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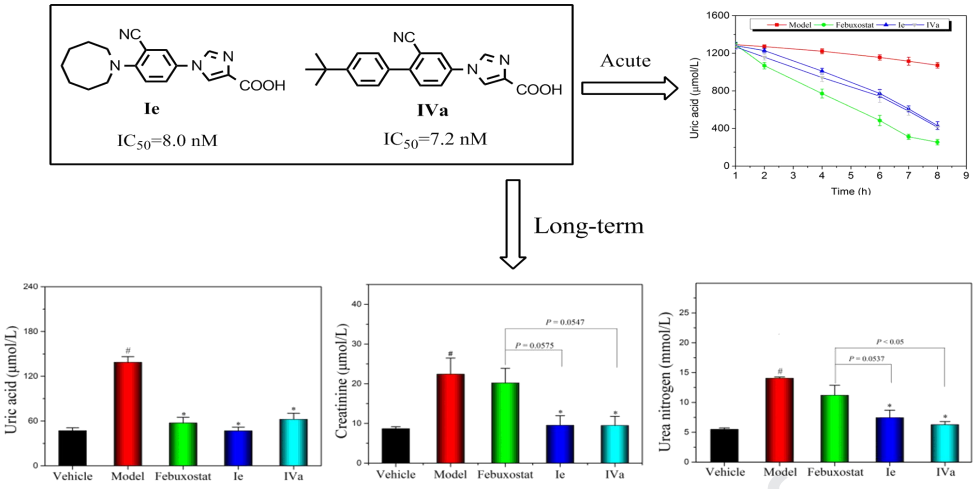

Synthesis and bioevaluation of 1-phenylimidazole-4-carboxylic acid derivatives as novel xanthine oxidoreductase inhibitors

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Abstract

As part of a continuing study, we designed and synthesized four series of 1-phenylimidazole-4-carboxylic acid derivatives as xanthine oxidoreductase (XOR) inhibitors, evaluated their in vitro inhibitory potencies against XOR and hypouricemic effects in mice, and determined their structure-activity relationships (SARs). Most of the compounds exhibited in vitro XOR inhibition at the nanomolar level. In comparison to febuxostat (half-maximal inhibitory concentration [IC_{50}] value of 7.0 nM), compounds **Ie** and **IVa** exhibited the most promising XOR inhibitory effects with IC_{50} values of 8.0 and 7.2 nM, respectively. In the potassium oxonate/hypoxanthine-induced acute and long-term hyperuricemia mouse models, compounds **Ie** and **IVa** displayed significant hypouricemic potencies ($P < 0.05$), that were slightly weaker than and similar to febuxostat, respectively. More interestingly, both compounds showed a capacity to improve kidney damage by decreasing creatinine and urea nitrogen levels compared to the long-term hyperuricemia mouse group ($P < 0.05$), while febuxostat showed no significant effect.

Keywords: 1-phenylimidazole-4-carboxylic acid, xanthine oxidoreductase inhibitors, hypouricemic

1. Introduction

Hyperuricemia is a common metabolic disorder caused by continuously elevated serum uric acid, which is produced as the final oxidation product of purine catabolism in humans and induces the formation of monosodium urate crystal deposits that eventually cause gout [1–3]. In purine metabolism, xanthine oxidoreductase (XOR) is

a key enzyme that catalyzes the oxidation of hypoxanthine and xanthine to generate the final product uric acid. Therefore, XOR is currently considered the most promising target for blocking uric acid production in hyperuricemia treatment [4–6].

The representative purine XOR inhibitor allopurinol (Fig. 1) has been the cornerstone of the treatment of gout and hyperuricemia in the USA for several decades. Despite its potent urate-lowering properties, allopurinol reportedly induces several common and severe adverse reactions such as renal failure and allopurinol hypersensitivity syndrome [7–9]. Hence, it is crucial to develop new XOR inhibitors with fewer side effects.

Non-purine XOR inhibitors such as febuxostat (Fig. 1) and topiroxostat (FYX-051, Fig. 1) have been investigated to avoid the side effects of allopurinol. Febuxostat, a phenylthiazole derivative with outstanding and longer-lasting XOR inhibitory potency than allopurinol, was approved for the treatment of gout in the USA in 2009 [10, 11]. The triazole derivative topiroxostat was approved for the clinical treatment of hyperuricemia in Japan in 2013 [12, 13]. Furthermore, other structural XOR inhibitors are being studied, such as imidazoles, pyrazoles (e.g., Y-700, Fig. 1) [14], flavonoids [15], fraxamoside [16], *N*-(1,3-diaryl-3-oxopropyl)amides [17], *N*-acetylpyrazolines [18], quinazolines [19], pyrimidines [20], isoxazoles [21], isocytosines [22], selenazoles [23], 1,2,3-triazoles [24], and 1,3,4-oxadiazoles [25].

Similarly, we obtained pyrazole derivatives such as WN1703 (Fig. 1) through the lead optimization of febuxostat via the bioisosterism concept. WN1703 showed similar XOR inhibitory activity in vitro and similar hypouricemic potency in the potassium oxonate/hypoxanthine-induced acute and long-term hyperuricemia mouse models as compared with febuxostat [26 and supplementary material 1]. We also

explored structure–activity relationships (SARs) via the molecular docking and three-dimensional quantitative structure–activity relationship (3D-QSAR) approaches [27]. Based on the above work, we conducted further optimization experiments (Fig. 2). Since some simple imidazole analogues are good XOR inhibitors [28], we designed compounds in which the pyrazole moiety of WN1703 was replaced by imidazole. To facilitate the discussion of the SARs in the following articles, these compounds are classified into four series according to functional group substitutions: series **I** could be considered as imidazole analogues of WN1703; series **II** and **III** were further modifications of series **I** that focused on substitution of the C4 of the phenyl moiety and the coupling mode between the phenyl and imidazole groups, respectively. While referring to our previous findings on the SARs of the XOR inhibitors [27] and the three-ring structure of topiroxostat, series **IV** was designed to determine the hydrophobic group size that can be accommodated at the entrance of the active site. Consequently, 22 novel XOR inhibitors were synthesized, some of which were found to exhibit in vitro XOR inhibition and potent hypouricemic effects in mice.

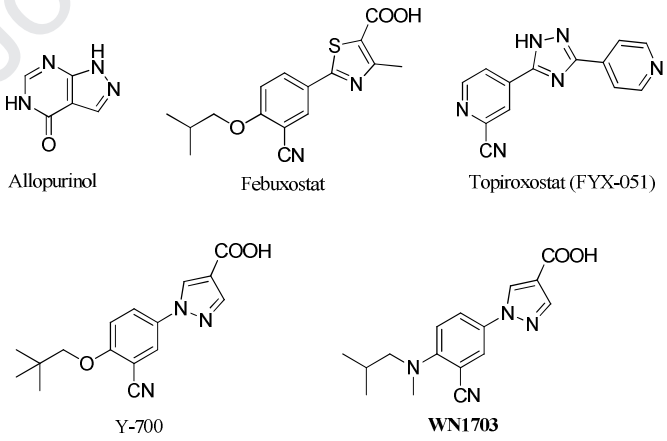


Fig. 1. Chemical structures of allopurinol, febuxostat, topiroxostat, Y-700, and WN1703.

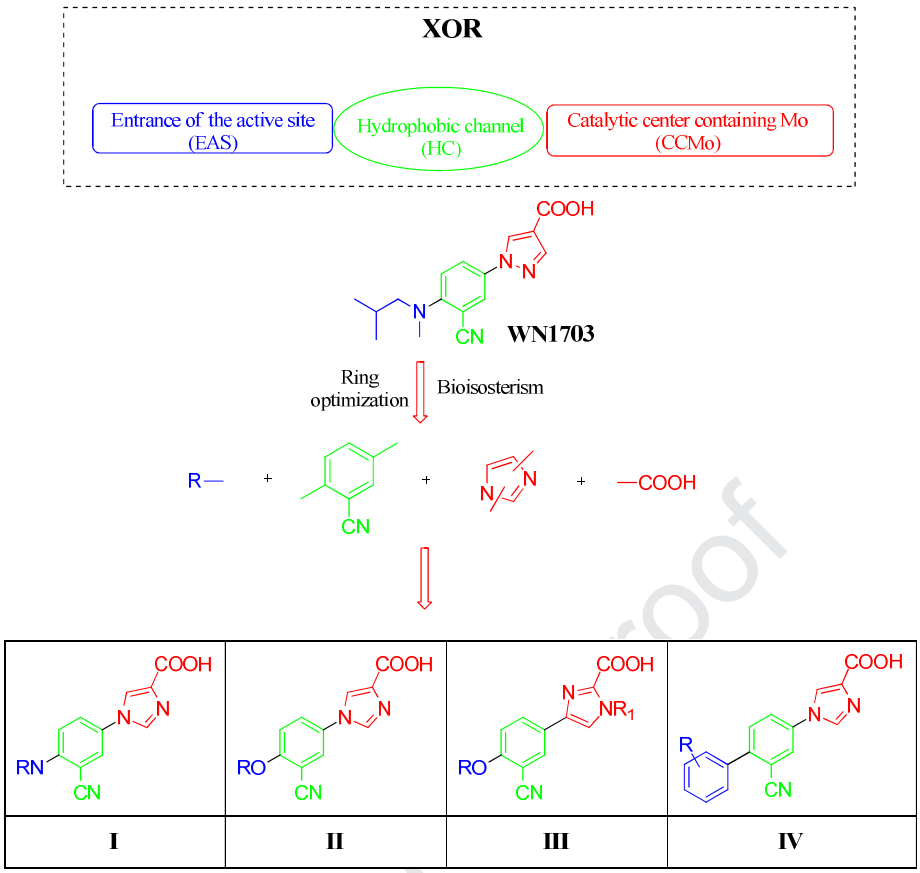
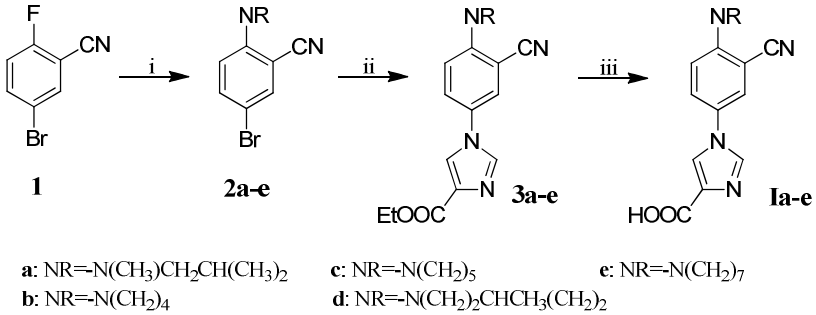


Fig. 2. Design of compounds **I**–**IV**.

2. Results and discussion

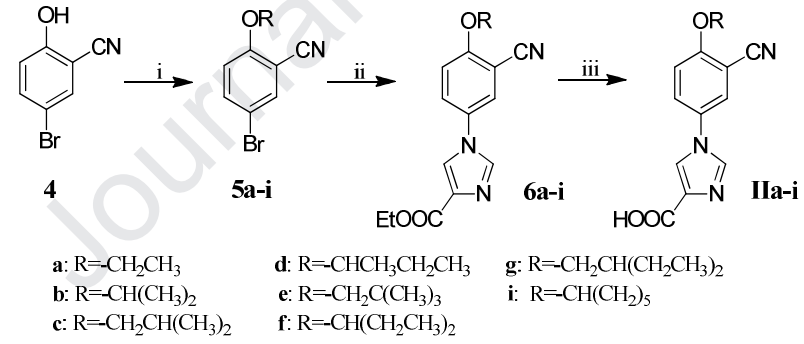
2.1. Chemistry

The series **I** derivatives were synthesized using the strategy depicted in Scheme 1. Compound **1** was alkylated using the corresponding alkylamine in the presence of K_2CO_3 to produce **2a**–**e**, followed by a coupling reaction with 1*H*-imidazole-4-ethyl formate to obtain **3a**–**e**, subsequent hydrolyzation with NaOH, and then acidification to yield **Ia**–**e**.



Scheme 1. Reagents and conditions: (i) alkylamine, K_2CO_3, and DMF at 70–80 °C; (ii) CuI, K_2CO_3, *trans*-*N*,*N*′-dimethyl-1,2-cyclohexanediamine, 1*H*-imidazole-4-ethyl formate, and DMF at 120 °C; (iii) NaOH aq. (1 M), THF/EtOH (1:1), 65 °C, HCl aq. (1 M), rt.

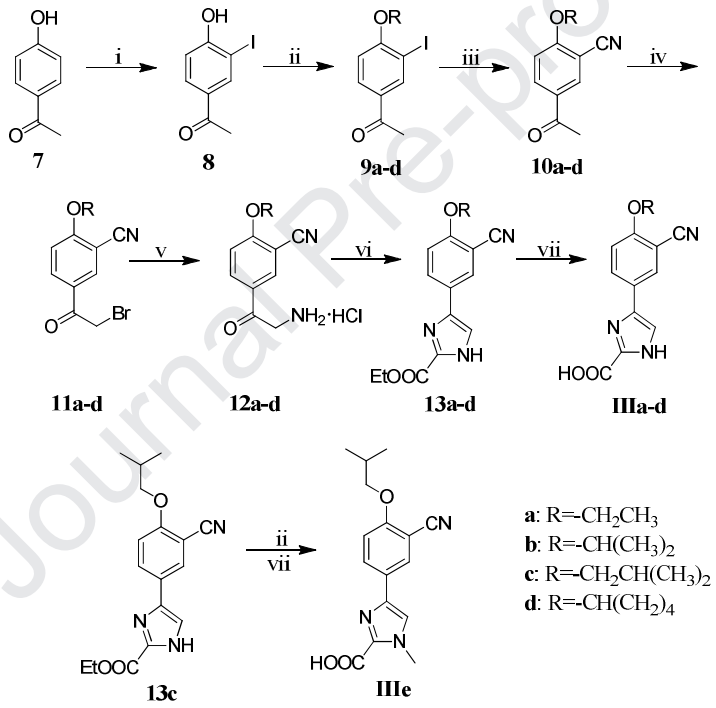
Similarly, compounds **IIa**–**i** were synthesized as presented in Scheme 2. Compound **4** was alkylated with the corresponding alkyl bromide or alkyl iodide in the presence of K_2CO_3 to afford **5a**–**i**, followed by a coupling reaction with 1*H*-imidazole-4-ethyl formate to furnish **6a**–**i**, which were hydrolyzed to yield **IIa**–**i**.



Scheme 2. Reagents and conditions: (i) K_2CO_3, alkyl halide, DMF, 70–80 °C; (ii) CuI, K_2CO_3, *trans*-*N*,*N*′-dimethyl-1,2-cyclohexanediamine, 1*H*-imidazole-4-ethyl formate, and DMF at 120 °C; (iii) NaOH aq. (1 M), THF/EtOH (1:1), 65 °C, HCl aq. (1 M), rt.

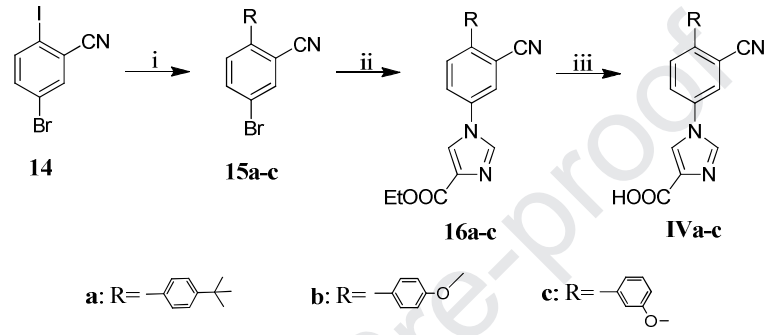
The synthesis of the target derivatives **IIIa**–**e** was accomplished as outlined in Scheme 3. Commercially available 1-(4-hydroxyphenyl)ethanone (**7**) was iodinated with I_2 and KI to generate intermediate **8**, which was alkylated with the appropriate C2–C5 alkyl bromide/iodide in dimethylformamide (DMF) in the presence of

anhydrous K_2CO_3 to obtain **9a–d**, followed by cyano substitution to afford **10a–d**. Compounds **10a–d** were treated with bromine solution or $CuBr_2$ to obtain **11a–d**. The Delépine reaction of **11a–d** was performed through hexamethylenetetramine treatment, followed by acidic hydrolysis with HCl (conc.) to yield the key intermediates **12a–d**. Cyclization of **12a–d** with ethyl thiooxamate in the presence of trimethyloxonium tetrafluoroborate and CH_3COONa delivered **13a–d**, which were hydrolyzed with NaOH and then acidified to yield **IIIa–d**. In addition, **13c** was alkylated with CH_3I and then hydrolyzed to afford **IIIe**.



