Bioorganic & Medicinal Chemistry 24 (2016) 2549–2558

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and biological evaluation of benzyloxyphenyl-methylaminophenol derivatives as STAT3 signaling pathway inhibitors

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ARTICLE INFO

Article history:
Received 12 January 2016
Revised 20 March 2016
Accepted 11 April 2016
Available online 12 April 2016

Keywords:
STAT3 signaling pathway
Inhibitors
Benzyloxyphenyl-methylaminophenol derivatives
Structure–activity relationship

ABSTRACT

STAT3 signaling pathway has been validated as a vital therapeutic target for cancer therapy. Based on the novel STAT3 inhibitor of a benzyloxyphenyl-methylaminophenol scaffold hit (**1**) discovered through virtual screening, a series of analogues had been designed and synthesized for more potent inhibitors. The preliminary SAR had been discussed and the unique binding site in SH2 domain was predicted by molecular docking. Among them, compounds **4a** and **4b** exhibited superior activities than hit compound (**1**) against IL-6/STAT3 signaling pathway with IC_{50} values as low as 7.71 μM and 1.38 μM, respectively. Compound **4a** also displayed potent antiproliferative activity against MDA-MB-468 cell line with an IC_{50} value of 9.61 μM. We believe that these benzyloxyphenyl-methylaminophenol derivatives represent a unique mechanism for interrogating STAT3 as well as a potential structure type for further exploration.

1. Introduction

Signal transducers and activators of transcription (STATs) protein comprise seven members (STAT1–STAT4, STAT5A, STAT5B, and STAT6) that regulate cell proliferation, differentiation, apoptosis, immune and inflammatory response, and angiogenesis.[1–6] Among all of these STAT proteins, STAT3 is the most studied member for its important function in cell proliferation, differentiation, apoptosis, angiogenesis, immune responses and metastasis.[7–10] Furthermore, newly identified cancer-promoting functions of STAT3 (mitochondria, epigenetic regulation, obesity, and pre-metastatic niches) further highlight the importance of targeting STAT3.[11–14] STAT3 is activated by receptor tyrosine kinases (EGFR, VEGFR, PDGFR, FGFR, etc), receptor associated kinases (JAK) and non-receptor tyrosine kinases (Src and Abl) at tyrosine residue (Tyr705).[15] Upon Tyr705 phosphorylation, STAT3 forms homodimerization, then translocates into the nucleus and binds to STAT3-specific DNA binding element, activating the gene transcription.[16] Persistent activation of STAT3 signaling has been demonstrated to induce cell proliferation and prevent apoptosis in human cancer cells, such as ovarian, cervical, breast, head and neck squamous cell carcinoma, prostate, and leukemias.[17–19] Hence STAT3 signaling is an attractive target for cancer therapy.

Our previous work by using virtual screening approach had discovered several benzyloxyphenyl-methylaminophenol scaffolds STAT3 inhibitors as promising anticancer agents. The hit compound (**1**) was identified as an inhibitor of IL-6/STAT3 signaling pathway with an IC_{50} value of 26.68 μM and no obvious effects on closely related IFN-γ/STAT1, TNF-α/NF-κB signaling pathway, and upstream JAK2 and Src kinases.[20] In order to obtain more potent inhibitors, several series of hit **1** derivatives were designed, synthesized and tested against STAT3 signaling pathway and breast cancer MDA-MB-468 cell line. As shown in Figure 1, we first focused on the optimization on the ring A, B (**4a**–**r**) and then the linker (**8t** and **10u**) among these rings in hit **1**. Conformational restriction strategy was also used to reduce the molecular flexibility (**16v** and **16w**).

2. Results and discussion

2.1. Chemistry

The benzyloxyphenyl-methylaminophenol derivatives were prepared according to the synthetic route as outlined in Scheme 1.[21] By reacting with 4-chlorobenzyl chloride in the presence of potassium carbonate, substituted salicylaldehydes **2a**–**k**

Abbreviations: STAT3, signal transducers and activators of transcription 3; IL-6, interleukin-6; JAK, Janus kinase; SAR, structure–activity relationships.

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http://dx.doi.org/10.1016/j.bmc.2016.04.022
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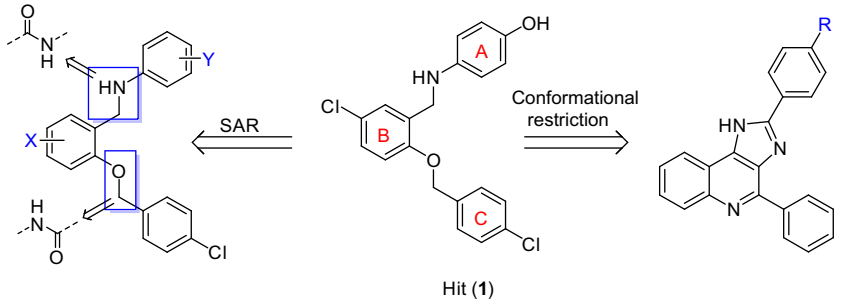
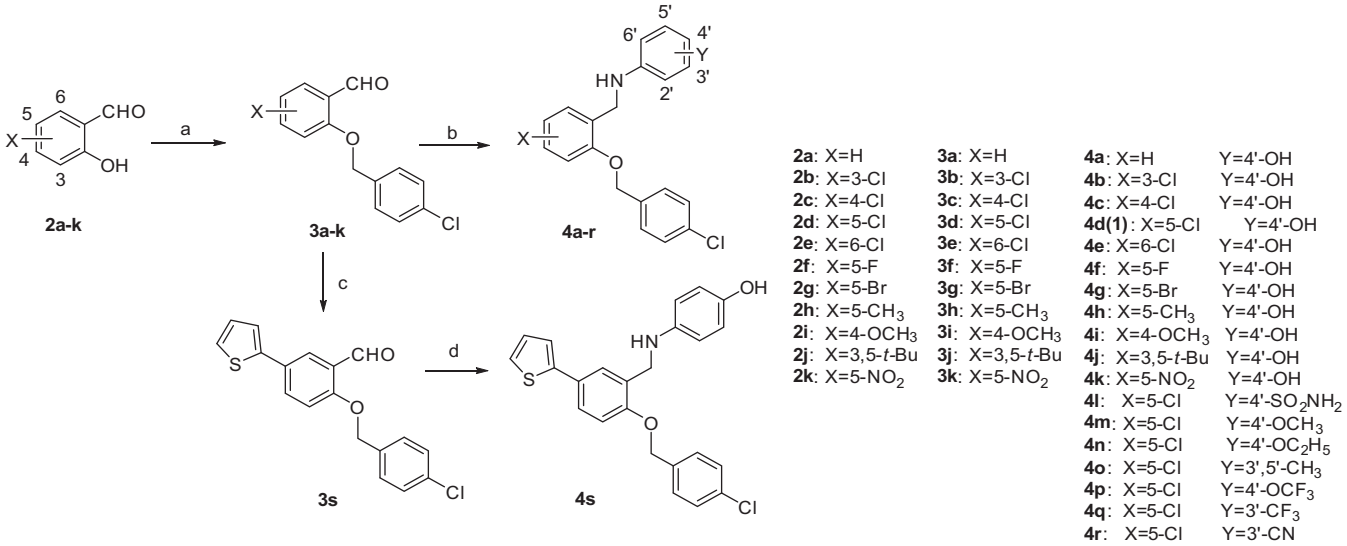


Figure 1. Design of target compounds.

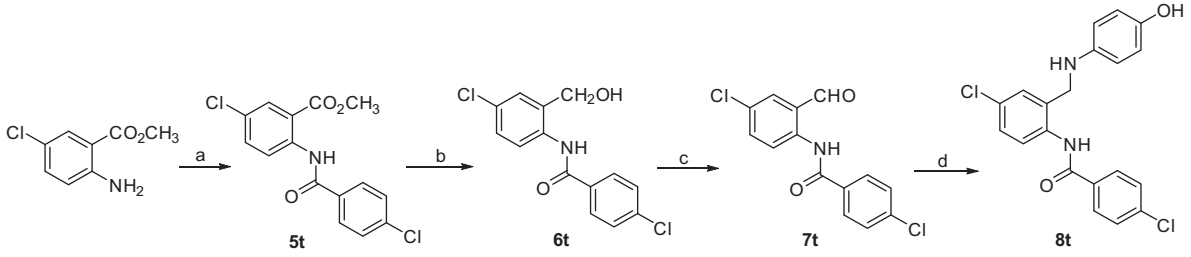


Scheme 1. Reagents and conditions: (a) 4-chlorobenzyl chloride, K_2CO_3, DMF, 120 °C, 2 h, 75.0–99.0%; (b) substituted anilines, $NaBH_3CN$, AcOH, CH_3OH, rt, 5 h, 70.0–100%. (c) **3g**, 2-thiopheneboronic acid, Cs_2CO_3, $Pd(PPh_3)_4$, dioxane/H_2O (3:1), 120 °C, microwave, 2 h; (d) 4-aminophenol, $NaBH_3CN$, AcOH, CH_3OH, rt, 5 h, 77.9%.

