop-wise $NaNO_2$ (0.27 g, 3.0 mmol) in H_2O (5 mL) at 0 °C. After the completion of addition, the reaction mixture was stirred at this temperature for 30 min. Ethyl acetylacetate (0.39 g, 3 mmol), anhydrous sodium acetate (1.6 g, 20 mmol), and EtOH (10 mL) was added to the reaction mixture at 0 °C. Then, the mixture was filtered, and the residue was dried to afford yellow solids (**20d**) in 90–95% yields. The collected solid was used directly in the next step without further purification.

10. General procedure for the preparation of compounds 22a–h and 23a–h

A mixture of corresponding compound **20** or **21** (0.63 mmol), ammonium acetate (0.092 g, 1.2 mmol), $CuBr_2$ (0.011 g, 0.05 mmol) in DMF (5 mL) and HOAc (5 mL) was heated at 110 °C for 5 h. The reaction mixture was diluted with H_2O (20 mL) then extracted with EtOAc (3 × 20 mL) and the combined organic phases were washed with brine (20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (0–25% EtOAc in hexanes).

10.1. Ethyl 2-(3-cyano-4-propoxyphenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylate (22a). Light yellow solid, (yield: 81%), mp: 121–122 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, J = 2.7 Hz, 1H), 8.25 (dd, J = 9.2, 2.7 Hz, 1H), 7.05 (d, J = 9.2 Hz, 1H), 4.47 (m, 2H), 4.10 (t, J = 6.4 Hz, 2H), 2.61 (s, 3H), 1.96–1.87 (m, 2H), 1.42–1.48 (m, 3H), 1.11 (t, J = 7.4 Hz, 3H). ESI-MS m/z 315.1 $[M + H]^+$.

10.2. Ethyl 2-(3-cyano-4-isopropoxyphenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylate (22b). Light yellow, (yield: 84%), mp: 71–75 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, J = 2.7 Hz, 1H), 8.25 (dd, J = 9.2, 2.7 Hz, 1H), 7.06 (d, J = 9.2 Hz, 1H), 4.72 (m, 1H), 4.47 (m, 2H), 2.61 (s, 3H), 1.47–1.43 (m, 3H), 1.46–1.43 (m, 6H). ESI-MS m/z 315.1 $[M + H]^+$.

10.3. Ethyl 2-(4-butoxy-3-cyanophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylate (22c). White solid, (yield: 77%), mp: 67–68 °C, ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.31 (d, J = 2.7 Hz, 1H), 8.25 (dd, J = 9.2, 2.7 Hz, 1H), 7.06 (d, J = 9.2 Hz, 1H), 4.47 (q, J = 7.1 Hz, 2H), 4.13 (m, 3H), 2.61 (s, 3H), 1.90–1.85 (m, 2H), 1.59–1.53 (m, 2H), 1.45 (t, J = 7.1 Hz, 3H), 1.01 (t, J = 7.4 Hz, 3H). ESI-MS m/z 329.2 $[M + H]^+$.

10.4. Ethyl 2-(3-cyano-4-isobutoxyphenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylate (22d). White solid, (yield: 86%), mp: 77–79 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, J = 2.7 Hz, 1H), 8.25 (dd, J = 9.2, 2.7 Hz, 1H), 7.04 (d, J = 9.2 Hz, 1H), 4.47 (m, 2H), 3.89 (d, J = 6.5 Hz, 2H), 2.61 (s, 3H), 2.23 (m, 1H), 1.45 (t, J = 7.1 Hz, 3H), 1.10 (d, J = 6.7 Hz, 6H). ESI-MS m/z 329.2 $[M + H]^+$.

10.5. Ethyl 2-(3-cyano-4-(amyloxy)phenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylate (22e). White solid, (yield: 79%), mp: 82–83 °C, ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.21–8.17 (m, 2H), 7.40 (dd, J = 8.3, 1.4 Hz, 1H), 4.34 (m, 2H), 4.17 (t, J = 6.5 Hz, 2H), 3.32 (s, 2H), 2.50 (s, 3H), 1.79–1.74 (m, 2H), 1.42 (dd, J = 9.1, 6.5 Hz, 2H), 1.38–1.35 (m, 2H), 1.33 (t, J = 7.1 Hz, 3H), 0.90 (t, J = 7.2 Hz, 3H). ESI-MS m/z 343.2 $[M + H]^+$.