Scheme 3. Reagents and conditions: (i) KI, I_2, and NH_4OH followed by acidification with HCl (conc.); (ii) K_2CO_3, DMF, and alkyl halide, 70–80 °C; (iii) CuCN and DMF, 140 °C; (iv) Br_2 aq. (1 M), or $CuBr_2$ and dioxane, 120 °C; (v) $CHCl_3$, Et_2O, and HMTA followed by acidic hydrolysis with HCl (conc.); (vi) ethyl thiooxamate, trimethyloxonium tetrafluoroborate, CH_2Cl_2, CH_3COOH, CH_3COONa, and dioxane, 70 °C; (vii) NaOH aq. (1 M) and THF/EtOH (1:1), 65 °C, followed by acidification with HCl aq. (1 M).

Compounds **IVa–c** were synthesized as outlined in Scheme 4. Commercially available 5-bromo-2-iodobenzonitrile (**14**) was coupled with the corresponding alkylboronic acid to produce **15a–c**, followed by a coupling reaction with 1*H*-imidazole-4-ethyl formate to obtain **16a–c**. Subsequently, **16a–c** were hydrolyzed using NaOH in tetrahydrofuran (THF)/ethanol (EtOH) and then acidified to produce **IVa–c**.



Scheme 4. Reagents and conditions: (i) alkylboronic acid, K_2CO_3, $Pd[P(C_6H_5)_3]_4$, and DMF, 120 °C; (ii) CuI, K_2CO_3, *trans*-*N*,*N*′-dimethyl-1,2-cyclohexanediamine, 1*H*-imidazole-4-ethyl formate, and DMF, 120 °C; (iii) NaOH aq. (1 M) and THF/EtOH (1:1), 65 °C, followed by acidification with HCl aq. (1 M).

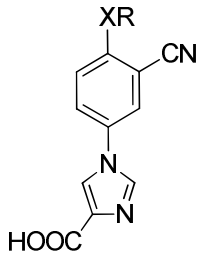
2.2. Biological activity in vitro

The in vitro bovine XOR inhibitory activities of target compounds **Ia–e**, **IIa–i**, **IIIa–e**, and **IVa–c** were measured by determining uric acid levels at 295 nm, with febuxostat as the reference compound [26].

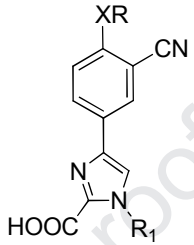
The XOR inhibitory activity of Compounds **Ia–e, IIa–i**, and **IIIa–e** are shown in Table 1. In general, most of compounds **I** and **II** (with the exception of **IIa** and **IIb**) displayed certain XOR inhibitory activity with half-maximal inhibitory concentration (IC_{50}) values <100 nM, which were significantly superior to the XOR inhibition of

compounds **III**. SARs were revealed with reference to their XOR inhibitory activities. (1) The XOR inhibitory activity of the alkylamine-containing derivatives was superior to that of the alkoxy-containing derivatives, which reconfirmed our previous study [26]. (2) Comparison of **IIa**–**g** revealed that inhibitory activity gradually increased with carbon chain length. Generally, when substituents at the phenyl 4-position (R) possessed the same number of carbon atoms, the inhibitory activity of chain-alkane-substituted derivatives was superior to that of cyclic-alkane-substituted derivatives, such as **Ia** versus **Ic** and **IIf** versus **IIh**. (3) Compound **Ie**, which contains a cycloheptylamine substituent at the phenyl 4-position (R), exhibited the strongest inhibitory activity, which was equivalent to that of febuxostat (*t* test, $P > 0.05$), suggesting that the most appropriate molecular volume could maximize inhibitory activity. (4) The coupling mode between the phenyl and imidazole groups affected XOR inhibitory activity. Obviously, when XR was the same, compounds **III** showed lower XOR inhibition than compounds **II**, for example, **IIa** and **IIIa**, **IIb** and **IIIb**, **IIc** and **IIIc**, and **IIh** and **IIId**. (5) A methyl group introduced at the R_1 position led to a fourfold reduction in XOR inhibitory activity as compared **IIIe** with **IIIc**, further indicating that although those compounds can be considered as structural modifications of febuxostat, methyl is not necessary for their XOR inhibitory activity after the bioisosterism of five-membered heterocycles. Taken together, the results in Table 1 suggest that imidazole analogues were generally tolerated for the structural optimization of WN1703; however, we did not obtain compounds with significantly higher XOR inhibitory activity, except **Ie** with XOR inhibition similar to febuxostat.

Table 1. In vitro XOR inhibitory activities of designed compounds **Ia**–**e**, **IIa**–**i** and **IIIa**–**e** (*n* = 3).



I, **II**

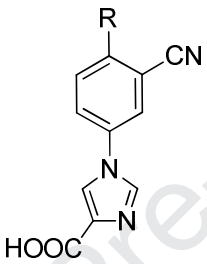


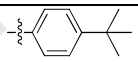
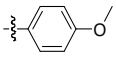
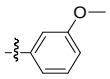
III

Compd.	-XR	IC_{50} (nM)	Compd.	-XR	IC_{50} (nM)	Compd.	-XR	$-R_1$	IC_{50} (nM)
Ia	$-N(CH_3)CH_2CH(CH_3)_2$	27.1	**IIa**	$-OCH_2CH_3$	226.3	**IIIa**	$-OCH_2CH_3$	-H	948.0
Ib	$-N(CH_2)_4$	34.1	**IIb**	$-OCH(CH_3)_2$	197.9	**IIIb**	$-OCH(CH_3)_2$	-H	750.0
Ic	$-N(CH_2)_5$	55.6	**IIc**	$-OCH_2CH(CH_3)_2$	94.5	**IIIc**	$-OCH_2CH(CH_3)_2$	-H	510.0
Id	$-N(CH_2)_4CHCH_3$	30.5	**IId**	$-OCH(CH_3)CH_2CH_3$	73.9	**IIId**	$-OCH(CH_2)_4$	-H	387.0
Ie	$-N(CH_2)_7$	8.0	**IIe**	$-OCH_2C(CH_3)_3$	67.9	**IIIe**	$-OCH_2CH(CH_3)_2$	$-CH_3$	2158.0
			IIf	$-OCH(CH_2CH_3)_2$	40.6				
			IIg	$-OCH_2CH(CH_2CH_3)_2$	29.8				
			IIh	$-OCH(CH_2)_4$	63.0				
Febuxostat	—	7.0	**IIi**	$-OCH(CH_2)_5$	47.4				

As shown in Table 2, **IVa** was the most potent XOR inhibitor with an IC_{50} value of 7.2 nM, and its inhibitory activity was equivalent to that of febuxostat (*t* test, $P > 0.05$). The inhibitory activity of **IVb** was approximately twofold higher than that of **IVc**, indicating that para substitution of the benzene ring was superior to ortho substitution. Although compounds **IVa–c** displayed potent inhibitory activity against XOR, more derivatives of series **IV** are needed for future discussions regarding SARs.

Table 2. In vitro XOR inhibitory activities of designed compounds **IVa–c** ($n = 3$).

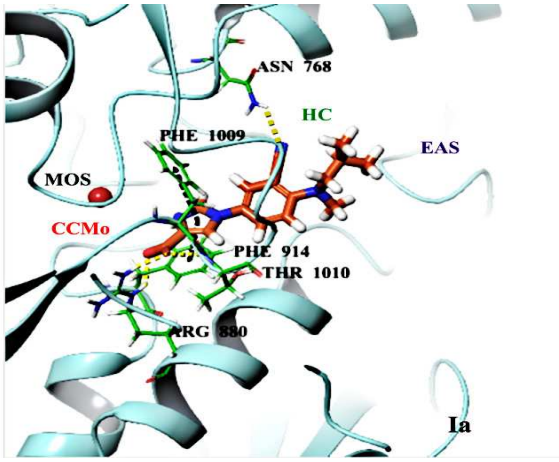


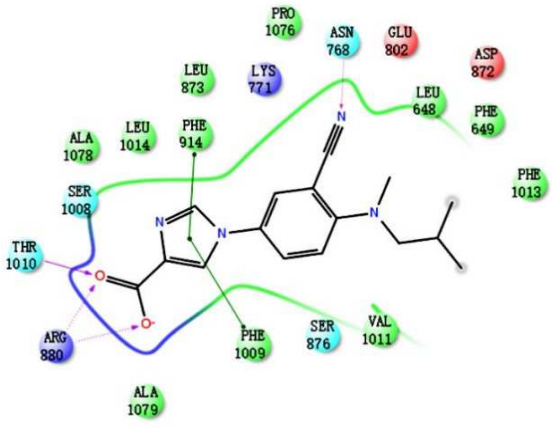
Compd.	R	IC_{50} (nM)
IVa		7.2
IVb		34.5
IVc		74.4
Febuxostat	-	7.0

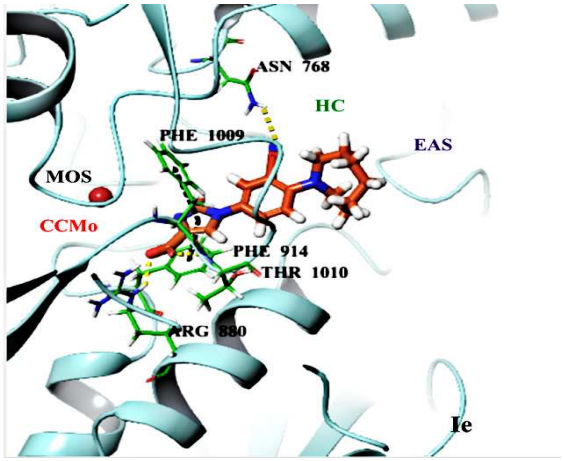
2.3. Molecular modeling

To further study the binding mode, molecular docking was performed between several compounds (**Ia**, **Ie**, **IVa**, WN1703, and febuxostat) and XOR using Schrodinger Glide 10.1 software [29–31]. As shown in Fig. 3A, the binding model of the compounds and XOR had three main features: (1) the carboxyl group on the heterocyclic ring interacted with Arg880 (O–H–N: 2.33 Å, O–H–N: 1.97 Å) and Thr1010 (O–H–N: 2.22 Å) through hydrogen bonds; (2) hydrogen bonds were also formed between the cyano groups and Asn768 (N–H–N: 2.44 Å); (3) the five-membered heterocyclic rings formed a slanted sandwich between Phe914 and

Phe1009 by π–π stacking. However, there were still subtle differences in their binding patterns. (1) As shown in Fig. 3B, to match the XOR binding cavity, the dihedral angle between the benzene ring and five-membered heterocyclic ring of these compounds was not exactly the same, which might have altered the π–π interaction between the five-membered heterocyclic ringsand Phe914 and Phe1009. Although the dihedral angle data of these compounds cannot show clear rules, it further suggests that bioisosterism substitution of different five-membered heterocycles would affect the spatial conformation of the whole molecule, thus affecting its affinity for XOR. (2) The substituent at the C4 position of the phenyl moiety mainly provided hydrophobic interactions at the initial part of the XOR hydrophobic channel, so the size of the substituent affected compound inhibitory activity to some extent. For example, it may be that **Ie** fitted more neatly into the hydrophobic pocket than **Ia**, which gave it stronger inhibitory potency. (3) **IVa** with a hydrophobic benzene ring substituted at the C4 position of the phenyl moiety exhibited an extra π–π interaction between the benzene ring and Phe649, which might explain its superior XOR inhibitory activity among these compounds. The above results of virtual molecular docking essentially agree with the in vitro XOR inhibitory activity data, which may help clarify the SARs of these compounds.



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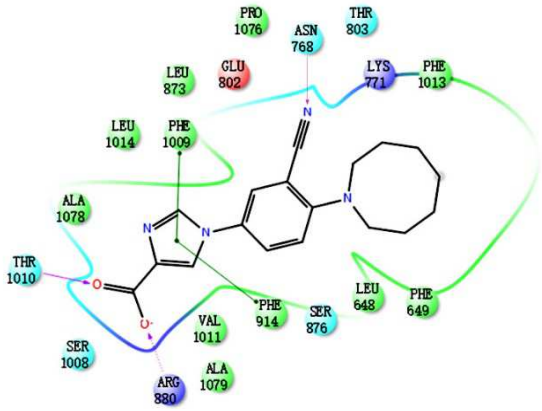
Ie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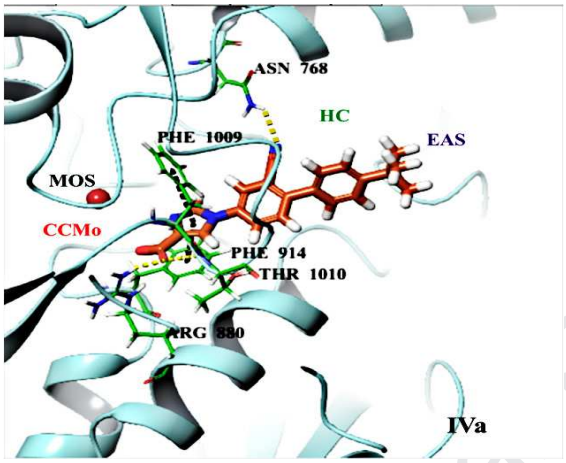
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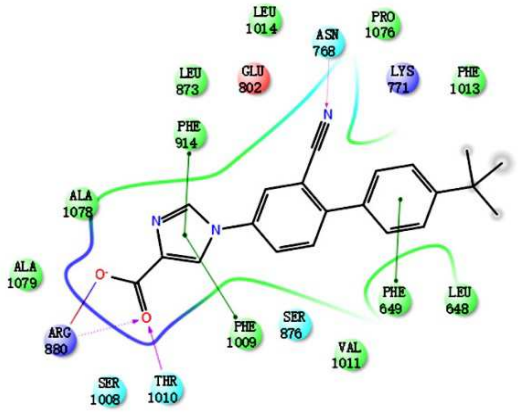
IVa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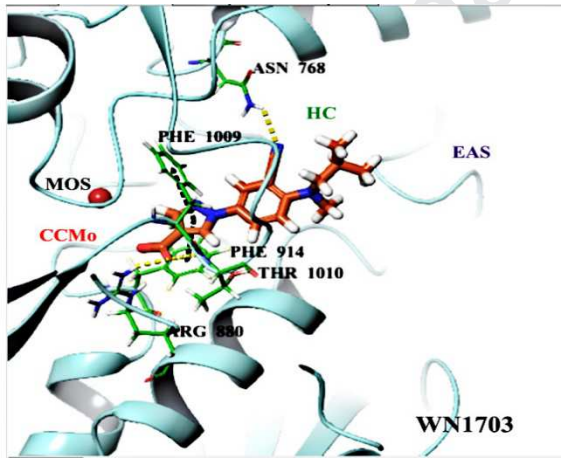
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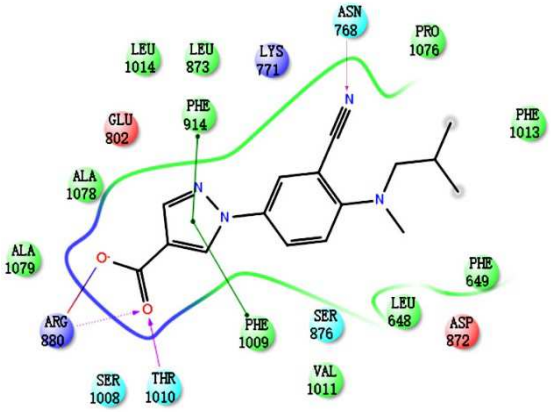
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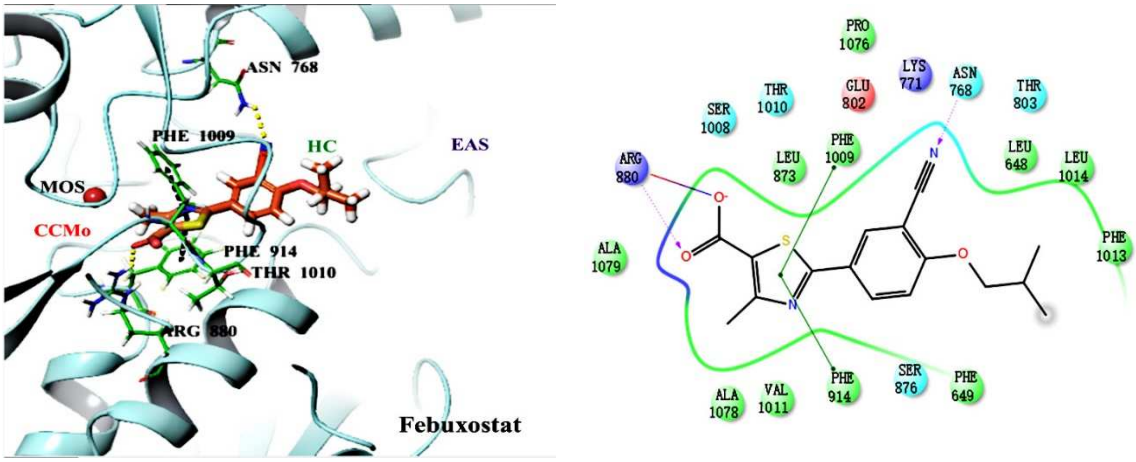
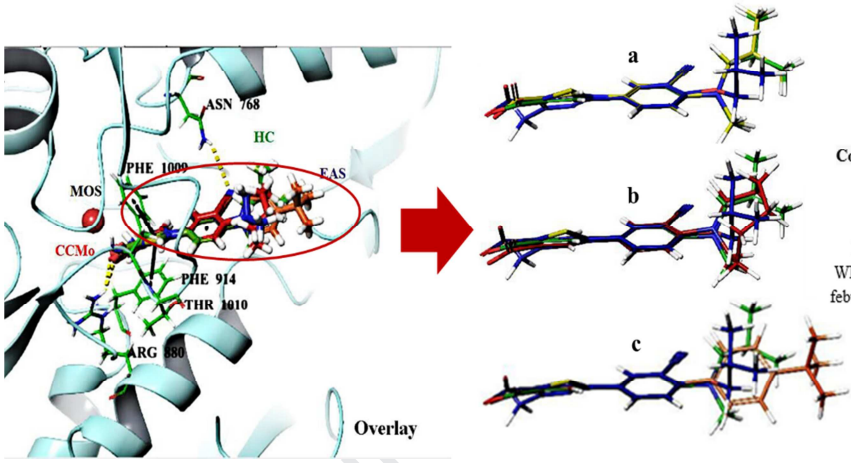


