European Journal of Medicinal Chemistry 103 (2015) 343–353

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Synthesis and evaluation of 1-hydroxy/methoxy-4-methyl-2-phenyl-1*H*-imidazole-5-carboxylic acid derivatives as non-purine xanthine oxidase inhibitors

Shaolei Chen [a, 1], Tingjian Zhang [b, 1], Jian Wang [a], Fangyang Wang [c], Handong Niu [a], Chunfu Wu [c], Shaojie Wang [a, *]

[a] Key Laboratory of Structure-Based Drugs Design & Discovery of Ministry of Education, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, 103 Culture Road, Shenhe District, Shenyang 110016, China
[b] School of Pharmacy, China Medical University, 77 Puhe Road, North New Area, Shenyang 110122, China
[c] Department of Pharmacology, Shenyang Pharmaceutical University, 103 Culture Road, Shenhe District, Shenyang 110016, China

ARTICLE INFO

Article history:
Received 4 February 2015
Received in revised form
27 August 2015
Accepted 28 August 2015
Available online 3 September 2015

Keywords:
4-Methyl-2-phenyl-1*H*-imidazole-5-carboxylic acid
Gout
Xanthine oxidase inhibitor

ABSTRACT

Xanthine oxidase is a key enzyme that catalyses hypoxanthine and xanthine to uric acid, whose overproduction leads to the gout-causing hyperuricemia. In this study, a series of 1-hydroxy/methoxy-4-methyl-2-phenyl-1*H*-imidazole-5-carboxylic acid derivatives (**4a**–**4k** and **6a**–**6k**) was synthesized and evaluated for their inhibitory potency against xanthine oxidase. The 1-hydroxyl substituted derivatives **4a**–**4k** showed excellent inhibitory potency with IC_{50} values ranging from 0.003 μM to 1.2 μM, with compounds **4d** (IC_{50} = 0.003 μM), **4e** (IC_{50} = 0.003 μM), and **4f** (IC_{50} = 0.006 μM) manifesting the most potent xanthine oxidase inhibitory potency that were comparable with that of Febuxostat (IC_{50} = 0.01 μM). Lineweaver–Burk plot analysis revealed that representative compound **4f** acted as a mixed-type inhibitor for xanthine oxidase. The basis of significant inhibition of xanthine oxidase by **4f** was rationalized by its molecular docking into the active site of xanthine dehydrogenase.

1. Introduction

Gout is a common metabolic disorder characterized by gouty arthritis and caused by the deposition of monosodium urate crystals within joints. Gout affects millions worldwide and is increasing in prevalence [1]. As a key enzyme in the last two steps of the purine metabolic pathway, xanthine oxidase (XO) could catalyze the oxidation of hypoxanthine to xanthine, and then to uric acid. The overproduction of uric acid leads to hyperuricemia, which is a key cause of gout. Therefore, XO is considered the most promising target for treating this condition [2,3]. Allopurinol (Fig. 1), a prototypical inhibitor of XO, 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 of allopurinol and its analogs with purine backbone have been reported, such as fulminant hepatitis, renal failure, and Stevens–Johnson syndrome [4]. Therefore, identifying some novel non-purine XO inhibitors with potent XO inhibitory potency and fewer side effects is extremely necessary. In fact, some excellent non-purine XO inhibitors have been reported in the recent literature, including carboxyl moiety containing inhibitors, such as Febuxostat [5], Y-700 [6], selenazoles [7], isoxazoles [8] and 2-(indol-5-yl)thiazoles [9]; and non-carboxyl moiety containing inhibitors, like Topiroxostat [10], isocytosines [11–13], *N*-(1,3-diaryl-3-oxopropyl)amides [14], *N*-1-acetyl-3,5-diaryl-4,5-dihydro- (1*H*) pyrazoles [15], naphthoflavones [16], 2-amino-5-alkylidene-thiazol-4-ones [17], azaflavones [18], naphthopyrans [19], 4,6-diaryl/heteroarylpyrimidin-2(1*H*)-ones [20], and 2,4-diaryl- pyrano[3,2-c] chromen-5(4*H*)-one [21] (Fig. 1). Among them, Febuxostat and Topiroxostat were successfully introduced into the market in 2009 in the USA and 2013 in Japan, respectively. Indeed, non-purine XO inhibitors have drawn extensive attention from pharmaceutical industries worldwide.

In continuation of our previous efforts on finding novel non-purine XO inhibitors [8], we had designed and synthesized a series of 1-hydroxy/methoxy-4-methyl-2-phenyl-1*H*-imidazole-5-

* Corresponding author.
E-mail address: sjwang_99@163.com (S. Wang).
[1] These two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ejmech.2015.08.056
0223-5234/

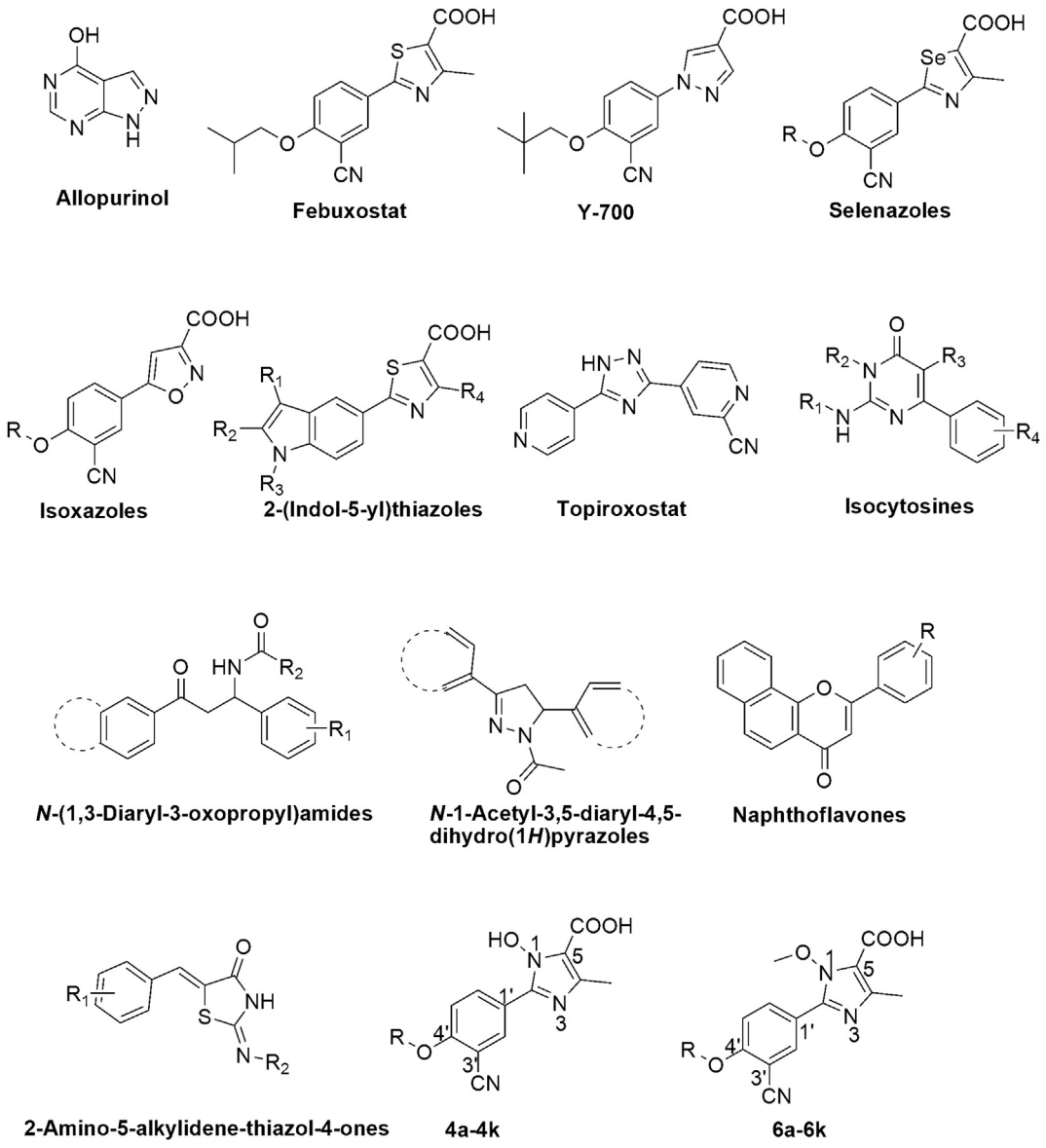


Fig. 1. Chemical structures of Allopurinol, non-purine XO inhibitors, and designed compounds **4a**–**4k** and **6a**–**6k.**

carboxylic acid derivatives (Fig. 1) and tested their XO inhibitory potency.

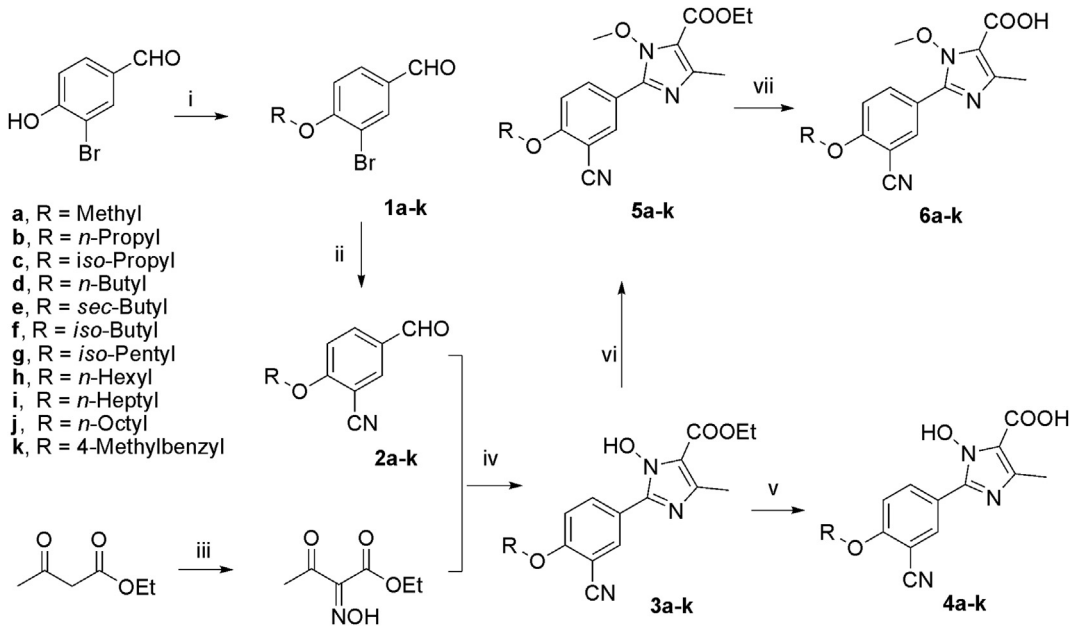
2. Results and discussion

2.1. Chemistry

The synthesis of the target 1-hydroxy/methoxy-4-methyl-2-phenyl-1*H*-imidazole-5-carboxylic acid derivatives **4a**–**4k** and **6a**–**6k** was performed as outlined in Scheme 1. The commercially available 3-bromo-4-hydroxybenzaldehyde was alkylated with dimethyl sulfate or appropriate alkyl bromides in DMF in the presence of potassium carbonate to provide 3-bromo-4-alkyloxybenzaldehydes **1a**–**1k**, which were treated with cuprous cyanide in DMF to obtain 2-alkyloxy-5-formylbenzonitriles **2a**–**2k** [8]. The ethyl 2-(4-alkyloxy-3-cyanophenyl)-1- hydroxy-4-methyl-1*H*-imidazole-5-carboxylates **3a**–**3k**, as key intermediates, were prepared through cyclization of the corresponding 2-alkyloxy-5-formylbenzonitriles with ethyl 2-hydroxyimino-3-oxobutanoate in the presence of ammonium acetate [22]. 2-Hydroxyimino-3-oxobutanoate was obtained by nitrosation of ethyl 3-oxobutanoate with sodium nitrite in acetic acid [23].

Key intermediates **3a**–**3k** were hydrolyzed with sodium hydroxide followed by acidification to obtain 1-hydroxyimidazole series compounds **4a**–**4k**. The methylation of compounds **3a**–**3k** with dimethyl sulfate to give compounds **5a**–**5k** [24], which were then hydrolyzed with sodium hydroxide and then acidified to provide 1-methoxyimidazole series compounds **6a**–**6k** [8].

