

Article

Discovery of Novel Biphenyl Carboxylic Acid Derivatives as Potent URAT1 Inhibitors

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Abstract: Urate transporter 1 (URAT1) is a clinically validated target for the treatment of hyperuricemia and gout. Due to the absence of protein structures, the molecular design of new URAT1 inhibitors generally resorts to ligand-based approaches. Two series of biphenyl carboxylic acids were designed based on the structures of URAT1 inhibitors Epaminurad and Telmisartan via a strategy of pharmacophore fusion. Fifty-one novel compounds were synthesized and most of them showed obvious inhibition against human URAT1. **A1** and **B21** were identified as the most potent URAT1 inhibitors in series A and B, respectively. They exhibited IC_{50} values of 0.93 μ M and 0.17 μ M, which were comparable or superior to the clinical uricosuric drug benzbromarone. The results confirmed the effectiveness of ligand-based approaches in identifying novel and potent URAT1 inhibitors.

Keywords: URAT1; biphenyl carboxylic acid; hyperuricemia; gout

1. Introduction

High serum uric acid concentration is an important risk factor for the development of gout. Chronically elevated uric acid levels, which is termed hyperuricemia, can lead to deposition of monosodium urate crystals in articular and non-articular structures, and hyperuricemia is the major cause of gout flares [1,2]. Besides anti-inflammatory treatment during gout attacks, effective control of the uric acid level is a key strategy for the prevention and treatment of gout. Urate lowering therapies (ULTs) aim to reverse hyperuricemia, which thereby dissolve monosodium urate crystals and prevent gout attacks in the long term.

Insufficient urate excretion is the primary reason for high serum urate (SU) levels, and urate excretion is predominantly regulated by urate transporters in the kidneys, specifically URAT1 (SLC22A12) [3–6], GLUT9 (SLC2A9) [5,7], and ABCG2 [8]. Therefore, uricosuric agents represented by URAT1 inhibitors are principal components of currently available small-molecule ULTs [9–11].

URAT1 inhibitors can inhibit urate reabsorption, promote urate excretion, and thus lower the serum urate level. Hence, URAT1 inhibitors have drawn extensive attention in recent years as an effective urate-lowering therapy [11,12]. Currently, four URAT1 inhibitors, namely Probenecid, Benzbromarone, Lesinurad, and Dotinurad (Figure 1), are used in clinic for the management of hyperuricemia. Probenecid exerts its effects by inhibiting both URAT1 and GLUT9. Despite its poor efficacy, Probenecid is still an option for patients who cannot tolerate allopurinol or fail to achieve target SU in monotherapy with xanthine oxidase (XO) inhibitors [9]. Benzbromarone is a highly active URAT1 inhibitor with an IC_{50} at submicromolar level. Although it is much more effective than Probenecid, occurrence of liver toxicity has been occasionally reported. Therefore, liver function monitoring is required when prescribing this drug [9,13,14]. Lesinurad is a relatively weak URAT1 inhibitor with an IC_{50} at micromolar level. Due to its poor efficacy, Lesinurad is usually used in clinic in combination with XO inhibitors to achieve reasonable urate lowering effects [15,16].



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Dotinurad is a selective urate reabsorption inhibitor (SURI). It inhibits URAT1 selectively, yet only shows minimal effects on ABCG2, OAT1, and OAT3. Dotinurad was approved in Japan for the treatment of hyperuricemia and gout in 2020 [17–19]. The clinic application of the marketed URAT1 inhibitors is more or less limited due to poor efficacy and potential toxicity associated with these drugs. Thus, safe and effective management of hyperuricemia is still an unmet clinic need. Most URAT1 inhibitors currently in clinical investigations are structurally derived from the marketed drugs, which might inherently share similar pharmacological and safety profiles with the previous drugs. Therefore, it is imperative to discover new chemical prototypes of URAT1 inhibitors [20,21].

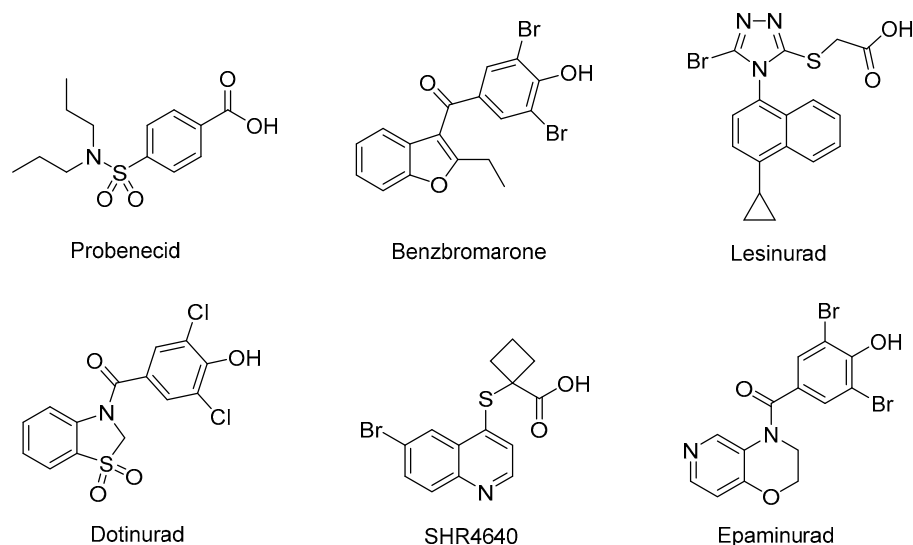


Figure 1. The structures of representative URAT1 Inhibitors.

Due to the transmembrane nature of URAT1, its structure has not been resolved, and the design of new URAT1 inhibitors mainly relies on ligand-based approaches [22–25]. Among the diverse URAT1 inhibitors reported, SHR4640 and Epaminurad (Figure 1) are highlighted as representative inhibitors progressed to clinical investigations. SHR4640 is a potent URAT1 inhibitor exhibiting good pharmacokinetic properties [26]. In a Phase II clinical trial in China, SHR4640 exhibited obvious urate lowering effects and good tolerability with a regimen of 5 mg or 10 mg once daily by oral administration for five weeks. SHR4640 is currently undergoing a phase III clinical trial [27,28]. Epaminurad (UR-1102) is a highly selective URAT1 inhibitor derived from benzbromarone, yet it has selectivity and safety profiles superior to benzbromarone [13,29,30].

Drug repurposing is not only a promising field to identify new therapeutic uses for existing drugs, but also an effective approach to recognize potential leads in drug discovery [31]. For example, the angiotensin II-receptor blockers (ARBs) Losartan, Irbesartan, and Telmisartan (Figure 2) are therapeutic drugs to treat hypertension. However, urate-lowering effects were observed during their clinical application, and they were later recognized as weak URAT1 inhibitors [32], which provided a new chemical prototype for URAT1 inhibitors.

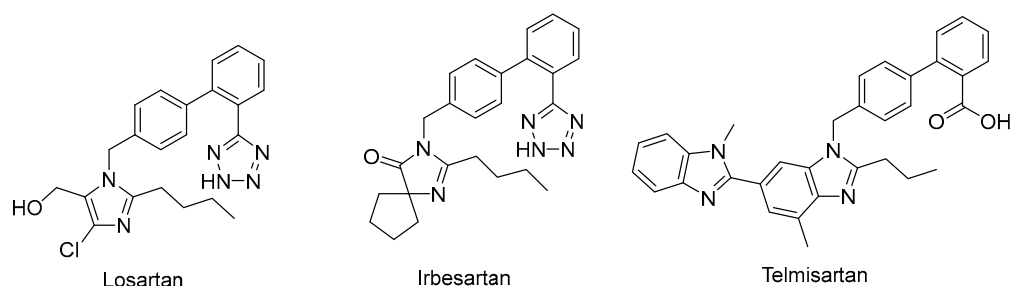


Figure 2. Representative angiotensin II receptor blockers with URAT1 inhibitory activity.

Careful examination of the structures of representative URAT1 inhibitors revealed the pharmacophore of URAT1 inhibitors, which includes anionic and hydrophobic components. To identify novel URAT1 inhibitors with better therapeutic profiles, we exploited a pharmacophore fusion strategy based on the structures of Telmisartan and Epaminurad (Figure 3). It was reported that the 3,5-dibromo-4-hydroxyphenyl fragment might be responsible for metabolic activation and resultant hepatic toxicity of Benzbromarone derivatives [14]. The biphenyl carboxylic acid fragment from Telmisartan was thus taken as the anionic component, while fragments similar to the 3,4-dihydro-2H-pyrido [4,3-b][1,4]oxazine part in Epaminurad were introduced as the hydrophobic component. The two parts are fused together through amide bonds to provide target compounds of series A (Figure 3), which were designed to reduce toxicity (compared to Epaminurad) and improve chemical accessibility (compared to Telmisartan) simultaneously. The amide bond in series A compounds was replaced with 1,2,4-oxadiazol via a bioisosteric strategy, and a variety of aromatic moieties were incorporated as the hydrophobic part to obtain target compounds of series B (Figure 3). These two series of compounds were both featured by the presence of the biphenyl carboxylic acid moiety, and we conducted preliminary structure–activity relationship (SAR) exploration on these compound classes to discover new chemical prototypes as potent URAT1 inhibitors.