A



Compd.	Dihedral angle of benzene ring and five-membered heterocyclic ring
Ia	27.5°
Ie	17.6°
IVa	23.6°
WN1703	18.1°
febuxostat	33.4°

B

Fig. 3. A: 3D (left) and 2D (right) docking modes of XOR with compounds **Ia**, **Ie**, **IVa**, WN1703, and febuxostat. **B**: The overlays of five compounds (a: **Ia** (yellow), WN1703 (green) and febuxostat (blue); b: **Ie** (red), WN1703, and febuxostat; c: **IVa** (orange), WN1703, and febuxostat). The hydrogen bonds are represented by yellow and purple in 3D and 2D maps, respectively. Salt bridges are represented by red-violet solid lines in 2D maps. π–π bonds are represented by black and green dotted arrows in 3D and 2D maps, respectively. CCMo: catalytic center containing Mo; EAS: entrance of the active site; HC: hydrophobic channel.

2.4. Hypouricemic effect in vivo

The in vivo hypouricemic effects of compounds **Ie** and **IVa** were further verified by measuring serum uric acid levels in an acute hyperuricemia mouse model (Fig. 4). The acute hyperuricemia mouse model was based on our previous research methods. Compared with the normal group (data not shown as they were below the detection limit), serum uric acid levels in the model group effectively increased and were maintained throughout the experiment [26, 32]. Compared with the hyperuricemia model group, compounds **Ie** and **IVa** significantly reduced serum uric acid levels at a dosage of 5 mg/kg during the entire test period after drug administration ($P < 0.05$). Although the uric-acid-lowering effects of these two compounds were slightly weaker than that of febuxostat, this experiment confirmed that both have uric-acid-lowering effects.

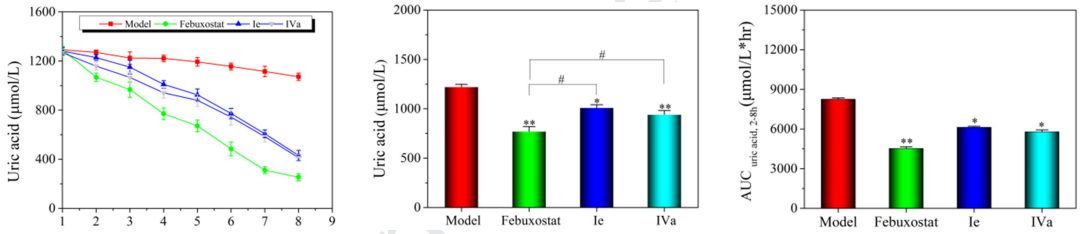
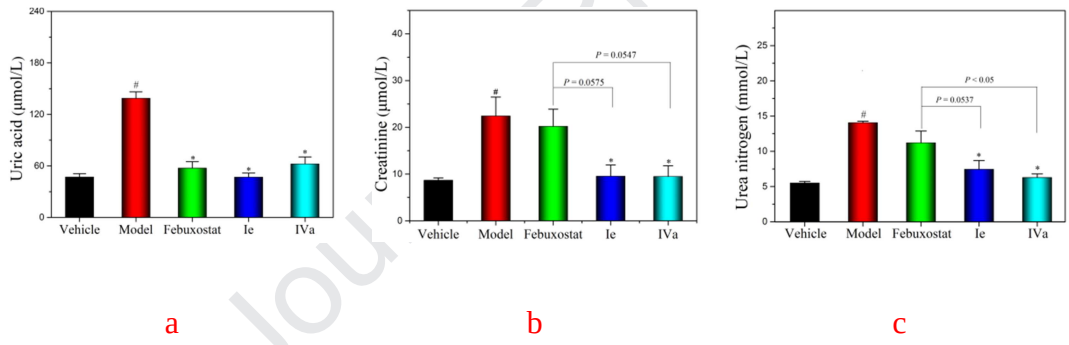


Fig. 4. Hypouricemic effects of compounds **Ie** and **IVa** at a dosage of 5 mg/kg in the in vivo potassium oxonate/hypoxanthine-induced acute hyperuricemia mouse model ($n = 8$). (a) Time-dependent changes in serum uric acid levels after oral administration of **Ie**, **IVa**, or febuxostat. (b) Serum uric acid levels 4 h after oral administration of **Ie**, **IVa**, or febuxostat. (c) Areas under the curve (uric acid, 2–8 h, AUC) after oral administration of **Ie**, **IVa**, or febuxostat. Data are expressed as mean ± S.D. *$P < 0.05$, **$P < 0.01$ vs model group, $^{\#}P < 0.05$ vs febuxostat group.

Furthermore, we explored the hypouricemic effects of compounds **Ie** and **IVa** compared with febuxostat in a long-term hyperuricemia mouse model. To prevent mouse death caused by intolerance during model establishment, the dose used (250 mg/kg potassium oxonate + 150 mg/kg hypoxanthine) was lower than that in the acute hyperuricemia model (250 mg/kg

potassium oxonate + 400 mg/kg hypoxanthine). As expected, **Ie** and **IVa** displayed continuous and potent hypouricemic effects compared with the hyperuricemia group at a dosage of 5 mg/kg ($P < 0.05$), and the hypouricemic effects of both compounds were similar to that of febuxostat. In addition, some studies suggest that hyperuricemia may lead to renal injury [33–36], but another group did not find evidence of a direct relationship [37]. In the current experiment, creatinine and urea nitrogen levels in the hyperuricemia group were significantly higher than those in the vehicle group, suggesting that renal injury occurred. More interestingly, **Ie** and **IVa** decreased creatinine and urea nitrogen levels compared to the hyperuricemia group ($P < 0.05$). This effect was not observed for febuxostat or WN1703 [supplementary material 1], suggesting that **Ie** and **IVa** could alleviate hyperuricemia-induced renal injury to some extent.



a b c

Fig. 5. Levels of uric acid (a), creatinine (b), and urea nitrogen (c) after oral administration of **Ie**, **IVa**, or febuxostat at a dosage of 5 mg/kg in the potassium oxonate/hypoxanthine-induced long-term hyperuricemia mouse model ($n = 8$, $^{*}P < 0.05$ vs model group, $^{\#}P < 0.05$ vs vehicle group).

3. Conclusions

Based on our previous me-too optimization with febuxostat as the lead compound, a series of 1-phenylimidazole-4-carboxylic acid derivatives were designed as novel XOR inhibitors. Among these compounds, **Ie** and **IVa** emerged as the most potent XOR inhibitors, with IC_{50}

values similar to those of febuxostat. Molecular modeling studies of selected compounds provided some explanations for their binding model to XOR and inhibitory activity in vitro; the results also clarified their SARs. Notably, while **Ie** and **IVa** exhibited weaker hypouricemic effects than febuxostat in the acute hyperuricemia mouse model, both exhibited considerable hypouricemic effects similar to febuxostat in the long-term hyperuricemia mouse model. Moreover, the creatinine- and urea nitrogen-lowering effects suggested that **Ie** and **IVa** could mitigate kidney injury; this point needs further examination and confirmation in our future research.

4. Experimental protocols

4.1. Chemistry

Unless otherwise indicated, reagents and solvents were purchased from commercial suppliers and used without further purification. All of the reactions were monitored via thin-layer chromatography using silica gel glass plates (Qingdao Ocean Chemical, Qingdao, Shandong, P. R. China) with an ultraviolet indicator at 254 nm.

Column chromatography was performed using silica gel (200–300 mesh) from Qingdao Ocean Chemical. ^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker Avance III HD 400 or 600 MHZ spectrometers (Bruker, Germany) using tetramethylsilane as the internal standard. High-resolution mass spectrometry (HRMS) spectra were determined on an Agilent 1290 instrument operating in electrospray ionization (ESI) mode (Agilent Technologies, USA).

*4.1.1. General procedure for the preparation of compounds **2a**–**e***

A mixture of compound **1** (5.0 mmol), alkylamine (15.0 mmol), and K_2CO_3 (15.0 mmol) in DMF (10 mL) was reacted at 70–80 °C for 3–5 h. After the reaction was complete, the mixture was poured into H_2O (100 mL) and extracted with ethyl acetate (100 mL × 2). The organic layer was collected, washed with brine (100 mL × 3), dried over anhydrous Na_2SO_4, and concentrated under vacuum to yield the crude product, which was purified by flash column chromatography (0–15% ethyl acetate in petroleum ether).

*4.1.1.1. 5-bromo-2-(isobutyl(methyl)amino)-benzonitrile (**2a**)* Yellow oil (yield: 93%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, 1H, *J* = 1.3 Hz, ArH), 7.42 (dd, 1H, *J* = 9.1, 3.3 Hz, ArH), 6.75 (d, 1H, *J* = 9.1 Hz, ArH), 3.19 (d, 2H, *J* = 7.5 Hz, -$NCH_3C\underline{H}_2CH(CH_3)_2$), 3.05 (s, 3H, -$NC\underline{H}_3CH_2CH(CH_3)_2$), 2.01 (m, 1H, -$NCH_3CH_2C\underline{H}(CH_3)_2$), 0.89 (d, 6H, *J* = 6.6 Hz, -$NCH_3CH_2CH(C\underline{H}_3)_2$).

*4.1.1.2. 5-bromo-2-pyrrolidin-benzonitrile (**2b**)* Yellow solid (yield: 90%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (d, *J* = 2.5 Hz, 1H, ArH), 7.36 (dd, *J* = 9.2, 2.5 Hz, 1H, ArH), 6.51 (d, *J* = 9.2 Hz, 1H, ArH), 3.62-3.54 (m, 4H, -$N(C\underline{H}_2)_2(CH_2)_2$), 2.04-1.98 (m, 4H, -$N(CH_2)_2(C\underline{H}_2)_2$).

*4.1.1.3. 5-bromo-2-(piperidin-1-yl)-benzonitrile (**2c**)* White solid (yield: 92%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.62 (dd, *J* = 2.3, 0.7 Hz, 1H, ArH), 7.52 (ddd, *J* = 8.8, 2.3, 0.9 Hz, 1H, ArH), 6.86 (d, *J* = 8.9 Hz, 1H, ArH), 3.18-3.12 (m, 4H, -$N(C\underline{H}_2)_5$), 1.76 (m, 4H, -$N(C\underline{H}_2)_5$), 1.63-1.56 (m, 2H, -$N(C\underline{H}_2)_5$).

*4.1.1.4. 5-bromo-2-(4-methylpiperidin-1-yl)-benzonitrile (**2d**)* White solid (yield: 90%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.62 (d, *J* = 2.4 Hz, 1H, ArH), 7.52 (dd, *J* = 8.9, 2.4 Hz, 1H, ArH), 6.86 (d, *J* = 8.9 Hz, 1H, ArH), 3.54 (d, *J* = 12.3 Hz, 2H, -$N(C\underline{H}_2)_2(CH_2)_2CHCH_3$), 2.78 (t, *J* = 13.0 Hz, 2H, -$N(C\underline{H}_2)_2(CH_2)_2CHCH_3$), 1.76 (d, *J* = 12.6 Hz, 2H, -$N(CH_2)_2(C\underline{H}_2)_2CHCH_3$), 1.53 (m, 1H, -$N(CH_2)_2(CH_2)_2C\underline{H}CH_3$), 1.48-1.41 (m, 2H, -$N(CH_2)_2(C\underline{H}_2)_2CHCH_3$), 1.00 (d, *J* = 6.4 Hz, 3H, -$N(CH_2)_2(CH_2)_2CHC\underline{H}_3$).

*4.1.1.5. 5-bromo-2-(azocan-1-yl)- benzonitrile (**2e**)* White solid (yield: 93%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (s, 1H, ArH), 7.37 (d, *J* = 9.2 Hz, 1H, ArH), 6.68 (d, *J* = 9.3 Hz, 1H, ArH), 3.69 (s, 4H, -N(C<u>H</u>$_2$)$_7$), 1.80 (s, 2H, -N(C<u>H</u>$_2$)$_7$), 1.60 (d, *J* = 10.1 Hz, 8H, -N(C<u>H</u>$_2$)$_7$).

*4.1.2. General procedure for the preparation of compounds **3a**–**e***

A mixture of compound **2a**–**e** (2.4 mmol), CuI (0.2 mmol), 1*H*-imidazole-4-ethyl carboxylate (2.0 mmol), *trans*-*N*,*N*-dimethyl-1,2-cyclohexanediamine (0.4 mmol), and K_2CO_3 (4.2 mmol) in DMF (5 mL) was stirred at 120 °C for 24 h under a nitrogen atmosphere. Subsequently, the reaction was diluted with H_2O (30 mL) and extracted with ethyl acetate (20 mL × 2). The organic phases were combined, washed with brine (30 mL × 3), dried over anhydrous Na_2SO_4, and concentrated under vacuum. The residue was purified by flash column chromatography (12–30% ethyl acetate in petroleum ether).

