European Journal of Medicinal Chemistry 220 (2021) 113453

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Design, synthesis, and biological evaluation of novel dual inhibitors targeting lysine specific demethylase 1 (LSD1) and histone deacetylases (HDAC) for treatment of gastric cancer

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ARTICLE INFO

Article history:
Received 10 October 2020
Received in revised form
14 March 2021
Accepted 15 March 2021
Available online 25 April 2021

Keywords:
LSD1
HDAC
Dual inhibitor
Anticancer

ABSTRACT

LSD1 and HDAC are physical and functional related to each other in various human cancers and simultaneous pharmacological inhibition of LSD1 and HDAC exerts synergistic anti-cancer effects. In this work, a series of novel LSD1/HDAC bifunctional inhibitors with a styrylpyridine skeleton were designed and synthesized based on our previously reported LSD1 inhibitors. The representative compounds **5d** and **5m** showed potent activity against LSD1 and HDAC at both molecular and cellular level and displayed high selectivity against MAO-A/B. Moreover, compounds **5d** and **5m** demonstrated potent antiproliferative activities against MGC-803 and HCT-116 cancer cell lines. Notably, compound **5m** showed superior *in vitro* anticancer potency against a panel of gastric cancer cell lines than **ORY-1001** and **SP-2509** with IC_{50} values ranging from 0.23 to 1.56 μM. Compounds **5d** and **5m** significantly modulated the expression of Bcl-2, Bax, Vimentin, ZO-1 and E-cadherin, induced apoptosis, reduced colony formation and suppressed migration in MGC-803 cancer cells. In addition, preliminary absorption, distribution, metabolism, excretion (ADME) studies revealed that compounds **5d** and **5m** showed acceptable metabolic stability in human liver microsomes with minimal inhibition of cytochrome P450s (CYPs). Those results indicated that compound **5m** could be a promising lead compound for further development as a therapeutic agent in gastric cancers *via* LSD1 and HDAC dual inhibition.

1. Introduction

Epigenetic regulation has played an important role in the progression of cancers [1]. Thus, anticancer drug discovery targeting epigenetic modifiers has attracted a lot of interest in the last years, leading to the approval of seven anticancer drugs including two DNA methyltransferase (DNMT) inhibitors and five histone deacetylases (HDAC) inhibitors as well as a number of clinical candidates [2–4].

Lysine-specific demethylase 1 (LSD1) is a critical epigenetic modifier associated with histone 3 methylation modification [5]. Through a flavin adenine dinucleotide (FAD) dependent oxidative reaction, LSD1 specifically demethylates H3K4me1/2, which are crucial marks relating to transcriptional activation, thereby regulating various key cellular processes including cell cycle, proliferation, differentiation and embryonic pluripotency. In specific context, LSD1 can catalyze H3K9me1/2 demethylation, changing its role from a transcriptional repressor to an activator [6]. In addition to histone, LSD1 also demethylates several other non-histone substrates, such as p53 [7], hypoxia-inducible factor 1α (HIF-1α) [8], E2F transcription factor 1 (E2F1) [9] and myosin phosphatase-targeting subunit 1 (MYPT1) [10]. Aberrant elevation of LSD1 is frequently observed and correlates with poor prognosis in various

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https://doi.org/10.1016/j.ejmech.2021.113453
0223-5234/

human solid cancers and leukemia [11,12]. Hence, targeting LSD1 represents a highly attractive strategy in modern medicinal chemistry research [13,14]. Over the past few years, numerous small-molecule LSD1 inhibitors have been reported and several of them are in various human clinical trials or preclinical stages for cancer therapy [15–23].

HDAC are a class of epigenetic enzymes that mediate transcriptional repression by removing acetyl group from lysine of histones and other proteins. Up to now, eighteen types of HDAC have been identified in human and they are divided into four groups, including the class I HDAC (HDAC1, 2, 3, and 8), Class II HDAC (HDAC4-7, 9 and 10), Class III HDAC (referred to as sirtuins), and class IV HDAC (only HDAC11) [24]. Class I, II and IV HDAC are Zn^{2+} dependent enzymes, while class III HDAC is NAD^{+} dependent enzymes. HDAC modulate multiple cellular processes in eukaryotes, including apoptosis, the cell cycle, growth and differentiation [25]. The dysregulation of HDAC, especially class I HDAC, are believed to be responsible for the onset and progression of various cancers [26]. In the past decades, HDACs have been one of the most intensively pursued targets for cancer therapy [27]. Up to now, five HDAC inhibitors have been approved by U.S. Food and Drug Administration (FDA) (vorinostat, romidepsin, panobinostat and belinostat) and China Food and Drug Administration (CFDA) (chidamide), respectively [28–31].

