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Novel Human Urate Transporter 1 Inhibitors as Hypouricemic Drug Candidates with Favorable Druggability

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Abstract

Lesinurad, a URAT1 inhibitor approved as a medication for the treatment of hyperuricemia associated with gout in 2015, can cause liver and renal toxicity. Here, we modified all three structural components of lesinurad by applying scaffold hopping, bioisosterism and substituent decorating strategies. In a mouse model of acute hyperuricemia, 21 of the synthesized compounds showed increased SUA-reducing activity; SUA was about 4-fold lower in animals treated with **44**, **54** and **83**, compared with lesinurad or benzbromarone. In URAT1 inhibition assay, **44** was over 8-fold more potent than lesinurad (IC_{50}: 1.57 μM vs. 13.21 μM). Notably, **83** also displayed potent inhibitory activity (IC_{50} = 31.73 μM) against GLUT9. Furthermore, we also preliminarily explored

the effect of chirality on the potency of the promising derivatives **44** and **54**. Compounds **44**, **54** and **83** showed favorable drug-like pharmacokinetics, and appear to be promising candidates for the treatment of hyperuricemia and gout.

Introduction

Uric acid, the final product of purine catabolism, is physiologically excreted in the urine. Endogenously produced uric acid accounts for approximately 80% of the total serum uric acid (SUA), while the remainder is derived from dietary purines. Most bacteria and mammals decompose uric acid *via* uricase-mediated metabolism to afford water-soluble allantoin. However, humans lack uricase , and uric acid is predominantly eliminated *via* renal excretion.[1–6] Thus, overproduction of uric acid or insufficient renal elimination causes hyperuricemia, which is generally defined as a serum level of uric acid > 6.8 mg/dL. Hyperuricemia can be asymptomatic, but when the serum uric acid concentration exceeds 6.8 mg/dL, monosodium urate crystallizes and is deposited in joints or surrounding tissues -- this is the cause of gout, a severe disease that affects millions of people worldwide, especially adult men.[7,8] Hyperuricemia is also associated with a range of chronic diseases, including hypertension, diabetes mellitus, metabolic syndrome, and renal and cardiovascular diseases.[9–11]

For a long time, the preferred drugs for clinical treatment of hyperuricemia were xanthine oxidase (XO) inhibitors, such as allopurinol (**1**), oxypurinol (**2**) and febuxostat (**3**) (**Figure 1**),[12] which reduce the rate of uric acid synthesis.[13,14] However, an appreciable proportion of patients do not respond well to XO inhibitors due to serious side effects and the development of resistance. For example, allopurinol (**1**) can cause severe allergic rash, stomach bleeding, fever, abdominal

pain, diarrhea and thrombocytopenia, while febuxostat can cause severe liver damage and cardiovascular side effects. Furthermore, in 2017, the U.S. Food and Drug Adminstration (FDA) warned that febuxostat might increase the risk of sudden cardiac death.[15–17] Uricosuric drugs are another option for patients who are intolerant of XO inhibitors. These agents reduce reabsorption and thus increase the excretion of uric acid. Multiple transporters are involved in reabsorption. For example, human urate transporter 1 (URAT1, encoded by SLC22A12) and glucose transporter 9 (GLUT9, encoded by SLC2A9) are both involved in reabsorption of urate, and high levels of URAT1 and GLUT9 expression may cause hyperuricemia or gout.[18–20] URAT1 mainly regulates tubular reabsorption of urate through an ion-exchange mechanism with organic anions at the apical membrane of renal proximal tubule cells.[21] Inhibition of URAT1 or GLUT9 blocks reabsorption of urate anion, thereby enhancing renal uric acid excretion, and mutations in hURAT1 have been shown to reduce the urate-transporting activity, leading to hypouricemia.[7,22,23] So far, four major uricosuric drugs have come onto the market, namely probenecid (**4**), sulfinpyrazone (**5**), benzbromarone (**6**) and lesinurad (**7**). Lesinurad, approved in 2015 by the FDA, increases the excretion of uric acid by inhibiting URAT1 (**Figure 2**).[21] Thus, URAT1 is an attractive target for the development of novel uricosuric agents. Nevertheless, uricosuric drugs can also cause side effects and may exhibit poor efficacy. For example, benzbromarone had to be withdrawn from the European market due to its severe liver and kidney toxicity and potent inhibition of cytochrome P450 (CYP) metabolic enzymes.[24] Also, the FDA has advised that lesinurad should be used with caution because of its severe renal toxicity and liver toxicity. This is problematic, because the SUA-lowering activity of lesinurad is not potent. Furthermore, lesinurad can only used in combination with the XO inhibitor febuxostat with the approval of the FDA,[25-27]

because the cardiovascular toxicity of febuxostat increases the risk of co-administration.[28,29] Therefore, there is still an urgent need to develop safe, potent and specific URAT1 inhibitors as novel hypouricemic agents.

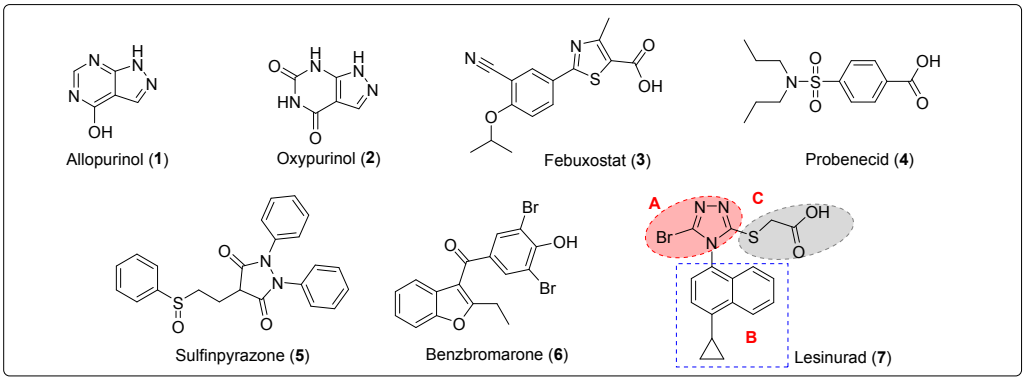


Figure 1. The structures of representative drugs used in the treatment of gout.

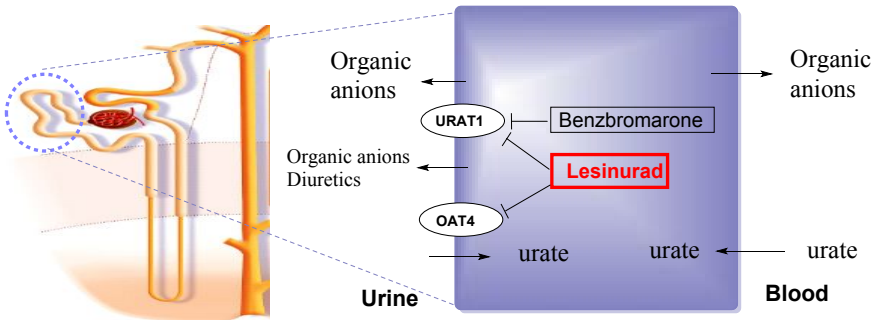


Figure 2. Mechanism of action of lesinurad.

Thus, although lesinurad is the newly approved URAT1 inhibitor and has a clear mechanism of action, it has many shortcomings. In this context, we decided to conside it as a lead compound and we set out to optimize its structure for improved safety and activity. Lesinurad contains three structural components, namely the triazole ring (zone A), cyclopropyl naphthalene (zone B) and the sulfhydryl side chain containing a carboxyl group (zone C). As regards zone A, recent work on selective uric acid reabsorption inhibitors has suggested that the triazole moiety might mainly serve as a scaffold to orient the pharmacophores in the proper geometry for binding to the target enzyme, and we suspected that the brominated triazole moiety in the parent structure might contribute to the toxicity and serious side effects. It has been reported that differences in the

electronic and conformational features of the heterocyclic core influence the binding of inhibitors to URAT1, as well as the toxicity.[30,31] Nevertheless, there has been relatively little recent work on the structure-activity relationship (SAR) and structural modifications of zone A.[32] A classical strategy in contemporary medicinal chemistry for 'follow-on'-based drug discovery to obtain novel hits, as well as to improve drug-like and safety properties, is scaffold refining *via* the replacement of the central core in existing bioactive lead compounds, combined with the introduction of privileged substituents.[33,34] However, in work reported so far, the original triazole ring has only been replaced with different five- or six-membered heterocyclic scaffolds.[35] Thus, there is still a large chemical space to be explored in zone A; in particular, the triazole moiety can be readily modified in terms of its ability to form hydrogen bonds, and scaffold hopping and bioisosterism strategies are expected be applicable.[32] As for zone B, cyclopropyl naphthalene is considered to be a functional pharmacophore, having a hydrophobic or van der Waals' interaction with the binding pocket of URAT1.[36] Therefore, exploration of the SAR of this part by means of structural diversification should be a promising approach to obtain better activity and safety.[37,38] As for zone C, the carboxylic acid moiety may be a critical feature, since URAT1 is an anion transporter. However, this group contributes to the strong acidity and high polarity of lesinurad, which may be related to the safety. Therefore, we decided to adopt a strategy of bioisosterism to replace the carboxylic acid in order to reduce the molecular polarity and toxicity.[39, 41-42] During the implementation of our project, some medicinal chemists have reported exploratory modifications of zones B and C, and several promising lead compounds were obtained, as exemplified by **7a-c** (**Figure 3**).[36-40] Nevertheless, we envision that a more detailed and systematic evaluation of the SAR and structure-toxicity relationships is likely to be fruitful. Therefore, in the present work, we examined the effects of structural modification in all three zones, especially zone C, aiming to find novel URAT1 inhibitors with lower toxicity, greater potency, and possibly new mechanisms of action.

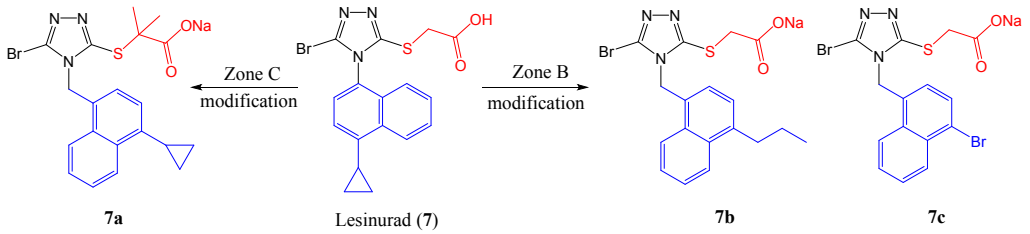


Figure 3. Reported modifications of zones B and C.

Based on these ideas, we focused on scaffold hopping and bioisosterism strategies to explore the SARs of unexploited scaffolds bearing privileged peripheral substituents (**Figure 4**). Firstly, we designed a set of synthetically accessible heterocyclic-fused cores, i.e., 3*H*-imidazole[4,5-*b*]pyridine, 1*H*-imidazole[4,5-*c*]pyridine and 1*H*-imidazole[4,5-*b*]pyridine, to replace zone A in lesinurad. Then, we investigated the effect of changing the aromatic substituent in zone B to mesitylene, naphthalene or cyanonaphthalene. Lastly, we modified zone C to obtain diverse substituted acylsulfonamides *via* a bioisosterism approach. We also made various structural modifications in zone C (increasing the length of the side chain, substituting the carbon atom at the *ortho*-position of the sulfur atom with a methyl, dimethyl or cyclobutyl group, and esterifying the terminal carboxyl group) that were expected to improve the pharmacokinetic properties. All the newly prepared compounds were evaluated in a well developed mouse model of acute hyperuricemia induced with hypoxanthine and potassium cyanate. A pharmacokinetics study of the most promising compounds **44**, **54** and **83** in rats indicated that they have favorable, drug-like pharmacokinetics.

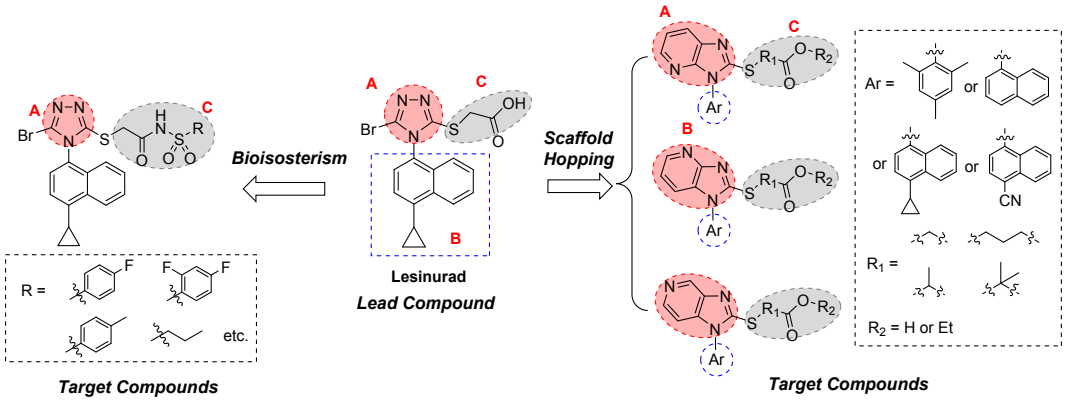


Figure 4. Design of target compounds *via* scaffold hopping and bioisosterism strategies.

Based on the bioisosterism strategy, we also designed and synthesized a series of *N*-acylbenzene sulfonamide analogues derived from the carboxylic acid moiety (zone C) (**Figure 4**). Some of the prepared compounds showed more potent activity than lesinurad, and a pharmacokinetics study of the most potent compound **83** was carried out. As expected, **83** showed favorable, drug-like pharmacokinetics. This compound also appears to be a promising candidate for the treatment of hyperuricemia and gout.

Chemistry

The synthetic protocols for the newly designed derivatives are outlined in **Schemes 1−6**. As shown in **Scheme 1**, 3*H*-imidazole[4,5-*b*]pyridine derivatives were synthesized by well-established methods from commercially available 2-chloro-3-nitropyridine (**8a**). Treatment of **8a** with naphthalen-1-amine (**9a**) afforded the intermediate **10a**. The nitro group of **10a** was reduced via Pd/C catalytic hydrogenation to form **11a**, followed by cyclization with potassium ethylxanthate and sodium bicarbonate to afford **12a**. Nucleophilic substitution reactions of **12a** afforded **13-16**, and hydrolysis with lithium hydroxide gave **17-20** (**Scheme 1**). The preparation method of compound **21-28** and **29-36** were similar to those of **13-20** except that the amines (**9b** and **9c**) used as starting material were different. [43,44]

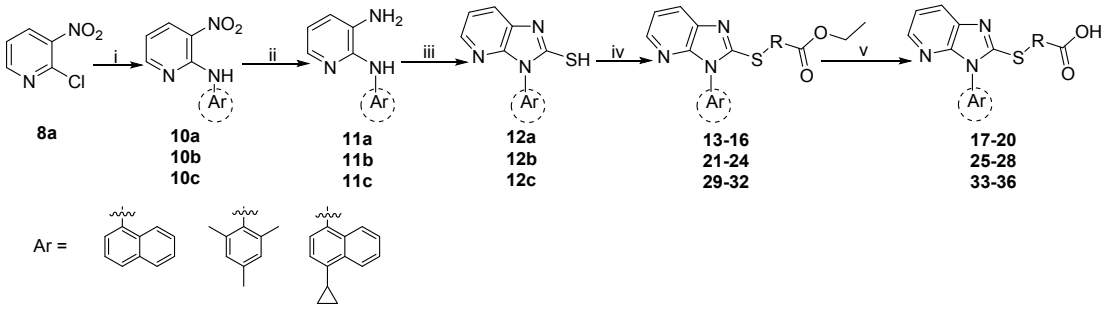
The synthetic route to **37-46** is shown in **Scheme 2**. 3-Chloro-2-nitropyridine (**8b**) was treated with 4-cyclopropyl-1-naphthylamine (**9c**) to afford intermediate **10d** *via* Buchwald-Hartwig coupling reaction. Then, hydrogenation reduction in the presence of Pd/C gave **11d**, which was cyclized with 1,1'-thiocarbonyldiimidazole to give the key intermediate **12d**, followed by nucleophilic substitution and hydrolysis to provide **37-46**.

As shown in **Scheme 3**, nucleophilic substitution of 4-chloro-3-nitropyridine (**8c**) with 4-cyclopropylnaphthalen-1-amine (**9c**) gave the intermediate **10e**, which afforded **47-56** via similar procedures to those shown in Scheme 1.[45]

The synthetic route to **57-80** is shown in **Scheme 4**. 4-Bromo-1-naphthonitrile (**9d**) was treated with 2-nitropyridin-3-amine (**8d**) or 3-nitropyridin-4-amine (**8e**) to afford intermediate **10f** and **10g** *via* coupling reaction. Then, hydrogenation reduction in the presence of Pd/C gave **11f** and **11g**, which were cyclized with 1,1'-thiocarbonyldiimidazole to give the key intermediates **12f** and **12g**, followed by nucleophilic substitution and hydrolysis to provide **57-80**.[46]

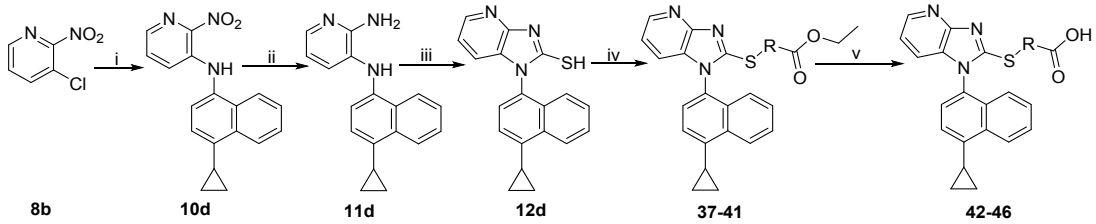
The synthetic routes to **81-96** are depicted in **Scheme 5**. The starting material, 4-bromonaphthalen-1-amine (**9e**), was converted to 4-cyclopropylnaphthalen-1-amine (**9c**) *via* a Suzuki coupling reaction in a toluene/water mixture, and **9c** was reacted with di(1*H*-imidazol-1-yl)methanethione to obtain the key intermediate 1-cyclopropyl-4-isothiocyanatonaphthalene (**10h**). Treatment of **10h** with hydrazinecarboximidamide afforded the intermediate 5-amino-4-(4-cyclopropylnaphthalen-1-yl)-4*H*-1,2,4-triazole-3-thiol (**11h**), which was subjected to substitution and bromination reaction to give the intermediate methyl 2-((5-amino-4-(4-cyclopropylnaphthalen-1-yl)-4*H*-1,2,4-triazol-3-yl)thio)acetate (**12h**) and brominated product **13h**. **13h** was hydrolyzed to provide lesinurad (**7**). Finally, compounds **81-96** were obtained *via* condensation reaction with lesinurad (**7**).[44]

All novel derivatives were fully characterized by high-resolution mass spectrometry (HR-MS), proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy, and carbon nuclear magnetic resonance (^{13}C-NMR) spectroscopy.



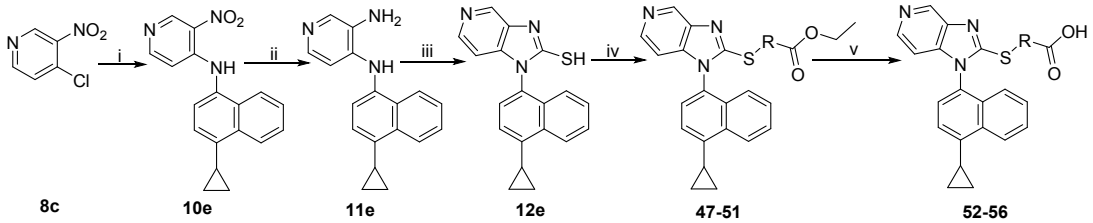
Scheme 1. Synthetic route to 3*H*-imidazole[4,5-*b*]pyridine derivatives

(i) KF, naphthalen-1-amine or 2,4,6-trimethylaniline or 4-cyclopropylnaphthalen-1-amine, 120 ℃; (ii) Pd/C, H_2, EtOH, r.t.; (iii) $EtOCS_2K$, $NaHCO_3$, H_2O, EtOH, 70 ℃; (iv) ester, K_2CO_3, DMF, r.t.; (v) LiOH, THF, EtOH, r.t..



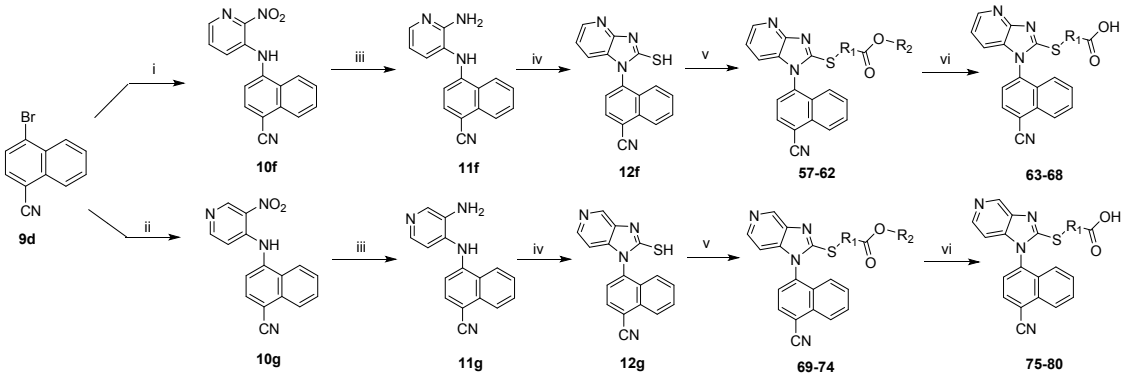
Scheme 2. Synthetic route to 1*H*-imidazole [4,5-*b*]pyridine derivatives

(i) 4-cyclopropylnaphthalen-1-amine, $Pd(OAc)_2$, XantPhos, Cs_2CO_3, N_2, 1,4-dioxane, 90 ℃; (ii) Pd/C, H_2, EtOH, r.t.; (iii) TCDI, Et_3N, THF, 60 ℃; (iv) ester, K_2CO_3, DMF, r.t.; (v) LiOH, THF, EtOH, r.t..



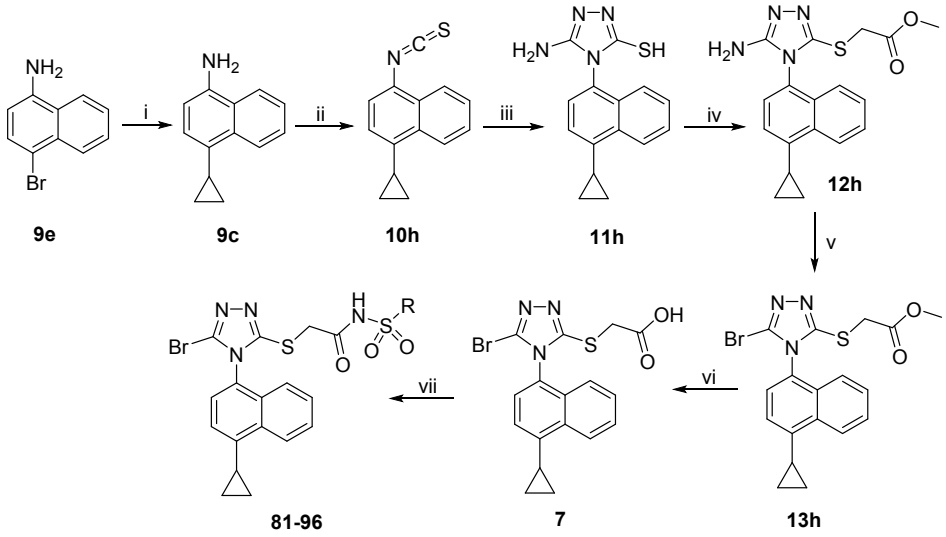
Scheme 3. Synthetic route to 1*H*-imidazole[4,5-*c*]pyridine derivatives

(i) 4-cyclopropylnaphthalen-1-amine, $NaHCO_3$, EtOH, 60 ℃; (ii) Pd/C, H_2, EtOH, r.t.; (iii) $EtOCS_2K$, $NaHCO_3$, H_2O, EtOH, 70 ℃; (iv) ester, K_2CO_3, DMF, r.t.; (v) LiOH, THF, EtOH, r.t..



Scheme 4. Synthetic route to 4-(2-mercapto-1*H*-imidazo[4,5-*b*]pyridin-1-yl)-1-naphthonitrile and 4-(2-mercapto-1*H*-imidazo[4,5-*c*]pyridin-1-yl)-1-naphthonitrile derivatives

(i) 2-nitropyridin-3-amine, NaOH, CaO, DMAC, 110 ℃; (ii) 3-nitropyridin-4-amine, NaOH, CaO, DMAC, 110 ℃;(iii) Pd/C, H_2, EtOH, r.t.; (iv) TCDI, Et_3N, THF, 60 ℃; (v) ester, K_2CO_3, DMF, r.t.; (vi) LiOH, THF, EtOH, r.t..



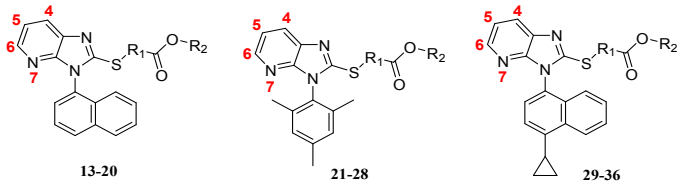
Scheme 5. Synthetic route to *N*-acylsulfonamide derivatives

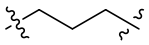
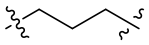
(i) cyclopropylboronic acid, $Ca_3(PO_4)_2$, $Pd(PPh_3)_4$, toluene/water (25:1), N_2, 100 ℃; (ii) di(1H-imidazol-1-yl)methanethione, CH_2Cl_2, r.t.; (iii) hydrazinecarboximidamide hydrochloride, DIEA, DMF, 50 ℃; (iv) methyl chloroacetate, K_2CO_3, DMF, r.t.; (v) $NaNO_2$, TEBA, $CHBr_3$, dichloroacetic acid, r.t.; (vi) LiOH, THF, EtOH, r.t.; (vii) sulfonamides, EDC·HCl, DMAP, DCM, 0 °C to r.t..

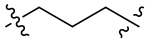
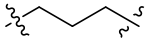
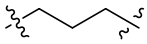
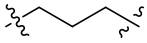
Results and Discussion

The activity of the synthesized compounds to lower serum uric acid (SUA) was evaluated using our established acute hyperuricemia model in mice, in which high SUA levels are maintained for more than 6 hours.[47] Benzbromarone and lesinurad were used as positive control drugs. The results are summarized in **Tables 1-4**.