10.6. Ethyl 2-(3-cyano-4-isoamyloxyphenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylate(22f). White solid, (yield: 93%), mp: 75–77 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, J = 2.7 Hz, 1H), 8.25 (dd, J = 9.2, 2.7 Hz, 1H), 7.06 (d, J = 9.2 Hz, 1H), 4.47 (q, J = 7.1 Hz, 2H), 4.16 (t, J = 6.6 Hz, 2H), 2.61 (s, 3H), 1.98–1.88 (m, 1H), 1.78 (q, J = 6.6 Hz, 2H), 1.45 (t, J = 7.1 Hz, 3H), 1.00 (d, J = 6.6 Hz, 6H). ESI-MS m/z 343.2 $[M + H]^+$.

10.7. Ethyl 2-(4-benzyloxy-3-cyanophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylate (22g). White solid, (yield: 67%), mp: 150–152 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, J = 2.7 Hz, 1H), 8.24 (dd, J = 9.2, 2.7 Hz, 1H), 7.50–7.32 (m, 5H), 7.11 (d, J = 9.2 Hz, 1H), 5.28 (s, 2H), 4.46 (m, 2H), 2.60 (s, 3H), 1.44 (m, 3H). ESI-MS m/z 363.1 $[M + H]^+$.

10.8. Ethyl 2-(4-allyloxy-3-cyanophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylate (22h). White solid, (yield: 73%), mp: 102–104 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (d, J = 2.7 Hz, 1H), 8.26 (dd, J = 9.2, 2.7 Hz, 1H), 7.06 (d, J = 9.2 Hz, 1H), 6.07 (ddd, J = 10.6, 5.1 Hz, 1H), 5.51 (dd, J = 1.2 Hz, 1H), 5.38 (dd, J = 10.6, 1.2 Hz, 1H), 4.73 (d, J = 5.1 Hz, 2H), 4.47 (q, J = 7.1 Hz, 2H), 2.61 (s, 3H), 1.45 (t, J = 7.1 Hz, 3H). ESI-MS m/z 313.1 $[M + H]^+$.

10.9. Ethyl 5-methyl-2-(3-nitro-4-propoxyphenyl)-2*H*-1,2,3-triazole-4-carboxylate (23a). Yellow solid, (yield: 86%), mp: 119–120 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, J = 2.7 Hz, 1H), 8.26

(dd, J = 9.2, 2.7 Hz, 1H), 7.17 (d, J = 9.2 Hz, 1H), 4.47 (q, J = 7.1 Hz, 2H), 4.13 (t, J = 6.6 Hz, 2H), 2.61 (s, 3H), 1.90 (m, 2H), 1.45 (t, J = 7.1 Hz, 3H), 1.09 (m, 3H). ESI-MS m/z 335.1 $[M + H]^+$.

10.10. Ethyl 2-(4-isopropoxy-3-nitrophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylate (23b). Yellow solid, (yield: 78%), mp: 87–89 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (d, J = 2.7 Hz, 1H), 8.23 (dd, J = 9.2, 2.7 Hz, 1H), 7.17 (d, J = 9.2 Hz, 1H), 4.74 (m, 1H), 4.49–4.43 (m, 2H), 2.61 (s, 3H), 1.46–1.42 (m, 3H), 1.44–1.42 (m, 6H). ESI-MS m/z 335.1 $[M + H]^+$.

10.11. Ethyl 2-(4-butoxy-3-nitrophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylate (23c). Yellow solid, (yield: 79%), mp: 77–79 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, J = 2.7 Hz, 1H), 8.26 (dd, J = 9.2, 2.7 Hz, 1H), 7.17 (d, J = 9.2 Hz, 1H), 4.47 (q, J = 7.1 Hz, 2H), 4.17 (m, 2H), 2.61 (s, 3H), 1.88–1.81 (m, 2H), 1.54 (dq, J = 14.7, 7.4 Hz, 2H), 1.45 (t, J = 7.1 Hz, 3H), 0.99 (t, J = 7.4 Hz, 3H). ESI-MS m/z 349.1 $[M + H]^+$.