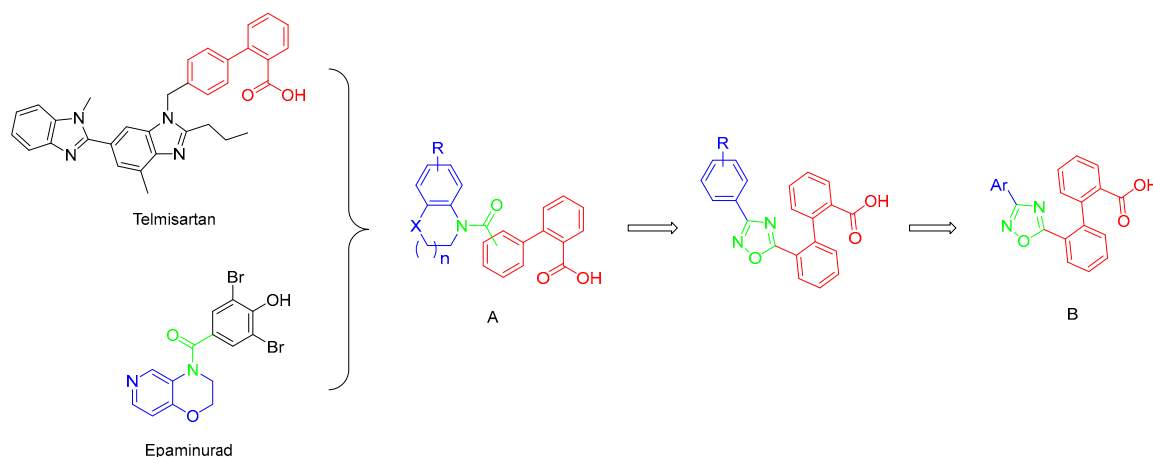


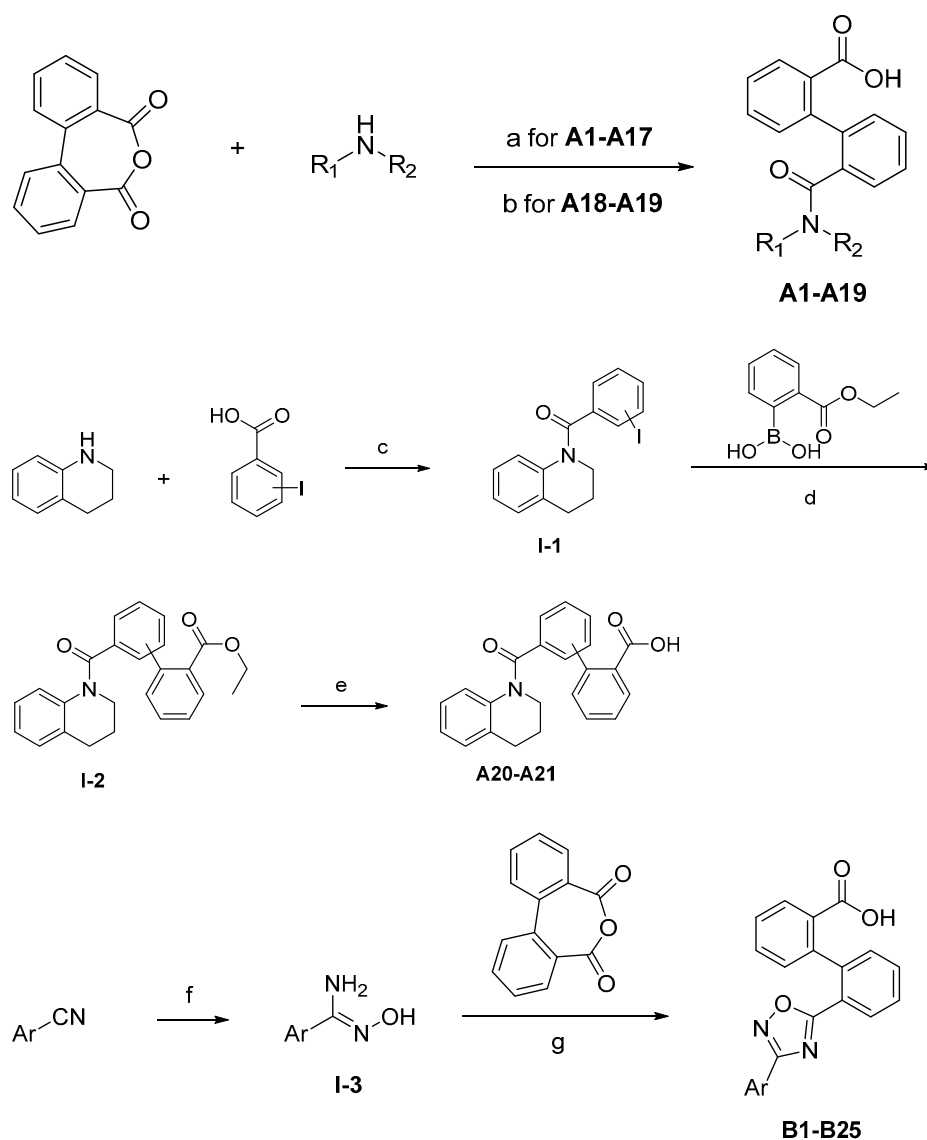
Figure 3. Novel biphenyl carboxylic acid-based URAT1 inhibitors designed by pharmacophore fusion. The anionic (presented in red) and hydrophobic (presented in blue) components were derived from Telmisartan and Epaminurad, respectively. The linker between the two parts was presented in green. A and B represent the general formula for compounds in Series A and B, respectively.

2. Results and Discussion

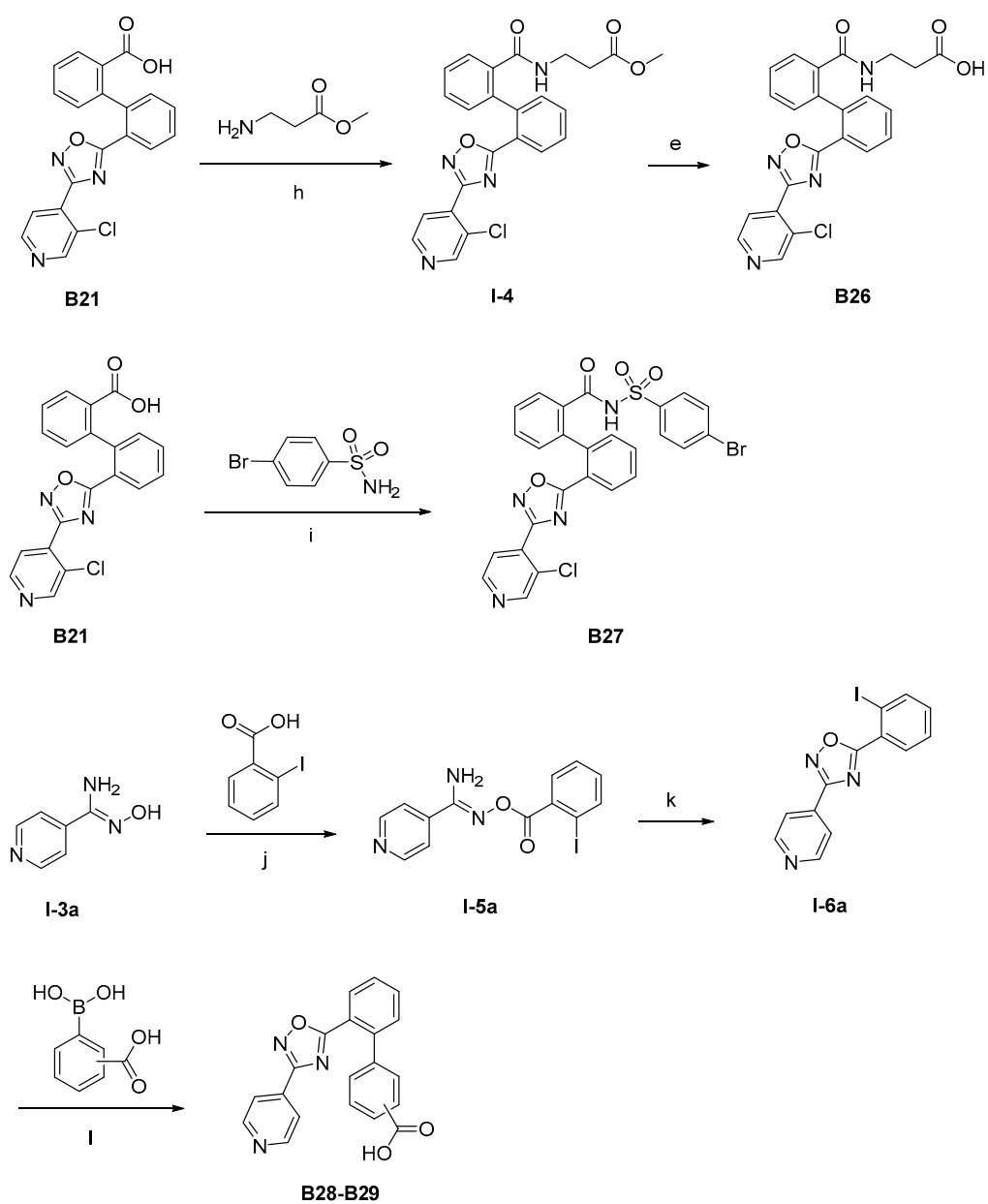
2.1. Chemistry

The synthesis of the target compounds were demonstrated in Scheme 1. Briefly, diphenic anhydride underwent amide condensation with substituted amines to form compounds **A1**–**A19**. Intermediate **I-1** was obtained through condensation of iodine substituted benzoic acid with 1,2,3,4-tetrahydroquinoline. **I-1** underwent a coupling reaction with

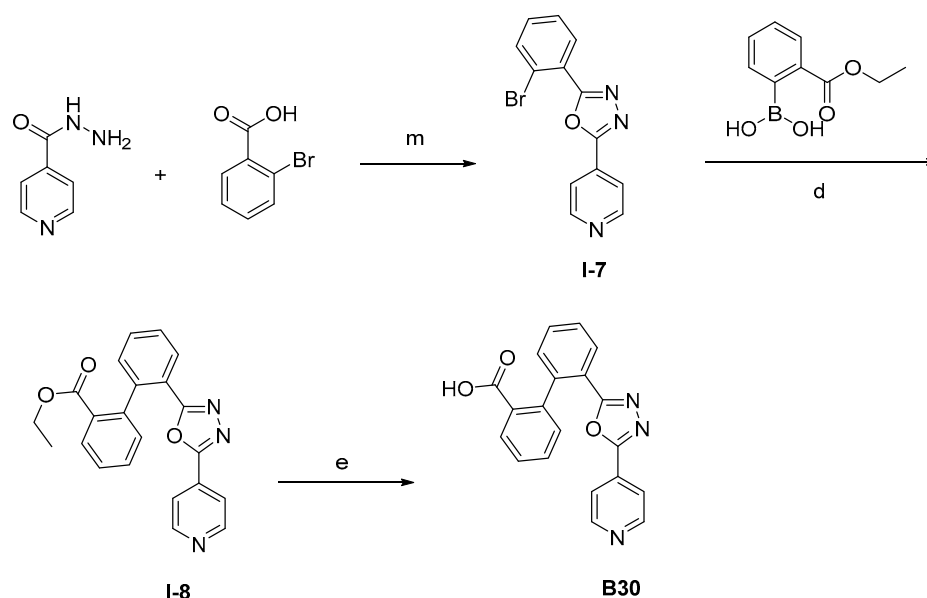
carboxylic ester substituted phenylboronic acid to give intermediate **I-2**, which is then hydrolyzed to provide compounds **A20–A21**. Cyanides reacted with hydroxylamine hydrochloride to afford intermediate **I-3**, which reacted with diphenic anhydride to furnish the oxadiazole ring and provide compounds **B1–B25**. Compound **B21** was subjected to successive amidation and hydrolysis to obtain compound **B26**. Compound **B27** was also obtained from **B21** by reacting with 4-bromobenzenesulfonamide. Intermediate **I-3a** condensed with iodine substituted benzoic acid to get intermediate **I-5a**, which was then cyclized to give the oxadiazole intermediate **I-6a**. **I-6a** underwent Suzuki reaction with substituted phenylboronic acid to produce compounds **B28** and **B29**. Intermediate **I-7** furnished the 1,3,4-oxadiazole ring through the hydrazine and carboxylic acid condensation. **I-7** then underwent Suzuki reaction with substituted phenylboronic acid to form intermediate **I-8**, and subsequently hydrolyzed to obtain compound **B30**.



Scheme 1. Cont



Scheme 1. Cont



Scheme 1. Synthetic routes of target compounds **A1–A21**, **B1–B30**. Reagents and conditions: (a) Toluene, 120 °C; (b) Triethylamine, DMAP, CH₂Cl₂, r.t., then 1N aq. HCl; (c) HATU, Triethylamine, Acetonitrile, r.t.; (d) K₂CO₃, Pd(PPh₃)₄, Toluene:95%Ethanol (1:1), 110 °C, N₂; (e) LiOH·H₂O, Ethanol:H₂O (4:1), r.t., then 1N aq. HCl; (f) NH₂OH·HCl, Na₂CO₃, Ethanol:H₂O (4:1), 60 °C; (g) DMSO, r.t., then NaOH, r.t., then 1N aq. HCl; (h) Triethylamine, HATU, DMF, 60 °C; (i) EDCI, DMAP, CH₂Cl₂, r.t.; (j) Triethylamine, HATU, CH₂Cl₂, r.t.; (k) Cs₂CO₃, DMSO, r.t.; (l) K₂CO₃, Pd(PPh₃)₄, Toluene:95%Ethanol (1:1), 110 °C, N₂, then 1N aq. HCl; (m) POCl₃, 110 °C. DMAP: 4-dimethylaminopyridine; HATU: 2-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate; EDCI: 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride.