*4.1.2.1. ethyl 1-(3-cyano-4-(isobutyl(methyl)amino)phenyl)-1H-imidazole-4- carboxylate (**3a**).* White solid (yield: 42%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.84 (d, *J* = 1.3 Hz, 1H, -NCH), 7.74 (d, *J* = 1.2 Hz, 1H, -NCH), 7.50 (d, *J* = 2.7 Hz, 1H, ArH), 7.39 (dd, *J* = 9.1, 2.8 Hz, 1H, ArH), 6.96 (d, *J* = 9.2 Hz, 1H, ArH), 4.40 (q, *J* = 7.1 Hz, 2H, -COOC<u>H</u>$_2$CH$_3$), 3.33 (d, *J* = 7.5 Hz, 2H, -NCH$_3$C<u>H</u>$_2$CH(CH$_3$)$_2$), 3.18 (s, 3H, -NC<u>H</u>$_3$CH$_2$CH(CH$_3$)$_2$), 2.12-2.02 (m, 1H, -NCH$_3$CH$_2$C<u>H</u>(CH$_3$)$_2$), 1.41 (t, *J* = 7.1 Hz, 3H, -COOCH$_2$C<u>H</u>$_3$), 0.95 (d, *J* = 6.6 Hz, 6H, -NCH$_3$CH$_2$CH(C<u>H</u>$_3$)$_2$).

*4.1.2.2. ethyl 1-(3-cyano-4-(pyrrolidin-1-yl)phenyl)-1H-imidazole-4-carboxylate (**3b**).* White solid (yield: 43%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.82 (s, 1H, -NCH), 7.72 (s, 1H, -NCH), 7.46 (d, *J* = 2.7 Hz, 1H, ArH), 7.34 (dd, *J* = 9.2, 2.6 Hz, 1H, ArH), 6.73 (d, *J* = 9.2 Hz, 1H,

ArH), 4.40 (q, *J* = 7.1 Hz, 2H, -COOC$\underline{H}_2$CH$_3$), 3.67 (t, *J* = 6.5 Hz, 4H, -N(C$\underline{H}_2$)$_2$(CH$_2$)$_2$), 2.09-2.03 (m, 4H, -N(CH$_2$)$_2$(C$\underline{H}_2$)$_2$), 1.41 (t, *J* = 7.1 Hz, 3H, -COOCH$_2$C$\underline{H}_3$).

*4.1.2.3. ethyl 1-(3-cyano-4-(piperidin-1-yl)phenyl)-1H-imidazole-4-carboxylate (**3c**).* White solid (yield: 40%); ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.86 (d, *J* = 1.3 Hz, 1H, -NCH), 7.77 (d, *J* = 1.3 Hz, 1H, -NCH), 7.57 (d, *J* = 2.7 Hz, 1H, ArH), 7.48 (dd, *J* = 8.9, 2.7 Hz, 1H, ArH), 7.09 (d, *J* = 8.9 Hz, 1H, ArH), 4.41 (q, *J* = 7.1 Hz, 2H, -COOC$\underline{H}_2$CH$_3$), 3.28-3.23 (m, 4H, -N(C$\underline{H}_2$)$_2$(CH$_2$)$_2$CH$_2$), 1.83-1.77 (m, 4H, -N(CH$_2$)$_2$(CH$_2$)$_2$C$\underline{H}_2$), 1.65 (t, *J* = 11.8 Hz, 2H, -N(CH$_2$)$_2$(CH$_2$)$_2$C$\underline{H}_2$), 1.41 (t, *J* = 7.1 Hz, 3H, -COOCH$_2$C$\underline{H}_3$).

*4.1.2.4. ethyl 1-(3-cyano-4-(4-methylpiperidin-1-yl)phenyl)-1H-imidazole-4-carboxylate (**3d**).* White solid (yield: 47%); ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.86 (d, *J* = 1.4 Hz, 1H, -NCH), 7.77 (d, *J* = 1.4 Hz, 1H, -NCH), 7.57 (d, *J* = 2.7 Hz, 1H, ArH), 7.47 (dd, *J* = 8.9, 2.7 Hz, 1H, ArH), 7.09 (d, *J* = 8.9 Hz, 1H, ArH), 4.41 (q, *J* = 7.1 Hz, 2H, -COOC$\underline{H}_2$CH$_3$), 3.66 (d, *J* = 12.3 Hz, 2H, -N(C$\underline{H}_2$)$_4$CHCH$_3$), 2.89 (m, -N(C$\underline{H}_2$)$_4$CHCH$_3$), 1.81 (dd, *J* = 12.7, 2.0 Hz, 2H, -N(C$\underline{H}_2$)$_4$CHCH$_3$), 1.62-1.54 (m, 1H, -N(CH$_2$)$_4$C$\underline{H}$CH$_3$), 1.51-1.44 (m, 2H, -N(C$\underline{H}_2$)$_4$CHCH$_3$), 1.41 (t, *J* = 7.1 Hz, 3H, -COOCH$_2$C$\underline{H}_3$), 1.02 (d, *J* = 6.5 Hz, 3H, -N(CH$_2$)$_4$CHC$\underline{H}_3$).

*4.1.2.5. ethyl 1-(4-(azocan-1-yl)-3-cyanophenyl)-1H-imidazole-4-carboxylate (**3e**).* White solid (yield: 40%); ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.83 (d, *J* = 1.3 Hz, 1H, -NCH), 7.73 (d, *J* = 1.3 Hz, 1H, -NCH), 7.48 (d, *J* = 2.8 Hz, 1H, ArH), 7.35 (dd, *J* = 9.3, 2.8 Hz, 1H, ArH), 6.89 (d, *J* = 9.4 Hz, 1H, ArH), 4.40 (q, *J* = 7.1 Hz, 2H, -COOC$\underline{H}_2$CH$_3$), 3.82-3.75 (m, 4H, -N(C$\underline{H}_2$)$_7$), 1.91-1.81 (m, 4H, -N(C$\underline{H}_2$)$_7$), 1.68-1.55 (m, 6H, -N(C$\underline{H}_2$)$_7$), 1.41 (t, *J* = 7.1 Hz, 3H, -COOCH$_2$C$\underline{H}_3$).

*4.1.3. General procedure for the preparation of compounds **Ia–e***

THF/ethanol (1:1, 10 mL) was added to **3a**–**e** (1.0 mmol), and the resulting mixture was maintained at 70 °C for 2 h. After completion of the reaction, the mixture was diluted with H_2O (100 mL), acidified to pH 2 with 1 M HCl solution, and stirred in an ice bath for 30 min. The resulting solid was filtered, washed with water, and then purified by recrystallization from methanol to yield the desired product.

*4.1.3.1. 1-(3-cyano-4-(isobutyl(methyl)amino)phenyl)-1H-imidazole-4-carboxylic acid (**Ia**).* White solid (yield: 95%); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.35 (d, *J* = 1.3 Hz, 1H, -NCH), 8.28 (d, *J* = 1.3 Hz, 1H, -NCH), 8.02 (d, *J* = 2.8 Hz, 1H, ArH), 7.83 (dd, *J* = 9.2, 2.8 Hz, 1H, ArH), 7.17 (d, *J* = 9.3 Hz, 1H, ArH), 3.28 (d, *J* = 7.5 Hz, 2H, -$NCH_3C\underline{H}_2CH(CH_3)_2$), 3.06 (s, 3H, -$NC\underline{H}_3CH_2CH(CH_3)_2$), 1.99 (m, 1H, -$NCH_3CH_2C\underline{H}(CH_3)_2$), 0.87 (d, *J* = 6.6 Hz, 6H, -$NCH_3CH_2CH(C\underline{H}_3)_2$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 163.90, 153.28, 137.13, 134.80, 127.77, 127.47, 127.26, 124.51, 119.33, 119.22, 99.64, 61.50, 41.91, 27.01, 20.21. HRMS(ESI): Calcd for $C_{16}H_{18}N_4O_2$ $[M+H]^+$ 299.1508, Found 299.1494.

*4.1.3.2. 1-(3-cyano-4-(pyrrolidin-1-yl)phenyl)-1H-imidazole-4-carboxylic acid (**Ib**).* White solid (yield: 97%); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.31 (d, *J* = 1.3 Hz, 1H, -NCH), 8.25 (d, *J* = 1.3 Hz, 1H, -NCH), 7.92 (d, *J* = 2.8 Hz, 1H, ArH), 7.75 (dd, *J* = 9.3, 2.8 Hz, 1H, ArH), 6.87 (d, *J* = 9.3 Hz, 1H, ArH), 3.56 (t, *J* = 6.5 Hz, 4H, -$N(C\underline{H}_2)_2(CH_2)_2$), 1.98-1.94 (m, 4H, -$N(CH_2)_2(C\underline{H}_2)_2$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 163.92, 149.39, 137.04, 134.65, 127.64, 127.44, 125.73, 124.50, 120.32, 116.30, 93.79, 50.28, 25.70. HRMS(ESI): Calcd for $C_{15}H_{14}N_4O_2$ $[M+H]^+$ 283.1195, Found 283.1179.

*4.1.3.3. 1-(3-cyano-4-(piperidin-1-yl)phenyl)-1H-imidazole-4-carboxylic acid (**Ic**).* White solid (yield: 95%); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.39 (s, 1H, -NCH), 8.33 (s, 1H, -NCH), 8.16 (d, *J* = 2.7 Hz, 1H, ArH), 7.93 (dd, *J* = 8.9, 2.7 Hz, 1H, ArH), 7.26 (d, *J* = 9.0 Hz, 1H, ArH), 3.17-3.15 (m, 4H, -$N(C\underline{H}_2)_2(CH_2)_2CH_2$), 1.72 -1.67 (m, 4H, -$N(CH_2)_2(C\underline{H}_2)_2CH_2$), 1.57

(dt, *J* = 11.4, 5.8 Hz, 2H, -N(CH_2)$_2$(CH_2)$_2$C<u>H_2</u>). ^{13}C NMR (151 MHz, DMSO-d_6) δ 163.85, 155.69, 137.17, 134.95, 130.08, 127.21, 126.82, 124.50, 120.69, 117.91, 105.57, 52.93, 26.08, 23.85. HRMS(ESI): Calcd for $C_{16}H_{16}N_4O_2$ $[M+H]^+$ 297.1352, Found 297.1337.

*4.1.3.4. 1-(3-cyano-4-(4-methylpiperidin-1-yl)phenyl)-1H-imidazole-4-carboxylic acid (**Id**).* White solid (yield: 95%); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.39 (d, *J* = 1.2 Hz, 1H, -NCH), 8.32 (d, *J* = 1.2 Hz, 1H, -NCH), 8.15 (d, *J* = 2.3 Hz, 1H, ArH), 7.93 (dd, *J* = 9.0, 2.7 Hz, 1H, ArH), 7.26 (d, *J* = 9.0 Hz, 1H, ArH), 3.52 (d, *J* = 12.2 Hz, 2H, -N(C<u>H_2</u>)$_4$CHCH$_3$), 2.84 (dd, *J* = 11.9, 10.3 Hz, 2H, -N(C<u>H_2</u>)$_4$CHCH$_3$, 1.76 (d, *J* = 10.9 Hz, 2H, -N(C<u>H_2</u>)$_4$CHCH$_3$), 1.58-1.50 (m, 1H, -N(CH_2)$_4$C<u>H</u>CH$_3$), 1.32 (m, 2H, -N(C<u>H_2</u>)$_4$CHCH$_3$, 0.97 (d, *J* = 6.5 Hz, 3H, -N(CH_2)$_4$CHC<u>H_3</u>). ^{13}C NMR (151 MHz, DMSO-d_6) δ 163.86, 155.46, 137.16, 134.96, 130.05, 127.19, 126.80, 124.48, 120.73, 117.92, 105.49, 52.21, 34.35, 30.23, 22.10. HRMS(ESI): Calcd for $C_{17}H_{18}N_4O_2$ $[M+Na]^+$ 333.1327, Found 333.1316.

*4.1.3.5. 1-(4-(azocan-1-yl)-3-cyanophenyl)-1H-imidazole-4-carboxylic acid (**Ie**).* White solid (yield: 98%); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.32 (d, *J* = 1.1 Hz, 1H, -NCH), 8.26 (d, *J* = 1.2 Hz, 1H, -NCH), 7.93 (d, *J* = 2.9 Hz, 1H, ArH), 7.75 (dd, *J* = 9.4, 2.9 Hz, 1H, ArH), 7.07 (d, *J* = 9.5 Hz, 1H, ArH), 3.76-3.71 (m, 4H, -N(C<u>H_2</u>)$_2$(CH_2)$_2$(CH_2)$_3$), 1.77-1.73 (m, 4H, -N(CH_2)$_2$(C<u>H_2</u>)$_2$(CH_2)$_3$), 1.59-1.46 (m, 6H,-N(CH_2)$_2$(CH_2)$_2$(C<u>H_2</u>)$_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 164.00, 149.60, 137.08, 134.75, 128.38, 127.63, 125.90, 124.46, 120.16, 117.23, 94.44, 52.54, 27.33, 26.81, 24.82. HRMS(ESI): Calcd for $C_{18}H_{20}N_4O_2$ $[M+H]^+$ 325.1665, Found 325.1661.

*4.1.4. General procedure for the preparation of compounds **5a**–**i***

K_2CO_3 (15.3 mmol) was added to compound **4** (5.1 mmol) in DMF and stirred at room temperature (rt) for 1 h. Alkyl halide (15.3 mmol) was added to the mixture and heated at 60–100 °C for 3–10 h. Subsequently, the mixture was cooled to rt, diluted with H_2O (150 mL), and extracted with ethyl acetate (150 mL × 2). The organic phases were combined, washed with H_2O (200 mL × 2) and brine (200 mL × 2), dried over anhydrous Na_2SO_4, evaporated under vacuum, and purified by flash column chromatography (0–10% ethyl acetate in petroleum ether).

*4.1.4.1. 5-bromo-2-ethoxy-benzonitrile (**5a**).* White solid (yield: 97%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, *J* = 2.4 Hz, 1H, ArH), 7.60 (dd, *J* = 9.0, 2.5 Hz, 1H, ArH), 6.85 (d, *J* = 9.0 Hz, 1H, ArH), 4.14 (q, *J* = 7.0 Hz, 2H, -OC$\underline{H}_2$CH$_3$), 1.48 (t, *J* = 7.0 Hz, 3H, -OCH$_2$C$\underline{H}_3$).

*4.1.4.2. 5-bromo-2-isopropoxy-benzonitrile (**5b**).* White solid (yield: 85%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66-7.56 (m, 2H, ArH), 6.85 (d, *J* = 9.0 Hz, 1H, ArH), 4.67-4.57 (m, 1H, -OC$\underline{H}$(CH$_3$)$_2$), 1.40 (d, *J* = 6.1 Hz, 6H, -OCH(C$\underline{H}_3$)$_2$).

*4.1.4.3. 5-bromo-2-isobutoxy-benzonitrile (**5c**).* Colourless oil (yield: 92%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, *J* = 2.4 Hz, 1H, ArH), 7.59 (dd, *J* = 9.0, 2.5 Hz, 1H, ArH), 6.84 (d, *J* = 9.0 Hz, 1H, ArH), 3.81 (d, *J* = 6.5 Hz, 2H, -OC$\underline{H}_2$CH(CH$_3$)$_2$), 2.23-2.09 (m, 1H, -OCH$_2$C$\underline{H}$(CH$_3$)$_2$), 1.06 (d, *J* = 6.7 Hz, 6H, -OCH$_2$CH(C$\underline{H}_3$)$_2$).

*4.1.4.4. 5-bromo-2-(sec-butoxy)-benzonitrile (**5d**).* Colourless oil (yield: 87%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66-7.56 (m, 2H, ArH), 6.84 (d, *J* = 9.0 Hz, 1H, ArH), 4.43-4.34 (m, 1H, -OC$\underline{H}$CH$_3$CH$_2$CH$_3$), 1.88-1.64 (m, 1H, -OCHCH$_3$C$\underline{H}_2$CH$_3$), 1.35 (d, *J* = 6.1 Hz, 2H, -OCHC$\underline{H}_3$CH$_2$CH$_3$), 1.00 (t, *J* = 7.4 Hz, 2H, -OCHCH$_3$CH$_2$C$\underline{H}_3$).

*4.1.4.5. 5-bromo-2-(neopentyloxy)-benzonitrile (**5e**).* White solid (yield: 90%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (d, 1H, *J* = 2.5 Hz, ArH), 7.58 (dd, 1H, *J* = 8.9 and 2.5 Hz, ArH), 6.83 (d, 1H, *J* = 8.9 Hz, ArH), 3.69 (s, 2H, -OC$\underline{H}_2$C$(CH_3)_3$), 1.07 (s, 9H, -OCH$_2$C(C$\underline{H}_3)_3$).