Interestingly, recent studies have confirmed that there are intimate physical and functional relationship between LSD1 and HDACs [32]. LSD1 and HDAC1/2 often coexist in the same repressive transcriptional complex as integral subunits including nucleosome remodeling and deacetylase (NuRD) [33], REST corepressor 1 (CoREST) [34] and Sin3A [35], and exert their demethylase and deacetylase activities in a mutual dependent manner. For example, LSD1 regulated pluripotency of embryonic stem and carcinoma cells through HDAC1 mediated deacetylation of H4K16 in an interdependent manner through CoREST complex [36]. LSD1 coordinates with the Sin3A/HDAC complex in repressing a series of pro-apoptotic genes and maintaining sensitivity to chemotherapy in breast cancer [35]. In addition, HDAC1 deacetylated LSD1 at K374 in its substrate binding domain, which facilitated the LSD1/H3 productive binding and promoted its demethylation activity and repressed the expression of target genes [37]. In breast cancer, LSD1 and HDAC5 were coordinately overexpressed, meanwhile, HDAC5 physically interacted with LSD1 and promoted its stability and demethylation activity by upregulating USP28, a bona fide deubiquitinase of LSD1 [38], contributing to the breast cancer development and progression. Conversely, depletion of HDAC5 resulted in LSD1 destabilization and suppressed the breast cancer cell proliferation and invasion [39,40]. Treatment with HDAC inhibitors suppressed the expression of LSD1 and promoted the H3K4 methylation as well as H3 acetylation [41], while loss of LSD1 sensitized cells to HDAC inhibitors. Co-administration of LSD1 and HDAC inhibitors displayed promising synergistic anticancer effect in various types of malignancies, including rhabdomyosarcoma [42], glioblastoma [43,44], breast cancer [40,45] Ewing sarcoma [46] and acute myelocytic leukemia (AML) [47].

Based on these evidence that LSD1 and HDAC are important cooperated players in cancers, and simultaneous inhibition of both targets synergistically blocked cancer cell proliferation, development of dual LSD1/HDAC inhibitors has emerged as a promising therapeutic approach for cancers. However, to date, only a few dual LSD1/HDAC inhibitors have been developed, and most of dual LSD1/HDAC inhibitors were designed by tethering known hydroxamic acid or benzamide group of HDAC inhibitors to the covalent LSD1 inhibitor tranylcypromine, as shown in Fig. 1. Compound **I**, **corin**, a hybrid derivative of tranylcypromine and entinostat developed by Kalin et al., simultaneously blocked CoREST complex LSD1 and HDAC1 activity with submicromolar potency and showed stronger anti-proliferative activity than single-target inhibitors alone or in combination in a panel of melamoma cells [48]. More recently, Shi's group found that co-inhibiting LSD1 and HDAC with **corin** potently impaired diffuse intrinsic pontine glioma growth *in vitro* and in vivo by re-programing chromatin to block cell cycle while promoting death and differentiation [49]. Our previous work has identified compound **II** as an effective dual LSD1/HDAC inhibitor, with stronger antitumor potency than SAHA against several tested human cancer cell lines [50]. A series of tranylcypromine-based LSD1/HDAC dual inhibitors was reported by Milelli's group, among which compound **III** exhibited good inhibitory activity towards HDAC1 and LSD1 with a K_i of 42.52 nM and an IC_{50} value of 3.85 μM, respectively. In vitro cell growth inhibition assay demonstrated that compound **III** exhibited more potent anti-proliferative effect than SAHA at 70 μM in MCF-7 cancer cell line [51]. **4SC-202** (compound **IV**) is the only one non-tranylcypromine based dual LSD1/HDAC inhibitor reported so far, which is currently being investigated in a phase I clinical trial for advanced hematological malignancies. **4SC-202** has also demonstrated potent activity against colorectal cancer [52], pancreatic cancer [53], hepatocellular cancer [54], medulloblastoma [55] and urothelial carcinoma [56]. Notably, a recent research revealed that in cutaneous T cell lymphoma cells, the anticancer activity of **4SC-202** was however not only due to LSD1/HDAC inhibition, but also its potent microtubule destabilizing activity [57].