Structures of the synthesized target compounds were elucidated by ESI-HRMS, IR, ^{1}H NMR, and ^{13}C NMR analyses. All spectral data were in accordance with assumed structures. In the ESI-HRMS analysis, **4a** showed an $[M + Na]^{+}$ ion peak, whereas the other compounds showed $[M + H]^{+}$ ion peaks. The IR spectra of target compounds displayed cyano group stretching vibrations at 2223–2236 cm^{-1}. In ^{1}H NMR spectra, the 4-methyl group of imidazole was observed as a singlet at around 2.51 and 2.39 ppm



Scheme 1. Reagents and conditions: (i) Me_2SO_4, K_2CO_3, 0 °C, 2 h or RBr, K_2CO_3, KI, DMF, 8 h; (ii) CuCN, DMF, 150 °C, 6 h; (iii) $NaNO_2$, AcOH, 0 °C, 3 h; (iv) NH_4OAc, AcOH, 50 °C, 24 h; (v) NaOH, THF, EtOH, H_2O, 50 °C, 8 h, then 1 M HCl; (vi) Me_2SO_4, K_2CO_3, 0 °C, 1.5 h; (vii) NaOH, THF, EtOH, H_2O, 50 °C, 8 h, then 1 M HCl.

for most of the 1-hydroxy substituted compounds **4a**–**4k** and 1-methoxy substituted compounds **6a**–**6k**, respectively. Moreover, the 1-methoxy group of imidazole was observed as a singlet at around 3.95 ppm for 1-methoxy substituted compounds **6a**–**6k**.

2.2. Biological activity

In vitro bovine XO inhibitory potency for target compounds **4a**–**4k** and **6a**–**6k** were spectrophotometrically measured by following uric acid levels at 295 nm [25]. Febuxostat was included as a reference compound. Results are shown in Table 1.

In general, the 1-hydroxyl substituted compounds **4a**–**4k** exhibited excellent inhibitory potency with IC_{50} values ranging from 0.003 μM to 1.2 μM. In particular, compounds **4d** (IC_{50} = 0.003 μM, pIC_{50} = 8.5), **4e** (IC_{50} = 0.003 μM, pIC_{50} = 8.5), and **4f** (IC_{50} = 0.006 μM, pIC_{50} = 8.2), bearing a *n*-butoxy, *sec*-butoxy, and *iso*-butoxy substituent at the 4′-position of phenyl moiety, respectively, emerged as the most potent XO inhibitors, and their potencies were 3.3-fold, 3.3-fold, and 1.7-fold higher than that of Febuxostat (IC_{50} = 0.01 μM, pIC_{50} = 8.0), respectively. However, compounds **4d**, **4e, 4f** and Febuxostat were in the same order of magnitude according to their pIC_{50} values.

As shown in Table 1, the XO inhibitory potency for the 1-hydroxyl substituted compounds was influenced by the size of the carbon chain at the 4′-position. Potencies decreased when butoxy groups (*n*-butoxy, *sec*-butoxy, and *iso*-butoxy) were replaced with methoxy (**4a**, IC_{50} = 0.55 μM), *n*-propoxy (**4b**, IC_{50} = 0.13 μM), or *iso*-propoxy (**4c**, IC_{50} = 0.17 μM). Moreover, it was also found that the inhibitory potency gradually declined with the increased size of 4′-position groups from butoxy to octyloxy. The exceptions were compounds **4g** and **4h**, bearing *iso*-pentyloxy and *n*-hexyloxy, respectively, which had the same inhibitory potency. In addition, to discuss the potency of benzyloxy substitution at the 4′-position, the 4-methoxybenzyloxy-substituted **4k** (IC_{50} = 0.11 μM) was synthesized and found to have only moderate potency. Therefore, the presence of a butoxy group (*n*-butoxy, *sec*-butoxy, or *iso*-butoxy) at the 4′-position benefited the XO inhibitory potency of 1-hydroxy-4-methyl-2-phenyl-1*H*-imidazole-5-carboxylic acid derivatives. This finding was also true for 1-phenylpyrazoles [6] and 5-phenylisoxazoles [8] XO inhibitors. A dose-dependent inhibition of representative compound **4f** on XO was exhibited (Fig. 2) and Lineweaver–Burk plot analysis revealed

Table 1
In vitro XO inhibitory potency of designed compounds.

Compound	Inhibition rate at 10 μg/mL (%)	IC_{50} (μM)[a]	pIC_{50}	Compound	Inhibition rate at 10 μg/mL (%)	IC_{50} (μM)[a]	pIC_{50}
4a	90.14 ± 2.01	0.55	6.3	**6a**	10.68 ± 0.09	n.a.[b]	n.a.[b]
4b	89.77 ± 1.19	0.13	6.9	**6b**	18.34 ± 0.63	n.a.	n.a.
4c	91.04 ± 0.23	0.17	6.8	**6c**	32.91 ± 0.98	n.a.	n.a.
4d	89.57 ± 0.54	0.003	8.5	**6d**	41.19 ± 2.00	n.a.	n.a.
4e	88.33 ± 1.44	0.003	8.5	**6e**	83.13 ± 0.70	1.5	5.8
4f	94.08 ± 0.89	0.006	8.2	**6f**	31.91 ± 0.97	n.a.	n.a.
4g	91.25 ± 0.73	0.03	7.5	**6g**	59.55 ± 0.84	8.0	5.1
4h	92.51 ± 0.71	0.03	7.5	**6h**	80.34 ± 1.26	3.9	5.4
4i	89.93 ± 1.37	0.11	7.0	**6i**	26.80 ± 1.97	n.a.	n.a.
4j	88.17 ± 0.11	1.2	5.9	**6j**	19.00 ± 0.75	n.a.	n.a.
4k	91.65 ± 0.88	0.11	7.0	**6k**	86.72 ± 1.59	1.1	5.9
Febuxostat	90.89 ± 0.16	0.01	8.0				

[a] IC_{50} values: the concentration of inhibitor required to produce 50% inhibition of xanthine oxidase (mean ± SD; n = 4).
[b] n.a.: not active (<50% inhibition at 10 μg/mL).

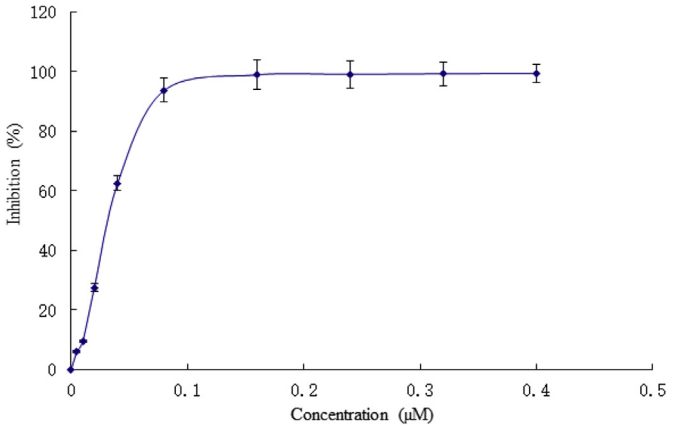


Fig. 2. The inhibition of compound **4f** on XO. Values are means ± SD, n = 3.

that **4f** acted as a mixed-type inhibitor for XO (Fig. 3).

To explore the influence of bulk at the 1-position of imidazole moiety [5], the 1-methoxyl-substituted compounds **6a**–**6k** were designed and synthesized. Biological data presented in Table 1 showed that the inhibitory potency of all these compounds obviously decreased compared with the corresponding compounds **4a**–**4k.** The majority of these compounds were inactive (<50% inhibition at 10 μg/mL), except **6e** (IC_{50} = 1.5 μM), **6g** (IC_{50} = 8.0 μM), **6h** (IC_{50} = 3.9 μM) and **6k** (IC_{50} = 1.1 μM), their IC_{50} values were 497-fold, 268-fold, 129-fold and 10-fold lower than the corresponding **4e** (IC_{50} = 0.003 μM), **4g** (IC_{50} = 0.03 μM), **4h** (IC_{50} = 0.03 μM) and **4k** (IC_{50} = 0.11 μM). These results suggested that 1-hydroxy group was much acceptable than 1-methoxyl group.

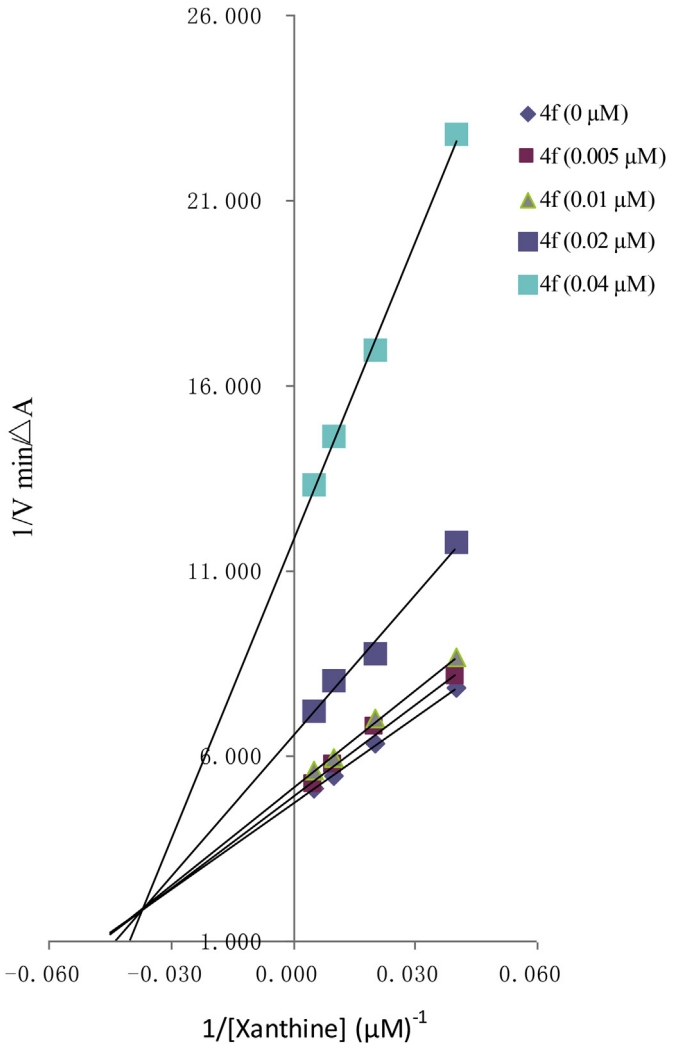


Fig. 3. Lineweaver–Burk plot of the inhibition of xanthine oxidase by compound **4f**.

2.3. Molecular modeling

XO is a form of molybdoflavin protein xanthine oxidoreductase [26], and bovine and human sequences of xanthine oxidoreductase are characterized by 90% identity [27]. The crystal structure of the complex of bovine xanthine dehydrogenase (XDH) with Febuxostat shows a highly specific binding pocket, presenting a long, narrow cavity leading toward the molybdenum-pterin center [5]. Moreover, molybdenum-pterin sites of both XO and XDH are structurally equivalent [27].

To rationalize the structure–activity relationships observed and to gain an insight into the binding mode of synthesized compounds with XO, docking simulations of **4f** and **6f** into the binding pocket of the bovine milk XDH/Febuxostat complex (PDB entry code: 1N5X) were performed [5]. The simulations were carried out with AutoDock 4 software package [28], and carboxyl groups of **4f** and **6f** were calculated in dissociated form. The energy-minimized structure of Febuxostat was preliminarily docked into XDH to examine how closely the AutoDock 4 algorithm can reproduce the binding modes observed in the crystallographic structure. A superposition of docked Febuxostat onto the crystallographic geometry yielded a root mean square deviation (RMSD) of 0.95 Å. The hydrogen bonds predicted by AutoDock 4 were virtually identical to those found in the crystal structure.

As shown in Fig. 4, compound **4f** interacted very closely with original ligand (Febuxostat) by hydrogen bonds that involved the carboxylate moiety interacting with Arg880 and Thr1010, the nitrogen atom of imidazole forming hydrogen bond with Glu802, and the nitrile group of phenyl unit forming hydrogen bond with Asn768 [5]. More importantly, 1-hydroxyl group of **4f** exhibited an extra hydrogen bond interaction with Thr1010, which may help **4f** to anchor onto the binding site more tightly. Furthermore, the calculated pKa of **4f** was 2.01, lower than that of Febuxostat (2.39), which may benefit the interaction of carboxylate group with guanidinium side chain of Arg880 [5,13]. These observations could provide a rational explanation for the inhibitory potency difference between **4f** and Febuxostat and also indicated that introducing a hydroxyl group into the position between phenyl unit and carboxyl moiety may be favorable for Febuxostat analogues to display better XO inhibitory potency.

Meanwhile, **6f**, which may have bumped with protein residues for the presence of 1-methoxy group, interacted in a distinct way with XDH by inserting the *iso*-butoxy moiety into the channel instead of carboxylate segment. Consequently, carboxylate moiety showed an interaction with Lys771, and the nitrile group of phenyl unit formed a hydrogen bond with Thr1010. These two interactions may help fix the molecule at the binding site of XO. However, interactions between **6f** and XO may be too weak compared with those of **4f** and Febuxostat to produce any apparent inhibitory potency in the experimental test. As exemplified by **6f**, other compounds in the 1-methoxy series may interact with XO in a similar pattern, which could reasonably explain their inferior potencies compared with those of their counterparts in the 1-hydroxy series.