*4.1.4.6. 5-bromo-2-(pentan-3-yloxy)-benzonitrile (**5f**).* Colourless oil (yield: 96%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66-7.54 (m, 2H, ArH), 6.84 (d, *J* = 9.0 Hz, 1H, ArH), 4.21 (p, *J* = 5.8 Hz, 1H, -OC$\underline{H}(CH_2)_2(CH_3)_2$), 1.79-1.69 (m, 4H, -OCH(C$\underline{H}_2)_2(CH_3)_2$), 0.98 (t, *J* = 7.4 Hz, 6H, -OCH$(CH_2)_2$(C$\underline{H}_3)_2$).

*4.1.4.7. 5-bromo-2-(2-ethylbutoxy)-benzonitrile (**5g**).* Colourless oil (yield: 96%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.64 (d, *J* = 2.5 Hz, 1H, ArH), 7.60 (dd, *J* = 9.0, 2.5 Hz, 1H, ArH), 6.86 (d, *J* = 9.0 Hz, 1H, ArH), 3.94 (d, *J* = 5.6 Hz, 2H, -OC$\underline{H}_2$CH$(CH_2)_2(CH_3)_2$), 1.73 (m, 1H, -OCH$_2$C$\underline{H}(CH_2)_2(CH_3)_2$), 1.55-1.46 (m, 4H, -OCH$_2$CH(C$\underline{H}_2)_2(CH_3)_2$), 0.94 (t, *J* = 7.5 Hz, 6H, -OCH$_2$CH$(CH_2)_2$(C$\underline{H}_3)_2$).

*4.1.4.8. 5-bromo-2-(cyclopentyloxy)-benzonitrile (**5h**).* Colourless oil (yield: 96%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 2.2 Hz, 1H, ArH), 8.18-8.14 (m, 1H, ArH), 7.07-7.04 (m, 1H, ArH), 5.01-4.92 (m, 1H, -OC$\underline{H}(CH_2)_4$), 4.35 (s, 2H, -OCH(C$\underline{H}_2)_4$), 2.05-1.81 (m, 6H, -OCH(C$\underline{H}_2)_4$).

*4.1.4.9. 5-bromo-2-(cyclohexyloxy)-benzonitrile (**5i**).* Colourless oil (yield: 92%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, *J* = 2.5 Hz, 1H, ArH), 7.57 (dd, *J* = 9.0, 2.5 Hz, 1H, ArH), 6.86 (d, *J* = 9.0 Hz, 1H, ArH), 4.42-4.32 (m, 1H, -OC$\underline{H}(CH_2)_5$), 1.98-1.76 (m, 4H, -OCH(C$\underline{H}_2)_5$), 1.67 (m, 2H, -OCH(C$\underline{H}_2)_5$), 1.56-1.33 (m, 4H, -OCH(C$\underline{H}_2)_5$).

*4.1.5. General procedure for the preparation of compounds **6a**–**i***

A mixture of compound **5a**–**i** (2.4 mmol), CuI (0.2 mmol), ethyl 1*H*-imidazole-4-carboxylate (2.0 mmol), *trans*-*N*,*N*-dimethyl-1,2-cyclohexanediamine (0.4 mmol), and K_2CO_3 (4.2 mmol) in DMF (5 mL) was stirred at 120 °C for 24 h under a nitrogen atmosphere. Subsequently, the reaction was diluted with H_2O (30 mL) and extracted with ethyl acetate (20 mL × 2). The organic phases were combined, washed with brine (30 mL × 3), dried over anhydrous Na_2SO_4, and concentrated under vacuum. The residue was purified by flash column chromatography (12–30% ethyl acetate in petroleum ether).

*4.1.5.1. ethyl 1-(3-cyano-4-ethoxyphenyl)-1H-imidazole-4-carboxylate (**6a**).* White solid (yield: 32%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90-7.54 (m, 4H, -NCH, ArH), 7.10 (d, *J* = 8.9 Hz, 1H, ArH), 4.46-4.18 (m, 4H, -COOC$\underline{H}_2$CH$_3$, -OC$\underline{H}_2$CH$_3$), 1.58-1.34 (m, 6H, -COOCH$_2$C$\underline{H}_3$, -OCH$_2$C$\underline{H}_3$).

*4.1.5.2. ethyl 1-(3-cyano-4-isopropoxyphenyl)-1H-imidazole-4-carboxylate (**6b**).* White solid (yield: 35%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, *J* = 1.4 Hz, 1H, -NCH), 7.77 (d, *J* = 1.4 Hz, 1H, -NCH), 7.63-7.53 (m, 2H, ArH), 7.10 (d, *J* = 9.1 Hz, 1H, ArH), 4.77-4.66 (m, 1H, -OC$\underline{H}$(CH$_3$)$_2$), 4.41 (q, *J* = 7.1 Hz, 2H, -COOC$\underline{H}_2$CH$_3$), 1.45 (d, *J* = 6.1 Hz, 6H, -OCH(C$\underline{H}_3$)$_2$), 1.41 (t, *J* = 7.1 Hz, 3H, -COOCH$_2$C$\underline{H}_3$).

*4.1.5.3. ethyl 1-(3-cyano-4-isobutoxyphenyl)-1H-imidazole-4-carboxylate (**6c**).* White solid (yield: 45%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, *J* = 1.3 Hz, 1H, -NCH), 7.77 (d, *J* = 1.3 Hz, 1H, -NCH), 7.62 (d, *J* = 2.7 Hz, 1H, ArH), 7.56 (dd, *J* = 9.0, 2.8 Hz, 1H, ArH), 7.09 (d, *J* = 9.0 Hz, 1H, ArH), 4.41 (q, *J* = 7.1 Hz, 2H, -COOC$\underline{H}_2$CH$_3$), 3.90 (d, *J* = 6.5 Hz, 2H, -OC$\underline{H}_2$CH(CH$_3$)$_2$), 2.28-2.14 (m, 1H, -OCH$_2$C$\underline{H}$(CH$_3$)$_2$), 1.41 (t, *J* = 7.1 Hz, 3H, -COOCH$_2$C$\underline{H}_3$), 1.10 (d, *J* = 6.7 Hz, 6H, -OCH$_2$CH(C$\underline{H}_3$)$_2$).

*4.1.5.4. ethyl 1-(4-(sec-butoxy)-3-cyanophenyl)-1H-imidazole-4-carboxylate (**6d**).* White solid (yield: 36%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, *J* = 1.4 Hz, 1H, -NCH), 7.77 (d, *J*

= 1.4 Hz, 1H, -NCH), 7.61 (d, *J* = 2.7 Hz, 1H, ArH), 7.55 (dd, *J* = 9.0, 2.8 Hz, 1H, ArH), 7.09 (d, *J* = 9.1 Hz, 1H, ArH), 4.53-4.44 (m, 1H, -OC<u>H</u>$(CH_3)CH_2CH_3$), 4.41 (q, *J* = 7.1 Hz, 2H, -COOC<u>H_2</u>CH_3), 1.92-1.71 (m, 2H, -OCH(CH_3)C<u>H_2</u>CH_3), 1.41 (dd, *J* = 10.0, 4.2 Hz, 6H, -OCH(C<u>H_3</u>$)CH_2CH_3$, -COOCH_2C<u>H_3</u>), 1.04 (t, *J* = 7.4 Hz, 3H, -OCH$(CH_3)CH_2$C<u>H_3</u>).

*4.1.5.5. ethyl 1-(3-cyano-4-(neopentyloxy)phenyl)-1H-imidazole-4-carboxylate (**6e**).* White solid (yield: 34%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.87 (d, *J* = 1.4 Hz, 1H, -NCH), 7.77 (d, *J* = 1.4 Hz, 1H, -NCH), 7.62 (d, *J* = 2.7 Hz, 1H, ArH), 7.56 (dd, *J* = 9.0, 2.8 Hz, 1H, ArH), 7.09 (d, *J* = 9.0 Hz, 1H, ArH), 4.41 (q, *J* = 7.1 Hz, 2H, -COOC<u>H_2</u>CH_3), 3.76 (s, 2H, -OC<u>H_2</u>$C(CH_3)_3$), 1.41 (t, *J* = 7.1 Hz, 3H, -COOC<u>H_2</u>CH_3), 1.12 (s, 9H, -OCH_2C(C<u>H_3</u>$)_3$).

*4.1.5.6. ethyl 1-(3-cyano-4-(pentan-3-yloxy)phenyl)-1H-imidazole-4-carboxylate (**6f**).* White solid (yield: 38%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.86 (d, *J* = 1.3 Hz, 1H, -NCH), 7.77 (d, *J* = 1.2 Hz, 1H, -NCH), 7.61 (d, *J* = 2.7 Hz, 1H, ArH), 7.56 (dd, *J* = 9.0, 2.8 Hz, 1H, ArH), 7.11 (d, *J* = 9.0 Hz, 1H, ArH), 4.41 (q, *J* = 7.1 Hz, 2H, -COOC<u>H_2</u>CH_3), 4.04 (d, *J* = 5.6 Hz, 2H), 1.78 (m, 1H, -OC<u>H</u>$(CH_2)_2(CH_3)_2$), 1.58-1.48 (m, 4H, -OCH(C<u>H_2</u>$)_2(CH_3)_2$), 1.41 (t, *J* = 7.1 Hz, 3H, -COOCH_2C<u>H_3</u>), 0.97 (t, *J* = 7.5 Hz, 6H, -OCH$(CH_2)_2$(C<u>H_3</u>$)_2$).

*4.1.5.7. ethyl 1-(3-cyano-4-(2-ethylbutoxy)phenyl)-1H-imidazole-4-carboxylate (**6g**).* White solid (yield: 42%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.86 (d, *J* = 1.3 Hz, 1H, -NCH), 7.77 (d, *J* = 1.2 Hz, 1H, -NCH), 7.61 (d, *J* = 2.7 Hz, 1H, ArH), 7.56 (dd, *J* = 9.0, 2.8 Hz, 1H, ArH), 7.11 (d, *J* = 9.0 Hz, 1H, ArH), 4.41 (q, *J* = 7.1 Hz, 2H, -COOC<u>H_2</u>CH_3), 4.04 (d, *J* = 5.6 Hz, 2H, -OC<u>H_2</u>$CH(CH_2)_2(CH_3)_2$), 1.78 (m, 1H, -OCH_2C<u>H</u>$(CH_2)_2(CH_3)_2$), 1.57-1.49 (m, 4H, -OCH_2CH(C<u>H_2</u>$)_2(CH_3)_2$), 1.41 (t, *J* = 7.1 Hz, 3H, -COOC<u>H_2</u>CH_3), 0.97 (t, *J* = 7.5 Hz, 6H, -$OCH_2CH(CH_2)_2$(C<u>H_3</u>$)_2$).

*4.1.5.8. ethyl 1-(3-cyano-4-(cyclopentyloxy)phenyl)-1H-imidazole-4-carboxylate (**6h**).* White solid (yield: 42%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (s, 1H, -NCH), 7.78 (s, 1H, -NCH),

7.62-7.51 (m, 2H, ArH), 7.11 (d, *J* = 9.0 Hz, 1H, ArH), 4.97-4.86 (m, 1H, -OC<u>H</u>$(CH_2)_4$), 4.42 (q, *J* = 7.1 Hz, 2H, -COOC<u>H_2</u>CH_3), 2.02-1.84 (m, 6H, -OCH(C<u>H_2</u>$)_4$), 1.75-1.69 (m, 2H, -OCH(C<u>H_2</u>$)_4$), 1.42 (t, *J* = 7.1 Hz, 3H, -COOC<u>H_2</u>CH_3).

*4.1.5.9. ethyl 1-(3-cyano-4-(cyclohexyloxy)phenyl)-1H-imidazole-4-carboxylate (**6i**).* White solid (yield: 45%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, *J* = 1.3 Hz, 1H, -NCH), 7.77 (d, *J* = 1.2 Hz, 1H, -NCH), 7.61 (d, *J* = 2.7 Hz, 2H, ArH), 7.54 (dd, *J* = 9.0, 2.8 Hz, 1H, ArH), 7.11 (d, *J* = 8.8 Hz, 1H, ArH), 4.48 (m, 1H, -OC<u>H</u>$(CH_2)_5$), 4.41 (q, *J* = 7.1 Hz, 2H, -COOC<u>H_2</u>CH_3), 2.02-1.70 (m, 2H, -OCH$(CH_2)_5$), 1.59-1.37 (m, 7H, -OCH(C<u>H_2</u>$)_5$, -COOCH$_2$C<u>H_3</u>).

*4.1.6. General procedure for the preparation of compounds **IIa–i***

THF/ethanol (1:1, 10 mL) was added to **6a**–**i** (1.0 mmol), and the resulting mixture was maintained at 70 °C for 2 h. After completion of the reaction, the mixture was diluted with H_2O (100 mL) and acidified to pH 2 with 1 M HCl solution, and the resulting suspension was stirred in an ice bath for 30 min. The resulting solid was filtered, washed with water, and purified by recrystallization from methanol to yield the desired product.

*4.1.6.1. 1-(3-cyano-4-ethoxyphenyl)-1H-imidazole-4-carboxylic acid (**IIa**).* White solid (yield: 95%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.63 (s, 1H, -NCH), 8.52 (s, 1H, -NCH), 8.25 (d, *J* = 2.7 Hz, 1H, ArH), 8.06 (dd, *J* = 9.2, 2.8 Hz, 1H, ArH), δ 7.42 (d, *J* = 9.2 Hz, 1H, ArH), 4.27 (q, *J* = 6.9 Hz, 2H, -OC<u>H_2</u>CH_3), 1.40 (t, *J* = 6.9 Hz, 3H, -OCH$_2$C<u>H_3</u>). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.10, 163.92, 156.65, 137.35, 134.79, 129.32, 125.79, 124.79, 123.67, 123.40, 115.17, 65.04, 14.98. HRMS(ESI): Calcd for $C_{13}H_{11}N_3O_3$ $[M+Na]^+$ 280.0698, Found 280.0691.

*4.1.6.2. 1-(3-cyano-4-isopropoxyphenyl)-1H-imidazole-4-carboxylic acid (**IIb**).* White solid (yield: 90%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (d, *J* = 1.4 Hz, 1H, -NCH), 8.33 (d, *J* = 1.4 Hz, 1H, -NCH), 8.21 (d, *J* = 2.8 Hz, 1H, ArH), 8.01 (dd, *J* = 9.2, 2.9 Hz, 1H, ArH), 7.44 (d, *J* = 9.3 Hz, 1H, ArH), 4.92-4.83 (m, 1H, -OC<u>H</u>$(CH_3)_2$), 1.34 (d, *J* = 6.0 Hz, 6H, -OCH(C<u>H</u>$_3)_2$). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.88, 158.91, 137.27, 134.95, 129.81, 128.12, 126.66, 124.66, 116.10, 115.92, 102.70, 72.55, 22.04. HRMS(ESI): Calcd for $C_{14}H_{13}N_3O_3$ $[M+H]^+$ 272.1035, Found 272.1025.

*4.1.6.3. 1-(3-cyano-4-isobutoxyphenyl)-1H-imidazole-4-carboxylic acid (**IIc**).* White solid (yield: 100%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (s, 1H, -NCH), 8.33 (s, 1H, -NCH), 8.22 (d, *J* = 2.6 Hz, 1H, ArH), 8.03 (dd, *J* = 9.1, 2.6 Hz, 1H, ArH), 7.40 (d, *J* = 9.2 Hz, 1H, ArH), 3.97 (dd, *J* = 13.3, 6.5 Hz, 2H, -OC<u>H</u>$_2$CH$(CH_3)_2$), 2.08 (m, 1H, -OCH$_2$C<u>H</u>$(CH_3)_2$), 1.02 (d, *J* = 6.7 Hz, 6H, -OCH$_2$CH(C<u>H</u>$_3)_2$). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.87, 159.91, 137.25, 134.95, 129.93, 128.14, 126.44, 124.65, 115.87, 114.82, 101.85, 75.67, 28.06, 19.20. HRMS(ESI): Calcd for $C_{15}H_{15}N_3O_3$ $[M+Na]^+$ 308.1011, Found 308.1017.