10.12. Ethyl 2-(4-isobutoxy-3-nitrophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylate (23d). Yellow solid, (yield: 84%), mp: 104–106 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (d, J = 2.7 Hz, 1H), 8.26 (dd, J = 9.2, 2.7 Hz, 1H), 7.16 (d, J = 9.2 Hz, 1H), 4.47 (q, J = 7.1 Hz, 2H), 3.92 (m, 2H), 2.61 (s, 3H), 2.18 (m, 1H), 1.45 (t, J = 7.1 Hz, 3H), 1.08 (d, J = 6.7 Hz, 6H). ESI-MS m/z 349.1 $[M + H]^+$.

10.13. Ethyl 5-methyl-2-(4-amyloxy-3-nitrophenyl)-2*H*-1,2,3-triazole-4-carboxylate (23e). Yellow solid, (yield: 84%), mp: 84–85 °C, ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.58 (d, J = 2.7 Hz, 1H), 8.25 (dd, J = 9.1, 2.7 Hz, 1H), 7.17 (d, J = 9.2 Hz, 1H), 4.47 (q, J = 7.2 Hz, 2H), 4.16 (t, J = 6.5 Hz, 2H), 2.60 (d, J = 4.6 Hz, 3H), 1.89–1.84 (m, 2H), 1.51–1.44 (m, 5H), 1.40 (dt, J = 14.8, 7.2 Hz, 2H), 0.94 (t, J = 7.3 Hz, 3H). ESI-MS m/z 363.2 $[M + H]^+$.

10.14. Ethyl 2-(4-isoamyloxy-3-nitrophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylate (23f). Yellow solid, (yield: 80%), mp: 85–86 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64–8.56 (m, 1H), 8.28 (dd, J = 9.2, 2.7 Hz, 1H), 7.20 (d, J = 9.2 Hz, 1H), 4.48 (q, J = 7.1 Hz, 2H), 4.21 (t, J = 6.6 Hz, 2H), 2.63 (s, 3H), 1.96–1.85 (m, 1H), 1.78 (q, J = 6.6 Hz, 2H), 1.47 (t, J = 7.1 Hz, 3H), 1.00 (d, J = 6.6 Hz, 6H). ESI-MS m/z 363.2 $[M + H]^+$.

10.15. Ethyl 2-(4-benzyloxy-3-nitrophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylate (23g). Yellow solid, (yield: 75%), mp: 90–91 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, J = 2.7 Hz, 1H), 8.27 (dd, J = 9.1, 2.7 Hz, 1H), 7.51–7.35 (m, 5H), 7.25 (d, J = 9.1 Hz, 1H), 5.33 (s, 2H), 4.49 (q, J = 7.1 Hz, 2H), 2.63 (s, 3H), 1.47 (t, J = 7.1 Hz, 3H). ESI-MS m/z 383.1 $[M + H]^+$.

10.16. Ethyl 2-(4-allyloxy-3-nitrophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylate (23h). Yellow solid, (yield: 68%), mp: 153–155 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, J = 2.7 Hz, 1H), 8.28 (dd, J = 9.2, 2.7 Hz, 1H), 7.20 (d, J = 9.2 Hz, 1H), 6.13–6.03 (m, 1H), 5.54 (dd, J = 17.3, 1.2 Hz, 1H), 5.40 (dd, J = 10.6, 1.2 Hz, 1H), 4.79–4.74 (m, 2H), 4.49 (q, J = 7.1 Hz, 2H), 2.63 (s, 3H), 1.46 (t, J = 7.1 Hz, 3H). ESI-MS m/z 333.1 $[M + H]^+$.

11. General procedure for the preparation of compounds 7a–h and 8a–h

A mixture of corresponding compound **22** or **23** (0.3 mmol), 1 M NaOH solution (3.0 mL) in THF/EtOH (1 : 1) (5.0 mL) was heated at 45 °C for 1.5 h. After the reaction was completed, the solvent was removed under reduced pressure. The residue was dissolved in water (20 mL) and acidified with a 1 M HCl solution to pH 3. The solid was collected by filtration, washed with water and purified by flash column chromatography (0–35% EtOAc in hexanes) to yield the desired product.