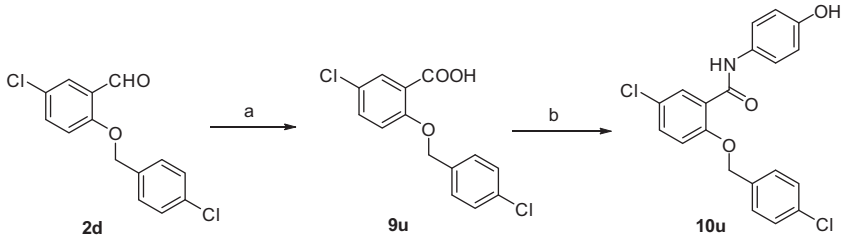
were transformed to intermediates **3a–k**. In addition, compound **3g** was coupled with 2-thiopheneboronic acid via Suzuki reaction to afford **3s**. Subsequently, in the presence of $NaBH_3CN$ and substituted anilines, **3a–k** and **3s** in methanol were transformed into target compounds **4a–4r** and **4s** via reductive amination (Scheme 1).

Compounds **8t** and **10u**, with more rigid linkers between these rings, were synthesized as described in Scheme 2 and 3. Treatment of the methyl 2-amino-5-chlorobenzoate with 4-chlorobenzoyl chloride and potassium carbonate in dichloromethane at room temperature resulted in the formation of **5t**. Then it was reduced with lithium aluminumhydride in anhydrous THF, providing the corresponding alcohol **6t**, which followed by oxidization using active manganese dioxide and formed **7t**. Finally, the compound **7t** was transformed to **8t** using the same reductive amination procedure as described above. For the synthesis of compound **10u**, the intermediate **2d** was oxidized to carboxylic acid **9u** using $NaClO_2$–$NaH_2PO_4{\cdot}2H_2O$ system, which was then coupled with 4-aminophenol in the presence of HATU/HOAT/DIPEA to give the target compound.

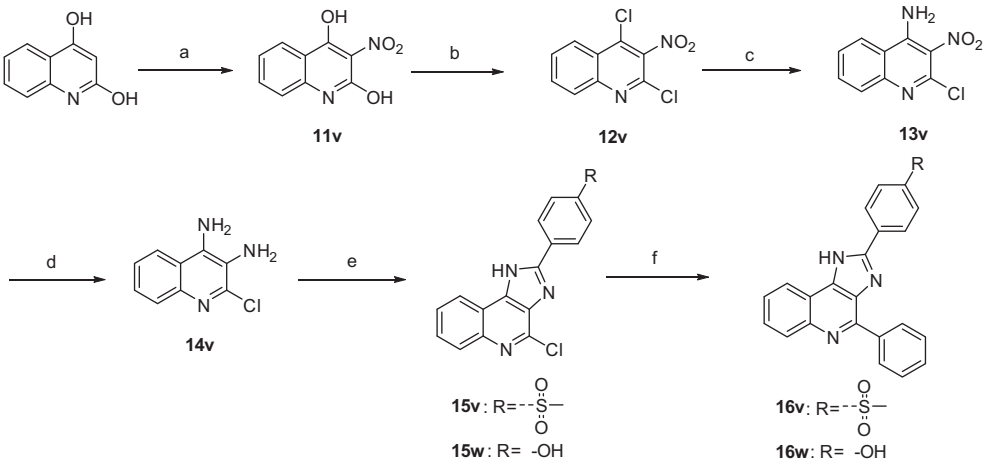
The synthesis of conformation restricted compounds were described in Scheme 4. Quinoline-2,4-diol was treated with nitric acid to afford 3-nitroquinoline-2,4-diol (**11v**). Compound **11v** was converted into 2,4-dichloro-3-nitroquinoline (**12v**) with phosphorus oxychloride, which subsequently reacted with 28% NH_3(aq) to form 2-chloro-3-nitroquinolin-4-amine (**13v**). This compound was treated with iron powder to give the diaminopyridine (**14v**), which was cyclized to give the 4-chloro-1*H*-imidazo[4,5-*c*]quinolones (**15v** and **15w**) in the presence of sodium pyrosulfate. Finally,



Scheme 2. Reagents and conditions: (a) 4-chlorobenzoyl chloride, K_2CO_3, DCM, rt, 2 h, 80.1%; (b) $LiAlH_4$, THF, 0 °C, 0.5–1 h, 67.2%; (c) MnO_2, DCM, reflux, 2 h, 82.8%; (d) 4-aminophenol, $NaBH_3CN$, AcOH, CH_3OH, rt, 2 h, 90.0%.



Scheme 3. Reagents and conditions: (a) $NaClO_2$, $NaH_2PO_4{\cdot}2H_2O$, 2-methyl-2-butene, *tert*-butanol, 95.0%; (b) 4-aminophenol, HATU, HOAT, DIPEA, THF, 84.8%.



Scheme 4. Reagents and conditions: (a) HNO_3, 75 °C, 98.5%; (b) $POCl_3$, 108 °C, 54.0%; (c) $NH_3{\cdot}H_2O$, CH_3CN, 50 °C, 87.0%; (d) Fe powder, NH_4Cl, CH_3CH_2OH/H_2O, 94.7%; (e) **15v**: 4-methylsulfonyl benzaldehyde, $Na_2S_2O_7$, THF (dry), reflux, 70.5%; **15w**: 4-hydroxybenzaldehyde, $Na_2S_2O_7$, THF (dry), reflux, 65.7%; (f) phenylboronic acid, $Pd(PPh_3)_4$, Cs_2CO_3, dioxane/H_2O (3:1), 120 °C, microwave, 2 h, 89.6–100%.

Suzuki coupling reaction was used to afford **16v** and **16w**, respectively.

2.2. Inhibitory activity on STAT3 transcription

We firstly tested the inhibitory activity against STAT3 signaling pathway using a STAT3 luciferase reporter method. As shown in Table 1, the target compounds displayed greatly varied inhibitory activities in HepG2 cell at 10 μM after 24 h incubation. The IC_{50} values of some potent compounds were listed in Table 2 and Figure 2. In light of these data, removing the chlorine atom at C-5 in ring B of hit compound (**1**) improved the luciferase activity from 26.68 μM to 7.71 μM (**1** vs **4a**). The replacement of a fluorine atom at C-5 position in ring B was favorable while the bromine atom was detrimental (**1** vs **4f**, **4g**). Incorporation of bigger steric substituents (tertiary butyl and thiophen-2-yl) at C-5 position rendered the compound inactive (**4j**, **4s**). These indicated that the size of substituents at C-5 position in ring B affected the activities. Changing the position of chlorine in ring B from C-5 to C-4 and C-6 led to the loss of inhibitory activity (**4c**, **4e**), while moving the chlorine from C-5 to C-3 resulted in a significant elevated luciferase activity (**1** vs **4b**), and as a result compound **4b** had an IC_{50} value of 1.38 μM. Introduction of electron-withdrawing nitro group at ring B (**4k**) displayed similar activity to hit compound (**1**) with an IC_{50} value of 24.09 μM. However, electron-donating groups made the activity decrease or miss (**4h**, **4i**). For ring A moiety, the hydroxyl group at C-4′ position was critical for the activities (**4m–r**). When the hydroxyl group was replaced by sulfamine (**4l**), the luciferase activity IC_{50} value dropped to 35.67 μM. Unfortunately, none of these conformation restricted compounds displayed inhibitory activities at concentration of 10 μM (**8t**, **10u**, **16v**, **16w**). Taken together, we found that compounds **4a**, **4b** and **4f** showed superior luciferase activities than hit compound **1**.

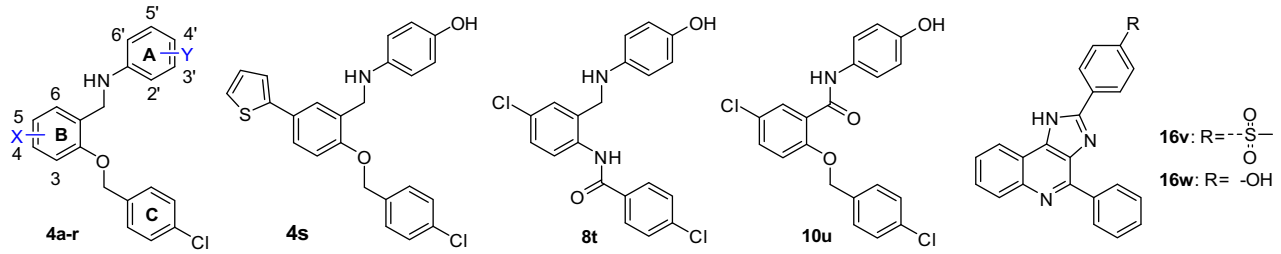
2.3. Cellular potency

Some selected compounds were further evaluated the antiproliferative activity in MDA-MB-468 cell line which harbors constitutively active STAT3. As shown in Table 2, all of these selected compounds exhibited micromole IC_{50} values against the tested cancer cell. Among them, **4a**, **4g**, **4k** displayed further enhanced anticancer activities when compared with hit compound **1** with IC_{50} values of 9.61 μM, 9.85 μM and 6.15 μM, respectively. Compounds **4b** and **4l** showed the similar activity to hit compound **1**.

2.4. Western blot analysis of compounds 4a and 4b

To further investigate the blockage of compounds **4a** and **4b** against the activation of STAT3 signaling pathway, we examined the STAT3 phosphorylation in MDA-MB-468 cell line using Western blot analysis. As shown in Figure 3, compounds **4a** and **4b** dose-dependently inhibited Tyr705 phosphorylation, whereas the expression of total STAT3 remained unchanged after 2 h incubation. This indicates that the decrease of Tyr705 phosphorylated STAT3 is not due to a constitutional decrease of total STAT3 protein. It is noteworthy that compounds **4a** and **4b** had no effect on the level of STAT1 or Tyr701 phosphorylated STAT1 which suggested that **4a** and **4b** had a good selectivity against the tumor suppressor STAT1. In addition, compounds **4a** and **4b** displayed no effects on the phosphorylation of upstream tyrosine kinase (Src), which participates in activating STAT3 signaling pathway. Above results prove that compounds **4a** and **4b** could down regulated STAT3 phosphorylation in an independent manner.

Table 1
Inhibitory activity on STAT3 transcription



Compound	IL-6/STAT3 pathway[a] 10 μM (%)	Compound	IL-6/STAT3 pathway[a] 10 μM (%)
4a	24.38	**4m**	−4.30
4b	39.51	**4n**	−28.07
4c	−2.98	**4o**	1.95
1	30.83	**4p**	3.13
4e	−4.30	**4q**	2.61
4f	37.18	**4r**	0.78
4g	32.54	**4s**	−12.58
4h	−8.42	**8t**	−220.37
4i	11.96	**10u**	−28.73
4j	−31.33	**16v**	7.17
4k	33.31	**16w**	5.28
4l	11.96	Curcumin	12.90

[a] STAT3-dependent luciferase reporter gene assay in HepG2 cell. Percent inhibition at 10 μM.

Table 2
Inhibitory activity on STAT3 transcription and human breast cancer cell lines of selected compounds

Compounds	IL-6/STAT3 pathway[a] (IC_{50} μM)	MDA-MB-468[b] (IC_{50} μM)
4a	7.71	9.61
4b	1.38	19.70
1	26.68	18.83
4f	11.04	57.25
4g	168.4	9.85
4k	24.09	6.15
4l	35.67	24.34

[a] STAT3-dependent luciferase reporter gene assay in HepG2 cell.
[b] Cell viability assay against MDA-MB-468 (MTT).

2.5. The selectivity against related IFN-γ/STAT1 and TNF-α/NF-κB signaling pathway

The selectivity of compounds **4a** and **4b** against related IFN-γ/STAT1 and TNF-α/NF-κB signaling pathway was investigated using a STAT1 luciferase reporter gene assay in HepG2 cells and a NF-κB luciferase reporter gene assay in 293 cells after the addition of IFN-γ and TNF-α respectively. As Table 3 shows, compounds **4a** did not displayed any of IFN-γ-induced STAT1 and TNF-α-induced NF-κB activations with a concentration as high as 100 μM. Compound **4b**, which displayed the most inhibitory activity against STAT3 signaling pathway, had IC_{50} values 40.19 μM and 77.96 μM against IFN-γ/STAT1 and TNF-α/NF-κB signaling pathway respectively. These results demonstrate that compounds **4a** and **4b** suppressive effects are highly selective for the IL-6/STAT3 signaling pathway over STAT1 and NF-κB signaling pathways.

2.6. Discussion

As **4a** and **4b** exhibited better inhibitory activity on STAT3 transcription, a molecular docking (Schrodinger, Maestro suite) model of compounds **4a** and **4b** bound to STAT3-SH2 domain was generated on the basis of the crystal structure of STAT3β homo dimer (PDB entry 1BG1). The results reveal that they bind to a unique site of STAT3-SH2 domain rather than the pTyr705 binding site surrounded by Lys591, Arg609, and Ser611 as illustrated in

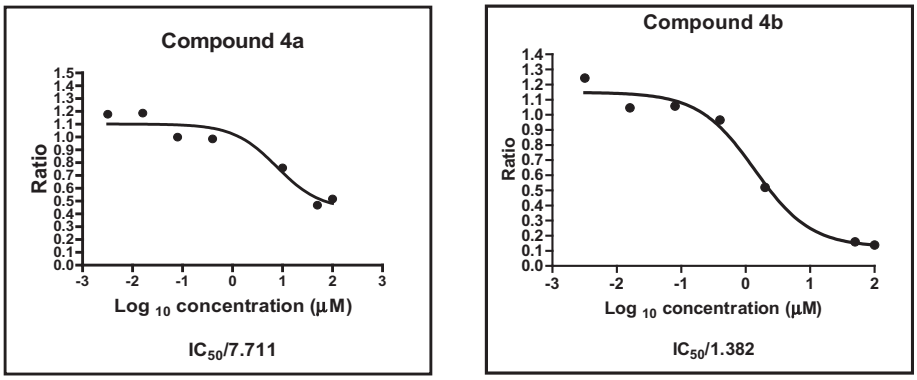


Figure 2. Dose-dependent inhibition of compounds **4a** and **4b** on the IL-6-induced STAT3-dependant luciferase activity. HepG2/STAT3-luciferase cells were pretreated with compounds **4a** and **4b** at the indicated concentrations for 1 h, and then were stimulated with IL-6 (10 ng/ml) for 5.5 h and the luciferase activity was measured following the 5.5 h stimulation.

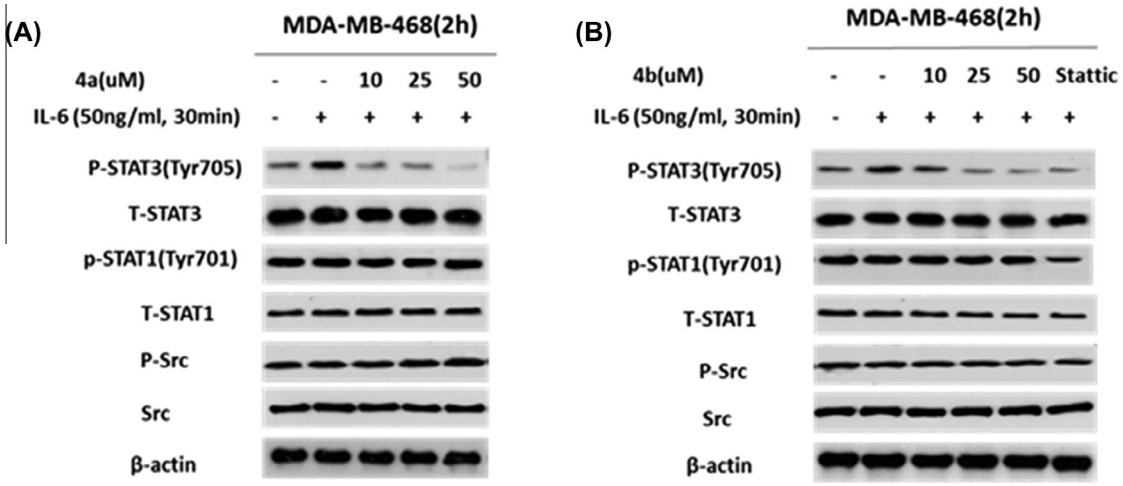


Figure 3. Western blot analysis of inhibition of STAT3 activity and selective inhibition against STAT1 and Src by compounds **4a** (A) and **4b** (B). Compounds **4a** and **4b** showed inhibition of STAT3 phosphorylation but not STAT1 and Src phosphorylation in MDA-MB-468. Cells were treated with stattic or **4a** and **4b** for 2 h, levels of pSTAT3, STAT3, pSTAT1, STAT1, pSrc, Src were probed by specific antibodies. β-Actin was used as the loading control.

Table 3
Inhibitory activity against related IFN-γ/STAT1 and TNF-α/NF-κB signaling pathway

Compounds	IL-6/STAT3 pathway[a] (IC_{50} μM)	IFN-γ/STAT1[b] (IC_{50} μM)	TNF-α/NF-κB[c] (IC_{50} μM)
4a	7.71	>100	>100
4b	1.38	40.19	77.96
1	26.68	>100	>100

[a] STAT3-dependent luciferase reporter gene assay in HepG2 cell.
[b] STAT1-dependent luciferase reporter gene assay in HepG2 cell.
[c] NF-κB-dependent luciferase reporter gene assay in 293 cell.

Figure 4. The ring A in **4a** and **4b** insert into a hydrophobic cleft which was provided by Tyr640, Ile653. It is a similar manner as Phe710 of the phosphotyrosine peptide (APpYLKTKF). Additionally, the hydroxyl group in ring A forms a hydrogen-bonding interaction with Cys712, which plays a vital role in activity. Changing the hydroxyl group to methoxyl group or ethyoxyl group decline activities (**4m**, **4n**). The activities also lost (**4o**–**4r**) after removing the hydroxyl group. Furthermore, it is predicted that the NH between ring A and ring B is able to form a hydrogen bond interaction with residue Gln644. Also, the size of substituent at C-5 position in ring B is crucial for interaction. Bulky groups, such as bromo, tertiary butyl and thiophen-2-yl, may have a steric clash with the surface of the binding pocket. Furthermore, only chlorine atom at C-3 and C-5 position in ring B is tolerate (**4b**, **1**). Changing the substitution position of chlorine atom in ring B to C-4 or C-6 led to possible clashes with the surface of the protein (**4c**, **4e**). More interestingly, ring B and ring C bound to the target protein like a

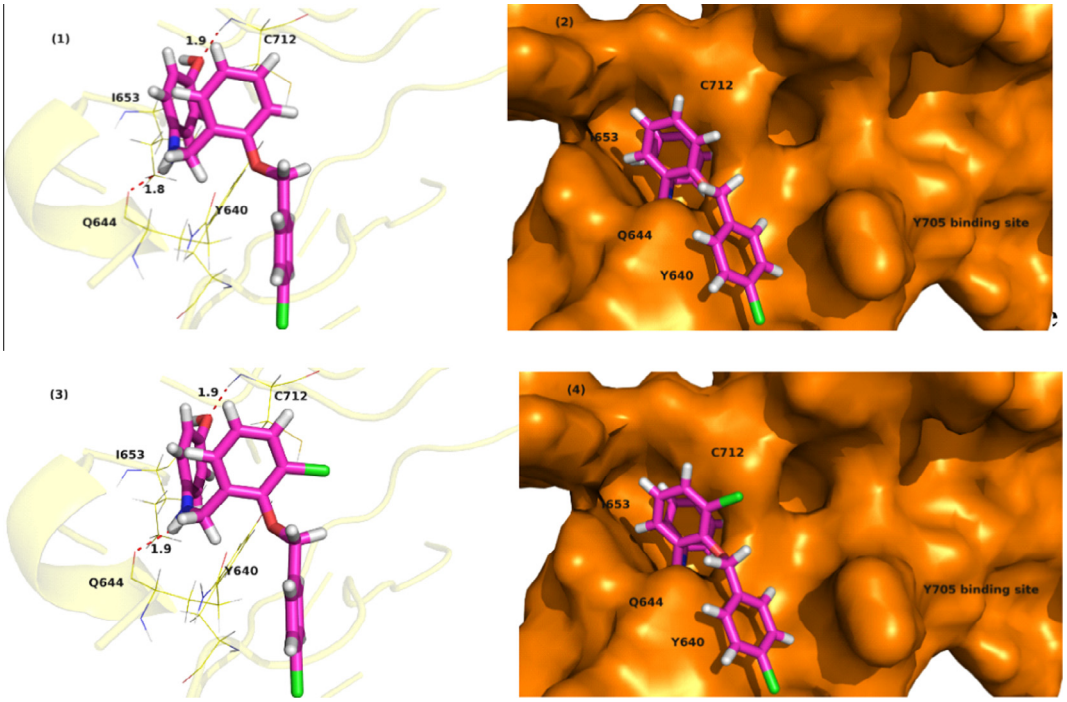


Figure 4. Predicted binding modes for compound **4a** (1–2) and compound **4b** (3–4) with STAT3 SH2 domain (PDB entry 1BG1), generated by Schrodinger, Maestro suite. The figures were generated using Pymol.

'clip'. Therefore, the flexible linker was necessary, as the conformational restricted compounds (**8t**, **10u**, **16v**, **16w**) could not bind to the predicted site so that the activities are missing.

3. Conclusion

Herein, we reported the structure–activity relationship study of a benzyloxyphenyl-methylaminophenol scaffold as STAT3 signaling pathway inhibitors. Finally, we discovered **4a** and **4b** potently inhibit activation and transactivation of STAT3 signaling pathway and explained the possible reasons combined with the docking results. Furthermore, **4a** could effectively inhibit the viability of tumor cells MDA-MB-468 harboring constitutively active STAT3 with an IC_{50} value of 9.61 μM. The present study provided more structural reference for the development of STAT3 signaling pathway. Additional in vivo antitumor activity studies on these compounds are undergoing and will be reported in due course.

4. Experiment

4.1. Biological evaluation

4.1.1. Cell lines and culture

HepG2/STAT3 and HepG2/STAT1 cells, gifts of Prof. Xinyuan Fu (National University of Singapore, Singapore), were HepG2 cells stably transfected with a STAT-responsive firefly luciferase reporter plasmid. 293/NF-κB cells were 293 cells stably transfected with NF-κB-responsive firefly luciferase reporter plasmid. All other cell lines were obtained from the American Type Culture Collection (USA).

4.1.2. Luciferase assay

Procedure is similar to published methods.[20]

4.1.3. MTT assay

Procedure is similar to published methods.[22]

4.1.4. Western blot analysis

Procedure is similar to published methods.[20]

4.2. Chemistry

4.2.1. General information

All solvents and chemical used were reagent grade. Purity and characterization of compounds were established by a combination of LC–MS and NMR. Flash column chromatography was performed on silica gel (200–300 mesh, Qingdao, China). ^{1}H NMR spectra were recorded with a 400 MHz Varian spectrometer and ^{13}C NMR spectra were recorded with a 600 MHz Bruker spectrometer. Chemical shifts (δ) are expressed in ppm, and coupling constants (J) are expressed in Hz. All reactions were performed under nitrogen unless otherwise stated. High resolution mass spectrometry data were given by AB 5600+Q TOF. The purity of all the designed compounds was analyzed using an Agilent 1200 HPLC system with a G1311B quaternary pump, a G1329B ALS and a G4212B DAD detector. The HPLC method consisted of the following: Agilent C18 RP column (250 mm × 4.6 mm, 5 μm); column temperature 25 °C; inject volume 2.0 μL; HPLC solvent H_2O (0.1% TFA)/CH_3OH = 60/40 (v/v); flow rate of 1.2 mL/min; detector wavelength of 275 nm.

4.2.2. General procedure for the synthesis of 3a–3k

To a solution of substituted salicylaldehyde (1.0 mmol) in anhydrous DMF was added 4-chlorobenzyl chloride (139 μL, 1.1 mmol), K_2CO_3 (553 mg, 4.0 mmol). The reaction mixture was stirred at 120 °C for 2 h and monitored by LC/MS analysis. Upon completion, the mixture was cooled to room temperature. Water and ethyl acetate were added and the mixture was extracted with ethyl acetate twice. The combined extracts were washed with water and brine, dried over Na_2SO_4. After filtration, the filtrate was removed in vacuo. The residue was purified by silica gel column chromatography to give **3a**–**3k** (75.0–99.0%).

4.2.2.1. 2-((4-Chlorobenzyl)oxy)benzaldehyde (3a). The title compound was obtained as a white solid, yield: 84.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.55 (s, 1H), 7.88 (dd, J = 7.7, 1.6 Hz, 1H), 7.59–7.52 (m, 1H), 7.41–7.38 (m, 4H), 7.10–7.05 (m, 1H), 7.03 (d, J = 8.4 Hz, 1H), 5.18 (s, 2H). ESI-MS: calcd for $[M+H]^+$ m/z: 247.0, found: 247.0

4.2.2.2. 3-Chloro-2-((4-chlorobenzyl)oxy) benzaldehyde (3b). The title compound was obtained as a white solid, yield: 99.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.15 (s, 1H), 7.72 (d, J = 7.1 Hz, 1H), 7.66 (d, J = 7.2 Hz, 1H), 7.19 (t, J = 7.5 Hz, 1H), 5.09 (s, 2H). ESI-MS: calcd for $[M+H]^+$ m/z: 281.0, found: 281.0.

4.2.2.3. 4-Chloro-2-((4-chlorobenzyl)oxy)benzaldehyde (3c). The title compound was obtained as a white solid, yield: 98.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.44 (s, 1H), 7.80 (d, J = 7.9 Hz, 1H), 7.44–7.34 (m, 4H), 7.05 (d, J = 8.0 Hz, 1H), 7.04 (s, 1H), 5.14 (s, 2H). ESI-MS: calcd for $[M+H]^+$ m/z: 281.0, found: 281.0.

4.2.2.4. 5-Chloro-2-((4-chlorobenzyl)oxy)benzaldehyde (3d). The title compound was obtained as a white solid, yield: 98.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.32 (s, 1H), 7.69 (dd, J = 8.9, 2.8 Hz, 1H), 7.63 (d, J = 2.8 Hz, 1H), 7.53 (d, J = 8.4 Hz, 2H), 7.45 (d, J = 8.4 Hz, 2H), 7.34 (d, J = 9.0 Hz, 1H), 5.28 (s, 2H). ESI-MS: calcd for $[M+H]^+$ m/z: 281.0, found: 281.0.

4.2.2.5. 2-Chloro-6-((4-chlorobenzyl)oxy)benzaldehyde (3e). The title compound was obtained as a white solid, yield: 75.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.55 (s, 1H), 7.42–7.33 (m, 5H), 7.05 (d, J = 8.0 Hz, 1H), 6.92 (d, J = 8.5 Hz, 1H), 5.14 (s, 2H). ESI-MS: calcd for $[M+H]^+$ m/z: 281.0, found: 281.0.

4.2.2.6. 2-((4-Chlorobenzyl)oxy)-5-fluorobenzaldehyde (3f). The title compound was obtained as a white solid, yield: 81.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.46 (d, J = 3.1 Hz, 1H), 7.52 (dd, J = 8.2, 3.2 Hz, 1H), 7.40–7.34 (m, 4H), 7.27–7.20 (m, 1H), 6.99 (dd, J = 9.1, 3.8 Hz, 1H), 5.14 (s, 2H). ESI-MS: calcd for $[M+H]^+$ m/z: 265.0, found: 265.0.

4.2.2.7. 5-Bromo-2-((4-chlorobenzyl)oxy)benzaldehyde (3g). The title compound was obtained as a white solid, yield: 98.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.45 (s, 1H), 7.96 (d, J = 2.5 Hz, 1H), 7.62 (dd, J = 8.8, 2.5 Hz, 1H), 7.40 (d, J = 8.5 Hz, 2H), 7.37 (d, J = 8.7 Hz, 2H), 6.93 (d, J = 8.9 Hz, 1H), 5.16 (s, 2H). ESI-MS: calcd for $[M+H]^+$ m/z: 325.0, found: 325.0.