Table 1. Structures of the series I compounds and serum uric acid concentrations in acute hyperuricemia model mice treated with these compounds



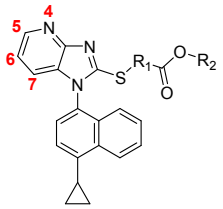
Compds	R_1	R_2	SUA (μM) [a,b]	Decrease ratio (DR) % [c]
13		Et	1187.60 ± 97.49	4.03
14		Et	815.40 ± 131.62	37.38
15		Et	1253.17 ± 92.61	-1.84
16		Et	1137.33 ± 125.76	8.54
17		H	1207.50 ± 130.25	2.25
18		H	1217.00 ± 136.92	1.40
19		H	1252.50 ± 75.27	-.178
20		H	1229.00 ± 150.16	0.32

21		Et	1255.20 ± 183.98	-2.20
22		Et	1107.75 ± 195.15	11.19
23		Et	983.25 ± 122.64	22.34
24		Et	768.80 ± 61.17	41.56
25		H	1028.75 ± 43.87	18.26
26		H	1087.00 ± 101.37	13.05
27		H	819.25 ± 100.77	37.04
28		H	1288.00 ± 211.53	-4.96
29		Et	764.33 ± 49.56	41.96
30		Et	967.80 ± 86.63	23.73
31		Et	782.40 ± 67.21	40.34
32		Et	1203.50 ± 94.83	2.61
33		H	745.00 ± 76.95	43.69
34		H	1220.67 ± 139.62	1.07
35		H	763.20 ± 248.75	42.06
36		H	1241.83 ± 36.75	-0.83

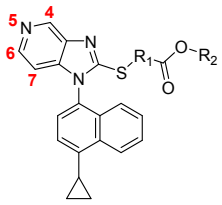
Model [a]	-	-	1232.60 ± 55.74	
Vehicle [b]	-	-	116.50 ± 9.85	
Benzbromarone	-	-	717.60 ± 65.35	46.14
Lesinurad	-	-	818.40 ± 87.15	37.11

[a] Model: mice with acute hyperuricemia induced with xanthine and potassium oxonate, and not treated with test compound (i.e., untreated hyperuricemic mice). [b] Vehicle: Mice not treated with xanthine and potassium oxonate, or test compound (i.e, untreated normal mice). [c] Decrease ratio = (Model SUA – Compound SUA) ÷ (Model SUA – Vehicle SUA)

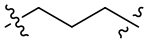
Table 2. Structures of the series II and III compounds and serum uric acid concentrations in acute hyperuricemia model mice treated with these compounds

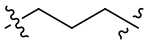
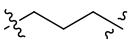
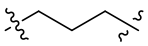


37-46



47-56

Compds	R_1	R_2	SUA (μM)	Decrease ratio (DR) % [c]
37		Et	777.20 ± 59.87	33.56
38		Et	776.80 ± 45.97	33.60
39		Et	759.40 ± 45.08	35.37

40		Et	658.80 ± 78.90	45.58
41		Et	760.70 ± 106.64	47.90
42		H	768.00 ± 39.05	34.50
43		H	759.20 ± 128.97	35.39
44		H	192.80 ± 49.81	92.89
45		H	994.80 ± 41.38	11.47
46		H	495.30 ± 57.42	62.18
47		Et	706.00 ± 46.34	40.79
48		Et	641.20 ± 94.12	47.37
49		Et	620.60 ± 123.7	49.46
50		Et	431.60 ± 114.6	68.65
51		Et	699.60 ± 48.61	41.35
52		H	692.40 ± 68.60	42.17
53		H	553.60 ± 126.7	56.26
54		H	225.00 ± 89.18	89.62

55		H	734.60 ± 142.0	37.89
56		H	388.80 ± 72.69	72.99
Model [a]	-	-	1108.00 ± 43.93	-
Vehicle [b]	-	-	122.80 ± 11.96	-
Benzbromarone	-	-	753.20 ± 32.86	36.00
Lesinurad	-	-	841.20 ± 55.16	27.06

[a] Model: mice with acute hyperuricemia induced with xanthine and potassium oxonate, and not treated with test compound (i.e., untreated hyperuricemic mice). [b] Vehicle: Mice not treated with xanthine and potassium oxonate, or test compound (i.e, untreated normal mice). [c] Decrease ratio = (Model SUA – Compound SUA) ÷ (Model SUA – Vehicle SUA)

First, we examined the effects of mesitylene, naphthalene and 1-cyclopropylnaphthalene substitution (zone B) at the imidazole nitrogen atom on the activity, in combination with a range of R_1 and R_2 substituents in the side chain (**13** - **36**). As shown in **Table 1**, some compounds exhibited high potency for reducing SUA. In particular, **24** (768.8 μM), **27** (768.8 μM), **29** (764.33 μM), **31** (782.40 μM), **33** (745.00 μM) and **35** (763.20 μM) were as potent as or more potent than lesinurad (818.40 μM). Examination of the SAR indicates that the order of potency for replacement of the aromatic ring was as follows: 1-cyclopropylnaphthalene > mesitylene > naphthalene.

Next, we retained the preferred 1-cyclopropylnaphthalene structure of Zone B, and applied a

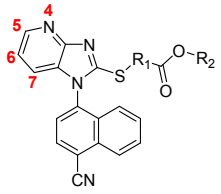
bioisosterism strategy to modify Zone A, using 1*H*-imidazole[4,5-*c*]pyridine (series II) and 1*H*-imidazole (series III) as replacements for 3*H*-imidazole[4,5-*b*]pyridine. As shown in **Table 2**, all the novel 1*H*-imidazole[4,5-*c*]pyridine and 1*H*-imidazole derivatives except **45** showed significant SUA-lowering activity, and eight of them were more active than lesinurad and benzbromarone. Among them, **44** (192.80 μM) and **54** (225.00 μM) were the most potent SUA-reducers, being over three times more potent than the reference drugs. SAR analysis indicated that the position of the N atom in the fused ring significantly influenced the activity. Thus, when the N atom is at the 4 or 5 position of pyridimazole, the potency of the derivatives is significantly greater than that of the counterparts with the N atom at the 7 position, and the 5-position is superior to the 4-position; for example, **48** > **38** > **30**, while **50** > **40** > **32**, **53** > **43** > **34** and **56** > **46** (**Table 2**).

We next investigated the effect of the side chain on the activity by altering its length and modifying the substituents (Zone C in **Figure 3**). We found that increasing the length of the side chain did not greatly affect the potency. For example, compounds **18**, **22**, **26**, **38**, **43** and **48**, which contain a propyl group, show comparable SUA-reducing activity to the corresponding short-chain analogues **17**, **21**, **25**, **37**, **42** and **47**, respectively. On the other hand, the presence of a methyl group or a dimethyl group on the carbon atom adjacent to the sulfur atom (R_1) did influence the activity. For example, among the acids (R_2 = H), methyl substitution was the best choice, since **27**, **35**, **44** and **54** were more potent SUA reducers than the unsubstituted or dimethyl-substituted compounds (**28**, **36**, **45** and **54**). On the other hand, among the esters (R_2 = Et), the order of potency for substitution at the side chain was as follows: $-C^*(CH_3)_2 > C^*HCH_3 > -CH_2-$ (**24** > **23** > **21**, **40** > **39** > **37**, and **50** > **49** > **47**). In other words, greater steric size of the substituent is

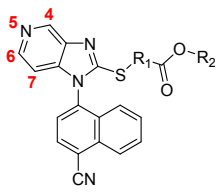
associated with greater SUA-reducing activity. A plausible explanation is that the conformation in the binding pocket of URAT1 might be stabilized via increased hydrophobic interaction.

Interestingly, most of the acid-containing compounds were more potent than the esters in the mouse model of acute hyperuricemia (e.g., **49** > **44**, **49** > **54**, **23** > **27**). This suggests that the esters were not hydrolyzed in this animal model, at least on the time scale of this experiment.

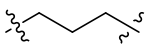
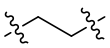
Table 3. Structures of the series II and III compounds and serum uric acid concentrations in acute hyperuricemia model mice treated with these compounds

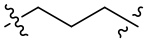
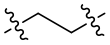
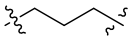
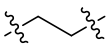
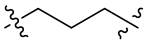
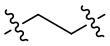


57-68



69-80

Compds	R_1	R_2	SUA (μM)	Decrease ratio (DR) %[c]
57		Me	970.60 ± 67.77	9.51
58		Me	992.80 ± 66.09	7.17
59		Me	1012.20 ± 82.74	5.12
60		Me	937.20 ± 82.21	13.03
61		Et	940.20 ± 61.73	12.71
62		Et	732.88 ± 166.66	34.56

63		H	568.20 ± 93.82	51.91
64		H	868.20 ± 54.99	20.30
65		H	381.20 ± 117.06	71.62
66		H	159.20 ± 19.61	95.01
67		H	1026.00 ± 73.51	3.67
68		H	484.40 ± 148.80	60.74
69		Me	922.80 ± 74.55	14.54
70		Me	975.80 ± 34.42	8.96
71		Me	952.00 ± 92.01	11.47
72		Me	891.00 ± 139.99	17.90
73		Me	974.60 ± 69.33	9.09
74		Et	923.00 ± 70.58	14.44
75		H	494.80 ± 121.30	59.22
76		H	954.60 ± 78.59	11.19
77		H	575.60 ± 61.80	51.13
78		H	796.60 ±40.44	27.84

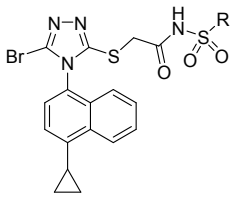
79		H	916.20 ± 50.08	15.24
80		H	801.20 ± 88.54	27.36
Model [a]	-	-	1060.83 ± 113.13	-
Vehicle [b]	-	-	111.83 ± 11.47	-
Benzbromarone	-	-	587.13 ± 66.86	49.94
Lesinurad	-	-	757.33 ± 93.05	31.93

[a] Model: mice with acute hyperuricemia induced with xanthine and potassium oxonate, and not treated with test compound (i.e., untreated hyperuricemic mice). [b] Vehicle: Mice not treated with xanthine and potassium oxonate, or test compound (i.e, untreated normal mice). [c] Decrease ratio = (Model SUA – Compound SUA) ÷ (Model SUA – Vehicle SUA)

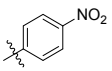
Next, we tested the activity of compounds bearing a cyano group instead of the cyclopropyl moiety (Series **IV**). As shown in Table 3, some compounds exhibited high potency in reducing SUA, but the overall activity was not significantly improved compared with that of compounds in Series II and III. Among them, **66** (159.20 μM), with the N atom was at the 4 position of pyridimazole, was the most potent derivative, being 2.8 times more potent than lesinurad. Meanwhile, most of the acid-containing compounds were more potent than the esters, which is consistent with the results in series I-III (e.g. **63** > **57**, **65** > **59**). Among the acids, replacement of the hydrogen in the *ortho*-position of sulfydryl resulted in more potent activity. However, among derivatives having the N atom at the 5 position of pyridimazole, most were inactive, and only a

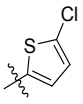
few compounds, such as compounds **75** (494.80 μM), **77** (575.60 μM) and **78** (796.60 μM), showed moderate SUA-reducing potency. Thus, the position of the N atom appears to be critical for the interaction of these compounds with URAT1.

Table 4. Structures of the series V compounds and serum uric acid concentrations in acute hyperuricemia model mice treated with these compounds



81-96

Compds	R	SUA (μM)	Decrease ratio (DR) %[c]
81		474.28 ± 40.84	64.65%
82		366.17 ± 43.93	75.17%
83		133.30 ± 49.72	97.84%
84		175.02 ± 53.97	93.78%
85		352.95 ± 41.47	76.46%
86		453.07 ± 69.66	66.71%
87		322.65 ± 66.18	79.41%
88		481.57 ± 60.43	63.94%

89		589.48 ± 108.17	53.44%
90		300.93 ± 34.21	81.53%
91		1025.08 ± 70.15	11.04%
92		891.30 ± 91.42	24.06%
93		1015.30 ± 89.90	11.99%
94		1077.43 ± 110.11	5.94%
95		958.25 ± 122.49	17.54%
96		983.80 ± 294.7	15.1%
Model [a]	-	1138.48 ± 125.93	-
Vehicle [b]	-	111.13 ± 13.89	-
Lesinurad	-	726.77 ± 105.94	40.07%

[a] Model: mice with acute hyperuricemia induced with xanthine and potassium oxonate, and not treated with test compound (i.e., untreated hyperuricemic mice). [b] Vehicle: Mice not treated with xanthine and potassium oxonate, or test compound (i.e, untreated normal mice). [c] Decrease ratio = (Model SUA – Compound SUA) ÷ (Model SUA – Vehicle SUA)

Finally, we tested the SUA-lowering activity of the newly synthesized acyl sulfonamide compounds *in vivo*. Ten compounds showed significant activity. Among them, compounds **83** and **84** were the most potent, reducing SUA to 133.30 μM and 175.02 μM, respectively (reduction

ratios of 97.84% and 93.78%, respectively).

Our results demonstrate that most of the compounds modified with an aromatic ring or a thiophene ring at the end of acylsulfonamide moiety have greater activity than those modified with a fatty acyl chain (**83** > **91**, **84** > **92**, etc.). For example, a benzene ring containing a substituent increased the potency: **83** > **81**, **84** > **81**, and **87** > **81**. The presence of halogens on the benzene ring resulted in much higher activity than the presence of a methyl group (**83** > **87**). Derivatives having a thiophene ring or a chlorothiophene ring also showed considerable activity.

In general, the modification of the acylsulfonamide moiety appears to be an effective approach to enhance the SUA-reducing activity of lead compounds.

Evaluation of serum uric acid-lowering activity in rats

Some representative derivatives were further evaluated for the ability to reduce SUA in another animal species (rats), in order to confirm their potency. Lesinurad was selected as positive control drug. The results are showed in **Table 5**.

Table 5. Serum uric acid concentrations in rats treated with compounds 44 and 54

Compds	SUA (μM)	Decrease ratio (DR) %
44	220.16 ± 82.75	83.96
54	240.42 ± 77.76	81.42
Lesinurad	467.32 ± 85.26	52.99
Model	890.30 ± 166.18	-
Vehicle	92.14 ± 17.66	-

Compounds **44** (220.16 μM) and **54** (240.42 μM) both appear to be promising candidates, being

about 1.6 times more potent than the reference drug lesinurad. The results in rats were consistent with those in mice.

Inhibition of human URAT1

We next examined the inhibition of human URAT1 by some representative compounds. As shown in **Table 6**, **44** inhibited the URAT1-mediated uptake of ^{14}C-UA with an IC_{50} of 1.57 μM, being more than 8-fold more potent than lesinurad (IC_{50} = 13.21 μM). The inhibitory activities of all the compounds tested were consistent with their relative activities in the acute hyperuricemia model in mice, supporting the idea that URAT1 is the target molecule of these compounds for hypouricemic activity, as expected. Furthermore, the representative acyl-sulfonamide compounds **83** and **84** showed good inhibitory activity against URAT1 with IC_{50} values of 4.17 μM and 7.29 μM respectively. Notably, **83** and **84** also exhibited significant inhibitory activity towards the transporter GLUT9, which plays an important synergistic role in the reabsorption of uric acid, with IC_{50} values of 31.73 μM and 32.20 μM, respectively. This may be one important reason why compounds **83** and **84** showed good activity *in vivo*. Thus, we have identified acylsulfonamide compounds as dual-target inhibitors.

Table 6. IC_{50} values of some representative compounds for URAT1-mediated ^{14}C-UA transport, and inhibitory activity towards GLUT9

Transporter	Substrate	Compds	IC_{50} (μM)
URAT1	^{14}C-UA	**Lesinurad**	13.21 ± 1.35

		35	>30
		54	6.14 ± 0.61
		44	1.57 ± 0.38
		83	4.17 ± 0.29
		84	7.29 ± 0.59
GLUT9	-	**83**	31.73 ± 3.92
	-	**84**	32.20 ± 2.26

CYP-Inhibitory Activity

Drug discovery efforts generally include in vitro assays early in the screening funnel for measurement of cytochrome P450 (CYP) inhibition. The most effective compounds **44**, **54** and **83** were evaluated for the ability to inhibit CYP drug-metabolizing enzymes in vitro. As summarized in **Table 7**, **44** displayed no significant inhibition of CYP1A2 (IC_{50} > 50.0 μM), CYP2C9 (IC_{50} = 20.0 μM), CYP2C19 (IC_{50} = 48.7 μM), CYP2D6 (IC_{50} > 50.0 μM) or CYP3A4M (IC_{50} > 50.0), and **54** also showed no significant inhibition of CYP1A2 (IC_{50} > 50.0 μM), CYP2C19 (IC_{50} > 50.0 μM), CYP2D6 (IC_{50} > 50.0 μM) or CYP3A4M (IC_{50} = 36.5), though it did inhibit CYP2C9 (IC_{50} = 2.39 μM). Similarly, compound **83** does not inhibit the activity of CYP1A2 (IC_{50} > 50.0 μM), CYP2C19 (IC_{50} = 20.6 μM) or CYP2D6 (IC_{50} > 50.0 μM). Importantly, more than half of all drugs are metabolized by CYP3A4, and thus inhibition of CYP3A4 by any new therapeutic is highly undesirable due to the potential for drug-drug interactions (DDI) in patients, the results of

investigation of the inhibition of hepatic CYP drug-metabolizing enzymes suggest that that **44**, **54** and **83** have low potential for hepatotoxicity and can be considered for further in vivo evaluation in rats.

Table 7. Inhibitory effects of 44 and 54 on CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4M

CYP isozyme	standard inhibitor	IC_{50} (μM)	Compd	IC_{50} (μM)	Compd	IC_{50} (μM)	Compd	IC_{50} (μM)
1A2	**α-Naphthoflavone**	0.216	**44**	> 50	**54**	> 50	**83**	> 50
2C9	**Sulfaphenazole**	0.609	**44**	20.0	**54**	2.39	**83**	0.636
2C19	**(+)-N-3-benzylnirvanol**	0.227	**44**	48.7	**54**	> 50	**83**	20.6
2D6	**Quinidine**	0.134	**44**	> 50	**54**	> 50	**83**	> 50
3A4M	**Ketoconazole**	0.0375	**44**	> 50	**54**	36.5	**83**	> 50

In Vivo Pharmacokinetic Study

The pharmacokinetic profiles of compounds **44**, **54** and **83** were examined in Wistar rats (**Table 8** and **Figure 5**). After a single 1 mg/kg iv dose of **44** and **54**, the half-life ($t_{1/2}$), mean clearance rate (CL) and mean residence time (MRT) values were 3.05 h, 264.0 mL h^{-1} kg^{-1}, 2.70 h and 2.27 h, 318.3 mL h^{-1} kg^{-1}, 2.86 h, respectively. At the dose of 20 mg/kg orally, compound **44** was rapidly absorbed with a time-to-maximum-concentration (T_{max}) of 2 h, a $t_{1/2}$ of 3.4 h, a MRT of 5.3 h, a maximum concentration (C_{max}) of 15.54 μg/mL, and an area under curve (AUC_{0-t}) of 53153.7 ng/mL•h. The corresponding values for **54** were 0.5 h, 3.1 h, and 4.1 h, 21.6 μg/mL and 58249.1 ng/mL•h. Notably, **44** and **54** exhibited extremely high oral bioavailability of 76.3% and 93.6%,

respectively. After a single 1 mg/kg iv dose of **83**, the $t_{1/2}$ value, CL value and MRT value were 5.05 h, 327.6 mL h^{-1} kg^{-1} and 5.05 h. Administration of **83** at 10 mg/kg resulted in T_{max} of 1.83 h with a C_{max} of 3390 ng/mL. The $t_{1/2}$ value of **83** was 3.43 h, and the MRT was 5.55 h. In addition, the oral bioavailability of **83** was 63.4%, which is sufficient for a drug candidate. Overall, **44**, **54** and **83** appear to have favorable drug-like properties.

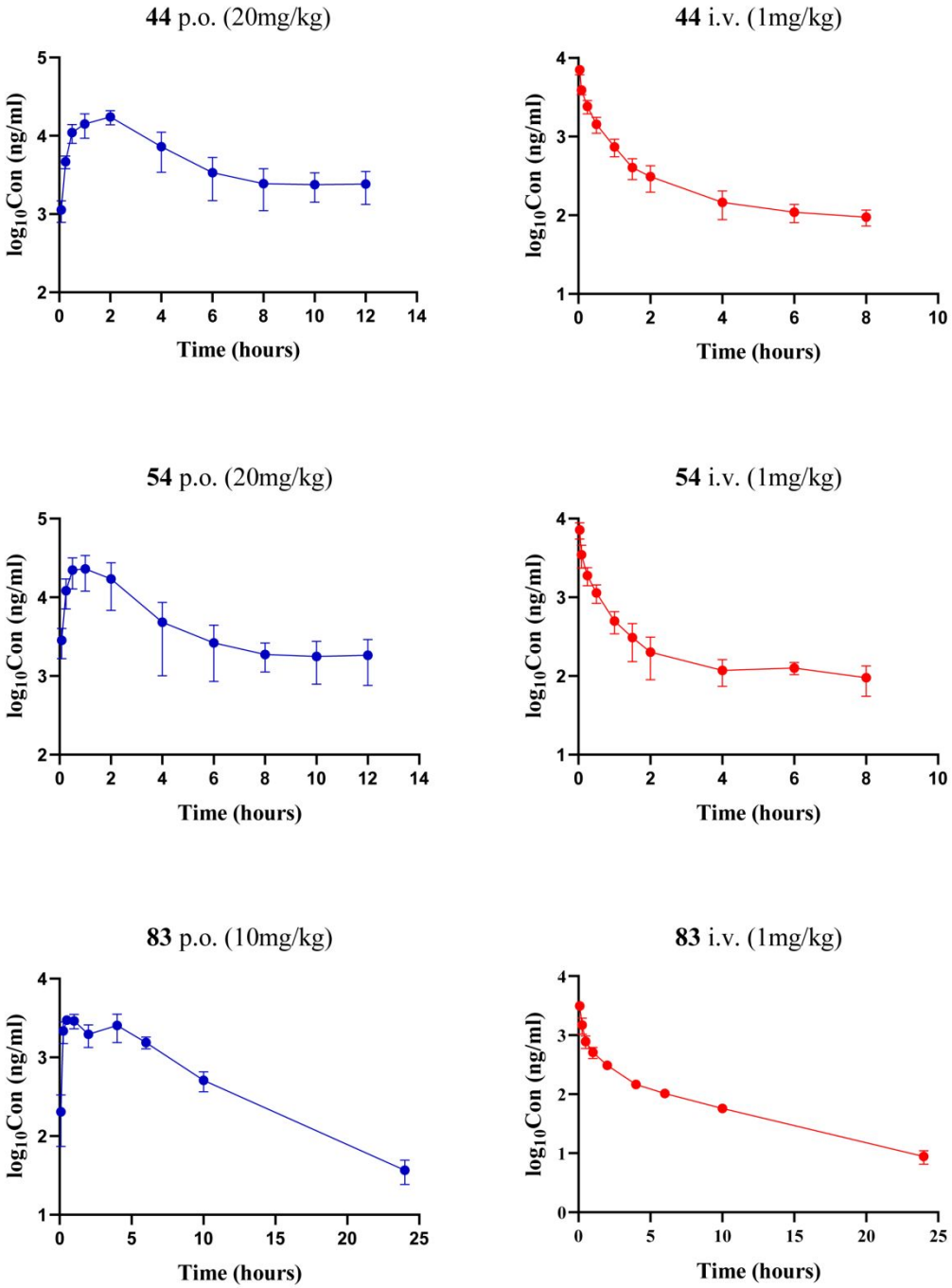


Figure 5. Plasma concentration−time profiles of **44**, **54** and **83** in rats following oral or intravenous administration.

Table 8. Pharmacokinetic parameters of 44, 54 and 83[a]

Parameter	Unit	p.o.		
Compound	-	**44**[b]	**54**[b]	**83**[c]
AUC(0-t)	ng/mL•h	53153.7	58249.1	19425.3
AUC(0-∞)	ng/mL•h	88846.0	65123.8	19612.7
MRT(0-∞)	h	5.3	4.6	5.6
$t_{1/2}$	h	3.4	3.1	3.4
T_{max}	h	2	0.5	1.8
C_{max}	μg/mL	15.5	21.6	3.4
F	%	76.3	93.6	63.4
Parameter	**Unit**	**i.v.**[d]		
Compounds	-	**44**	**54**	**83**
AUC(0-t)	ng/mL•h	3484.9	3111.7	3029.0
AUC(0-∞)	ng/mL•h	3911.3	3324.4	3094.3
MRT(0-∞)	h	2.7	2.86	4.3
$t_{1/2}$	h	3.1	2.3	5.0
CL	mL/h/kg	264.0	318.3	327.6

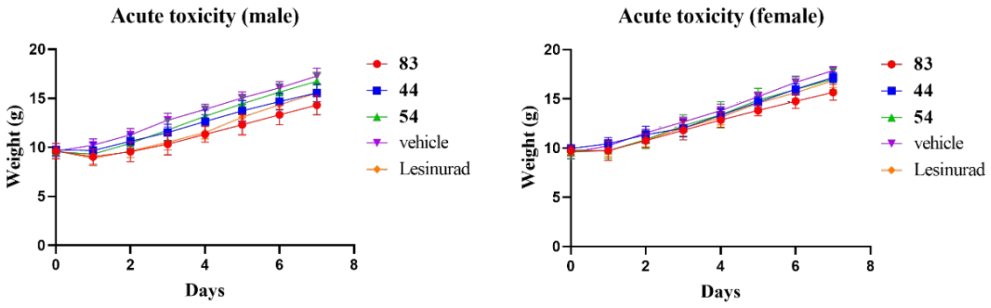
[a] PK parameters (mean ± SD, n = 5). [b] Dosed orally at 20 mg/kg. [c] Dosed orally at 10 mg/kg.

[d] Dosed intravenously at 1 mg/kg

Safety Assessment

Acute Toxicity Assessment of 44, 54 and 83 in Healthy Mice

To examine *in vivo* safety, we performed a single-dose acute toxicity test of **44, 54** and **83** in healthy Kunming mice. After intragastric administration of **44, 54** and **83** at the dose of 500 mg/kg, no mouse died up to one week, whereas 20% of the mice died in the group given 250 mg/kg lesinurad. These results suggest that the three candidate compounds show lower acute toxicity than the approved drug lesinurad, at least under the conditions of our study. We also observed no behavioral abnormality of the treated animals (lethargy, clonic convulsion, anorexia, or ruffled fur). In addition, there was no marked difference in weight gain of male or female mice over the 1-week period (**Figure 6**), compared with the vehicle control ($P > 0.05$), supporting the idea that **44, 54** and **83** show low toxicity.

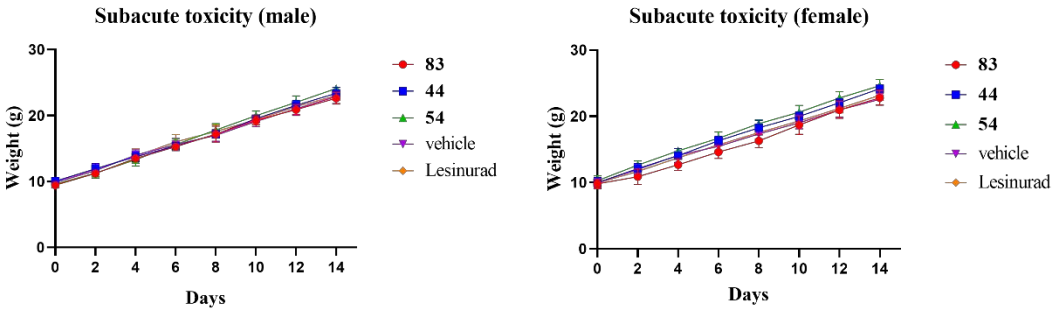


(A) (B)

Figure 6. Time courses of body weight of (A) male mice and (B) female mice after administration of test compounds.

Subacute Toxicity Assessment of 44, 54 and 83 in Healthy Mice

We next investigated the subacute toxicity of our three candidates in healthy mice. Each compound was administered p.o. at a dose of 50 mg·kg^{-1} every second day for 14 days. No deaths and no abnormal behaviors (lethargy, clonic convulsion, anorexia, or ruffled fur) were observed among the treated mice. Furthermore, the increase of body weight in test groups during the treatment period was the same as in the vehicle control ($P > 0.05$) for both males and females (**Figure 7**).



(A) (B)

Figure 7. Time courses of body weight of (A) male mice and (B) female mice after administration of test compounds.

Plasma Exposure to 44 and Lesinurad in KM Mice

The plasma exposure to compound **44** and lesinurad was examined in male KM mice (**Table 9 and Figure 8**). Each compound was administered p.o. at a dose of 2 mg/kg, 50 mg/kg or 500 mg/kg.