11.1. 2-(3-Cyano-4-propoxyphenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acid (7a). White solid, (yield: 88%), mp: 166–169 °C, ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.52 (s, 1H), 8.27–8.19 (m, 2H), 7.44 (d, J = 8.9 Hz, 1H), 4.17 (t, J = 6.4 Hz, 2H), 2.52 (s, 3H), 1.83–1.77 (m, 2H), 1.03 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.99, 159.91, 148.47, 138.80, 131.92, 125.34, 123.56, 115.20, 114.27, 101.32, 70.79, 21.76, 11.30, 10.15. HRMS: calcd for $C_{14}H_{14}N_4O_3$ $[M - H]^-$ 285.0993, found 285.0990.

11.2. 2-(3-Cyano-4-isopropoxyphenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acid (7b). White solid, (yield: 90%), mp: 180–182 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, J = 2.7 Hz, 1H), 8.29 (dd, J = 9.2, 2.7 Hz, 1H), 7.10 (d, J = 9.2 Hz, 1H), 4.74 (dq, J = 6.2 Hz, 1H), 2.67 (s, 3H), 1.48 (d, J = 6.2 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.33, 158.75, 148.99, 136.36, 131.11, 123.97, 123.66, 114.31, 113.07, 102.68, 71.69, 20.78, 10.57. HRMS: calcd for $C_{14}H_{14}N_4O_3$ $[M - H]^-$ 285.0993, found 285.0975.

11.3. 2-(4-Butoxy-3-cyanophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acid (7c). White solid, (yield: 83%), mp: 157–158 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (d, J = 2.6 Hz, 1H), 8.29 (dd, J = 9.2, 2.6 Hz, 1H), 7.09 (d, J = 9.2 Hz, 1H), 4.17 (t, J = 6.4 Hz, 2H), 2.66 (s, 3H), 1.93–1.85 (m, 2H), 1.58 (dq, J = 14.7, 7.4 Hz, 2H), 1.03 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.03, 160.90, 150.26, 137.63, 132.49, 125.29, 124.72, 115.34, 113.06, 103.01, 69.74, 31.09, 19.32, 13.99, 11.81. HRMS: calcd for $C_{15}H_{16}N_4O_3$ $[M - H]^-$ 299.1150, found 299.1146. IR_{max}/cm^{-1} 3426, 1700, 1287 (COOH), 2232 (CN), 2962, 2928, 2875, 1468, 1437, 1384 (CH_3, CH_2), 1614, 1531, 1505 (Ar), 1259 (C–O–C) (KBr).

11.4. 2-(3-Cyano-4-isobutoxyphenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acid (7d). White solid, (yield: 77%), mp: 184–186 °C, ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.34 (d, J = 2.7 Hz, 1H), 8.28 (dd, J = 9.1, 2.7 Hz, 1H), 7.07 (d, J = 9.2 Hz, 1H), 3.91 (d, J = 6.6 Hz, 2H), 2.65 (s, 3H), 2.22 (dt, 6.6 Hz, 1H), 1.10 (d, J = 2.9 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.98, 160.01, 148.49, 138.86, 131.97, 125.40, 123.58, 115.14, 114.36, 101.37, 75.23, 28.61, 18.72, 11.30. HRMS: calcd for $C_{15}H_{16}N_4O_3$ $[M - H]^-$ 299.1150, found 299.1134. IR_{max}/cm^{-1} 3430, 1704, 1288 (COOH), 2232 (CN), 2961, 2929, 2877, 1469, 1438, 1384 (CH_3, CH_2), 1614, 1533, 1506 (Ar), 1259 (C–O–C) (KBr).