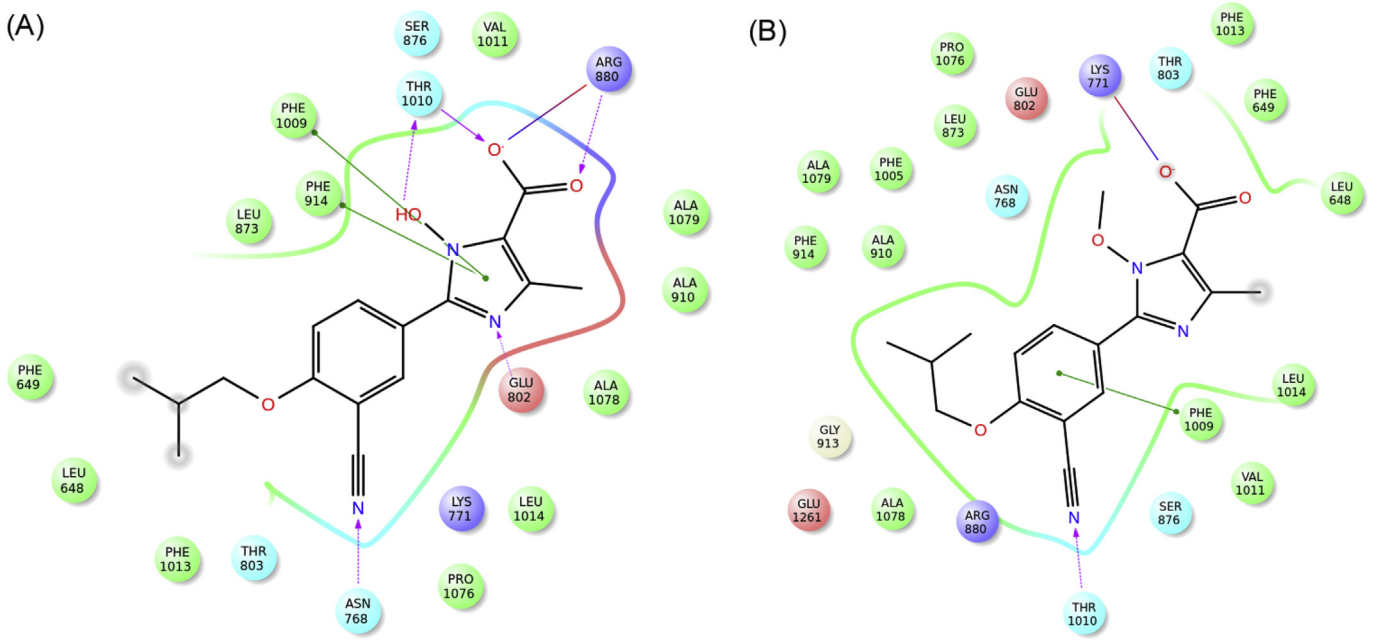


Fig. 4. Binding modes of **4f** (A) and **6f** (B) within the protein binding pocket.

3. Conclusions

We report the synthesis and in vitro XO inhibitory potency of 1-hydroxy/methoxy-4-methyl-2-phenyl-1*H*-imidazole-5-carboxylic acid derivatives. 1-Hydroxyl substituted derivatives **4a**-**4k** were found to have excellent inhibitory potency, with IC_{50} values ranging from 0.003 μM to 1.2 μM. Among them, compounds **4d** (IC_{50} = 0.003 μM), **4e** (IC_{50} = 0.003 μM), and **4f** (IC_{50} = 0.006 μM) emerged as the most potent XO inhibitors comparable with Febuxostat (IC_{50} = 0.01 μM). 1-Hydroxy group was much acceptable than 1-methoxyl group. Molecular docking studies rationalized the structure–activity relationships observed and provided insight into the binding mode of the synthesized compounds with XO. Lineweaver–Burk plot analysis revealed that representative compound **4f** acted as a mixed-type inhibitor for xanthine oxidase. The most potent compounds **4d**, **4e**, and **4f** appeared to be promising candidates for treating hyperuricemia, gout, and other diseases involving XO. Moreover, our research indicated that introducing a hydroxyl group into the position between phenyl unit and carboxyl moiety may be favorable for Febuxostat analogues to display better XO inhibitory potency.

4. Experimental protocols

4.1. Chemistry

Reagents and solvents were purchased from commercial sources, and some were redistilled under a positive pressure of dry nitrogen atmosphere in the presence of an appropriate desiccant when necessary. Melting points were obtained using an YRT-3 melting apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 300, 400, or 600 MHz spectrometer. Chemical shifts were expressed in parts per million using tetramethylsilane as an internal reference and DMSO-d_6 or $CDCl_3$ as solvent. IR spectra were determined as KBr pellets on a Bruker IFS-55 spectrometer and expressed in per centimeter. ESI-MS data were obtained using an Agilent 1100 instrument, and the ESI-HRMS data were obtained using a Bruker microTOF-Q instument. TLC was carried out using silica gel plates (Qingdao Makall Group Co., Ltd., Qingdao, China), and spots were visualized under UV light at 254 nm.

4.1.1. Synthesis of 3-bromo-4-methoxybenzaldehyde (**1a**)

Dimethyl sulfate (6.6 g, 0.053 mol) was added dropwise at a rate that maintained <0 °C reaction temperature to a stirred solution of 3-bromo-4-hydroxybenzaldehyde (10 g, 0.05 mol) and anhydrous potassium carbonate (10.28 g, 0.075 mol) in DMF (40 mL). Upon completion of addition, the mixture was stirred at 25 °C for another 2 h. The reaction mixture was slowly poured into cold water (100 mL) and stirred for 20 min. The solid was separated by filtration as a gray powder (9.7 g) in 90.6% yield, mp 40.1–41.0 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.84 (s, 1H, CHO), 8.08 (d, 1H, J = 2.0 Hz, Ar–H), 7.80 (dd, 1H, J = 2.0 & 8.5 Hz, Ar–H), 6.98 (d, 1H, J = 8.5 Hz, Ar–H), 3.86 (s, 3H, OCH_3).

4.1.2. General procedure for the synthesis of 3-bromo-4-alkoxybenzaldehydes **1b**–**1k**

A solution of 3-bromo-4-hydroxybenzaldehyde (50 mmol), anhydrous potassium carbonate (65 mmol), potassium iodide (1 mmol), and alkyl bromide (75 mmol) in DMF (40 mL) was stirred at 50 °C under nitrogen atmosphere for 8 h. After filtering the reaction mixture, the filtrate was concentrated in a vacuum. Ethyl acetate was added to the residue, and the ethyl acetate layer was sequentially washed with water and brine. After drying with anhydrous sodium sulfate and evaporation, the crude product was purified by column chromatography (petroleum ether) to provide the corresponding 4-alkoxy-3-bromobenzaldehydes **1b**–**1k**.

*4.1.2.1. 3-Bromo-4-propoxybenzaldehyde (**1b**).* A yellow powder, yield 80.5%, mp 38.3–39.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.83 (s, 1H, CHO), 8.07 (d, 1H, J = 1.9 Hz, Ar–H), 7.79 (dd, 1H, J = 2.0 & 8.5 Hz, Ar–H), 6.98 (d, 1H, J = 8.5 Hz, Ar–H), 4.08 (t, 2H, J = 6.4 Hz, OCH_2), 1.90 (m, 2H, CH_2), 1.10 (t, 3H, J = 7.4 Hz, CH_3).

*4.1.2.2. 3-Bromo-4-iso-propoxybenzaldehyde (**1c**).* A pale yellow oil, yield 77.0%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.83 (s,1H, CHO), 8.08 (d,

1H, J = 2.0 Hz, Ar–H), 7.78 (dd, 1H, J = 2.0 & 8.5 Hz, Ar–H), 7.00 (d, 1H, J = 8.6 Hz, Ar–H), 4.71 (m, 1H, OCH), 1.43 (d, 6H, J = 6.1 Hz, $2CH_3$).

*4.1.2.3. 3-Bromo-4-butoxybenzaldehyde (**1d**).* A white waxy solid, yield 90.0%, mp 37.2–38.6 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.83 (s, 1H, CHO), 8.08 (d, 1H, J = 2.0 Hz, Ar–H), 7.79 (dd, 1H, J = 2.0 & 8.5 Hz, Ar–H), 6.98 (d, 1H, J = 8.5 Hz, Ar–H), 4.12 (t, 2H, J = 6.4 Hz, OCH_2), 1.86 (m, 2H, CH_2), 1.56 (m, 2H, CH_2), 1.01 (t, 3H, J = 7.4 Hz, CH_3).

*4.1.2.4. 3-Bromo-4-sec-butoxybenzaldehyde (**1e**).* A yellow oil, yield 80.5%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.82 (s, 1H, CHO), 8.07 (d, 1H, J = 2.0 Hz, Ar–H), 7.77 (dd, 1H, J = 2.0 & 8.5 Hz, Ar–H), 6.97 (d, 1H, J = 8.5 Hz, Ar–H), 4.48 (m, 1H, OCH), 1.78 (m, 2H, CH_2), 1.38 (d, 3H, J = 6.1 Hz, CH_3), 1.02 (t, 3H, J = 7.4 Hz, CH_3).

*4.1.2.5. 3-Bromo-4-iso-butoxybenzaldehyde (**1f**).* A yellow oil, yield 64.7%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.83 (s, 1H, CHO), 8.07 (d, 1H, J = 1.9 Hz, Ar–H), 7.79 (dd, 1H, J = 2.0 & 8.5 Hz, Ar–H), 6.97 (d, 1H, J = 8.5 Hz, Ar–H), 3.88 (d, 2H, J = 6.4 Hz, OCH_2), 2.12 (m, 1H, CH), 1.09 (d, 6H, J = 6.7 Hz, $2CH_3$).

*4.1.2.6. 3-Bromo-4-iso-pentyloxybenzaldehyde (**1g**).* A pale yellow powder, yield 98.0%, mp 35.5–35.9 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.84 (s, 1H, CHO), 8.08 (d, 1H, J = 2.0 Hz, Ar–H), 7.80 (dd, 1H, J = 2.0 & 8.5 Hz, Ar–H), 6.99 (d, 1H, J = 8.5 Hz, Ar–H), 4.15 (t, 2H, J = 6.5 Hz, OCH_2), 1.90 (m, 1H, CH), 1.78 (m, 2H, CH_2), 0.98 (d, 6H, J = 6.5 Hz, $2CH_3$).

*4.1.2.7. 3-Bromo-4-hexyloxybenzaldehyde (**1h**).* A pale yellow powder, yield 90.9%, mp 36.8–37.4 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.84 (s, 1H, CHO), 8.08 (d, 1H, J = 2.0 Hz, Ar–H), 7.79 (dd, 1H, J = 2.0 & 8.5 Hz, Ar–H), 6.98 (d, 1H, J = 8.5 Hz, Ar–H), 4.12 (t, 2H, J = 6.5 Hz, OCH_2), 1.88 (m, 2H, CH_2), 1.52 (m, 2H, CH_2), 1.39–1.34 (m, 4H, $2CH_2$), 0.92 (t, 3H, J = 7.0 Hz, CH_3).

*4.1.2.8. 3-Bromo-4-heptyloxybenzaldehyde (**1i**).* A white powder, yield 98.0%, mp 49.3–50.1 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.84 (s, 1H, CHO), 8.08 (d, 1H, J = 1.9 Hz, Ar–H), 7.79 (dd, 1H, J = 1.9 & 8.5 Hz, Ar–H), 6.98 (d, 1H, J = 8.5 Hz, Ar–H), 4.12 (t, 2H, J = 6.5 Hz, OCH_2), 1.87 (m, 2H, CH_2), 1.49 (m, 2H, CH_2), 1.42–1.34 (m, 6H, $3CH_2$), 0.90 (t, 3H, J = 6.5 Hz, CH_3).

*4.1.2.9. 3-Bromo-4-octyloxybenzaldehyde (**1j**).* A yellow powder, yield 97.4%, mp 44.3–45.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.83 (s, 1H, CHO), 8.08 (d, 1H, J = 2.0 Hz, Ar–H), 7.80 (dd, 1H, J = 2.0 & 8.5 Hz, Ar–H), 6.98 (d, 1H, J = 8.5 Hz, Ar–H), 4.12 (t, 2H, J = 6.5 Hz, OCH_2), 1.88 (m, 2H, CH_2), 1.54 (m, 2H, CH_2), 1.42–1.34 (m, 8H, $4CH_2$), 0.89 (t, 3H, J = 6.5 Hz, CH_3).

*4.1.2.10. 3-Bromo-4-(4-methylbenzyloxy)benzaldehyde (**1k**).* A white powder, yield 88.0%, mp 49.3–50.1 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.85 (s, 1H, CHO), 8.08 (d, 1H, J = 1.9 Hz, Ar–H), 7.78 (dd, 1H, J = 1.9 & 8.5 Hz, Ar–H), 7.38 (d, 2H, J = 8.0 Hz, Ar–H), 7.24 (d, 2H, J = 7.8 Hz, Ar–H), 6.98 (d, 1H, J = 8.5 Hz, Ar–H), 5.32 (s, 2H, CH_2), 2.32 (s, 3H, CH_3).