2.2. In Vitro Evaluation and Structure–Activity Relationships

All the target compounds were evaluated for their URAT1 inhibitory activities in HEK293-URAT1 cells using ¹⁴C-labeled uric acid as the substrate. The activity data are listed in Tables 1 and 2. Currently available data revealed some SAR clues. The SAR exploration on series A compounds mainly focused on the hydrophobic component. Regioisomers **A1**, **A20** and **A21** were first investigated to determine the regioselectivity for URAT1 inhibition, and the ortho-substituted pattern (**A1**) emerged to be optimal. With the ortho-substituted biphenyl carboxylic acid fixed, a variety of hydrophobic components were introduced as R. This molecular area seemed sensitive to steric hindrance. Moieties with sizes similar to tetrahydroquinoline in **A1** are prone to maintain certain URAT1 inhibitory activities (**A2–A11**), though the activity was more or less decreased. However, moieties with sizes much larger (**A12**, **A14**) or smaller (**A16–A19**) than tetrahydroquinoline generally led to significantly decreased inhibitory activity. **A13** and **A15** were exceptions. The introduction of an electron-deficient pyridyl retained the URAT1 inhibitory activity (**A13**), while tetrahydroisoquinoline as R caused significant loss of activity (**A15**). These results implied the involvement of factors other than steric hindrance in affecting the inhibitory activity. For series B compounds, the ortho-substituted biphenyl carboxylic acid was initially kept, and various aromatic components were incorporated (**B1–B25**). Substituted phenyl (**B1–B7**), furanyl (**B8**), thienyl (**B9** and **B10**), pyrimidyl (**B11**), and pyridyl (**B12–B14**) were initially attempted, and only compound **B14** with 4-pyridyl showed significant inhibitory activity. Different substituents were introduced onto the 4-pyridyl (**B15–B21**). Except dimethyl substitution (**B20**), all the other substitution patterns led to increased inhibitory activities. In contrast, quinolyl (**B22–B24**) or methoxyl substituted naphthyl (**B25**) resulted in decreased URAT1 inhibition. Extending the carboxyl acid with β-alanine (**B26**) caused dramatic loss of activity, yet amidation of the carboxyl acid with 4-bromobenzenesulfonamide (**B27**) retained considerable inhibition. Regioisomers **B28–B30**

were also explored, and ortho-substituted biphenyl carboxylic acid and 1,2,4-oxadiazole were preferred.

Table 1. Structures and URAT1 inhibitory activities of compounds A1–A21.

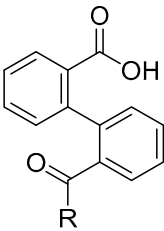
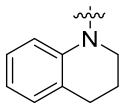
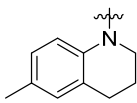
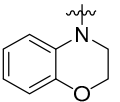
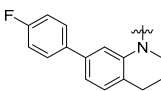
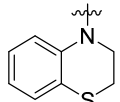
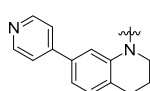
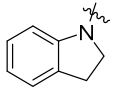
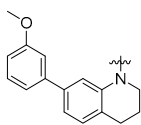
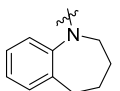
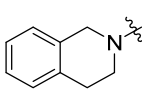
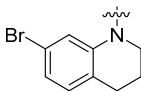
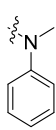
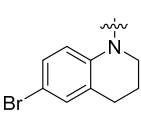
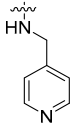
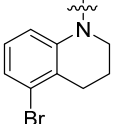
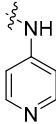
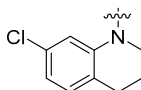
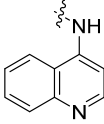
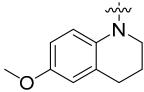
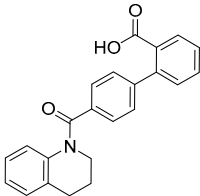
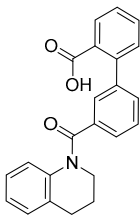
							
Cmpd.	R	Inhibition% *	IC ₅₀ (μM)	Cmpd.	R	Inhibition% *	IC ₅₀ (μM)
A1		89	0.93	A11		91	2.1
A2		75	3.9	A12		29	ND
A3		78	ND	A13		86	1.5
A4		77	4.7	A14		58	ND
A5		72	ND	A15		25	ND
A6		84	1.1	A16		13	ND
A7		94	1.1	A17		<10	ND
A8		90	1.6	A18		11	ND
A9		85	2.2	A19		24	ND

Table 1. Cont

Cmpd.	R	Inhibition% *	IC ₅₀ (μM)	Cmpd.	R	Inhibition% *	IC ₅₀ (μM)
A10		88	1.8				
A20		<10	ND				
A21		<10	ND				
Benz		-	-			-	0.36

* The percentage inhibition was measured at the concentration of 10 μM and was represented as the mean of three measurements. ND: not determined. Benz: benzbromarone.

Table 2. Structures and URAT1 inhibitory activities of compounds B1–B30.

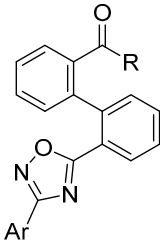
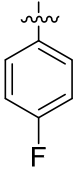
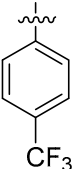
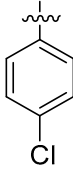
				
Cmpd.	Ar	R	Inhibition% *	IC ₅₀ (μM)
B1		OH	56	ND
B2		OH	39	ND
B3		OH	55	ND

Table 2. Cont

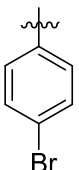
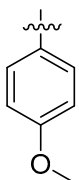
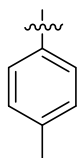
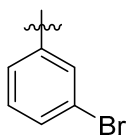
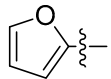
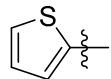
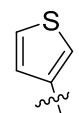
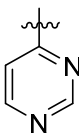
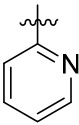
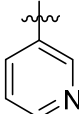
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B5		OH	68	ND
B6		OH	45	ND
B7		OH	44	ND
B8		OH	56	ND
B9		OH	68	ND
B10		OH	69	ND
B11		OH	30	ND
B12		OH	27	ND
B13		OH	64	ND

Table 2. Cont

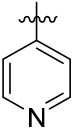
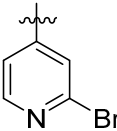
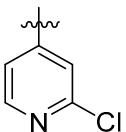
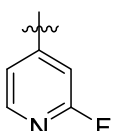
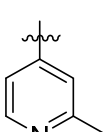
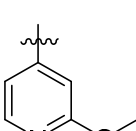
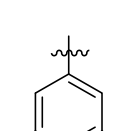
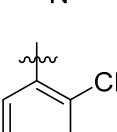
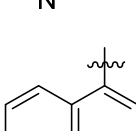
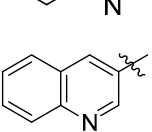
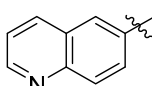
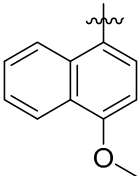
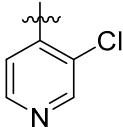
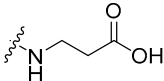
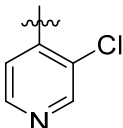
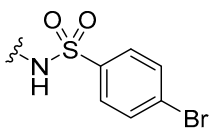
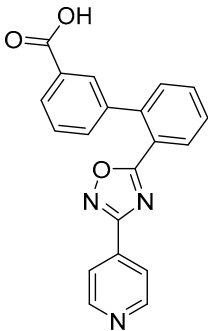
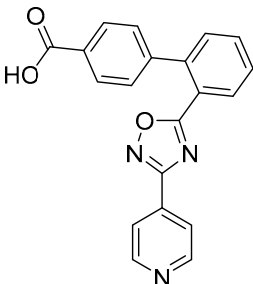
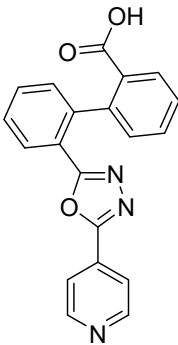
Cmpd.	Ar	R	Inhibition% *	IC ₅₀ (μM)
B14		OH	88	1.8
B15		OH	97	0.25
B16		OH	97	0.27
B17		OH	90	0.98
B18		OH	97	0.28
B19		OH	96	0.42
B20		OH	76	3.1
B21		OH	98	0.17
B22		OH	89	1.1
B23		OH	74	6.1
B24		OH	67	ND

Table 2. Cont

Cmpd.	Ar	R	Inhibition% *	IC ₅₀ (μM)
B25		OH	42	ND
B26			35	ND
B27			96	1.3
B28			29	ND
B29			18	ND
B30			33	ND
Benz	-	-	-	0.36

* The percentage inhibition was measured at the concentration of 10 μM and was represented as the mean of three measurements. ND: not determined. Benz: benzbromarone.

3. Materials and Methods

3.1. Chemistry

High-resolution mass spectra (HR-MS) were measured with an Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS system equipped with electrospray ionization (ESI). ^1H NMR and ^{13}C NMR spectra were recorded on a Quantum-I 400 or AVANCE NEO 700 spectrometer with TMS as the internal standard. All reagents and solvents were commercially obtained from local suppliers and were used directly without further purification.

General preparation of compounds **A1–A17**. The diphenic anhydride (1.0 eq) and substituted amines (1.0 eq) were mixed in 20 mL toluene. The mixture was stirred at 110 °C for 12 h, and the solvent was evaporated in a vacuum. The residue was purified by column chromatography to give **A1–A17**.

Compound **A1**: Yield 61%; white solid; mp: 147–149 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 7.84–7.77 (m, 1H), 7.50–7.37 (m, 5H), 7.21–7.15 (m, 1H), 7.14–7.09 (m, 1H), 7.03–6.96 (m, 2H), 6.92 (ddd, J = 14.6, 7.1, 1.8 Hz, 2H), 3.45 (t, J = 6.0 Hz, 2H), 2.43 (t, J = 6.9 Hz, 2H), 1.59 (p, J = 6.6 Hz, 2H); ^{13}C NMR (176 MHz, DMSO- d_6) δ 168.07, 167.40, 139.05, 137.64, 137.03, 135.38, 130.89, 130.08, 129.13, 128.06, 126.97, 126.73, 124.36, 123.47, 25.22, 22.05; HR-ESI-MS: m/z = 358.1455 $[\text{M} + \text{H}]^+$, calculated for $\text{C}_{23}\text{H}_{20}\text{NO}_3$: 358.1438.

Compound **A2**: Yield 63%; white solid; mp: 174–176 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 12.36 (s, 1H), 7.87–7.74 (m, 1H), 7.55–7.37 (m, 6H), 7.24 (dd, J = 6.3, 2.3 Hz, 1H), 7.08 (s, 1H), 6.95 (td, J = 7.8, 1.6 Hz, 1H), 6.81–6.62 (m, 2H), 3.83 (s, 2H), 3.70–3.42 (m, 2H); HR-ESI-MS: m/z = 360.1252 $[\text{M} + \text{H}]^+$, calculated for $\text{C}_{22}\text{H}_{18}\text{NO}_4$: 360.1230.

Compound **A3**: Yield 51%; white solid; mp: 193–195 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 12.22 (s, 1H), 8.01–7.78 (m, 1H), 7.55–7.36 (m, 5H), 7.20 (d, J = 7.3 Hz, 1H), 7.09–7.02 (m, 2H), 7.00–6.94 (m, 1H), 6.92–6.77 (m, 2H), 3.71 (s, 2H), 2.93 (t, J = 5.5 Hz, 2H); HR-ESI-MS: m/z = 376.1001 $[\text{M} + \text{H}]^+$, calculated for $\text{C}_{22}\text{H}_{18}\text{NO}_3\text{S}$: 376.1002.

Compound **A4**: Yield 66%; white solid; mp: 195–196 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 12.72 (s, 1H), 7.93 (d, J = 8.0 Hz, 1H), 7.77 (dd, J = 7.7, 1.4 Hz, 1H), 7.57–7.43 (m, 4H), 7.37 (dt, J = 8.9, 7.2 Hz, 2H), 7.28 (d, J = 6.9 Hz, 1H), 7.21–7.08 (m, 2H), 6.99 (d, J = 7.7 Hz, 1H), 3.79 (s, 2H), 2.87 (s, 2H); HR-ESI-MS: m/z = 344.1277 $[\text{M} + \text{H}]^+$, calculated for $\text{C}_{22}\text{H}_{18}\text{NO}_3$: 344.1281.