11.5. 2-(4-Amyloxy-3-cyanophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acid (7e). White solid, (yield: 91%), mp: 155–157 °C, ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.23–8.17 (m, 1H), 7.42 (d, J = 9.3 Hz, 1H), 4.18 (t, J = 6.5 Hz, 2H), 2.50 (s, 3H), 1.80–1.72 (m, 2H), 1.47–1.39 (m, 2H), 1.39–1.31 (m, 2H), 0.90 (t, J = 7.2 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 161.95, 159.95, 148.50, 138.74, 131.91, 125.37, 123.61, 115.20, 114.29, 101.32, 69.42, 28.97, 27.46, 21.78, 13.89, 11.29. HRMS: calcd for $C_{16}H_{18}N_4O_3$ $[M - H]^-$ 313.1306, found 313.1305. IR_{max}/cm^{-1} 3426, 1708, 1289 (COOH), 2232 (CN), 2958, 2929, 2860, 1467, 1436, 1384 (CH_3, CH_2), 1614, 1529, 1506 (Ar), 1258 (C–O–C) (KBr).

11.6. 2-(3-Cyano-4-isoamyloxyphenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acid (7f). White solid, (yield: 92%), mp:

185–186 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (d, J = 2.7 Hz, 1H), 8.30 (dd, J = 9.2, 2.7 Hz, 1H), 7.10 (d, J = 9.2 Hz, 1H), 4.19 (t, J = 6.6 Hz, 2H), 2.67 (s, 3H), 1.95 (m, 1H), 1.81 (q, J = 6.6 Hz, 2H), 1.02 (d, J = 6.6 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.97, 159.92, 148.47, 138.77, 131.91, 125.31, 123.55, 115.19, 114.26, 101.30, 67.99, 36.99, 24.62, 22.38, 11.29. HRMS: calcd for $C_{16}H_{18}N_4O_3$ $[M - H]^-$ 313.1306, found 313.1288. IR_{max}/cm^{-1} 3432, 1704, 1289 (COOH), 2231 (CN), 2957, 2927, 1466, 1437, 1384 (CH_3, CH_2), 1615, 1532, 1506 (Ar), 1259 (C–O–C) (KBr).

11.7. 2-(4-Benzyloxy-3-cyanophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acid (7g). White solid, (yield: 86%), mp: 199–201 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (d, J = 2.6 Hz, 1H), 8.28 (dd, J = 9.2, 2.7 Hz, 1H), 7.51–7.40 (m, 5H), 7.15 (d, J = 9.2 Hz, 1H), 5.32 (s, 2H), 2.67 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.20, 163.19, 150.55, 149.12, 139.14, 136.56, 133.92, 131.10, 127.83, 127.51, 126.06, 123.22, 115.93, 114.89, 70.70, 10.53. HRMS: calcd for $C_{18}H_{14}N_4O_3$ $[M - H]^-$ 333.0933, found 333.1100.

11.8. 2-(4-Allyloxy-3-cyanophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acid (7h). White solid, (yield: 84%), mp: 212–215 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, J = 2.7 Hz, 1H), 8.30 (dd, J = 9.3, 2.7 Hz, 1H), 7.10 (d, J = 9.3 Hz, 1H), 6.09 (ddd, J = 22.3, 10.6, 5.1 Hz, 1H), 5.54 (dd, J = 17.3, 1.1 Hz, 1H), 5.41 (d, J = 10.6 Hz, 1H), 4.77 (d, J = 5.1 Hz, 2H), 2.67 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.16, 160.05, 150.05, 137.35, 132.60, 131.33, 124.98, 124.59, 118.92, 115.05, 113.35, 103.18, 70.13, 10.54. HRMS: calcd for $C_{14}H_{12}N_4O_3$ $[M - H]^-$ 283.0837, found 283.0832.

11.9. 5-Methyl-2-(3-nitro-4-propoxyphenyl)-2*H*-1,2,3-triazole-4-carboxylic acid (8a). White solid, (yield: 80%), mp: 204–205 °C, ^{1}H NMR (600 MHz, DMSO-d_6) 8.42 (d, J = 2.7 Hz, 1H), 8.21 (dd, J = 9.2, 2.7 Hz, 1H), 7.55 (d, J = 9.2 Hz, 1H), 4.18 (t, J = 6.3 Hz, 2H), 2.52 (s, 3H), 1.80–1.72 (m, 2H), 0.99 (t, J = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 162.93, 151.12, 148.65, 139.09, 138.96, 131.09, 124.29, 116.48, 115.58, 71.19, 21.78, 11.30, 10.19. HRMS: calcd for $C_{13}H_{14}N_4O_5$ $[M - H]^-$ 305.0891, found 305.0874.