Given the potential utility of co-targeting LSD1 and HDACs to treat cancers and the limited dual LSD1/HDAC inhibitors have been found, the identification of novel potent dual LSD1/HDAC inhibitors is urgently in need.

2. Results and discussion

2.1. Design of novel LSD1/HDAC dual inhibitors

Our group has been devoted to the development of novel small molecule LSD1 inhibitors [58–61]. Recently, we reported the discovery of a novel stilbene-based reversible FAD-competitive LSD1 inhibitor **GY-046** (depicted as compound **8c** in our previous article), which exhibiting an IC_{50} value of 283 nM (Fig. 2) [62]. Considering the chemical similarity between amidoxime and hydroxamic acid moiety, a well-known pharmacophore of HDAC inhibitors, we speculated that replacing the amidoxime of compound **GY-046** with a hydroxamic acid moiety would be tolerated while achieving HDAC inhibitory activity. Enzyme assays showed that compound **5a** displayed moderate LSD1 inhibition with an IC_{50} value of 3.55 μM, less potent than **GY-046** (IC_{50} = 0.283 μM). Consistent with our speculation, compound **5a** also exhibited moderate inhibitory activity against HDAC1/2/6 with IC_{50} values of 1.51, 2.06 and 0.40 μM in the enzymatic assay, respectively, while compound **GY-046** showed no obvious HDAC1/2 inhibitory activity at up to 100 μM. Their inhibitory activities against HCT-116 human lung cancer cells and MGC-803 human gastric cancer cells were evaluated using a CellTiter-Glo luminescent cell viability assay 72 h post-treatment (Fig. 2). Excitingly, compound **5a** showed better anticancer potency (IC_{50} = 3.89, 2.71 μM, respectively) than **GY-046** (43.3%, 35.5% inhibition at 10 μM, respectively), although its LSD1 inhibitory activities was about 10-fold less potent than that of **GY-046**. These results indicated that the cytotoxicity of compound **5a** might be the consequence of the LSD1/HDAC dual inhibition. Encouraged by the promising LSD1/HDACs dual inhibitory activity and anticancer efficacy, further structural optimization of compound **5a** was conducted. These studies led to the discovery of novel dual LSD1/HDAC inhibitors exhibiting excellent *in vitro* antitumor potency.

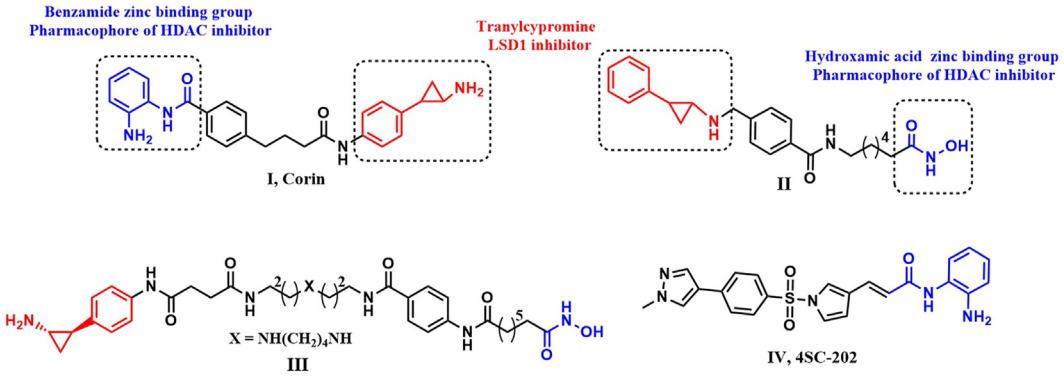


Fig. 1. Reported dual LSD1/HDAC inhibitors.

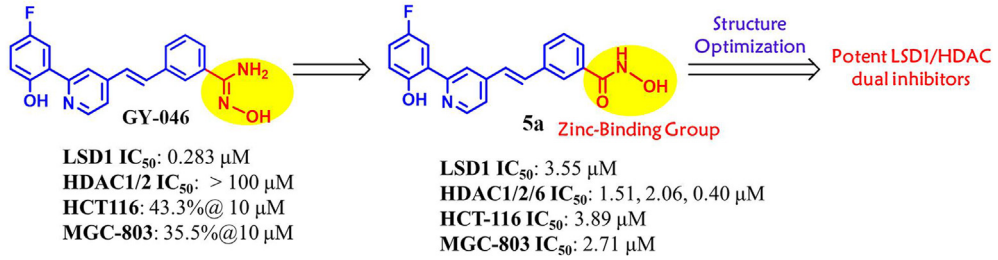
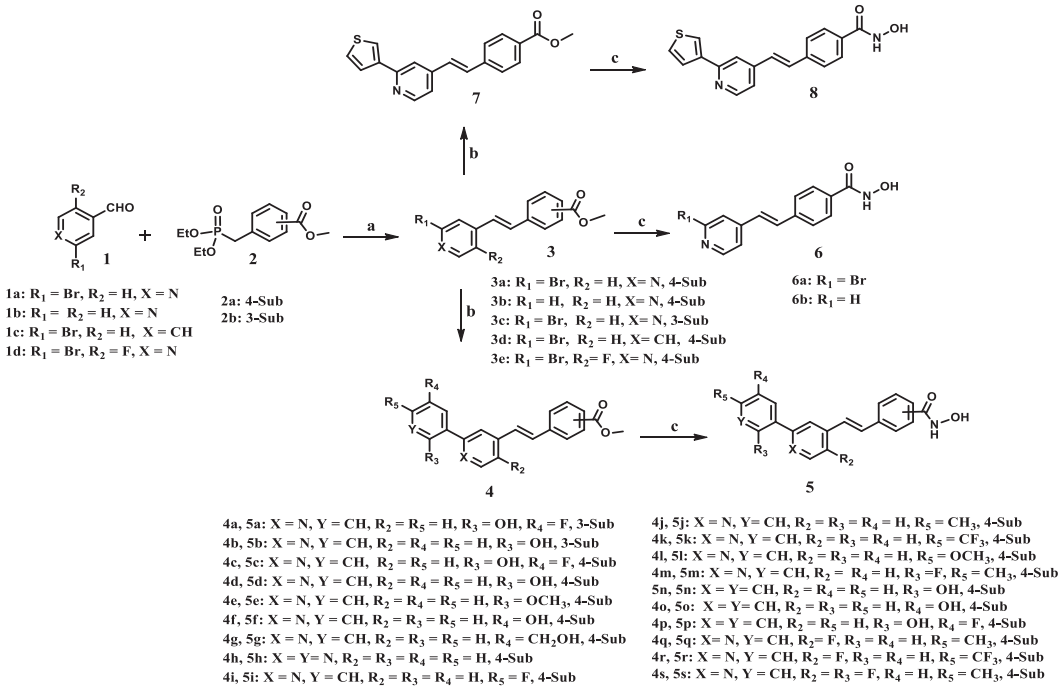


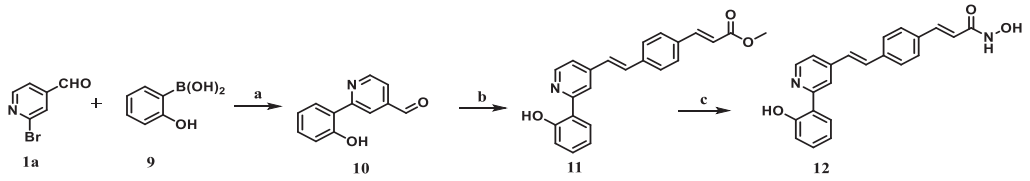
Fig. 2. The rationale design of LSD1/HDAC dual inhibitors.