*4.1.6.4. 1-(4-(sec-butoxy)-3-cyanophenyl)-1H-imidazole-4-carboxylic acid (**IId**).* White solid (yield: 94%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (s, 1H, -NCH), 8.33 (s, 1H, -NCH), 8.20 (d, *J* = 2.8 Hz, 1H, ArH), 8.01 (dd, *J* = 9.2, 2.8 Hz, 1H, ArH), 7.44 (d, *J* = 9.3 Hz, 1H, ArH), 4.72-4.64 (m, 1H, -OC<u>H</u>$(CH_3)CH_2CH_3$), 1.77-1.61 (m, 2H, -OCH(CH_3)C<u>H</u>$_2CH_3$), 1.31 (d, *J* = 6.1 Hz, 3H, -OCH(C<u>H</u>$_3)$C<u>H</u>$_2CH_3$), 0.96 (t, *J* = 7.4 Hz, 3H, -OCH$(CH_3)CH_2$C<u>H</u>$_3$). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.85, 159.24, 137.30, 134.89, 129.78, 128.18, 126.71, 124.69, 116.04, 115.86, 102.65, 77.18, 28.82, 19.27, 9.63. HRMS(ESI): Calcd for $C_{15}H_{15}N_3O_3$ $[M+Na]^+$ 308.1011, Found 308.1013.

*4.1.6.5. 1-(3-cyano-4-(neopentyloxy)phenyl)-1H-imidazole-4-carboxylic acid (**IIe**).* White solid (yield: 92%); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.40 (d, *J* = 1.3 Hz, 1H, -NCH), 8.33 (d,

J = 1.3 Hz, 1H, -NCH), 8.22 (d, *J* = 2.8 Hz, 1H, ArH), 8.03 (dd, *J* = 9.1, 2.8 Hz, 1H, ArH), 7.39 (d, *J* = 9.2 Hz, 1H, ArH), 3.87 (s, 2H, -OC$\underline{H}_2$C(CH_3)$_3$), 1.04 (s, 9H, -OCH_2C(C$\underline{H}_3$)$_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 163.85, 160.21, 137.24, 134.98, 129.94, 128.14, 126.37, 124.64, 115.77, 114.81, 101.89, 79.16, 32.24, 26.89, 26.57. HRMS(ESI): Calcd for $C_{16}H_{17}N_3O_3$ $[M+H]^+$ 300.1348, Found 300.1344.

*4.1.6.6. 1-(3-cyano-4-(pentanyloxy)phenyl)-1H-imidazole-4-carboxylic acid (**IIf**).* White solid (yield: 96%); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.56 (s, 1H, -NCH), δ 8.48 (s, 1H, -NCH), 8.22 (d, *J* = 2.9 Hz, 1H, ArH), 8.01 (dd, *J* = 9.2, 2.9 Hz, 1H, ArH), 7.46 (d, *J* = 9.3 Hz, 1H, ArH), 4.58-4.52 (m, 1H, -OC$\underline{H}$(CH_2CH_3)$_2$), 1.74-1.62 (m, 4H, -OCH(C$\underline{H}_2$$CH_3$)$_2$), 0.94 (t, *J* = 7.4 Hz, 6H, -OCH(CH_2C$\underline{H}_3$)$_2$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 163.23, 159.96, 137.38, 133.74, 129.49, 128.46, 127.02, 124.86, 115.95, 115.87, 102.60, 81.98, 25.88, 9.50. HRMS(ESI): Calcd for $C_{16}H_{17}N_3O_3$ $[M+Na]^+$ 322.1168, Found 322.1158.

*4.1.6.7. 1-(3-cyano-4-(2-ethylbutoxy)phenyl)-1H-imidazole-4-carboxylic acid (**IIg**).* White solid (yield: 98%); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.40 (d, *J* = 1.3 Hz, 1H, -NCH), 8.33 (d, *J* = 1.3 Hz, 1H, -NCH), 8.21 (d, *J* = 2.8 Hz, 1H, ArH), 8.03 (dd, *J* = 9.1, 2.8 Hz, 1H, ArH), 7.43 (d, *J* = 9.2 Hz, 1H, ArH), 4.10 (d, *J* = 5.7 Hz, 2H, -OC$\underline{H}_2$ CH(C_2H_5)$_2$), 1.68 (m, 1H, -OCH_2C$\underline{H}$(C_2H_5)$_2$), 1.52-1.39 (m, 4H, -OCH_2CH(C$\underline{H}_2$$CH_3$)$_2$), 0.92 (t, *J* = 7.5 Hz, 6H, -OCH_2CH(CH_2C$\underline{H}_3$)$_2$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 163.87, 160.05, 137.24, 135.04, 129.95, 128.16, 126.46, 124.63, 115.83, 114.80, 101.89, 71.92, 23.26, 11.39. HRMS(ESI): Calcd for $C_{17}H_{19}N_3O_3$ $[M+Na]^+$ 336.1324, Found 336.1307.

*4.1.6.8. 1-(3-cyano-4-(cyclopentyloxy)phenyl)-1H-imidazole-4-carboxylic acid (**IIh**).* White solid (yield: 95%); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.40 (s, 1H, -NCH), 8.33 (s, 1H, -NCH), 8.20 (d, *J* = 2.7 Hz, 1H, ArH), 8.01 (dd, *J* = 9.1, 2.7 Hz, 1H, ArH), 7.40 (d, *J* = 9.2 Hz, 1H, ArH), 5.11-5.04 (m, 1H, -OC$\underline{H}$(CH_2)$_4$), 1.97 (dd, *J* = 12.7, 7.2 Hz, 2H, -OCH(C$\underline{H}_2$)$_4$), 1.84-

1.58 (m, 6H, -OCH(C<u>H</u>$_2$)$_4$). ^{13}C NMR (151 MHz, DMSO-d$_6$) δ 163.88, 158.94, 137.28, 134.93, 129.79, 128.04, 126.63, 124.67, 116.00, 115.89, 102.56, 81.65, 32.66, 32.60, 23.97, 23.93. HRMS(ESI): Calcd for $C_{16}H_{15}N_3O_3$ $[M+Na]^+$ 320.1011, Found 320.1003.

*4.1.6.9. 1-(3-cyano-4-(cyclohexyloxy)phenyl)-1H-imidazole-4-carboxylic acid (**IIi**).* White solid (yield: 95%); ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.40 (s, 1H, -NCH), 8.34 (s, 1H, -NCH), 8.20 (d, *J* = 2.7 Hz, 1H, ArH), 8.00 (dd, *J* = 9.1, 2.7 Hz, 1H, ArH), 7.46 (d, *J* = 9.3 Hz, 1H, ArH), 4.74-4.55 (m, 1H, -OC<u>H</u>(CH$_2$)$_5$), 1.96-1.65 (m, 4H, -OCH(C<u>H</u>$_2$)$_5$), 1.60-1.35 (m, 6H, -OCH(C<u>H</u>$_2$)$_5$). ^{13}C NMR (151 MHz, DMSO-d$_6$) δ 163.86, 158.74, 137.31, 134.88, 129.82, 128.09, 126.63, 124.70, 116.19, 116.07, 102.79, 76.62, 31.14, 25.38, 23.02. HRMS(ESI): Calcd for $C_{17}H_{17}N_3O_3$ $[M+Na]^+$ 312.1348, Found 312.1339.

*4.1.7. Synthesis of 1-(4-hydroxy-3-iodophenyl)ethanone **8***

Compound **7** (2.0 g, 14.7 mmol) was dissolved in $NH_3 \cdot H_2O$ and added to a solution of KI (12 g, 73.5 mmol) and I_2 (3.7 g, 14.7 mmol) in H_2O (200 mL). The reaction mixture was stirred at rt overnight and then filtered. The filtrate was acidified to pH 2.0–3.0 with HCl to obtain a yellow precipitate, which was purified by recrystallization from methanol:H_2O (7:3). Yellow solid (yield: 81%), ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H, -OH), 7.90 (d, *J* = 8.3 Hz, 1H, ArH), 7.06 (d, *J* = 8.5 Hz, 1H, ArH), 5.99 (s, 1H, ArH), 2.57 (s, 3H, -C=OC<u>H</u>$_3$).

*4.1.8. General procedure for the preparation of compounds **9a–d***

K_2CO_3 (15.3 mmol) was added to compound **8** (5.1 mmol) in DMF and stirred at rt for 1 h. Alkyl halide (15.3 mmol) was added to the mixture and heated at 70–80 °C for 3–5 h. After completion of the reaction, the mixture was cooled to rt, diluted with H_2O (150 mL), and

extracted with ethyl acetate (150 mL × 2). The organic phases were combined, washed with H_2O (200 mL × 2) and brine (200 mL × 2), dried over anhydrous Na_2SO_4, evaporated under vacuum, and purified by flash column chromatography (0–10% ethyl acetate in petroleum ether).

*4.1.8.1. 1-(4-ethoxy-3-iodophenyl)-ethanone (**9a**).* Colourless oil (yield: 86.4%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H, ArH), 7.93 (d, *J* = 8.6 Hz, 1H, ArH), 6.81 (d, *J* = 8.6 Hz, 1H, ArH), 4.17 (q, *J* = 6.9 Hz, 2H, -OC<u>H</u>$_2$CH$_3$), 2.55 (s, 3H, -C=OC<u>H</u>$_3$), 1.52 (t, *J* = 7.0 Hz, 3H, -OCH$_2$C<u>H</u>$_3$).

*4.1.8.2. 1-(3-iodo-4-isopropoxyphenyl)-ethanone (**9b**).* Colourless oil (yield: 85%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H, ArH), 7.91 (d, *J* = 8.6 Hz, 1H, ArH), 6.82 (d, *J* = 8.6 Hz, 1H, ArH), 4.78-4.57 (m, 1H, -OC<u>H</u>(CH$_3$)$_2$), 2.54 (s, 3H, -C=OC<u>H</u>$_3$), 1.42 (d, *J* = 5.8 Hz, 6H, -OCH(C<u>H</u>$_3$)$_2$).

*4.1.8.3. 1-(3-iodo-4-isobutoxyphenyl)-ethanone (**9c**).* Colourless oil (yield: 82%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, 1H, ArH), 7.92 (d, *J* = 8.6 Hz, 1H, ArH), 6.79 (d, *J* = 8.5 Hz, 1H, ArH), 3.85 (d, *J* = 6.1 Hz, 2H, -OC<u>H</u>$_2$CH(CH$_3$)$_2$), 2.54 (s, 3H, -C=OC<u>H</u>$_3$), 2.18 (m, 1H, -OCH$_2$C<u>H</u>(CH$_3$)$_2$), 1.10 (d, *J* = 6.6 Hz, 6H, -OCH$_2$CH(C<u>H</u>$_3$)$_2$).

*4.1.8.4. 1-(4-(cyclopentyloxy)-3-iodophenyl)-ethanone (**9d**).* Colourless oil (yield: 84%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (d, *J* = 2.2 Hz, 1H, ArH), 7.93 (dd, *J* = 8.6, 2.2 Hz, 1H, ArH), 6.84 (d, *J* = 8.7 Hz, 1H, ArH), 4.94-4.88 (m, 1H, -OC<u>H</u>(CH$_2$)$_4$), 2.56 (s, 3H, -C=OC<u>H</u>$_3$), 1.99-1.87 (m, 6H, -OCH(C<u>H</u>$_2$)$_4$), 1.72-1.66 (m, 2H, -OCH(C<u>H</u>$_2$)$_4$).

*4.1.9. General procedure for the preparation of compounds **10a–d***

CuCN (30.6 mmol) was added to compound **9a**–**d** (19.1 mmol) in DMF (20 mL) and stirred at 140 °C for 7 h. After completion of the reaction, the mixture was cooled to rt and diluted with H_2O. The precipitate was collected via filtration and dried to afford a yellow-brown solid. The yellow-brown solid was then dissolved in hot $CHCl_3$, any insoluble solid was filtered out, and the filtrate was concentrated to remove the solvent. The residue was purified by recrystallization from methanol:H_2O (7:3).

*4.1.9.1. 5-acetyl-2-ethoxy-benzonitrile (**10a**).* Yellow-green solid (yield: 59.8%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H, ArH), 8.15 (d, *J* = 9.1 Hz, 1H, ArH), 7.02 (d, *J* = 8.8 Hz, 1H, ArH), 4.25 (q, *J* = 6.9 Hz, 2H, -OC<u>H</u>$_2$CH$_3$), 2.58 (s, 3H, -C=OC<u>H</u>$_3$), 1.53 (t, *J* = 6.9 Hz, 3H, -OCH$_2$C<u>H</u>$_3$).

*4.1.9.2. 5-acetyl-2-isopropoxybenzonitrile (**10b**).* Yellow-green solid (yield: 58.6%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, *J* = 2.2 Hz, 1H, ArH), 8.13 (dd, *J* = 8.9, 2.2 Hz, 1H, ArH), 7.02 (d, *J* = 8.9 Hz, 1H, ArH), 4.81-4.72 (m, 1H, -OC<u>H</u>(CH$_3$)$_2$), 2.57 (s, 3H, -C=OC<u>H</u>$_3$), 1.45 (d, *J* = 6.1 Hz, 6H, -OCH(C<u>H</u>$_3$)$_2$).

*4.1.9.3. 5-acetyl-2-isobutoxy-benzonitrile (**10c**).*Yellow-green solid (yield: 63.5%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H, ArH), 8.14 (d, *J* = 8.8 Hz, 1H, ArH), 7.01 (d, *J* = 8.7 Hz, 1H, ArH), 3.92 (d, *J* = 6.3 Hz, 2H, -OC<u>H</u>$_2$CH(CH$_3$)$_2$), 2.57 (s, 3H, -C=OC<u>H</u>$_3$), 2.21 (m, 1H, -OCH$_2$C<u>H</u>(CH$_3$)$_2$), 1.09 (d, *J* = 6.4 Hz, 6H, -OCH$_2$CH(C<u>H</u>$_3$)$_2$).

*4.1.9.4. 5-acetyl-2-(cyclopentyloxy)-benzonitrile (**10d**).*Yellow-green solid (yield: 57.8%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18-8.10 (m, 2H, ArH), 7.02 (d, *J* = 8.9 Hz, 1H, ArH), 4.97-4.92 (m, 1H, -OC<u>H</u>(CH$_2$)$_4$), 2.57 (s, 3H, -C=OC<u>H</u>$_3$), 2.02-1.81 (m, 6H, -OCH(C<u>H</u>$_2$)$_4$), 1.73-1.64 (m, 1H, -OCH(C<u>H</u>$_2$)$_4$).

*4.1.10. General procedure for the preparation of compounds **11a–d***

Br_2 (13.7 mmol) was slowly added dropwise over 30 min into compound **10a**–**d** (10.6 mmol) in dioxane and stirred at rt for 2 h. After completion of the reaction, saturated Na_2SO_3 was poured into the reaction mixture and stirred for 10 min. The water phase was extracted with ethyl acetate (100 mL × 2). The combined organic phases were washed with H_2O (100 mL × 2) and brine (100 mL × 2), dried over anhydrous Na_2SO_4, evaporated under vacuum, *and purified by recrystallization from methanol:H2O (3:7).*

4.1.10.1. 5-(2-bromoacetyl)-2-ethoxy-benzonitrile (11a). Yellow solid (yield: 95%); 1H NMR (400 MHz, CDCl3) δ 8.43-8.16 (m, 2H, ArH), 7.11-7.00 (m, 1H, ArH), 4.36 (s, 2H, -COCH2Br), 4.29-4.23 (m, 2H, -OCH2CH3), 1.57-1.51 (m, 3H, -OCH2CH3).

4.1.10.2. 5-(2-bromoacetyl)-2-isopropoxy-benzonitrile (11b). Yellow solid (yield: 92%); 1H NMR (400 MHz, CDCl3) δ 8.23 (s, 1H, ArH), 8.19 (d, J = 9.0 Hz, 1H, ArH), 7.06 (d, J = 9.0 Hz, 1H, ArH), 4.80 (m, 1H, -OCH(CH3)2), 4.37 (s, 2H, -C=OCH2Br), 1.47 (t, J = 5.4 Hz, 6H, -OCH($C\underline{H}_3$)$_2$).