At the dose of 2 mg/kg, compound **44** was rapidly absorbed with a time-to-maximum-concentration (T_{max}) of 0.5 h, a half-life ($t_{1/2}$) of 4.24 h, and an area under the

curve (AUC) value of 12978 ng/mL•h. The corresponding values for lesinurad were 0.667 h, 3.08 h, and 7079 ng/mL•h. When the dose was 50 mg/kg, administration of **44** resulted in T_{max} of 1.67 h with a maximum concentration (C_{max}) of 37914 ng/mL. The $t_{1/2}$ value of **44** was 7.06 h, and the AUC value was 334901 ng/mL•h. Lesinurad exhibited an AUC value of 143855 ng/mL•h at the dose of 50 mg/kg, which is almost one-half that of compound **44**. We evaluated only **44** at the dose of 500 mg/kg, because most of the mice given lesinurad at this dose died. The AUC value of **44** reached 2958126 ng/mL•h, which is high for a drug candidate.

Notably, **44** was rapidly absorbed and the AUC value increased significantly with increasing dose. In addition, the concentration of **44** in the blood was much higher than that of Lesinurad at the same dose. These results suggest that compound **44** may have a wide therapeutic window, and a good safety profile, with low toxicity.

Table 9. Plasma exposure data of compounds 44 and lesinurad

Parameter	**Unit**	**p.o.**				
Dose	**-**	**2 mg/kg**		**50 mg/kg**		**500 mg/kg**
Compound	**-**	**44**	**Lesinurad**	**44**	**Lesinurad**	**44**
AUC(0-t)	ng/mL•h	12978	7079	334901	143855	2958126
AUC(0-∞)	ng/mL•h	13022	7118	335049	144648	2958248
MRT(0-∞)	h	6.44	5.09	6.16	6.35	15.7
$t_{1/2}$	h	4.24	3.08	7.06	7.46	3.90
T_{max}	h	0.500	0.667	1.67	1.0	18.7
C_{max}	ng/mL	2075	1497	37914	26469	148712

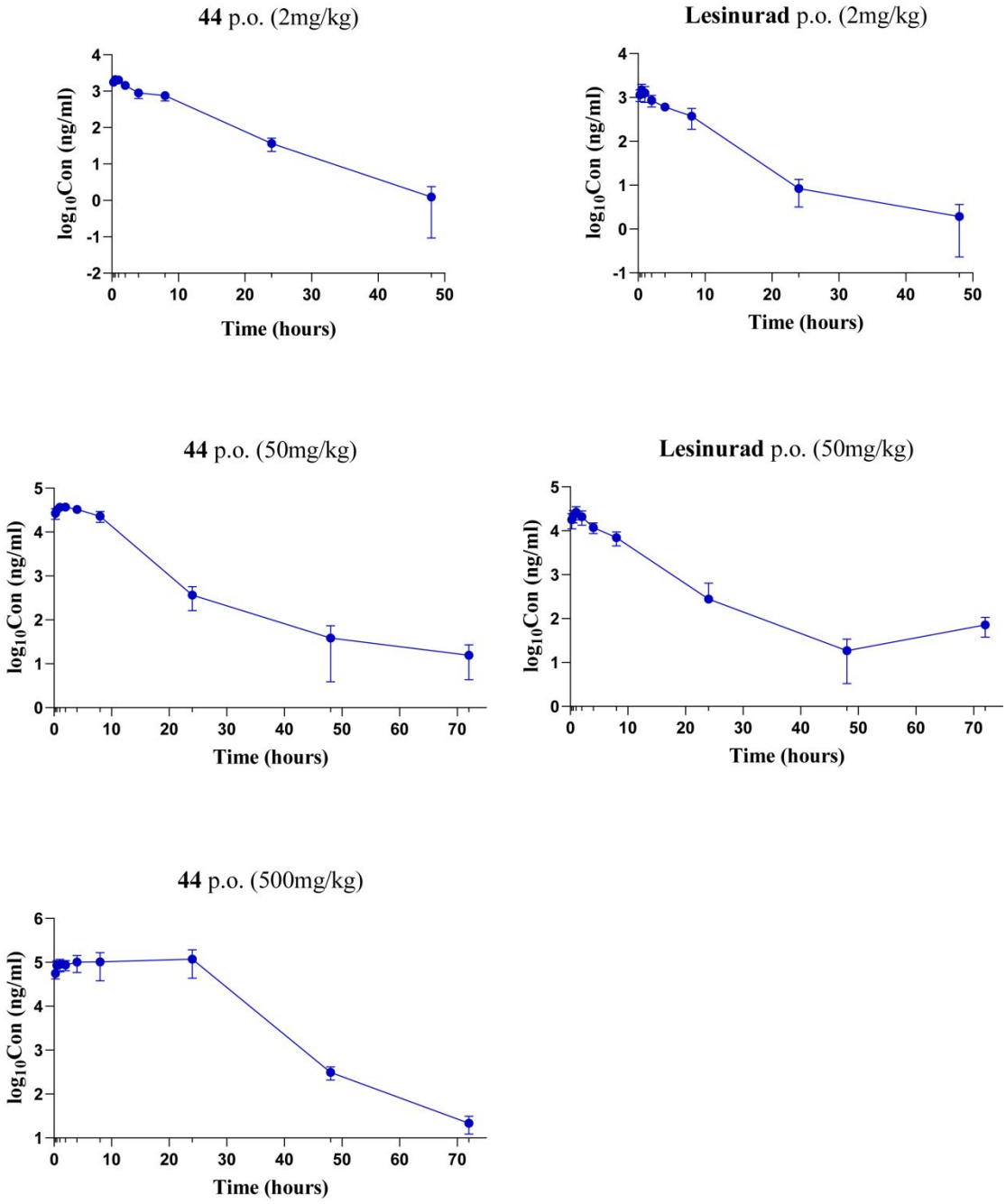


Figure 8. Plasma concentration−time profiles of **44** and lesinurad in mice following oral administration.

Hematoxylin and Eosin Staining

We also carried out hematoxylin and eosin staining of vital organs (**Figure 9**). No marked pathological abnormalities were seen, except in the lesinurad group. As indicated by the blue arrows, lesinurad caused mild congestion in the hepatic central vein, hydropic degeneration of hepatocytes, and extensive ballooning degeneration and hepatocellular condensation of the liver. We observed slight edema in the proximal tubules in the groups given lesinurad and compound **46**. All of these outcomes support the view that **44, 54** and **83** are promising, orally bioavailable candidates for treatment of hyperuricemia and gout.

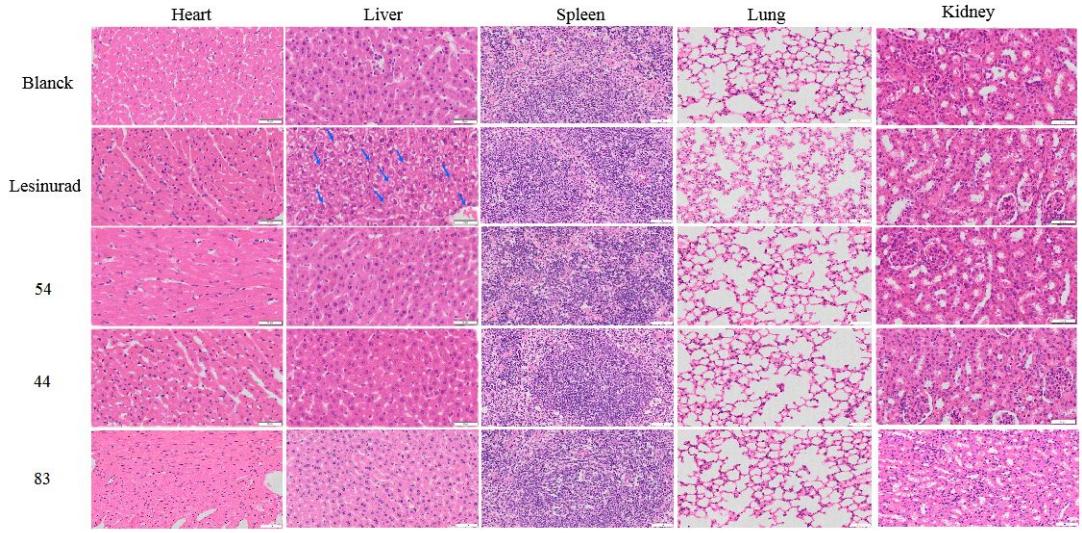


Figure 9. Histopathology study of treated mice. Heart, liver, and kidney were sectioned and stained with hematoxylin and eosin. Blue arrows indicate lesions in the liver.

Assessment of hERG-inhibitory Activity

Compounds with high affinity for the hERG potassium channels may show severe cardiotoxicity due to induction of prolonged QT intervals. Therefore, we tested the hERG-inhibitory activity of **44** *in vitro* using a manual patch-clamp method, with cisapride monohydrate as a reference drug.

Compound **44** did not inhibit the potassium channel (IC_{50} > 30 μM).

Chiral separation and *in vivo* activity evaluation of chiral isomers

The enantiomers of chiral compounds often have different pharmacological activities and toxicity. Therefore, we separated the enantiomers of the two chiral candidate compounds with the best activity and screened them separately (**Fig. 10**).

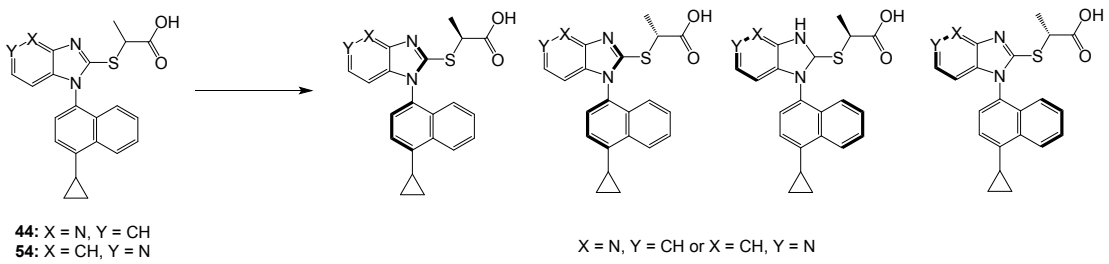


Figure 10. Chiral separation of compounds **44** and **54**

We used supercritical fluid chromatography (SFC) to separate compounds **44** and **54** under different conditions. Since there are not only chiral centers, but also chiral axes in the two derivatives, four distinct chiral isomers were obtained for each compound. The activity of these eight compounds was examined in the same way as described above (note that we have simply numbered these compounds **44a-d** and **54a-d** in the order of their elution).

The chiral isomers **44a-d** all had different *in vivo* activities, as shown in **Table 10**. Among them, **44d** (135.80 μM) exhibited the most potent SUA-lowering activity, which corresponded well to that of the racemic **44**, and the SUA was almost reduced to the blank level within 4 hours. Compounds **44b** and **44c** showed moderate SUA-reducing potency with SUA values of 669.60 μM and 758.40 μM respectively, while **44a** showed only weak activity. Similarly, among **54a-d**, **54c** showed the most potent SUA-reducing activity with an SUA value of 173.20 μM, which is

comparable to that of racemic **54**. The other three chiral isomers had weak to moderate activity.

Thus, chirality has a significant effect on the activity of the candidate compounds, and one of the four chiral isomers had potent *in vivo* activity in each case. The chirality may affect the binding mode to the target sites. Further study of this issue is underway.

Table 10. Serum uric acid concentrations in acute hyperuricemia model mice treated with chiral isomers of 44 and 54

Compound	SUA (μM)	Decrease ratio (DR) % [c]
44a	927.00±193.71	12.87
44b	669.60±102.81	40.58
44c	758.40±91.18	31.02
44d	135.80±12.93	98.04
54a	507.00±70.36	58.08
54b	745.20±168.21	32.44
54c	177.60±68.69	93.54
54d	508.80±148.06	57.89
44	173.20±54.44	94.02
54	206.00±73.16	90.48
Lesinurad	626.20±86.44	45.25
Model [a]	1046.60±98.85	-
Vehicle [b]	117.60±23.93	-

[a] Model: mice with acute hyperuricemia induced with xanthine and potassium oxonate, and not treated with test compound (i.e., untreated hyperuricemic mice). [b] Vehicle: Mice not treated with

xanthine and potassium oxonate, or test compound (i.e, untreated normal mice). [c] Decrease ratio = (Model SUA − Compound SUA) ÷ (Model SUA − Vehicle SUA)

Conclusion

We applied scaffold hopping, substituent decorating and bioisosterism strategies to the clinically used drug lesinurad as a lead compound, and explored the SAR of the heterocyclic core and peripheral substituents. The SAR indicated that methyl (or dimethyl) substitution and a carboxylic acid group in the side chain, with a 1-cyclopropylnaphthalene core, are favorable for SUA-lowering activity. Among the synthesized compounds, 21 derivatives showed potent SUA-lowering activity in a mouse model of hyperuricemia, being superior to lesinurad. Compounds **44**, **54** and **83** showed the greatest activity *in vivo*, and **44** was a markedly more potent inhibitor of URAT1 as compared with lesinurad (IC_{50} = 1.57 μM vs. 13.21 μM). An especially noteworthy finding is that **83** (IC_{50} for URAT1: 4.17 μM) also displayed potent inhibitory activity (IC_{50} = 31.73 μM) against GLUT9, which contributes to uric acid reabsorption together with URAT1. These three compounds displayed favorable pharmacokinetic properties and high oral bioavailability in rats. Furthermore, acute and subacute toxicity studies, assessment of hERG-inhibitory activity and histopathological study suggested that **44**, **54** and **83** are less toxic than lesinurad. Based on these *in vitro* and *in vivo* results, we consider that **44**, **54** and **83** are promising candidates for the treatment of hyperuricemia and gout.

Experimental Section

Chemistry

All melting points were determined on a micromelting point apparatus (RY-1G, Tianjin TianGuang Optical Instruments) and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded in DMSO-d_6 or $CDCl_3$ on a Bruker AV-400 spectrometer with tetramethylsilane (TMS) as the internal standard. Coupling constants are given in hertz, and chemical shifts are reported in δ values (ppm) from TMS; signals are abbreviated as s (singlet), d (doublet), t (triplet), q (quarter), and m (multiplet). A G1313A Standard LC autosampler (Agilent) was used to collect samples for measurement of mass spectra. The temperature of the reaction mixture was monitored with a mercury thermometer. All reactions were routinely monitored by thin layer chromatography (TLC) on silica gel GF254 for TLC (Merck), and spots were visualized with iodine vapor or by irradiation with UV light (λ = 254 nm). After completion of each reaction, the mixture was brought to ambient temperature via air-jet cooling. Flash column chromatography was performed on columns packed with silica gel (200−300 mesh), purchased from Qingdao Haiyang Chemical Company. Solvents were purified and dried by means of standard methods when necessary. Organic solutions were dried over anhydrous sodium sulfate and concentrated with a rotary evaporator under reduced pressure. Other reagents were obtained commercially and were used without further purification. Analysis of sample purity was performed on a Shimadzu SPD-20A/20AV HPLC system with a Inertsil ODS-SP, 5 μm C18 column (150 mm × 4.6 mm). HPLC conditions: methanol/water with 80:20; flow rate 1.0 mL/min; UV detection from 210 to 400 nm; temperature, ambient; injection volume, 10μL. The purity of representative final compounds was checked by high performance liquid chromatography (HPLC) and all was > 95%.

*N-(Naphthalen-1-yl)-3-nitropyridin-2-amine (**10a**).* A mixture of 2-chloro-3-nitropyridine (1 g,

6.33 mmol), 1-naphthylamine (1.81 g, 12.66 mmol), and potassium (0.55 g, 9.495 mmol) was heated at 120 °C for 12 h (monitored by TLC). After cooling to room temperature, 50 mL of water was added and the mixture was extracted with dichloromethane (3 × 10 mL). The organic phase was washed with saturated brine, dried over with anhydrous sodium sulfate and evaporated to dryness, The residue was recrystallized from ethanol-petroleum ether to give crude N-(naphthalen-1-yl)-3-nitropyridin-2-amine (**10a**) as a yellow solid. Yield 70.0%. Melting point: 154-156 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.26 (s, 1H, NH), 8.57 (dd, J = 8.3, 1.6 Hz, 1H, Pyr-H), 8.34 (dd, J = 4.4, 1.6 Hz, 1H, Pyr-H), 7.99 (d, J = 8.9 Hz, 1H, Naph-H), 7.94 (d, J = 8.8 Hz, 1H, Naph-H), 7.87 (d, J = 8.2 Hz, 1H, Naph-H), 7.75 (d, J = 7.3 Hz, 1H, Naph-H), 7.58-7.51 (m, 3H, Naph-H), 6.92 (dd, J = 8.3, 4.5 Hz, 1H, Pyr-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.8, 151.7, 135.8, 134.8, 134.3, 129.8, 129.0, 128.7, 126.8, 126.6, 126.5, 126.2, 124.0, 123.1, 114.4. $C_{15}H_{11}N_3O_2$ (Exact Mass: 265.0851).

*N2-(Naphthalen-1-yl)pyridine-2,3-diamine (**11a**)*. The intermediate **10a** (1 g, 3.77 mmol) was dissolved in ethanol (30 mL), and palladium 10% on carbon (0.1 g, 0.94 mmol) was added to the solution. The mixture was stirred under a hydrogen atmosphere at room temperature overnight (monitored by TLC), and then filtered. The filtrate was concentrated under reduced pressure. The residue was recrystallized from ethyl acetate to obtain N_2-(naphthalen-1-yl)pyridine-2,3-diamine (**11a**) as a white solid. Yield 85.3%. Melting point: 171-172.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.05 (d, J = 7.8 Hz, 1H, Naph-H), 7.88 (d, J = 8.9 Hz, 1H, Naph-H), 7.76 (s, 1H, NH), 7.57 (d, J = 4.3 Hz, 1H, Pyr-H), 7.55 (d, J = 3.3 Hz, 1H, Naph-H), 7.50-7.38 (m, 4H, Naph-H), 6.97 (dd, J = 7.6, 1.3 Hz, 1H, Pyr-H), 6.64 (dd, J = 7.6, 4.8 Hz, 1H, Pyr-H), 5.11 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 145.5, 138.3, 135.3, 134.5, 132.9, 128.5, 128.0, 126.3, 126.0, 125.3, 123.5,

122.5, 120.5, 118.1, 116.6. $C_{15}H_{13}N_3$ (Exact Mass: 235.1109).

*3-(Naphthalen-1-yl)-3H-imidazo[4,5-b]pyridine-2-thiol **(12a)***. N2-(Naphthalen-1-yl)pyridine-2,3-diamine (**11a**) (0.61 g, 2.6 mmol), potassium ethylxanthogenate (0.5 g, 3.1 mmol) and sodium bicarbonate (0.05 g, 0.6 mmol) were dissolved in 48 mL ethanol/water (5:1). The reaction mixture was refluxed for 5 h, and then cooled to room temperature. Water (10 ml) and 2 M sodium hydroxide solution (4 ml) were added, and the mixture was filtered. The pH value of the filtrate was adjusted to 7 with 2 M hydrochloric acid solution, and the resulting precipitate was collected by filtration to afford crude 3-(naphthalen-1-yl)-3H-imidazo[4,5-b]pyridine-2-thiol (**12a**) as a white solid. Yield 92.8%. Melting point: 241-244 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.37 (s, 1H, SH), 8.16 (d, J = 8.3 Hz, 1H, Pyr-H), 8.10 (d, J = 8.2 Hz, 1H, Naph-H), 8.01 (dd, J = 5.0, 1.3 Hz, 1H, Pyr-H), 7.74-7.68 (m, 2H, Naph-H), 7.63-7.57 (m, 2H, Naph-H), 7.49-7.45 (m, 1H, Pyr-H), 7.28-7.24 (m, 2H, Naph-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.6, 147.6, 142.9, 134.4, 131.6, 130.4, 130.1, 128.8, 128.5, 127.5, 126.9, 126.2, 125.46, 123.2, 119.7, 117.4. $C_{16}H_{11}N_3S$ (Exact Mass: 277.07).

*N-Mesityl-3-nitropyridin-2-amine (**10b**)*. The synthetic method was similar to that described for **10a,** except that the starting material 2-chloro-3-nitropyridine (1.00 g, 6.33 mmol) was reacted with trimethylaniline. Yellow solid, yield 67.1%. Melting point: 158-160 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H, NH), 8.50 (dd, J = 8.3, 1.7 Hz, 1H, Pyr-H), 8.32 (dd, J = 4.4, 1.7 Hz, 1H, Pyr-H), 6.94 (s, 2H, Ph-H), 6.83 (dd, J = 8.3, 4.4 Hz, 1H, Pyr-H), 2.27 (s, 3H, CH_3), 2.06 (s, 6H, 2 × CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.3, 151.6, 136.1, 135.9, 135.8, 133.9, 128.8, 128.2, 113.3, 21.0, 18.5. $C_{14}H_{15}N_3O_2$ (Exact Mass: 257.1164).

*N2-Mesitylpyridine-2,3-diamine (**11b**)*. The synthetic method was similar to that described for **11a**. White solid, yield 70.8%. Melting point: 172.5-174 ° C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.19 (dd, J = 4.9, 1.4 Hz, 1H, Pyr-H), 6.85 (s, 2H, Ph-H), 6.81 (s, H, NH), 6.78-6.76 (m, 2H, Pyr-H), 6.40 (dd, J = 7.4, 4.9 Hz, 1H), 4.90 (s, 2H, NH_2), 2.23 (s, 3H CH_3), 2.04 (s, 6H, 2 × CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.6, 136.5, 135.5, 135.1, 133.9, 130.9, 128.7, 118.6, 113.9, 20.9, 18.7. $C_{14}H_{17}N_3$ (Exact Mass: 227.1422).

*3-Mesityl-3H-imidazo[4,5-b]pyridine-2-thiol (**12b**)*. The synthetic method was similar to that described for **12a**. White solid, Yield 75.1%. Melting point: 274-280 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.23 (s, 1H, SH), 8.07 (dd, J = 5.0, 1.3 Hz, 1H, Pyr-H), 7.63 (dd, J = 7.9, 1.3 Hz, 1H, Pyr-H), 7.24 (dd, J = 7.9, 5.0 Hz, 1H, Pyr-H), 7.06 (s, 2H, Ph-H), 2.33 (s, 3H, CH_3), 1.85 (s, 6H, 2 × CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.0, 146.0, 143.1, 139.0, 136.6, 130.4, 129.2, 125.3, 119.5, 117.4, 21.1, 17.9. $C_{15}H_{15}N_3S$ (Exact Mass: 269.0987).

*N-(4-Cyclopropylnaphthalen-1-yl)-3-nitropyridin-2-amine (**10c**)*. The synthetic method was similar to that described for **10a** except that the starting material 2-chloro-3-nitropyridine (1 g, 6.33 mmol) was reacted with 4-cyclopropyl-1-naphthylamine (**9c**). Yellow solid, yield 68.8%. Melting point: 159-160.5℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.16 (s, 1H, NH), 8.55 (dd, J = 8.3, 1.7 Hz, 1H, Pyr-H), 8.46 (d, J = 8.3 Hz, 1H, Naph-H), 8.30 (dd, J = 4.4, 1.7 Hz, 1H, Pyr-H), 7.95 (d, J = 8.2 Hz, 1H, Naph-H), 7.64-7.52 (m, 3H, Naph-H), 7.30 (d, J = 7.6 Hz, 1H, Naph-H), 6.89 (dd, J = 8.3, 4.5 Hz, 1H, Pyr-H), 2.46-2.39 (m, 1H, CH), 1.10-1.06 (m, 2H, CH_2), 0.77-0.74 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.8, 151.9, 137.7, 135.8, 134.0, 133.2, 130.0, 128.9, 126.6, 126.5, 125.0, 124.0, 123.7, 123.5, 114.2, 13.3, 7.1. $C_{18}H_{15}N_3O_2$ (Exact Mass: 305.1164).

*N2-(4-Cyclopropylnaphthalen-1-yl)pyridine-2,3-diamine (**11c**)*. The synthetic method was similar to that described for **11a**. White solid, yield 56.4%. Melting point: 171-172 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.39 (d, J = 8.3 Hz, 1H, Naph-H), 8.03 (d, J = 8.3 Hz, 1H, Naph-H), 7.71 (s, 1H, NH), 7.56 (t, J = 8.0 Hz, 1H, Naph-H), 7.47 (t, J = 7.6 Hz, 1H, Naph-H), 7.41 (d, J = 7.7 Hz, 1H, Naph-H), 7.31 (dd, J = 4.8, 1.4 Hz, 1H, Pyr-H), 7.20 (d, J = 7.7 Hz, 1H, Naph-H), 6.94 (dd, J = 7.6, 1.5 Hz, 1H, Pyr-H), 6.59 (dd, J = 7.5, 4.8 Hz, 1H, Pyr-H), 5.12 (s, 2H, NH_2), 2.37-2.30 (m, 1H, CH), 1.05-1.00 (m, 2H, CH_2), 0.71-0.67 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.0, 136.6, 135.0, 134.1, 133.7, 132.5, 128.7, 126.0, 125.2, 124.8, 124.2, 123.9, 120.2, 119.0, 116.1, 13.2, 6.8. $C_{18}H_{17}N_3$ (Exact Mass: 275.1422).

*3-(4-Cyclopropylnaphthalen-1-yl)-3H-imidazo[4,5-b]pyridine-2-thiol (**12c**)*. The synthetic method was similar to that described for **12a**. White solid, yield 86.2%. Melting point: 294-296 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.36 (s, 1H, SH), 8.54 (d, J = 8.5 Hz, 1H, Pyr-H), 7.99 (dd, J = 5.0, 1.3 Hz, 1H, Pyr-H), 7.66 (ddd, J = 16.7, 8.1, 1.2 Hz, 2H, Naph-H), 7.50-7.42 (m, 3H, Naph-H + Pyr-H), 7.26-7.23 (m, 2H, Naph-H), 2.57-2.52 (m, 1H, CH), 1.19-1.11 (m, 2H, CH2), 0.90-0.81 (m, 2H, CH2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.7, 147.6, 142.8, 141.4, 134.1, 130.3, 129.9, 128.1, 127.2, 126.9, 125.4, 125.2, 123.7, 123.3, 119.6, 117.4, 13.4, 7.5, 7.3. $C_{19}H_{15}N_3S$ (Exact Mass: 317.0987).

*General Procedure for the Preparation of **13-16**, **21-24** and **29-32***. Compound **12a** (or **12b**, **12c**) was dissolved in DMF (10 mL) in the presence of potassium carbonate (0.31 g, 2.232 mmol), followed by addition of the appropriate substituted benzyl chloride (bromide) (1.1 equiv). After stirring for 15 min, the appropriate substituted ester (2.787 mmol) was added dropwise and the mixture was stirred at room temperature for 4 h (monitored by TLC). The solvent was evaporated

under reduced pressure and the residue was taken up in ethyl acetate (30 mL). The organic solution was washed with saturated aqueous sodium chloride solution (3 × 10 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was purified by flash column chromatography. The product was recrystallized from ethyl acetate (EA) to afford the target compounds **13-16**, **21-24** and **29-32**.

Ethyl 2-((3-(naphthalen-1-yl)-3H-imidazo[4,5-b]pyridin-2-yl)thio)acetate ***(13)***. Recrystallized from EA as a white solid, yield 67.1%, Melting point: 116.5-117 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.25 (d, J = 8.8 Hz, 1H, Pyr-H), 8.16 (d, J = 8.2 Hz, 1H, Naph-H), 8.12-8.09 (m, 2H, Naph-H), 7.78-7.72 (m, 2H, Naph-H), 7.64 (t, J = 8.0 Hz, 1H, Naph-H), 7.51 (t, J = 8.0 Hz, 1H, Pyr-H), 7.31 (dd, J = 7.9, 4.9 Hz, 1H, Naph-H), 7.09 (d, J = 8.4 Hz, 1H, Pyr-H), 4.25 (d, J = 2.6 Hz, 2H, CH_2), 4.13 (q, J = 7.1 Hz, 2H, CH_2), 1.18 (t, J = 7.1 Hz, 3H, CH_3).^{13}C NMR (100 MHz, DMSO-d_6) δ 168.5, 154.7, 150.9, 143.3, 135.4, 134.5, 131.0, 130.1, 130.1, 129.0, 128.1, 127.9, 127.4, 126.3, 125.8, 122.5, 119.1, 61.7, 33.4, 14.4. HR-MS: m/z 364.1111 $[M + H]^+$. $C_{20}H_{17}N_3O_2S$ (Exact Mass: 363.1041). HPLC purity: 99.19 %.