2.2. Chemistry

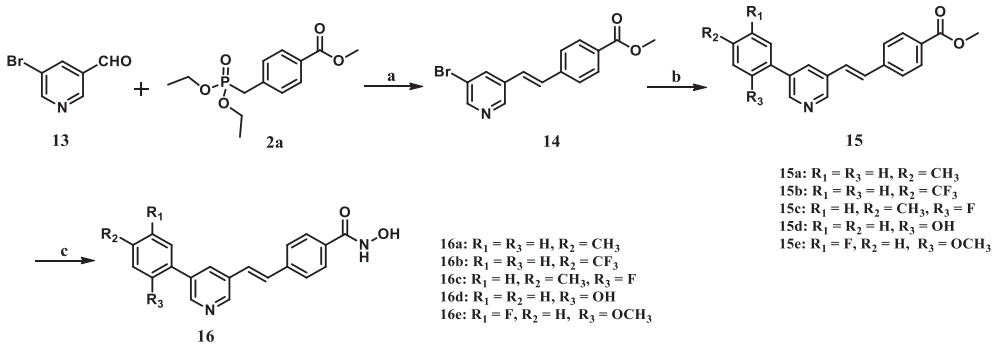
The general synthetic routes of the target compounds are shown in Schemes 1–3. Compounds **5a-s**, **6a-b** and **8** were prepared by the procedure presented in Scheme 1. Compounds **3a-e** were synthesized utilizing Horner-Wadsworth-Emmons (HWE) reaction of commercially available compounds **1a-d** with substituted diethyl benzylphosphonate (**2a-b**). A Suzuki coupling reaction between compounds **3a**, **3c-e** and different boronic acid compounds gave compounds **7** and **4a-s**. The treatment of **3a-b**, **4a-s** and **7** with



Scheme 1. Synthesis of compounds **3**–**8**. Reagents and conditions: (a) t-BuOK, anhydrous DMF, 0°C-rt, 0.5–1.5 h, yield 45.4%–76.1%; (b) R–$B(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, toluene, EtOH, H_2O, 95 °C, 4–6 h, yield 51.1%–81.2%; (c) $NH_2OH{\cdot}HCl$, KOH, anhydrous CH_3OH–CH_2Cl_2, rt, 1–2 h, yield 36.6%–87.3%.



Scheme 2. Synthesis of compounds **10**–**12**. Reagents and conditions: (a) $Pd(PPh_3)_4$, K_2CO_3, toluene, EtOH, H_2O, 95 °C, 4 h, yield 76%; (b) (E)-methyl 3-(4-((diethoxyphosphoryl) methyl) phenyl) acrylate, t-BuOK, anhydrous DMF, 0°C-rt, 1.5 h, yield 58.7%; (c) $NH_2OH{\cdot}HCl$, KOH, anhydrous CH_3OH–CH_2Cl_2, rt, 1.5 h, yield 72.9%.



Scheme 3. Synthesis of compounds **14**–**16**. Reagents and conditions: (a) t-BuOK, anhydrous DMF, 0°C-rt, 0.5 h, yield 80.1%; (b) R–$B(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, toluene, EtOH, H_2O, 95 °C, 1.0–3.5 h, yield 69.2%–81.7%; (c) $NH_2OH{\cdot}HCl$, KOH, anhydrous CH_3OH–CH_2Cl_2, rt, 1–2 h, yield 61.2%-.

NH_2OK in dry CH_3OH yielded target hydroxamic acids **6a-b**, **5a-s** and **8**. The synthesis of compound **12** was reported in Scheme 2. 2-bromoisonicotinaldehyde (**1a**) was subjected to a Suzuki coupling reaction with (2-hydroxyphenyl)boronic acid to obtain compound **10**. Subsequent HWE reaction of compound **10** with (E)-methyl 3-(4-((diethoxyphosphoryl)methyl) phenyl)acrylate provided the compound **11**, which was subsequently converted to **12** with freshly prepared NH_2OK methanol solution. Compounds **16a-e** were synthesized using the strategy similar to that of **5a-s** (Scheme 3). In this case, HWE condensation of 5-bromonicotinaldehyde 1**3** with compound **2a** led to compound **14**, which was coupled with corresponding boronic acid compounds via Suzuki coupling reaction to obtain compounds **15a-e**. Compounds **15a-e** were converted to the desired final hydroxamic acids **16a-e** using hydroxylamine hydrochloride in the presence of KOH.

2.3. In vitro LSD1 and HDAC1/5/6 inhibition assay

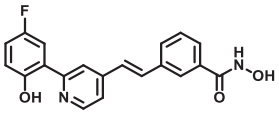
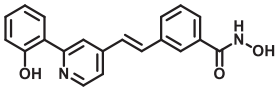
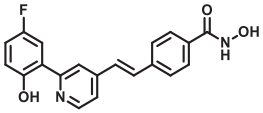
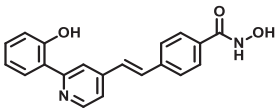
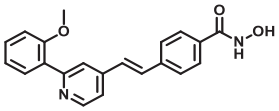
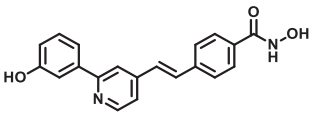
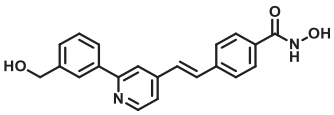
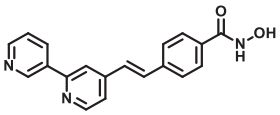
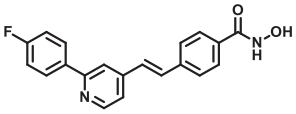
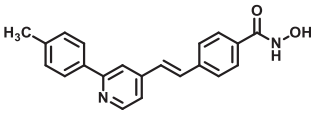
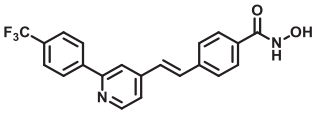
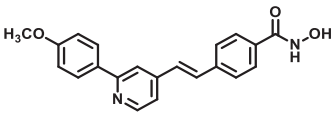
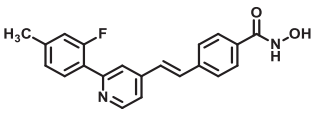
All the target compounds were evaluated against LSD1 and HDAC1/5/6, using LSD1 inhibitor **ORY-1001**, HDACs inhibitors **SAHA** and **LBH589** as the positive controls, respectively. As shown in Table 1, these target compounds generally showed good inhibitory activity against LSD1 and HDAC1/6 with IC_{50} values in the nano-micromolar to micromolar range, while showed no obvious activity against HDAC5 at 10 μM. Compound **5a** and **5b** was initially synthesized and evaluated against LSD1 and HDAC1/2/6 to validate our design strategy. Excitingly, compounds **5a** and **5b** showed good activity against HDAC1/2/6 (IC_{50} range: 0.39–2.06 μM), while maintaining moderate potency for LSD1 (IC_{50} = 3.57 and 2.26 μM, respectively). Changing the hydroxamate acid group of compounds **5a**-**5b** form *meta*-position to *para*-position resulted in **5c** and **5d**, respectively, which showed enhanced activities against both LSD1 and HDAC1/6. Installation of an electron-withdrawing group on the phenyl group at pyridine ring led to decreased LSD1 and HDAC1 inhibition, as exemplified by compound **5i** and **5k**. Converting the 2-phenyl group at pyridine ring of compound **5d** to pyridine or thiophene was generally tolerated for LSD1 and HDAC1/6 inhibition, leading to compound **5h** and **8** with potent activities toward LSD1 (IC_{50} = 1.37 and 2.03 μM, respectively) and HDAC1/6 (IC_{50} range: 0.014–0.58 μM). Introducing a fluorine in pyridine ring (**5q-5s**) generally gave remarkable reduced LSD1 inhibition activity compared with compounds **5j-5k** and **5m**. Compounds **5n-5p** were synthesized by replacing the pyridine motif with a phenyl ring. Unfortunately, their inhibitory activities toward both targets were attenuated. Compound **12**, with an *N*-hydroxycinnamamide fragment was synthesized to explore the effects of linker on LSD1 and HDAC1 inhibition. Compared with compound **5d**, compound **12** exhibited comparable HDAC1 activity, however, its inhibitory activity toward LSD1 was dramatically decreased (22.9% inhibition at 10 μM), indicating that a longer linker was detrimental for LSD1 inhibition. Switching the nitrogen atom at pyridine ring to *meta*-position of phenyl group greatly reduced the potency for both LSD1 and HDAC1/6 (**5j** vs **16a**, **5m** vs **16c**). Among all compounds tested, compounds **5d** and **5m** showed the most balanced inhibitory activities against LSD1 and HDAC1/6, with IC_{50} values of 0.93, 1.64, 0.22, 0.37, 0.047 and 0.015 μM, respectively.

Considering that the target compounds featured with a phenolic ring may interfere with the HRP used for the LSD1 assays, twelve compounds with IC_{50} values less than 2 μM were subjected to LSD1 inhibitor screening assay with H_2O_2 instead of LSD1 recombinant, so that false positive compounds that can react with H_2O_2 or HRP can be excluded. The result showed that all the tested compounds did not interfere with the enzymatic assays.