11.10. 2-(4-Isopropoxy-3-nitrophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acid (8b). White solid, (yield: 94%), mp: 201–203 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, J = 2.7 Hz, 1H), 8.28 (dd, J = 9.3, 2.7 Hz, 1H), 7.22 (d, J = 9.3 Hz, 1H), 4.78 (m, 1H), 2.67 (s, 3H), 1.46 (d, J = 6.1 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.93, 149.51, 148.64, 140.12, 131.02, 123.99, 117.63, 115.52, 72.80, 21.57, 11.31. HRMS: calcd for $C_{13}H_{14}N_4O_5$ $[M - H]^-$ 305.0891, found 305.0878.

11.11. 2-(4-Butoxy-3-nitrophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acid (8c). White solid, (yield: 91%), mp: 183–185 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (d, J = 2.7 Hz, 1H), 8.30 (dd, J = 9.2, 2.7 Hz, 1H), 7.21 (d, J = 9.2 Hz, 1H), 4.20 (t, J = 6.4 Hz, 2H), 2.67 (s, 3H), 1.88 (tt, J = 12.5, 6.4 Hz, 2H), 1.61–1.51 (m, 2H), 1.02 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.86, 151.13, 148.60, 138.98, 138.88, 131.03, 124.21, 116.42, 115.49, 69.50, 30.37, 18.52, 13.55, 11.26. HRMS: calcd for $C_{14}H_{16}N_4O_5$ $[M - H]^-$ 319.1048, found 319.1041.

11.12. 2-(4-Isobutoxy-3-nitrophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acid (8d). White solid, (yield: 86%), mp: 203–205 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, J = 2.7 Hz, 1H), 8.30 (dd, J = 9.2, 2.7 Hz, 1H), 7.20 (d, J = 9.2 Hz, 1H), 3.96 (d, J = 6.4 Hz, 2H), 2.70 (s, 3H), 2.26–2.15 (m, 1H), 1.10 (d, J = 6.7 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.91, 150.24, 147.64, 137.89, 130.03, 123.29, 115.38, 114.56, 74.57, 26.65, 17.71, 10.29. HRMS: calcd for $C_{14}H_{16}N_4O_5$ $[M - H]^-$ 319.1048, found 319.1029.

11.13. 2-(4-Isoamyloxy-3-nitrophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acid (8e). Light yellow, (yield: 89%), mp: 183–184 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (d, J = 2.6 Hz, 1H), 8.30 (dd, J = 9.2, 2.6 Hz, 1H), 7.21 (d, J = 9.2 Hz, 1H), 4.19 (t, J = 6.4 Hz, 2H), 2.67 (s, 3H), 1.94–1.84 (m, 2H), 1.55–1.47 (m, 2H), 1.41 (dt, J = 14.3, 7.2 Hz, 2H), 0.97 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.73, 152.41, 150.30, 139.86, 137.64, 131.73, 124.43, 116.98, 115.19, 100.12, 70.42, 28.71, 28.07, 22.45, 14.08, 11.73. HRMS: calcd for $C_{15}H_{18}N_4O_5$ $[M - H]^-$ 333.1204, found 333.1187. IR_{max}/cm^{-1} 3425, 1702, 1279 (COOH), 1540 (NO_2), 2959, 2933, 2873, 1469, 1433, 1383 (CH_3, CH_2), 1625, 1590, 1497 (Ar), 1255 (C–O–C) (KBr).