E*thyl 4-((3-(naphthalen-1-yl)-3H-imidazo[4,5-b]pyridin-2-yl)thio)butanoate* ***(14)***. Recrystallized from EA as a white solid, yield 72.3%, Melting point: 100-103 ° C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (dd, J = 6.7, 2.6 Hz, 1H, Pyr-H), 8.15-8.09 (m, 3H, Naph-H), 7.76-7.71 (m, 2H, Naph-H), 7.62 (t, J = 7.9 Hz, 1H, Naph-H), 7.49 (t, J = 7.7 Hz, 1H, Pyr-H), 7.30 (dd, J = 7.9, 4.9 Hz, 1H, Naph-H), 7.05 (d, J = 8.4 Hz, 1H, Pyr-H), 4.02 (q, J = 7.1 Hz, 2H, CH_2), 3.37-3.33 (m, 2H, CH_2), 2.39 (t, J = 7.3 Hz, 2H, CH_2), 1.99 (p, J = 7.2 Hz, 2H, CH_2), 1.14 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.6, 155.5, 150.9, 143.1, 135.5, 134.4, 130.8, 130.4, 130.2, 129.0, 128.1, 127.9, 127.3, 126.3, 125.6, 122.5, 119.0, 60.3, 32.6, 30.6, 24.7,

14.5. HR-MS: m/z 392.1431 $[M + H]^+$. $C_{22}H_{21}N_3O_2S$ (Exact Mass: 391.1354). HPLC purity: 99.17%.

Ethyl 2-((3-(naphthalen-1-yl)-3H-imidazo[4,5-b]pyridin-2-yl)thio)propanoate ***(15)***. Recrystallized from EA as a white solid, yield 69.5%, Melting point: 144-145 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (dd, J = 6.6, 2.6 Hz, 1H, Pyr-H), 8.16-8.11 (t, 3H, Naph-H), 7.77-7.70 (m, 2H, Naph-H), 7.64 (t, J = 8.0 Hz, 1H, Naph-H), 7.50 (t, J = 8.2 Hz, 1H, Pyr-H), 7.34 – 7.31 (m, 1H, Naph-H), 7.05 (t, J = 7.7 Hz, 1H, Pyr-H), 4.77-4.70 (m, 1H, CH), 4.09 (q, J = 7.1 Hz, 2H, CH_2), 1.57 (t, J = 7.6 Hz, 3H, CH_3), 1.19-1.08 (m, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.3, 153.8, 150.7, 143.5, 135.4, 134.4, 131.0, 130.2, 130.1, 129.0, 128.1, 127.9, 127.4, 126.2, 125.9, 122.4, 119.2, 61.7, 43.6, 18.2, 14.3. HR-MS: m/z 378.1275 $[M + H]^+$. $C_{21}H_{19}N_3O_2S$ (Exact Mass: 377.1189). HPLC purity: 98.75%.

Ethyl 2-methyl-2-((3-(naphthalen-1-yl)-3H-imidazo[4,5-b]pyridin-2-yl)thio)propanoate ***(16)***. Recrystallized from EA as a white solid, yield 72.6%, Melting point: 135-136 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (d, J = 8.1 Hz, 1H, Pyr-H), 8.15-8.09 (m, 3H, Naph-H), 7.74 (t, J = 7.7 Hz, 1H, Naph-H), 7.68 (d, J = 6.9 Hz, 1H, Naph-H), 7.63 (t, J = 7.4 Hz, 1H, Naph-H), 7.50 (t, J = 7.5 Hz, 1H, Pyr-H), 7.31 (dd, J = 8.0, 4.8 Hz, 1H, Naph-H), 7.00 (d, J = 8.4 Hz, 1H, Pyr-H), 4.12-4.10 (m, 2H, CH_2), 1.70 (s, 3H, CH_3), 1.63 (s, 3H, CH_3), 1.10 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8, 153.1, 150.1, 143.7, 135.4, 134.4, 130.8, 130.3, 130.2, 129.0, 128.1, 127.9, 127.4, 126.2, 126.1, 122.4, 119.2, 61.7, 53.1, 26.7 (2 × C), 14.30. HR-MS: m/z 392.1433 $[M + H]^+$. $C_{22}H_{21}N_3O_2S$ (Exact Mass: 391.1354). HPLC purity: 97.89%.

Ethyl 2-((3-mesityl-3H-imidazo[4,5-b]pyridin-2-yl)thio)acetate ***(21)***.Recrystallized from EA as

a white solid, yield 67.1%, Melting point: 115-116.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (dd, J = 4.8, 1.4 Hz, 1H, Pyr-H), 8.02 (dd, J = 8.0, 1.4 Hz, 1H, Pyr-H), 7.27 (dd, J = 8.0, 4.8 Hz, 1H, Pyr-H), 7.14 (s, 2H, PhH), 4.26 (s, 2H, CH_2), 4.13 (q, J = 7.1 Hz, 2H, CH_2), 2.36 (s, 3H, CH_3), 1.85 (s, 6H, 2 × CH_3), 1.17 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.5, 153.9, 149.3, 143.3, 140.1, 136.8, 135.4, 129.7, 129.2, 125.6, 118.8, 61.7, 32.9, 21.2, 17.5, 14.4. HR-MS: m/z 356.1429 $[M + H]^+$. $C_{19}H_{21}N_3O_2S$ (Exact Mass: 355.1354). HPLC purity: 98.87%.

Ethyl 4-((3-mesityl-3H-imidazo[4,5-b]pyridin-2-yl)thio)butanoate ***(22)***. Purified by flash column chromatography as a yellow oil, yield 71.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (dd, J = 4.8, 1.4 Hz, 1H, Pyr-H), 8.03 (dd, J = 8.0, 1.4 Hz, 1H, Pyr-H), 7.27 (dd, J = 8.0, 4.8 Hz, 1H, Pyr-H), 7.12 (s, 2H, Ph-H), 4.04 (q, J = 7.1 Hz, 2H, CH_2), 3.38-3.34 (2H, CH_2), 2.43 (t, J = 7.3 Hz, 2H, CH_2), 2.35 (s, 3H, CH_3), 2.00 (p, J = 7.2 Hz, 2H, CH_2), 1.81 (s, 6H, 2 × CH_3), 1.15 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.6, 154.5, 149.3, 143.1, 139.9, 136.7, 135.5, 129.6, 129.4, 125.4, 118.7, 60.3, 32.6, 30.1, 24.9, 21.1, 17.5, 14.5. HR-MS: m/z 384.1744 $[M + H]^+$. $C_{21}H_{25}N_3O_2S$ (Exact Mass: 383.1667). HPLC purity: 99.86%.

Ethyl 2-((3-mesityl-3H-imidazo[4,5-b]pyridin-2-yl)thio)propanoate ***(23)***. Recrystallized from EA as a white solid, yield 68.5%. Melting point: 70-70.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (dd, J = 4.8, 1.4 Hz, 1H, Pyr-H), 8.03 (dd, J = 8.0, 1.4 Hz, 1H, Pyr-H), 7.28 (dd, J = 8.0, 4.8 Hz, 1H, Pyr-H), 7.13 (s, 2H, Ph-H), 4.73 (q, J = 7.3 Hz, 1H, CH), 4.12 (q, J = 7.1 Hz, 2H, CH_2), 2.35 (s, 3H, CH_3), 1.83 (s, 6H, 2 × CH_3), 1.60 (d, J = 7.3 Hz, 3H, CH_3), 1.14 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.3, 153.1, 149.0, 143.5, 140.1, 136.8, 136.7, 135.4, 129.8, 129.7, 129.2, 125.7, 118.9, 61.8, 43.0, 21.1, 18.1, 17.6, 17.5, 14.3. HR-MS: m/z 370.1582

$[M + H]^+$. $C_{20}H_{23}N_3O_2S$ (Exact Mass: 369.1511). HPLC purity: 97.34%.

Ethyl 2-((3-mesityl-3H-imidazo[4,5-b]pyridin-2-yl)thio)-2-methylpropanoate ***(24)***. Recrystallized from EA as a white solid, yield 73.6%. Melting point: 93-95 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (dd, J = 4.8, 1.3 Hz, 1H, Pyr-H), 7.99 (dd, J = 8.0, 1.3 Hz, 1H, Pyr-H), 7.26 (dd, J = 8.0, 4.8 Hz, 1H, Pyr-H), 7.12 (s, 2H, Ph-H), 4.10 (q, J = 7.1 Hz, 2H, CH_2), 2.35 (s, 3H, CH_3), 1.82 (s, 6H, 2 × CH_3), 1.73 (s, 6H, 2 × CH_3), 1.05 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9, 152.8, 148.5, 143.4, 140.0, 136.7, 135.5, 129.6, 129.3, 125.6, 118.8, 61.7, 52.5, 26.8, 21.1, 17.5, 14.2. HR-MS: m/z 384.1742 $[M + H]^+$. $C_{21}H_{25}N_3O_2S$ (383.1667). HPLC purity: 96.69%.

Ethyl 2-((3-(4-cyclopropylnaphthalen-1-yl)-3H-imidazo[4,5-b]pyridin-2-yl)thio)acetate ***(29)***. Recrystallized from EA as a white solid, yield 67.1%. Melting point: 141-143.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (d, J = 8.5 Hz, 1H, Pyr-H), 8.10 (s, 1H, Naph-H), 8.08 (d, J = 1.8 Hz, 1H, Naph-H), 7.70 (t, J = 8.2 Hz, 1H, Naph-H), 7.61 (d, J = 7.6 Hz, 1H, Pyr-H), 7.51 (t, J = 7.6 Hz, 1H, Pyr-H), 7.47 (d, J = 7.5 Hz, 1H, Naph-H), 7.31-7.28 (m, 1H, Naph-H), 7.07 (d, J = 8.3 Hz, 1H, Naph-H), 4.24 (d, J = 2.3 Hz, 2H, CH_2), 4.13 (q, J = 7.0 Hz, 2H, CH_2), 2.61-2.54 (m, 1H, CH), 1.20-1.15 (m, 5H, CH_2+CH_3), 0.93-0.82 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.5, 154.9, 151.0, 143.3, 142.5, 135.3, 134.2, 130.0, 128.3, 127.8, 127.5, 127.3, 125.8, 125.4, 123.3, 123.1, 119.1, 61.7, 33.4, 14.4, 13.4, 7.7, 7.4. HR-MS: m/z 404.1432 $[M + H]^+$. $C_{23}H_{21}N_3O_2S$ (Exact Mass: 403.1354). HPLC purity: 98.61%.

Ethyl 4-((3-(4-cyclopropylnaphthalen-1-yl)-3H-imidazo[4,5-b]pyridin-2-yl)thio)butanoate ***(30)***. Recrystallized from EA as a white solid, yield 71.9%. Melting point: 79-80.5 °C. ^{1}H NMR (400

MHz, DMSO-d_6) δ 8.57 (d, J = 8.5 Hz, 1H, Pyr-H), 8.11-8.06 (m, 2H, Naph-H), 7.68 (ddd, J = 8.3, 6.9, 1.1 Hz, 1H, Naph-H), 7.60 (d, J = 7.6 Hz, 1H, Naph-H), 7.51-7.47 (m, 1H, Pyr-H), 7.44 (d, J = 7.5 Hz, 1H, Naph-H), 7.29 (dd, J = 8.0, 4.9 Hz, 1H, Pyr-H), 7.03 (d, J = 8.3 Hz, 1H, Naph-H), 4.02 (q, J = 7.1 Hz, 2H, CH_2), 3.36-3.32 (m, 2H, CH_2), 2.59-2.54 (d, J = 22.2 Hz, 1H, CH), 2.39 (t, J = 7.3 Hz, 2H, CH_2), 2.02-1.95 (m, 2H, CH_2), 1.17-1.11 (m, 5H, CH_2+CH_3), 0.91-0.81 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.6, 155.6, 151.0, 143.1, 142.3, 135.5, 134.1, 130.1, 128.6, 127.8, 127.6, 127.3, 125.5, 125.4, 123.2, 123.0, 118.9, 60.3, 40.6, 40.4, 40.1, 39.9, 39.7, 39.5, 39.3, 32.6, 30.5, 24.7, 14.5, 13.4, 7.7, 7.4. HR-MS: m/z 432.1742 $[M + H]^+$. $C_{25}H_{25}N_3O_2S$ (Exact Mass: 431.1667). HPLC purity: 99.66%.

*Ethyl 2-((3-(4-cyclopropylnaphthalen-1-yl)-3H-imidazo[4,5-b]pyridin-2-yl)thio)propanoate **(31)***. Recrystallized from EA as a white solid, yield 68.5%. Melting point: 111.5-112 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (d, J = 8.5 Hz, 1H, Pyr-H), 8.10 (d, J = 7.1 Hz, 2H, Naph-H), 7.69 (t, J = 8.2 Hz, 1H, Naph-H), 7.59 (t, J = 8.2 Hz, 1H, Naph-H), 7.50 (t, J = 8.3 Hz, 1H, Naph-H), 7.45 (dd, J = 7.5, 3.1 Hz, 1H, Pyr-H), 7.32-7.29 (m, 1H, Pyr-H), 7.03 (t, J = 7.4 Hz, 1H, Naph-H), 4.75-4.66 (m, 1H, CH), 4.18-4.06 (m, 2H, CH_2), 2.59-2.53 (m, 1H, CH), 1.55 (t, J = 7.2 Hz, 3H, CH_3), 1.18-1.10 (m, 5H, CH_2 + CH_3), 0.91-0.82 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.3, 153.9, 150.7, 143.5, 142.6, 135.4, 134.1, 130.0, 128.3, 127.9, 127.6, 127.4, 125.9, 125.4, 123.2, 122.9, 119.2, 61.8, 43.5, 18.1, 14.3, 13.4, 7.7, 7.4. HR-MS: m/z 418.1589 $[M + H]^+$. $C_{24}H_{23}N_3O_2S$ (Exact Mass: 417.1511). HPLC purity: 97.17%.

*Ethyl 2-((3-(4-cyclopropylnaphthalen-1-yl)-3H-imidazo[4,5-b]pyridin-2-yl)thio)-2-methylpropanoate **(32)***. Recrystallized from EA as a white solid, yield 73.6%. Melting point: 117.5-118 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (d, J = 8.5 Hz, 1H, Pyr-H), 8.11-8.07 (m,

2H, Naph-H), 7.71-7.67 (m, 1H, Naph-H), 7.56 (d, J = 7.6 Hz, 1H, Naph-H), 7.51 (t, J = 8.1 Hz, 1H, Pyr-H), 7.45 (d, J = 7.6 Hz, 1H, Naph-H), 7.30 (dd, J = 8.0, 4.8 Hz, 1H, Pyr-H), 6.98 (d, J = 8.3 Hz, 1H, Naph-H), 4.14-4.06 (m, 2H, CH_2), 2.60-2.53 (m, 1H, CH), 1.70 (s, 3H, CH_3), 1.63 (s, 3H, CH_3), 1.19-1.14 (m, 2H, CH_2), 1.10 (t, J = 7.1 Hz, 3H, CH_3), 0.93-0.81 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8, 153.3, 150.2, 143.6, 142.4, 135.4, 134.1, 130.1, 128.6, 127.8, 127.5, 127.3, 126.0, 125.4, 123.2, 122.9, 119.2, 61.7, 53.0, 26.8, 26.7, 14.3, 13.4, 7.7, 7.4. HR-MS: m/z 432.1735 $[M + H]^+$. $C_{25}H_{25}N_3O_2S$ (Exact Mass: 431.1667). HPLC purity: 99.41%.

*General Procedure for the Preparation of **17-20, 25-28** and **33-36**.* Compound **13-16, 21-24** or **29-32** was dissolved in a mixture of 5 mL tetrahydrofuran and 5 mL ethanol. Lithium hydroxide (0.2 g, 8.26 mmol) was dissolved in a small amount of water and added dropwise to the above solution, and then the mixture was stirred at 0 °C for 1 h. After the reaction was completed, the solvent was removed by rotary evaporation under reduced pressure. 10 mL of water was added to the residue, and 2 M HCl solution was added dropwise to adjust the pH to 3-4. The product was collected by filtration and recrystallized from ethanol to obtain the target compound.

*2-((3-(Naphthalen-1-yl)-3H-imidazo[4,5-b]pyridin-2-yl)thio)acetic acid (**17**).* Recrystallized from EA as a white solid, yield 87.1%. Melting point: 129-130 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (d, J = 7.6 Hz, 1H), 8.10-8.16 (m, 3H), 7.80-7.69 (m, 2H), 7.64 (t, J = 7.4 Hz, 1H), 7.50 (t, J = 7.6 Hz, 1H), 7.31 (dd, J = 7.8, 5.0 Hz, 1H), 7.09 (d, J = 8.4 Hz, 1H), 4.19 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.7, 155.1, 150.9, 143.2, 135.4, 134.5, 130.9, 130.2, 130.1,129.0, 128.1, 127.9, 127.5, 126.3, 125.7, 122.6, 119.1, 34.0. HR-MS: m/z 336.0800 $[M + H]^+$. $C_{18}H_{13}N_3O_2S$ (Exact Mass: 335.0728). HPLC purity: 99.70%.

*4-((3-(Naphthalen-1-yl)-3H-imidazo[4,5-b]pyridin-2-yl)thio)butanoic acid (**18**).* Recrystallized from EA as a white solid, yield 92.3%. Melting point: 129-130 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.13 (s, 1H, OH), 8.22 (dd, J = 6.6, 2.7 Hz, 1H, Pyr-H), 8.15-8.08 (m, 3H, Naph-H), 7.76-7.71 (m, 2H, Naph-H), 7.62 (t, J = 7.9 Hz, 1H, Naph-H), 7.49 (t, J = 8.1 Hz, 1H, Pyr-H), 7.30 (dd, J = 8.0, 4.9 Hz, 1H, Naph-H), 7.05 (d, J = 8.4 Hz, 1H, Pyr-H), 3.36-3.33 (m, 2H, CH_2), 2.33 (t, J = 7.3 Hz, 2H, CH_2), 2.00-1.95 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.2, 155.5, 150.9, 143.1, 135.5, 134.4, 130.8, 130.4, 130.2, 129.0, 128.1, 128.0, 127.3, 126.3, 125.6, 122.5, 119.0, 32.8, 30.7, 24.7. HR-MS: m/z 364.1118 $[M + H]^+$. $C_{20}H_{17}N_3O_2S$ (Exact Mass: 363.1041). HPLC purity: 99.90%.

2-((3-(Naphthalen-1-yl)-3H-imidazo[4,5-b]pyridin-2-yl)thio)propanoic acid (***19***). Recrystallized from EA as a white solid, yield 91.5%. Melting point: 110-112 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (d, J = 7.5 Hz, 1H, Pyr-H), 8.15-8.11 (m, 3H, Naph-H), 7.76-7.70 (m, 2H, Naph-H), 7.63 (t, J = 7.5 Hz, 1H, Naph-H), 7.52-7.47 (m, 1H, Pyr-H), 7.31 (dd, J = 7.9, 4.9 Hz, 1H, Naph-H), 7.06 (d, J = 8.5 Hz, 1H, Pyr-H), 4.68 (qd, J = 7.2, 2.5 Hz, 1H, CH), 1.59 (dd, J = 18.5, 7.2 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8, 154.4, 150.7, 143.4, 135.5, 134.4, 130.9, 130.2, 129.0, 128.2, 127.9, 127.9, 127.4, 126.3, 125.8, 122.5, 119.1, 44.7, 18.9. HR-MS: m/z 350.0954 $[M + H]^+$. $C_{19}H_{15}N_3O_2S$ (Exact Mass: 349.0885). HPLC purity: 99.89%.

2-Methyl-2-((3-(naphthalen-1-yl)-3H-imidazo[4,5-b]pyridin-2-yl)thio)propanoic acid (***20***). Recrystallized from EA as a white solid, yield 93.7%. Melting point: 165-166 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (d, J = 8.2 Hz, 1H, Pyr-H), 8.15-8.11 (m, 3H, Naph-H), 7.73 (t, J = 7.7 Hz, 1H, Naph-H), 7.67 (dd, J = 7.2, 1.1 Hz, 1H, Naph-H), 7.62 (t, J = 8.0 Hz, 1H, Naph-H), 7.49 (t, J = 8.1 Hz, 1H, Pyr-H), 7.33-7.30 (m, 1H, Naph-H), 7.00 (d, J = 8.4 Hz, 1H, Pyr-H), 1.68 (s,

6H, 2 × CH_3).^{13}C NMR (100 MHz, DMSO-d_6) δ 174.4, 153.4, 150.1, 143.7, 135.5, 134.4, 130.7, 130.5, 130.3, 128.9, 128.1, 128.0, 127.3, 126.2, 126.1, 122.4, 119.2, 53.9, 26.8, 26.8. HR-MS: m/z 364.1116 $[M + H]^+$. $C_{20}H_{17}N_3O_2S$ (Exact Mass: 363.1041). HPLC purity: 99.90%.

2-((3-Mesityl-3H-imidazo[4,5-b]pyridin-2-yl)thio)acetic acid (***25***). Recrystallized from EA as a white solid, yield 94.2%. Melting point: 190-200.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (dd, J = 4.8, 1.4 Hz, 1H, Pyr-H), 8.03 (dd, J = 8.0, 1.4 Hz, 1H, Pyr-H), 7.27 (dd, J = 8.0, 4.8 Hz, 1H, Pyr-H), 7.14 (s, 2H, Ph-H), 4.19 (s, 2H, CH_2), 2.36 (s, 3H, CH_3), 1.85 (s, 6H, 2 × CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.7, 154.2, 149.3, 143.2, 140.0, 136.9, 135.4, 129.6, 129.3, 125.5, 118.7, 33.4, 21.2, 17.5. HR-MS: m/z 328.1116 $[M + H]^+$. $C_{17}H_{21}N_3O_2S$ (Exact Mass: 327.1041). HPLC purity: 99.34%.

*4-((3-Mesityl-3H-imidazo[4,5-b]pyridin-2-yl)thio)butanoic acid (**26**)*. Recrystallized from EA as a white solid, yield 90.0%. Melting point: 138-140 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (dd, J = 4.8, 1.3 Hz, 1H, Pyr-H), 8.03 (dd, J = 8.0, 1.3 Hz, 1H, Pyr-H), 7.27 (dd, J = 8.0, 4.8 Hz, 1H, Pyr-H), 7.12 (s, 2H, Ph-H), 3.47-3.42 (m, 2H, CH_2), 2.38-2.35 (m, 5H, CH_2+CH_3), 2.01-1.94 (m, 2H, CH_2), 1.82 (s, 6H, 2 × CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.2, 154.6, 149.3, 143.1, 139.9, 136.7, 135.6, 129.6, 129.4, 125.4, 118.7, 32.8, 30.2, 24.9, 21.1, 17.5. HR-MS: m/z 356.1432 $[M + H]^+$. $C_{19}H_{21}N_3O_2S$ (Exact Mass: 355.1354). HPLC purity: 98.74%.

*2-((3-Mesityl-3H-imidazo[4,5-b]pyridin-2-yl)thio)propanoic acid (**27**)*. Recrystallized from EA as a white solid, yield 93.3%. Melting point: 148-149.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.14 (s, 1H, OH), 8.16 (dd, J = 4.8, 1.3 Hz, 1H, Pyr-H), 8.05 (dd, J = 8.0, 1.3 Hz, 1H, Pyr-H), 7.28 (dd, J = 8.0, 4.8 Hz, 1H, Pyr-H), 7.13 (s, 2H, Ph-H), 4.69 (q, J = 7.2 Hz, 1H, CH), 2.36 (s, 3H, CH_3), 1.83 (s, 6H, 2 × CH_3), 1.61 (d, J = 7.3 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ

172.8, 153.5, 149.1, 143.4, 140.0, 136.8, 136.7, 135.5, 129.7, 129.6, 129.3, 125.7, 118.8, 43.7, 21.1, 18.6, 17.5, 17.5. HR-MS: m/z 342.1275 $[M + H]^+$. $C_{18}H_{19}N_3O_2S$ (Exact Mass: 341.1198). HPLC purity: 99.43%.

*2-((3-Mesityl-3H-imidazo[4,5-b]pyridin-2-yl)thio)-2-methylpropanoic acid (**28**).* Recrystallized from EA as a white solid, yield 93.9%. Melting point: 180-183 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (dd, J = 4.8, 1.4 Hz, 1H, Pyr-H), 8.02 (dd, J = 8.0, 1.4 Hz, 1H, Pyr-H), 7.26 (dd, J = 8.0, 4.8 Hz, 1H, Pyr-H), 7.11 (s, 2H, Ph-H), 2.35 (s, 3H, CH_3), 1.81 (s, 6H, 2 × CH_3), 1.74 (s, 6H, 2 × CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.4, 153.1, 148.5, 143.4, 139.9, 136.7, 135.5, 129.6, 125.7, 118.7, 53.1, 26.8, 21.1, 17.5. HR-MS: m/z 356.1428 $[M + H]^+$. $C_{19}H_{21}N_3O_2S$ (Exact Mass: 355.1354). HPLC purity: 99.76%.

*2-((3-(4-Cyclopropylnaphthalen-1-yl)-3H-imidazo[4,5-b]pyridin-2-yl)thio)acetic acid (**33**).* Recrystallized from EA as a white solid, yield 94.2%. Melting point: 202-204 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.99 (s, 1H, OH), 8.59 (d, J = 8.5 Hz, 1H, Pyr-H), 8.11-8.08 (d, J = 13.0 Hz, 2H, Naph-H), 7.70 (ddd, J = 8.3, 6.9, 1.0 Hz, 1H, Naph-H), 7.61 (d, J = 7.6 Hz, 1H, Naph-H), 7.53-7.49 (m, 1H, Pyr-H), 7.46 (d, J = 7.6 Hz, 1H, Naph-H), 7.30 (dd, J = 7.9, 4.9 Hz, 1H, Pyr-H), 7.07 (d, J = 8.3 Hz, 1H, Naph-H), 4.18 (s, 2H, CH_2), 2.61-2.54 (m, 1H, CH), 1.19-1.14 (m, 2H, CH_2), 0.93-0.83 (d, J = 42.1 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.7, 155.2, 151.0, 143.2, 142.5, 135.4, 134.1, 130.0, 128.4, 127.8, 127.5, 127.3, 125.7, 125.4, 123.3, 123.1, 119.0, 33.9, 13.4, 7.7, 7.4. HR-MS: m/z 376.1110 $[M + H]^+$. $C_{21}H_{17}N_3O_2S$ (Exact Mass: 375.1041). HPLC purity: 99.41%.

*4-((3-(4-Cyclopropylnaphthalen-1-yl)-3H-imidazo[4,5-b]pyridin-2-yl)thio)butanoic acid (**34**).*

Recrystallized from EA as a white solid, yield 90.9%. Melting point: 103-105 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.57 (d, J = 8.5 Hz, 1H, Pyr-H), 8.12-8.07 (m, 2H, Naph-H), 7.68 (t, J = 8.1 Hz, 1H, Naph-H), 7.60 (d, J = 7.6 Hz, 1H, Naph-H), 7.50 (t, J = 7.9 Hz, 1H, Pyr-H), 7.44 (d, J = 7.6 Hz, 1H, Naph-H), 7.30 (dd, J = 8.0, 4.9 Hz, 1H, Pyr-H), 7.04 (d, J = 8.4 Hz, 1H, Naph-H), 4.05-4.00 (m, 2H, CH_2), 2.59-2.54 (m, 1H, CH), 2.32 (t, J = 7.3 Hz, 2H, CH_2), 1.99-1.92 (m, 2H, CH_2), 1.18-1.14 (m, 2H, CH_2), 0.91-0.81 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.2, 155.7, 150.9, 143.1, 142.4, 135.4, 134.1, 130.1, 128.5, 127.8, 127.6, 127.3, 125.5, 125.4, 123.3, 123.0, 119.0, 32.8, 30.7, 24.7, 13.4, 7.7, 7.4. HR-MS: m/z 404.1433 $[M + H]^+$. $C_{23}H_{21}N_3O_2S$ (Exact Mass: 403.1354). HPLC purity: 99.15%.