*4.1.3. General procedure for the synthesis of 2-alkoxy-5-formylbenzonitriles **2a**–**2k***

A mixture of 4-alkoxy-3-bromobenzaldehyde (100 mmol), CuCN (110 mmol), and DMF (100 mL) was stirred at 150 °C under nitrogen atmosphere for 8 h. The reaction mixture was cooled to room temperature and diluted with CH_2Cl_2. The insoluble substance was filtered off, and the organic layer was sequentially washed with ammonia water, water, and brine. After drying with anhydrous sodium sulfate and evaporation, the crude product was purified by column chromatography (petroleum ether) to give the corresponding 2-alkoxy-5-formylbenzonitriles **2a**–**2k**.

*4.1.3.1. 5-Formyl-2-methoxybenzonitrile (**2a**).* A white powder, yield 56.5%, mp 70.1–72.0 °C ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.88 (s, 1H, CHO), 8.10 (d, 1H, J = 2.0 Hz, Ar–H), 8.06 (dd, 1H, J = 2.1 & 8.67 Hz, Ar–H), 7.10 (d, 1H, J = 8.7 Hz, Ar–H), 3.85 (s, 3H, OCH_3).

*4.1.3.2. 5-Formyl-2-propoxybenzonitrile (**2b**).* A yellow powder, yield 91.6%, mp 51.1–54.0 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.89 (s, 1H, CHO), 8.09 (d, 1H, J = 1.6 Hz, Ar–H), 8.06 (dd, 1H, J = 1.6 & 8.76 Hz, Ar–H), 7.10 (d, 1H, J = 8.7 Hz, Ar–H), 4.15 (t, 2H, J = 6.5 Hz, OCH_2), 1.92 (m, 2H, CH_2), 1.11 (t, 3H, J = 7.4, CH_3).

*4.1.3.3. 5-Formyl-2-iso-propoxybenzonitrile (**2c**).* A pale yellow powder, yield 85.3%, mp 51.5–52.6 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.88 (s, 1H, CHO), 8.09 (d, 1H, J = 2.0 Hz, Ar–H), 8.05 (dd, 1H, J = 2.1 & 8.7 Hz, Ar–H), 7.10 (d, 1H, J = 8.8 Hz, Ar–H), 4.80 (m, 1H, OCH), 1.46 (d, 6H, J = 6.1 Hz, $2CH_3$).

*4.1.3.4. 2-Butoxy-5-formylbenzonitrile (**2d**).* A white waxy solid, yield 88.0%, mp 21.8–23.0 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.89 (s, 1H, CHO), 8.09 (d, 1H, J = 2.0 Hz, Ar–H), 8.06 (dd, 1H, J = 2.1 & 8.6 Hz, Ar–H), 7.10 (d, 1H, J = 8.7 Hz, Ar–H), 4.20 (t, 2H, J = 6.4 Hz, OCH_2), 1.87 (m, 2H, CH_2), 1.56 (m, 2H, CH_2), 1.03 (t, 3H, J = 7.4, CH_3).

*4.1.3.5. 2-sec-Butoxy-5-formylbenzonitrile (**2e**).* A yellow oil, yield 83.9%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.88 (s, 1H, CHO), 8.09 (d, 1H, J = 1.8 Hz, Ar–H), 8.04 (dd, 1H, J = 1.8 & 8.8 Hz, Ar–H), 7.09 (d, 1H, J = 8.8 Hz, Ar–H), 4.56 (m, 1H, OCH), 1.79 (m, 2H, CH_2), 1.41 (d, 3H, J = 6.1 Hz, CH_3), 1.01 (t, 3H, J = 7.4, CH_3).

*4.1.3.6. 2-iso-Butoxy-5-formylbenzonitrile (**2f**).* A white powder, yield 77.8%, mp 52.8–53.9 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.89 (s, 1H, CHO), 8.10 (d, 1H, J = 2.0 Hz, Ar–H), 8.06 (dd, 1H, J = 2.1 & 8.7 Hz, Ar–H), 7.09 (d, 1H, J = 8.7 Hz, Ar–H), 3.95 (t, 2H, J = 6.5 Hz, OCH_2), 2.22 (m, 1H, CH), 1.01 (d, 6H, J = 6.8, $2CH_3$).

*4.1.3.7. 5-Formyl-2-iso-pentyloxybenzonitrile (**2g**).* A white powder, yield 81.3%, mp 46.1–47.8 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.90 (s, 1H, CHO), 8.09 (s, 1H, Ar–H), 8.06 (d, 1H, J = 8.8 Hz, Ar–H), 7.11 (d, 1H, J = 8.8 Hz, Ar–H), 4.21 (t, 2H, J = 6.5 Hz, OCH_2), 1.91 (m, 1H, CH), 1.80 (m, 2H, CH_2), 1.00 (d, 6H, J = 6.6, $2CH_3$).

*4.1.3.8. 5-Formyl-2-hexyloxybenzonitrile (**2h**).* A white waxy solid, yield 86.0%, mp 20.7–21.1 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.89 (s, 1H, CHO), 8.10 (d, 1H, J = 1.6 Hz, Ar–H), 8.06 (dd, 1H, J = 2.0 & 8.70 Hz, Ar–H), 7.10 (d, 1H, J = 8.7 Hz, Ar–H), 4.12 (t, 2H, J = 6.5 Hz, OCH_2), 1.90 (m, 2H, CH_2), 1.50 (m, 2H, CH_2), 1.37–1.34 (m, 4H, $2CH_2$), 0.92 (t, 3H, J = 6.9 Hz, CH_3).

*4.1.3.9. 5-Formyl-2-heptyloxybenzonitrile (**2i**).* A yellow oil, yield 64.6%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.89 (s, 1H, CHO), 8.09 (s, 1H, Ar–H), 8.06 (d, 1H, J = 8.7 Hz, Ar–H), 7.10 (d, 1H, J = 8.6 Hz, Ar–H), 4.13 (t, 2H, J = 6.5 Hz, OCH_2), 1.88 (m, 2H, CH_2), 1.51–1.32 (m, 8H, $4CH_2$), 0.90 (t, 3H, J = 6.3 Hz, CH_3).

*4.1.3.10. 5-Formyl-2-octyloxybenzonitrile (**2j**).* A yellow powder, yield 75.0%, mp 36.2–36.8 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.89 (s, 1H, CHO), 8.09 (d, 1H, J = 1.4 Hz, Ar–H), 8.06 (dd, 1H, J = 1.4 & 8.8 Hz, Ar–H), 7.09 (d, 1H, J = 8.8 Hz, Ar–H), 4.18 (t, 2H, J = 6.5 Hz, OCH_2), 1.90 (m, 2H, CH_2), 1.50 (m, 2H, CH_2),1.38–1.25 (m, 8H, $4CH_2$), 0.90 (t, 3H, J = 6.6 Hz, CH_3).

*4.1.3.11. 5-Formyl-2-(4-methylbenzyloxy)benzonitrile (**2k**).* A gray powder, yield 81.0%, mp 146.1–147.8 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.90 (s, 1H, CHO), 8.08 (s, 1H, Ar–H), 8.06 (d, 1H, J = 8.8 Hz, Ar–H), 7.38 (d, 2H, J = 7.9 Hz, Ar–H), 7.25 (d, 2H, J = 7.9 Hz, Ar–H), 7.10 (d, 1H, J = 8.8 Hz, Ar–H), 5.32 (s, 2H, CH_2), 2.32 (s, 3H, CH_3).

*4.1.4. General procedure for the synthesis of ethyl 2-(4-alkoxy- 3-cyanophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylates **3a**–**3k***

A mixture of 2-alkoxy-5-formylbenzonitrile (43 mmol), ethyl 2-hydroxyimino-3-oxobutanoate (52 mmol), ammonium acetate (430 mmol), and acetic acid (176 mL) was stirred at 50 °C under nitrogen atmosphere for 24 h. The reaction mixture was cooled to room temperature and then slowly poured into cold water (500 mL). The resulting precipitate was filtered, dried, and washed with ethyl acetate to obtain the corresponding ethyl 2-(4-alkoxy- 3-cyanophenyl)-1-hydroxy-4-methyl-1*H*-imidazole-5-carboxylates **3a**–**3k**.

*4.1.4.1. Ethyl 2-(3-cyano-4-methoxyphenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylate (**3a**).* A white crystalline powder, yield 65.8%, mp 173.2–175.1 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.32 (d, 1H, J = 2.1 Hz, Ar–H), 8.21 (dd, 1H, J = 2.1 & 9.0 Hz, Ar–H), 7.14 (d, 1H, J = 8.1 Hz, Ar–H), 4.41 (q, 2H, J = 6.9 Hz, CH_2), 3.94 (s, 3H, CH_3), 2.19 (s, 3H, CH_3), 1.26 (t, 3H, J = 6.9 Hz, CH_3). ESI-MS m/z 302.3 $[M + H]^+$.

*4.1.4.2. Ethyl 2-(3-cyano-4-propoxyphenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylate (**3b**).* A white crystalline powder, yield 62.1%, mp 152.2–154.1 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.29 (dd, 1H, J = 2.1 & 8.9 Hz, Ar–H), 8.26 (s, 1H, Ar–H), 7.40 (d, 1H, J = 9.0 Hz, Ar–H), 4.38 (q, 2H, J = 7.2 Hz, CH_2), 4.22 (t, 2H, J = 6.6 Hz, CH_2), 2.25 (s, 3H, CH_3), 1.84 (m, 2H, CH_2), 1.35 (t, 3H, J = 7.3 Hz, CH_3), 1.03 (t, 3H, J = 7.1 Hz, CH_3). ESI-MS m/z 330.4 $[M + H]^+$.

*4.1.4.3. Ethyl 2-(3-cyano-4-iso-propoxyphenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylate (**3c**).* A white crystalline powder, yield 70.1%, mp 150.2–151.9 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.26 (dd, 1H, J = 2.1 & 8.8 Hz, Ar–H), 8.23 (d, 1H, J = 2.1 Hz, Ar–H), 7.40 (d, 1H, J = 8.9 Hz, Ar–H), 4.88 (m, 1H, CH), 4.35 (q, 2H, J = 7.2 Hz, CH_2), 2.42 (s, 3H, CH_3), 1.38 (d, 6H, J = 6.3 Hz, $2CH_3$), 1.35 (t, 3H, J = 7.0 Hz, CH_3). ESI-MS m/z 330.4 $[M + H]^+$.

*4.1.4.4. Ethyl 2-(4-butoxy-3-cyanophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylate (**3d**).* A white crystalline powder, yield 71.0%, mp 149.8–150.5 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.16 (s, 1H, OH), 8.28 (dd, 1H, J = 2.1 & 8.7 Hz, Ar–H), 8.26 (d, 1H, J = 2.4 Hz, Ar–H), 7.39 (d, 1H, J = 9.6 Hz, Ar–H), 4.30 (q, 2H, J = 7.2 Hz, CH_2), 4.21 (t, 2H, J = 6.3 Hz, CH_2), 2.37 (s, 3H, CH_3), 1.77 (m, 2H, CH_2), 1.48 (m, 2H, CH_2), 1.32 (t, 3H, J = 6.9 Hz, CH_3), 0.96 (t, 3H, J = 7.5 Hz, CH_3). ESI-MS m/z 344.4 $[M + H]^+$.

*4.1.4.5. Ethyl 2-(4-sec-butoxy-3-cyanophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylate (**3e**).* A white crystalline powder, yield 69.8%, mp 159.8–162.3 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.11 (s, 1H, OH), 8.28 (s, 1H, Ar–H), 8.24 (d, 1H, J = 9.1 Hz, Ar–H), 7.41 (d, 1H, J = 9.0 Hz, Ar–H), 4.67 (m, 1H, CH), 4.29 (q, 2H, J = 9.1 Hz, CH_2), 2.38 (s, 3H, CH_3), 1.77 (m, 2H, CH_2), 1.30 (m, 6H, $2CH_3$), 0.96 (t, 3H, J = 7.4 Hz, CH_3). ESI-MS m/z 344.4 $[M + H]^+$.

*4.1.4.6. Ethyl 2-(4-iso-butoxy-3-cyanophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylate (**3f**).* A white crystalline powder, yield 69.4%, mp 147.4–148.3 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.12 (s, 1H, OH), 8.30 (d, 1H, J = 2.2 Hz, Ar–H), 8.28 (dd, 1H, J = 2.2 & 9.6 Hz, Ar–H), 7.39 (d, 1H, J = 9.6 Hz, Ar–H), 4.29 (q, 2H, J = 7.1 Hz, CH_2), 4.00 (d, 2H, J = 6.5 Hz, CH_2), 2.38 (s, 3H, CH_3), 2.08 (m, 1H, CH), 1.32 (t, 3H, J = 7.1 Hz, CH_3), 1.02 (d, 6H, J = 6.7 Hz, $2CH_3$). ESI-MS m/z 344.4 $[M + H]^+$.