*4.1.10.3. 5-(2-bromoacetyl)-2-isobutoxy-benzonitrile (**11c**).* Yellow solid (yield: 95.6%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H, ArH), 8.18 (d, *J* = 8.7 Hz, 1H, ArH), 7.04 (d, *J* = 8.7 Hz, 1H, ArH), 4.36 (s, 2H, -C=OC$\underline{H}_2$Br), 3.94 (d, *J* = 6.0 Hz, 2H, -OC$\underline{H}_2$CH(CH_3)$_2$), 2.28-2.13 (m, 1H, -OCH$_2$C$\underline{H}$(CH_3)$_2$), 1.09 (d, *J* = 6.3 Hz, 6H, -OCH$_2$CH(C$\underline{H}_3$)$_2$).

*4.1.10.4. 5-(2-bromoacetyl)-2-(cyclopentyloxy)-benzonitrile (**11d**).* Yellow solid (yield: 95.7%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 2.2 Hz, 1H, ArH), 8.16 (dd, *J* = 8.9, 2.3 Hz, 1H, ArH), 7.05 (d, *J* = 8.9 Hz, 1H, ArH), 5.01-4.92 (m, 1H, -OC$\underline{H}$(CH_2)$_4$), 4.36 (s, 2H, -C=OC$\underline{H}_2$Br), 2.05-1.82 (m, 6H, -OCH(C$\underline{H}_2$)$_4$), 1.74-1.64 (m, 2H, -OCH(C$\underline{H}_2$)$_4$).

*4.1.11. General procedure for the preparation of compounds **12a–d***

Et_2O (30 mL) was added quickly into a mixture of hexamethylenetetramine (3.7 mmol) and compound **11a–d** (3.7 mmol) in $CHCl_3$ (15 mL) and stirred for 30 min under a nitrogen atmosphere. The resulting precipitate was collected by filtration, washed with Et_2O, and dried to produce a white solid. The product was dissolved in methanol, HCl (55.5 mmol) was added, and the resulting mixture was stirred for 15 min in an ice bath and then at rt for 3days. The insoluble solid was then removed by filtration, and the filtrate was concentrated to remove the solvent. The residue was treated with hot ethanol, the insoluble solid was removed by filtration, and the filtrate was evaporated under vacuum. The newly formed solid was then washed with cold ether to obtain the desired product.

*4.1.11.1. 5-(2-aminoacetyl)-2-ethoxy-benzonitrile hydrochloride (**12a**).* White solid (yield: 97.4%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.46 (s, 3H, ArH, -$CH_2N\underline{H}_2$), 8.28 (d, *J* = 9.0 Hz, 1H, ArH), 7.44 (d, *J* = 9.1 Hz, 1H, ArH), 4.57 (s, 2H, -$C\underline{H}_2NH_2$), 4.34 (q, *J* = 6.8 Hz, 2H, -$OC\underline{H}_2CH_3$), 1.41 (t, *J* = 6.9 Hz, 3H, -$OCH_2C\underline{H}_3$).

*4.1.11.2. 5-(2-aminoacetyl)-2-isopropoxy-benzonitrile hydrochloride (**12b**).* White solid (yield: 96.5%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (s, 1H, -$CH_2N\underline{H}_2$, ArH), 8.25 (d, *J* = 9.0 Hz, 1H, ArH), 7.48 (d, *J* = 9.6 Hz, 1H, ArH), 4.99 (m, 1H, -$OC\underline{H}(CH_3)_2$), 4.57 (s, 1H, -$C\underline{H}_2NH_2$), 1.36 (d, *J* = 5.9 Hz, 6H, -$OCH(C\underline{H}_3)_2$).

*4.1.11.3. 5-(2-aminoacetyl)-2-isobutoxy-benzonitrile hydrochloride (**12c**).* White solid (yield: 99%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (s, 3H, -$CH_2N\underline{H}_2$, ArH), 8.27 (d, *J* = 8.7 Hz, 1H, ArH), 7.43 (s, 1H, ArH), 4.57 (s, 2H, -$C\underline{H}_2NH_2$), 4.07 (d, *J* = 6.0 Hz, 2H, -$OC\underline{H}_2CH(CH_3)_2$), 2.14-2.02 (m, 1H, -$OCH_2C\underline{H}(CH_3)_2$), 1.02 (d, *J* = 6.4 Hz, 6H, -$OCH_2CH(C\underline{H}_3)_2$).

*4.1.11.4. 5-(2-aminoacetyl)-2-(cyclopentyloxy)-benzonitrile hydrochloride (**12d**).* White solid (yield: 96.4%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (s, 2H, -$CH_2N\underline{H}_2$), 8.44 (d, *J* = 2.2 Hz, 1H, ArH), 8.26 (dd, *J* = 9.0, 2.2 Hz, 1H, ArH), 7.45 (d, *J* = 9.1 Hz, 1H, ArH), 5.17 (m, 1H, -$OC\underline{H}(CH_2)_4$), 4.57 (s, 2H, -$C\underline{H}_2NH_2$), 2.01 (m, 2H, -$OCH(C\underline{H}_2)_4$), 1.83-1.57 (m, 6H, -$OCH(C\underline{H}_2)_4$).

*4.1.12. General procedure for the preparation of compounds **13a–d***

Trimethyloxonium tetrafluoroborate (12.5 mmol) was slowly added into ethyl thiooxamate (18.3 mmol) in CH_2Cl_2 and stirred for 30 min in an ice bath under a nitrogen atmosphere. Subsequently, the mixture was stirred for 1 h after removing the ice bath and then evaporated under vacuum. Then, CH_3COONa (41.6 mmol), compound **12a–d** (8.3 mmol), CH_3COOH (5 mL), and dioxane were added and reacted at 65 °C for 3 h. The reaction was neutralized with saturated sodium bicarbonate, diluted with H_2O (100 mL), extracted with ethyl acetate (100 mL × 3), washed with H_2O (150 mL × 2) and brine (150 mL × 2), dried over anhydrous Na_2SO_4, and evaporated under vacuum. The residue was purified by flash column chromatography (5–25% ethyl acetate in petroleum ether).

*4.1.12.1. ethyl 4-(3-cyano-4-ethoxyphenyl)-1H-imidazole-2-carboxylate (**13a**).* White solid (yield: 30%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.53 (s, 1H, -NH), 8.03 (d, *J* = 2.2 Hz, 2H, ArH, -NCH), 8.01 (s, 1H, ArH), 7.43 (d, *J* = 2.2 Hz, 1H, ArH), 6.98 (d, *J* = 8.5 Hz, 1H, ArH), 4.49 (q, *J* = 7.1 Hz, 2H, -$COOC\underline{H}_2CH_3$), 4.18 (q, *J* = 7.0 Hz, 2H, -$OC\underline{H}_2CH_3$), 1.49 (t, *J* = 7.0 Hz, 3H, -$COOCH_2C\underline{H}_3$), 1.45 (t, *J* = 7.1 Hz, 3H, -$OCH_2C\underline{H}_3$).

*4.1.12.2. ethyl 4-(3-cyano-4-isopropoxyphenyl)-1H-imidazole-2-carboxylate (**13b**).* White solid (yield: 28%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.83 (s, 1H, -NH), 8.07-7.72 (m, 2H, -

NCH, ArH), 7.44 (d, *J* = 8.4 Hz, 1H, ArH), 7.00 (m, 1H, ArH), 4.68 (dd, *J* = 10.4, 5.2 Hz, 1H, -OC<u>H</u>$(CH_3)_2$), 4.54-4.41 (m, 2H, -COOC<u>H</u>$_2$$CH_3$), 1.56-1.21 (m, 9H, -OCH(C<u>H</u>$_3$)$_2$, -$COOCH_2$C<u>H</u>$_3$).

*4.1.12.3. ethyl 4-(3-cyano-4-isobutoxyphenyl)-1H-imidazole-2-carboxylate (**13c**).* White solid (yield: 32%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.75 (s, 1H, -NH), 8.03 (2H, m, ArH, -NCH), 7.45 (s, 1H, ArH), 6.99 (d, *J* = 8.7 Hz, 1H, ArH), 4.50 (q, *J* = 7.0 Hz, 2H, -COOC<u>H</u>$_2$$CH_3$), 3.87 (d, *J* = 6.4 Hz, 2H, -OC<u>H</u>$_2$$CH(CH_3)_2$), 2.27-2.12 (m, 1H, -$OCH_2$C<u>H</u>$(CH_3)_2$), 1.46 (t, *J* = 7.1 Hz, 3H, -$COOCH_2$C<u>H</u>$_3$), 1.09 (d, *J* = 6.6 Hz, 6H, -OCH_2CH(C<u>H</u>$_3$)$_2$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.74, 158.78, 141.01, 137.72, 131.55, 129.80, 127.53, 117.87, 116.72, 113.95, 101.33, 75.25, 61.32, 28.12, 19.25, 14.69. HRMS(ESI): Calcd for $C_{17}H_{19}N_3O_3$ $[M+Na]^+$ 314.1505, Found 314.1507.

*4.1.12.4. ethyl 4-(3-cyano-4-(cyclopentyloxy)phenyl)-1H-imidazole-2-carboxylate (**13d**).* White solid (yield: 26%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.09 (s, 1H, -NH), 8.02-7.95 (m, 2H, -C=CH, ArH), 7.46 (d, *J* = 17.4 Hz, 1H, ArH), 7.04-6.93 (m, 1H, ArH), 4.88 (dd, *J* = 8.1, 4.1 Hz, 1H, -OC<u>H</u>$(CH_2)_4$), 4.47 (q, *J* = 7.1 Hz, 2H, -COOC<u>H</u>$_2$$CH_3$), 2.03-1.80 (m, 8H, -OCH(C<u>H</u>$_2$)$_4$), 1.42 (t, *J* = 7.1 Hz, 3H, -$COOCH_2$C<u>H</u>$_3$).

*4.1.13. General procedure for the preparation of compounds **IIIa–d***

THF/ethanol (1:1, 10 mL) was added to **13a–d** (1.0 mmol), and the resulting mixture was maintained at 70 °C for 2 h. After completion of the reaction, the mixture was diluted with H_2O (100 mL) and acidified to pH 2 with 1 M HCl solution, and the resulting suspension was stirred in an ice bath for 30 min. The resulting solid was collected by filtration, washed with water, and then purified by recrystallization from methanol to yield the desired product.

*4.1.13.1. 4-(3-cyano-4-ethoxyphenyl)-1H-imidazole-2-carboxylic acid (**IIIa**).* White solid (yield: 96%); H NMR (400 MHz, DMSO-d_6) δ 8.17 (s, 1H, -NCH), 8.11 (d, *J* = 8.6 Hz, 1H, ArH), 7.80 (s, 1H, ArH), 7.27 (d, *J* = 8.8 Hz, 1H, ArH), 4.22 (dd, *J* = 13.9, 6.9 Hz, 2H, -OC<u>H</u>$_2$CH$_3$), 1.38 (t, *J* = 6.9 Hz, 3H, -OCH$_2$C<u>H</u>$_3$).^{13}C NMR (101 MHz, DMSO-d_6) δ 159.38, 131.60, 131.09, 129.94, 129.27, 116.93 (s, 11H), 113.77, 101.21, 65.1, 65.06, 14.86. HRMS(ESI): Calcd for $C_{13}H_{11}N_3O_3$ $[M-H]^-$ 256.0746, Found 256.0722.

*4.1.13.2. 4-(3-cyano-4-ethoxyphenyl)-1H-imidazole-2-carboxylic acid (**IIIb**).* White solid (yield: 97.8%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (s, 2H, -NH, -NCH), 8.06-7.98 (m, 2H, ArH), 7.24 (d, *J* = 8.4 Hz, 1H, ArH), 4.92-4.76 (m, 1H, -OC<u>H</u>(CH$_3$)$_2$), 1.32 (d, *J* = 4.9 Hz, 3H, -OCH(C<u>H</u>$_3$)$_2$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.83, 159.77, 158.73, 155.10, 139.10, 131.58, 130.10, 129.22, 128.03, 124.25, 116.98, 115.28, 102.15, 71.73, 22.24. HRMS(ESI): Calcd for $C_{14}H_{13}N_3O_3$ $[M-H]^-$ 270.0879, Found 270.0889.

*4.1.13.3. 4-(3-cyano-4-isobutoxyphenyl)-1H-imidazole-2-carboxylic acid (**IIIc**).* White solid (yield: 96.5%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (s, 1H, ArH), 8.12 (d, *J* = 8.8 Hz, 1H, ArH), 7.87 (s, 1H, -NHC<u>H</u>), 7.29 (d, *J* = 8.8 Hz, 1H, ArH), 3.95 (d, *J* = 6.4 Hz, 2H, -OC<u>H</u>$_2$CH(CH$_3$)$_2$), 2.13-2.03 (m, 1H, -OCH$_2$C<u>H</u>(CH$_3$)$_2$), 1.02 (d, *J* = 6.6 Hz, 6H, -OCH$_2$CH(C<u>H</u>$_3$)$_2$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.91, 159.76, 139.35, 136.52, 131.63, 131.09, 129.90, 129.18, 126.89, 116.90, 116.70, 114.01, 113.94, 101.37, 75.29, 75.23, 28.11, 19.26, 19.24. HRMS(ESI): Calcd for $C_{17}H_{19}N_3O_3$ $[M+Na]^+$ 308.1011, Found 308.1025.

*4.1.13.4. 4-(3-cyano-4-(cyclopentyloxy)phenyl)-1H-imidazole-2-carboxylic acid (**IIId**).* White solid (yield: 100%); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.15 (d, *J* = 2.2 Hz, 1H, -NHC<u>H</u>), 8.10 (dd, *J* = 8.8, 2.2 Hz, 1H, ArH), 7.87 (s, 1H, ArH), 7.30 (s, 1H, ArH), 5.03 (dd, *J* = 9.8, 3.9 Hz, 1H, -OC<u>H</u>(CH$_2$)$_4$), 1.99-1.92 (m, 2H, -OC<u>H</u>(CH$_2$)$_4$), 1.76 (dt, *J* = 10.6, 5.8 Hz, 4H, -OCH(C<u>H</u>$_2$)$_4$), 1.66-1.58 (m, 2H, -OCH(C<u>H</u>$_2$)$_4$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.84,

158.81, 139.12, 131.50, 130.08, 116.83, 115.18, 102.12, 81.21, 32.67, 23.94. HRMS(ESI): Calcd for $C_{16}H_{15}N_3O_3$ $[M+H]^+$ 298.1192, Found 298.1181.

*4.1.14. Synthesis of ethyl 4-(3-cyano-4-isobutoxyphenyl)-1-methyl-1H-imidazole-2-carboxylic acid (**IIIe**)*

K_2CO_3 (2.9 mmol) was added to compound **13c** (0.96 mmol) in DMF and stirred at rt for 0.5 h. CH_3I (2.9 mmol) was added to the mixture and heated at 70 °C for 3 h. After completion of the reaction, the mixture was cooled to rt, diluted with H_2O (150 mL), and then extracted with ethyl acetate (100 mL × 2). The organic phases were combined, washed with H_2O (100 mL × 2) and brine (100 mL × 2), dried over anhydrous Na_2SO_4, evaporated under vacuum, and purified by flash column chromatography (0–10% ethyl acetate in petroleum ether) to afford a white solid (yield: 95%); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.06 (d, *J* = 2.1 Hz, 1H, ArH), 8.04 (dd, *J* = 8.7, 2.2 Hz, 1H, ArH), 7.99 (s, 1H, -NCH), 7.29 (d, *J* = 8.8 Hz, 1H, ArH), 4.33 (q, *J* = 7.1 Hz, 2H, -COOC$\underline{H}_2$CH$_3$), 3.95 (s, 3H, -NCH$_3$), 3.94 (d, *J* = 6.5 Hz, 2H, -OC$\underline{H}_2$CH(CH$_3$)$_2$), 2.12–2.03 (m, 1H, -OCH$_2$C$\underline{H}$(CH$_3$)$_2$), 1.34 (t, *J* = 7.1 Hz, 3H, -COOCH$_2$C$\underline{H}_3$), 1.02 (d, *J* = 6.7 Hz, 6H, -OCH$_2$CH(C$\underline{H}_3$)$_2$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.82, 159.05, 138.67, 136.63, 131.41, 129.63, 127.11, 123.98, 116.65, 114.04, 101.37, 75.27, 61.25, 36.34, 28.11, 19.24, 14.63. HRMS(ESI): Calcd for $C_{18}H_{21}N_3O_3$ $[M + Na]^+$ 350.1481, Found 350.1486.