*2-((3-(4-Cyclopropylnaphthalen-1-yl)-3H-imidazo[4,5-b]pyridin-2-yl)thio)propanoic acid (**35**).* Recrystallized from EA as a white solid, yield 93.3%. Melting point: 122-128 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.18 (s, 1H, OH), 8.58 (d, J = 8.5 Hz, 1H, Naph-H), 8.14-8.09 (m, 2H, Naph-H), 7.69 (t, J = 7.7 Hz, 1H, Naph-H), 7.61 (dd, J = 7.6, 4.3 Hz, 1H, Pyr-H), 7.53-7.49 (m, 1H, Naph-H), 7.46 (dd, J = 7.6, 2.4 Hz, 1H, Pyr-H), 7.31 (ddd, J = 8.0, 4.9, 0.6 Hz, 1H, Naph-H), 7.05 (d, J = 8.4 Hz, 1H, Pyr-H), 4.69 (qd, J = 7.2, 3.1 Hz, 1H, CH), 2.60-2.55 (m, 1H, CH), 1.59 (dd, J = 17.5, 7.2 Hz, 3H, CH_3), 1.18-1.14 (m, 2H, CH_2), 0.92-0.83 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8, 154.4, 150.7, 143.4, 142.5, 135.4, 134.1, 130.0, 128.4, 127.9, 127.5, 127.3, 125.8, 125.4, 123.3, 123.0, 119.1, 44.3, 18.8, 13.4, 7.7, 7.4. HR-MS: m/z 390.1273 $[M + H]^+$. $C_{22}H_{19}N_3O_2S$ (Exact Mass: 389.1198). HPLC purity: 99.30%.

2-((3-(4-Cyclopropylnaphthalen-1-yl)-3H-imidazo[4,5-b]pyridin-2-yl)thio)-2-methylpropanoic acid (***36***). Recrystallized from EA as a white solid, yield 93.9%. Melting point: 171-175 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.57 (d, J = 8.5 Hz, 1H, Pyr-H), 8.11 (d, J = 1.0 Hz, 1H, Naph-H),

8.10 (s, 1H, Naph-H), 7.68 (t, J = 8.2 Hz, 1H, Pyr-H), 7.55 (d, J = 7.6 Hz, 1H, Naph-H), 7.50 (t, J = 7.6 Hz, 1H, Naph-H), 7.44 (d, J = 7.6 Hz, 1H, Naph-H), 7.32-7.29 (m, 1H, Pyr-H), 6.99 (d, J = 8.3 Hz, 1H, Naph-H), 2.59-2.53 (m, 1H, CH), 1.68 (s, 6H, 2 × CH_3), 1.18-1.14 (m, 2H, CH_2), 0.93-0.81 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.4, 153.5, 150.2, 143.6, 142.2, 135.4, 134.1, 130.2, 128.7, 127.8, 127.6, 127.2, 126.1, 125.4, 123.2, 123.0, 119.1, 53.6, 26.8, 13.4, 7.7, 7.3. HR-MS: m/z 404.1428 $[M + H]^+$. $C_{23}H_{21}N_3O_2S$ (Exact Mass: 403.1354). HPLC purity: 99.27%.

*N-(4-Cyclopropylnaphthalen-1-yl)-3-nitropyridin-4-amine (**10e**).* 1-Naphthylamine (20.0 g, 90 mmol), cyclopropylboronic acid (10.0 g, 116 mmol), phosphoric acid (64.0 g, 300 mmol) and palladium tetrakistriphenylphosphine (7.0 g, 6 mmol) were added to 100 mL toluene and 4 mL water, and the mixture was heated at 100 ° C for 12 h under nitrogen. After the reaction was completed, the mixture was cooled to room temperature and 100 mL H_2O was added to it. The resulting mixture was extracted with ethyl acetate (EA) and the organic solution was dried over sodium sulfate, filtered and concentrated under reduced pressure to give 13.8 g of crude 4-cyclopropyl-1-naphthylamine, yield 83.6%. Chloropyridine (1 g, 6.33 mmol), 4-cyclopropyl-1-naphthylamine (1.4 g, 7.6 mmol) and sodium bicarbonate (1.6 g, 18.9 mmol) were dissolved in 50 mL ethanol, and the solution was refluxed at 60 ° C for 10 h, then cooled to room temperature. Dichloromethane (30 ml) was added, and the mixture was washed with saturated sodium chloride (3 × 10 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. Flash column chromatography of the residue gave N-(4-cyclopropylnaphthalen-1-yl)-3-nitro-4-amine (**10e**). Yellow solid, yield 69.8%. Melting point: 116-118 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.06 (s, 1H, NH), 9.14 (s, 1H,

Pyr-H), 8.51 (d, *J* = 8.3 Hz, 1H, Pyr-H), 8.08 (d, *J* = 6.1 Hz, 1H, Naph-H), 7.91 (d, *J* = 8.1 Hz, 1H, Pyr-H), 7.69-7.65 (m, 1H, Naph-H), 7.59-7.55 (m, 1H, Naph-H), 7.45 (d, *J* = 7.6 Hz, 1H, Naph-H), 7.36 (d, *J* = 7.6 Hz, 1H, Naph-H), 6.29 (s, 1H, Naph-H), 2.50-2.45 (m, 1H, CH), 1.14-1.09 (m, 2H, CH_2), 0.81-0.77 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.3, 148.8, 148.5, 139.8, 134.3, 132.2, 130.3, 130.1, 127.2, 127.1, 125.5, 125.3, 123.7, 123.6, 110.4, 13.3, 7.3 (2 × C). ESI-MS: m/z 306.4 $[M+H]^+$. $C_{18}H_{15}N_3O_2$ (Exact Mass: 305.12).

*N4-(4-Cyclopropylnaphthalen-1-yl)pyridine-3,4-diamine (**11e**).* The intermediate **10e** (1 g, 3.28 mmol) was dissolved in ethanol (30 mL) and palladium on carbon (0.1 g) was added to the solution. The mixture was stirred under a hydrogen atmosphere at room temperature overnight, then filtered and concentrated under reduced pressure. The residue was recrystallized from EA to give N_4-(4-cyclopropylnaphthalen-1-yl)pyridine-3,4-diamine (**11e**) as a white solid. Yield 76.2%. Melting point: 192-193 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 (d, *J* = 8.2 Hz, 1H, Naph-H), 7.98 (d, *J* = 7.9 Hz, 1H, Naph-H), 7.89 (s, 1H, NH), 7.63-7.48 (m, 4H, Naph-H), 7.25 (d, *J* = 7.6 Hz, 1H, Pyr-H), 7.19 (d, *J* = 7.6 Hz, 1H, Pyr-H), 6.23 (d, *J* = 5.3 Hz, 1H, Pyr-H), 4.99 (s, 2H, NH_2), 2.37 (m, 1H, CH), 1.08-1.06 (dm, 2H, CH_2), 0.74-0.70 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 139.4, 139.3, 136.4, 135.9, 135.5, 134.4, 133.1, 129.0, 126.6, 125.9, 125.1, 124.0, 120.2, 108.9, 56.5, 13.2, 7.0 (2 × C). ESI-MS: m/z 276.4 $[M+H]^+$. $C_{18}H_{17}N_3$ (Exact Mass: 275.14).

1-(4-Cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-c]pyridine-2-thiol (***12e***). A solution of N_4-(4-cyclopropylnaphthalen-1-yl)pyridine-3,4-diamine (**11e**) (0.61 g, 2.6 mmol), potassium ethylxanthogenate (0.5 g, 3.1 mmol) and sodium bicarbonate (0.05 g, 0.6 mmol) in 12 mL ethanol/water (5:1) was refluxed for 5 h, and cooled to room temperature. Then 5 mL water and 2 mL 2 M sodium hydroxide solution were added, and the mixture was filtered. The pH of the

filtrate was adjusted to 7 with 2M hydrochloric acid solution, and the resulting precipitate was collected by filtration to afford 1-(4-cyclopropylnaphthalen-1-yl)-1*H*-imidazo[4,5-*c*]pyridine-2-thiol (**12e**). White solid. Yield 79.8%. Melting point: 199.5-201 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.89 (s, 1H, Pyr-H), 8.59 (d, J = 8.5 Hz, 1H, Pyr-H), 8.42 (d, J = 6.3 Hz, 1H, Naph-H), 7.71 (t, J = 8.0 Hz, 1H, Naph-H), 7.63 (d, J = 7.6 Hz, 1H, Naph-H), 7.54 (t, J = 7.4 Hz, 1H, Naph-H), 7.48 (d, J = 7.6 Hz, 1H, Naph-H), 7.42 (d, J = 8.4 Hz, 1H, Pyr-H), 7.05 (d, J = 6.3 Hz, 1H, Naph-H), 2.60-2.54 (m, 1H, CH), 1.18-1.15 (m, 2H, CH_2), 0.89-0.86 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.2, 145.0, 142.7, 137.5, 134.2, 130.9, 129.3, 128.5, 127.8, 127.7, 127.4, 125.5, 123.9, 123.4, 123.3, 106.4, 13.4, 7.7, 7.6. ESI-MS: m/z 318.2 $[M+H]^+$. $C_{18}H_{17}N_3$ (Exact Mass: 317.10).

General procedure for the preparation of **47-51**. The synthetic method was similar to that described for **13-16** except that the starting material 1-(4-cyclopropylnaphthalen-1-yl)-1*H*-imidazo[4,5-*c*]pyridine-2-thiol (**12e**) was reacted with appropriate substituted esters.

*Ethyl 2-((1-(4-cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)acetate (**47**).* Recrystallized from EA as a white solid, yield 76.1%. Melting point: 117-120 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.97 (s, 1H, Pyr-H), 8.61 (d, J = 8.5 Hz, 1H, Pyr-H), 8.22 (d, J = 5.5 Hz, 1H, Naph-H), 7.72 (t, J = 7.7 Hz, 1H, Naph-H), 7.66 (d, J = 7.6 Hz, 1H, Naph-H), 7.55 (t, J = 7.6 Hz, 1H, Naph-H), 7.48 (d, J = 7.6 Hz, 1H, Naph-H), 7.05 (d, J = 8.2 Hz, 1H, Pyr-H), 6.90 (dd, J = 5.5, 0.9 Hz, 1H, Naph-H), 4.24 (d, J = 1.9 Hz, 2H, CH_2), 4.13 (q, J = 7.0 Hz, 2H, CH_2), 2.62-2.55 (m, 1H, CH), 1.20-1.15 (m, 5H, CH_2+CH_3), 0.90-0.87 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.4, 155.4, 143.0, 143.0, 142.6, 140.5, 140.3, 134.3, 129.4, 128.2, 128.0, 127.6, 127.1, 125.7,

123.3, 122.6, 105.6, 61.7, 34.0, 14.4, 13.4, 7.8, 7.7. HR-MS: m/z 404.1425 $[M+H]^+$. $C_{23}H_{21}N_3O_2S$ (Exact Mass: 403.1354). HPLC purity: 95.79%.

*Ethyl 4-((1-(4-cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)butanoate (**48**).* Purified by flash column chromatography as a yellow oil, yield 69.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.98 (s, 1H, Pyr-H), 8.59 (d, J = 8.5 Hz, 1H, Pyr-H), 8.21 (d, J = 5.5 Hz, 1H, Naph-H), 7.70 (t, J = 7.7 Hz, 1H, Naph-H), 7.65 (d, J = 7.6 Hz, 1H, Naph-H), 7.52 (t, J = 7.7 Hz, 1H, Naph-H), 7.45 (d, J = 7.6 Hz, 1H, Naph-H), 7.01 (d, J = 8.3 Hz, 1H, Pyr-H), 6.86 (d, J = 6.3 Hz, 1H, Naph-H), 4.02 (q, J = 7.1 Hz, 2H, CH_2), 3.34 (t, J = 7.1 Hz, 2H, CH_2), 2.60-2.53 (m, 1H, CH), 2.38 (t, J = 7.3 Hz, 2H, CH_2), 1.98 (p, J = 7.2 Hz, 2H, CH_2), 1.18-1.11 (m, 5H, CH_2 + CH_3), 0.89-0.86 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.6, 156.1, 143.0, 142.8, 142.4, 140.7, 140.1, 134.2, 129.4, 128.3, 128.1, 127.5, 127.2, 125.6, 123.3, 122.5, 105.5, 60.3, 32.5, 31.2, 24.6, 14.5, 13.4, 7.7, 7.6. HR-MS: m/z 432.1743 $[M + H]^+$. $C_{25}H_{25}N_3O_2S$ (Exact Mass: 431.1667). HPLC purity: 98.86%.

*Ethyl 2-((1-(4-cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)propanoate (**49**).* Purified by flash column chromatography as a yellow oil, yield 68.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99 (s, 1H, Pyr-H), 8.61 (d, J = 8.5 Hz, 1H, Pyr-H), 8.23 (d, J = 6.4 Hz, 1H, Naph-H), 7.72 (t, J = 8.0 Hz, 1H, Pyr-H), 7.66 (t, J = 7.9 Hz, 1H, Naph-H), 7.58-7.52 (m, 1H, Naph-H), 7.47 (dd, J = 7.5, 2.6 Hz, 1H, Naph-H), 7.02 (t, J = 7.7 Hz, 1H, Naph-H), 6.91 (ddd, J = 5.5, 2.6, 0.9 Hz, 1H, Naph-H), 4.74-4.66 (m, 1H, CH), 4.17-4.07 (m, 2H, CH_2), 2.61-2.55 (m, 1H, CH), 1.56 (t, J = 7.2 Hz, 3H, CH_3), 1.18-1.10 (m, 5H, CH_2+CH_3), 0.90-0.87 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.2, 154.4, 143.0, 142.8, 142.6, 140.6, 140.4, 134.2, 129.4, 128.3, 128.0, 127.6, 127.1, 125.7, 123.3, 122.5, 105.7, 61.8, 44.3, 18.3, 18.1, 14.3, 13.4, 7.7.

HR-MS: m/z 418.1584 $[M + H]^+$. $C_{24}H_{23}N_3O_2S$ (Exact Mass: 417.1511). HPLC purity: 99.56%.

*Ethyl2-((1-(4-cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)-2-met hylpropanoate (**50**).* Recrystallized from EA as a white solid, yield 71.4%. Melting point: 140-142 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.97 (s, 1H, Pyr-H), 8.60 (d, J = 8.5 Hz, 1H, Pyr-H), 8.22 (d, J = 5.5 Hz, 1H, Naph-H), 7.71 (t, J = 8.2 Hz, 1H, Naph-H), 7.62 (d, J = 7.6 Hz, 1H, Naph-H), 7.54 (t, J = 8.0 Hz, 1H, Naph-H), 7.46 (d, J = 7.6 Hz, 1H, Naph-H), 6.97 (d, J = 8.3 Hz, 1H, Pyr-H), 6.87 (d, J = 6.3 Hz, 1H, Naph-H), 4.15-4.07 (m, 2H, CH_2), 2.61-2.54 (m, 1H, CH), 1.70 (s, 3H, CH_3), 1.63 (s, 3H, CH_3), 1.20-1.15 (m, 2H, CH_2), 1.11 (t, J = 7.1 Hz, 3H, CH_3), 0.90-0.86 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 153.5, 142.8, 142.7, 142.1, 140.7, 140.6, 134.2, 129.5, 128.3, 128.2, 127.5, 127.1, 125.6, 123.3, 122.4, 105.7, 61.7, 53.3, 26.7, 14.2, 13.4, 7.7. HR-MS: m/z 432.1737 $[M + H]^+$. $C_{25}H_{25}N_3O_2S$ (Exact Mass: 431.1667). HPLC purity: 99.98%.

*Ethyl 1-((1-(4-cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)cyclobutane-1-carboxylate (**51**).* Recrystallized from EA as a white solid, yield 71.4%. Melting point: 140-142 °C. HR-MS: m/z 444.1737 $[M + H]^+$. $C_{26}H_{25}N_3O_2S$ (Exact Mass: 443.1667).

*General procedure for the preparation of **52-56**.* Compounds **47-51** were each dissolved in 5 mL tetrahydrofuran and 5 mL ethanol. Lithium hydroxide (0.3 g, 12.5 mmol) was dissolved in a small amount of water and added dropwise, and the mixture was stirred at 0 °C for 1 h. After the reaction was completed, the solvent was removed by rotary evaporation under reduced pressure. 10 mL water was added to the residue, and the pH was adjusted to 3-4 by dropwise addition of 2 M HCl solution. The resulting precipitate was collected by filtration and recrystallized from

ethanol to obtain the target compound.

*2-((1-(4-Cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)acetic acid (**52**).* Recrystallized from EA as a white solid, yield 95.1%. Melting point: 164.8-167.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.98 (s, 1H, Pyr-H), 8.61 (d, J = 8.5 Hz, 1H, Pyr-H), 8.22 (d, J = 5.4 Hz, 1H, Naph-H), 7.72 (t, J = 7.7 Hz, 1H, Naph-H), 7.66 (d, J = 7.6 Hz, 1H, Naph-H), 7.54 (t, J = 7.5 Hz, 1H, Naph-H), 7.48 (d, J = 7.6 Hz, 1H, Naph-H), 7.06 (d, J = 8.4 Hz, 1H, Pyr-H), 6.89 (d, J = 5.4 Hz, 1H, Naph-H), 4.18 (s, 2H, CH_2), 2.62-2.55 (m, 1H, CH), 1.20-1.15 (m, 2H, CH_2), 0.91-0.87 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.6, 155.9, 143.0, 142.9, 142.4, 140.6, 140.2, 134.3, 129.4, 128.2, 128.1, 127.6, 127.1, 125.6, 123.4, 122.6, 105.5, 34.9, 13.4, 7.7, 7.6. HR-MS: m/z 376.1118 $[M + H]^+$. $C_{21}H_{17}N_3O_2S$ (Exact Mass: 375.1041). HPLC purity: 96.33%.

*4-((1-(4-Cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)butanoic acid (**53**).* Recrystallized from EA as a white solid, yield 94.2%. Melting point: 213-214 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.50 (s, 1H, Pyr-H), 8.63 (d, J = 8.5 Hz, 1H, Pyr-H), 8.50 (d, J = 6.5 Hz, 1H, Naph-H), 7.79 (d, J = 7.6 Hz, 1H, Naph-H), 7.78 – 7.73 (m, 1H, Naph-H), 7.59-7.55 (m, 1H, Naph-H), 7.49 (dd, J = 14.4, 7.0 Hz, 2H, Naph-H), 7.19 (d, J = 8.4 Hz, 1H, Pyr-H), 3.41 (td, J = 7.0, 1.8 Hz, 2H, CH_2), 2.64-2.57 (m, 1H, CH), 2.34 (t, J = 7.3 Hz, 2H, CH_2), 2.00 (p, J = 7.1 Hz, 2H, CH_2), 1.21-1.17 (m, 2H, CH_2), 0.92-0.88 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.1, 163.0, 147.6, 143.8, 140.8, 134.9, 134.2, 132.5, 129.0, 128.5, 127.8, 127.4, 126.9, 125.7, 123.3, 122.4, 107.9, 32.7, 31.6, 24.4, 13.4, 7.9, 7.8. HR-MS: m/z 404.1428 $[M + H]^+$. $C_{23}H_{21}N_3O_2S$ (Exact Mass: 403.1354). HPLC purity: 99.58%.

*2-((1-(4-Cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)propanoic acid (**54**)*. Recrystallized from EA as a white solid, yield 92.3%. Melting point: 160.5-163.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (s, 1H, Pyr-H), 8.62 (d, J = 8.5 Hz, 1H, Pyr-H), 8.30 (d, J = 5.7 Hz, 1H, Naph-H), 7.75-7.67 (m, 2H, Naph-H), 7.57-7.53 (m, 1H, Naph-H), 7.48 (d, J = 7.6 Hz, 1H, Pyr-H), 7.06 (t, J = 6.4 Hz, 2H, Naph-H), 4.68 (d, J = 25.1 Hz, 1H, CH), 2.59 (d, J = 27.6 Hz, 1H, CH), 1.60 (dd, J = 16.0, 7.2 Hz, 3H, CH_3), 1.18 (d, J = 17.7 Hz, 2H, CH_2), 0.89 (d, J = 14.1 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.6, 156.5, 143.9, 143.2, 140.8, 140.6, 138.6, 134.2, 129.3, 128.3, 127.7, 127.6, 127.2, 125.7, 123.3, 122.5, 106.2, 45.1, 18.7, 13.4, 7.8, 7.6. HR-MS: m/z 390.1276 $[M + H]^+$. $C_{22}H_{19}N_3O_2S$ (Exact Mass: 389.1198). HPLC purity: 98.15%.

*2-((1-(4-Cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)-2-methylpropanoic acid (**55**)*. Recrystallized from EA as a white solid, yield 94.0%. Melting point: 227-229 °C. HR-MS: m/z 404.1428 $[M + H]^+$. $C_{23}H_{21}N_3O_2S$ (Exact Mass: 403.1354).

*1-((1-(4-Cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)cyclobutane-1-carboxylic acid (**56**)*. Recrystallized from EA as a white solid, yield 72.0%. Melting point: 227-229 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.75 (s, 1H, COOH), 9.09 (d, J = 5.1 Hz, 1H, Naph-H), 8.65 – 8.53 (m, 1H, Pyr-H), 8.28 (d, J = 4.9 Hz, 1H, Naph-H), 7.78 – 7.65 (m, 1H, Naph-H), 7.55 (t, J = 7.0 Hz, 1H, Naph-H), 7.51 – 7.42 (m, 1H, Naph-H), 7.24 (d, J = 8.5 Hz, 1H, Pyr-H), 7.10 – 6.99 (m, 1H, Pyr-H), 6.64 (d, J = 4.7 Hz, 1H, Naph-H), 3.67 (s, 1H, CH), 3.15 – 2.64 (m, 2H, CH_2), 2.56 (s, 1H, CH), 2.22 (d, J = 85.5 Hz, 2H, CH_2), 1.17 (s, 2H, CH_2), 0.90 (s, 2H, CH_2). HR-MS: m/z 414.1328 $[M - H]^-$. $C_{24}H_{21}N_3O_2S$ (Exact Mass: 415.1354). HPLC purity: 99.40%.

*N-(4-Cyclopropylnaphthalen-1-yl)-2-nitropyridin-3-amine (**10d**)*. Palladium acetate (0.007 g,

0.0 315 mmol) and 4,5-bis (diphenylphosphino)-9,9-dimethylxanthene (0.036 g, 0.063 mmol) were dissolved in 2 mL dioxane and the solution was stirred for 15 min. 3-Chloro-2-nitropyridine (0.1 g, 0.63 mmol), 4-cyclopropyl-1-naphthylamine (0.13 g, 0.76 mmol) and cesium carbonate (0.41 g, 1.26 mmol) were dissolved in 10 mL dioxane. The two solutions were mixed and refluxed at 90 °C for 12 h under nitrogen, then cooled to room temperature, and 30 mL dichloromethane and saturated aqueous sodium chloride (3 × 10 mL) were added. The organic layer was separated, dried over anhydrous sodium sulfate and filtered. The product was purified by flash column chromatography to give N-(4-cyclopropylnaphthalen-1-yl)-2-nitropyridin-3-amine (**10d**). Yellow solid, yield 60.6%. Melting point: 76-79 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.45 (s, 1H, NH), 8.50 (d, J = 8.4 Hz, 1H, Pyr-H), 7.95 (d, J = 8.4 Hz, 1H, Pyr-H), 7.90 (d, J = 5.2 Hz, 1H, Naph-H), 7.66 (t, J = 7.6 Hz, 1H, Naph-H), 7.56 (t, J = 8.0 Hz, 1H, Pyr-H), 7.45-7.40 (m, 2H, Naph-H), 7.34 (d, J = 7.6 Hz, 1H, Naph-H), 7.00 (d, J = 8.6 Hz, 1H, Naph-H), 2.48-2.43 (m, 1H, CH), 1.12-1.08 (m, 2H, CH_2), 0.80-0.76 (m, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.2, 140.6, 139.6, 136.9, 134.7, 131.8, 130.1, 129.9, 126.9, 126.7, 126.0, 125.3, 123.9, 123.8, 122.7, 13.3, 6.6. ESI-MS: m/z 306.4 $[M+H]^+$. $C_{18}H_{15}N_3O_2$ (Exact Mass: 305.12).

*N3-(4-Cyclopropylnaphthalen-1-yl)pyridine-2,3-diamine (**11d**).* The synthetic method was similar to that described for **11e**. Yellow solid, yield 73.2%. Melting point: 143.5-144 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.37 (d, J = 7.8 Hz, 1H, Naph-H), 8.16 (d, J = 8.2 Hz, 1H, Naph-H), 7.67 (dd, J = 4.9, 1.6 Hz, 1H, Naph-H), 7.60-7.56 (m, 1H, Pyr-H), 7.53-7.49 (m, 1H, Naph-H), 7.34 (s, 1H, NH), 7.11 (d, J = 7.7 Hz, 1H, Pyr-H), 6.95 (dd, J = 7.6, 1.5 Hz, 1H, Naph-H) , 6.62 (d, J = 7.7 Hz, 1H, Pyr-H), 6.49 (dd, J = 7.5, 4.9 Hz, 1H, Naph-H), 5.64 (s, 2H, NH_2), 2.30-2.23 (m, 1H, CH), 1.01-0.97 (m, 2H, CH_2), 0.65-0.61 (m, 2H, CH_2). ^{13}C NMR (100

MHz, DMSO-d_6) δ 153.6, 141.5, 139.1, 134.4, 131.4, 127.6, 126.5, 126.3, 125.6, 125.1, 124.9, 124.4, 123.6, 113.2, 112.5, 13.1, 6.7. ESI-MS: m/z 276.1 $[M+H]^+$. $C_{18}H_{17}N_3$ (Exact Mass: 275.14).

1-(4-Cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-b]pyridine-2-thiol ***(12d)***. N3-(4-Cyclopropylnaphthalen-1-yl)pyridine-2,3-diamine (**12e**) (0.1 g, 0.36 mmol), 1,1'-thiocarbonyldiimidazole (0.1 g, 0.58 mmol) and triethylamine (0.08 mL) were dissolved in 30 mL tetrahydrofuran. The solution was refluxed at 60 °C for 5 h, then cooled to room temperature, and the solvent was evaporated under reduced pressure. The residue was washed with 30 mL dichloromethane and saturated aqueous sodium chloride (3 × 10 mL), and the organic layer was separated, dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by flash column chromatography to give crude 1-(4-cyclopropylnaphthalen-1-yl)-1*H*-imidazo[4,5-*b*]pyridine-2-thiol (**12d**) as a yellow solid, yield 70.9%. Melting point: 246-247 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.71 (s, 1H, SH), 8.56 (d, J = 8.5 Hz, 1H, Naph-H), 8.21 (dd, J = 5.0, 1.3 Hz, 1H, Pyr-H), 7.69-7.65 (m, 1H, Naph-H), 7.56 (d, J = 7.6 Hz, 1H, Naph-H), 7.52-7.48 (m, 1H, Naph-H), 7.44 (d, J = 7.6 Hz, 1H, Naph-H), 7.27 (d, J = 8.3 Hz, 1H, Naph-H), 7.07 (dd, J = 7.9, 5.0 Hz, 1H, Pyr-H), 6.91 (dd, J = 7.9, 1.3 Hz, 1H, Pyr-H), 2.57-2.53 (m, 1H, CH), 1.19-1.11 (m, 2H, CH_2), 0.89-0.82 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.0, 146.0, 143.6, 141.7, 134.3, 129.8, 129.7, 128.5, 127.7, 127.5, 127.1, 125.4, 123.4, 123.4, 118.8, 116.7, 13.4, 7.6, 7.4. ESI-MS: m/z 318.4 $[M+H]^+$. $C_{18}H_{17}N_3$ (Exact Mass: 317.10).

General Procedure for the Preparation of ***37-41***. The synthetic method was similar to that described for **47-51** except that the starting material

1-(4-cyclopropylnaphthalen-1-yl)-1*H*-imidazo[4,5-*b*]pyridine-2-thiol (**12d**) was reacted with appropriate substituted esters.

*Ethyl 2-((1-(4-cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)acetate (**37**).* Purified by flash column chromatography as a yellow oil, yield 77.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.61 (d, J = 8.5 Hz, 1H, Naph-H), 8.40 (dd, J = 4.8, 1.5 Hz, 1H, Pyr-H), 7.72 (ddd, J = 8.3, 6.9, 1.1 Hz, 1H, Naph-H), 7.67 (d, J = 7.6 Hz, 1H, Naph-H), 7.55 (t, J = 8.1 Hz, 1H, Naph-H), 7.47 (d, J = 7.6 Hz, 1H, Naph-H), 7.26 (dd, J = 8.0, 1.5 Hz, 1H, Pyr-H), 7.13 (dd, J = 8.0, 4.8 Hz, 1H, Pyr-H), 7.09 (d, J = 8.2 Hz, 1H, Naph-H), 4.14 (q, J = 7.1 Hz, 2H, CH_2), 2.62-2.55 (m, 1H, CH), 1.20 (d, J = 7.1 Hz, 3H, CH_3), 1.06 (t, J = 7.0 Hz, 4H, 2 × CH_2), 0.91-0.87 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.5, 156.6, 155.7, 144.2, 142.9, 134.3, 131.0, 129.5, 128.3, 128.1, 127.6, 127.2, 125.6, 123.4, 122.7, 118.4, 117.7, 61.7, 19.0, 14.4, 13.4, 7.7, 7.6. HR-MS: m/z 404.1425 $[M + H]^+$. $C_{23}H_{21}N_3O_2S$ (Exact Mass: 403.1354). HPLC purity: 98.56%.

*Ethyl 4-((1-(4-cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)butanoate (**38**).* Purified by flash column chromatography as a yellow oil, yield 71.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (d, J = 8.5 Hz, 1H, Pyr-H), 8.40 (dd, J = 4.8, 1.5 Hz, 1H, Pyr-H), 7.71 (ddd, J = 9.6, 5.7, 2.3 Hz, 1H, Naph-H), 7.65 (t, J = 6.4 Hz, 1H, Naph-H), 7.52 (ddd, J = 8.1, 5.2, 1.0 Hz, 1H, Naph-H), 7.45 (d, J = 7.6 Hz, 1H, Naph-H), 7.22 (dd, J = 8.0, 1.5 Hz, 1H, Pyr-H), 7.14-7.09 (m, 1H, Naph-H), 7.05 (d, J = 8.1 Hz, 1H, Naph-H), 4.03 (q, J = 7.1 Hz, 2H,CH_2), 2.62-2.53 (m, 1H, CH), 2.44-2.36 (m, 2H, CH_2), 2.05-1.95 (m, 2H, CH_2), 1.21-1.11 (m, 5H, CH_2 + CH_3), 1.07 (t, J = 7.0 Hz, 2H, CH_2), 0.92-0.82 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.6, 157.4, 156.0, 144.1, 142.6, 134.2, 131.0, 129.6, 128.6, 128.1, 127.5, 127.3, 125.6, 123.3, 122.6, 118.2,

117.5, 60.3, 32.6, 31.2, 24.8, 19.0, 14.5, 7.7, 7.6. HR-MS: m/z 432.1736 $[M + H]^+$. $C_{25}H_{25}N_3O_2S$ (Exact Mass: 431.1667). HPLC purity: 96.34%.

Ethyl 2-((1-(4-cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)propanoate ***(39)***. Purified by flash column chromatography as a yellow oil, yield 69.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (d, J = 8.5 Hz, 1H, Pyr-H), 8.42 (dd, J = 4.8, 0.8 Hz, 1H, Pyr-H), 7.75-7.68 (m, 1H, Naph-H), 7.66 (t, J = 8.0 Hz, 1H, Naph-H), 7.58 – 7.50 (m, 1H, Naph-H), 7.46 (dd, J = 7.4, 2.8 Hz, 1H, Naph-H), 7.27 (ddd, J = 8.0, 2.5, 1.5 Hz, 1H, Naph-H), 7.14 (ddd, J = 8.0, 4.8, 1.3 Hz, 1H, Pyr-H), 7.05 (dd, J = 8.1, 5.4 Hz, 1H, Naph-H), 4.77-4.69 (m, 1H, CH), 4.16-4.07 (m, 2H, CH_2), 2.61-2.54 (m, 1H, CH), 1.60-1.56 (m, 3H, CH_3), 1.19-1.12 (m, 5H, CH_2 + CH_3), 0.90-0.87 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.2, 155.7, 144.4, 142.9, 134.2, 130.8, 129.5, 128.3, 128.2, 128.1, 127.6, 127.3, 125.6, 123.4, 122.6, 118.5, 117.9, 61.8, 18.4, 18.2, 14.3, 13.4, 7.7, 7.6. HR-MS: m/z 418.1583 $[M + H]^+$. $C_{24}H_{23}N_3O_2S$ (Exact Mass: 417.1511). HPLC purity: 95.69%.

Ethyl 2-((1-(4-cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)-2-methylpropanoate (***40***). Purified by flash column chromatography as a yellow oil, yield 74.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (d, J = 8.5 Hz, 1H, Naph-H), 8.41 (dd, J = 4.7, 1.5 Hz, 1H, Pyr-H), 7.72-7.68 (m, 1H, Naph-H), 7.62 (d, J = 7.6 Hz, 1H, Naph-H), 7.55-7.51 (m, 1H, Naph-H), 7.45 (d, J = 7.5 Hz, 1H, Naph-H), 7.23 (dd, J = 8.0, 1.5 Hz, 1H, Pyr-H), 7.12 (dd, J = 8.0, 4.8 Hz, 1H, Pyr-H), 7.00 (d, J = 8.3 Hz, 1H, Naph-H), 4.16-4.08 (m, 2H, CH_2), 2.60-2.53 (m, 1H, CH), 1.71 (s, 3H, CH_3), 1.65 (s, 3H, CH_3), 1.20-1.15 (m, 2H, CH_2), 1.12 (t, J = 7.1 Hz, 3H, CH_3), 0.90-0.86 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 155.8, 154.8, 144.4, 142.7, 134.2, 130.2, 129.6,

128.5, 128.1, 127.5, 127.2, 125.6, 123.3, 122.5, 118.5, 117.9, 61.7, 53.3, 26.7, 14.3, 13.4, 7.7, 7.6. HR-MS: m/z 432.1737 $[M + H]^+$. $C_{25}H_{25}N_3O_2S$ (Exact Mass: 431.1667). HPLC purity: 99.04%.

Ethyl 1-((1-(4-cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)cyclobutane -1-carboxylate (***41***). Purified by flash column chromatography as a yellow oil, yield 74.8%. HR-MS: m/z 444.1737 $[M + H]^+$. $C_{26}H_{25}N_3O_2S$ (Exact Mass: 443.1667).

General Procedure for the Preparation of ***42-46***. The synthetic method was similar to that described for **17-20**.

*2-((1-(4-Cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)acetic acid (**42**).* Recrystallized from EA as a white solid, yield 93.3%. Melting point: 151.5-153 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (d, J = 8.5 Hz, 1H, Pyr-H), 8.38 (dd, J = 4.8, 1.4 Hz, 1H, Pyr-H), 7.73-7.69 (m, 1H, Naph-H), 7.65 (d, J = 7.6 Hz, 1H, Naph-H), 7.53 (t, J = 7.6 Hz, 1H, Naph-H), 7.47 (d, J = 7.6 Hz, 1H, Naph-H), 7.23 (dd, J = 8.0, 1.4 Hz, 1H, Pyr-H), 7.12-7.07 (m, 2H, Naph-H), 4.13 (s, 2H, CH_2), 2.61-2.54 (m, 1H, CH), 1.19-1.14 (m, 2H, CH_2), 0.90-0.87 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.6, 157.6, 155.9, 144.0, 142.7, 134.3, 130.9, 129.6, 128.6, 128.1, 127.5, 127.2, 125.6, 123.4, 122.8, 118.1, 117.5, 36.3, 13.4, 7.7, 7.5. HR-MS: m/z 376.1114 $[M + H]^+$. $C_{21}H_{17}N_3O_2S$ (Exact Mass: 375.1041). HPLC purity: 98.33%.

*4-((1-(4-Cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)butanoic acid (**43**).* Recrystallized from EA as a white solid, yield 94.5%. Melting point: 94-97 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (d, J = 8.5 Hz, 1H, Naph-H), 8.38 (dd, J = 4.7, 1.3 Hz, 1H, Pyr-H), 7.69 (t, J = 7.5 Hz, 1H, Naph-H), 7.64 (d, J = 7.6 Hz, 1H, Naph-H), 7.51 (t, J = 7.5 Hz, 1H, Naph-H), 7.44 (d, J = 7.6 Hz, 1H, Naph-H), 7.20 (dd, J = 7.9, 1.3 Hz, 1H, Pyr-H), 7.10 (dd, J = 7.9, 4.8 Hz,

1H, Pyr-H), 7.04 (d, J = 8.3 Hz, 1H, Naph-H), 4.08 (s, 2H, CH_2), 2.59-2.54 (m, 1H, CH), 2.20 (t, J = 7.2 Hz, 2H, CH_2), 1.95-1.88 (m, 2H, CH_2), 1.18-1.13 (m, 2H, CH_2), 0.88-0.85 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.63, 156.06, 144.09, 142.64, 134.26, 130.97, 129.6, 128.6, 128.1, 127.5, 127.2, 125.6, 123.4, 122.6, 118.1, 117.4, 34.4, 31.6, 25.38, 13.4, 7.7, 7.6. HR-MS: m/z 404.1427 $[M + H]^+$. $C_{23}H_{21}N_3O_2S$ (Exact Mass: 403.1354). HPLC purity: 99.05%.

*2-((1-(4-Cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)propanoic acid (**44**).* Recrystallized from EA as a white solid, yield 93.9%. Melting point: 156-158 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (d, J = 8.5 Hz, 1H, Naph-H), 8.39 (d, J = 4.5 Hz, 1H, Pyr-H), 7.70 (t, J = 7.7 Hz, 1H, Naph-H), 7.64 (d, J = 7.6 Hz, 1H, Naph-H), 7.55-7.50 (m, 1H, Naph-H), 7.46 (d, J = 7.6 Hz, 1H, Naph-H), 7.22 (dd, J = 7.9, 1.4 Hz, 1H, Pyr-H), 7.11 (dd, J = 7.9, 4.8 Hz, 1H, Pyr-H), 7.04 (dd, J = 8.2, 4.4 Hz, 1H, Naph-H), 4.59-4.54 (m, 1H, CH), 2.60-2.54 (m, 1H, CH), 1.64-1.57 (m, 3H, CH_3), 1.16 (d, J = 8.8 Hz, 2H, CH_2), 0.88 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9, 157.4, 156.0, 144.1, 142.6, 134.2, 130.6, 129.6, 128.6, 128.1, 127.5, 127.3, 125.6, 123.4, 122.6, 118.1, 117.5, 47.4, 19.8, 13.4, 7.7, 7.5. HR-MS: m/z 390.1275 $[M + H]^+$. $C_{22}H_{19}N_3O_2S$ (Exact Mass: 389.1198). HPLC purity: 99.36%.

*2-((1-(4-Cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)-2-methylpropanoic acid (**45**).* Recrystallized from EA as a white solid, yield 90.9%. Melting point: 160-165 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (d, J = 8.5 Hz, 1H, Naph-H), 8.42 (dd, J = 4.7, 1.4 Hz, 1H, Pyr-H), 7.71 (t, J = 7.6 Hz, 1H, Naph-H), 7.62 (d, J = 7.6 Hz, 1H, Naph-H), 7.53 (t, J = 7.6 Hz, 1H, Naph-H), 7.45 (d, J = 7.6 Hz, 1H, Naph-H), 7.23 (dd, J = 8.0, 1.4 Hz, 1H, Pyr-H), 7.13 (dd, J = 8.0, 4.8 Hz, 1H, Pyr-H), 7.00 (d, J = 8.4 Hz, 1H, Naph-H), 2.60-2.54 (m, 1H, CH), 1.70 (s, 6H, 2 × CH_3), 1.17 (dd, J = 8.4, 1.8 Hz, 2H, CH_2), 0.88 (q, J = 5.6 Hz, 2H, CH_2). ^{13}C NMR (100 MHz,

DMSO-d_6) δ 174.3, 155.8, 155.1, 144.4, 142.6, 134.2, 130.1, 129.7, 128.6, 128.1, 127.5, 127.3, 125.6, 123.3, 122.5, 118.5, 117.9, 53.9, 26.7, 13.4, 7.7, 7.6. HR-MS: m/z 404.1424 $[M+H]^+$. $C_{23}H_{21}N_3O_2S$ (Exact Mass: 403.1354). HPLC purity: 96.85%.

*1-((1-(4-Cyclopropylnaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)cyclobutane-1-carboxylic acid (**46**)*. Recrystallized from EA as a white solid, yield 90.9%. Melting point: 160-165 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.89 (s, 1H, COOH), 8.60 (d, J = 7.4 Hz, 1H, Naph-H), 8.39 (s, 1H, Pyr-H), 7.74 (d, J = 22.7 Hz, 1H, Naph-H), 7.64 (d, J = 6.4 Hz, 1H, Naph-H), 7.53 (s, 1H, Naph-H), 7.46 (d, J = 6.3 Hz, 1H, Naph-H), 7.23 (d, J = 6.7 Hz, 1H, Pyr-H), 7.12 (s, 1H, Pyr-H), 7.05 (d, J = 7.3 Hz, 1H, Naph-H), 2.80 (s, 2H, CH_2), 2.57 (s, 1H, CH), 2.41 (s, 2H, CH_2), 2.13 – 1.96 (m, 2H, CH_2), 1.17 (s, 2H, CH_2), 0.88 (s, 2H, CH_2). HR-MS: m/z 414.1324 $[M-H]^-$. $C_{24}H_{21}N_3O_2S$ (Exact Mass: 415.1354). HPLC purity: 98.43%.

*4-((2-Nitropyridin-3-yl)amino)-1-naphthonitrile (**10f**)*. 4-Bromo-1-naphthonitrile (**9d**) (0.56 g, 2.40 mmol), 2-nitropyridin-3-amine (0.5 g, 3.59 mmol), sodium hydroxide (0.20 g, 4.8 mmol) and calcium oxide (0.18 g, 3.12 mmol) were added to 100 mL dimethylacetamide. The reaction mixture was heated at 110 ° C for 12 h under nitrogen, then cooled to room temperature, and 200 mL H_2O was added to it. Filtration afforded crude 4-((2-nitropyridin-3-yl)amino)-1-naphthonitrile as a brown solid, yield 66.8%. Melting point: 169-171 °C. ESI-MS: m/z 291.3 $[M+H]^+$. $C_{16}H_{10}N_4O_2$ (Exact Mass: 290.08).

*4-((2-Aminopyridin-3-yl)amino)-1-naphthonitrile (**11f**)*. The synthetic method was similar to that described for **11e.** yellow solid, Yield 87.9%. Melting point: 164-166 °C. ESI-MS: m/z 261.4 $[M+H]^+$. $C_{16}H_{12}N_4$ (Exact Mass: 260.11).

*4-(2-Mercapto-1H-imidazo[4,5-b]pyridin-1-yl)-1-naphthonitrile (**12f**)*. The synthetic method was similar to that described for **12d.** yellow solid, Yield 50.5%. Melting point: 196-197 °C. ESI-MS: m/z 301.1 $[M - H]^-$. $C_{17}H_{10}N_4S$ (Exact Mass: 302.06).

General procedure for the preparation of **57-62**. The synthetic method was similar to that described for **13-16** except that the starting material 4-(2-mercapto-1H-imidazo[4,5-b]pyridin-1-yl)-1-naphthonitrile (**12f**) was reacted with appropriate substituted esters.

Methyl 2-((1-(4-cyanonaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)acetate (***57***). Purified by flash column chromatography as a yellow oil, yield 35.5%. Melting point: 110-113 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (d, J = 7.5 Hz, 1H, Naph-H), 8.44 (d, J = 4.3 Hz, 1H, Pyr-H), 8.34 (d, J = 8.4 Hz, 1H, Naph-H), 8.04 (d, J = 7.6 Hz, 1H, Naph-H), 7.96 (t, J = 7.5 Hz, 1H, Naph-H), 7.76 (t, J = 7.6 Hz, 1H, Naph-H), 7.39 (d, J = 8.0 Hz, 1H, Pyr-H), 7.33 (d, J = 8.4 Hz, 1H, Naph-H), 7.20-7.17 (m, 1H, Pyr-H), 4.31 (s, 2H, CH_2), 3.68 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.0, 155.9, 155.7, 144.7, 135.1, 134.1, 133.3, 130.9, 130.8, 130.2, 129.5, 127.5, 125.8, 123.7, 118.7, 118.1, 117.3, 112.1, 53.1, 34.1. HR-MS: m/z 375.0913 $[M + H]^+$. $C_{20}H_{14}N_4O_2S$ (Exact Mass: 374.0837). HPLC purity: 96.49%.

Methyl 4-((1-(4-cyanonaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)butanoate (***58***). Purified by flash column chromatography as a gray solid, yield 50.0%. Melting point: 111-113 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.46 (d, J = 7.6 Hz, 1H, Naph-H), 8.43 (d, J = 4.7 Hz, 1H, Pyr-H), 8.33 (d, J = 8.4 Hz, 1H, Naph-H), 8.03 (d, J = 7.6 Hz, 1H, Naph-H), 7.94 (t, J = 7.6 Hz, 1H, Naph-H), 7.73 (t, J = 7.7 Hz, 1H, Naph-H), 7.35 (d, J = 7.9 Hz, 1H, Pyr-H), 7.30 (d, J = 8.5 Hz, 1H, Naph-H), 7.18-7.15 (m, 1H, Pyr-H), 3.57 (s, 3H, CH_3), 3.38 (t, J = 6.9 Hz, 2H, CH_2), 2.42

(t, J = 7.2 Hz, 2H, CH_2), 2.06-1.98 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.1, 156.7, 156.0, 144.5, 135.4, 134.0, 133.3, 130.9, 130.7, 130.2, 129.6, 127.6, 125.8, 123.7, 118.5, 117.8, 117.3, 111.9, 51.8, 32.4, 31.5, 24.7. HR-MS: m/z 403.1223 $[M + H]^+$. $C_{22}H_{18}N_4O_2S$ (Exact Mass: 402.1150). HPLC purity: 97.42%.

Methyl 3-((1-(4-cyanonaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)propanoate (***59***). Purified by flash column chromatography as a white solid, yield 50.0%. Melting point: > 200 °C. ^1H NMR (400 MHz, DMSO-d_6) δ 8.47-8.43 (m, 2H, Naph-H + Pyr-H), 8.33 (d, J = 8.4 Hz, 1H, Naph-H), 8.01 (d, J = 7.6 Hz, 1H, Naph-H), 7.94 (t, J = 7.7 Hz, 1H, Naph-H), 7.74 (t, J = 7.7 Hz, 1H, Naph-H), 7.36 (d, J = 8.0 Hz, 1H, Pyr-H), 7.32 (d, J = 8.5 Hz, 1H, Naph-H), 7.19-7.15 (m, 1H, Pyr-H), 3.58 (s, 3H, CH_3), 3.54 (t, J = 6.8 Hz, 2H, CH_2), 2.92 (t, J = 6.8 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.1, 156.5, 156.0, 144.6, 135.3, 134.0, 133.3, 130.9, 130.7, 130.2, 129.6, 127.5, 125.8, 123.7, 118.6, 117.9, 117.3, 111.9, 52.1, 34.0, 27.4. HR-MS: m/z 389.1067 $[M + H]^+$. $C_{21}H_{16}N_4O_2S$ (Exact Mass: 388.0994). HPLC purity: 98.32%.

Methyl 2-((1-(4-cyanonaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)propanoate (***60***). Purified by flash column chromatography as a white solid, yield 47.0%. Melting point: 100-102 °C. ^1H NMR (400 MHz, DMSO-d_6) δ 8.48-8.45 (m, 2H, Naph-H + Pyr-H), 8.34 (d, J = 8.4 Hz, 1H, Naph-H), 8.08-8.01 (m, 1H, Naph-H), 7.95 (t, J = 7.6 Hz, 1H, Naph-H), 7.76 (t, J = 7.6 Hz, 1H, Naph-H), 7.40 (d, J = 8.0 Hz, 1H, Pyr-H), 7.30 (dd, J = 15.3, 8.5 Hz, 1H, Naph-H), 7.21-7.18 (m, 1H, Pyr-H), 4.79 (q, J = 8.7, 8.0 Hz, 1H, CH), 3.67 (d, J = 13.7 Hz, 3H, CH_3), 1.60 (t, J = 6.2 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.7, 155.7, 154.9, 144.8, 135.1, 134.0, 133.3, 130.7, 130.7, 130.2, 129.6, 127.6, 125.8, 123.5, 118.9, 118.2, 117.3, 112.1, 53.2, 44.4, 18.2. HR-MS: m/z 389.1065 $[M + H]^+$. $C_{21}H_{16}N_4O_2S$ (Exact Mass: 388.0994). HPLC purity: 98.96%.

Ethyl 2-((1-(4-cyanonaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)-2-methylpropanoate (***61***). Purified by flash column chromatography as a white solid, yield 32.6%. Melting point: 139-142 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48-8.45 (m, 2H, Naph-H + Pyr-H), 8.34 (d, J = 8.4 Hz, 1H, Naph-H), 7.99 (d, J = 7.6 Hz, 1H, Naph-H), 7.95 (t, J = 7.6 Hz, 1H, Naph-H), 7.75 (t, J = 7.7 Hz, 1H, Naph-H), 7.39 (d, J = 8.0 Hz, 1H, Pyr-H), 7.23 (d, J = 8.5 Hz, 1H, Naph-H), 7.21-7.18 (m, 1H, Pyr-H), 4.11 (q, J = 7.0 Hz, 2H, CH_2), 1.71 (s, 3H, CH_3), 1.65 (s, 3H, CH_3), 1.12 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.6, 155.8, 153.9, 145.0, 135.3, 134.0, 133.3, 130.7, 130.2, 130.1, 129.7, 127.6, 125.9, 123.4, 119.0, 118.3, 117.3, 111.9, 61.8, 53.9, 26.7, 14.3. HR-MS: m/z 417.1381 $[M + H]^+$. $C_{23}H_{20}N_4O_2S$ (Exact Mass: 416.1307). HPLC purity: 99.05%.

Ethyl 1-((1-(4-cyanonaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)cyclobutane-1-carboxylate (***62***). Purified by flash column chromatography as a white solid, yield 20.7%. Melting point: > 200 °C. HR-MS: m/z 429.1381 $[M + H]^+$. $C_{24}H_{20}N_4O_2S$ (Exact Mass: 428.1307).

General Procedure for the Preparation of ***63-68***. The synthetic method was similar to that described for **17-20**.

2-((1-(4-Cyanonaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)acetic acid (***63***). Recrystallized from EA as a yellow solid, yield 62.0%. Melting point: 142-144 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (d, J = 7.7 Hz, 1H, Naph-H), 8.43 (d, J = 4.6 Hz, 1H, Pyr-H), 8.34 (d, J = 8.4 Hz, 1H, Naph-H), 8.02 (d, J = 7.3 Hz, 1H, Naph-H), 7.95 (t, J = 7.5 Hz, 1H, Naph-H), 7.75 (t, J = 7.7 Hz, 1H, Naph-H), 7.38 (d, J = 8.0 Hz, 1H, Pyr-H), 7.34 (d, J = 8.5 Hz, 1H, Naph-H), 7.19-7.16 (m, 1H, Pyr-H), 4.23 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.6, 156.3,

155.8, 144.6, 135.2, 134.0, 133.3, 130.9, 130.7, 130.2, 129.5, 127.5, 125.8, 123.7, 118.6, 118.0, 117.3, 112.0, 34.8. HR-MS: m/z 359.0608 $[M - H]^-$. $C_{19}H_{12}N_4O_2S$ (Exact Mass: 360.0681). HPLC purity: 96.69%.

4-((1-(4-Cyanonaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)butanoic acid (***64***). Recrystallized from EA as a white solid, yield 50.0%. Melting point: 95-97 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.24 (s, 1H, OH), 8.46 (d, J = 7.6 Hz, 1H, Naph-H), 8.43 (d, J = 4.3 Hz, 1H, Pyr-H), 8.33 (d, J = 8.4 Hz, 1H, Naph-H), 8.03 (d, J = 7.7 Hz, 1H, Naph-H), 7.94 (t, J = 7.5 Hz, 1H, Naph-H), 7.74 (t, J = 7.6 Hz, 1H, Naph-H), 7.35 (d, J = 7.9 Hz, 1H, Pyr-H), 7.30 (d, J = 8.5 Hz, 1H, Naph-H), 7.18-7.15 (m, 1H, Pyr-H), 3.38 (t, J = 6.6 Hz, 2H, CH_2), 2.34-2.31 (m, 2H, CH_2), 2.02-1.96 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.4, 146.2, 143.8, 136.7, 134.0, 133.3, 131.4, 130.3, 129.9, 129.5, 128.4, 128.2, 128.0, 127.4, 125.6, 124.5, 119.0, 111.2, 35.7, 32.6, 19.6. HR-MS: m/z 423.0688 $[M + Cl]^-$. $C_{21}H_{16}N_4O_2S$ (Exact Mass: 388.0994). HPLC purity: 99.42%.

3-((1-(4-Cyanonaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)propanoic acid (***65***). Recrystallized from EA as a yellow solid, yield 45.0%. Melting point: 199-200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.88 (s, 1H, OH), 8.44 (d, J = 7.4 Hz, 1H, Naph-H), 8.30 (d, J = 8.4 Hz, 1H, Pyr-H), 8.25 (d, J = 4.4 Hz, 1H, Naph-H), 7.93-7.89 (m, 2H, 2 × Naph-H), 7.71 (t, J = 7.6 Hz, 1H, Naph-H), 7.54 (d, J = 8.4 Hz, 1H, Pyr-H), 7.11 (d, J = 4.9 Hz, 1H, Naph-H), 7.10-7.06 (m, 1H, Pyr-H), 3.38-3.29 (m, 2H, CH_2), 1.54-1.23 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.7, 146.2, 143.9, 136.7, 134.0, 133.3, 130.3, 129.9, 129.6, 128.3, 128.2, 128.0, 125.6, 124.5, 119.0, 117.5, 116.9, 111.2, 35.3, 24.2. HR-MS: m/z 373.0765 $[M - H]^-$. $C_{20}H_{14}N_4O_2S$ (Exact Mass: 374.0837). HPLC purity: 97.73%.

2-((1-(4-Cyanonaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)propanoic acid (***66***). Recrystallized from EA as a yellow solid, yield 70.0%. Melting point: > 200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.19 (s, 1H, OH), 8.48-8.45 (m, 2H, Naph-H + Pyr-H), 8.34 (d, J = 8.4 Hz, 1H, Naph-H), 8.02 (t, J = 8.2 Hz, 1H, Naph-H), 7.95 (t, J = 7.6 Hz, 1H, Naph-H), 7.75 (t, J = 7.5 Hz, 1H, Naph-H), 7.39 (d, J = 7.3 Hz, 1H, Pyr-H), 7.33-7.29 (m, 1H, Naph-H), 7.21-7.17 (m, 1H, Pyr-H), 4.72-4.69 (m, 1H, CH), 1.63-1.59 (m, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.6, 155.8, 144.7, 135.2, 134.0, 133.3, 130.7, 130.2, 130.2, 129.6, 127.6, 127.5, 125.8, 123.6, 118.8, 118.1, 117.3, 112.0, 45.3, 18.7. HR-MS: m/z 373.0763 $[M - H]^-$. $C_{20}H_{14}N_4O_2S$ (Exact Mass: 374.0837). HPLC purity: 97.22%.