*4.1.4.7. Ethyl 2-(3-cyano-4-iso-pentyloxyphenyl)-1-hydroxy-4-methyl-1H-imidazole-5- carboxylate (**3g**).* A white crystalline powder, yield 69.7%, mp 148.8–150.1 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.41 (s, 1H, Ar–H), 8.25 (dd, 1H, J = 2.1 & 8.7 Hz, Ar–H), 7.21 (d, 1H, J = 9.0 Hz, Ar–H), 4.20 (q, 2H, J = 6.9 Hz, CH_2), 4.18 (t, 2H, J = 6.3 Hz, CH_2), 2.24 (s, 3H, CH_3), 1.85 (m, 1H, CH), 1.68 (q, 2H, J = 6.6 Hz, CH_2), 1.27 (t, 3H, J = 6.9 Hz, CH_3), 0.98 (d, 6H, J = 6.6 Hz, $2CH_3$). ESI-MS m/z 358.4 $[M + H]^+$.

*4.1.4.8. Ethyl 2-(3-cyano-4-hexyloxyphenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylate (**3h**).* A white crystalline powder, yield 64.1%, mp 109.3–112.8 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.14 (s, 1H, OH), 8.29 (s, 1H, Ar–H), 8.27 (s, 1H, Ar–H), 7.38 (d, 1H, J = 8.6 Hz, Ar–H), 4.29 (q, 2H, J = 7.1 Hz, CH_2), 4.20 (t, 2H, J = 6.4 Hz, CH_2), 2.50 (s, 3H, CH_3), 1.77 (m, 2H, CH_2), 1.46 (m, 2H, CH_2), 1.35–1.29 (m, 7H, $2CH_2$ & CH_3), 0.87 (t, 3H, J = 6.9 Hz, CH_3). ESI-MS m/z 372.4 $[M + H]^+$.

*4.1.4.9. Ethyl 2-(3-cyano-4-heptyloxyphenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylate (**3i**).* A white crystalline powder, yield 66.8%, mp 139.5–142.2 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.27 (d, 1H, J = 2.0 Hz, Ar–H), 8.24 (dd, 1H, J = 2.2 & 8.6 Hz, Ar–H), 7.39 (d, 1H, J = 8.9 Hz, Ar–H), 4.24 (q, 2H, J = 7.1 Hz, CH_2), 4.18 (t, 2H, J = 6.4 Hz, CH_2), 2.31 (s, 3H, CH_3), 1.75 (m, 2H, CH_2), 1.48–1.28 (m, 11H, $4CH_2$ & CH_3), 0.88 (t, 3H, J = 6.7 Hz, CH_3). ESI-MS m/z 386.5 $[M + H]^+$.

*4.1.4.10. Ethyl 2-(3-cyano-4-octyloxyphenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylate (**3j**).* A white crystalline powder, yield 72.1%, mp 143.2–145.1 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.35 (d, 1H, J = 1.8 Hz, Ar–H), 8.19 (dd, 1H, J = 2.1 & 9.0 Hz, Ar–H), 7.26 (d, 1H, J = 8.9 Hz, Ar–H), 5.24 (s, 2H, CH_2), 4.14 (q, 2H, J = 7.2 Hz, CH_2), 2.33 (s, 3H, CH_3), 1.79 (m, 2H, CH_2), 1.45–1.20 (m, 13H, $5CH_2$ & CH_3), 0.90 (t, 3H, J = 6.7 Hz, CH_3). ESI-MS m/z 400.4 $[M + H]^+$.

*4.1.4.11. Ethyl 2-[3-cyano-4-(4-methylbenzyloxy)phenyl]-1-hydroxy-4-methyl-1H- imidazole-5-carboxylate (**3k**).* A white crystalline powder, yield 70.0%, mp 150.3–151.5 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.27 (dd, 1H, J = 2.1 & 7.2 Hz, Ar–H), 8.26 (s, 1H, Ar–H), 7.51 (d, 1H, J = 7.2 Hz, Ar–H), 7.39 (d, 2H, J = 8.1 Hz, Ar–H), 7.24 (d, 2H, J = 7.8 Hz, Ar–H), 5.32 (s, 2H, CH_2), 4.31 (q, 2H, J = 7.2 Hz, CH_2), 2.40 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 1.33 (t, 3H, J = 7.2 Hz, CH_3). ESI-MS m/z 392.4 $[M + H]^+$.

*4.1.5. General procedure for the synthesis of 2-(4-alkoxy- 3-cyanophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylic acids **4a**–**4k***

A mixture of ethyl 2-(4-alkoxy-3-cyanophenyl)-1-hydroxy-4-methyl-1*H*-imidazole-5- carboxylate (2.9 mmol), 1 M NaOH aqueous (11 mL), THF (5 mL), and ethanol (5 mL) was stirred at 50 °C for 8 h. The solvent was concentrated in a vacuum, and the residue was acidified with dilute hydrochloric acid to pH 1. The resulting precipitate was filtered, dried, and recrystallized with a mixture of methanol and ethyl acetate (2:1) to yield the corresponding 2-(4-alkoxy- 3-cyanophenyl)-1-hydroxy-4-methyl-1*H*-imidazole-5-carboxylic acids **4a**–**4k**.

*4.1.5.1. 2-(3-Cyano-4-methoxyphenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylic acid (**4a**).* A white crystalline powder, yield 52.5%. HPLC: 98.15%. mp 214.1–214.5 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.60 (d, 1H, J = 2.0 Hz, Ar–H), 8.44 (dd, 1H, J = 2.2 & 8.8 Hz,

Ar–H), 7.27 (d, 1H, 8.9 Hz, Ar–H), 3.88 (s, 3H, CH_3), 2.42 (s, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.95, 160.37, 142.98, 139.84, 132.55, 131.20, 117.28, 116.67, 115.58, 113.69, 101.00, 70.70, 21.72. ESI-HRMS calcd. for $C_{13}H_{11}N_3NaO_4$ $[M+Na]^+$ 296.0642, found: 296.0645. IR (KBr, cm^{-1}): 3459.7, 2967.7, 2230.2, 1611.0, 1502.3, 1376.5, 1304.5, 1001.4.

*4.1.5.2. 2-(3-Cyano-4-propoxyphenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylic acid (**4b**).* A white crystalline powder, yield 62.5%. HPLC: 96.12%. mp 191.9–193.0 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 13.69 (s, 1H, COOH), 8.54 (s, 1H, Ar–H), 8.46 (d, 1H, $J = 8.7$ Hz, Ar–H), 7.45 (d, 1H, $J = 8.8$ Hz, Ar–H), 4.18 (t, 2H, $J = 6.3$ Hz, CH_2), 2.50 (s, 3H, CH_3), 1.80 (m, 2H, CH_2), 1.03 (t, 3H, $J = 7.4$ Hz, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.00, 159.78, 142.97, 139.78, 133.65, 132.22, 120.34, 116.42, 115.75, 114.48, 101.88, 66.81, 21.54, 15.75, 10.96. ESI-HRMS calcd. for $C_{15}H_{16}N_3O_4$ $[M+H]^+$ 302.1135, found: 302.1132. IR (KBr, cm^{-1}): 3460.1, 2983.5, 2231.5, 1610.7, 1493.3, 1376.5, 1294.8, 1102.8.

*4.1.5.3. 2-(3-Cyano-4-iso-propoxyphenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylic acid (**4c**).* A white crystalline powder, yield 64.6%. HPLC: 96.25%. mp 190.8–191.2 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 13.65 (s, 1H, COOH), 8.54 (s, 1H, Ar–H), 8.48 (d, 1H, $J = 8.7$ Hz, Ar–H), 7.52 (d, 1H, $J = 8.8$ Hz, Ar–H), 4.91 (m, 1H, CH), 2.51 (s, 3H, CH_3), 1.37 (d, 6H, $J = 5.9$ Hz, $2CH_3$). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.37, 160.09, 142.95, 139.82, 132.48, 131.44, 117.28, 116.43, 115.76, 114.54, 101.68, 72.10, 21.55, 10.99. ESI-HRMS calcd. for $C_{15}H_{16}N_3O_4$ $[M + H]^+$ 302.1135, found: 302.1139. IR (KBr, cm^{-1}): 3433.3, 2983.3, 2231.4, 1610.4, 1490.8, 1376.2, 1293.2, 1103.8.

*4.1.5.4. 2-(4-Butoxy-3-cyanophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylic acid (**4d**).* A white crystalline powder, yield 68.7%. HPLC: 98.15%. mp 193.7–195.3 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 8.56 (s, 1H, Ar–H), 8.49 (d, 1H, $J = 7.0$ Hz, Ar–H), 7.45 (d, 1H, $J = 8.9$ Hz, Ar–H), 4.22 (t, 2H, $J = 6.2$ Hz, CH_2), 2.51 (s, 3H, CH_3), 1.77 (m, 2H, CH_2), 1.48 (m, 2H, CH_2), 0.97 (t, 3H, $J = 7.3$ Hz, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.94, 160.32, 142.88,139.81, 132.44, 131.11, 117.27, 116.65, 115.56, 113.63, 100.97, 69.06, 30.32, 18.55, 13.62, 10.95. ESI-HRMS calcd. for $C_{16}H_{18}N_3O_4$ $[M + H]^+$ 316.1292, found: 316.1291. IR (KBr, cm^{-1}): 3430.4, 2959.0, 2938.1, 2229.9, 1610.3, 1294.1.

*4.1.5.5. 2-(4-sec-Butoxy-3-cyanophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylic acid (**4e**).* A white crystalline powder, yield 65.6%. HPLC: 96.17%. mp 190.9–191.5 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 8.55 (s, 1H, Ar–H), 8.48 (d, 1H, $J = 8.8$ Hz, Ar–H), 7.52 (d, 1H, $J = 9.1$ Hz, Ar–H), 4.72 (m, 1H, CH), 2.51 (s, 3H, CH_3), 1.71 (m, 2H, CH_2), 1.33 (d, 3H, $J = 5.9$ Hz, CH_3), 0.97 (t, 3H, $J = 7.3$ Hz, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.38, 159.75, 142.95, 139.84, 132.45, 131.40, 120.54, 117.27, 115.71, 114.53, 101.69, 76.71, 28.36, 18.76, 10.98, 9.20. ESI-HRMS calcd. for $C_{16}H_{18}N_3O_4$ $[M + H]^+$ 316.1292, found: 316.1292. IR (KBr, cm^{-1}): 3432.1, 2959.9, 2934.0, 2230.7, 1610.5, 1498.4, 1383.5, 1294.1.

*4.1.5.6. 2-(4-iso-Butoxy-3-cyanophenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylic acid (**4f**).* A white crystalline powder, yield 65.2%. HPLC: 95.24%. mp 191.6–192.2 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 8.55 (s, 1H, Ar–H), 8.47 (d, 1H, $J = 8.8$ Hz, Ar–H), 7.46 (d, 1H, $J = 9.0$ Hz, Ar–H), 4.00 (d, 2H, $J = 6.4$ Hz, CH_2), 2.51 (s, 3H, CH_3), 2.10 (m, 1H, CH), 1.03 (d, 6H, $J = 6.7$ Hz, $2CH_3$). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.47, 159.02, 141.95, 140.75, 133.91, 132.09, 121.34, 117.94, 115.92, 113.30, 100.71, 67.65, 24.62, 22.37, 15.71. ESI-HRMS calcd. for $C_{16}H_{18}N_3O_4$ $[M+H]^+$ 316.1292, found: 316.1291. IR (KBr, cm^{-1}): 3219.9, 2957.5, 2229.6, 1711.5, 1469.7, 1293.8, 1104.3.

*4.1.5.7. 2-(3-Cyano-4-iso-pentyloxyphenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylic acid (**4g**).* A white crystalline powder, yield 64.6%. HPLC: 97.36%. mp 194.7–194.9 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 8.54 (s, 1H, Ar–H), 8.48 (s, 1H, Ar–H), 7.50 (s, 1H, Ar–H), 4.25 (s, 2H, CH_2), 2.51 (s, 3H, CH_3), 1.82 (s, 1H, CH), 1.70 (s, 2H, CH_2), 0.97 (s, 6H, $2CH_3$). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.89, 160.33, 142.94, 139.80, 132.34, 131.03, 117.27, 116.59, 115.54, 113.57, 100.96, 69.33, 30.86, 28.21, 24.91, 22.04. ESI-HRMS calcd. for $C_{17}H_{20}N_3O_4$ $[M + H]^+$ 330.1148, found: 330.1149. IR (KBr, cm^{-1}): 3458.6, 2930.4, 2230.1, 1610.7, 1495.4, 1373.9, 1294.4.

*4.1.5.8. 2-(3-Cyano-4-hexyloxyphenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylic acid (**4h**).* A white crystalline powder, yield 75.0%. HPLC: 99.74%. mp 190.8–191.1 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 8.53 (s, 1H, Ar–H), 8.45 (d, 1H, $J = 8.7$ Hz, Ar–H), 7.45 (d, 1H, $J = 8.9$ Hz, Ar–H), 4.21 (t, 2H, $J = 5.9$ Hz, CH_2), 2.50 (s, 3H, CH_3), 1.78 (m, 2H, CH_2), 1.46 (m, 2H, CH_2), 1.34 (m, 4H, $2CH_2$), 0.89 (s, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.92, 160.31, 142.94, 139.80, 132.40, 131.08, 120.54, 117.28, 115.54, 113.61, 100.98, 69.34, 31.22, 28.34, 25.23, 22.03, 13.92, 10.92. ESI-HRMS calcd. for $C_{18}H_{22}N_3O_4$ $[M + H]^+$ 344.1605, found: 344.1596. IR (KBr, cm^{-1}): 3485.4, 2928.1, 2229.8, 1610.8, 1502.3, 1374.0, 1302.6.