Compound **A5**: Yield 64%; white solid; mp: 215–217 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 7.86 (d, J = 7.6 Hz, 1H), 7.63–6.93 (m, 10H), 6.75–6.59 (m, 1H), 3.25 (s, 2H), 2.56 (s, 1H), 1.57 (m, 4H); HR-ESI-MS: m/z = 372.1594 $[\text{M} + \text{H}]^+$, calculated for $\text{C}_{24}\text{H}_{22}\text{NO}_3$: 372.1594.

Compound **A6**: Yield 66%; white solid; mp: 231–233 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 12.29 (s, 1H), 7.86–7.77 (m, 1H), 7.52–7.36 (m, 6H), 7.25–7.20 (m, 1H), 7.12 (dd, J = 8.2, 2.0 Hz, 1H), 7.05–6.99 (m, 1H), 6.96 (d, J = 8.2 Hz, 1H), 3.43 (t, J = 6.0 Hz, 2H), 2.39 (d, J = 6.9 Hz, 2H), 1.56 (p, J = 6.5 Hz, 2H); HR-ESI-MS: m/z = 436.0553 $[\text{M} + \text{H}]^+$, calculated for $\text{C}_{23}\text{H}_{19}\text{BrNO}_3$: 436.0543.

Compound **A7**: Yield 65%; white solid; mp: 261–262 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 12.27 (s, 1H), 7.81 (dd, J = 6.7, 2.3 Hz, 1H), 7.51–7.37 (m, 5H), 7.20 (q, J = 2.8 Hz, 2H), 7.13–7.01 (m, 3H), 3.43 (t, J = 6.0 Hz, 2H), 2.44 (d, J = 6.9 Hz, 2H), 1.58 (p, J = 6.5 Hz, 2H); HR-ESI-MS: m/z = 436.0544 $[\text{M} + \text{H}]^+$, calculated for $\text{C}_{23}\text{H}_{19}\text{BrNO}_3$: 436.0543.

Compound **A8**: Yield 61%; white solid; mp: 201–202 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 12.26 (s, 1H), 7.82 (dt, J = 6.7, 2.1 Hz, 1H), 7.52–7.37 (m, 5H), 7.27 (d, J = 7.9 Hz, 1H), 7.17 (dt, J = 6.5, 2.0 Hz, 1H), 7.04 (d, J = 8.3 Hz, 1H), 6.88 (tt, J = 9.9, 8.1, 3.5 Hz, 2H), 3.45 (t, J = 5.8 Hz, 2H), 2.40 (t, J = 7.2 Hz, 2H), 1.59 (t, J = 6.5 Hz, 2H); HR-ESI-MS: m/z = 436.0552 $[\text{M} + \text{H}]^+$, calculated for $\text{C}_{23}\text{H}_{19}\text{BrNO}_3$: 436.0543.

Compound **A9**: Yield 65%; white solid; mp: 233–234 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 12.27 (s, 1H), 7.83 (dt, J = 7.4, 1.7 Hz, 1H), 7.53–7.38 (m, 5H), 7.31–7.19 (m, 2H), 7.08–6.93 (m, 3H), 3.43 (t, J = 6.1 Hz, 2H), 2.43 (t, J = 6.9 Hz, 2H), 1.57 (t, J = 6.4 Hz, 2H); HR-ESI-MS: m/z = 392.1064 $[\text{M} + \text{H}]^+$, calculated for $\text{C}_{23}\text{H}_{19}\text{ClNO}_3$: 392.1048.

Compound **A10**: Yield 65%; white solid; mp: 198–200 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 12.27 (s, 1H), 7.87–7.74 (m, 1H), 7.48–7.35 (m, 5H), 7.18 (dq, J = 4.2, 2.4 Hz, 1H), 7.05 (s,

2H), 6.58 (d, $J = 2.9$ Hz, 1H), 6.52 (dd, $J = 8.9, 2.9$ Hz, 1H), 3.69 (s, 3H), 3.41 (t, $J = 6.1$ Hz, 2H), 2.41 (d, $J = 8.5$ Hz, 2H), 1.57 (p, $J = 6.4$ Hz, 2H); HR-ESI-MS: $m/z = 388.1536$ $[M + H]^+$, calculated for $C_{24}H_{22}NO_4$: 388.1543.

Compound A11: Yield 67%; white solid; mp: 220–222 °C; 1H NMR (400 MHz, DMSO- d_6) δ 7.89–7.72 (m, 1H), 7.41 (dq, $J = 5.3, 2.5, 1.9$ Hz, 5H), 7.26–7.15 (m, 1H), 7.01 (s, 2H), 6.81 (d, $J = 2.0$ Hz, 1H), 6.73 (dd, $J = 8.3, 2.1$ Hz, 1H), 3.42 (t, $J = 6.1$ Hz, 2H), 2.40 (t, $J = 6.9$ Hz, 2H), 2.19 (s, 3H), 1.57 (p, $J = 6.5$ Hz, 2H); HR-ESI-MS: $m/z = 372.1588$ $[M + H]^+$, calculated for $C_{24}H_{22}NO_3$: 372.1594.

Compound A12: Yield 55%; white solid; mp: 245–246 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.07 (s, 1H), 7.82 (dd, $J = 7.0, 2.2$ Hz, 1H), 7.59–7.12 (m, 12H), 7.05 (d, $J = 7.9$ Hz, 1H), 6.85 (d, $J = 6.9$ Hz, 1H), 3.50 (t, $J = 6.0$ Hz, 2H), 2.40 (d, $J = 6.8$ Hz, 2H), 1.59 (p, $J = 6.4$ Hz, 2H); HR-ESI-MS: $m/z = 452.1649$ $[M + H]^+$, calculated for $C_{29}H_{23}FNO_3$: 452.1656.

Compound A13: Yield 55%; white solid; mp: 266–268 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.22 (s, 1H), 8.62–8.40 (m, 2H), 7.96–7.76 (m, 1H), 7.58 (dd, $J = 7.0, 2.0$ Hz, 1H), 7.49 (dtd, $J = 14.1, 7.4, 1.7$ Hz, 2H), 7.44–7.31 (m, 4H), 7.28 (d, $J = 5.1$ Hz, 2H), 7.19 (dd, $J = 7.0, 1.9$ Hz, 1H), 7.11 (d, $J = 7.9$ Hz, 1H), 6.85 (s, 1H), 3.49 (d, $J = 6.1$ Hz, 2H), 2.40 (s, 2H), 1.58 (p, $J = 6.4$ Hz, 2H); HR-ESI-MS: $m/z = 435.1696$ $[M + H]^+$, calculated for $C_{28}H_{23}N_2O_3$: 435.1703.

Compound A14: Yield 54%; white solid; mp: 177–179 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.19 (s, 1H), 7.81 (dd, $J = 7.4, 1.7$ Hz, 1H), 7.60–7.53 (m, 1H), 7.51–7.44 (m, 2H), 7.43–7.34 (m, 2H), 7.28 (t, $J = 7.9$ Hz, 1H), 7.20 (ddd, $J = 13.1, 7.5, 2.2$ Hz, 3H), 7.04 (d, $J = 7.9$ Hz, 1H), 6.93–6.75 (m, 4H), 3.80 (s, 3H), 3.50 (s, 2H), 2.38 (s, 2H), 1.58 (t, $J = 6.4$ Hz, 2H); HR-ESI-MS: $m/z = 464.1849$ $[M + H]^+$, calculated for $C_{30}H_{26}NO_4$: 464.1856.

Compound A15: Yield 62%; white solid; mp: 171–173 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.74 (s, 1H), 7.76 (dd, $J = 7.7, 1.5$ Hz, 1H), 7.50–6.48 (m, 11H), 4.29 (s, 2H), 3.33 (s, 2H), 2.73–2.17 (m, 2H); HR-ESI-MS: $m/z = 358.1448$ $[M + H]^+$, calculated for $C_{23}H_{20}NO_3$: 358.1438.

Compound A16: Yield 53%; white solid; mp: 169–172 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.64 (s, 1H), 7.85 (d, $J = 7.7$ Hz, 1H), 7.69–6.66 (m, 12H), 3.04 (s, 3H); HR-ESI-MS: $m/z = 332.1284$ $[M + H]^+$, calculated for $C_{21}H_{18}NO_3$: 332.1281.

Compound A17: Yield 58%; white solid; mp: 118–120 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.74 (s, 1H), 8.42 (t, $J = 6.1$ Hz, 1H), 8.39–8.31 (m, 2H), 7.81 (dd, $J = 7.5, 1.7$ Hz, 1H), 7.59–7.41 (m, 5H), 7.16 (td, $J = 6.4, 2.4$ Hz, 2H), 6.90–6.83 (m, 2H), 4.25 (d, $J = 5.9$ Hz, 2H); HR-ESI-MS: $m/z = 333.1246$ $[M + H]^+$, calculated for $C_{20}H_{17}N_2O_3$: 333.1234.

General preparation of compounds A18 and A19. The diphenic anhydride (1.0 eq), aromatic amines (1.0 eq), triethylamine (3.0 eq), and 4-dimethylaminopyridine (0.2 eq) were mixed in 20 mL dichloromethane. The mixture was stirred at room temperature for 10 h, and the solvent was evaporated in a vacuum. The reaction mixture was poured into water (50 mL), 1 M HCl solution was added dropwise to separated water phase to adjust the pH to 6 and the aqueous residue was extracted with dichloromethane. The combined organic solution was washed with saturated aqueous sodium chloride solution (30 mL $\times$ 3) and dried over anhydrous Na_2SO_4 . The residue was purified by column chromatography to **A18** and **A19**.

Compound A18: Yield 55%; white solid; mp: 190–191 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.63 (s, 1H), 8.33–8.25 (m, 2H), 7.68–7.56 (m, 2H), 7.52–7.43 (m, 2H), 7.39–7.35 (m, 2H), 7.27 (tt, $J = 7.4, 5.7$ Hz, 2H), 7.16–7.05 (m, 1H), 6.99–6.91 (m, 1H); HR-ESI-MS: $m/z = 319.1092$ $[M + H]^+$, calculated for $C_{19}H_{14}N_2O_3$: 319.1077.

Compound A19: Yield 53%; white solid; mp: 176–178 °C; 1H NMR (400 MHz, DMSO- d_6) δ 8.68 (d, $J = 5.0$ Hz, 1H), 7.88 (dd, $J = 12.7, 8.5$ Hz, 2H), 7.82 (d, $J = 5.0$ Hz, 1H), 7.70–7.61 (m, 2H), 7.52–7.45 (m, 3H), 7.39 (t, $J = 7.7$ Hz, 1H), 7.24 (dd, $J = 6.2, 2.8$ Hz, 2H), 7.10 (dd, $J = 5.9, 3.0$ Hz, 1H), 7.05 (d, $J = 4.0$ Hz, 1H); HR-ESI-MS: $m/z = 369.1253$ $[M + H]^+$, calculated for $C_{23}H_{17}N_2O_3$: 369.1234.

General preparation of intermediate I-1. Iodine substituted benzoic acid (1.2 eq) and 1,2,3,4-tetrahydroquinoline (1.0 eq) were dissolved in 20 mL acetonitrile, then HATU (1.5 eq) and triethylamine (2.0 eq) were added. The mixture was stirred at room temperature for

10 h, and the solvent was evaporated in a vacuum. The residue was purified by column chromatography to give intermediate **I-1**.

General preparation of intermediate **I-2**. The intermediate **I-1** (1.0 eq), substituted phenylboronic acids (1.0 eq), potassium carbonate (3.0 eq), and tetrakis(triphenylphosphine)palladium (0.2 eq) were added into 20 mL of a mixed solvent of toluene: 95% ethanol (1:1). The mixture was stirred at 110 °C under N₂ for 12 h. The solvent was then evaporated in a vacuum and the residue was purified by column chromatography to get intermediate **I-2**.

General preparation of compounds **A20** and **A21**. The intermediate **I-2** (1.0 eq) and lithium hydroxide monohydrate (2.0 eq) were dissolved in 20 mL of a mixed solvent of ethanol: H₂O (4:1). The mixture was stirred at room temperature for 8 h, and the solvent was evaporated in a vacuum. The reaction mixture was poured into water (50 mL). 1 M HCl solution was added dropwise to adjust the pH to 2. The mixture was extracted with ethyl acetate (50 mL × 3). The combined organic solution was washed with saturated aqueous sodium chloride solution (30 mL × 3) and dried over anhydrous Na₂SO₄. The residue was purified by column chromatography to **A20** and **A21**.

Compound **A20**: Yield 59%; white solid; mp: 165–167 °C; ¹H NMR (400 MHz, DMSO-d₆) δ 7.74 (dd, *J* = 7.7, 1.4 Hz, 1H), 7.66–7.53 (m, 2H), 7.47 (td, *J* = 7.5, 1.3 Hz, 1H), 7.42–7.34 (m, 3H), 7.33–7.28 (m, 2H), 7.23–7.15 (m, 1H), 7.01 (td, *J* = 7.3, 1.6 Hz, 1H), 6.93 (dd, *J* = 14.9, 7.8 Hz, 2H), 3.77 (t, *J* = 6.6 Hz, 2H), 2.83 (t, *J* = 6.6 Hz, 2H), 1.96 (p, *J* = 6.6 Hz, 2H); ¹³C NMR (176 MHz, DMSO-d₆) δ 168.71, 168.60, 142.00, 139.68, 138.20, 134.56, 130.49, 130.35, 129.79, 128.67, 128.12, 127.94, 127.49(2C), 127.27(2C), 127.04, 124.83, 124.32, 123.68, 44.09, 25.62, 22.96; HR-ESI-MS: *m/z* = 358.1445 [M + H]⁺, calculated for C₂₃H₂₀NO₃: 358.1438.

Compound **A21**: Yield 62%; white solid; mp: 100–102 °C; ¹H NMR (400 MHz, DMSO-d₆) δ 12.85 (s, 1H), 7.74 (dd, *J* = 7.7, 1.4 Hz, 1H), 7.53 (td, *J* = 7.5, 1.5 Hz, 1H), 7.45 (td, *J* = 7.6, 1.3 Hz, 1H), 7.39–7.34 (m, 2H), 7.33–7.25 (m, 2H), 7.18 (ddd, *J* = 9.5, 7.5, 1.5 Hz, 2H), 7.02 (td, *J* = 7.4, 1.4 Hz, 1H), 6.94 (td, *J* = 7.7, 7.2, 1.6 Hz, 1H), 6.86 (d, *J* = 8.2 Hz, 1H), 3.77 (t, *J* = 6.6 Hz, 2H), 2.81 (t, *J* = 6.6 Hz, 2H), 1.95 (p, *J* = 6.6 Hz, 2H); ¹³C NMR (176 MHz, DMSO-d₆) δ 168.78, 168.51, 140.16, 139.60, 138.24, 135.55, 130.69, 130.24, 129.71, 129.29, 128.67, 127.87, 127.36 (d, *J* = 3.3 Hz), 126.96, 126.17, 124.84, 124.50, 123.68, 43.96, 25.62, 22.99; HR-ESI-MS: *m/z* = 358.1443 [M + H]⁺, calculated for C₂₃H₂₀NO₃: 358.1438.

General preparation of intermediate **I-3**. The aromatic cyanides (1.0 eq), hydroxylamine hydrochloride (1.5 eq), and sodium carbonate (2.0 eq) were added into 20 mL of a mixed solvent of ethanol: H₂O (4:1). The mixture was stirred at 60 °C for 6 h. The solvent was then evaporated in a vacuum and the residue was purified by column chromatography to provide intermediate **I-3**.

General preparation of compounds **B1–B25**. The diphenic anhydride (1.0 eq) and intermediate **I-3** (1.0 eq) were mixed in 20 mL dimethyl sulfoxide. The mixture was stirred at room temperature for 2 h, then sodium hydroxide (2.0 eq) was added to the reaction solution, the mixture was stirred at room temperature for 1 h. The reaction mixture was poured into water (50 mL), 1 M HCl solution was added dropwise to separated water phase to adjust the pH to 5, and the solid precipitates were filtered. The residue was purified by column chromatography to afford **B1–B25**.

Compound **B1**: Yield 55%; white solid; mp: 150–153 °C; ¹H NMR (400 MHz, DMSO-d₆) δ 12.54 (s, 1H), 8.18 (d, *J* = 7.8 Hz, 1H), 7.96 (d, *J* = 7.7 Hz, 1H), 7.94–7.86 (m, 2H), 7.76–7.67 (m, 1H), 7.68–7.59 (m, 2H), 7.55 (t, *J* = 7.6 Hz, 1H), 7.38 (t, *J* = 8.9 Hz, 3H), 7.28 (d, *J* = 7.5 Hz, 1H); ¹³C NMR (101 MHz, DMSO-d₆) δ 175.95, 167.61, 166.67, 163.92 (d, *J* = 249.1 Hz), 142.53, 141.07, 132.28, 131.58, 130.77 (2C), 130.71, 129.69 (2C), 129.44, 129.35, 129.25, 127.85 (d, *J* = 13.9 Hz, 2C), 122.66 (d, *J* = 2.4 Hz), 122.33, 116.39 (d, *J* = 22.2 Hz, 2C); HR-MS(ESI) *m/z* = 361.0983 [M + H]⁺, calculated for C₂₁H₁₄FN₂O₃: 361.0983.

Compound **B2**: Yield 59%; white solid; mp: 173–175 °C; ¹H NMR (400 MHz, DMSO-d₆) δ 12.56 (s, 1H), 8.20 (d, *J* = 7.3 Hz, 1H), 8.06 (d, *J* = 8.1 Hz, 2H), 7.97 (d, *J* = 7.5 Hz, 1H), 7.90 (d, *J* = 8.3 Hz, 2H), 7.72 (t, *J* = 7.5 Hz, 1H), 7.63 (t, *J* = 7.5 Hz, 2H), 7.55 (t, *J* = 7.5 Hz, 1H), 7.40 (d, *J* = 7.4 Hz, 1H), 7.29 (d, *J* = 7.1 Hz, 1H); ¹³C NMR (101 MHz, DMSO-d₆) δ 176.37, 167.64, 166.54, 142.59, 141.00, 132.43, 131.61, 131.39 (d, *J* = 32.1 Hz), 130.77, 130.75, 129.94,

129.73, 129.34, 127.90 (d, $J = 15.6$ Hz, 2C), 127.72 (3C), 126.21 (d, $J = 3.5$ Hz, 2C), 123.80 (d, $J = 272.6$ Hz), 122.22; HR-ESI-MS: $m/z = 411.0946$ $[M + H]^+$, calculated for $C_{22}H_{14}F_3N_2O_3$: 411.0951.

Compound **B3**: Yield 59%; white solid; mp: 154–156 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.55 (s, 1H), 8.18 (d, $J = 7.8$ Hz, 1H), 7.96 (d, $J = 7.7$ Hz, 1H), 7.86 (d, $J = 8.7$ Hz, 2H), 7.72 (t, $J = 7.3$ Hz, 1H), 7.66–7.59 (m, 4H), 7.55 (t, $J = 7.4$ Hz, 1H), 7.39 (d, $J = 7.6$ Hz, 1H), 7.28 (d, $J = 7.5$ Hz, 1H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 176.09, 167.61, 166.68, 142.53, 141.03, 136.30, 132.32, 131.59, 130.77, 130.72 (2C), 129.71, 129.39 (2C), 129.29, 128.65 (2C), 127.94, 127.79, 124.94, 122.30; HR-ESI-MS: $m/z = 377.0684$ $[M + H]^+$, calculated for $C_{21}H_{14}ClN_2O_3$: 377.0687.

Compound **B4**: Yield 51%; white solid; mp: 148–150 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.54 (s, 1H), 8.18 (d, $J = 7.7$ Hz, 1H), 7.95 (d, $J = 7.6$ Hz, 1H), 7.79 (d, $J = 8.5$ Hz, 2H), 7.74 (d, $J = 8.5$ Hz, 2H), 7.70 (d, $J = 7.5$ Hz, 1H), 7.62 (t, $J = 7.2$ Hz, 2H), 7.55 (t, $J = 7.5$ Hz, 1H), 7.39 (d, $J = 7.5$ Hz, 1H), 7.28 (d, $J = 7.4$ Hz, 1H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 176.10, 167.61, 166.79, 142.53, 141.02, 132.31 (3C), 131.59, 130.77, 130.72, 129.71, 129.30, 128.81 (3C), 127.94, 127.79, 125.28, 125.16, 122.29; HR-ESI-MS: $m/z = 421.0187$ $[M + H]^+$, calculated for $C_{21}H_{14}BrN_2O_3$: 421.0182.

Compound **B5**: Yield 51%; white solid; mp: 154–157 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.53 (s, 1H), 8.17 (dd, $J = 1.2, 7.8$ Hz, 1H), 7.97 (dd, $J = 1.3, 7.7$ Hz, 1H), 7.83–7.75 (m, 2H), 7.70 (td, $J = 1.3, 7.6$ Hz, 1H), 7.65–7.58 (m, 2H), 7.55 (td, $J = 1.3, 7.6$ Hz, 1H), 7.38 (dd, $J = 1.1, 7.6$ Hz, 1H), 7.27 (dd, $J = 1.2, 7.5$ Hz, 1H), 7.10–7.02 (m, 2H), 3.81 (s, 3H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 175.50, 167.61, 167.18, 161.68, 142.52, 141.21, 132.10, 131.55, 130.76 (2C), 130.66, 129.71, 129.18, 128.57 (2C), 127.87, 127.73, 122.49, 118.38, 114.58 (2C), 55.38; HR-ESI-MS: $m/z = 373.1179$ $[M + H]^+$, calculated for $C_{22}H_{17}N_2O_4$: 373.1183.

Compound **B6**: Yield 60%; white solid; mp: 151–153 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.52 (s, 1H), 8.18 (d, $J = 7.7$ Hz, 1H), 7.96 (d, $J = 7.6$ Hz, 1H), 7.75 (d, $J = 8.0$ Hz, 2H), 7.71 (t, $J = 7.5$ Hz, 1H), 7.67–7.59 (m, 2H), 7.55 (t, $J = 7.5$ Hz, 1H), 7.38 (d, $J = 7.6$ Hz, 1H), 7.33 (d, $J = 8.0$ Hz, 2H), 7.28 (d, $J = 7.5$ Hz, 1H), 2.36 (s, 3H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 175.69, 167.60, 167.43, 142.52, 141.48, 141.17, 132.15, 131.56, 130.77, 130.74, 130.67, 129.71 (3C), 129.21, 127.88, 127.75, 126.83 (2C), 123.33, 122.47, 21.06; HR-ESI-MS: $m/z = 357.1229$ $[M + H]^+$, calculated for $C_{22}H_{17}N_2O_3$: 357.1234.

Compound **B7**: Yield 55%; white solid; mp: 119–121 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.55 (s, 1H), 8.19 (d, $J = 7.8$ Hz, 1H), 8.00–7.93 (m, 2H), 7.86 (d, $J = 7.8$ Hz, 1H), 7.81–7.75 (m, 1H), 7.72 (td, $J = 1.2, 7.6$ Hz, 1H), 7.67–7.59 (m, 2H), 7.56 (td, $J = 1.1, 7.6$ Hz, 1H), 7.49 (t, $J = 7.9$ Hz, 1H), 7.40 (d, $J = 7.6$ Hz, 1H), 7.29 (d, $J = 7.5$ Hz, 1H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 176.05, 167.61, 166.31, 142.61, 141.06, 134.30, 132.40, 131.60, 131.47, 130.78 (2C), 130.69, 129.65, 129.48, 129.25, 128.23, 127.90, 127.81, 125.75, 122.27, 122.17; HR-ESI-MS: $m/z = 421.0177$ $[M + H]^+$, calculated for $C_{21}H_{14}BrN_2O_3$: 421.0182.

Compound **B8**: Yield 58%; white solid; mp: 171–175 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.56 (s, 1H), 8.15 (d, $J = 7.7$ Hz, 1H), 7.94 (d, $J = 9.2$ Hz, 2H), 7.75–7.67 (m, 1H), 7.65–7.58 (m, 2H), 7.58–7.50 (m, 1H), 7.38 (d, $J = 7.5$ Hz, 1H), 7.27 (d, $J = 7.5$ Hz, 1H), 7.06 (d, $J = 3.4$ Hz, 1H), 6.77–6.67 (m, 1H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 175.83, 167.60, 160.49, 146.43, 142.48, 141.39, 140.93, 132.33, 131.59, 130.78, 130.72, 130.67, 129.76, 129.38, 127.96, 127.79, 122.25, 114.34, 112.22; HR-ESI-MS: $m/z = 333.0869$ $[M + H]^+$, calculated for $C_{19}H_{13}N_2O_4$: 333.0870.

Compound **B9**: Yield 58%; white solid; mp: 140–142 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.54 (s, 1H), 8.16 (d, $J = 7.5$ Hz, 1H), 7.96 (d, $J = 7.7$ Hz, 1H), 7.84 (d, $J = 4.9$ Hz, 1H), 7.71 (t, $J = 7.6$ Hz, 1H), 7.67–7.58 (m, 3H), 7.54 (t, $J = 7.4$ Hz, 1H), 7.38 (d, $J = 7.5$ Hz, 1H), 7.28 (d, $J = 7.4$ Hz, 1H), 7.23 (t, $J = 4.4$ Hz, 1H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 175.77, 167.59, 163.64, 142.56, 141.02, 132.32, 131.58, 130.76 (2C), 130.69, 130.67, 129.79, 129.75, 129.29, 128.45, 127.93, 127.78, 127.34, 122.21; HR-ESI-MS: $m/z = 349.0635$ $[M + H]^+$, calculated for $C_{19}H_{13}N_2O_3S$: 349.0641.

Compound **B10**: Yield 50%; white solid; mp: 146–148 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.53 (s, 1H), 8.16 (d, $J = 7.7$ Hz, 1H), 8.10 (d, $J = 2.8$ Hz, 1H), 7.95 (d, $J = 7.5$ Hz,

1H), 7.74 (dd, $J = 3.0, 5.0$ Hz, 1H), 7.70 (t, $J = 7.6$ Hz, 1H), 7.62 (t, $J = 7.4$ Hz, 2H), 7.54 (t, $J = 7.6$ Hz, 1H), 7.45 (d, $J = 5.0$ Hz, 1H), 7.38 (d, $J = 7.5$ Hz, 1H), 7.27 (d, $J = 7.3$ Hz, 1H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 175.61, 167.61, 164.11, 142.51, 141.09, 132.18, 131.55, 130.77 (2C), 130.67, 129.72, 129.25, 128.63, 128.37, 127.92, 127.74, 127.45, 125.55, 122.41; HR-ESI-MS: $m/z = 349.0636$ $[\text{M} + \text{H}]^+$, calculated for $\text{C}_{19}\text{H}_{13}\text{N}_2\text{O}_3\text{S}$: 349.0641.

Compound **B11**: Yield 58%; white solid; mp: 167–169 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 12.59 (s, 1H), 9.38 (d, $J = 1.4$ Hz, 1H), 9.07 (d, $J = 5.1$ Hz, 1H), 8.21 (dd, $J = 7.8, 1.4$ Hz, 1H), 7.96 (dd, $J = 7.7, 1.5$ Hz, 1H), 7.90 (dd, $J = 5.1, 1.4$ Hz, 1H), 7.74 (td, $J = 7.6, 1.4$ Hz, 1H), 7.64 (tdd, $J = 7.5, 2.5, 1.5$ Hz, 2H), 7.56 (td, $J = 7.6, 1.4$ Hz, 1H), 7.40 (dd, $J = 7.7, 1.3$ Hz, 1H), 7.30 (dd, $J = 7.6, 1.4$ Hz, 1H); ^{13}C NMR (176 MHz, DMSO- d_6) δ 177.47, 168.14, 166.91, 159.90, 159.60, 153.03, 142.99, 141.29, 133.09, 132.16, 131.28, 131.16, 130.24, 129.98, 128.56, 128.39, 122.60, 120.13; HR-ESI-MS: $m/z = 345.0996$ $[\text{M} + \text{H}]^+$, calculated for $\text{C}_{19}\text{H}_{13}\text{N}_4\text{O}_3$: 345.0982.

Compound **B12**: Yield 50%; white solid; mp: 190–193 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 12.56 (s, 1H), 8.76–8.70 (m, 1H), 8.20 (d, $J = 7.8$ Hz, 1H), 8.03–7.93 (m, 2H), 7.86 (dd, $J = 0.8, 8.0$ Hz, 1H), 7.72 (t, $J = 7.5$ Hz, 1H), 7.63 (t, $J = 7.4$ Hz, 2H), 7.60–7.52 (m, 2H), 7.39 (d, $J = 7.6$ Hz, 1H), 7.30 (d, $J = 7.5$ Hz, 1H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 176.24, 167.64, 167.53, 150.27 (2C), 145.69, 142.47, 141.03, 137.62, 132.28, 131.61, 130.80, 130.74 (2C), 129.76, 129.37, 127.96, 127.81, 126.07, 123.18, 122.45; HR-ESI-MS: $m/z = 344.1026$ $[\text{M} + \text{H}]^+$, calculated cd for $\text{C}_{20}\text{H}_{14}\text{N}_3\text{O}_3$: 344.1030.

Compound **B13**: Yield 52%; white solid; mp: 210–212 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 12.56 (s, 1H), 9.00 (s, 1H), 8.75 (s, 1H), 8.20 (d, $J = 7.5$ Hz, 2H), 7.97 (d, $J = 7.7$ Hz, 1H), 7.72 (t, $J = 7.5$ Hz, 1H), 7.63 (t, $J = 7.3$ Hz, 2H), 7.60–7.52 (m, 2H), 7.40 (d, $J = 7.5$ Hz, 1H), 7.29 (d, $J = 7.4$ Hz, 1H); ^{13}C NMR (176 MHz, DMSO- d_6) δ 175.58, 167.04, 165.16, 151.74, 147.08, 142.02, 140.42, 133.78, 131.86, 131.03, 130.19, 130.18, 130.14, 129.09, 128.70, 127.37, 127.24, 123.69, 121.74, 121.53; HR-ESI-MS: $m/z = 344.1028$ $[\text{M} + \text{H}]^+$, calculated for $\text{C}_{20}\text{H}_{14}\text{N}_3\text{O}_3$: 344.1030.

Compound **B14**: Yield 65%; white solid; mp: 188–191 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 12.56 (s, 1H), 8.80–8.75 (m, 2H), 8.20 (dd, $J = 1.3, 7.8$ Hz, 1H), 7.96 (dd, $J = 1.4, 7.7$ Hz, 1H), 7.81–7.76 (m, 2H), 7.73 (td, $J = 1.4, 7.6$ Hz, 1H), 7.68–7.61 (m, 2H), 7.56 (td, $J = 1.3, 7.6$ Hz, 1H), 7.41 (dd, $J = 1.2, 7.6$ Hz, 1H), 7.30 (dd, $J = 1.2, 7.5$ Hz, 1H); ^{13}C NMR (176 MHz, DMSO- d_6) δ 176.00, 167.03, 165.58, 150.25, 141.99, 140.31, 132.72, 131.93, 131.04, 130.19, 130.16, 130.14, 129.11, 128.76, 127.41, 127.26, 121.50, 120.18; HR-ESI-MS: $m/z = 344.1027$ $[\text{M} + \text{H}]^+$, calculated for $\text{C}_{20}\text{H}_{14}\text{N}_3\text{O}_3$: 344.1030.

Compound **B15**: Yield 39%; white solid; mp: 165–168 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 12.57 (s, 1H), 8.59 (d, $J = 5.0$ Hz, 1H), 8.21 (d, $J = 7.7$ Hz, 1H), 7.96 (d, $J = 7.7$ Hz, 1H), 7.93 (s, 1H), 7.84 (d, $J = 5.1$ Hz, 1H), 7.74 (t, $J = 7.5$ Hz, 1H), 7.64 (t, $J = 7.4$ Hz, 2H), 7.56 (t, $J = 7.1$ Hz, 1H), 7.41 (d, $J = 7.6$ Hz, 1H), 7.29 (d, $J = 7.5$ Hz, 1H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 176.72, 167.63, 165.05, 151.80 (2C), 142.67, 142.21, 140.88, 136.32, 132.68, 131.65, 130.79 (2C), 129.65, 129.37, 127.98, 127.88, 124.98, 121.88, 120.36; HR-ESI-MS: $m/z = 422.0133$ $[\text{M} + \text{H}]^+$, calculated for $\text{C}_{20}\text{H}_{13}\text{BrN}_3\text{O}_3$: 422.0135.

Compound **B16**: Yield 61%; white solid; mp: 145–147 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 12.57 (s, 1H), 8.69–8.59 (m, 1H), 8.22 (dd, $J = 7.8, 1.3$ Hz, 1H), 7.96 (dd, $J = 7.8, 1.4$ Hz, 1H), 7.84–7.79 (m, 2H), 7.74 (td, $J = 7.6, 1.4$ Hz, 1H), 7.64 (tt, $J = 7.5, 1.4$ Hz, 2H), 7.56 (td, $J = 7.6, 1.4$ Hz, 1H), 7.41 (dd, $J = 7.7, 1.2$ Hz, 1H), 7.30 (dd, $J = 7.6, 1.3$ Hz, 1H); ^{13}C NMR (176 MHz, DMSO- d_6) δ 176.16, 167.04, 164.61, 150.80, 150.71, 142.07, 140.27, 136.13, 132.09, 131.07, 130.19, 130.18, 129.07, 128.79, 127.41, 127.29, 121.28, 120.75, 119.57; HR-ESI-MS: $m/z = 378.0712$ $[\text{M} + \text{H}]^+$, calculated for $\text{C}_{20}\text{H}_{13}\text{ClN}_3\text{O}_3$: 378.0640.

Compound **B17**: Yield 61%; white solid; mp: 98–100 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 12.57 (s, 1H), 8.46 (d, $J = 5.1$ Hz, 1H), 8.21 (dd, $J = 7.7, 1.3$ Hz, 1H), 7.96 (dd, $J = 7.6, 1.4$ Hz, 1H), 7.78–7.70 (m, 2H), 7.68–7.60 (m, 2H), 7.56 (td, $J = 7.6, 1.4$ Hz, 1H), 7.51 (s, 1H), 7.41 (dd, $J = 7.6, 1.4$ Hz, 1H), 7.30 (dd, $J = 7.6, 1.3$ Hz, 1H); ^{13}C NMR (176 MHz, DMSO- d_6) δ 176.18, 167.04, 164.75 (d, $J = 3.9$ Hz), 162.89 (d, $J = 236.0$ Hz), 148.80, 148.72, 142.06, 140.25, 138.39 (d, $J = 8.7$ Hz), 132.08, 131.06, 130.19, 129.08, 128.79, 127.43, 127.29, 121.31,

118.71 (d, $J = 4.2$ Hz), 106.65, 106.42; HR-ESI-MS: $m/z = 362.0945$ $[M + H]^+$, calculated for $C_{20}H_{13}FN_3O_3$: 362.0935.

Compound **B18**: Yield 61%; white solid; mp: 228–230 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.56 (s, 1H), 8.63 (dd, $J = 5.2, 0.9$ Hz, 1H), 8.20 (dd, $J = 7.8, 1.4$ Hz, 1H), 7.96 (dd, $J = 7.8, 1.5$ Hz, 1H), 7.73 (td, $J = 7.6, 1.4$ Hz, 1H), 7.68–7.60 (m, 3H), 7.60–7.52 (m, 2H), 7.40 (dd, $J = 7.6, 1.3$ Hz, 1H), 7.29 (dd, $J = 7.5, 1.4$ Hz, 1H), 2.55 (s, 3H); ^{13}C NMR (176 MHz, DMSO- d_6) δ 175.90, 167.07, 165.69, 158.77, 149.57, 142.03, 140.33, 132.95, 131.89, 130.99, 130.26, 130.17, 130.13, 129.11, 128.73, 127.36, 127.23, 121.55, 119.49, 117.31, 23.45; HR-ESI-MS: $m/z = 358.1198$ $[M + H]^+$, calculated for $C_{21}H_{16}N_3O_3$: 358.1186.

Compound **B19**: Yield 57%; white solid; mp: 154–156 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.54 (s, 1H), 8.34 (d, $J = 5.3$ Hz, 1H), 8.19 (dd, $J = 7.8, 1.4$ Hz, 1H), 7.96 (dd, $J = 7.7, 1.5$ Hz, 1H), 7.73 (td, $J = 7.6, 1.4$ Hz, 1H), 7.63 (td, $J = 7.5, 1.5$ Hz, 2H), 7.56 (td, $J = 7.6, 1.4$ Hz, 1H), 7.42–7.37 (m, 2H), 7.29 (dd, $J = 7.6, 1.3$ Hz, 1H), 7.12 (t, $J = 1.1$ Hz, 1H), 3.90 (s, 3H); ^{13}C NMR (176 MHz, DMSO- d_6) δ 175.81, 167.03, 165.42, 163.61, 147.84 (d, $J = 2.4$ Hz), 142.05, 140.37, 135.70, 131.93, 131.03, 130.19, 130.18, 130.15, 129.07, 128.69, 127.38, 127.25, 121.42, 113.42, 107.36, 53.01; HR-ESI-MS: $m/z = 374.1152$ $[M + H]^+$, calculated for $C_{21}H_{16}N_3O_4$: 374.1135.

Compound **B20**: Yield 57%; white solid; mp: 214–216 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.55 (s, 1H), 8.19 (dd, $J = 7.8, 1.4$ Hz, 1H), 7.96 (dd, $J = 7.7, 1.4$ Hz, 1H), 7.73 (td, $J = 7.6, 1.4$ Hz, 1H), 7.63 (tdd, $J = 7.6, 2.7, 1.4$ Hz, 2H), 7.56 (td, $J = 7.6, 1.4$ Hz, 1H), 7.45 (s, 2H), 7.40 (dd, $J = 7.7, 1.3$ Hz, 1H), 7.29 (dd, $J = 7.5, 1.3$ Hz, 1H), 2.49 (s, 6H); ^{13}C NMR (176 MHz, DMSO- d_6) δ 176.88, 168.19, 166.81, 159.16, 142.96, 141.41, 134.47, 133.05, 132.23, 131.23, 131.10, 130.15, 129.77, 128.49, 128.37, 122.56, 117.81, 24.24(2C); HR-ESI-MS: $m/z = 372.1361$ $[M + H]^+$, calculated for $C_{22}H_{18}N_3O_3$: 372.1343.

Compound **B21**: Yield 59%; white solid; mp: 233–235 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.57 (s, 1H), 8.85 (d, $J = 0.6$ Hz, 1H), 8.70 (d, $J = 5.0$ Hz, 1H), 8.20 (dd, $J = 7.9, 1.3$ Hz, 1H), 7.96 (dd, $J = 7.8, 1.4$ Hz, 1H), 7.77 (dd, $J = 5.0, 0.6$ Hz, 1H), 7.74 (td, $J = 7.6, 1.5$ Hz, 1H), 7.64 (tdd, $J = 7.5, 3.8, 1.4$ Hz, 2H), 7.55 (td, $J = 7.6, 1.4$ Hz, 1H), 7.40 (dd, $J = 7.7, 1.3$ Hz, 1H), 7.30 (dd, $J = 7.6, 1.3$ Hz, 1H); ^{13}C NMR (176 MHz, DMSO- d_6) δ 175.33, 166.99, 164.27, 150.18, 148.02, 142.05, 140.35, 131.98, 131.69, 131.08, 130.20, 130.18, 130.01, 129.23, 128.79, 128.41, 127.38, 127.28, 123.89, 121.36; HR-ESI-MS: $m/z = 378.0698$ $[M + H]^+$, calculated for $C_{20}H_{13}ClN_3O_3$: 378.0640.

Compound **B22**: Yield 58%; white solid; mp: 258–259 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.56 (s, 1H), 9.06 (d, $J = 4.4$ Hz, 1H), 8.42 (dd, $J = 8.6, 1.4$ Hz, 1H), 8.25 (dd, $J = 7.8, 1.4$ Hz, 1H), 8.14 (dd, $J = 8.5, 1.3$ Hz, 1H), 8.00 (dd, $J = 7.8, 1.5$ Hz, 1H), 7.96 (d, $J = 4.4$ Hz, 1H), 7.87 (ddd, $J = 8.4, 6.8, 1.4$ Hz, 1H), 7.76 (td, $J = 7.5, 1.4$ Hz, 1H), 7.72–7.64 (m, 3H), 7.61 (td, $J = 7.5, 1.4$ Hz, 1H), 7.43 (dd, $J = 7.7, 1.3$ Hz, 1H), 7.36 (dd, $J = 7.5, 1.4$ Hz, 1H); ^{13}C NMR (176 MHz, DMSO- d_6) δ 176.23, 168.19, 167.29, 150.82, 148.72, 143.09, 141.47, 133.05, 132.26, 131.33, 131.30, 131.15, 131.12, 130.65, 130.32, 130.18, 129.82, 128.61, 128.51, 128.44, 126.14, 124.37, 122.57, 122.28; HR-ESI-MS: $m/z = 394.1208$ $[M + H]^+$, calculated for $C_{24}H_{16}N_3O_3$: 394.1186.

Compound **B23**: Yield 52%; white solid; mp: 236–238 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.57 (s, 1H), 9.27 (d, $J = 2.1$ Hz, 1H), 8.90 (dd, $J = 2.2, 0.8$ Hz, 1H), 8.26 (dd, $J = 7.8, 1.4$ Hz, 1H), 8.17 (dd, $J = 8.3, 1.5$ Hz, 1H), 8.11 (dd, $J = 8.4, 1.1$ Hz, 1H), 8.00 (dd, $J = 7.7, 1.5$ Hz, 1H), 7.90 (ddd, $J = 8.4, 6.9, 1.5$ Hz, 1H), 7.74 (qd, $J = 7.2, 1.3$ Hz, 2H), 7.66 (td, $J = 7.7, 1.4$ Hz, 2H), 7.59 (td, $J = 7.6, 1.4$ Hz, 1H), 7.43 (dd, $J = 7.6, 1.3$ Hz, 1H), 7.32 (dd, $J = 7.5, 1.4$ Hz, 1H); ^{13}C NMR (176 MHz, DMSO- d_6) δ 175.64, 165.27, 147.91, 147.12, 134.47, 131.86, 131.05, 130.90, 130.19, 130.16, 129.12, 128.75, 128.47, 128.33, 127.38, 127.20, 126.26, 121.59, 118.88; HR-ESI-MS: $m/z = 394.1180$ $[M + H]^+$, calculated for $C_{24}H_{16}N_3O_3$: 394.1186.

Compound **B24**: Yield 51%; white solid; mp: 264–266 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.57 (s, 1H), 9.00 (dd, $J = 4.2, 1.7$ Hz, 1H), 8.57 (d, $J = 1.8$ Hz, 1H), 8.53 (dd, $J = 8.3, 1.7$ Hz, 1H), 8.25 (dd, $J = 7.9, 1.4$ Hz, 1H), 8.20–8.11 (m, 2H), 8.00 (dd, $J = 7.8, 1.5$ Hz, 1H), 7.73 (dd, $J = 7.6, 1.4$ Hz, 1H), 7.69–7.62 (m, 3H), 7.59 (td, $J = 7.6, 1.4$ Hz, 1H), 7.42 (dd, $J = 7.7, 1.3$ Hz, 1H), 7.32 (dd, $J = 7.5, 1.4$ Hz, 1H); ^{13}C NMR (176 MHz, DMSO- d_6) δ 176.67, 168.16,

167.59, 152.73, 149.09, 143.04, 141.55, 137.48, 132.87, 132.17, 131.29, 131.23, 131.19, 130.57, 130.25, 129.83, 128.50, 128.32, 128.27, 128.16, 127.38, 124.48, 123.02, 122.83; HR-ESI-MS: $m/z = 394.1205$ $[M + H]^+$, calculated for $C_{24}H_{16}N_3O_3$: 394.1186.

Compound **B25**: Yield 51%; white solid; mp: 169–171 °C; 1H NMR (400 MHz, DMSO- d_6) δ 12.55 (s, 1H), 8.60–8.45 (m, 1H), 8.29–8.24 (m, 1H), 8.22 (dd, $J = 7.8, 1.4$ Hz, 1H), 8.07–7.96 (m, 2H), 7.73 (td, $J = 7.5, 1.4$ Hz, 1H), 7.69–7.54 (m, 5H), 7.41 (dd, $J = 7.6, 1.3$ Hz, 1H), 7.34 (dd, $J = 7.5, 1.4$ Hz, 1H), 7.10 (d, $J = 8.3$ Hz, 1H), 4.05 (s, 3H); ^{13}C NMR (176 MHz, DMSO- d_6) δ 174.95, 168.55, 168.16, 157.97, 143.00, 141.72, 132.65, 132.18, 131.29, 131.20, 131.19, 131.11, 130.84, 130.29, 129.65, 128.53, 128.39, 128.32, 126.39, 125.97, 125.39, 122.94, 122.50, 115.42, 104.52, 56.49; HR-ESI-MS: $m/z = 421.1184$ $[M-H]^-$, calculated for $C_{26}H_{17}N_2O_4$: 421.1183.

The procedure for the synthesis of intermediate **I-4**. Compound **B21** (1.0 eq) and methyl 3-aminopropionate hydrochloride (1.2 eq) were dissolved in 20 mL *N,N*-dimethylformamide, then HATU (1.5 eq) and triethylamine (2 eq) were added. The mixture was stirred at 60 °C for 10 h, and the solvent was evaporated in a vacuum. The reaction mixture was poured into water (50 mL). The mixture was extracted with ethyl acetate (50 mL $\times$ 3). The combined organic solution was washed with saturated aqueous sodium chloride solution (30 mL $\times$ 3) and dried over anhydrous Na_2SO_4 . The residue was purified by column chromatography to **I-4**.

The procedure for the synthesis of compound **B26**. The intermediate **I-4** (1.0 eq) and lithium hydroxide monohydrate (2.0 eq) were dissolved in 20 mL of a mixed solvent of ethanol: H_2O (4:1). The mixture was stirred at room temperature for 8 h, and the solvent was evaporated in a vacuum. The reaction mixture was poured into water (50 mL). 1 M HCl solution was added dropwise to adjust the pH to 5. The mixture was extracted with ethyl acetate (50 mL $\times$ 3). The combined organic solution was washed with saturated aqueous sodium chloride solution (30 mL $\times$ 3) and dried over anhydrous Na_2SO_4 . The residue was purified by column chromatography to **B26**.