2-((1-(4-Cyanonaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)-2-methylpropanoic acid (***67***). Recrystallized from EA as a white solid, yield 69.0%. Melting point: 101-104 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48-8.45 (m, 2H, Naph-H + Pyr-H), 8.33 (d, J = 8.4 Hz, 1H, Naph-H), 7.98 (d, J = 8.4 Hz, 1H, Naph-H), 7.95-7.93 (m, 1H, Naph-H), 7.74 (t, J = 7.7 Hz, 1H, Naph-H), 7.40 (d, J = 8.0 Hz, 1H, Pyr-H), 7.24 (d, J = 8.6 Hz, 1H, Naph-H), 7.22-7.20 (m, 1H, Pyr-H), 1.70 (s, 6H, 2 × CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.2, 155.6, 154.4, 144.6, 135.4, 134.0, 133.2, 130.7, 130.2, 130.2, 129.7, 127.6, 125.8, 123.5, 119.0, 118.6, 117.3, 111.9, 54.5, 26.7. HR-MS: m/z 387.0921 $[M - H]^-$. $C_{21}H_{16}N_4O_2S$ (Exact Mass: 388.0994). HPLC purity: 98.00%.

1-((1-(4-Cyanonaphthalen-1-yl)-1H-imidazo[4,5-b]pyridin-2-yl)thio)cyclobutane-1-carboxylic acid (***68***). Recrystallized from EA as a gray solid, yield 82.5%. Melting point: > 200 °C. HR-MS: m/z 399.0921 $[M - H]^-$. $C_{22}H_{16}N_4O_2S$ (Exact Mass: 400.0994).

*4-((3-Nitropyridin-4-yl)amino)-1-naphthonitrile (**10g**)*. The synthetic method was similar to that described for ***10f***. Brown solid, yield 45.0%. Melting point: 162-163 °C. ESI-MS: m/z 291.3

$[M+H]^+$. $C_{16}H_{10}N_4O_2$ (Exact Mass: 290.08).

*4-((3-Aminopyridin-4-yl)amino)-1-naphthonitrile (**11g**).* The synthetic method was similar to that described for **11e.** yellow solid, Yield 81.0%. Melting point: 188-190 °C. ESI-MS: m/z 261.3 $[M + H]^+$. $C_{16}H_{12}N_4$ (Exact Mass: 260.11).

*4-(2-Mercapto-1H-imidazo[4,5-c]pyridin-1-yl)-1-naphthonitrile (**12g**).* The synthetic method was similar to that described for **12d.** White solid, Yield 42.1%. Melting point: 199-201 °C. ESI-MS: m/z 301.1 $[M - H]^-$. $C_{17}H_{10}N_4S$ (Exact Mass: 302.06).

General procedure for the preparation of **69-74**. The synthetic method was similar to that described for **13-16** except that the starting material *4-(2-mercapto-1H-imidazo[4,5-c]pyridin-1-yl)-1-naphthonitrile (**12g**)* was reacted with appropriate substituted esters.

Methyl 2-((1-(4-cyanonaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)acetate (***69***). Purified by flash column chromatography as a white solid, yield 27.6%. Melting point: 142-144 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (d, J = 31.8 Hz, 1H, Pyr-H), 8.46-8.44 (m, 1H, Pyr-H + Naph-H), 8.18 (d, J = 8.0 Hz, 1H, Naph-H), 8.12 (d, J = 7.3 Hz, 1H, Naph-H), 7.96-7.82 (m, 4H, Pyr-H + 3 × Naph-H), 5.48 (s, 2H, CH_2), 5.27 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.1, 140.1, 139.8, 137.1, 134.8, 133.3, 132.3, 131.5, 131.1, 129.6, 128.7, 127.5, 127.4, 125.6, 125.6, 117.9, 110.4, 109.5, 59.9, 33.2.HR-MS: m/z 375.0913 $[M + H]^+$. $C_{20}H_{14}N_4O_2S$ (Exact Mass: 374.0837). HPLC purity: 99.58%.

Methyl 4-((1-(4-cyanonaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)butanoate (***70***). Purified by flash column chromatography as a slight yellow solid, yield 30.0%. Melting point:

115-117 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (s, 1H, Pyr-H), 8.47 (d, J = 7.5 Hz, 1H, Naph-H), 8.34 (d, J = 8.3 Hz, 1H, Pyr-H), 8.26 (d, J = 5.2 Hz, 1H, Naph-H), 8.04 (d, J = 7.5 Hz, 1H, Naph-H), 7.95 (t, J = 7.6 Hz, 1H, Naph-H), 7.74 (t, J = 7.7 Hz, 1H, Naph-H), 7.27 (d, J = 8.5 Hz, 1H, Pyr-H), 7.00 (d, J = 5.3 Hz, 1H, Naph-H), 3.57 (s, 3H, CH_3), 3.38 (t, J = 6.7 Hz, 2H, CH_2), 2.42 (t, J = 7.1 Hz, 2H, CH_2), 2.04-1.97 (m, 2H, CH_2). ^{13}C NMR (101 MHz, DMSO) δ 173.1, 155.4, 142.8, 142.8, 140.8, 140.4, 135.1, 134.0, 133.3, 130.7, 130.2, 129.5, 127.5, 125.8, 123.5, 117.3, 112.0, 105.6, 51.8, 32.4, 31.5, 24.6. HR-MS: m/z 403.1223 $[M + H]^+$. $C_{22}H_{18}N_4O_2S$ (Exact Mass: 402.1150). HPLC purity: 99.04%.

Methyl 3-((1-(4-cyanonaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)propanoate (***71***). Purified by flash column chromatography as a white solid, yield 33.8%. Melting point: > 200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.96 (s, 1H, Pyr-H), 8.45 (d, J = 7.6 Hz, 1H, Naph-H), 8.32-8.28 (m, 2H, Pyr-H + Naph-H), 7.92-7.89 (m, 2H, 2 × Naph-H), 7.71 (t, J = 7.7 Hz, 1H, Naph-H), 7.49 (d, J = 8.5 Hz, 1H, Pyr-H), 6.82 (d, J = 5.3 Hz, 1H, Naph-H), 4.69 (t, J = 6.9 Hz, 2H, CH_2), 3.63 (s, 3H, CH_3), 3.03 (t, J = 6.9 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.6, 171.5, 144.4, 138.9, 136.6, 134.1, 133.4, 132.4, 130.4, 130.2, 129.7, 129.6, 128.1, 125.7, 124.4, 117.5, 111.6, 105.2, 52.2, 41.1, 31.9. HR-MS: m/z 389.1067 $[M + H]^+$. $C_{21}H_{16}N_4O_2S$ (Exact Mass: 388.0994). HPLC purity: 98.98%.

Methyl 2-((1-(4-cyanonaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)propanoate (***72***). Purified by flash column chromatography as a white solid, yield 40.0%. Melting point: 161-163 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.06 (s, 1H, Pyr-H), 8.47 (d, J = 7.6 Hz, 1H, Naph-H), 8.34 (d, J = 8.4 Hz, 1H, Pyr-H), 8.29 (d, J = 5.5 Hz, 1H, Naph-H), 8.03 (t, J = 7.3 Hz, 1H, Naph-H), 7.96 (t, J = 7.6 Hz, 1H, Naph-H), 7.75 (t, J = 7.4 Hz, 1H, Naph-H), 7.30-7.26 (m, 1H, Pyr-H),

7.05 (d, J = 5.0 Hz, 1H, Naph-H), 4.67 (p, J = 6.2 Hz, 1H, CH), 3.64 (s, 3H, CH_3), 1.63-1.58 (m, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.5, 154.2, 142.6, 140.7, 140.3, 134.8, 134.0, 133.3, 130.8, 130.3, 129.4, 127.4, 126.1, 125.9, 123.5, 117.3, 112.2, 105.9, 63.1, 45.4, 18.8. HR-MS: m/z 389.1067 $[M + H]^+$. $C_{21}H_{16}N_4O_2S$ (Exact Mass: 388.0994). HPLC purity: 97.77%.

Methyl 2-((1-(4-cyanonaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)-2-methylpropanoate (***73***). Purified by flash column chromatography as a slight yellow solid, yield 41.9%. Melting point: > 200 °C. ^1H NMR (400 MHz, DMSO-d_6) δ 9.03 (s, 1H, Pyr-H), 8.47 (d, J = 7.6 Hz, 1H, Naph-H), 8.34 (d, J = 8.4 Hz, 1H, Pyr-H), 8.28 (d, J = 5.4 Hz, 1H, Naph-H), 8.00 (d, J = 7.6 Hz, 1H, Naph-H), 7.95 (t, J = 7.6 Hz, 1H, Naph-H), 7.76 (t, J = 7.7 Hz, 1H, Naph-H), 7.20 (d, J = 8.5 Hz, 1H, Pyr-H), 7.01 (d, J = 5.5 Hz, 1H, Naph-H), 3.65 (s, 3H, CH_3), 1.68 (s, 3H, CH_3), 1.63 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.2, 152.3, 143.1, 141.9, 141.1, 140.7, 135.1, 134.0, 133.2, 130.7, 130.3, 129.6, 127.5, 125.8, 123.4, 117.3, 112.1, 106.0, 53.9, 53.3, 26.7, 26.7. HR-MS: m/z 403.1223 $[M + H]^+$. $C_{22}H_{18}N_4O_2S$ (Exact Mass: 402.1150). HPLC purity: 98.58%.

Ethyl 1-((1-(4-cyanonaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)cyclobutane-1-carboxylate (***74***). Purified by flash column chromatography as a white solid, yield 38.0%. Melting point: > 200 °C. ^1H NMR (400 MHz, DMSO-d_6) δ 8.99 (s, 1H, Pyr-H), 8.49 (d, J = 7.0 Hz, 1H, Naph-H), 8.35 (d, J = 7.7 Hz, 1H, Pyr-H), 8.28-8.27 (m, 1H, Naph-H), 8.03 (d, J = 6.9 Hz, 1H), 7.97 (t, J = 6.7 Hz, 1H, Naph-H), 7.77 (t, J = 6.6 Hz, 1H, Naph-H), 7.24 (d, J = 8.0 Hz, 1H, Pyr-H), 7.03-7.02 (m, 1H, Naph-H), 4.11 (d, J = 6.1 Hz, 2H, CH_2), 2.92-2.76 (m, 2H, CH_2), 2.37-2.29 (m, 2H, CH_2), 2.10-1.98 (m, 2H, CH_2), 1.09 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO) δ 171.8, 153.2, 143.0,

142.2, 140.8, 135.0, 134.0, 133.3, 130.8, 130.2, 129.5, 129.1, 127.4, 125.9, 123.4, 117.3, 112.1, 105.8, 61.8, 54.0, 32.7, 32.5, 17.0, 14.3. HR-MS: m/z 429.1376 $[M + H]^+$. $C_{24}H_{20}N_4O_2S$ (Exact Mass: 428.1307). HPLC purity: 99.23%.

*General Procedure for the Preparation of **75-80***. The synthetic method was similar to that described for **17-20**.

2-((1-(4-Cyanonaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)acetic acid (***75***). Recrystallized from EA as a yellow solid, yield 62.0%. Melting point: 140-143 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.27 (s, 1H, OH), 8.77 (s, 1H, Pyr-H), 8.40 (d, J = 7.3 Hz, 1H, Naph-H), 8.22-8.19 (m, 2H, Pyr-H + Naph-H), 8.13-8.12 (m, 1H, Naph-H), 7.88-7.87 (m, 2H, 2 × Naph-H), 7.55 (d, J = 5.6 Hz, 1H, Pyr-H), 6.84 (s, 1H, Naph-H), 3.71 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.6, 144.9, 141.7, 140.6, 133.4, 132.3, 131.2, 131.1, 130.8, 129.5, 129.4, 128.6, 128.4, 127.3, 127.0, 118.1, 110.2, 109.3, 32.8. HR-MS: m/z 359.0608 $[M - H]^-$. $C_{19}H_{12}N_4O_2S$ (Exact Mass: 360.0681). HPLC purity: 97.11%.

4-((1-(4-Cyanonaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)butanoic acid (***76***). Recrystallized from EA as a white solid, yield 44.5%. Melting point: 112-113 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.15 (s, 1H, OH), 9.03 (s, 1H, Pyr-H), 8.47 (d, J = 7.5 Hz, 1H, Naph-H), 8.34 (d, J = 8.4 Hz, 1H, Pyr-H), 8.26 (d, J = 5.2 Hz, 1H, Naph-H), 8.04 (d, J = 7.6 Hz, 1H, Naph-H), 7.95 (t, J = 7.6 Hz, 1H, Naph-H), 7.74 (t, J = 7.6 Hz, 1H, Naph-H), 7.27 (d, J = 8.5 Hz, 1H, Pyr-H), 6.99 (d, J = 5.3 Hz, 1H, Naph-H), 3.38-3.35 (m, 2H, CH_2), 2.33 (t, J = 7.1 Hz, 2H, CH_2), 1.99-1.95 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.2, 155.4, 142.8, 142.7, 140.8, 140.3, 135.1, 134.0, 133.3, 130.7, 130.2, 129.5, 127.5, 125.8, 123.5, 117.3, 112.0, 105.6, 32.8, 31.7, 24.7. HR-MS: m/z 423.0688 $[M + Cl]^-$. $C_{21}H_{16}N_4O_2S$ (Exact Mass: 388.0994). HPLC purity:

98.42%.

3-((1-(4-Cyanonaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)propanoic acid (***77***). Recrystallized from EA as a white solid, yield 37.0%. Melting point: 199-200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.58 (s, 1H, OH), 8.98 (s, 1H, Pyr-H), 8.45 (d, J = 7.7 Hz, 1H, Naph-H), 8.31-8.27 (d, J = 9.1 Hz, 2H, Pyr-H + Naph-H), 8.23 (d, J = 5.3 Hz, 1H, Naph-H), 7.93-7.89 (m, 3H, Pyr-H + 2 × Naph-H), 7.51 (s, 1H, Naph-H), 4.65 (t, J = 6.6 Hz, 2H, CH_2), 2.95 (t, J = 6.7 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 171.5, 144.2, 143.7, 136.6, 136.4, 134.0, 133.3, 132.4, 131.6, 130.4, 129.6, 128.2, 128.1, 125.6, 124.3, 117.5, 111.6, 41.3, 32.1. HR-MS: m/z 409.0531 $[M + Cl]^-$. $C_{20}H_{14}N_4O_2S$ (Exact Mass: 374.0837). HPLC purity: 98.08%.

2-((1-(4-Cyanonaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)propanoic acid (***78***). Recrystallized from EA as a slight yellow solid, yield 77.1%. Melting point: > 200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.04 (s, 1H, Pyr-H), 8.47 (d, J = 7.4 Hz, 1H, Naph-H), 8.34 (d, J = 8.5 Hz, 1H, Pyr-H), 8.27 (d, J = 4.7 Hz, 1H, Naph-H), 8.18 (d, J = 5.4 Hz, 1H, Naph-H), 7.95 (t, J = 8.2 Hz, 1H, Naph-H), 7.83-7.79 (m, 1H, Naph-H), 7.27 (d, J = 7.4 Hz, 1H, Pyr-H), 7.02 (d, J = 4.9 Hz, 1H, Naph-H), 4.66-4.62 (m, 1H, CH), 1.63-1.57 (m, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.7, 142.9, 141.1, 140.7, 140.6, 140.2, 134.0, 133.3, 132.5, 130.7, 128.5, 127.5, 126.2, 125.9, 123.5, 117.3, 112.1, 105.8, 54.2, 27.8. HR-MS: m/z 373.0765 $[M - H]^-$. $C_{20}H_{14}N_4O_2S$ (Exact Mass: 374.0837). HPLC purity: 96.63%.

2-((1-(4-Cyanonaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)-2-methylpropanoic acid (***79***). Recrystallized from EA as a white solid, yield 43.0%. Melting point: > 200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ13.05 (s, 1H, OH), 9.05 (s, 1H, Pyr-H), 8.46 (d, J = 7.6 Hz, 1H, Naph-H), 8.34 (d, J = 8.4 Hz, 1H, Pyr-H), 8.28 (d, J = 5.4 Hz, 1H, Naph-H), 7.98 (d, J = 8.2 Hz, 1H,

Naph-H), 7.94 (d, *J* = 7.7 Hz, 1H, Naph-H), 7.74 (t, *J* = 7.7 Hz, 1H, Naph-H), 7.20 (d, *J* = 8.5 Hz, 1H, Pyr-H), 7.01 (d, *J* = 5.4 Hz, 1H, Naph-H), 1.67 (s, 6H, 2 × CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.2, 152.9, 142.9, 142.0, 140.9, 140.7, 135.2, 134.0, 133.2, 130.7, 130.2, 129.6, 127.5, 125.8, 123.4, 117.3, 112.0, 106.0, 54.5, 26.8, 26.7. HR-MS: m/z 389.1067 $[M + H]^+$. $C_{21}H_{16}N_4O_2S$ (Exact Mass: 388.0994). HPLC purity: 96.44 %.

1-((1-(4-Cyanonaphthalen-1-yl)-1H-imidazo[4,5-c]pyridin-2-yl)thio)cyclobutane-1-carboxylic acid (***80***). Recrystallized from EA as a white solid, yield 79.6%. Melting point: > 200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.98 (s, 1H, OH), 8.93 (s, 1H, Pyr-H), 8.40 (d, *J* = 7.3 Hz, 1H, Naph-H), 8.27 (d, *J* = 8.0 Hz, 1H, Pyr-H), 8.19 (d, *J* = 3.7 Hz, 1H), 7.94 (d, *J* = 7.3 Hz, 1H, Naph-H), 7.88 (t, *J* = 7.2 Hz, 1H, Naph-H), 7.67 (t, *J* = 7.3 Hz, 1H, Naph-H), 7.18 (d, *J* = 8.2 Hz, 1H, Pyr-H), 6.93 (d, *J* = 3.7 Hz, 1H, Naph-H), 2.82-2.62 (m, 2H, CH_2), 2.37-2.21 (m, 2H, CH_2), 2.06-1.84 (m, 2H, CH_2). ^{13}C NMR (101 MHz, DMSO) δ 173.4, 153.6, 142.9, 142.1, 140.8, 135.2, 134.0, 133.3, 130.7, 130.2, 129.5, 127.4, 125.8, 123.5, 117.3, 112.0, 105.8, 54.6, 32.7, 32.6, 17.0. HR-MS: m/z 399.0921 $[M - H]^-$. $C_{22}H_{16}N_4O_2S$ (Exact Mass: 400.0994). HPLC purity: 98.02%.

General procedure for the preparation of final compounds ***81-96***. Lesinurad (0.100 g, 0.25 mmol) was dissolved in 10 ml of dry dichloromethane and the solution was stirred for 3 min under an ice bath. Then 4-dimethylaminopyridine (DMAP) (0.036 g,0.30 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC · HCl) (0.057 g,0.30 mmol) were added, and stirring was continued in the ice bath for 30 min. Finally, the appropriate sulfonamide (0.030 mmol) was added. After the reaction was completed (12 h), the solvent was evaporated under reduced pressure. The residue was taken up in 30 mL dichloromethane and washed with saturated aqueous sodium chloride (3 × 10 mL). The organic layer was separated,

dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated and the residue was purified by flash column chromatography to give the target compound **81-96**.

*2-((5-Bromo-4-(4-cyclopropylnaphthalen-1-yl)-4H-1,2,4-triazol-3-yl)thio)-N-(phenylsulfonyl)acetamide (**81**)*. Recrystallized from EA as a white solid, yield 40.1 %. Melting point: 181-183 °C. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.58 (d, J = 8.5 Hz, 1H, Naph-H), 7.99 (d, J = 9.0 Hz, 2H, Naph-H), 7.71 – 7.63 (m, 2H, Naph-H), 7.58 (d, J = 7.9 Hz, 2H, Ph-H), 7.54 (d, J = 7.4 Hz, 1H, Ph-H), 7.40 (s, 2H, Ph-H), 7.02 (d, J = 8.3 Hz, 1H, Naph-H), 3.94 (s, 2H, CH_2), 2.48 (t, J = 8.4 Hz, 1H, CH), 1.18 (q, J = 6.1 Hz, 2H, CH_2), 0.84 (q, J = 5.2, 4.7 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, CD_3OD_SPE) δ 166.3, 154.1, 143.9, 139.1, 134.1, 133.5, 131.8, 131.6, 129.0, 128.6, 128.6, 127.8, 126.9, 126.5, 126.4, 125.7, 125.0, 122.5, 121.5, 35.2, 12.8, 6.3(2C). HR-MS: m/z 541.0009 [M – H]$^-$. $C_{23}H_{19}BrN_4O_3S_2$ (Exact Mass: 542.0082). HPLC purity: 98.91%.

*2-((5-Bromo-4-(4-cyclopropylnaphthalen-1-yl)-4H-1,2,4-triazol-3-yl)thio)-N-((4-fluorophenyl)sulfonyl)acetamide (**82**)*. Recrystallized from EA as a white solid, yield 56.6 %. Melting point: 195-197 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.63 (s, 1H, NH), 8.57 (d, J = 8.5 Hz, 1H, Naph-H), 7.99 (dd, J = 8.8, 5.1 Hz, 2H, Ph-H), 7.74 (t, J = 7.6 Hz, 1H, Naph-H), 7.63 (t, J = 7.6 Hz, 1H, Naph-H), 7.57 (d, J = 7.6 Hz, 1H, Naph-H), 7.46 (t, J = 8.8 Hz, 2H, Ph-H), 7.41 (d, J = 7.6 Hz, 1H, Naph-H), 7.01 (d, J = 8.4 Hz, 1H, Naph-H), 4.03 (s, 2H, CH_2), 2.55 (td, J = 9.1, 4.7 Hz, 1H, CH), 1.14 (d, J = 10.1 Hz, 2H, CH_2), 0.86 (d, J = 4.0 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d6) δ 166.7, 143.6, 133.9, 131.9, 131.3, 131.2, 129.0, 128.6, 127.8, 127.2, 126.9, 125.6, 123.1, 122.1, 116.8, 116.6, 36.3, 13.4, 7.8, 7.7. HR-MS: m/z 558.9966 [M – H]$^-$. $C_{23}H_{18}BrFN_4O_3S_2$ (Exact Mass: 559.9988). HPLC purity: 99.47%.

*2-((5-Bromo-4-(4-cyclopropylnaphthalen-1-yl)-4H-1,2,4-triazol-3-yl)thio)-N-((4-bromophenyl) sulfonyl)acetamide (**83**)*. Recrystallized from EA as a white solid, yield 60.0 %. Melting point: > 200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.70 (s, 1H, NH), 8.57 (d, J = 8.5 Hz, 1H, Naph-H), 7.84 (s, 4H, Ph-H), 7.74 (t, J = 7.5 Hz, 1H, Naph-H), 7.63 (t, J = 7.6 Hz, 1H, Naph-H), 7.57 (d, J = 7.6 Hz, 1H, Naph-H), 7.41 (d, J = 7.6 Hz, 1H, Naph-H), 6.98 (d, J = 8.3 Hz, 1H, Naph-H), 4.13 – 3.96 (m, 2H, CH_2), 2.59 – 2.52 (m, 1H, CH), 1.15 (d, J = 8.3 Hz, 2H, CH_2), 0.86 (d, J = 3.9 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.6 , 166.7 , 153.5 , 143.7, 138.6, 133.9, 132.6, 132.0, 130.1, 129.0, 128.6, 128.3, 127.8, 127.2, 126.9, 125.6, 123.1, 122.1, 36.0, 21.5, 13.4, 7.8, 7.7. HR-MS: m/z 618.9114 $[M - H]^-$. $C_{23}H_{18}Br_2N_4O_3S_2$ (Exact Mass: 619.9187). HPLC purity: 96.46%.

*2-((5-Bromo-4-(4-cyclopropylnaphthalen-1-yl)-4H-1,2,4-triazol-3-yl)thio)-N-((2,4-difluorophen yl)sulfonyl)acetamide (**84**)*. Recrystallized from EA as a white solid, yield 40.0 %. Melting point: 195-197 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.98 (s, 1H, NH), 8.57 (d, J = 8.5 Hz, 1H, Naph-H), 8.06 – 7.95 (m, 1H, Ph-H), 7.73 (t, J = 7.6 Hz, 1H, Naph-H), 7.63 (t, J = 7.6 Hz, 1H, Ph-H), 7.60 – 7.51 (m, 2H, Naph-H), 7.41 (d, J = 7.7 Hz, 1H, Naph-H), 7.33 (t, J = 8.5 Hz, 1H, Naph-H), 7.02 (d, J = 8.4 Hz, 1H, Ph-H), 4.14 – 3.99 (m, 2H, CH_2), 2.54 (t, J = 5.4 Hz, 1H, CH), 1.14 (d, J = 10.1 Hz, 2H, CH_2), 0.85 (d, J = 3.9 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.6, 153.5, 143.6, 133.9, 131.9, 129.0, 128.6, 127.7, 127.2, 126.9, 125.6, 123.1, 122.1, 112.7, 112.5, 106.9, 106.6, 106.4, 36.1, 21.5, 13.4, 7.8, 7.8. HR-MS: m/z 576.9821 $[M - H]^-$. $C_{23}H_{17}BrF_2N_4O_3S_2$ (Exact Mass: 577.9894). HPLC purity: 99.77%.

*2-((5-Bromo-4-(4-cyclopropylnaphthalen-1-yl)-4H-1,2,4-triazol-3-yl)thio)-N-((4-chlorophenyl) sulfonyl)acetamide (**85**)*. Recrystallized from EA as a white solid, yield 36.0 %. Melting point: >

200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.68 (s, 1H, NH), 8.57 (d, J = 8.5 Hz, 1H, Naph-H), 7.91 (s, 2H, Naph-H), 7.75 (d, J = 7.3 Hz, 1H, Naph-H), 7.70 (d, J = 8.7 Hz, 2H, Ph-H), 7.61 (dd, J = 25.5, 7.7 Hz, 2H, Ph-H), 7.41 (d, J = 7.6 Hz, 1H, Naph-H), 6.99 (d, J = 8.3 Hz, 1H, Naph-H), 4.11 – 3.98 (m, 2H, CH_2), 2.54 (t, J = 5.4 Hz, 1H, CH), 1.19 – 1.11 (m, 2H, CH_2), 0.86 (q, J = 5.4 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.7, 153.5, 143.7, 139.2, 138.2, 133.9 132.0, 130.1, 129.7, 129.5, 129.0, 128.6, 128.1, 127.8, 127.2, 126.9, 125.6, 123.1, 122.1, 36.0, 13.4, 7.8, 7.7. HR-MS: m/z 574.9619 $[M - H]^-$. $C_{23}H_{18}ClN_4O_3S_2$ (Exact Mass: 575.9692). HPLC purity: 98.02%.

*2-((5-Bromo-4-(4-cyclopropylnaphthalen-1-yl)-4H-1,2,4-triazol-3-yl)thio)-N-((4-nitrophenyl)sulfonyl)acetamide (**86**)*. Recrystallized from EA as a white solid, yield 38.0 %. Melting point: > 200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.74 (s, 1H, NH), 8.56 (d, J = 8.5 Hz, 1H, Naph-H), 8.43 (d, J = 8.9 Hz, 2H, Ph-H), 8.18 (d, J = 8.9 Hz, 2H, Ph-H), 7.73 (t, J = 7.3 Hz, 1H, Naph-H), 7.63 (t, J = 7.6 Hz, 1H, Naph-H), 7.57 (d, J = 7.6 Hz, 1H, Naph-H), 7.41 (d, J = 7.6 Hz, 1H, Naph-H), 6.96 (d, J = 8.3 Hz, 1H, Naph-H), 4.16 – 3.97 (m, 2H, CH_2), 2.59 – 2.52 (m, 1H, CH), 1.14 (q, J = 5.8 Hz, 2H, CH_2), 0.85 (q, J = 5.5 Hz, 2H, CH_2).^{13}C NMR (100 MHz, DMSO-d_6) δ 167.0, 153.5, 150.7, 144.7, 143.7, 133.9, 131.9, 129.8, 129.0, 128.6, 127.7, 127.2, 126.9, 125.6, 124.8, 123.1, 122.1, 36.1, 13.3, 7.8, 7.7. HR-MS: m/z 585.9861 $[M - H]^-$. $C_{23}H_{18}BrN_5O_5S_2$ (Exact Mass: 586.9933). HPLC purity: 98.40%.

*2-((5-Bromo-4-(4-cyclopropylnaphthalen-1-yl)-4H-1,2,4-triazol-3-yl)thio)-N-tosylacetamide (**87**)*. Recrystallized from EA as a white solid, yield 45.0%. Melting point: > 200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.47 (s, 1H, NH), 8.56 (d, *J* = 8.5 Hz, 1H, Naph-H), 7.79 (d, *J* = 8.2 Hz, 2H, Ph-H), 7.73 (t, *J* = 7.5 Hz, 1H, Naph-H), 7.61 (t, *J* = 7.6 Hz, 1H, Naph-H), 7.56 (d, *J* = 7.6 Hz,

1H, Naph-H), 7.42 (s, 1H, Naph-H), 7.41 (d, *J* = 6.1 Hz, 2H, Ph-H), 7.00 (d, *J* = 8.4 Hz, 1H, Naph-H), 4.12 – 3.96 (m, 2H, CH_2), 2.54 (t, *J* = 3.4 Hz, 1H, CH), 2.38 (s, 3H, CH_3), 1.14 (d, *J* = 10.1 Hz, 2H, CH_2), 0.85 (d, *J* = 4.0 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.3, 153.5, 144.8, 143.6, 136.6, 133.9, 131.9, 130.0, 129.8, 129.0, 128.6, 128.1, 127.7, 127.2, 126.9, 126.1, 125.6, 123.1, 122.1, 36.1, 21.6, 13.4, 7.8, 7.8. HR-MS: m/z 555.0166 $[M - H]^-$. $C_{24}H_{21}BrN_4O_3S_2$ (Exact Mass: 556.0238). HPLC purity: 99.00%.

*2-((5-Bromo-4-(4-cyclopropylnaphthalen-1-yl)-4H-1,2,4-triazol-3-yl)thio)-N-(thiophen-2-ylsulfonyl)acetamide (**88**)*. Recrystallized from EA as a white solid, yield 60.0%. Melting point: > 200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.68 (s, 1H, NH), 8.58 (d, *J* = 8.4 Hz, 1H, Naph-H), 8.11 – 8.00 (m, 1H, Naph-H), 7.83 – 7.71 (m, 2H, Naph-H), 7.65 (t, *J* = 7.5 Hz, 1H, Naph-H), 7.59 (d, *J* = 7.6 Hz, 1H, Thiophene-H), 7.42 (d, *J* = 7.7 Hz, 1H, Naph-H), 7.25 – 7.17 (m, 1H, Thiophene-H), 7.09 (d, *J* = 8.3 Hz, 1H, Thiophene-H), 4.06 (d, *J* = 2.3 Hz, 2H, CH_2), 2.62 – 2.52 (m, 1H, CH), 1.2–1.08 (m, 2H, CH_2), 0.86 (q, *J* = 5.4 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.4, 153.5,143.7, 139.7, 135.4, 134.8, 133.9, 131.9, 129.0, 128.6, 128.0, 127.8, 127.3, 126.9, 125.6, 123.1, 122.2, 36.2, 13.4, 7.8, 7.7. HR-MS: m/z 546.9573 $[M - H]^-$. $C_{21}H_{17}BrN_4O_3S_3$ (Exact Mass: 547.9646). HPLC purity: 96.00%.

*2-((5-Bromo-4-(4-cyclopropylnaphthalen-1-yl)-4H-1,2,4-triazol-3-yl)thio)-N-((5-chlorothiophen-2-yl)sulfonyl)acetamide (**89**)*. Recrystallized from EA as a white solid, yield 40.9%. Melting point: > 200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.52 (s, 1H, NH), 8.58 (d, *J* = 8.4 Hz, 1H, Naph-H), 7.75 (t, *J* = 7.5 Hz, 1H, Naph-H), 7.71 – 7.63 (m, 2H, Naph-H), 7.61 (d, *J* = 7.6 Hz, 1H, Naph-H), 7.43 (d, *J* = 7.6 Hz, 1H, Naph-H), 7.28 (d, *J* = 4.1 Hz, 1H, Thiophene-H), 7.09 (d, *J* = 8.3 Hz, 1H, Thiophene-H), 4.13 – 4.00 (m, 2H, CH_2), 2.63 – 2.52 (m, 1H, CH), 1.22 – 1.09 (m,

2H, CH_2), 0.87 (q, J = 5.3 Hz, 2H, CH_2).^{13}C NMR (100 MHz, DMSO-d_6) δ 167.0, 153.6, 143.7, 137.8, 137.3, 134.6, 134.0, 131.9, 129.0, 128.6, 128.1, 127.8, 127.2, 126.9, 125.6, 123.1, 122.2, 36.2, 13.4, 7.8, 7.8. HR-MS: m/z 580.9184 $[M - H]^-$. $C_{21}H_{16}BrClN_4O_3S_3$ (Exact Mass: 581.9256). HPLC purity: 98.87%.

*2-((5-Bromo-4-(4-cyclopropylnaphthalen-1-yl)-4H-1,2,4-triazol-3-yl)thio)-N-(methylsulfonyl)acetamide (**90**)*. Recrystallized from EA as a white solid, yield 60.6%. Melting point: 171-173 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.09 (s, 1H, NH), 8.59 (d, J = 8.4 Hz, 1H, Naph-H), 7.75 (t, J = 7.4 Hz, 1H, Naph-H), 7.72 – 7.62 (m, 2H, Naph-H), 7.45 (d, J = 7.6 Hz, 1H, Naph-H), 7.17 (d, J = 8.3 Hz, 1H, Naph-H), 4.07 (s, 2H, CH_2), 3.22 (s, 3H, CH_3), 1.92 (s, 1H, CH), 1.19 – 1.13 (m, 2H, CH_2), 0.90 – 0.84 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.4, 153.7, 143.7, 134.0, 132.1, 129.1, 128.7, 127.8, 127.3, 127.0, 125.7, 123.2, 122.2, 41.3, 36.3, 13.4, 7.8, 7.8. HR-MS: m/z 478.9853 $[M - H]^-$. $C_{18}H_{17}BrN_4O_3S_2$ (Exact Mass: 479.9925). HPLC purity: 99.21%.

*2-((5-Bromo-4-(4-cyclopropylnaphthalen-1-yl)-4H-1,2,4-triazol-3-yl)thio)-N-(ethylsulfonyl)acetamide (**91**)*. Recrystallized from EA as a white solid, yield 40.4%. Melting point: > 200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.01 (s, 1H, NH), 8.59 (d, J = 8.4 Hz, 1H, Naph-H), 7.76 (t, J = 7.3 Hz, 1H, Naph-H), 7.72 – 7.63 (m, 2H, Naph-H), 7.45 (d, J = 7.6 Hz, 1H, Naph-H), 7.15 (d, J = 8.3 Hz, 1H, Naph-H), 4.08 (s, 2H, CH_2), 3.35 – 3.31 (m, 2H, CH_2), 2.62 – 2.53 (m, 1H, CH), 1.25 (t, J = 7.3 Hz, 3H, CH_3), 1.16 (dd, J = 8.4, 1.9 Hz, 2H, CH_2), 0.89 – 0.85 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.3, 153.7, 143.7, 134.0, 132.1, 129.1, 128.7, 127.8, 127.3, 126.9, 125.7, 123.1, 122.1, 47.1, 36.1, 13.4, 8.2, 7.8, 7.8. HR-MS: m/z 493.0009 $[M - H]^-$. $C_{19}H_{19}BrN_4O_3S_2$ (Exact Mass: 494.0082). HPLC purity: 96.65%.

*2-((5-Bromo-4-(4-cyclopropylnaphthalen-1-yl)-4H-1,2,4-triazol-3-yl)thio)-N-(propylsulfonyl)acetamide (**92**)*. Recrystallized from EA as a white solid, yield 70.0%. Melting point: 183-184 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.03 (s, 1H, NH), 8.59 (d, J = 8.4 Hz, 1H, Naph-H), 7.75 (t, J = 7.6 Hz, 1H, Naph-H), 7.67 (dd, J = 13.2, 7.8 Hz, 2H, Naph-H), 7.45 (d, J = 7.6 Hz, 1H, Naph-H), 7.14 (d, J = 8.3 Hz, 1H, Naph-H), 4.13 – 4.03 (m, 2H, CH_2), 3.31 (d, J = 7.8 Hz, 2H, CH_2), 2.56 (ddd, J = 14.2, 8.6, 5.9 Hz, 1H, CH), 1.72 (h, J = 7.4 Hz, 2H, CH_2), 1.19 – 1.12 (m, 2H, CH_2), 0.97 (t, J = 7.4 Hz, 3H, CH_3), 0.91 – 0.82 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.2, 153.7, 143.7, 134.0, 132.0, 129.1, 128.7, 127.8, 127.3, 126.9, 125.7, 123.2, 122.1, 54.0, 36.1, 17.1, 13.41, 12.9, 7.8, 7.8. HR-MS: m/z 507.0166 $[M - H]^-$. $C_{20}H_{21}BrN_4O_3S_2$ (Exact Mass: 508.0238). HPLC purity: 99.43%.

*2-((5-Bromo-4-(4-cyclopropylnaphthalen-1-yl)-4H-1,2,4-triazol-3-yl)thio)-N-(isopropylsulfonyl)acetamide (**93**)*. Recrystallized from EA as a white solid, yield 50.2%. Melting point: 195-197 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.91 (s, 1H, NH), 8.59 (d, J = 8.4 Hz, 1H, Naph-H), 7.75 (t, J = 7.6 Hz, 1H, Naph-H), 7.66 (dd, J = 14.0, 7.5 Hz, 2H, Naph-H), 7.44 (d, J = 7.6 Hz, 1H, Naph-H), 7.15 (d, J = 8.3 Hz, 1H, Naph-H), 4.08 (s, 2H, CH_2), 3.56 (Hept, J = 6.8 Hz, 1H, CH), 2.56 (ddd, J = 13.9, 8.5, 5.6 Hz, 1H, CH), 1.30 (dd, J = 6.7, 4.6 Hz, 6H, $CH_3 \times 2$), 1.18 – 1.13 (m, 2H, CH_2), 0.93 – 0.83 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.2, 153.7, 143.7, 134.0, 132.0, 129.1, 128.7, 127.8, 127.3, 126.9, 125.7, 123.2, 122.2, 53.1, 36.1, 15.9, 15.9, 13.4, 7.9, 7.8. HR-MS: m/z 507.0166 $[M - H]^-$. $C_{20}H_{21}BrN_4O_3S_2$ (Exact Mass: 508.0238). HPLC purity: 97.74%.

*2-((5-Bromo-4-(4-cyclopropylnaphthalen-1-yl)-4H-1,2,4-triazol-3-yl)thio)-N-(tert-butylsulfonyl)acetamide (**94**)*. Recrystallized from EA as a white solid, yield 70.1%. Melting point: 165-168 °C.

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.48 (s, 1H, NH), 8.52 (d, J = 8.4 Hz, 1H, Naph-H), 7.68 (t, J = 7.3 Hz, 1H, Naph-H), 7.64 – 7.55 (m, 2H, Naph-H), 7.38 (d, J = 7.6 Hz, 1H, Naph-H), 7.06 (d, J = 8.3 Hz, 1H, Naph-H), 4.02 (d, J = 2.7 Hz, 2H, CH_2), 2.50 (dq, J = 8.2, 4.2, 2.8 Hz, 1H, CH), 1.28 (s, 9H, CH_3 × 3), 1.09 (dd, J = 8.4, 1.9 Hz, 2H, CH_2), 0.80 (q, J = 5.2 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.3, 153.8, 143.7, 134.0, 132.0, 129.0, 128.7, 127.8, 127.3, 126.9, 125.7, 123.2, 122.1, 61.7, 36.7, 24.4, 24.1, 13.4, 7.8, 7.8. HR-MS: m/z 521.0322 $[M - H]^-$. $C_{21}H_{23}BrN_4O_3S_2$ (Exact Mass: 522.0395). HPLC purity: 99.90%.

*2-((5-Bromo-4-(4-cyclopropylnaphthalen-1-yl)-4H-1,2,4-triazol-3-yl)thio)-N-(cyclopropylsulfonyl)acetamide (**95**)*. Recrystallized from EA as a white solid, yield 70.9%. Melting point: > 200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.06 (s, 1H, NH), 8.59 (d, J = 8.4 Hz, 1H, Naph-H), 7.75 (t, J = 7.4 Hz, 1H, Naph-H), 7.67 (dd, J = 12.3, 7.8 Hz, 2H, Naph-H), 7.45 (d, J = 7.6 Hz, 1H, Naph-H), 7.16 (d, J = 8.3 Hz, 1H, Naph-H), 4.09 (s, 2H, CH_2), 2.90 (ddd, J = 12.9, 7.7, 5.2 Hz, 1H, CH), 2.57 (dq, J = 8.0, 4.1, 2.5 Hz, 1H, CH), 1.21–1.13 (m, 2H, CH_2), 1.06 (dd, J = 11.6, 3.8 Hz, 4H, CH_2 × 2), 0.90 – 0.86 (m, 2H, CH_2). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.0, 153.6, 143.7, 137.3, 134.6, 134.0, 131.9, 129.0, 128.6, 128.1, 127.8, 127.2, 126.9, 125.6, 123.1, 122.2, 36.2, 13.4, 7.8. HR-MS: m/z 505.0009 $[M - H]^-$. $C_{20}H_{19}BrN_4O_3S_2$ (Exact Mass: 506.0082). HPLC purity: 99.16%.

*2-((5-bromo-4-(4-cyclopropylnaphthalen-1-yl)-4H-1,2,4-triazol-3-yl)thio)-N-((4-(tert-butyl)phenyl)sulfonyl)acetamide (**96**)*. Recrystallized from EA as a white solid, yield 40.7%. Melting point: 195-196 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.53 (s, 1H, NH), 8.56 (d, J = 8.5 Hz, 1H, Naph-H), 7.84 (d, J = 8.6 Hz, 2H, Naph-H), 7.73 (t, J = 7.6 Hz, 1H, Naph-H), 7.67 – 7.52 (m, 4H, Ph-H), 7.41 (d, J = 7.6 Hz, 1H, Naph-H), 6.97 (d, J = 8.4 Hz, 1H, Naph-H), 4.12 – 4.00 (m, 2H,

CH_2), 2.55 (dd, J = 8.3, 5.5 Hz, 1H, CH), 1.28 (s, 9H, $CH_3 \times 3$), 1.17 – 1.11 (m, 2H, CH_2), 0.85 (q, J = 5.6 Hz, 2H, CH_2).^{13}C NMR (100 MHz, DMSO-d_6) δ 166.4, 157.3, 153.6, 143.6, 136.6, 133.9, 131.9, 129.0, 128.6, 127.9, 127.7, 127.2, 126.9, 126.4, 125.6, 123.1, 122.2, 36.0, 35.4, 31.2, 13.4, 7.8, 7.8. HR-MS: m/z 597.0635 $[M - H]^-$. $C_{27}H_{27}BrN_4O_3S_2$ (Exact Mass: 598.0708). HPLC purity: 97.41%.

Establishment of the mouse model of hyperuricemia. Healthy male Kunming mice were provided by Experimental Animal Center of Shandong University and maintained with free access to standard food and water. The mice were divided into a vehicle group, a model group, an active-control group and an experimental group. The vehicle group consisted of mice not treated with xanthine and potassium oxonate, or test compound (i.e, untreated normal mice). The model group consisted of mice with acute hyperuricemia induced with 0.2 mL xanthine intragastric injection and 0.2 mL potassium oxonate subcutaneous injection, and not treated with test compound (i.e., untreated hyperuricemic mice). The active-control group consisted of hyperuricemic mice treated with benzbromarone or lesinurad (2 mg/kg, dissolved in 0.5% CMC-Na) as a positive control. The experimental group consisted of hyperuricemic mice treated with a test compound (2 mg/kg, dissolved in 0.5% CMC-Na). Positive control drugs or test compounds were administered 3 h before xanthine/potassium oxonate. At 6 h after induction of hyperuricemia, blood was collected and analyzed immunoenzymatically with a UniCel DxC 800 Synchron automatic biochemical analyzer (Beckman Coulter), kindly made available by the Second Hospital of Shandong University.

Animal care and handling were conducted in compliance with the Guide for the Care and Use of Laboratory Animals (U.S. Department of Health and Human Services, NIH Publication No.

93-23, revised 1985), as well as the Animal Experiment Ethics Review Committee of Shandong University and the Animal Management Rules of the Ministry of Health of the People's Republic of China.

IC_{50} assay. ^{14}C-Uric acid (55mCi/mmol) was purchased from American Radiolabeled Chemicals (ARC, St. Louis, MO). HEK293T cells were purchased from ATCC and grown in DMEM/high glucose supplemented with 10% FBS at 37 ℃ in 5% CO_2. The human URAT1 (SLC22A12, GenBank BC053348.1) gene was subcloned into the pcDNA3.1(+)-EGFP vector (Sangon, Shanghai, CHN) to express hURAT1.

A transient expression system for hURAT1 in HEK293T cells was constructed for evaluation of ^{14}C-uric acid transport. In brief, 96-well plates were pre-incubated with poly-D-lysine solution (0.1 mg/ml) for 12 h to improve cell adhesion. Then cells were seeded into the plates, and when the cells reached 70 to 80% confluence, 100 ng/well DNA plasmid was transferred into the plates with Lipofectamine 3000 according to the manufacturer's instructions. After transfection for 24 h, the culture medium was removed and the cells were washed with PBS twice. The cells were incubated with uric acid uptake buffer (containing 125 mM sodium gluconate, 4.8 mM potassium gluconate, 1.2 mM monobasic potassium phosphate, 1.2 mM magnesium sulfate, 1.3 mM calcium gluconate and 5.6 mM glucose) in the presence and absence of test compounds for 15 min. The uric acid uptake was initiated by the addition of 50 μM ^{14}C-uric acid. Cells were washed three times with ice-cold DPBS to terminate the reaction, and lysed with 40 μl of 0.1 M sodium hydroxide. Intracellular radioactivity was determined with a liquid scintillation counter (PerkinElmer, Boston, MA) after the addition of 0.2 ml of scintillant. Each treatment was measured in triplicate.[36,46]

Cell culture. HEK293T cells were sourced from ATCC. All cultured cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) FBS, 1% penicillin and 1% streptomycin at 37 °C in a humidified incubator. The cultures were checked periodically and found to be free of mycoplasma contamination.[48]

Pharmacokinetics study. Ten male Sprague-Dawley rats were randomly divided into two groups to receive intravenous (1 $mL \cdot kg^{-1}$) or oral administration (20 $mL \cdot kg^{-1}$, 10 $mL \cdot kg^{-1}$) of a test drug. Solutions of **44, 54** and **83** were prepared by dissolving each compound in a mixture of polyethylene glycol (PEG) 400/normal saline (70/30, V/V) before the experiment. Blood samples of the intravenous group were collected from the sinus jugularis into heparinized centrifugation tubes at 2 min, 5 min, 15 min, 30 min, 1 h, 1.5 h, 2 h, 4 h, 6 h, and 8 h after dosing, and blood samples of the oral administration group were collected at 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, 14 h, and 24 h after dosing (200 μL of blood each time). The samples were centrifuged at 2200 g for 10 min to separate plasma, which was stored at -20 °C until LC-MS analysis to determine the concentration of **44, 54** or **83**. Briefly, 50 μL of plasma was added to 50 μL of internal standard (**45**, **55** and dexamethasone) and 300 μL of methanol in a 5 mL centrifugation tube, which was centrifuged at 3000g for 10 min. The supernatant was collected, and a 20 μL aliquot was injected for LC-MS analysis. Standard curves for **44, 54** and **83** in blood were generated by the addition of various concentrations of **44, 54** and **83** together with internal standard into blank plasma. All samples were quantified with an Agilent 1200 LC/MSD (Agilent, USA). The mobile phase was methanol/1.5% glacial acetic acid (50:50, V/V) at a flow rate of 1.0 mL/min, and the test wavelength was 225 nm. All blood samples were centrifuged in an Eppendorf 5415D centrifuge and quantified with an Agilent 1200 LC/MSD (Agilent, USA).

Acute toxicity experiment. Kunming mice (10-12 g) were purchased from the Animal Experimental Center of Shandong University. Mice were fasted for 12 h, then a suspension of **44**, **54**, **83** or lesinurad in 5% CMC-Na at the concentration of 100 $mg \cdot mL^{-1}$ was administered intragastrically to provide a dose of 500 $mg \cdot kg^{-1}$. Each group consisted of 12 mice (6 males, 6 females).

Subacute toxicity experiment. Another batch of 8 male and 8 female Kunming mice (also purchased from the animal experimental center of Shandong University) were randomly divided into four groups (n = 4): male control group, female control group, male test group, and female test group. All mice were deprived of feed for 12 h, and then the mice in test groups were given 50 $mg \cdot kg^{-1}$ p.o. of **44**, **54**, **83** or lesinurad once every other day for 14 days (D0, D2, D4, D6, D8, D10, and D12), while the mice in control groups received the same volume of vehicle solution. The mice were weighed before each dosing. All the mice were killed and dissected at D14, and the heart, liver, spleen, lung, and kidney were extracted. These organs were examined by HE staining.

Plasma exposure experiment. Ten male Kunming mice were randomly divided into five groups to receive oral administration ($2mg \cdot kg^{-1}$, 50 $mg \cdot kg^{-1}$, 500 $mg \cdot kg^{-1}$) of a test drug. Solutions of **44** and lesinurad were prepared by dissolving each compound in a mixture of polyethylene glycol (PEG) 400/ normal saline (70/30, V/V) before the experiment. Blood samples of each group were collected from the submandibular or saphenous vein into pre-chilled commercial microcentrifuge tubes containing K_2EDTA as an anti-coagulant at 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 8 h, 24 h, 48 h and 72 h after dosing (30 μL of blood each time). The samples were centrifuged at 3200g at approximately 4 ℃ within half an hour of collection. Plasma samples were placed in polypropylene tubes, quick-frozen over dry ice and kept at -70 ℃ until LC-MS/MS analysis to

determine the concentration of **44** or lesinurad. Briefly, 6 μL of plasma was added to 120 μL of internal standard (dexamethasone) and 120 μL of acetonitrile in a 5 mL centrifugation tube, which was vortex-mixed for 10 min at 800 rpm and then centrifuged for 15 min at 3220 g. The supernatant was collected, and a 50 μL aliquot was injected for LC-MS analysis. Standard curves for **44** and lesinurad in blood were generated by the addition of various concentrations of **44** and lesinurad together with internal standard into blank plasma. All samples were quantified with an Agilent 1200 LC/MSD (Agilent, USA). The mobile phase was 0.1% FA and 2 mM $HCOONH_4$ in water/ACN (95:5, V/V) at a flow rate of 0.6 mL/min, and the test wavelength was 225 nm. All blood samples were centrifuged in an Eppendorf 5415D centrifuge and quantified with an Agilent 1200 LC/MSD (Agilent, USA).

Assay of CYP-inhibitory activity assay. CYP-inhibitory activities were measured by WuXi AppTec (Shanghai) Co., Ltd, China. Compounds **44** and **54** were prepared at 8 concentrations (0, 0.05, 0.15, 0.5, 1.5, 5.0, 15, 50 μM) and incubated with human liver microsomes (0.1 mg/mL) and cofactor NADPH (1 mM) in the presence of the corresponding probe substrates for 3-20 min at 37 ± 0.2 °C. Selective CYP inhibitors, α-naphthoflavone (CYP1A2), sulfaphenazole (CYP2C9), (+)-N-3-benzylnirvanol (CYP2C19), quinidine (CYP2D6) and ketoconazole (CYP3A4M), were screened as positive controls. 400 μL cold acetonitrile solution containing 200 ng/mL tolbutamide and 200 ng/mL labetalol was added to terminate the reaction. The sample solutions were centrifuged at 4000 rpm for 20 minutes to precipitate protein, and then 200 μL of the supernatant was diluted with 100 μL HPLC water and was shaken for 10 min. Finally, the mixtures were subjected to LC-MS/MS analysis.

Assay procedures for hERG activity HEK293 cells stably transfected with hERG cDNA were used to test the inhibitory effect of test compounds on the hERG potassium channel. HEK239 cells expressing hERG were plated in 35 mm dishes and maintained for at least 24 hours at 37 °C under 5% CO_2 before the experiment. A micropipette was drawn out from borosilicate glass to give a tip resistance between 3 ~ 5 MΩ. For each trial, a single dish of cells was removed from the incubator, washed twice with ECS and placed on the microscope stage. Whole-cell recordings were conducted with a commercial patch clamp amplifier.

Tail currents were evoked once every 30 s by a 3 s, -50 mV repolarizing pulse following a 2 s, +50 mV depolarizing pulse with a hold voltage of -80 mV. A 50 ms depolarizing pulse to -50 mV at the beginning of the voltage protocol served as a baseline for calculating the amplitude of the peak tail current. Only stable cells with recorded parameters exceeding the threshold were used for experiments. The hERG currents were allowed to stabilize over a 3-minute period with vehicle alone prior to test compound application. The cells were kept in the test solution until the peak tail current was stable (< 5% change) for ~5 sweeps. Peak tail amplitudes were plotted as a function of the sweep number. Five peak tail current measurements at the steady state before the test compound (**44**) application were averaged and used as the control current amplitude. Four or five peak tail current measurements at the steady state after test compound application were averaged and used as the remaining current amplitude after inhibition by the test compound. Percent inhibition was calculated according to the following equation:

$$\% \text{ inhibition} = \{1 - (\text{remaining current amplitude})/(\text{control current amplitude})\} * 100$$

Associated Content

The Supporting Information is available free of charge on the ACS Publications website at DOI:

Molecular formula strings and data (CSV)

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Notes

The authors declare that all experimental work complied with the institutional guidelines on animal studies (care and use of laboratory animals).

The authors declare no competing financial interest.

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Abbreviations Used

SUA, serum uric acid; SARs, structure-activity relationships; URAT1, human urate transporter 1; XO, xanthine oxidase; GLUT9, glucose transporter 9; hERG alpha subunit of human potassium ion channel.

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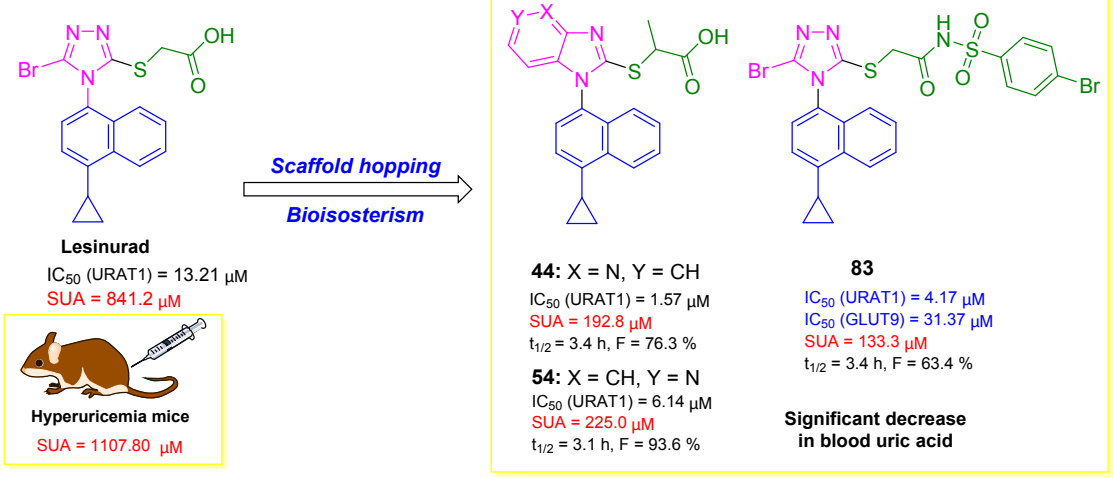
Table of Contents Graphic



Candidate drugs to treat hyperuricemia and gout