THF/ethanol (1:1, 10 mL) was added to the white solid (1.0 mmol), and the resulting mixture was maintained at 70 °C for 2 h. After completion of the reaction, the mixture was diluted with H_2O (100 mL) and acidified to pH 2 with 1 M HCl solution, and the resulting suspension was stirred in an ice bath for 30 min. The resulting solid was collected by

filtration, washed with water, and then purified by recrystallization from methanol to yield a white solid **IIIe** (yield: 98.9%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10–7.97 (m, 2H, -$NCH_3C\underline{H}$, ArH), 7.94 (s, 1H, ArH), 7.29 (d, *J* = 8.6 Hz, 1H, ArH), 3.94 (s, 5H, -$OC\underline{H}_2CH(CH_3)_2$, -$NCH_3$), 2.07 (m, 1H, -$OCH_2C\underline{H}(CH_3)_2$), 1.01 (d, *J* = 6.0 Hz, 6H, -$OCH_2CH(C\underline{H}_3)_2$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 159.20, 139.09, 139.02, 130.97, 129.04, 128.53, 117.36, 116.88, 114.06, 113.93, 101.23, 75.21, 33.60, 28.12, 19.25. HRMS(ESI): Calcd for $C_{18}H_{21}N_3O_3$ $[M + Na]^+$ 322.1162, Found 322.1181.

*4.1.15. General procedure for the preparation of compounds **15a–c***

A mixture of (4-(*tert*-butyl)phenyl)boronic acid (7.5 mmol), K_2CO_3 (20 mmol), $Pd[P(C_6H_5)_3]_4$ (0.25 mmol), and compound **14** (5 mmol) in DMF was stirred at 120 °C for 24 h under an argon atmosphere. The reaction was diluted with H_2O (30 mL) and extracted with ethyl acetate (20 mL × 2). The organic phases were combined, washed with brine (30 mL × 3), dried over anhydrous Na_2SO_4, and concentrated under vacuum to yield the crude product, which was purified by flash column chromatography (0–10% ethyl acetate in petroleum ether).

*4.1.15.1. 4-bromo-4'-(tert-butyl)-[1, 1'-biphenyl]-2-carbonitrile (**15a**).* White solid (yield: 82%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97-7.81 (m, 1H, ArH), 7.77-7.67 (m, 1H, ArH), 7.60-7.43 (m, 5H, ArH), 1.41-1.35 (m, 9H, -$C_6H_4C(C\underline{H}_3)_3$).

*4.1.15.2. 4-bromo-4'-methoxy-[1, 1'-biphenyl]-2-carbonitrile (**15b**).* White solid (yield: 70%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.86 (d, *J* = 2.1 Hz, 1H, ArH), 7.73 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.49-7.44 (m, 2H, ArH), 7.36 (d, *J* = 8.4 Hz, 1H, ArH), 7.04-7.00 (m, 2H, ArH), 3.86 (s, 3H, -$C_6H_4OC\underline{H}_3$).

*4.1.15.3. 4-bromo-3'-methoxy-[1, 1'-biphenyl]-2-carbonitrile (**15c**).* White solid (yield: 70%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.89 (d, *J* = 2.0 Hz, 1H, ArH), 7.76 (dd, *J* = 8.4, 2.1 Hz, 1H, ArH), 7.40 (d, *J* = 8.3 Hz, 2H, ArH), 7.10 (m, 1H, ArH), 7.06-7.04 (m, 1H, ArH), 7.00 (m, 1H, ArH), 3.87 (s, 3H, -$C_6H_4OC\underline{H}_3$).

*4.1.16. General procedure for the preparation of compounds **16a–c**.*

A mixture of compound **15a–c** (2.4 mmol), CuI (0.2 mmol), ethyl 1*H*-imidazole-4-carboxylate (2.0 mmol), *trans*-*N*,*N*-dimethyl-1,2-cyclohexanediamine (0.4 mmol), and K_2CO_3 (4.2 mmol) in DMF (5 mL) was stirred at 120 °C for 24 h under a nitrogen atmosphere. The reaction was then diluted with H_2O (30 mL) and extracted with ethyl acetate (20 mL × 2). The organic phases were combined, washed with brine (30 mL × 3), dried over anhydrous Na_2SO_4, and concentrated under vacuum. The residue was purified by flash column chromatography (12–30% ethyl acetate in petroleum ether).

4.1.16.1. ethyl 1-(4'-(tert-butyl)-2-cyano-[1, 1'-biphenyl]-4-yl)-1H-imidazole-4-carb

*-oxylate (**16a**)* White solid (yield: 35%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 1.1 Hz, 1H, ArH), 7.93 (d, *J* = 1.0 Hz, 1H, ArH), 7.83-7.81 (m, 1H, ArH), 7.72-7.67 (m, 2H, ArH), 7.58-7.52 (m, 4H, ArH), 4.43 (q, *J* = 7.1 Hz, 2H, -COOC$\underline{H}_2$CH$_3$), 1.43 (t, *J* = 7.1 Hz, 5H, -COOCH$_2$C$\underline{H}_3$), 1.38 (s, 9H, -C_6H_4(C$\underline{H}_3$)$_3$).

*4.1.16.2. ethyl 1-(2-cyano-4'-methoxy-[1, 1'-biphenyl]-4-yl)-1H-imidazole-4-carb- oxylate (**16b**)* White solid (yield: 32%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.99 (d, *J* = 1.3 Hz, 1H, -NCH), 7.92 (d, *J* = 1.3 Hz, 1H, -NCH), 7.80 (d, *J* = 2.1 Hz, 1H, ArH), 7.69-7.65 (m, 2H, ArH), 7.54 (d, *J* = 8.7 Hz, 2H, ArH), 7.06 (d, *J* = 8.7 Hz, 2H, ArH), 4.43 (q, *J* = 7.1 Hz, 2H, -COOC$\underline{H}_2$CH$_3$), 3.89 (s, 3H, -$C_6H_4OC\underline{H}_3$), 1.43 (t, *J* = 7.1 Hz, 3H, -COOCH$_2$C$\underline{H}_3$).

4.1.16.3. ethyl 1-(2-cyano-3'-methoxy-[1, 1'-biphenyl]-4-yl)-1H-imidazole-4-carb-

*oxylate (**16c**)* Brown solid (yield: 30%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.00 (s, 1H, -NCH), 7.93 (s, 1H, -NCH), 7.83 (s, 1H, ArH), 7.71 (s, 2H, ArH), 7.45 (t, *J* = 7.9 Hz, 1H, ArH), 7.15 (d, *J* = 7.6 Hz, 1H, ArH), 7.10 (d, *J* = 9.9 Hz, 1H, ArH), 7.04 (dd, *J* = 8.3, 2.2 Hz, 1H, ArH), 4.43 (q, *J* = 7.1 Hz, 2H, -COOC$\underline{H}_2$$CH_3$), 3.89 (s, 3H, -$C_6H_4OC\underline{H}_3$), 1.43 (t, *J* = 7.1 Hz, 3H, -COOC$\underline{H}_2$$CH_3$).

*4.1.17. General procedure for the preparation of compounds **IVa–c**.*

THF/ethanol (1:1, 10 mL) was added to **16a–c** (1.0 mmol), and the resulting mixture was maintained at 70 °C for 2 h. After completion of the reaction, the mixture was diluted with H_2O (100 mL) and acidified to pH 2 with 1 M HCl solution, and the resulting suspension was stirred in an ice bath for 30 min. The resulting solid was filtered, washed with water, and purified by recrystallization from methanol to yield the desired product.

*4.1.17.1. 1-(4'-(tert-butyl)-2-cyano-[1, 1'-biphenyl]-4-yl)-1H-imidazole-4-carboxylic acid (**IVa**).* White solid (yield: 90%); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.58 (d, *J* = 1.1 Hz, 1H, -NCH), 8.52 (d, *J* = 1.1 Hz, 1H, -NCH), 8.46 (d, *J* = 2.4 Hz, 1H, ArH), 8.18 (dd, *J* = 8.5, 2.4 Hz, 1H, ArH), 7.78 (d, *J* = 8.5 Hz, 1H, ArH), 7.60-7.56 (m, 4H, ArH), 1.35 (s, 9H, -$C_6H_4C(C\underline{H}_3)_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 163.80, 152.10, 143.68, 137.30, 136.01, 135.39, 134.42, 132.02, 128.89, 126.13, 126.07, 125.94, 124.38, 118.31, 111.74, 34.96, 31.60, 31.50. HRMS(ESI): Calcd for $C_{21}H_{19}N_3O_2$ $[M+Na]^+$ 368.1375, Found 368.1362.

*4.1.17.2. 1-(4'-(tert-butyl)-2-cyano-[1, 1'-biphenyl]-4-yl)-1H-imidazole-4-carboxylic acid (**IVb**).* White solid (yield: 80%); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.56 (s, 1H, -NCH), 8.51 (s, 1H, -NCH), 8.44 (d, *J* = 2.3 Hz, 1H, ArH), 8.16 (dd, *J* = 8.5, 2.3 Hz, 1H, ArH), 7.74 (d, *J*

= 8.5 Hz, 1H, ArH), 7.58 (d, *J* = 8.7 Hz, 2H, ArH), 7.12 (d, *J* = 8.7 Hz, 2H, ArH), 3.84 (s, 3H, -$C_6H_4OC\underline{H}_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 163.87, 160.43, 143.58, 137.21, 135.75, 135.62, 131.84, 130.53, 129.51, 125.95, 125.86, 124.23, 118.37, 114.78, 111.67, 55.81. HRMS(ESI): Calcd for $C_{18}H_{13}N_3O_3$ $[M+H]^+$ 320.1035, Found 320.1025.

*4.1.17.3. 1-(2-cyano-3'-methoxy-[1, 1'-biphenyl]-4-yl)-1H-imidazole-4-carboxylic acid(**IVc**).* Brown solid (yield: 90%); ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.61 (d, *J* = 4.7 Hz, 2H, -NCH), 8.49 (d, *J* = 1.8 Hz, 1H, ArH), 8.20 (dd, *J* = 8.4, 1.9 Hz, 1H, ArH), 7.81 (d, *J* = 8.5 Hz, 1H, ArH), δ 7.48 (t, *J* = 7.9 Hz, 1H, ArH), 7.19 (d, *J* = 7.2 Hz, 1H, ArH), 7.11-7.08 (m, 1H, ArH), 3.84 (s, 2H, -$C_6H_4OC\underline{H}_3$). ^{13}C NMR (151 MHz, DMSO-d_6) δ 163.60, 159.84, 143.70, 138.60, 137.35, 136.17, 135.04, 132.07, 130.45, 126.04, 125.89, 124.43, 121.41, 118.11, 115.14, 114.76, 112.02, 55.77. HRMS(ESI): Calcd for $C_{18}H_{13}N_3O_3$ $[M+H]^+$ 320.1035, Found 320.1026.

4.2. XOR inhibitory activity in vitro

The assay for XOR activity with xanthine as the substrate was previously described by Li et al. [26] and Fukunari et al. [31]. Phosphate-buffered saline (1× PBS), xanthine (0.5 mM), and XOR (0.5 μL/100 μL) (from bovine milk, Sigma, USA) were prepared. The tested compounds were also diluted with PBS to the required concentrations. The specific procedure was as follows. PBS, enzyme solution (100 μL), and sample were added to 96-well plates and incubated at 37 °C for 3 min. Next, the prepared xanthine solution was immediately added to the wells. The plates were then scanned at 295 nm on SpectraMax190 (Molecular Devices, USA) once every 30 s for 5 min. Three replicates were performed for each concentration. The IC_{50} values were calculated using Excel 2007 (Microsoft, USA) and GraphPad 6.0 software (GraphPad Inc., USA).

4.3. Molecular modeling

Molecular docking was carried out using the Glide module of the Schrodinger suite. **Ia**, **Ie**, **IVa**, WN1703, and febuxostat data were imported and prepared using the Ligprep module. The X-ray crystal structure of the Y-700/XOR complex (PBD entry code 1VDV) was used as the target receptor to dock, add hydrogen, delete water, fill the missing loops, and refine by energy minimization and optimization with the OPLS-2005 force field. The active site was determined through the centroid of the co-crystallized ligand of the receptor, and the grid box was generated using the Receptor grid generation module. Docking runs were then performed with the prepared compounds, and the results are shown based on docking scores [30, 31].

4.4. Assay of hypouricemic effects in vivo

4.4.1. Reagents

Carboxymethylcellulose sodium (CMC-Na), hypoxanthine, and potassium oxonate were purchased from Aladdin (Shanghai, P. R. China), febuxostat was obtained from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, P. R. China), and the uric acid assay kit was from SanNuo (Changsha, P. R. China). Blood uric acid, creatinine, and urea nitrogen diagnostic kits were purchased from JianCheng Bioengineering Institute (Nanjing, P. R. China).

4.4.2. Animals

ICR mice (18–22 g) were purchased from SJA Laboratory Animals (Hunan, P. R. China). They were allowed at least 1 week to adapt to the environment before being used in

experiments. Animals were housed in plastic cages with a standard chow diet and water at 22 ± 2 °C with a relative humidity of 55 ± 5%, under a normal 12 h light/dark cycle (light period 7:00 AM to 7:00 PM). Eight mice were allocated to each group. All studies adhered to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication 85-23, revised 1986) and the institutional committee of the South China University of Technology.

4.4.3. Hypouricemic effect in acute hyperuricemia mice

Acute hyperuricemia was induced in mice via subcutaneous injection of 250 mg/kg potassium oxonate with intraperitoneal injection of 400 mg/kg hypoxanthine, according to a modified version of the procedure reported by Li et al. [26] and Xu et al. [32]. Potassium oxonate and hypoxanthine were dissolved in saline. Febuxostat, **Ie**, and **IVa** were suspended in 0.5% CMC-Na solution for intragastric administration at a dosage of 5 mg/kg. The animals were treated with potassium oxonate and hypoxanthine, and one drop of blood was collected from the tail into a heparin-coated tube for analysis with a uric acid assay kit at 1 h (marked as 1 h in Fig. 4). Next, drugs were administered to the test groups. The model and blank groups received the corresponding solvent, and blood samples were taken and analyzed using a uric acid assay kit within 2–8 h.

4.4.4. Hypouricemic effect in long-term hyperuricemia mice

Potassium oxonate and hypoxanthine were dissolved in saline. Febuxostat, **Ie**, and **IVa** were suspended in 0.5% CMC-Na solution for intragastric administration at a dosage of 5 mg/kg. Long-term hyperuricemic mice were generated via subcutaneous injection of 250 mg/kg potassium oxonate with intraperitoneal injection of 150 mg/kg at 9:00 AM once daily

for 7 consecutive days. Subsequently, the mice were administered blank solvents, febuxostat, **Ie**, or **IVa** at 10:00 AM. After 7 days of treatment, blood was collected and centrifuged at 4000 r/min for 10 min to obtain serum; this was stored at −80 °C prior to analysis. The levels of uric acid, creatinine, and urea nitrogen were measured using appropriate diagnostic kits [38].

All data are expressed as mean ± SEM, and statistical analyses were performed using Student's *t* tests. The figures were produced using GraphPad Prism 6.0 and OriginPro 9.0 software.

Conflict of interest

The authors have declared no conflict of interest.

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Highlights

- Compounds **Ie** and **IVa** endowed with comparable activity with febuxostat in vitro.
- Compounds **Ie** and **IVa** displayed significant hypouricemic potency compared to hyperuricemia mouse.
- Compounds **Ie** and **IVa** showed renoprotection in animal models.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: