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Discovery of *meta*-amido bromophenols as new antitubercular agents

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Abstract

A series of *m*-amido bromophenol derivatives were designed and synthesized. The compounds were found to potently inhibit the growth of *Mycobacterium tuberculosis* H37Ra. They also exhibited moderate inhibitory activity against *Mycobacterium tuberculosis* H37Rv and multidrug-resistant strains. The compounds did not show inhibitory activity against normal Gram-positive and Gram-negative bacteria. Moderate cytotoxicities and good metabolic stability were observed for the selected compounds. The results demonstrated *meta*-amido bromophenols as a new class of antitubercular agents with good potentials.

Keywords

Mycobacterium tuberculosis, meta-amido bromophenol, antitubercular agent

1. Introduction

Tuberculosis (TB), a chronic infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*), has plagued people for thousands of years and remains a huge threat to public health. According to the report of the World Health Organization (WHO), it is estimated that over 1.3 million people died from TB in 2017.[1] One third of the population in the world carries latent TB, which can be activated upon immune system suppressed or disrupted, such as aging and HIV co-infection.[2] Moreover, *Mtb* has developed the resistance to many first-line anti-TB drugs including rifampicin, isoniazid, streptomycin, ethambutol and pyrazinamide etc.[3,4] Rather limited drugs are applicable for the patients infected with multidrug-resistant (MDR) *Mtb* and extensive drug-resistant (XDR) *Mtb*.[5] The treatment period is extremely long (typically 18-24 months) for MDR-TB and the cure rates are less than 50%.[6] In recent four decades, only two new anti-TB drugs, bedaquiline[7] and delamanid[8], were approved for the treatment of MDR-TB and XDR-TB. The two drugs are still beset by the side effects. For these reasons, anti-TB drugs with novel structures and action mechanisms are urgently desired.[9]

In the recent study, we found that *m*-amido phenol derivatives exhibit good inhibitory activity against *Mtb in vitro*.[10] However, the privileged compound **YZ-7** did not exert *in vivo* efficacy attributed to the fast clearance in the liver. The 3-benzyl group in compound **YZ-7** was supposed to be liable to the oxidation catalyzed by CYP450. The further structural modifications were made to increase the metabolic stability (**Chart 1**). The replacement of 3-benzyl with a bromine led to the decreased activity. The shift of the bromine to 2-position resulted in the complete loss of the antitubercular activity. However, to our delight, the 4-bromo derivative **3** showed good inhibitory activity, which is comparable with **YZ-7**. In this paper, we report the design, synthesis and biological evaluation of a series of *meta*-amido bromophenols as new antitubercular agents.

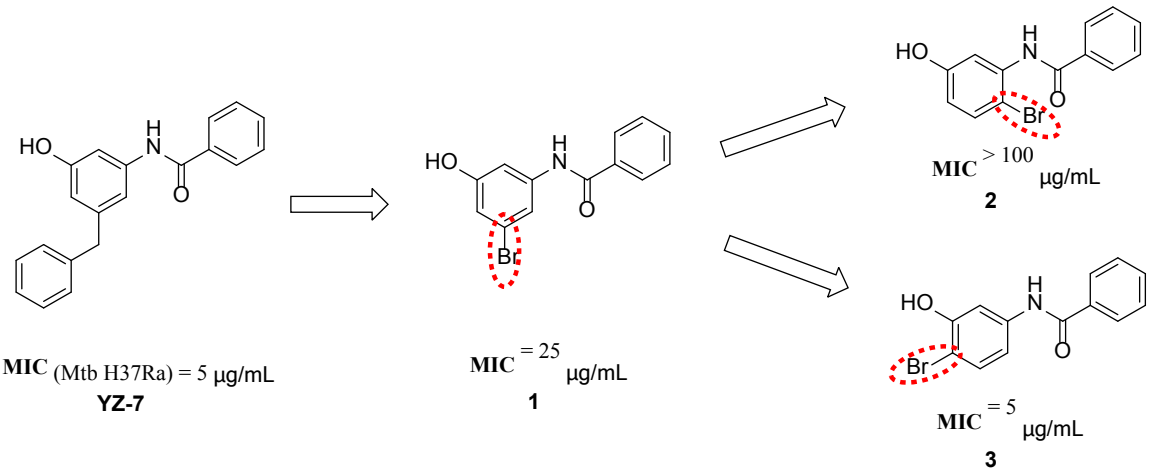


Chart 1. Structural modifications of compound **YZ-7**.

2. Results and discussion

2.1 Design of new derivatives

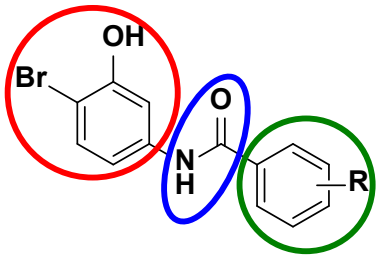


Chart 2. Design of *meta*-amido bromophenols.

A series of *meta*-amido bromophenols were designed based on the structure of the lead compound **3**. The structural modifications were made at the left-side bromophenol moiety, the amide linker, and the right-side phenyl group respectively (**Chart 2**). For the modification of bromophenol moiety, the hydroxyl was removed or methylated to provide compounds **4a**, **4b**. The bromine was replaced with chlorine, fluorine, methyl, methoxy, ester, acetyl, and cyano to afford derivatives **4c**-**4k**. For the modification of the amide linker, the shift of amide group to *para*- or *ortho*-position of the hydroxyl group led to compounds **5a** and **5b**. Furthermore, the transpositional analog **5c** and *N*-benzyl analog **5d** were prepared. For the structural modification of the right-side phenyl group, the replacement of the phenyl with alkyl, vinyl, heteroaryl, and the introduction of various substitutents on the phenyl ring led to the compounds **6a**-**6u**.

2.2 Synthesis of the target compounds

The synthesis of compounds **1-3** and **4a-4k** is outlined in **Chart 3**. The reaction of substituted anilines with benzoyl chloride provided the target compounds in good yields. Compound **4i** was obtained after the hydrolysis of **4h** in aqueous sodium hydroxide.

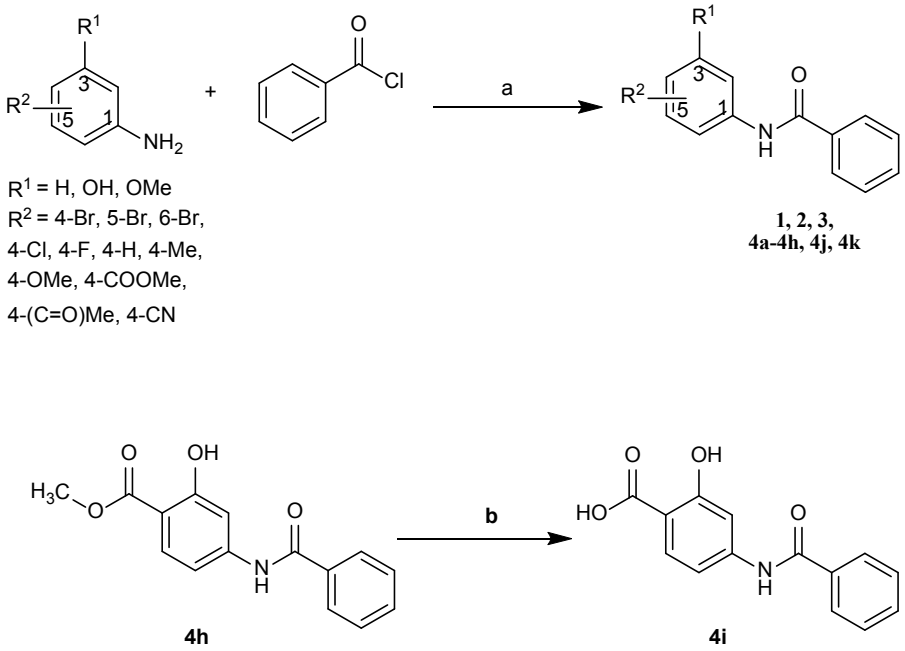


Chart 3. Synthesis of compounds **1**, **2**, **3**, and **4a-4k.** Reagents and conditions: (a) Et_3N, THF, 0°C to rt, 67-87%; (b) NaOH, CH_3OH, H_2O, 50°C, 73%.

The synthesis of compounds **5a-5d** is outlined in **Chart 4**. Compound **5a** and **5b** were prepared via the reaction of 4-amino-2-bromophenol or 2-amino-6-bromophenol with benzoic acids in the presence of HATU and DIPEA. Compound **5c** was obtained via the condensation of 4-bromo-3-methoxybenzoic acid and aniline. Compound **5d** was prepared via the benzylation of 4-bromo-3-methoxyaniline and subsequent demethylation.

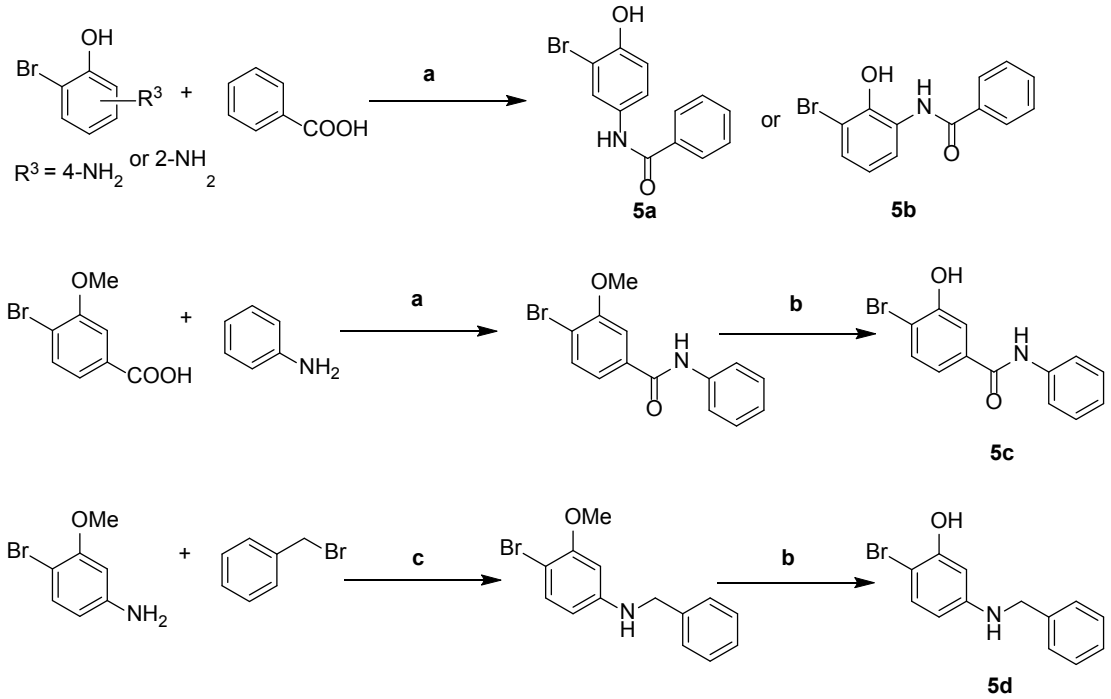


Chart 4. Synthesis of compounds **5a-5d**. Reagents and conditions: (a) HATU, DIPEA, DMF, rt, 71-90%; (b) BBr_3, CH_2Cl_2, -20°C to 0°C, 38-44%; (c) K_2CO_3, DMF, 50°C, 72%.

The synthesis of compounds **6a-6u** is outlined in **Chart 5**. 4-Bromo-3-methoxyaniline was treated with BBr_3 to give 4-bromo-3-hydroxyaniline. The subsequent reaction with acyl chlorides or the direct condensation with acids in the presence of HATU and DIPEA provided compounds **6a-6m**.

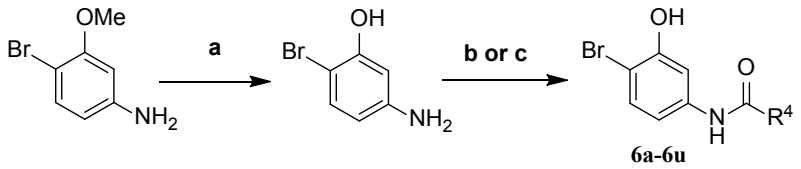


Chart 5. Synthesis of **6a-6u.** Reagents and conditions: (a) BBr_3, CH_2Cl_2, 0°C to rt, 88%; (b) R^4COCl, Et_3N, THF, 0°C to rt, 42%-83%; (c) R^4COOH, HATU, DIPEA, DMF, rt, 76-80%.

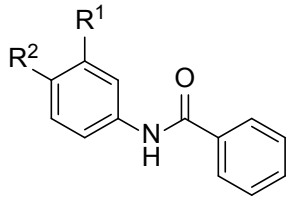
2.3 Biological study

2.3.1 Inhibitory activity *in vitro*

Inhibitory activities of compounds **4a-4k** were evaluated against autoluminescent Mtb H37Ra strain.[11] The bacteria growth was determined by the bioluminescence intensity and the results are summarized in **Table 1**. The existence of free hydroxyl is crucial for the antitubercular

activity. Both the deletion and the methylation led to the complete loss of the inhibitory activity (**Table 1**, entries 2-3). The replacement of bromine with chlorine or fluorine resulted in the decreased activity (**Table 1**, entries 4-5). 4-Unsubstitutued derivative **4e** is inactive (**Table 1**, entry 6). The results confirmed that the 4-substitution is indispensable. Non-halogen substituents such as methyl, methoxy, ester, carboxyl, acetyl, and cyano are incompatible (**Table 1**, entries 8-13). The bromine is demonstrated to be unique for the antitubercular activity, probably due to its suitably steric and electronic effect.

Table 1. Inhibitory activity of compounds 4a-4k against *Mtb* H37Ra.

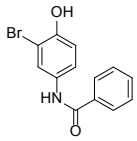
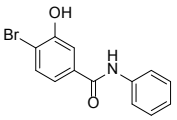
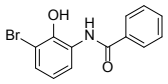
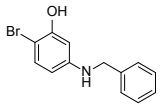


Compound	R^1	R^2	MIC (μg/mL)	Compound	R^1	R^2	MIC (μg/mL)
3	-OH	Br	5	**4f**	-OH	Me	>100
4a	-H	Br	>100	**4g**	-OH	OMe	>100
4b	-OMe	Br	>100	**4h**	-OH	COOMe	>100
4c	-OH	Cl	12.5	**4i**	-OH	COOH	>100
4d	-OH	F	50	**4j**	-OH	COMe	>100
4e	-OH	H	>100	**4k**	-OH	CN	>100
INH			0.1	**EMB**			2

INH: isoniazid; EMB: ethambutol

The compounds **5a-5d** with the modifications of amide linker were investigated and the results are summarized in **Table 2**. The placement of amide group at the *meta*-position of hydroxyl group is crucial, since the compounds **5a** and **5b** almost lose antitubercular activities (**Table 2**, entries 1-2). The compounds **5c** and **5d** with the transposition of amide group or *N*-benzylation showed lower activity (**Table 2**, entries 3-4).

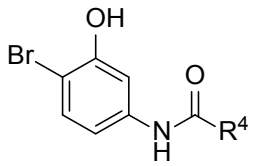
Table 2. Inhibitory activity of compounds 5a-5d against *Mtb* H37Ra.

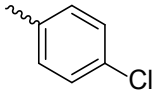
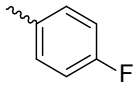
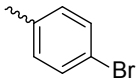
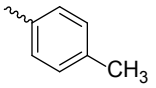
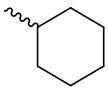
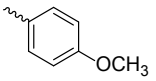
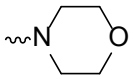
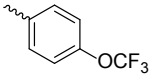
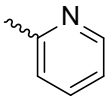
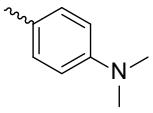
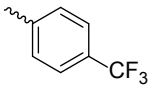
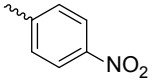
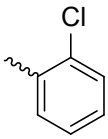
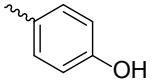
Compound	Structure	MIC (μg/mL)	Compound	Structure	MIC (μg/mL)
5a		>100	**5c**		12.5
5b		50	**5d**		12.5
INH		0.1	**EMB**		2

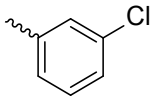
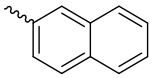
Furthermore, the antitubercular activity of compounds **6a-6u** were determined and the results are summarized in **Table 3**. The replacement of phenyl with methyl, vinyl and heptyl led to the significant loss of the activity (**Table 3**, entries 2-4). However, the cyclohexyl derivative **6d** showed certain antitubercular activity. The morpholine derivative **6e** showed poor activity. The results suggested the strongly hydrophobic group is favorable herein. The lower activities of heteroaryl derivatives **6f-6h** (**Table 3**, entries 6-9) also support this hypothesis. The effect of the substitution on the phenyl group was examined (**Table 3**, entries 10-23). The substitution of chlorine at *ortho*- and *meta*-position (**6i**, **6j**) afforded the comparable activity with compound **3**, but *para*-chloro derivative **6k** showed 4-folds increase in the activity. The *para*-fluoro derivative **6l** showed slightly lower activity. The *para*-bromo and *para*-methyl analogs **6m-6n** exhibited the comparable activity with **6k**. Other *para*-substitutents, such as MeO, CF_3O, and dimethylamino also afforded good activity. The substitution with the electron-withdrawing and hydrophobic CF_3 group (**6r**) showed superior activity. The MIC value is 20-folds lower than compound **3**. The *para*-nitro derivative **6s** exhibited lower activity. The *para*-hydroxyl substitution (**6t**) led to the complete loss of activity. The replacement phenyl with a 2-naphthyl (**6u**) afforded a better activity. The results demonstrated that the hydrophobic property of the *para*-substitutent is favorable for antitubercular activity. On the other hand, the activity is not sensitive to the electronic

property of the substitutent. Both electron-donating groups and electron-withdrawing groups provide good activity.

Table 3. Inhibitory activity of compounds 6a-6u against *Mtb* H37Ra.



Compound	R^4	MIC (μg/mL)	Compound	R^4	MIC (μg/mL)
3	Ph	5	**6k**		1.25
6a	-Me	100	**6l**		2.5
6b	-CH=CH_2	50	**6m**		1.25
6c		100	**6n**		1.25
6d		12.5	**6o**		2.5
6e		50	**6p**		0.5
6f		25	**6q**		2.5
6g		12.5	**6r**		0.25
6h		25	**6s**		6.25
6i		5	**6t**		>100

6j		5	**6u**		1.25
INH		0.1	**EMB**		2

2.3.2 Structure-activity relationship of *m*-amido bromophenol derivatives

The structure-activity relationship (SAR) of *meta*-amido bromophenol derivatives is summarized in **Chart 6**. The *ortho*-bromphenol moiety is a key pharmacophore for antitubercyular activity. The *meta*-amido linker is also important. The transposition of amide group or the replacement by *N*-benzyl group decreases the activity. For the right side moiety, the hydrophobic and π-π interaction are crucial. The phenyl with 4-hydophobic substitutent provides the best activity.

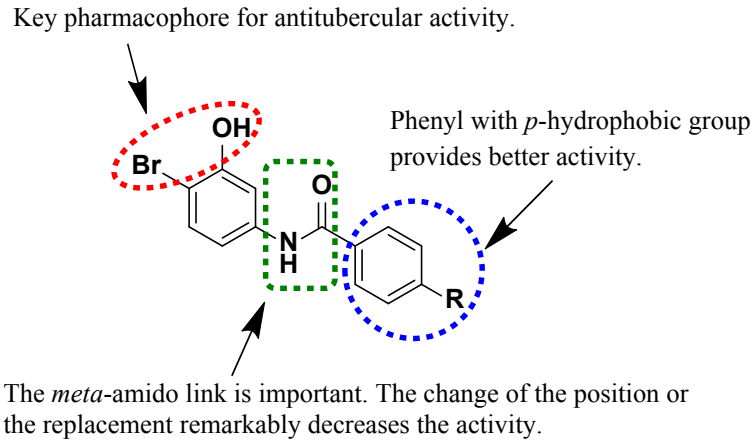


Chart 6. SAR of *m*-amido bromophenol derivatives.

2.3.3 Inhibitory activity against *Mtb* H37Rv and clinically isolated MDR-TB strains

Compounds **6p** and **6r** were selected for further evaluation against *Mtb* H37Rv and six clinically isolated MDR-TB strains. The results are summarized in Table **4**. Both compounds exhibited moderate inhibitory activity against *Mtb* H37Rv and MDR-TB strains (MIC = 4 - 16 μg/mL). The activities are significantly lower than those against H37Ra. The reason is elusive at the present stage.

Table 4. Inhibitory activity against *Mtb* H37Rv and clinically isolated MDR-TB strains.

Strains	MIC (μg/mL)				
	6p	6r	RIF	INH	EMB
H37Rv	8	16	0.03	0.41	2
V4	8	16	<1	>10	<2
K4	8	16	>50	>50	--
K5	8	16	>50	>50	--
K12	4	8	>10	>10	>4
K16	8	16	>10	>10	>4
K18	8	16	>10	>10	>4

RIF: rifampicin

2.3.4 Evaluation of antibacterial activity

Table 5. Antibacterial activity of compounds 6p and 6r.

Bacteria	MIC (μg/mL)		
	6p	6r	Amoxicillin
E. coli	>50	>50	1.56
S. aureus	>50	>50	0.78
E. faecalis	>50	>50	6.25
P. aeruginosa	>50	>50	--[a]

[a] unmeasured

The current treatment of TB requires the combinations of several antitubercular agents and a period of 6 months for drug-susceptible *Mtb*. Therefore, new antitubercular agents are expected to exclusively inhibit *Mtb* without influencing the normal intestinal flora.[12] The antibacterial activities of compounds **6p** and **6r** were examined against Gram-positive

bacteria *S. aureus*, *E. faecalis* and Gram-negative bacteria *E. coli, P. aeruginosa*. Amoxicillin was used as the positive control. The results are summarized in **Table 5**. Compounds **6p** and **6r** did not show inhibitory activity (MIC > 50 μg/mL) against the tested bacteria. This result demonstrated that the inhibitory activity of *meta*-amido bromophenols against *Mtb* is highly exclusive.

2.3.5 Evaluation of cytotoxicity

The cytotoxicities of compounds **6p**, **6r** against cell lines A549 and HepG2 were evaluated and the results are listed in **Table 6**. Low cytotoxicities (IC_{50} > 50 μg/mL) were observed against A549, but moderate cytotoxicities were determined against HepG2. The select indexs (SI = IC_{50}/MIC) are in a range of 47-200 and are acceptable for the further research.

Table 6. Cytotoxicity of compounds 6p, 6r.

Compound	IC_{50} (μg/mL)		MIC (H37Ra)
	A549 cells	HepG2	
6p	>50 (SI >100)	23.4 (SI = 47)	0.5
6r	50 (SI = 200)	23.3 (SI = 93)	0.25

2.3.6 Evaluation of metabolic stability in liver microsome

The stability of compounds **6r** in rat liver microsome was also examined. In comparison with compound **YZ-7** ($T_{1/2}$ = 16.18 min, CLint = 85.7 mL/min/kg), **6r** had a better metabolic stability ($T_{1/2}$ = 30.0 min, CLint = 69.9 mL/min/kg). The result confirmed that the replacement of benzyl group in **YZ-7** improves the metabolic stability.

3. Conclusion

We identified *meta*-amido bromophenols derivatives as a new class of antitubercular agents. Several compounds exhibited potent inhibitory activity against *Mtb* H37Ra. Furthermore, the selected compounds **6p** and **6r** showed moderate inhibitory activity against

Mtb H37Rv and clinically isolated MDR-TB strains. The compounds did not inhibit the representative G+ and G- bacteria. Moderate cytotoxicities were determined and the selective indexes are acceptable. In addition, good metabolic stability in rat liver microsome was observed. Considering the simple structure and readily modular property, *meta*-amido bromophenols hold the good potentials for the further development as new antitubercular agents.

4. Experimental

4.1 Chemistry

^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE 400 spectrometer. Chemical shifts of protons are reported in parts per million downfield from tetramethylsilane. Peaks are labeled as single (s), doublet (d), triplet (t), double doublet (dd), doublet of triplets (dt), multiplet (m). The high-resolution mass spectra were analyzed on a SHIMADZU LCMS-IT-TOF mass spectrometer. All chemicals were purchased from Sigma-Aldrich, Energy and Alfa Aesar chemical companies and were used without further purification.

4.1.1 Typical procedure for the synthesis of compounds 1-3, 4a-4k

A solution of BBr_3 in dichloromethane (1.0 M, 12 mL, 12 mmol) was added slowly to a solution of 4-bromo-3-methoxyaniline (800 mg, 3.96 mmol) in methylene chloride (15 mL) at 0°C. The resulting brown solution was warmed to room temperature and stirred for 24 h. After saturated aqueous $NaHCO_3$ (30 mL) was added at 0°C, the solution was extracted with EtOAc (20 mL × 3). The combined organic layer was dried with anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by flash chromatography over silica gel (petroleum/EtOAc = 2:1) to give 5-amino-2-bromophenol (665 mg, 88%).

To a solution of 5-amino-2-bromophenol (55 mg, 0.29 mmol) and triethylamine (53 μL, 0.38 mmol) in THF (3 mL) was added slowly benzoyl chloride (0.32 mmol) at 0 °C. The reaction mixture was then stirred at room temperature for 30 min. After the reaction was quenched with water (10 mL), the solution was extracted with EtOAc (10 mL × 2). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by column chromatography to afford the product **3** (74 mg,

87%).

***N*-(4-bromo-3-hydroxyphenyl)benzamide (3)**

White solid, m.p. 178.7 - 180.2 °C; **^{1}H-NMR** (400 MHz, DMSO-d_6) δ: 10.29 (s, 1H), 10.25 (s, 1H), 7.98 – 7.87 (m, 2H), 7.66 (d, J = 2.4 Hz, 1H), 7.61 – 7.56 (m, 1H), 7.52 (t, J = 7.3 Hz, 2H), 7.41 (d, J = 8.7 Hz, 1H), 7.12 (dd, J = 8.7, 2.4 Hz, 1H); **^{13}C-NMR** (101 MHz, DMSO-d_6) δ: 166.15, 154.42, 140.04, 135.33, 132.87, 132.11, 128.86, 128.15, 113.11, 108.68, 103.73; **ESI-MS** *m/z*: 291.9944 (Calcd for $C_{13}H_{10}NO_2Br$ $[M+H]^+$: 291.9968) .

***N*-(3-bromo-5-hydroxyphenyl)benzamide (1)**

The compound was synthesized via a similar procedure starting from 3-bromo-5-methoxyaniline. White solid, yield 80%. m.p. 184.3 - 186.2 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.25 (s, 1H), 9.96 (s, 1H), 7.94 – 7.89 (m, 2H), 7.62 – 7.57 (m, 1H), 7.55 – 7.50 (m, 2H), 7.49 (t, J = 1.8 Hz, 1H), 7.33 (t, J = 2.0 Hz, 1H), 6.68 (t, J = 2.0 Hz, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 166.24, 159.08, 141.93, 135.15, 132.23, 128.90, 128.16, 121.91, 113.96, 106.67; **ESI-MS** *m/z*: 291.9952 (Calcd for $C_{13}H_{10}NO_2Br$ $[M+H]^+$: 291.9968) .

***N*-(2-bromo-5-hydroxyphenyl)benzamide (2)**

The compound was synthesized via a similar procedure starting from 2-bromo-5-methoxyaniline. White solid, yield 52%. m.p. 195.5 - 197.2 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 9.86 (d, J = 27.5 Hz, 2H), 7.97 (d, J = 6.9 Hz, 2H), 7.62 – 7.58 (m, 1H), 7.53 (dd, J = 10.8, 3.8 Hz, 2H), 7.45 (dd, J = 8.7, 2.9 Hz, 1H), 7.07 (s, 1H), 6.72 – 6.57 (m, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 165.32, 157.20, 136.99, 134.16, 132.91, 131.97, 128.65, 127.70, 115.26, 115.22, 108.45; **ESI-MS** *m/z*: 291.9922 (Calcd for $C_{13}H_{10}NO_2Br$ $[M+H]^+$: 291.9968) .

***N*-(4-bromophenyl)benzamide (4a)**

The compound was synthesized via a similar procedure starting from 4-bromoaniline. White solid, yield 85%. m.p. 209.9 - 212.0 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.38 (s, 1H), 7.95 (d, J = 7.2 Hz, 2H), 7.77 (d, J = 8.8 Hz, 2H), 7.66 – 7.44 (m, 5H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 165.69, 138.60, 134.72, 131.75, 131.46, 128.45, 127.70, 122.21, 115.34; **ESI-MS** *m/z*: 297.9822 (Calcd for $C_{13}H_{10}NOBr$ $[M+Na]^+$: 297.9838) .

***N*-(4-bromo-3-methoxyphenyl)benzamide (4b)**

The compound was synthesized via a similar procedure starting from 4-bromo-3-methoxyaniline. White solid, yield 89%. m.p. 134.6 - 136.5 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.35 (s, 1H), 7.96 (d, J = 7.3 Hz, 2H), 7.67 (d, J = 10.1 Hz, 1H), 7.63 – 7.48 (m, 4H), 7.40 (dd, J = 8.6, 2.1 Hz, 1H), 3.85 (s, 3H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 166.13, 155.75, 140.57, 135.18, 133.03, 132.20, 128.89, 128.11, 113.98, 105.26, 104.75, 56.45; **ESI-MS** *m/z*: 306.0110 (Calcd for $C_{14}H_{12}NO_2Br$ $[M+H]^+$: 306.0124) .

***N*-(4-chloro-3-hydroxyphenyl)benzamide (4c)**

The compound was synthesized via a similar procedure starting from 5-amino-2-chlorophenol. White solid, yield 83%. m.p. 183.6 - 185.4 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.24 (d, J = 8.7 Hz, 2H), 7.92 (d, J = 7.2 Hz, 2H), 7.66 (d, J = 2.2 Hz, 1H), 7.56 (dt, J = 26.6, 7.2 Hz, 3H), 7.27 (d, J = 8.7 Hz, 1H), 7.17 (dd, J = 8.7, 2.2 Hz, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 166.09, 153.37, 139.39, 135.36, 132.07, 129.92, 128.84, 128.15, 114.59, 112.53, 108.88; **ESI-MS** *m/z*: 248.0462 (Calcd for $C_{13}H_{10}NO_2Cl$ $[M+H]^+$: 248.0473) .

***N*-(4-fluoro-3-hydroxyphenyl)benzamide (4d)**

The compound was synthesized via a similar procedure starting from 5-amino-2-fluorophenol. Pale yellow solid, yield 83%. m.p. 180.1 - 182.3 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.18 (s, 1H), 9.93 (s, 1H), 7.99 – 7.90 (m, 2H), 7.63 – 7.56 (m, 2H), 7.53 (t, J = 7.3 Hz, 2H), 7.13 (m, 2H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 165.48, 147.45 (d, J_{CF} = 237.4 Hz), 144.57 (d, J_{CF} = 13.1 Hz), 135.67(d, J_{CF} = 2.0 Hz), 135.03, 131.57, 128.42, 127.68, 115.70 (d, J_{CF} = 20.2 Hz), 111.19 (d, J_{CF} = 6.0 Hz), 110.09; **ESI-MS** *m/z*: 232.0748 (Calcd for $C_{13}H_{10}NO_2F$ $[M+H]^+$: 232.0768) .

***N*-(3-hydroxy-4-methylphenyl)benzamide (4f)**

The compound was synthesized via a similar procedure starting from 5-amino-2-methylphenol. White solid, yield 81%. m.p. 213.4 - 216.0 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.07 (s, 1H), 9.36 (s, 1H), 7.96 – 7.89 (m, 2H), 7.60 – 7.48 (m, 3H), 7.43 (d, J = 1.8 Hz, 1H), 7.05 (dd, J = 8.1, 1.9 Hz, 1H), 6.99 (d, J = 8.2 Hz, 1H), 2.09 (s, 3H);**^{13}C NMR** (101 MHz, DMSO-d_6) δ 165.32, 155.22, 137.78, 135.26, 131.39, 130.18, 128.35, 127.66,

119.24, 111.08, 107.17, 15.64; **ESI-MS** *m/z*: 228.1086 (Calcd for $C_{14}H_{13}NO_2$ $[M+H]^+$: 228.1019) .

***N*-(3-hydroxy-4-methoxyphenyl)benzamide (4g)**

The compound was synthesized via a similar procedure starting from 5-amino-2-methoxyphenol. White solid, yield 80%. m.p. 176.5 - 178.5 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.02 (s, 1H), 9.07 (s, 1H), 7.99 – 7.87 (m, 1H), 7.60 – 7.47 (m, 1H), 7.35 (d, J = 2.4 Hz, 1H), 7.14 (dd, J = 8.7, 2.4 Hz, 1H), 6.88 (d, J = 8.7 Hz, 1H), 3.74 (s, 1H);**^{13}C NMR** (101 MHz, DMSO-d_6) δ 165.09, 146.28, 144.14, 135.23, 132.73, 131.35, 128.36, 127.59, 112.26, 111.23, 108.92, 55.88; **ESI-MS** *m/z*: 244.0927 (Calcd for $C_{14}H_{13}NO_3$ $[M+H]^+$: 244.0968) .

methyl 4-benzamido-2-hydroxybenzoate (4h)

The compound was synthesized via a similar procedure starting from methyl 4-amino-2-hydroxybenzoate. White solid, yield 77%. m.p. 170.0 - 171.8 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.65 (s, 1H), 10.51 (s, 1H), 7.94 (dd, J = 7.4, 5.9 Hz, 2H), 7.77 (d, J = 8.8 Hz, 1H), 7.63 – 7.57 (m, 2H), 7.57 – 7.51 (m, 2H), 7.37 (dd, J = 8.8, 2.0 Hz, 1H), 3.88 (s, 3H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 169.09, 166.23, 161.11, 145.76, 134.57, 131.99, 130.63, 128.49, 127.87, 111.41, 107.77, 107.14, 52.29; **ESI-MS** *m/z*: 294.0727 (Calcd for $C_{15}H_{13}NO_4$ $[M+Na]^+$: 294.0737) .

Synthesis of 4-benzamido-2-hydroxybenzoic acid (4i)

To a solution of compound **4h** (90 mg, 0.33 mmol) in THF (2 mL) was added aqueous NaOH (1.5 M, 8 mL). The mixture was stirred at 50 °C for 2 h. After being cooled to room temperature, the solution was acidified with 2M HCl to pH = 3-4. The precipitation was collected to give the compound **4h** (62 mg, 73%). m.p. 263.1 - 264.7 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.48 (s, 1H), 8.05 – 7.89 (m, 2H), 7.76 (d, J = 8.7 Hz, 1H), 7.67 – 7.44 (m, 4H), 7.34 (dd, J = 8.7, 2.0 Hz, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 172.11, 166.64, 162.44, 146.08, 135.06, 132.40, 131.32, 128.93, 128.30, 111.59, 108.51, 107.38; **ESI-MS** *m/z*: 280.0563 (Calcd for $C_{14}H_{11}NO_4$ $[M+Na]^+$: 280.0580) .

***N*-(4-acetyl-3-hydroxyphenyl)benzamide (4j)**

The compound was synthesized via a similar procedure starting from 1-(4-amino-2-

hydroxyphenyl)ethan-1-one. Pale yellow solid, yield 82%. m.p. 204.9 - 206.6 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 12.34 (s, 1H), 10.54 (s, 1H), 7.95 (d, J = 8.0 Hz, 2H), 7.90 (d, J = 8.8 Hz, 1H), 7.65 – 7.59 (m, 1H), 7.59 – 7.50 (m, 3H), 7.37 (d, J = 8.8 Hz, 1H), 2.60 (s, 3H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 203.27, 166.31, 162.34, 146.29, 134.50, 132.42, 132.07, 128.52, 127.90, 115.83, 111.06, 106.96, 26.96; **ESI-MS** *m/z*: 278.0792 (Calcd for $C_{15}H_{13}NO_3$ $[M+Na]^+$: 278.0788) .

***N*-(4-cyano-3-hydroxyphenyl)benzamide (4k)**

The compound was synthesized via a similar procedure starting from 4-amino-2-methoxy benzonitrile. Yellow brown solid, yield 67%. m.p. 151.3 - 153.6 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.19 – 8.10 (m, 2H), 7.78 (t, J = 7.4 Hz, 1H), 7.63 (t, J = 7.7 Hz, 2H), 7.48 (d, J = 9.1 Hz, 1H), 6.60 – 6.54 (m, 2H), 6.45 (s, 2H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 164.22, 155.14, 154.15, 134.99, 134.50, 130.38, 129.65, 128.57, 117.47, 111.90, 107.27, 90.59; **ESI-MS** *m/z*: 261.0582 (Calcd for $C_{14}H_{10}N_2O_2$ $[M+Na]^+$: 261.0634) .

4.1.2 Synthesis of compounds 5a-5c

To a solution of benzoic acid (37 mg, 0.30 mmol) and HATU (138 mg, 0.36 mmol) in DMF (4 mL) was added DIPEA (63 μL, 0.36 mmol) and 4-amino-2-bromophenol (56 mg, 0.30 mmol) separately. The reaction mixture was stirred at room temperature for 4 h. After water (20 mL) was added, the mixture was extracted with EtOAc (10 mL × 2). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by flash chromatography over silica gel (petroleum/EtOAc = 10:1 to 5:1) to give the compound **5a.**

***N*-(3-bromo-4-hydroxyphenyl)benzamide (5a)**

White solid, yield 90%. m.p. 198.8 - 200.2 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.16 (s, 1H), 10.11 (s, 1H), 7.98 (d, J = 2.5 Hz, 1H), 7.92 (dd, J = 5.2, 3.3 Hz, 2H), 7.60 – 7.47 (m, 4H), 6.93 (d, J = 8.8 Hz, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 165.24, 150.44, 134.83, 131.86, 131.61, 128.48, 127.61, 124.95, 121.27, 116.03, 108.58; **ESI-MS** *m/z*: 291.9941 (Calcd for $C_{13}H_{10}NO_2Br$ $[M+H]^+$: 291.9968) .

***N*-(3-bromo-2-hydroxyphenyl)benzamide (5b)**

The compound was synthesized via a similar procedure starting from

2-amino-6-bromophenol. White solid, yield 71%. m.p. 127.4 - 128.6 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.08 (s, 1H), 9.76 (s, 1H), 8.01 (d, J = 7.3 Hz, 2H), 7.62 (t, J = 7.3 Hz, 1H), 7.55 (t, J = 7.5 Hz, 2H), 7.44 (t, J = 6.7 Hz, 2H), 6.84 (t, J = 8.0 Hz, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 166.81, 148.04, 134.23, 132.43, 130.44, 128.91, 128.39, 127.91, 125.78, 121.05, 112.13; **ESI-MS** *m/z*: 291.9932 (Calcd for $C_{13}H_{10}NO_2Br$ $[M+H]^+$: 291.9968) .

4-bromo-3-hydroxy-*N*-phenylbenzamide (5c)

The compound was synthesized via a similar procedure starting from 4-bromo-3-hydroxybenzoic acid and aniline. White solid, yield 77%. m.p. 188.5 - 190.7 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.64 (s, 1H), 10.25 (s, 1H), 7.74 (d, J = 7.6 Hz, 2H), 7.64 (d, J = 8.2 Hz, 1H), 7.47 (d, J = 2.0 Hz, 1H), 7.38 – 7.30 (m, 3H), 7.10 (t, J = 7.4 Hz, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 164.85, 154.15, 139.05, 135.66, 132.81, 128.67, 123.79, 120.39, 119.36, 115.64, 113.05; **ESI-MS** *m/z*: 291.9819 (Calcd for $C_{13}H_{10}NO_2Br$ $[M+H]^+$: 291.9968) .

Synthesis of 5-(benzylamino)-2-bromophenol (5d)

To a solution of 4-bromo-3-methoxyaniline (80 mg, 0.40 mmol) and K_2CO_3 (110 mg, 0.80 mmol) in DMF (5 mL) was added (bromomethyl)benzene (75 mg, 0.44mmol) at room temperature. The mixture was stirred at 55 °C for 24 h. After water (20 mL) was added, the mixture was extracted with EtOAc (10mL × 2). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified by flash chromatography over silica gel (petroleum/EtOAc = 10:1 to 5:1) to give *N*-benzyl-4-bromo-3-methoxyaniline (84 mg, 72%).

The compound **5d** was synthesized form *N*-benzyl-4-bromo-3-methoxyaniline via a similar procedure 4.1.1. White solid, yield 38%. m.p. 77.2 - 78.3 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 9.65 (s, 1H), 7.38 – 7.27 (m, 4H), 7.25 – 7.17 (m, 1H), 7.04 (d, J = 8.6 Hz, 1H), 6.36 (t, J = 5.9 Hz, 1H), 6.19 (d, J = 2.6 Hz, 1H), 6.02 (dd, J = 8.7, 2.6 Hz, 1H), 4.19 (d, J = 5.9 Hz, 2H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 154.70, 149.67, 140.37, 132.88, 128.76, 127.54, 127.13, 106.19, 100.62, 95.37, 46.91; **ESI-MS** *m/z*: 278.0149 (Calcd for $C_{13}H_{12}NOBr$ $[M+H]^+$: 278.0175) .

4.1.3 Synthesis of compounds 6a-6u

***N*-(4-bromo-3-hydroxyphenyl)acetamide (6a)**

The compound was synthesized via a similar procedure 4.1.1 using acetyl chloride. White solid, yield 54%. m.p. 215.7 - 216.5 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.22 (s, 1H), 9.93 (s, 1H), 7.47 (d, J = 2.4 Hz, 1H), 7.33 (d, J = 8.6 Hz, 1H), 6.94 – 6.79 (m, 1H), 2.01 (s, 3H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 168.41, 154.03, 139.74, 132.48, 111.25, 106.88, 102.39, 24.11; **ESI-MS** *m/z*: 229.9885 (Calcd for $C_8H_8NO_2Br$ $[M+H]^+$: 229.9811) .

***N*-(4-bromo-3-hydroxyphenyl)acrylamide (6b)**

The compound was synthesized via a similar procedure 4.1.1 using acryloyl chloride. Brown solid, yield 63%. m.p. 183.6 - 186.0 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.30 (s, 1H), 10.16 (s, 1H), 7.54 (d, J = 2.3 Hz, 1H), 7.38 (d, J = 8.6 Hz, 1H), 7.00 (dd, J = 8.7, 2.3 Hz, 1H), 6.43 (dd, J = 17.0, 10.1 Hz, 1H), 6.25 (dd, J = 17.0, 2.0 Hz, 1H), 5.76 (dd, J = 10.1, 2.0 Hz, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 163.12, 154.06, 139.37, 132.56, 131.76, 127.01, 111.64, 107.25, 103.04; **ESI-MS** *m/z*: 241.9805 (Calcd for $C_9H_8NO_2Br$ $[M+H]^+$: 241.9811) .

***N*-(4-bromo-3-hydroxyphenyl)nonanamide (6c)**

The compound was synthesized via a similar procedure 4.1.2 using octanoic acid. White solid, yield 76%. m.p. 181.8 - 183.3 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.18 (s, 1H), 9.84 (s, 1H), 7.47 (d, J = 2.3 Hz, 1H), 7.32 (d, J = 8.6 Hz, 1H), 6.89 (dd, J = 8.7, 2.4 Hz, 1H), 2.26 (t, J = 7.4 Hz, 2H), 1.61 – 1.49 (m, 2H), 1.26 (dd, J = 5.7, 2.5 Hz, 8H), 0.85 (d, J = 7.0 Hz, 3H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 171.36, 154.00, 139.74, 132.43, 111.33, 106.97, 102.31, 36.47, 31.19, 28.65, 28.48, 25.10, 22.09, 13.97; **ESI-MS** *m/z*: 314.0734 (Calcd for $C_{14}H_{20}NO_2Br$ $[M+H]^+$: 314.0750) .

***N*-(4-bromo-3-hydroxyphenyl)cyclohexanecarboxamide (6d)**

The compound was synthesized via a similar procedure 4.1.1 using cyclohexanecarbonyl chloride. White solid, yield 80%. m.p. 194.9 - 197.2 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.19 (s, 1H), 9.79 (s, 1H), 7.46 (d, J = 2.3 Hz, 1H), 7.31 (d, J = 8.7 Hz, 1H), 6.90 (dd, J = 8.7, 2.4 Hz, 1H), 2.33 – 2.24 (m, 1H), 1.82 – 1.69 (m, 4H), 1.43 – 1.32 (m, 2H), 1.31 – 1.09 (m, 4H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 174.44, 153.98, 139.90, 132.42, 111.49, 107.12, 102.33, 44.91, 29.13, 25.42, 25.25; **ESI-MS** *m/z*: 298.0391 (Calcd for $C_{13}H_{16}NO_2Br$ $[M+H]^+$: 298.0437) .

***N*-(4-bromo-3-hydroxyphenyl)morpholine-4-carboxamide (6e)**

To a solution of 5-amino-2-bromophenol (55 mg, 0.29 mmol) and triethylamine (53 μL, 0.38 mmol) in THF (3 mL) was added slowly morpholine-4-carbonyl chloride (0.32 mmol) at 0 °C. The mixture was stirred at 45 °C for 12 h. The reaction was quenched with water (10 mL) and the mixture was extracted with EtOAc (10 mL × 2). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by column chromatography to afford the product **6e** as a white solid (60 mg, 69%). m.p. 193.0 - 194.8 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.08 (s, 1H), 8.54 (s, 1H), 7.31 (d, J = 2.4 Hz, 1H), 7.27 (d, J = 8.7 Hz, 1H), 6.82 (dd, J = 8.7, 2.4 Hz, 1H), 3.62 – 3.56 (m, 4H), 3.42 – 3.38 (m, 4H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 155.36, 154.22, 141.39, 132.48, 112.31, 107.88, 101.65, 66.44, 44.62; **ESI-MS** *m/z*: 301.0182 (Calcd for $C_{11}H_{13}N_2O_3Br$ $[M+H]^+$: 301.0143) .

***N*-(4-bromo-3-hydroxyphenyl)picolinamide (6f)**

The compound was synthesized via a similar procedure 4.1.2 using picolinic acid. White solid, yield 80%. m.p. 202.5 - 204.4 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.61 (s, 1H), 10.33 (s, 1H), 8.79 – 8.69 (m, 1H), 8.14 (d, J = 7.8 Hz, 1H), 8.10 – 8.02 (m, 1H), 7.80 (d, J = 2.4 Hz, 1H), 7.71 – 7.63 (m, 1H), 7.42 (d, J = 8.7 Hz, 1H), 7.21 (dd, J = 8.7, 2.4 Hz, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 162.57, 154.03, 149.84, 148.45, 138.74, 138.16, 132.48, 126.99, 122.46, 112.79, 108.18, 103.67; **ESI-MS** *m/z*: 292.9910 (Calcd for $C_{12}H_9N_2O_2Br$ $[M+H]^+$: 292.9920) .

***N*-(4-bromo-3-hydroxyphenyl)thiophene-2-carboxamide (6g)**

The compound was synthesized via a similar procedure 4.1.1 using thiophene-2-carbonyl chloride. White solid, yield 66%. m.p. 222.7 - 224.5 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.31 (s, 1H), 10.21 (s, 1H), 8.01 (d, J = 3.1 Hz, 1H), 7.84 (d, J = 4.8 Hz, 1H), 7.57 (d, J = 2.2 Hz, 1H), 7.41 (d, J = 8.7 Hz, 1H), 7.24 – 7.16 (m, 1H), 7.09 (dd, J = 8.7, 2.2 Hz, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 159.88, 154.01, 139.91, 139.16, 132.50, 132.04, 129.29, 128.09, 112.60, 108.20, 103.40; **ESI-MS** *m/z*: 297.9494 (Calcd for $C_{11}H_8NO_2SBr$ $[M+H]^+$: 297.9532) .

***N*-(4-bromo-3-hydroxyphenyl)furan-2-carboxamide (6h)**

The compound was synthesized via a similar procedure 4.1.1 using furan-2-carbonyl chloride. White solid, yield 53%. m.p. 207.2 - 209.7 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.32 (s, 1H), 10.19 (s, 1H), 7.93 (dd, J = 1.6, 0.7 Hz, 1H), 7.61 (d, J = 2.4 Hz, 1H), 7.43 – 7.31 (m, 2H), 7.09 (dd, J = 8.7, 2.4 Hz, 1H), 6.70 (dd, J = 3.5, 1.7 Hz, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 156.22, 154.00, 147.38, 145.90, 139.00, 132.49, 114.96, 112.66, 112.22, 108.21, 103.43; **ESI-MS** *m/z*: 281.9753 (Calcd for $C_{11}H_8NO_3Br$ $[M+H]^+$: 281.9760) .

***N*-(4-bromo-3-hydroxyphenyl)-2-chlorobenzamide (6i)**

The compound was synthesized via a similar procedure 4.1.1 using 2-chlorobenzoyl chloride. White solid, yield 79%. m.p. 204.4 - 206.7 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.51 (s, 1H), 10.33 (s, 1H), 7.61 (d, J = 2.3 Hz, 1H), 7.56 (dd, J = 7.8, 2.5 Hz, 2H), 7.50 (m, J = 7.7, 1.7 Hz, 1H), 7.44 (m, J = 7.3, 1.1 Hz, 1H), 7.40 (d, J = 8.6 Hz, 1H), 7.01 (dd, J = 8.6, 2.3 Hz, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 165.02, 154.16, 139.30, 136.90, 132.66, 131.20, 129.91, 129.69, 128.95, 127.33, 111.93, 107.52, 103.43; **ESI-MS** *m/z*: 325.9586 (Calcd for $C_{13}H_9NO_2ClBr$ $[M+H]^+$: 325.9578) .

***N*-(4-bromo-3-hydroxyphenyl)-3-chlorobenzamide (6j)**

The compound was synthesized via a similar procedure 4.1.1 using 3-chlorobenzoyl chloride. White solid, yield 74%. m.p. 184.2 - 186.1 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.35 (d, J = 3.6 Hz, 2H), 7.98 (t, J = 1.7 Hz, 1H), 7.89 (d, J = 7.8 Hz, 1H), 7.70 – 7.61 (m, 2H), 7.56 (t, J = 7.9 Hz, 1H), 7.42 (d, J = 8.7 Hz, 1H), 7.11 (dd, J = 8.7, 2.4 Hz, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 164.17, 154.03, 139.33, 136.86, 133.22, 132.50, 131.49, 130.44, 127.48, 126.58, 112.67, 108.24, 103.56; **ESI-MS** *m/z*: 325.9562 (Calcd for $C_{13}H_9NO_2ClBr$ $[M+H]^+$: 325.9578) .

***N*-(4-bromo-3-hydroxyphenyl)-4-chlorobenzamide (6k)**

The compound was synthesized via the procedure similar to compound **6e**. White solid, yield 52%. m.p. 195.0 - 197.3 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.32 (s, 1H), 10.19 (s, 1H), 7.93 (dd, J = 1.6, 0.7 Hz, 1H), 7.61 (d, J = 2.4 Hz, 1H), 7.43 – 7.31 (m, 2H), 7.09 (dd, J = 8.7, 2.4 Hz, 1H), 6.70 (dd, J = 3.5, 1.7 Hz, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 156.22, 154.00, 147.38, 145.90, 139.00, 132.49, 114.96, 112.66, 112.22, 108.21, 103.43; **ESI-MS**

m/z: 325.9565 (Calcd for $C_{13}H_9NO_2ClBr$ $[M+H]^+$: 325.9578) .

***N*-(4-bromo-3-hydroxyphenyl)-4-fluorobenzamide (6l)**

The compound was synthesized via the procedure similar to compound **3**. White solid, yield 83%. m.p. 181.9 - 183.4 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.29 (d, J = 18.2 Hz, 2H), 8.11 – 7.95 (m, 2H), 7.64 (s, 1H), 7.38 (dt, J = 16.4, 8.7 Hz, 3H), 7.11 (d, J = 8.7 Hz, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 164.54, 164.12 (d, J_{CF} = 249.5 Hz), 154.01, 139.52, 132.46, 131.32, 130.50 (d, J_{CF} = 9.1 Hz), 115.36 (d, J_{CF} = 22.2 Hz), 112.65, 108.22, 103.35; **ESI-MS** *m/z*: 309.9838 (Calcd for $C_{13}H_9NO_2FBr$ $[M+H]^+$: 309.9873) .

4-bromo-*N*-(4-bromo-3-hydroxyphenyl)benzamide (6m)

The compound was synthesized via a similar procedure 4.1.1 using 4-bromobenzoyl chloride. White solid, yield 81%. m.p. 208.5 - 210.1 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.31 (s, 2H), 7.93 – 7.81 (m, 2H), 7.80 – 7.68 (m, 2H), 7.63 (d, J = 2.4 Hz, 1H), 7.41 (d, J = 8.7 Hz, 1H), 7.10 (dd, J = 8.7, 2.4 Hz, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 164.66, 154.02, 139.41, 133.94, 132.48, 131.43, 129.88, 125.44, 112.65, 108.23, 103.47; **ESI-MS** *m/z*: 369.9088 (Calcd for $C_{13}H_9NO_2Br_2$ $[M+H]^+$: 369.9073) .

***N*-(4-bromo-3-hydroxyphenyl)-4-methylbenzamide (6n)**

The compound was synthesized via a similar procedure 4.1.1 using 4-methylbenzoyl chloride. White solid, yield 62%. m.p. 195.4 - 197.2 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.30 (s, 1H), 10.17 (s, 1H), 7.85 (d, J = 8.2 Hz, 2H), 7.66 (d, J = 2.4 Hz, 1H), 7.40 (d, J = 8.7 Hz, 1H), 7.33 (d, J = 8.0 Hz, 2H), 7.11 (dd, J = 8.7, 2.4 Hz, 1H), 2.38 (s, 3H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 165.47, 153.98, 141.70, 139.71, 132.40, 132.01, 128.94, 127.78, 112.63, 108.20, 103.14, 21.07; **ESI-MS** *m/z*: 306.0111 (Calcd for $C_{14}H_{12}NO_2Br$ $[M+H]^+$: 306.0124) .

***N*-(4-bromo-3-hydroxyphenyl)-4-methoxybenzamide (6o)**

The compound was synthesized via a similar procedure 4.1.1 using 4-methoxybenzoyl chloride. White solid, yield 81%. m.p. 207.6 - 208.9 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.26 (s, 1H), 10.09 (s, 1H), 7.93 (d, J = 8.8 Hz, 2H), 7.65 (d, J = 2.3 Hz, 1H), 7.39 (d, J = 8.7 Hz, 1H), 7.11 (dd, J = 8.7, 2.3 Hz, 1H), 7.06 (t, J = 5.8 Hz, 2H), 3.83 (s, 3H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 164.96, 161.95, 153.93, 139.80, 132.34, 129.67, 126.88, 113.60, 112.60, 108.16, 102.96, 55.46; **ESI-MS** *m/z*: 322.0041 (Calcd for $C_{14}H_{12}NO_3Br$ $[M+H]^+$:

322.0073) .

***N*-(4-bromo-3-hydroxyphenyl)-4-(trifluoromethoxy)benzamide (6p)**

The compound was synthesized via a similar procedure 4.1.1 using 4-(trifluoromethoxy)benzoyl chloride. White solid, yield 80%. m.p. 189.1 - 190.1 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.35 (d, J = 10.6 Hz, 2H), 8.13 – 8.01 (m, 2H), 7.64 (d, J = 2.4 Hz, 1H), 7.52 (d, J = 8.0 Hz, 2H), 7.42 (d, J = 8.7 Hz, 1H), 7.11 (dd, J = 8.7, 2.4 Hz, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 164.49, 154.04, 139.41, 134.07, 132.51, 130.16, 120.75, 112.63, 108.21, 103.50, **^{13}C NMR** (101 MHz, DMSO-d_6) δ 164.49, 154.04, 150.50, 139.41, 134.07, 132.51, 130.16, 120.75, 112.63, 108.21, 103.50; **ESI-MS** *m/z*: 375.9757 (Calcd for $C_{14}H_9NO_3F_3Br$ $[M+H]^+$: 375.9791) .

***N*-(4-bromo-3-hydroxyphenyl)-4-(dimethylamino)benzamide (6q)**

The compound was synthesized via a similar procedure 4.1.1 using 4-(dimethylamino)benzoyl chloride. White solid, yield 71%. m.p. 238.3 - 240.7 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.22 (s, 1H), 9.87 (s, 1H), 7.85 (d, J = 8.9 Hz, 2H), 7.67 (d, J = 2.3 Hz, 1H), 7.37 (d, J = 8.7 Hz, 1H), 7.11 (dd, J = 8.7, 2.3 Hz, 1H), 6.74 (d, J = 9.0 Hz, 2H), 2.99 (s, 6H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 165.28, 153.89, 152.44, 140.19, 132.25, 130.96, 129.24, 120.93, 112.55, 110.76, 108.10, 102.52; **ESI-MS** *m/z*: 355.0345 (Calcd for $C_{15}H_{15}N_2O_2Br$ $[M+H]^+$: 335.0390) .

***N*-(4-bromo-3-hydroxyphenyl)-4-(trifluoromethyl)benzamide (6r)**

The compound was synthesized via a similar procedure 4.1.1 using 4-(trifluoromethyl)benzoyl chloride. White solid, yield 73%. m.p. 205.1 - 206.6 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.47 (s, 1H), 10.35 (s, 1H), 8.12 (d, J = 8.1 Hz, 2H), 7.90 (d, J = 8.3 Hz, 2H), 7.65 (d, J = 2.4 Hz, 1H), 7.43 (d, J = 8.7 Hz, 1H), 7.13 (dd, J = 8.7, 2.4 Hz, 1H); **^{13}C NMR** (101 MHz, DMSO) δ 164.95, 154.50, 139.70, 139.16, 132.96, 131.85 (d, J_{CF}= 32.3Hz), 129.10, 125.83 (d, J_{CF}= 3.0 Hz), 113.12, 108.72, 104.13; **ESI-MS** *m/z*: 359.9803 (Calcd for $C_{14}H_9NO_2F_3Br$ $[M+H]^+$: 359.9842) .