Compound **B26**: Yield 53%; white solid; mp: 240–242 °C; 1H NMR (400 MHz, DMSO- d_6) δ 8.84 (s, 1H), 8.70 (d, $J = 5.0$ Hz, 1H), 8.14 (dd, $J = 7.8, 1.4$ Hz, 1H), 8.00 (s, 1H), 7.82 (d, $J = 5.0$ Hz, 1H), 7.70 (td, $J = 7.6, 1.4$ Hz, 1H), 7.61 (td, $J = 7.6, 1.4$ Hz, 1H), 7.54–7.44 (m, 3H), 7.39 (dd, $J = 7.7, 1.3$ Hz, 1H), 7.29 (dd, $J = 7.8, 1.7$ Hz, 1H), 3.03 (d, $J = 7.0$ Hz, 2H), 1.83 (t, $J = 7.4$ Hz, 2H); ^{13}C NMR (176 MHz, DMSO- d_6) δ 176.54, 167.71, 165.22, 151.16, 149.12, 141.97, 138.92, 136.97, 132.92, 131.63, 130.70, 130.19, 129.86, 129.47, 128.47, 128.23, 127.46, 125.18, 122.89, 37.00, 36.91; HR-ESI-MS: $m/z = 449.1032$ $[M + H]^+$, calculated for $C_{23}H_{18}ClN_4O_4$: 449.1011.

The procedure for the synthesis of compound **B27**. The compound **B21** (1.0 eq) and 4-bromobenzenesulfonamide (1.2 eq) were dissolved in 20 mL *N,N*-dimethylformamide in an ice bath, then 4-dimethylaminopyridine (1.5 eq) and EDCI (1.2 eq) were added. The mixture was stirred at room temperature for 6 h. The solvent was evaporated in a vacuum. The residue was purified by column chromatography to give **B27**.

Compound **B27**: Yield 49%; white solid; mp: 188–190 °C; 1H NMR (400 MHz, DMSO- d_6) δ 8.84 (d, $J = 0.5$ Hz, 1H), 8.70 (d, $J = 5.0$ Hz, 1H), 8.11–8.04 (m, 1H), 7.86 (dd, $J = 4.9, 0.6$ Hz, 1H), 7.82–7.77 (m, 1H), 7.60–7.46 (m, 2H), 7.42–7.30 (m, 6H), 7.21–7.14 (m, 1H), 7.07–7.00 (m, 1H); ^{13}C NMR (176 MHz, DMSO- d_6) δ 175.62, 170.76, 163.81, 150.05, 147.93, 144.64, 143.32, 139.34, 138.44, 131.99, 131.26, 130.27, 129.59, 129.25, 128.62, 128.34, 128.17, 127.88, 127.51, 126.30, 126.15, 124.19, 122.23, 121.45; HR-ESI-MS: $m/z = 594.9864$ $[M + H]^+$, calculated for $C_{26}H_{17}BrClN_4O_4S$: 594.9837.

The procedure for the synthesis of Intermediate **I-5a**. Iodine substituted benzoic acid (1.2 eq) and intermediate **I-3a** (1.0 eq) were dissolved in 20 mL dichloromethane, then HATU (1.5 eq) and triethylamine (2 eq) were added. The mixture was stirred at room temperature for 10 h, and the solvent was evaporated in a vacuum. The residue was purified by column chromatography to give intermediate **I-5a**.

The procedure for the synthesis of intermediate **I-6a**. Intermediate **I-5a** (1.0 eq) and cesium carbonate (1.0 eq) were dissolved in 20 mL dimethyl sulfoxide. The mixture was stirred at room

temperature for 10 h, the reaction mixture was poured into water (50 mL), the mixture was extracted with ethyl acetate (50 mL $\times$ 3). The combined organic solution was washed with saturated aqueous sodium chloride solution (30 mL $\times$ 3) and dried over anhydrous Na₂SO₄. The residue was purified by column chromatography to intermediate **I-6a**.

General preparation of compounds **B28** and **B29**. The intermediate **I-6a** (1.0 eq), substituted phenylboronic acids (1.0 eq), potassium carbonate (3.0 eq), and tetrakis(triphenylphosphine)palladium (0.2 eq) were added into 20 mL of a mixed solvent of toluene: 95% ethanol (1:1). The mixture was stirred at 110 °C under N₂ for 12 h. The solvent was then evaporated in a vacuum, the reaction mixture was poured into water (50 mL). 1 M HCl solution was added dropwise to adjust the pH to 5. The mixture was extracted with ethyl acetate (50 mL $\times$ 3). The combined organic solution was washed with saturated aqueous sodium chloride solution (30 mL $\times$ 3) and dried over anhydrous Na₂SO₄. The residue was purified by column chromatography to **B28** and **B29**.

Compound **B28**: Yield 55%; white solid; mp: 288–289 °C; ¹H NMR (400 MHz, DMSO-d₆) δ 13.07 (s, 1H), 8.82–8.73 (m, 2H), 8.17 (dd, J = 7.8, 1.4 Hz, 1H), 7.98 (dt, J = 6.8, 1.9 Hz, 1H), 7.88–7.78 (m, 4H), 7.71 (td, J = 7.6, 1.3 Hz, 1H), 7.64 (dd, J = 7.7, 1.3 Hz, 1H), 7.60–7.52 (m, 2H); ¹³C NMR (176 MHz, DMSO-d₆) δ 176.20, 166.46, 165.81, 150.32(2C), 140.64, 139.18, 132.65, 132.52, 130.74, 130.23, 128.81, 128.09, 128.07, 128.02, 121.42, 120.26(2C); HR-ESI-MS: m/z = 344.1041 [M + H]⁺, calculated for C₂₀H₁₄N₃O₃: 344.1030.

Compound **B29**: Yield 54%; white solid; mp: 258–260 °C; ¹H NMR (400 MHz, DMSO-d₆) δ 8.85–8.75 (m, 2H), 8.08 (dd, J = 7.8, 1.4 Hz, 1H), 7.94–7.89 (m, 2H), 7.86–7.82 (m, 2H), 7.78 (td, J = 7.6, 1.4 Hz, 1H), 7.65 (td, J = 7.6, 1.3 Hz, 1H), 7.60 (dd, J = 7.8, 1.2 Hz, 1H), 7.19–7.14 (m, 2H); ¹³C NMR (176 MHz, DMSO-d₆) δ 176.78, 167.92, 165.81, 150.30(2C), 141.97, 140.00, 138.65, 132.74, 132.23, 130.56, 130.22, 128.38(2C), 127.33, 126.77(2C), 121.56, 120.34(2C); HR-ESI-MS: m/z = 344.1043 [M + H]⁺, calculated for C₂₀H₁₄N₃O₃: 344.1030.

The procedure for the synthesis of intermediate **I-7**. The isoniazid (1.0 eq) and 2-bromobenzoic acid (1.05 eq) were dissolved in 20 mL phosphorus oxychloride. The mixture was stirred at 110 °C for 10 h. Saturated sodium bicarbonate solution was added dropwise until there were no bubbles in the reaction solution. The mixture was extracted with ethyl acetate (50 mL $\times$ 3). The combined organic solution was washed with saturated aqueous sodium chloride solution (30 mL $\times$ 3) and dried over anhydrous Na₂SO₄. The residue was purified by column chromatography to obtain **I-7**.

The procedure for the synthesis of intermediate **I-8**. The intermediate **I-7** (1.0 eq), substituted phenylboronic acid (1.0 eq), potassium carbonate (3.0 eq), and tetrakis(triphenylphosphine)palladium (0.2 eq) were added into 20 mL of a mixed solvent of toluene: 95% ethanol (1:1). The mixture was stirred at 110 °C under N₂ for 12 h. The solvent was then evaporated in a vacuum and the residue was purified by column chromatography to intermediate **I-8**.

The procedure for the synthesis of compound **B30**. The intermediate **I-8** (1.0 eq) and lithium hydroxide monohydrate (2.0 eq) were dissolved in 20 mL of a mixed solvent of ethanol: H₂O (4:1). The mixture was stirred at room temperature for 8 h, and the solvent was evaporated in a vacuum. The reaction mixture was poured into water (50 mL). 1 M HCl solution was added dropwise to adjust the pH to 5. The mixture was extracted with ethyl acetate (50 mL $\times$ 3). The combined organic solution was washed with saturated aqueous sodium chloride solution (30 mL $\times$ 3) and dried over anhydrous Na₂SO₄. The residue was purified by column chromatography to **B30**.

Compound **B30**: Yield 58%; white solid; mp: 237–239 °C; ¹H NMR (400 MHz, DMSO-d₆) δ 12.60 (s, 1H), 8.80–8.67 (m, 2H), 8.19 (dt, J = 7.7, 1.8 Hz, 1H), 7.95 (dt, J = 7.7, 1.8 Hz, 1H), 7.72–7.56 (m, 4H), 7.51–7.46 (m, 2H), 7.40 (dd, J = 7.6, 1.4 Hz, 1H), 7.33 (dd, J = 7.6, 1.5 Hz, 1H); ¹³C NMR (176 MHz, DMSO-d₆) δ 168.24, 165.58, 162.46, 151.35, 142.00, 141.48, 132.12, 132.10, 131.36, 131.22, 131.10, 130.54, 130.11, 128.96, 128.45, 128.33, 122.26, 120.16; HR-ESI-MS: m/z = 344.1026 [M + H]⁺, calculated for C₂₀H₁₄N₃O₃: 344.1030.

Spectral data, including HR-ESI-MS, ¹H-NMR and ¹³C-NMR, for compounds **A1–A21** and **B1–B30** are provided in Supplementary Materials.

3.2. Biology

In Vitro URAT1 Inhibitory Assay. A transient expression system of human URAT1 in HEK293T cells was constructed to measure ^{14}C -uric acid transport. The HEK293T cells were obtained from ATCC (catalogue No.: CRL-11268). The cell cryovials were removed from liquid nitrogen storage, and immediately placed on dry ice prior to thawing. The frozen cell vials were placed briefly (30 s to 1 min) in a 37 °C water bath, until only small ice crystals left and the cell pellet was almost completely thawed. The cell suspension was transferred into a 15 mL centrifuge tube, and 10 mL complete medium (DMEM +10% FBS +500 µg/mL G418 +1% P/S) was added. After centrifugation at 1000 rpm for 5 min, the supernatant was discarded, and cell precipitation was resuspended in 5 mL complete medium and transferred to a T75 culture flask. 15 mL of the assay complete medium was added into a T75 flask and incubated at 37 °C and 5% CO_2 . The uptake assay test could be carried out after cell culture adherent to the well in the 96-well plate. Then, the cells were washed with 200 µL/well of pre-warmed Cl^- free HBSS buffer, and all buffer was removed at the last washing. 50 µL/well of Cl^- free HBSS Buffer containing Uric acid [$8\text{-}^{14}\text{C}$] (2.5 µCi/mL) and samples were added to the cells, and incubated for 8 min. Then, the cells were washed three times with Cl^- free HBSS Buffer and all buffer was removed at the last washing. 50 µL/well of lysis buffer (100 mM NaOH) was added to the lysate cells and stirred at 900 rpm for 5 min. 150 µL/well MicroScintTM-40 cocktail was added and agitated at 900 rpm for 5 min. Finally, the 96-plate was counted in a MicroBeta2 (PerkinElmer). The data were analyzed and IC_{50} values was calculated with GraphPad Prism 6 software.

The IC_{50} curves for the compounds are provided in Supplementary Materials.

4. Conclusions

Based on the known URAT1 inhibitors Epaminurad and Telmisartan, two series of biphenyl carboxylic acid-based URAT1 inhibitors were recognized, and SAR clues were provided. Both series afforded potent URAT1 inhibitors, and **A1** and **B21** exhibited IC_{50} values of 0.93 µM and 0.17 µM, respectively. These new inhibitors not only represent a new chemical prototype of potent URAT1 inhibitors, but are expected to have reduced toxicity and improved chemical accessibility compared to Epaminurad and Telmisartan. The results confirmed that ligand-based approaches could be an effective way to identify novel and potent URAT1 inhibitors.

5. Patents

A Chinese patent (202211251941.3) derived from results presented herein was applied in 2022.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules28217415/s1>.

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