*4.1.5.9. 2-(3-Cyano-4-heptyloxyphenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylic acid (**4i**).* A white crystalline powder, yield 70.8%. HPLC: 94.12%. mp 194.3–194.9 °C. ^1H NMR (600 MHz, DMSO-d_6): δ 13.75 (s, 1H, COOH), 8.55 (s, 1H, Ar–H), 8.48 (d, 1H, $J = 8.9$ Hz, Ar–H), 7.47 (d, 1H, $J = 9.1$ Hz, Ar–H), 4.22 (t, 2H, $J = 6.4$ Hz, CH_2), 2.51 (s, 3H, CH_3), 1.78 (m, 2H, CH_2), 1.45 (m, 2H, CH_2), 1.35 (m, 2H, CH_2), 1.29 (m, 4H, $2CH_2$), 0.88 (t, 3H, $J = 6.6$ Hz, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.90, 159.82, 142.99, 139.77, 133.74, 132.04, 120.55, 116.45, 115.74, 113.56, 101.23, 69.17, 31.26, 28.38, 28.32, 25.28, 22.07, 15.76, 13.93. ESI-HRMS calcd. for $C_{19}H_{24}N_3O_4$ $[M + H]^+$ 358.1761, found: 358.1758. IR (KBr, cm^{-1}): 3432.3, 2929.1, 2230.8, 1610.4, 1494.5, 1373.9, 1293.8.

*4.1.5.10. 2-(3-Cyano-4-octyloxyphenyl)-1-hydroxy-4-methyl-1H-imidazole-5-carboxylic acid (**4j**).* A white crystalline powder, yield 70.8%. HPLC: 97.38%. mp 186.5–187.5 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 13.76 (s, 1H, COOH), 8.57 (s, 1H, Ar–H), 8.51 (d, 1H, $J = 9.2$ Hz, Ar–H), 7.50 (d, 1H, $J = 9.1$ Hz, Ar–H), 4.23 (t, 2H, $J = 6.4$ Hz, CH_2), 2.52 (s, 3H, CH_3), 1.78 (m, 2H, CH_2), 1.44 (m, 2H, CH_2), 1.41–1.27 (m, 8H, $4CH_2$), 0.86 (t, 3H, $J = 6.4$ Hz, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.96, 160.38, 142.95, 139.85, 132.52, 131.19, 117.30, 116.69, 115.57, 113.68, 101.02, 69.35, 31.21, 28.65, 28.62, 28.24, 25.26, 22.09, 13.95, 10.99. ESI-HRMS calcd. for $C_{20}H_{26}N_3O_4$ $[M + H]^+$ 372.1918, found: 372.1914. IR (KBr, cm^{-1}): 3430.8, 2927.0, 2855.9, 2230.3, 1610.5, 1495.2, 1374.3, 1294.2.

*4.1.5.11. 2-[3-Cyano-4-(4-methylbenzyloxy)phenyl]-1-hydroxy-4-methyl-1H-imidazole-5- carboxylic acid (**4k**).* A white crystalline powder, yield 71.9%. HPLC: 99.14%. mp 199.2–199.8 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 13.81 (s, 1H, COOH), 8.58 (s, 1H, Ar–H), 8.51 (d, 1H, $J = 8.9$ Hz, Ar–H), 7.60 (d, 1H, $J = 9.1$ Hz, Ar–H), 7.39 (d, 2H, $J = 8.0$ Hz, Ar–H), 7.24 (d, 2H, $J = 7.9$ Hz, Ar–H), 5.33 (s, 2H, CH_2), 2.51 (s, 3H, CH_3), 2.32 (s, 3H, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.63, 160.36, 140.45, 139.39, 137.74, 132.52, 132.48, 131.33, 129.18, 127.96, 117.31, 116.95, 115.61, 114.23, 101.31, 70.63, 20.80, 13.87. ESI-HRMS calcd. for $C_{20}H_{18}N_3O_4$ $[M+H]^+$ 364.1296, found: 364.1295. IR (KBr, cm^{-1}): 3433.3, 3052.7, 2923.5, 2230.5, 1610.0, 1494.3, 1375.8, 1279.3.

4.1.6. General procedure for the synthesis of ethyl 2-(4-alkoxy-3-cyanophenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxylates ***5a–5k***

Dimethyl sulfate (53 mmol) was added dropwise at a rate that maintained <0 °C reaction temperature to a stirred mixture of ethyl 2-(4-alkoxy-3-cyanophenyl)-1-hydroxy-4-methyl-1*H*- imidazole-5-carboxylate (50 mmol), anhydrous potassium carbonate (75 mmol) and DMF (40 mL). Upon completion of addition, the mixture was stirred at 25 °C for another 1.5 h, and then the reaction mixture was slowly poured into cold water (100 mL). The resulting precipitate was filtered, dried, and recrystallized with ethyl acetate to yield the corresponding ethyl 2-(4-alkoxy- 3-cyanophenyl)-1-methoxy-4-methyl-1*H*-imidazole-5-carboxylates **5a**–**5k**.

*4.1.6.1. Ethyl 2-(3-cyano-4-methoxyphenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxylate (**5a**).* A white crystalline powder, yield 84.7%, mp 135.1–137.4 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.27 (dd, 1H, J = 2.3 & 8.8 Hz, Ar–H), 8.24 (d, 1H, J = 1.9 Hz, Ar–H), 7.41 (d, 1H, J = 8.9 Hz, Ar–H), 4.31 (m, 2H, J = 7.2 Hz, CH_2), 3.96 (s, 3H, CH_3), 3.88 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.33 (t, 3H, CH_3). ESI-MS m/z 316.4 $[M + H]^+$.

*4.1.6.2. Ethyl 2-(3-cyano-4-propoxyphenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxylate (**5b**).* A white crystalline powder, yield 78.8%, mp 143.2–144.5 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.27 (dd, 1H, J = 2.3 & 8.7 Hz, Ar–H), 8.24 (s, 1H, Ar–H), 7.42 (d, 1H, J = 8.8 Hz, Ar–H), 4.33 (q, 2H, J = 7.2 Hz, CH_2), 4.19 (t, 2H, J = 6.4 Hz, CH_2), 3.96 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.80 (m, 2H, CH_2), 1.33 (t, 3H, J = 7.1 Hz, CH_3), 1.03 (t, 3H, J = 7.3 Hz, CH_3). ESI-MS m/z 344.4 $[M + H]^+$.

*4.1.6.3. Ethyl 2-(3-cyano-4-iso-propoxyphenyl)-1-methoxy-4-methyl-1H-imidazole-5- carboxylate (**5c**).* A white crystalline powder, yield 80.8%, mp 170.4–172.8 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.27 (dd, 1H, J = 2.3 & 8.5 Hz, Ar–H), 8.24 (d, 1H, J = 2.3 Hz, Ar–H), 7.45 (d, 1H, J = 8.6 Hz, Ar–H), 4.90 (m, 1H, CH), 4.32 (q, 2H, J = 7.1 Hz, CH_2), 3.96 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.36 (d, 6H, J = 5.9 Hz, $2CH_3$), 1.31 (t, 3H, J = 7.1 Hz, CH_3). ESI-MS m/z 344.4 $[M + H]^+$.

*4.1.6.4. Ethyl 2-(4-butoxy-3-cyanophenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxylate (**5d**).* A white crystalline powder, yield 86.5%, mp 135.4–136.2 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.26 (dd, 1H, J = 2.3 & 8.8 Hz, Ar–H), 8.24 (d, 1H, J = 1.9 Hz, Ar–H), 7.42 (d, 1H, J = 8.9 Hz, Ar–H), 4.32 (q, 2H, J = 7.1 Hz, CH_2), 4.23 (t, 2H, J = 6.4 Hz, CH_2), 3.96 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 1.77 (m, 2H, CH_2), 1.48 (m, 2H, CH_2), 1.33 (t, 3H, J = 7.1 Hz, CH_3), 0.96 (t, 3H, J = 7.3 Hz, CH_3). ESI-MS m/z 358.4 $[M + H]^+$.

*4.1.6.5. Ethyl 2-(4-sec-butoxy-3-cyanophenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxylate (**5e**).* A white crystalline powder, yield 88.5%, mp 122.9–123.1 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.26 (dd, 1H, J = 2.1 & 8.8 Hz, Ar–H), 8.24 (s, 1H, Ar–H), 7.51 (d, 1H, J = 9.0 Hz, Ar–H), 4.69 (m, 1H, CH), 4.31 (q, 2H, J = 6.9 Hz, CH_2), 3.96 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.70 (m, 2H, CH_2), 1.34 (t, 3H, J = 6.9 Hz, CH_3), 1.31 (d, 3H, J = 6.0 Hz, CH_3), 0.96 (t, 3H, J = 7.2 Hz, CH_3). ESI-MS m/z 358.4 $[M + H]^+$.

*4.1.6.6. Ethyl 2-(4-iso-butoxy-3-cyanophenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxylate (**5f**).* A white crystalline powder, yield 92.9%, mp 123.8–124.9 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.30 (d, 1H, J = 2.2 Hz, Ar–H), 8.28 (dd, 1H, J = 2.2 & 9.6 Hz, Ar–H), 7.39 (d, 1H, J = 9.6 Hz, Ar–H), 4.31 (q, 2H, J = 6.9 Hz, CH_2), 4.01 (d, 2H, J = 6.4 Hz, CH_2), 3.96 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.10 (m, 1H, CH), 1.34 (t, 3H, J = 6.9 Hz, CH_3), 1.03 (d, 6H, J = 6.7 Hz, $2CH_3$). ESI-MS m/z 358.4 $[M + H]^+$.

*4.1.6.7. Ethyl 2-(3-cyano-4-iso-pentyloxyphenyl)-1-methoxy-4-methyl-1H-imidazole-5- carboxylate (**5g**).* A white crystalline powder, yield 73.7%, mp 138.1–139.6 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.27 (dd, 1H, J = 2.1 & 8.8 Hz, Ar–H), 8.24 (d, 1H, J = 2.0 Hz, Ar–H), 7.45 (d, 1H, J = 8.9 Hz, Ar–H), 4.33 (q, 2H, J = 7.1 Hz, CH_2), 4.26 (t, 2H, J = 6.6 Hz, CH_2), 3.96 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.82 (m, 1H, CH), 1.69 (m, 2H, CH_2), 1.33 (t, 3H, J = 7.1 Hz, CH_3), 0.96 (d, 6H, J = 6.6 Hz, $2CH_3$). ESI-MS m/z 372.4 $[M + H]^+$.

*4.1.6.8. Ethyl 2-(3-cyano-4-hexyloxyphenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxylate (**5h**).* A white crystalline powder, yield 71.8%, mp 118.7–120.6 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.26 (dd, 1H, J = 2.3 & 8.7 Hz, Ar–H), 8.24 (d, 1H, J = 2.0 Hz, Ar–H), 7.42 (d, 1H, J = 8.9 Hz, Ar–H), 4.32 (q, 2H, J = 7.1 Hz, CH_2), 3.96 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.78 (m, 2H, CH_2), 1.49–1.31 (m, 9H, $3CH_2$ & CH_3), 0.89 (t, 3H, J = 6.8 Hz, CH_3). ESI-MS m/z 386.5 $[M + H]^+$.

*4.1.6.9. Ethyl 2-(3-cyano-4-heptyloxyphenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxylate (**5i**).* A white crystalline powder, yield 76.8%, mp 166.2–166.6 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.26 (dd, 1H, J = 2.3 & 8.7 Hz, Ar–H), 8.24 (d, 1H, J = 2.0 Hz, Ar–H), 7.42 (d, 1H, J = 8.8 Hz, Ar–H), 4.32 (q, 2H, J = 7.1 Hz, CH_2), 4.22 (t, 2H, J = 6.4 Hz, CH_2), 3.96 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 1.78 (m, 2H, CH_2), 1.48–1.28 (m, 11H, $4CH_2$ & CH_3), 0.87 (t, 3H, J = 6.7 Hz, CH_3). ESI-MS m/z 400.5 $[M + H]^+$.

*4.1.6.10. Ethyl 2-(3-cyano-4-octyloxyphenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxylate (**5j**).* A white crystalline powder, yield 78.1%, mp 165.8–167.3 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.27 (dd, 1H, J = 2.0 & 8.8 Hz, Ar–H), 8.24 (d, 1H, J = 2.0 Hz, Ar–H), 7.45 (d, 1H, J = 8.9 Hz, Ar–H), 4.32 (q, 2H, J = 7.1 Hz, CH_2), 4.22 (t, 2H, J = 6.4 Hz, CH_2), 3.96 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 1.79 (m, 2H, CH_2), 1.45–1.20 (m, 13H, $5CH_2$ & CH_3), 0.90 (t, 3H, J = 6.7 Hz, CH_3). ESI-MS m/z 414.5 $[M + H]^+$.