4.2.2.8. 2-((4-Chlorobenzyl)oxy)-5-methylbenzaldehyde (3h). The title compound was obtained as a white solid, yield: 77.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.50 (s, 1H), 7.66 (d, J = 2.1 Hz, 1H), 7.38–7.36 (m, 4H), 7.34 (dd, J = 8.5, 2.3 Hz, 1H), 6.91 (d, J = 8.5 Hz, 1H), 5.13 (s, 2H), 2.32 (s, 3H). ESI-MS: calcd for $[M+H]^+$ m/z: 261.1, found: 261.1.

4.2.2.9. 2-((4-Chlorobenzyl)oxy)-4-methoxybenzaldehyde (3i). The title compound was obtained as a white solid, yield: 94.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.35 (s, 1H), 7.83 (d, J = 8.7 Hz, 1H), 7.38–7.36 (m, 4H), 6.57 (dd, J = 8.7, 2.1 Hz, 1H), 6.47 (d, J = 2.2 Hz, 1H), 5.12 (s, 2H), 3.85 (s, 3H). ESI-MS: calcd for $[M+H]^+$ m/z: 277.1, found: 277.1.

4.2.2.10. 3,5-Di-*tert*-butyl-2-((4-chlorobenzyl)oxy)benzaldehyde (3j). The title compound was obtained as a white solid. The crude product was used directly for the next step without further purification.

4.2.2.11. 2-((4-Chlorobenzyl)oxy)-5-nitrobenzaldehyde (3k). The title compound was obtained as a white solid, yield: 90.0%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.53 (s, 1H), 8.67 (dd, J = 9.2, 2.8 Hz, 1H), 8.61 (d, J = 2.7 Hz, 1H), 7.75 (d, J = 8.3 Hz, 2H), 7.70 (d, J = 9.2 Hz, 1H), 7.66 (d, J = 8.3 Hz, 2H), 5.62 (s, 2H). ESI-MS: calcd for $[M+H]^+$ m/z: 292.0, found: 292.0.

4.2.3. 2-((4-Chlorobenzyl)oxy)-5-(thiophen-2-yl)benzaldehyde (3s)

A mixture of **2g** (325 mg, 1 mmol), thiophen-2-ylboronic acid (192 mg, 1.5 mmol), $Pd(PPh_3)_4$ (116 mg, 0.1 mmol) and Cs_2CO_3 (1.30 g, 4 mmol) in 12 mL dioxane/Water(v/v = 3:1) was stirred at 120 °C by microwave for 2 h, then cooled to room temperature. After removing the dioxane, water and ethyl acetate were added and then the mixture was extracted with ethyl acetate twice. The combined extracts were washed with brine, dried over Na_2SO_4. After filtration, the filtrate was removed in vacuo. The crude product was used directly for the next step without further purification.

4.2.4. General procedure for the synthesis of 4a–4s

To a stirring solution of aldehyde (**3a**–**k**, **3s**) (1 mmol) and catalytic amount acetic acid (1 drop) in anhydrous CH_3OH (25 mL) was added phenylamine derivatives (1 mmol). After the solution was stirred at room temperature for 1 h, $NaBH_3CN$ (93.7 mg, 1.5 mmol) was added and stirred for another 2 h. After removing the methanol, water and ethyl acetate were added and then the organic was washed with saturated sodium bicarbonate, brine, dried over Na_2SO_4. After filtration, the filtrate was removed in vacuo. The residue was purified by silica gel column chromatography to give **4a**–**s**.

4.2.4.1. 4-((2-((4-Chlorobenzyl)oxy)benzyl)amino)phenol (4a). The title compound was obtained as a white solid, yield: 98.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.31 (m, 5H), 7.23 (t, J = 7.8 Hz, 1H), 6.97–6.89 (m, 2H), 6.67 (d, J = 8.7 Hz, 2H), 6.54 (d, J = 8.7 Hz, 2H), 5.09 (s, 2H), 4.33 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 155.65, 147.25, 141.63, 134.89, 133.13, 128.66, 128.20, 127.91, 127.71, 127.25, 120.49, 115.52, 114.20, 111.07, 68.59, 44.27. ESI-MS: calcd for $[M+H]^+$ m/z: 340.1, found: 340.1. ESI-HRMS $[M+H]^+$ calcd for $C_{20}H_{19}ClNO_2$: 340.1099, found: 340.1100. HPLC: t_R = 3.113 min.

4.2.4.2. 4-((3-Chloro-2-((4-chlorobenzyl)oxy)benzyl)amino)phenol (4b). The title compound was obtained as a brown solid, yield: 95.0%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (s, 1H), 7.51 (d, J = 8.5 Hz, 2H), 7.45 (d, J = 8.4 Hz, 2H), 7.37 (dd, J = 7.9, 1.3 Hz, 1H), 7.27 (d, J = 7.6 Hz, 1H), 7.09 (t, J = 7.8 Hz, 1H), 6.48 (d, J = 8.7 Hz, 2H), 6.32 (d, J = 8.7 Hz, 2H), 5.52 (t, J = 4.0 Hz, 1H), 4.99 (s, 2H), 4.14 (d, J = 4.0 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 151.89, 147.39, 141.25, 134.55, 134.41, 133.67, 129.17, 128.99, 128.17, 127.36, 127.17, 124.67, 115.60, 113.97, 73.78, 43.98. ESI-MS: calcd for $[M+H]^+$ m/z: 374.1, found: 374.1. ESI-HRMS $[M+H]^+$ calcd for $C_{20}H_{18}Cl_2NO_2$: 374.0709, found: 374.0707. HPLC: t_R = 3.441 min.

4.2.4.3. 4-((4-Chloro-2-((4-chlorobenzyl)oxy)benzyl)amino)phenol (4c). The title compound was obtained as a brown solid, yield: 95.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.30 (m, 4H), 7.24 (d, J = 8.5 Hz, 1H), 6.93–6.89 (m, 2H), 6.66 (d, J = 8.7 Hz, 2H), 6.50 (d, J = 8.7 Hz, 2H), 5.05 (s, 2H), 4.28 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 156.05, 147.23, 141.53, 134.16, 133.42, 132.81, 129.18, 128.30, 127.97, 126.03, 120.44, 115.53, 114.03, 111.72, 68.89, 43.62. ESI-MS: calcd for $[M+H]^+$ m/z: 374.1, found: 374.1. ESI-HRMS $[M+H]^+$ calcd for $C_{20}H_{18}Cl_2NO_2$: 374.0709, found: 374.0713. HPLC: t_R = 3.463 min.

4.2.4.4. 4-((2-Chloro-6-((4-chlorobenzyl)oxy)benzyl)amino)phenol (4e). The title compound was obtained as a white solid, yield: 95.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.29 (m, 4H), 7.14 (t, J = 8.2 Hz, 1H), 7.01 (d, J = 8.1 Hz, 1H), 6.80 (d, J = 8.3 Hz, 1H), 6.67–6.61 (m, 4H), 5.06 (s, 2H), 4.47 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.08, 147.56, 141.46, 134.91, 134.27, 133.40, 128.34, 128.30, 128.01, 125.57, 121.82, 115.38, 114.99, 110.03, 69.32, 41.22. ESI-MS: calcd for $[M+H]^+$ m/z: 374.1, found: 374.1. ESI-HRMS $[M+H]^+$ calcd for $C_{20}H_{18}Cl_2NO_2$: 374.0709, found: 374.0711. HPLC: t_R = 3.294 min.

4.2.4.5. 4-((2-((4-Chlorobenzyl)oxy)-5-fluorobenzyl)amino)phenol (4f). The title compound was obtained as a white solid, yield: 99.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.31 (m, 4H), 7.07 (dd, J = 8.9, 2.9 Hz, 1H), 6.91–6.80 (m, 2H), 6.68 (d, J = 8.7 Hz, 2H), 6.50 (d, J = 8.7 Hz, 2H), 5.05 (s, 2H), 4.30 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.61, 156.03, 151.49, 147.27, 141.40, 134.67, 133.28, 129.64, 129.61, 128.24, 127.98, 115.56, 115.04, 114.00, 113.13, 112.04, 69.31, 43.74. ESI-MS: calcd for $[M+H]^+$ m/z: 358.1, found: 358.1. ESI-HRMS $[M+H]^+$ calcd for $C_{20}H_{18}ClFNO_2$: 358.1005, found: 358.1007. HPLC: t_R = 3.099 min.

4.2.4.6. 4-((5-Bromo-2-((4-chlorobenzyl)oxy)benzyl)amino)phenol (4g). The title compound was obtained as a white solid, yield: 97.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, J = 2.4 Hz, 1H), 7.38–7.28 (m, 5H), 6.78 (d, J = 8.7 Hz, 1H), 6.71–6.65 (m, 2H), 6.54–6.48 (m, 2H), 5.06 (s, 2H), 4.29 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 154.59, 147.32, 141.41, 134.37, 133.36, 131.06, 130.18, 129.89, 128.28, 127.94, 115.58, 114.07, 112.96, 112.76, 68.90, 43.75. ESI-MS: calcd for $[M+H]^+$ m/z: 418.0, found: 418.0. ESI-HRMS $[M+H]^+$ calcd for $C_{20}H_{18}BrClNO_2$: 420.0183, found: 420.0186. HPLC: t_R = 3.426 min.

4.2.4.7. 4-((2-((4-Chlorobenzyl)oxy)-5-methylbenzyl)amino)phenol (4h). The title compound was obtained as a white solid, yield: 92.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36–7.32 (m, 4H), 7.14 (s, 1H), 7.01 (d, J = 8.2 Hz, 1H), 6.80 (d, J = 8.3 Hz, 1H), 6.68 (d, J = 8.7 Hz, 2H), 6.55 (d, J = 8.7 Hz, 2H), 5.05 (s, 2H), 4.28 (s, 2H), 2.26 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 153.55, 147.11, 141.97, 135.09, 133.04, 129.80, 129.46, 128.16, 127.89, 127.10, 115.50, 114.19, 114.10, 111.20, 68.78, 44.27, 19.95. ESI-MS: calcd for $[M+H]^+$ m/z: 354.1, found: 354.1. ESI-HRMS $[M+H]^+$ calcd for $C_{21}H_{21}ClNO_2$: 354.1255, found: 354.1263. HPLC: t_R = 3.413 min.

4.2.4.8. 4-((2-((4-Chlorobenzyl)oxy)-4-methoxybenzyl)amino)phenol (4i). The title compound was obtained as a white solid, yield: 94.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35–7.31 (m, 4H), 7.21 (d, J = 8.3 Hz, 1H), 6.67 (d, J = 8.6 Hz, 2H), 6.54 (d, J = 8.7 Hz, 2H), 6.49 (d, J = 2.0 Hz, 1H), 6.45 (dd, J = 8.3, 2.1 Hz, 1H), 5.05 (s, 2H), 4.24 (s, 2H), 3.77 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.41, 156.58, 147.24, 141.81, 134.72, 133.14, 129.37, 128.20, 127.90, 119.68, 115.48, 114.25, 103.85, 99.37, 68.61, 54.78, 43.97. ESI-MS: calcd for $[M+H]^+$ m/z: 370.1, found: 370.1. ESI-HRMS $[M+H]^+$ calcd for $C_{21}H_{21}ClNO_3$: 370.1204, found: 370.1197. HPLC: t_R = 3.265 min.

4.2.4.9. 4-((3,5-Di-*tert*-butyl-2-((4-chlorobenzyl)oxy)benzyl)amino)phenol (4j). The title compound was obtained as a white solid, yield: 94.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.39 (s, 1H), 7.50–7.43 (m, 4H), 7.34 (d, J = 2.3 Hz, 1H), 7.20 (d, J = 2.4 Hz, 1H), 6.49 (d, J = 8.8 Hz, 2H), 6.44 (d, J = 8.9 Hz, 2H), 5.39 (s, 1H), 4.91 (s, 2H), 4.14 (d, J = 5.2 Hz, 2H), 1.37 (s, 9H), 1.23 (s, 9H). ^{13}C NMR

(150 MHz, $CDCl_3$) δ 153.42, 147.21, 145.82, 141.94, 141.67, 135.69, 132.73, 131.19, 128.00, 127.70, 124.85, 123.29, 115.52, 113.81, 74.35, 44.73, 34.90, 33.96, 30.89, 30.63. ESI-MS: calcd for $[M+H]^+$ m/z: 452.2, found: 452.2. ESI-HRMS $[M+H]^+$ calcd for $C_{28}H_{35}ClNO_2$: 452.2351, found: 452.2354. HPLC: t_R = 4.646 min.

4.2.4.10. 4-((2-((4-Chlorobenzyl)oxy)-5-nitrobenzyl)amino)phenol (4k). The title compound was obtained as a brown solid, yield: 90.3%. ^1H NMR (400 MHz, DMSO-d_6) δ 8.46 (s, 1H), 8.14 (d, J = 8.6 Hz, 1H), 8.13 (s, 1H), 7.55 (d, J = 7.9 Hz, 2H), 7.48 (d, J = 8.1 Hz, 2H), 7.28 (d, J = 8.6 Hz, 1H), 6.52 (d, J = 8.3 Hz, 2H), 6.39 (d, J = 8.4 Hz, 2H), 5.72 (t, J = 4.8 Hz, 1H), 5.35 (s, 2H), 4.24 (d, J = 4.8 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.26, 147.46, 141.20, 141.01, 133.85, 133.32, 129.09, 128.48, 128.03, 123.97, 123.73, 115.64, 113.97, 110.46, 69.38, 43.49. ESI-MS: calcd for $[M+H]^+$ m/z 385.1, found: 385.1. ESI-HRMS $[M+H]^+$ calcd for $C_{20}H_{18}ClN_2O_4$: 385.0950, found: 385.0954. HPLC: t_R = 2.896 min.

4.2.4.11. 4-((5-Chloro-2-((4-chlorobenzyl)oxy)benzyl)amino) benzenesulfonamide (4l). The title compound was obtained as a white solid, yield: 83.6%. ^1H NMR (400 MHz, DMSO-d_6) δ 7.53–7.42 (m, 6H), 7.25 (dd, J = 8.7, 2.5 Hz, 1H), 7.15 (d, J = 2.5 Hz, 1H), 7.09 (d, J = 8.7 Hz, 1H), 6.94–6.87 (m, 3H), 6.58 (d, J = 8.7 Hz, 2H), 5.19 (s, 2H), 4.31 (d, J = 6.0 Hz, 2H). ^{13}C NMR (150 MHz, CD_3OD) δ 154.18, 151.11, 135.15, 132.82, 128.96, 128.78, 128.10, 127.70, 126.96, 126.78, 126.73, 125.01, 112.57, 110.48, 68.64, 40.41. ESI-MS: calcd for $[M+H]^+$ m/z: 437.0, found: 437.0. ESI-HRMS $[M+H]^+$ calcd for $C_{20}H_{19}Cl_2N_2O_3S$: 459.0307, found: 459.0311. HPLC: t_R = 4.064 min.

4.2.4.12. *N*-(5-Chloro-2-((4-chlorobenzyl)oxy)benzyl)-4-methoxyaniline (4m). The title compound was obtained as a white solid, yield: 97.5%. ^1H NMR (400 MHz, $CDCl_3$) δ 7.38–7.31 (m, 5H), 7.16 (dd, J = 8.7, 2.3 Hz, 1H), 6.82 (d, J = 8.7 Hz, 1H), 6.76 (d, J = 8.8 Hz, 2H), 6.56 (d, J = 8.8 Hz, 2H), 5.06 (s, 2H), 4.30 (s, 2H), 3.74 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 154.05, 151.71, 141.47, 134.43, 133.34, 129.58, 128.27, 128.15, 127.94, 127.11, 125.58, 114.27, 113.77, 112.26, 68.97, 55.16, 43.63. ESI-MS: calcd for $[M+H]^+$ m/z: 388.1, found: 388.1. ESI-HRMS $[M+H]^+$ calcd for $C_{21}H_{20}Cl_2NO_2$: 388.0866, found: 388.871. HPLC: t_R = 3.792 min.

4.2.4.13. *N*-(5-Chloro-2-((4-chlorobenzyl)oxy)benzyl)-4-ethoxyaniline (4n). The title compound was obtained as a white solid, yield: 98.1%. ^1H NMR (400 MHz, DMSO-d_6) δ 7.52–7.42 (m, 4H), 7.27–7.18 (m, 2H), 7.06 (d, J = 8.9 Hz, 1H), 6.65 (d, J = 8.8 Hz, 2H), 6.43 (d, J = 8.8 Hz, 2H), 5.75 (t, J = 6.2 Hz, 1H), 5.17 (s, 2H), 4.18 (d, J = 6.1 Hz, 2H), 3.83 (q, J = 6.9 Hz, 2H), 1.23 (t, J = 7.0 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 154.04, 150.98, 141.47, 134.45, 133.33, 129.64, 128.26, 128.15, 127.93, 127.10, 125.58, 115.12, 113.74, 112.25, 68.96, 63.45, 43.62, 14.40. ESI-MS: calcd for $[M+H]^+$ m/z: 402.1, found: 402.1. ESI-HRMS $[M+H]^+$ calcd for $C_{22}H_{22}Cl_2NO_2$: 402.1022, found: 402.1027. HPLC: t_R = 3.986 min.

4.2.4.14. *N*-(5-Chloro-2-((4-chlorobenzyl)oxy)benzyl)-3,5-dimethyl aniline (4o). The title compound was obtained as a colorless oil, yield: 90.5%. ^1H NMR (400 MHz, $CDCl_3$) δ 7.39–7.28 (m, 5H), 7.19–7.12 (m, 1H), 6.81 (d, J = 8.7 Hz, 1H), 6.38 (s, 1H), 6.24 (s, 2H), 5.05 (s, 2H), 4.32 (s, 2H), 2.21 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 154.03, 147.40, 138.31, 134.48, 133.32, 129.59, 128.27, 128.11, 127.94, 127.13, 125.60, 119.25, 112.23, 110.40, 68.95, 42.71, 20.90. ESI-MS: calcd for $[M+H]^+$ m/z: 386.1, found: 386.1. ESI-HRMS $[M+H]^+$ calcd for $C_{22}H_{21}Cl_2NO$: 408.0892, found: 408.0889. HPLC: t_R = 4.808 min.

4.2.4.15. *N*-(5-Chloro-2-((4-chlorobenzyl)oxy)benzyl)-4-(trifluoromethoxy)aniline (4p). The title compound was obtained as a colorless oil, yield: 91.8%. ^1H NMR (400 MHz, $CDCl_3$) δ 7.38–7.26 (m, 5H), 7.18 (d, J = 7.9 Hz, 1H), 7.00 (d, J = 7.6 Hz, 2H), 6.83 (d, J = 8.3 Hz, 1H), 6.53 (d, J = 8.1 Hz, 2H), 5.05 (s, 2H), 4.32 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 154.06, 145.87, 140.14, 134.30, 133.46, 128.65, 128.30, 127.98, 127.44, 125.63, 121.80, 112.72, 112.36, 69.02, 42.84. ESI-MS: calcd for $[M+H]^+$ m/z: 442.1, found: 442.1. ESI-HRMS $[M+H]^+$ calcd for $C_{21}H_{17}Cl_2F_3NO_2$: 442.0583, found: 442.0582. HPLC: t_R = 5.414 min.

4.2.4.16. *N*-(5-Chloro-2-((4-chlorobenzyl)oxy)benzyl)-3-(trifluoromethyl)aniline (4q). The title compound was obtained as a colorless oil, yield: 90.3%. ^1H NMR (400 MHz, $CDCl_3$) δ 7.38–7.26 (m, 5H), 7.17 (d, J = 7.9 Hz, 1H), 7.04–6.90 (m, 2H), 6.88–6.64 (m, 2H), 6.52 (d, J = 7.5 Hz, 1H), 5.05 (s, 2H), 4.33 (s, 2H). ^{13}C NMR (150 MHz, CD_3OD) δ 154.17, 148.29, 146.98, 139.20, 135.15, 132.79, 129.38, 128.65, 128.08, 128.01, 127.67, 126.98, 126.61, 125.06, 121.12, 114.67, 112.50, 111.97, 68.62, 41.29, 40.82. ESI-MS: calcd for $[M+H]^+$ m/z: 426.1, found: 426.1. ESI-HRMS $[M+H]^+$ calcd for $C_{21}H_{17}Cl_2F_3NO$: 426.0634, found: 426.0636. HPLC: t_R = 5.405 min.

4.2.4.17. 3-((5-Chloro-2-((4-chlorobenzyl)oxy)benzyl)amino) benzonitrile (4r). The title compound was obtained as a white solid, yield: 85.3%. ^1H NMR (400 MHz, $CDCl_3$) δ 7.33 (d, J = 2.9 Hz, 4H), 7.24–7.14 (m, 3H), 6.95 (d, J = 6.8 Hz, 1H), 6.84 (d, J = 8.5 Hz, 1H), 6.75 (s, 2H), 5.06 (s, 2H), 4.33 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 154.05, 147.30, 134.18, 133.54, 129.33, 128.35, 128.06, 127.76, 127.66, 125.65, 120.63, 118.76, 116.88, 114.58, 112.40. ESI-MS: calcd for $[M+H]^+$ m/z: 383.1, found: 383.1. ESI-HRMS $[M+H]^+$ calcd for $C_{21}H_{17}Cl_2N_2O$: 383.0712, found: 383.0712. HPLC: t_R = 5.060 min.

4.2.4.18. 4-((2-((4-Chlorobenzyl)oxy)-5-(thiophen-2-yl)benzyl) amino)phenol (4s). The title compound was obtained as a white solid, yield: 77.9%. ^1H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, J = 1.8 Hz, 1H), 7.45 (dd, J = 8.4, 2.1 Hz, 1H), 7.35 (s, 4H), 7.23–7.18 (m, 1H), 7.16 (d, J = 2.5 Hz, 1H), 7.03 (dd, J = 5.0, 3.6 Hz, 1H), 6.90 (d, J = 8.5 Hz, 1H), 6.68 (d, J = 8.6 Hz, 2H), 6.56 (d, J = 8.6 Hz, 2H), 5.10 (s, 2H), 4.34 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 155.20, 147.45, 143.46, 134.66, 133.25, 128.24, 127.95, 127.33, 127.07, 126.52, 125.31, 123.45, 121.77, 115.55, 114.37, 111.49, 68.81, 44.36.ESI-MS: calcd for $[M+H]^+$ m/z: 422.1, found: 422.1. ESI-HRMS $[M+H]^+$ calcd for $C_{24}H_{21}ClNO_2S$: 422.0976, found: 422.0975. HPLC: t_R = 3.823 min.

4.2.5. Synthesis of 4-chloro-*N*-(4-chloro-2-(((4-hydroxyphenyl) amino)methyl)phenyl)benzamide (8t)

4.2.5.1. Methyl 5-chloro-2-(4-chlorobenzamido)benzoate (5t). To a solution of methyl 2-amino-5-chlorobenzoate (1.0 g, 5.39 mmol) in dry CH_2Cl_2 (25 mL) was added 4-chlorobenzoyl chloride (826 μL, 6.47 mmol). Then the K_2CO_3 (1.86 g, 13.47 mmol) were added. Upon completion, the solvent was evaporated under reduce pressure. Water and ethyl acetate were added and the mixture was extracted with ethyl acetate. The combined extracts were washed with water and brine, dried over Na_2SO_4. After filtration, the filtrate was removed in vacuo. The residue was purified by silica gel column chromatography to give **5t** as a white solid (1.40 g, 80.1%). ^1H NMR (400 MHz, DMSO-d_6) δ 11.40 (s, 1H), 8.30 (s, 1H), 7.96 (d, J = 8.5 Hz, 2H), 7.83–7.55 (m, 4H), 3.82 (s, 3H). ESI-MS: calcd for $[M+H]^+$ m/z: 324.0, found: 324.0.

4.2.5.2. 4-Chloro-*N*-(4-chloro-2-(hydroxymethyl)phenyl)benzamide (6t). To a solution of **5t** (400 mg, 1.23 mmol) in anhydrous THF (10 mL) was added $LiAlH_4$ (93.6 mg, 2.47 mmol) slowly at 0 °C. Stirring was continued for another 15 min before treating with ice water dropwise. The resulting suspension was filtered and washed thoroughly with ethyl acetate. The combined organic

fractions were dried over Na_2SO_4. After filtration, the filtrate was removed in vacuo. The residue was purified by silica gel column chromatography to give **6t** as a white solid (310 mg, 84.8%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.13 (s, 1H), 7.93 (d, J = 8.1 Hz, 2H), 7.60 (d, J = 8.2 Hz, 3H), 7.48 (s, 1H), 7.34 (d, J = 8.4 Hz, 1H), 5.60 (s, 1H), 4.54 (s, 2H). ESI-MS: calcd for $[M+H]^+$ m/z 296.0, found: 296.0.

4.2.5.3. 4-Chloro-*N*-(4-chloro-2-formylphenyl)benzamide (7t). To a solution of **6t** (200 mg, 0.68 mmol) in anhydrous CH_2Cl_2 (10 mL) was treated with MnO_2 (177 mg, 2.04 mmol) slowly. Stirring was continued for another 3 h under reflux. The resulting suspension was filtered and washed thoroughly with CH_2Cl_2. The combined organic fractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give the crude compound **7t**. The purity of **7t** was quite sufficient to use for the following synthesis.

4.2.5.4. 4-Chloro-*N*-(4-chloro-2-(((4-hydroxyphenyl)amino)methyl) phenyl)benzamide (8t). Following the preparation protocol of compounds **4a**–**s**. To a stirring solution of **7t** and catalytic amount acetic acid (1 drop) in anhydrous CH_3OH (25 mL) was added 4-aminophenol (15.0 mg, 0.14 mmol). After the solution was stirred at room temperature for 1 h, $NaBH_3CN$ (13.0 mg, 0.27 mmol) was added and stirred for another 2 h. After removing the methanol, water and ethyl acetate were added and then the organic was washed with saturated sodium bicarbonate, brine, dried over Na_2SO_4. After filtration, the filtrate was removed in vacuo. The residue was purified by silica gel column chromatography to give **8t** as a white solid (25.0 mg, 47.5%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.43 (s, 1H), 8.50 (s, 1H), 7.94 (d, J = 8.3 Hz, 2H), 7.57 (d, J = 8.4 Hz, 3H), 7.39 (s, 1H), 7.32 (d, J = 8.2 Hz, 1H), 6.51 (d, J = 8.7 Hz, 2H), 6.41 (d, J = 8.7 Hz, 2H), 5.73 (t, J = 5.2 Hz, 1H), 4.18 (d, J = 5.2 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.12, 148.85, 140.60, 136.80, 136.47, 134.82, 132.76, 129.68, 129.31, 128.45, 127.13, 126.77, 126.53, 115.56, 113.93, 44.31. ESI-MS: calcd for $[M+H]^+$ m/z: 387.1, found: 386.8. ESI-HRMS $[M+H]^+$ calcd for $C_{20}H_{17}Cl_2N_2O_2$: 387.0662, found: 387.0668. HPLC: t_R = 2.942 min.

4.2.6. Synthesis of 5-chloro-2-((4-chlorobenzyl)oxy)-*N*-(4-hydroxyphenyl)benzamide (10u)

4.2.6.1. 5-Chloro-2-((4-chlorobenzyl)oxy)benzoic acid (9u). To a solution of **2d** (50 mg, 0.18 mmol) in *t*-BuOH (5 mL) and 2-methyl-2-butene (2.5 mL) were added $NaClO_2$ (96.6 mg, 1.07 mmol) and $NaH_2PO_4{\cdot}2H_2O$ (83.3 mg, 0.53 mmol) buffer solution (1.07 mL) at 0 °C. Then the mixture was stirred at room temperature. Upon completion, the solution was extracted with ethyl acetate and the organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude compound was purified by silica gel column chromatography to give **9u** (50.2 mg, 95.0%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.62 (d, J = 2.6 Hz, 1H), 7.52 (dd, J = 8.9, 2.6 Hz, 1H), 7.50–7.42 (m, 4H), 7.19 (d, J = 8.9 Hz, 1H), 5.18 (s, 2H). ESI-MS: calcd for $[M-H]^-$ m/z 295.0, found: 295.0.