***N*-(4-bromo-3-hydroxyphenyl)-4-nitrobenzamide (6s)**

The compound was synthesized via a similar procedure 4.1.1 using 4-nitrobenzoyl chloride. Yellow solid, yield 48%. m.p. 237.0 - 239.1 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.58 (s,

1H), 10.38 (s, 1H), 8.37 (d, *J* = 8.8 Hz, 2H), 8.16 (d, *J* = 8.8 Hz, 2H), 7.65 (d, *J* = 2.2 Hz, 1H), 7.45 (d, *J* = 8.7 Hz, 1H), 7.14 (dd, *J* = 8.7, 2.3 Hz, 1H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 164.45, 154.52, 149.62, 141.00, 139.58, 133.02, 129.74, 124.01, 113.11, 108.69, 104.28. **ESI-MS** *m/z*: 336.9884 (Calcd for $C_{13}H_9N_2O_4Br$ $[M+H]^+$: 336.9818) .

***N*-(4-bromo-3-hydroxyphenyl)-4-hydroxybenzamide (6t)**

The compound was synthesized by compound **6o** as the material and then the procedure similar to 4.1.1. White solid, yield 32%. m.p. 215.2 - 216.7 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.19 (d, *J* = 47.7 Hz, 2H), 9.99 (s, 1H), 7.82 (d, *J* = 8.3 Hz, 2H), 7.64 (s, 1H), 7.38 (d, *J* = 8.7 Hz, 1H), 7.09 (d, *J* = 8.7 Hz, 1H), 6.85 (d, *J* = 8.3 Hz, 2H); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 165.62, 161.06, 154.36, 140.37, 132.76, 130.25, 125.77, 115.36, 113.00, 108.56, 103.25; **ESI-MS** *m/z*: 307.9882 (Calcd for $C_{13}H_{10}NO_3Br$ $[M+H]^+$: 307.9917) .

Synthesis of *N*-(4-bromo-3-hydroxyphenyl)-2-naphthamide (6u)

The compound was synthesized via a similar procedure 4.1.1 using 2-naphthoyl chloride. White solid, yield 73%. m.p. 207.6 - 209.9 °C; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.43 (s, 1H), 10.32 (s, 1H), 8.56 (s, 1H), 8.11 – 7.97 (m, 4H), 7.72 (d, *J* = 2.4 Hz, 1H), 7.68 – 7.58 (m, 2H), 7.43 (d, *J* = 8.7 Hz, 1H), 7.17 (dd, *J* = 8.7, 2.4 Hz, 1H);**^{13}C NMR** (101 MHz, DMSO-d_6) δ 166.17, 154.48, 140.14, 134.75, 132.92, 132.67, 132.51, 129.44, 128.49, 128.34, 128.16, 127.35, 124.96, 113.11, 108.67, 103.76; **ESI-MS** *m/z*: 342.0097 (Calcd for $C_{17}H_{12}NO_2Br$ $[M+H]^+$: 342.0124) .

4.2 Biological assays

4.2.1 Evaluation of inhibitory activity against Mtb H37Ra *in vitro*

Selectable marker-free autoluminescent *Mtb* H37Ra (UAlRa)[13] broth culture in Middlebook 7H9 medium plus 0.05% Tween80 and 10% oleic acid albumin dextrose catalase (OADC) (7H9-Tw-OADC). Upon reaching an OD_{600} of 0.5-0.8, relative light unit (RLU) value was determined by luminometer. When the RLU of 200 μL broth culture reached to over 1 million, the test compounds were prepare with two-fold serial dilutions in a range of final concentration from 100 μg/mL to 0.0625 μg/mL per 200 μL UAlRa of broth culture (RLU were diluted to 2000-5000 per 200 μL). On the other hand, DMSO final concentration

of 2% was used as negative control while the compounds (2-fold decreasing concentration from 100 μg/mL to 0.0625 μg/mL) as well as isoniazid (0.1 μg/mL) and ethambutol (2 μg/mL) were used as positive controls. The RLU values were determined once daily until the 6th day. Data was analysed as MIC_{90} values which is basically the lowest drug concentration that achieved a RLU_{drug}/RLU_{DMSO} of less than 10% after incubation.

4.2.2 Evaluation of inhibitory activity against Mtb H37Rv and MDR-TB strains

The MICs of the test compounds were determined by the microplate alamar blue assay (MABA)[14] against *Mtb* H37Rv and clinically isolated MDR-TB strains isolated from the Guangzhou Chest Hospital. *Mtb* H37Rv and MDR-TB strains were cultured in 7H9-Tw-OADC. The test compounds were prepared with two-fold serial dilutions in a range of final concentration from 0.25 μg/mL to 128 μg/mL, two-fold dilutions of compounds were prepared in 7H9-OADC medium in a 100 μL volume in 96-well microplates. INH, RIF and EMB were used as the positive controls. Bacterial suspension of inoculum of 2×10^5 CFU/mL was added 100 μL per well into the microplates, and the plates incubated 6 days at 37℃. On the 7th day, 12.5 μL of 20% Tween 80 and 20 μL of alamar Blue (Bio-Rad) were added to the test plate, and incubated another 24h at 37℃, and then observed the color change. If a blue color in the well was deemed to no growth, and the blue color change to pink or violet was interpreted as growth. The MIC was defined as the lowest drug concentration which prevented a color change from blue to pink.

4.2.3 Determination of the antibacterial activity

To evaluated compounds exclusive of against *Mtb*, we selected **6p** and **6r** for determination of antibacterial activity against Gram-positive and Gram-negative bacteria. Initially, the test compounds were dissolved in DMSO to prepare the stock solutions (10 mg/mL), and then prepared a range of concentrations from 50 to 0.4 μg/mL by means of the standard two fold serial dilution method in 96-well microtest plates. Amoxicillin was used as the positive controls. The bacterial suspension was adjusted with sterile saline to a concentration of 1×10^5 CFU. 100 μL the bacterial suspension per well was added into the plates and incubated at 37℃ for 24h. The MIC of compound was defined as the lowest

concentration (the highest dilution) that completely inhibited the growth of bacteria after incubation at 37 ℃ for 18-24 h.[15]

4.2.4 Determination of the cytotoxicity

A549 cell and HepG2 cell were used for determination of the cytotoxicity of compounds. This two cell lines both were culture in RPMI 1640 Medium (with 5% fetal bovine serum (FBS) and 1% penicillin-streptomycin), and incubated at 37℃ in 5% CO_2 until they reach log phage. The test compounds were diluted with RPMI 1640 Medium by 2-fold dilution and the concentrations from 100 to 3.125 μg/mL. The cells suspension (1×10^5 cell/mL) of 100 μL were seeded in 96-well plates and then incubated at 37℃ in 5% CO_2 overnight.[16] 100 μL different concentrations of the test compound were added to the plate by the form of replace the cell medium and 100 μL RPMI 1640 Medium was used as the 0% inhibitor control. Each experiment was repeated three times. After being incubated for 48 h, 10 μL/well CCK8 was added in the plates, and another 1-2 hour incubation, the plates were read in the Envision MultilabelReader at 450 nm. Cell survival rate (activity%) was calculated as follow: activity%=100*($OD_{compound}$-OD_{blank})/ ($OD_{control}$-OD_{blank}). The cytotoxicities were reported as IC_{50} values, which were calculated by GraphPad Prism Software version 5.23.

4.2.5 Determination of the liver microsome stability

The rat liver microsome was purchased from Xenotech company. The final incubation volume was set at 45 μL, contained NADPH (2 mM), microsomes (0.5 mg/mL) and the tested compound (1 μM). The incubation times were 0, 5, 15, 30, 45 and 60 min. Ice-cold acetonitrile was added to terminate the reaction and then centrifuged at 4 ℃ for 15 min at 4000 rpm. The concentration of the tested compound in supernatant was determined by LC-MS.[17]

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Conflict of interest

The authors declare no conflict of interest.

Supplementary Materials

The online version of this article contains supplementary materials.

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