*4.1.6.11. Ethyl 2-[3-cyano-4-(4-methylbenzyloxy)phenyl]-1-methoxy-4-methyl-1H-imidazole-5-carboxylate (**5k**).* A white crystalline powder, yield 70.1%, mp 147.5–149.5 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.27 (dd, 1H, J = 2.1 & 7.2 Hz, Ar–H), 8.26 (s, 1H, Ar–H), 7.51 (d, 1H, J = 7.2 Hz, Ar–H), 7.39 (d, 2H, J = 8.1 Hz, Ar–H), 7.24 (d, 2H, J = 7.8 Hz, Ar–H), 5.32 (s, 2H, CH_2), 4.31 (q, 2H, J = 7.2 Hz, CH_2), 3.96 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 1.33 (t, 3H, J = 7.2 Hz, CH_3). ESI-MS m/z 405.5 $[M + H]^+$.

4.1.7. General procedure for the synthesis of 2-(4-alkoxy-3-cyanophenyl)-1-methoxy-4 -methyl-1H-imidazole-5-carboxylic acids ***6a–6k***

A mixture of ethyl 2-(4-alkoxy -3-cyanophenyl)-1-methoxy-4-methyl-1*H*-imidazole-5- carboxylate (2.8 mmol), 1 M NaOH aqueous (7 mL), THF (5 mL), and ethanol (5 mL) was stirred at 50 °C for 2 h. The solvent was concentrated in vacuum and the residue was acidified with dilute hydrochloric acid to pH 1, The resulting precipitate was filtered, dried, and recrystallized with mixture of methanol and ethyl acetate (1:2) to yield the corresponding 2-(4-alkoxy- 3-cyanophenyl)-1-methoxy-4-methyl-1*H*-imidazole-5-carboxylic acids **6a**–**6k**.

*4.1.7.1. 2-(3-Cyano-4-methoxyphenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxylic acid (**6a**).* A white crystalline powder, yield 50.4%. HPLC: 98.64%. mp 185.4–186.3 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.01 (s, 1H, COOH), 8.26 (dd, 1H, J = 2.0 & 9.0 Hz, Ar–H), 8.24 (s, 1H, Ar–H), 7.40 (d, 1H, J = 8.9 Hz, Ar–H), 3.95 (s, 3H, CH_3), 3.88 (s, 3H, CH_3),2.40 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-

d_6): δ 160.80, 159.79, 142.98, 139.84, 133.85, 132.16, 120.53, 116.43, 115.85, 113.62, 101.16, 70.70, 66.84, 21.72. ESI-HRMS calcd. for $C_{14}H_{14}N_3O_4$ $[M + H]^+$ 288.2710, found: 288.2715. IR (KBr, cm^{-1}): 3393.9, 2966.3, 2223.5, 1704.1, 1611.2, 1527.8, 1443.2, 1287.0, 1262.5, 1100.6.

*4.1.7.2. 2-(3-Cyano-4-propoxyphenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxylic acid (**6b**).* A white crystalline powder, yield 63.0%. HPLC: 97.42%. mp 193.1–194.0 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.06 (s, 1H, COOH), 8.28 (dd, 1H, J = 2.1 & 9.8 Hz, Ar–H), 8.24 (s, 1H, Ar–H), 7.41 (d, 1H, J = 8.9 Hz, Ar–H), 4.19 (t, 2H, J = 6.4 Hz, CH_2), 3.95 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 1.79 (m, 2H, CH_2), 1.03 (t, 3H, J = 7.3 Hz, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.92, 159.77, 142.95, 139.81, 133.79, 132.07, 120.54, 116.44, 115.73, 113.63, 101.20, 68.89, 66.80, 18.55, 15.77, 13.59. ESI-HRMS calcd. for $C_{16}H_{18}N_3O_4$ $[M + H]^+$ 316.1292, found: 316.1297. IR (KBr, cm^{-1}): 3442.5, 2980.4, 2936.9, 2231.5, 1661.8, 1464.4, 1297.6, 1108.6.

*4.1.7.3. 2-(3-Cyano-4-iso-propoxyphenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxylic acid (**6c**).* A white crystalline powder, yield 65.2%. HPLC: 96.98%. mp 189.0–189.3 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.06 (s, 1H, COOH), 8.27 (dd, 1H, J = 2.2 & 9.1 Hz, Ar–H), 8.24 (s, 1H, Ar–H), 7.40 (d, 1H, J = 9.0 Hz, Ar–H), 4.86 (m, 1H, CH), 3.93 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 1.37 (d, 6H, J = 6.0 Hz, $2CH_3$). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.01, 159.78, 142.95, 139.82, 133.70, 132.25, 120.37, 116.43, 115.90, 114.53, 101.89, 71.92, 66.82, 21.57, 15.73. ESI-HRMS calcd. for $C_{16}H_{18}N_3O_4$ $[M + H]^+$ 316.1292, found: 316.1295. IR (KBr, cm^{-1}): 3455.2, 2957.0, 2223.0, 1704.8, 1611.5, 1443.0, 1287.4, 1100.9.

*4.1.7.4. 2-(4-Butoxy-3-cyanophenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxylic acid (**6d**).* A white crystalline powder, yield 72.8%. HPLC: 95.96%. mp 193.1–194.0 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.28 (s, 1H, Ar–H), 8.24 (s, 1H, Ar–H), 7.41 (d, 1H, J = 8.9 Hz, Ar–H), 4.23 (t, 2H, J = 6.3 Hz, CH_2), 3.95 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 1.78 (m, 2H, CH_2), 1.50 (m, 2H, CH_2), 0.96 (t, 3H, J = 7.3 Hz, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.31, 159.77, 142.95, 139.84, 133.71, 132.24, 120.37, 116.42, 115.85, 114.53, 101.88, 76.57, 66.82, 28.38, 18.80, 15.73, 9.20. ESI-HRMS calcd. for $C_{17}H_{20}N_3O_4$ $[M + H]^+$ 330.1448, found: 330.1447. IR (KBr, cm^{-1}): 3429.4, 2959.0, 2938.1, 2229.9, 1664.5, 1613.3, 1465.6, 1295.8, 1108.4.

*4.1.7.5. 2-(4-sec-Butoxy-3-cyanophenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxylic acid (**6e**).* A white crystalline powder, yield 67.4%. HPLC: 98.55%. mp 181.5–182.3 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.06 (s, 1H, COOH), 8.27 (s, 1H, Ar–H), 8.24 (s, 1H, Ar–H), 7.44 (d, 1H, J = 8.8 Hz, Ar–H), 4.69 (m, 1H, CH), 3.97 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 1.67 (m, 2H, CH_2), 1.33 (d, 3H, J = 5.9 Hz, CH_3), 0.97 (t, 3H, J = 7.3 Hz, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.91, 159.75, 142.95, 139.84, 133.80, 132.08, 120.54, 116.42, 115.73, 113.66, 101.19, 67.74, 66.81, 36.95, 24.60, 22.35, 15.72. ESI-HRMS calcd. for $C_{17}H_{20}N_3O_4$ $[M + H]^+$ 330.1448, found: 330.1449. IR (KBr, cm^{-1}): 3440.0, 2954.8, 2229.6, 1663.0, 1465.6, 1296.1, 1107.8.

*4.1.7.6. 2-(4-iso-Butoxy-3-cyanophenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxylic acid (**6f**).* A white crystalline powder, yield 69.6%. HPLC: 99.10%. mp 190.8–191.3 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.06 (s, 1H, COOH), 8.27 (dd, 1H, J = 2.2 & 8.6 Hz, Ar–H), 8.24 (s, 1H, Ar–H), 7.41 (d, 1H, J = 8.8 Hz, Ar–H), 4.01 (d, 2H, J = 6.4 Hz, CH_2), 3.96 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.10 (m, 1H, CH), 1.03 (d, 6H, J = 6.7Hz, $2CH_3$). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.97, 159.79, 142.88, 139.81, 133.84, 132.02, 120.59, 116.51, 115.67, 113.73, 101.22, 74.97, 66.80, 27.57, 18.70, 15.71. ESI-HRMS calcd. for $C_{17}H_{20}N_3O_4$ $[M + H]^+$ 330.1448, found: 330.1445. IR (KBr, cm^{-1}): 3440.3, 2969.6, 2932.7, 2230.8, 1663.6, 1460.3, 1288.0, 1107.2.

*4.1.7.7. 2-(3-Cyano-4-iso-pentyloxyphenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxylic acid (**6g**).* A white crystalline powder, yield 63.0%. HPLC: 97.24%. mp 191.3–191.9 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.06 (s, 1H, COOH), 8.27 (dd, 1H, J = 2.2 & 8.9 Hz, Ar–H), 8.24 (d, 1H, J = 2.0 Hz, Ar–H), 7.44 (d, 1H, J = 8.9 Hz, Ar–H), 4.25 (t, 2H, J = 6.5 Hz, CH_2), 3.95 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.86 (m, 1H, CH), 1.70 (m, 2H, CH_2), 0.96 (d, 6H, J = 6.6 Hz, $2CH_3$). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.90, 159.77, 142.94, 139.80, 133.77, 132.05, 120.54, 116.44, 115.71, 113.62, 101.21, 69.17, 66.79, 30.82, 28.21, 24.91, 22.02. ESI-HRMS calcd. for $C_{18}H_{22}N_3O_4$ $[M + H]^+$ 344.1605, found: 344.1603. IR (KBr, cm^{-1}): 3383.3, 2956.4, 2924.0, 2231.0, 1702.2, 1606.7, 1461.1, 1397.6.

*4.1.7.8. 2-(3-Cyano-4-hexyloxyphenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxylic acid (**6h**).* A white crystalline powder, yield 64.5%. HPLC: 98.26%. mp 192.8–193.9 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.02 (s, 1H, COOH), 8.26 (dd, 1H, J = 2.2 & 9.0 Hz, Ar–H), 8.23 (d, 1H, J = 2.0 Hz, Ar–H), 7.41 (d, 1H, J = 8.9 Hz, Ar–H), 4.22 (t, 2H, J = 6.4 Hz, CH_2), 3.95 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 1.78 (m, 2H, CH_2), 1.46 (m, 2H, CH_2), 1.32 (m, 4H, $2CH_2$), 0.89 (t, 3H, J = 6.8 Hz, CH_3). ^{13}C NMR (150 MHz, DMSO-d_6): δ 160.90, 159.77, 142.94, 139.80, 133.76, 132.05, 120.54, 116.44, 115.71, 113.62, 101.21, 69.17, 66.78, 31.19, 28.29, 25.22, 21.99, 15.72, 13.89. ESI-HRMS calcd. for $C_{19}H_{24}N_3O_4$ $[M + H]^+$ 358.1761, found: 358.1757. IR (KBr, cm^{-1}): 3435.2, 2955.2, 2934.3, 2229.4, 1663.7, 1612.5, 1465.9, 1296.9.

*4.1.7.9. 2-(3-Cyano-4-heptyloxyphenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxylic acid (**6i**).* A white crystalline powder, yield 63.4%. HPLC: 98.10%. mp 193.9–194.2 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 13.05 (s, 1H, COOH), 8.26 (dd, 1H, J = 2.2 & 9.0 Hz, Ar–H), 8.23 (d, 1H, J = 2.2 Hz, Ar–H), 7.41 (d, 1H, J = 9.0 Hz, Ar–H), 4.21 (t, 2H, J = 6.4 Hz, CH_2), 3.95 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.79 (m, 2H, CH_2), 1.45 (m, 2H, CH_2), 1.36 (m, 2H, CH_2), 1.28 (m, 4H, $2CH_2$), 0.87 (t, 3H, J = 6.7 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.94, 159.80, 142.98, 139.84, 133.83, 132.09, 120.54, 116.44, 115.76, 113.67, 101.21, 69.18, 66.83, 31.23, 28.32, 28.28, 25.25, 22.03, 15.76, 13.95. ESI-HRMS calcd. for $C_{20}H_{26}N_3O_4$ $[M + H]^+$ 372.1918, found: 372.1914. IR (KBr, cm^{-1}): 3439.8, 2931.0, 2229.8, 1663.8, 1613.5, 1466.3, 1298.2, 1108.6.

*4.1.7.10. 2-(3-Cyano-4-octyloxyphenyl)-1-methoxy-4-methyl-1H-imidazole-5-carboxylic acid (**6j**).* A white crystalline powder, yield 66.7%. HPLC: 99.17%. mp 188.6–189.8 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.06 (s, 1H, COOH), 8.26 (dd, 1H, J = 2.2 & 8.9 Hz, Ar–H), 8.23 (d, 1H, J = 2.0 Hz, Ar–H), 7.41 (d, 1H, J = 8.9 Hz,Ar–H), 4.21 (t, 2H, J = 6.4 Hz, CH_2), 3.95 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 1.78 (m, 2H, CH_2), 1.45–1.27 (m, 10H, $5CH_2$), 0.86 (t, 3H, J = 6.3 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.94, 159.78, 142.95, 139.85, 133.85, 132.10, 120.54, 116.45, 115.75, 113.70, 101.20, 69.18, 66.83, 31.18, 28.62, 28.57, 28.24, 25.25, 22.07, 15.75, 13.95. ESI-HRMS calcd. for $C_{21}H_{28}N_3O_4$ $[M + H]^+$ 386.2704, found: 386.2706. IR (KBr, cm^{-1}): 3432.3, 2929.0, 2229.6, 1610.4, 1464.5, 1293.8.