2.4. In vitro antiproliferative assay

All synthesized compounds were evaluated their anti-proliferative activities against HCT-116 (human colon cancer) and MGC-803 (human gastric cancer) cancer cell lines by the CellTiter-Glo luminescent cell viability assay. SAHA was used as the positive control. As shown in Table 2, most target compounds showed moderate to good inhibitory activity against the tested two cancer cell lines. Among the tested compounds, compounds **5d** (IC_{50} = 1.45 μM), **5f** (IC_{50} = 1.61 μM), **5g** (IC_{50} = 1.70 μM), **5m**

Table 1
Structures and *in vitro* LSD1 and HDAC inhibition for target compounds.

Comp.	Structures	IC_{50} (μM) or inhibition % at 10 μM			
		LSD1[a]	HDAC1[d]	HDAC5[d]	HDAC6[d]
5a		3.57 ± 0.55	1.51 (2.06)[c]	>10	0.40
5b		2.26 ± 0.35	1.47 (1.87)[c]	>10	0.39
5c		2.20 ± 0.34	1.14	9.34	0.13
5d		0.93 ± 0.03	0.22	>10	0.047
5e		1.07 ± 0.03	1.04	>10	0.028
5f		1.83 ± 0.22	0.29	>10	0.019
5g		1.14 ± 0.06	0.28	>10	0.034
5h		1.37 ± 0.14	0.58	>10	0.014
5i		21.2%@10 μM	N.D.[b]	N.D.	N.D.
5j		2.70 ± 0.43	0.96	>10	0.15
5k		33%@10 μM	4.84	N.D.	N.D.
5l		1.90 ± 0.28	0.69	>10	0.11
5m		1.64 ± 0.21	0.37	>10	0.015

(*continued on next page*)

Table 1 (*continued*)

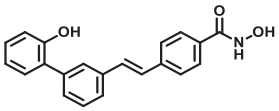
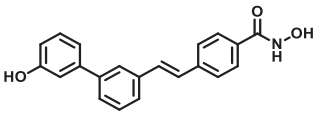
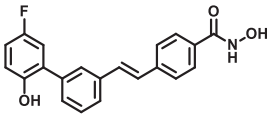
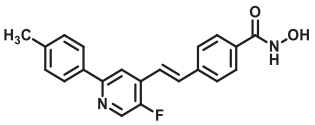
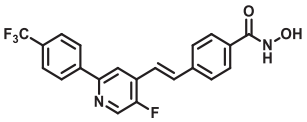
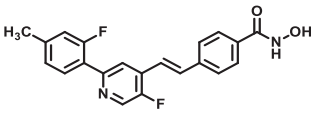
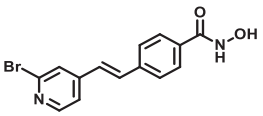
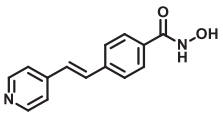
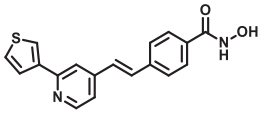
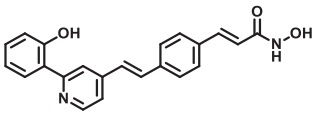
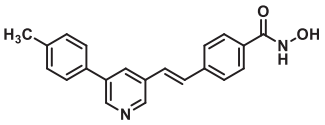
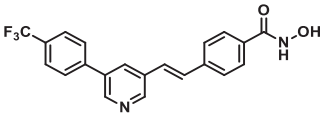
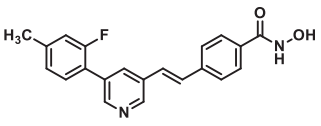
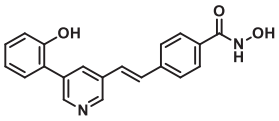
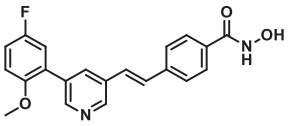
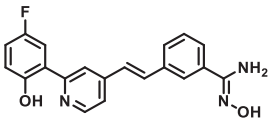
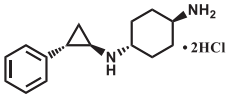
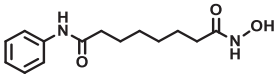
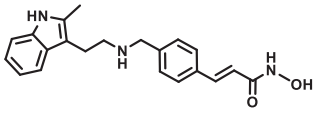
Comp.	Structures	IC_{50} (μM) or inhibition % at 10 μM			
		LSD1[a]	HDAC1[d]	HDAC5[d]	HDAC6[d]
5n		1.95 ± 0.29	0.93	>10	0.18
5o		3.65 ± 0.55	1.24	>10	0.20
5p		3.51 ± 0.54	2.12	>10	0.24
5q		20.7%@10 μM	1.18	N.D.	N.D.
5r		5.6%@10 μM	2.95	N.D.	N.D.
5s		55.2%@10 μM	0.88	>10	N.D.
6a		1.02 ± 0.008	3.66	>10	0.32
6b		0.85 ± 0.07	0.63	>10	0.13
8		2.03 ± 0.31	0.21	>10	0.054
12		22.9%@10 μM	0.47	N.D.	N.D.
16a		29.8%@10 μM	43.6	N.D.	N.D.
16b		5.6%@10 μM	N.D.	N.D.	N.D.
16c		3.06 ± 0.49	1.07	>10	0.15