11.14. 2-(4-Isoamyloxy-3-nitrophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acid (8f). Light yellow, (yield: 90%), mp: 200–201 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (d, J = 2.7 Hz, 1H), 8.30 (dd, J = 9.2, 2.7 Hz, 1H), 7.22 (d, J = 9.2 Hz, 1H), 4.22 (t, J = 6.5 Hz, 2H), 2.67 (s, 3H), 1.90 (td, J = 13.3, 6.6 Hz, 1H), 1.79 (q, J = 6.5 Hz, 2H), 1.01 (d, J = 6.6 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.63, 152.24, 150.13, 139.82, 137.45, 131.65, 124.27, 116.87, 115.05, 68.74, 37.52, 24.90, 22.47, 11.56. HRMS: calcd for $C_{15}H_{18}N_4O_5$ $[M - H]^-$ 333.1204, found 333.1205. IR_{max}/cm^{-1} 3428, 1704, 1281 (COOH), 1540 (NO_2), 2959, 2873, 1472, 1432, 1385 (CH_3, CH_2), 1625, 1591, 1497 (Ar), 1256 (C–O–C) (KBr).

11.15. 2-(4-Benzyloxy-3-nitrophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acid (8g). Light yellow, (yield: 93%), mp: 204–206 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (d, J = 2.7 Hz, 1H), 8.29 (dd, J = 9.1, 2.6 Hz, 1H), 7.51–7.38 (m, 5H), J = 9.1, 7.26 (d, 1H) 5.33 (s, 2H), 2.66 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.19, 150.55, 149.12, 139.14, 136.56, 133.92, 131.10, 127.83, 127.51, 126.06, 123.22, 115.93, 114.89, 70.70, 28.69, 10.53. HRMS: calcd for $C_{17}H_{14}N_4O_5$ $[M - H]^-$ 353.0891, found 353.0889.

11.16. 2-(4-Allyloxy-3-nitrophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carboxylic acid (8h). Light yellow, (yield: 91%), mp: 220–222 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, J = 2.7 Hz, 1H), 8.30 (dd, J = 9.2, 2.7 Hz, 1H), 7.22 (d, J = 9.2 Hz, 1H), 6.08 (ddd, J = 22.2, 10.4, 5.0 Hz, 1H), 5.58–5.51 (m, 1H), 5.41 (dd, J = 10.4, 1.2 Hz, 1H), 4.79 (d, J = 5.0 Hz, 2H), 2.67 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.89, 150.55, 148.69, 139.19, 138.95, 132.22, 131.33, 124.23, 118.17, 116.84, 115.67, 69.95, 11.29. HRMS: calcd for $C_{13}H_{12}N_4O_5$ $[M - H]^-$ 303.0736, found 303.0716.

12. General procedure for the preparation of compounds 9e–f and 10e–f

A mixture of the corresponding compound **22** or **23** (0.3 mmol), hydrazine hydrate (1.0 mL) in EtOH (10.0 mL) was heated at 55 °C for 5 h. After the reaction was completed, the solvent was then removed under reduced pressure. The residue was purified by flash column chromatography (0–5% methanol in DCM) to yield the desired product.

12.1. 2-(4-Amyloxy-3-cyanophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carbohydrazide (9e). White solid, (yield: 77%), mp: 113–115 °C, ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.30 (d, J = 2.7 Hz, 1H), 8.23 (dd, J = 9.2, 2.7 Hz, 1H), 7.45 (d, J = 9.2 Hz, 1H), 4.19 (t, J = 6.5 Hz, 2H), 2.50 (s, 3H), 1.80–1.74 (m, 2H), 1.46–1.40 (m, 2H), 1.36 (dt, J = 14.0, 7.2 Hz, 2H), 0.90 (t, J = 7.2 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.71, 159.60, 146.41, 139.50, 132.05, 124.97, 123.41, 115.39, 114.33, 101.27, 69.42, 27.98, 27.47, 21.78, 13.90, 10.81.

12.2 2-(3-Cyano-4-isopentyloxyphenyl)-5-methyl-2*H*-1,2,3-triazole-4-carbohydrazide (9f). White solid, (yield: 68%), mp: 124–126 °C, ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.30 (d, J = 2.7 Hz, 1H), 8.23 (dd, J = 9.2, 2.7 Hz, 1H), 7.47 (d, J = 9.2 Hz, 1H), 4.22 (t, J = 6.6 Hz, 2H), 2.50 (s, 3H), 1.85–1.78 (m, 1H), 1.67 (q, J = 6.6 Hz, 2H), 0.95 (d, J = 6.6 Hz, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.75, 159.63, 146.46, 139.54, 132.09, 125.03, 123.45, 115.44, 114.38, 101.29, 68.04, 24.66, 22.43, 20.52, 10.85. HRMS: calcd for $C_{16}H_{20}N_6O_2$ $[M + H]^+$ 329.1721, found 329.1721. IR_{max}/cm^{-1} 3423, 1669 ($CONHNH_2$), 2230 (CN), 2958, 2929, 1468, 1433, 1384 (CH_3, CH_2), 1625, 1543, 1508 (Ar), 1256 (C–O–C) (KBr).