*4.1.7.11. 2-[3-Cyano-4-(4-methylbenzyloxy)phenyl]-1-methoxy-4-methyl-1H-imidazole-5- carboxylic acid (**6k**).* A white crystalline powder, yield 58.1%. HPLC: 96.75%. mp 189.0–190.1 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.31 (s, 1H, Ar–H), 8.30 (dd, 1H, J = 2.2 & 8.1 Hz, Ar–H), 7.55 (d, 1H, J = 9.8 Hz, Ar–H), 7.40 (d, 2H, J = 8.0 Hz, Ar–H), 7.25 (d, 2H, J = 7.9 Hz, Ar–H), 5.34 (s, 2H, CH_2), 3.98 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 2.32 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.26, 159.22, 140.45, 139.39, 137.77, 134.39, 133.03, 132.54, 129.22, 127.96, 118.46, 116.91, 115.59, 114.34, 101.62, 70.68, 67.37, 20.83, 14.34. ESI-HRMS calcd. for $C_{21}H_{20}N_3O_4$ $[M+H]^+$ 378.1448, found: 378.1446. IR (KBr, cm^{-1}): 3405.1, 2659.0, 2235.6, 1719.9, 1609.7, 1499.7, 1456.6, 1306.3, 1288.3, 1266.7, 1115.3.

4.2. Assay of the in vitro XO inhibitory potency

The XO activity with xanthine as the substrate was measured spectrophotometrically, based on the procedure reported by Kong et al. [25], with modification. The 200 μL assay mixture consisted of 0.1 M sodium pyrophosphate buffer (pH 8.3), 0.3 mM Na_2EDTA, 0.2 mM xanthine (Sigma, X4002), 25 U/L XO (Sigma, X1875), and the test compound. After incubation at 25 °C for 120 min, the reaction was monitored at 295 nm on a SpectraMax Plus 384 reader (MD, USA). Test compounds were initially assayed for their inhibition of XO at 10 μg/mL. If >50% inhibition was observed, the compound was classified as active. Active compounds were then tested at five concentrations diluted ten times, with each concentration having four replicates. IC_{50} values were calculated using SPSS 17.0 software.

4.3. Molecular modeling

AutoDock 4 [28] was used to perform docking calculations. The crystal structure of XDH in complex with Febuxostat (PDB code: 1N5X) [5] was used in docking calculations. A 80 × 80 × 80 Å grid box with a grid spacing of 0.375 Å was generated to define the binding pocket. Affinity grid fields were generated using the auxiliary program AutoGrid 4. Ligand structures were built and minimized with Accelrys Discovery Studio 3.0 software package [29]. Flexible torsions in the ligands were assigned, and all dihedral angles were allowed to freely rotate.

The Lamarckian genetic algorithm was used to determine the appropriate binding positions, orientations, and conformations of ligands. The optimized AutoDocking parameters were as follows: the maximum number of energy evaluations was increased to 25 000 000 per run, the iterations of Solis & Wets local search were 3000, the number of individuals in population was 300, and the number of generations was 100. Results differing by < 2 Å in a positional root mean square deviation were clustered together. In each group, the lowest binding energy configuration with the highest percentage frequency was selected as the group representative. All other parameters were maintained as default. Accelrys Discovery Studio Visualizer 4.0 [29] was used for graphic display.

Acknowledgments

This work was supported by Program for Liaoning Excellent Talents in University (LR2012035) and the National Natural Science Foundation of China (J1210029). The authors are grateful to Wen Li, Aihua Song, and Dr. Ying Peng (Analytical Department of Shenyang Pharmaceutical University, Shenyang, China) for assistance in gathering ^1H NMR, ^{13}C NMR, IR, and ESI-HRMS spectroscopy data.

References

[1] E. Roddy, H.K. Choi, Epidemiology of gout, Rheum. Dis. Clin. North. Am. 40 (2014) 155–175.
[2] J.M. Lü, Q. Yao, C. Chen, 3, 4-Dihydroxy-5-nitrobenzaldehyde (DHNB) is a potent inhibitor of xanthine oxidase: a potential therapeutic agent for treatment of hyperuricemia and gout, Biochem. Pharmacol. 86 (2013) 1328–1337.
[3] E. Roddy, M. Doherty, Treatment of hyperuricaemia and gout, Clin. Med. 13 (2013) 400–403.
[4] P. Pacher, A. Nivorozhkin, C. Szabó, Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol, Pharmacol. Rev. 58 (2006) 87–114.
[5] K. Okamoto, B.T. Eger, T. Nishino, S. Kondo, E.F. Pai, An extremely potent inhibitor of xanthine oxidoreductase: crystal structure of the enzyme-inhibitor complex and mechanism of inhibition, J. Biol. Chem. 278 (2002) 1848–1855.
[6] S. Ishibuchi, H. Morimoto, T. Oe, T. Ikebe, H. Inoue, A. Fukunari, M. Kamezawa, I. Yamada, Y. Naka, Synthesis and structure–activity relationships of 1-phenylpyrazoles as xanthine oxidase inhibitors, Bioorg. Med. Chem. Lett. 11 (2001) 879–882.
[7] Q. Guan, Z. Cheng, X. Ma, L. Wang, D. Feng, Y. Cui, K. Bao, L. Wu, W. Zhang, Synthesis and bioevaluation of 2-phenyl-4-methyl-1,3-selenazole-5-carboxylic acids as potent xanthine oxidase inhibitors, Eur. J. Med. Chem. 85 (2014) 508–516.
[8] S. Wang, J. Yan, J. Wang, J. Chen, T. Zhang, Y. Zhao, M. Xue, Synthesis of some 5-phenyl- isoxazole-3-carboxylic acid derivatives as potent xanthine oxidase inhibitors, Eur. J. Med. Chem. 45 (2010) 2663–2670.
[9] J.U. Song, S.P. Choi, T.H. Kim, C.-K. Jung, J.-Y. Lee, S.-H. Jung, G.T. Kim, Design and synthesis of novel 2-(indol-5-yl)thiazole derivatives as xanthine oxidase inhibitors, Bioorg. Med. Chem. Lett. 25 (2015) 1254–1258.
[10] K. Matsumoto, K. Okamoto, N. Ashizawa, T. Nishino, FYX-051: a novel and potent hybrid-type inhibitor of xanthine oxidoreductase, J. Pharmacol. Exp. Ther. 336 (2010) 95–103.
[11] S. Khanna, S. Burudkar, K. Bajaj, P. Shah, A. Keche, U. Ghosh, A. Desai, A. Srivastava, A. Kulkarni-Almeida, N.J. Deshmukh, A. Dixit, M.K. Brahma, U. Bahirat, L. Doshi, K.V.S. Nemmani, P. Tannu, A. Damre, C. B-Rao, R. Sharma, H. Sivaramakrishnan, Isocytosine-based inhibitors of xanthine oxidase: design, synthesis, SAR, PK and in vivo efficacy in rat model of hyperuricemia, Bioorg. Med. Chem. Lett. 22 (2012) 7543–7546.
[12] K. Bajaj, S. Burudkar, P. Shah, A. Keche, U. Ghosh, P. Tannu, S. Khanna, A. Srivastava, N.J. Deshmukh, A. Dixit, Y. Ahire, A. Damre, K.V.S. Nemmani, A. Kulkarni-Almeida, C. B-Rao, R. Sharma, H. Sivaramakrishnan, Lead optimization of isocytosine-derived xanthine oxidase inhibitors, Bioorg. Med. Chem. Lett. 23 (2013) 834–838.
[13] J. Evenäs, F. Edfeldt, M. Lepistö, N. Svitacheva, A. Synnergren, B. Lundquist, M. Gränse, A. Rönnholm, M. Varga, J. Wright, M. Wei, S. Yue, J. Wang, C. Li, X. Li, G. Chen, Y. Liao, G. Lv, A. Tjörnebo, F. Narjes, HTS followed by NMR based counterscreening. Discovery and optimization of pyrimidones as reversible and competitive inhibitors of xanthine oxidase, Bioorg. Med. Chem. Lett. 24 (2014) 1315–1321.
[14] K. Nepali, A. Agarwal, S. Sapra, V. Mittal, R. Kumar, U.C. Banerjee, M.K. Gupta, N.K. Satti, O.P. Suri, K.L. Dhar, *N*-(1,3-Diaryl-3-oxopropyl)amides as a new template for xanthine oxidase inhibitors, Bioorg. Med. Chem. 19 (2011) 5569–5576.
[15] K. Nepali, G. Singh, A. Turan, A. Agarwal, S. Sapra, R. Kumar, U.C. Banerjee, P.K. Verma, N.K. Satti, M.K. Gupta, O.P. Suri, K.L. Dhar, A rational approach for the design and synthesis of 1-acetyl-3,5-diaryl-4,5-dihydro(1*H*)pyrazoles as a new class of potential non-purine xanthine oxidase inhibitors, Bioorg. Med. Chem. 19 (2011) 1950–1958.
[16] H. Singh, S. Sharma, R. Ojha, M.K. Gupta, K. Nepali, P.M.S. Bedi, Synthesis and evaluation of naphthoflavones as a new class of non purine xanthine oxidase inhibitors, Bioorg. Med. Chem. Lett. 24 (2014) 4192–4197.
[17] Z. Smelcerovic, A. Veljkovic, G. Kocic, D. Yancheva, Z. Petronijevic, M. Anderluh, A. Smelcerovic, Xanthine oxidase inhibitory properties and anti-inflammatory activity of 2-amino-5-alkylidene-thiazol-4-ones, Chem. Biol. Interact. 229 (2015) 73–81.
[18] R. Dhiman, S. Sharma, G. Singh, K. Nepali, P.M. Singh Bedi, Design and synthesis of aza-flavones as a new class of xanthine oxidase inhibitors, Arch. Pharm. Weinh. 346 (2013) 7–16.
[19] S. Sharma, K. Sharma, R. Ojha, D. Kumar, G. Singh, K. Nepali, P.M.S. Bedi, Microwave assisted synthesis of naphthopyrans catalysed by silica supported fluoroboric acid as a new class of non purine xanthine oxidase inhibitors, Bioorg. Med. Chem. Lett. 24 (2014) 495–500.
[20] S. Shukla, D. Kumar, R. Ojha, M.K. Gupta, K. Nepali, P.M.S. Bedi, 4,6-Diaryl/heteroaryl- pyrimidin-2(1*H*)-ones as a new class of xanthine oxidase inhibitors, Arch. Pharm. Weinh. 347 (2014) 486–495.
[21] H.S. Virdi, S. Sharma, S. Mehndiratta, P.M.S. Bedi, K. Nepali, Design, synthesis and evaluation of 2,4-diarylpyrano[3,2-c]chromen-5(4H)-one as a new class of non-purine xanthine oxidase inhibitors, J. Enzyme Inhib. Med. Chem. (2014) 1–7.
[22] M. Witschel, Design, synthesis and herbicidal activity of new iron chelating motifs for HPPD-inhibitors, Bioorg. Med. Chem. 17 (2009) 4221–4229.
[23] G. Mlostoń, M. Jasiński, H. Heimgartner, Straightforward access to (imidazol-2-yl)acetates by reaction of 2-unsubstituted imidazole 3-oxides with dimethyl acetylenedicarboxylate, Eur. J. Org. Chem. 2011 (2011) 2542–2547.
[24] A.R. Massah, M. Mosharafian, A.R. Momeni, H. Aliyan, H.J. Naghash, Solvent-free Williamson synthesis: an efficient, simple, and convenient method for chemoselective etherification of phenols and bisphenols, Synth. Commun. 37 (2007) 1807–1815.
[25] L.D. Kong, Y. Zhang, X. Pan, R.X. Tan, C.H. Cheng, Inhibition of xanthine oxidase by liquiritigenin and isoliquiritigenin isolated from *Sinofranchetia chinensis*, Cell. Mol. Life Sci. 57 (2000) 500–505.
[26] R. Hille, Molybdenum-containing hydroxylases, Arch. Biochem. Biophys. 433 (2005) 107–116.
[27] C. Enroth, B.T. Eger, K. Okamoto, T. Nishino, T. Nishino, E.F. Pai, Crystal structures of bovine milk xanthine dehydrogenase and xanthine oxidase: structure-based mechanism of conversion, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 10723–10728.
[28] G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A.J. Olson, Autodock4 and AutoDockTools4: automated docking with selective receptor flexibility, J. Comput. Chem. 30 (2009) 2785–2791.
[29] Accelrys Inc, 10188 Telesis Court, Suite 100 San Diego, CA 92121, USA, 2008.