4.2.6.2. 5-Chloro-2-((4-chlorobenzyl)oxy)-*N*-(4-hydroxyphenyl) benzamide (10u). To a solution of **9u** (50.0 mg, 0.17 mmol) in dry THF (10 mL) was added HATU (96.0 mg, 0.25 mmol), HOAT (34.5 mg, 0.25 mmol), DIPEA (89.0 μL, 0.50 mmol) at 0 °C. After stirred at room temperature for10 min, 4-aminophenol (18.6 mg, 0.17 mmol) was added. Upon completion, the solvent was evaporated under reduce pressure. The residue was dissolved in ethyl acetate and washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The crude compound was purified by silica gel column chromatography to give **10u** as a white solid (55.4 mg, 84.8%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 9.28 (s, 1H), 7.60 (d, J = 2.5 Hz, 1H), 7.56–7.48 (m, 3H), 7.42 (d, J = 8.3 Hz, 2H), 7.35 (d, J = 8.7 Hz, 2H), 7.24 (d, J = 8.9 Hz, 1H), 6.68 (d, J = 8.7 Hz, 2H), 5.20 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 162.05, 154.03, 153.52, 135.24, 132.57, 130.93, 130.28, 129.52, 128.88, 128.32, 127.17, 124.54, 120.97, 115.18, 114.93, 69.41. ESI-MS: calcd for $[M+H]^+$ m/z 388.0, found: 388.0. ESI-HRMS $[M+H]^+$ calcd for $C_{20}H_{16}Cl_2NO_3$: 388.0502, found: 388.0511. HPLC: t_R = 4.180 min.

4.2.7. Synthesis of 16v and 16w

4.2.7.1. 3-Nitroquinoline-2,4-diol (11v). Quinoline-2,4-diol (5 g, 31.0 mmol) was dissolved in 30 mL of nitric acid and stirred at room temperature for 10 min, followed by heating the reaction mixture at 75 °C for another 15 min. The reaction mixture was then allowed to cool down to room temperature and was added to ice-water mixture to precipitate the product. The yellow precipitate was filtered and dried. The crude product was used directly for the next step without further purification.

4.2.7.2. 2,4-Dichloro-3-nitroquinoline (12v). Compound **11v** (3 g, 14.5 mmol) was dissolved in 20 mL of phenylphosphonyl dichloride and heated at 135 °C for 3 h. The reaction mixture was poured into ice-water and stirred vigorously to obtain the precipitate, which was filtered and dried to afford the compound **12v** (1.90 g, 54.0%).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (d, J = 8.1 Hz, 1H), 8.11 (d, J = 8.1 Hz, 1H), 7.95 (t, J = 7.2 Hz, 1H), 7.81 (t, J = 7.2 Hz, 1H). ESI-MS: calcd for $[M+H]^+$ m/z 243.0, found: 243.0.

4.2.7.3. 2-Chloro-3-nitroquinolin-4-amine (13v). To a solution of **12v** (200 mg, 0.82 mmol) in CH_3CN (10.0 mL) was added 28% NH_3(aq) (350 μL, 6.59 mmol) at rt and then the mixture was stirred at 50 °C for 6.5 h. Upon completion, water and ethyl acetate were added and then the mixture was extracted with ethyl acetate twice. The combined extracts were washed with water and brine, dried over Na_2SO_4. After filtration, the filtrate was removed in vacuo. The residue was purified by silica gel column chromatography to give **13v** (160 mg, 87.0%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (d, J = 8.4 Hz, 1H), 8.15 (s, 2H), 7.83–7.74 (m, 2H), 7.59 (t, J = 7.5 Hz, 1H). ESI-MS: calcd for $[M+H]^+$ m/z 224.0, found: 224.0.

4.2.7.4. 2-Chloroquinoline-3,4-diamine (14v). To a solution of **13v** (145 mg, 0.65 mmol) in CH_3CH_2OH/H_2O (5 mL/5 mL) was added Fe powder (109 mg, 1.94 mmol) and NH_4Cl (104 mg, 1.94 mmol). Then the mixture was stirred at 75 °C for 2 h. After removing the ethanol, ethyl acetate was added. The mixture was extracted with ethyl acetate twice. The combined extracts were washed with water and brine, dried over Na_2SO_4. After filtration, the filtrate was removed in vacuo. The residue was purified by silica gel column chromatography to give **14v** (119 mg, 94.7%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.00 (d, J = 8.3 Hz, 1H), 7.56 (d, J = 8.3 Hz, 1H), 7.36 (t, J = 7.4 Hz, 1H), 7.30 (t, J = 7.5 Hz, 1H), 6.31 (s, 2H), 4.76 (s, 2H). ESI-MS: calcd for $[M+H]^+$ m/z 194.0, found: 194.0.

4.2.7.5. 4-Chloro-2-(4-(methylsulfonyl)phenyl)-1*H*-imidazo[4,5-*c*]quinoline (15v). A mixture of **14v** (30.0 mg, 0.16 mmol), 4-(methylsulfonyl)benzaldehyde (43.0 mg, 0.23 mmol), and sodium pyrosulfate (52.0 mg, 0.23 mmol) in anhydrous THF (5.0 mL) was stirred at 67 °C for 4 h. After removing the THF, ethyl acetate and water were added. The mixture was extracted with ethyl acetate twice. The combined extracts were washed with water and brine, dried over Na_2SO_4. After filtration, the solvent was removed in vacuo. The crude product was used directly for the next step without further purification.

4.2.7.6. 4-(4-Chloro-1*H*-imidazo[4,5-*c*]quinolin-2-yl)phenol (15w). Following the preparation protocol of compounds **15v**. Starting from 4-hydroxybenzaldehyde (47.0 mg, 0.39 mmol), **14v** (50 mg, 0.26 mmol) and sodium pyrosulfate (86.0 mg, 0.39 mmol), the compound **15w** was obtained (60.0 mg, 65.7%). ESI-MS: calcd for $[M+H]^+$ m/z 296.1, found: 296.0.

4.2.7.7. 2-(4-(Methylsulfonyl)phenyl)-4-phenyl-1*H*-imidazo[4,5-*c*]quinoline (16v). A mixture of **15v** (50.0 mg, 0.14 mmol), phenylboronic acid (25.6 mg, 0.21 mmol), $Pd(PPh_3)_4$ (16.2 mg, 0.01 mmol) and Cs_2CO_3 (137 mg, 0.42 mmol) in 4.0 mL Dioxane/Water (v/v = 3:1) was stirred at 120 °C by microwave for 2 h, then cooled to room temperature. After removing the dioxane, water and ethyl acetate were added and then the mixture was extracted with ethyl acetate twice. The combined extracts were washed with brine, dried over Na_2SO_4. After filtration, the filtrate was removed in vacuo. The residue was purified by silica gel column chromatography to give **16v** (42.2 mg, 89.6%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.91 (d, J = 7.4 Hz, 2H), 8.56 (d, J = 8.1 Hz, 2H), 8.50 (d, J = 7.5 Hz, 1H), 8.22–8.13 (m, 3H), 7.77–7.66 (m, 2H), 7.61 (t, J = 7.5 Hz, 2H), 7.57–7.50 (m, 1H), 3.30 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 149.20, 149.06, 143.30, 141.53, 137.48, 136.67, 135.48, 133.98, 129.66, 129.45, 129.33, 128.15, 127.85, 127.73, 127.27, 126.14, 121.60, 116.75, 43.33. ESI-MS: calcd for $[M+H]^+$ m/z 400.1, found: 400.1. ESI-HRMS $[M+H]^+$ calcd for $C_{23}H_{18}N_3O_2S$: 400.1114, found: 400.1117. HPLC: t_R = 2.623 min.

4.2.7.8. 4-(4-Phenyl-1*H*-imidazo[4,5-*c*]quinolin-2-yl)phenol (16w). Following the preparation protocol of compound **16v**. Starting from **15w** (30.0 mg, 0.16 mmol), the compound **16w** was obtained (57.0 mg, 100%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.71 (s, 1H), 10.06 (s, 1H), 8.94 (d, J = 7.3 Hz, 2H), 8.51–8.42 (m, 1H), 8.16 (d, J = 8.6 Hz, 2H), 8.14–8.09 (m, 1H), 7.70–7.62 (m, 2H), 7.58 (t, J = 7.4 Hz, 2H), 7.50 (t, J = 7.1 Hz, 1H), 6.99 (d, J = 8.6 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.06, 156.18, 153.17, 147.63, 142.54, 141.00, 140.22, 134.27, 134.15, 133.94, 133.16, 132.78, 131.89, 130.47, 126.16, 125.21, 121.47, 120.44. ESI-MS: calcd for $[M+H]^+$ m/z 338.1, found: 338.1. ESI-HRMS $[M+H]^+$ calcd for $C_{22}H_{16}N_3O$: 338.1288, found: 338.1286. HPLC: t_R = 2.944 min.

Acknowledgment

This work was financially supported by the National Natural Science Foundation of China (Grant No. 81473075).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2016.04.022.

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