12.3. 5-Methyl-2-(3-nitro-4-pentyloxyphenyl)-2*H*-1,2,3-triazole-4-carbohydrazide (10e). Light yellow, (yield: 60%), mp: 117–118 °C, ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.47 (d, J = 2.7 Hz, 1H), 8.23 (dd, J = 9.2, 2.7 Hz, 1H), 7.57 (d, J = 9.2 Hz, 1H), 4.24–4.18 (m, 2H), 2.51 (s, 3H), 1.76–1.71 (m, 2H), 1.43–1.37 (m, 2H), 1.37–1.30 (m, 2H), 0.88 (t, J = 7.2 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.55, 150.92, 146.62, 139.75, 139.16, 131.25, 124.01, 116.54, 115.33, 69.82, 28.03, 27.48, 21.76, 13.92, 10.85. HRMS: calcd for $C_{15}H_{20}N_6O_4$ $[M + H]^+$ 349.1619, found 349.1616.

12.4. 2-(4-Isopentyloxy-3-nitrophenyl)-5-methyl-2*H*-1,2,3-triazole-4-carbohydrazide (10f). Light yellow, (yield: 66%), mp: 120–122 °C, ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.83 (s, 1H), 8.46 (d, J = 2.6 Hz, 1H), 8.22 (dd, J = 9.2, 2.6 Hz, 1H), 7.59 (d, J = 9.2 Hz, 1H), 4.24 (t, J = 6.5 Hz, 2H), 2.51 (s, 3H), 1.83–1.75 (m, 1H), 1.64 (q, J = 6.5 Hz, 2H), 0.92 (d, J = 6.6 Hz, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 159.89, 151.25, 146.94, 140.08, 139.48, 131.59, 124.30, 116.87, 115.63, 68.71, 37.40, 24.87, 22.68, 11.19.

13. Assay of the *in vitro* xanthine oxidase inhibitory activity

The inhibitory activity of Bovine milk XO *in vitro* was assayed spectrophotometrically by measuring the absorbance of uric acid at 295 nm at 25 °C based on the procedure by Kalra *et al.* and Tamta *et al.* with some modification.[22,23] The enzyme assay mixture contained 50 mM phosphate buffer (pH 8.1), 100 mM xanthine (Sigma, X7375), optimal xanthine oxidase (Sigma, X4875), and the test compound at the concentration range of 50–500 nM, or absent for the purpose of the control reaction. The enzyme was preincubated for 5 min with the test compounds at various concentrations, and the reaction was initiated by the addition of xanthine and monitored at 295 nm after incubation for 30 min. The inhibitory activity of each test compound against XO was indicated in terms of IC_{50} value, which was calculated based on a non-linear regression analysis. All the experiments were repeated three to four times and values were expressed as means of several experiments.

14. Molecular modeling

Discovery Studio 3.0 was used to perform molecular docking. The crystal structure of XO (PDB code: 1N5X) with the corresponding entry was adopted as a target protein. In the docking process, the protein was prepared *via* several operations, which was illustrated in detail in our previous study.[14] Then, the compound **7f** was drawn with Chemdraw and the energy fully minimized using the CHARMm force field. Finally, it was docked into the binding site using the CDOCKER protocol with the default settings. The schematic diagrams of interactions between the active site of XO and compound **7f** were analyzed by Discovery Studio software package.

Acknowledgements

We gratefully acknowledge the National Natural Science Foundation of China (81502932, 81673293, 81602969). This work was also supported by Program for Innovative Research Team of the Ministry of Education and Program for Liaoning Innovative Research Team in University.

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