Table 1 (*continued*)

Comp.	Structures	IC_{50} (μM) or inhibition % at 10 μM			
		LSD1[a]	HDAC1[d]	HDAC5[d]	HDAC6[d]
16d		0.51 ± 0.03	3.37	>10	0.013
16e		1.60 ± 0.21	0.67	>10	0.093
GY-046		0.283 ± 0.002	>100	N.D.	N.D.
ORY-1001		85%@0.015	N.D.	N.D.	N.D.
SAHA		>100	0.014	N.D.	0.0092
LBH589		N.D.	N.D.	0.29	N.D.

[a] IC_{50} values are the mean of at least three independent assays, presented as mean ± SD.
[b] N.D.: Not determined.
[c] IC_{50} Value for HDAC2.
[d] Values are the mean of at least two independent experiments, and the SD values are <20% of the mean.

(IC_{50} = 0.80 μM) and **5s** (IC_{50} = 1.67 μM) showed comparable antiproliferative activity to that of SAHA (IC_{50} = 1.85 μM) in the HCT-116 cell line. Compounds **5m** was about two-fold more active than SAHA against MGC-803 cell line with IC_{50} value of 0.77 μM. Particularly, consistent with the balanced and potent inhibitory activities toward LSD1/HDAC1/6, compound **5d** and **5m** showed greater potency than SAHA against both tested cancer cell lines. Surprisingly, compound **5h** with potent LSD1 (IC_{50} = 1.37 μM) and HDAC1/6 (IC_{50} = 0.58, 0.014 μM, respectively) inhibitory activity displayed moderate antiproliferative activity against HCT-116 and MGC-803 cell lines.

Although LSD1 inhibitors in clinical trials and approved HDAC inhibitors have shown good efficacy against hematologic cancers, they displayed limited anticancer potency as monotherapy for solid tumors. Base on the encouraging findings that the compound **5m** potently inhibited LSD1/HDAC activity and showed potent anticancer potency against MGC-803, the antiproliferative capacity of compound **5m** against a panel of gastric cancer cell lines including AGS, BGC-823, MKN-1, MKN-45, NCI—N87 and SNU-1, was further evaluated. Irreversible LSD1 inhibitor **ORY-1001**, reversible LSD1 inhibitor **SP-2509** and HDAC inhibitor **SAHA** were used as positive controls. The results depicted in Table 3 revealed that compound **5m** was more potent than **ORY-1001** and **SP-2509** against all the cell lines employed, with IC_{50} values ranging from 0.23 to 1.56 μM. The combination of **SP-2509** and **SAHA** were also evaluated to investigate the synergic effect between LSD1 and HDAC inhibitors. Compounds **SP-2509** and **SAHA** (1:1) possessed improved anticancer potency than that of **SP-2509** and **SAHA** used alone against BGC-823, NCI—N87 and SNU-1 cells. It was noteworthy that compound **5m** showed 6-fold better antiproliferative activity than the combination of **SP-2509** and **SAHA** in NCI—N87 cells and similar activities in other two gastric cancer cell lines, indicating that compound **5m** deserved further development for gastric cancer therapy.

2.5. Inhibitory properties of compounds 5d and 5 m against LSD1

Considering the balanced inhibitory activity against LSD1/HDAC and excellent antiproliferative activity of compounds **5d** and **5m,** a direct measure of compounds **5d** and **5m** binding to LSD1 was tested by surface plasmon resonance (SPR). At steady state, compounds **5d** and **5m** displayed potent affinity for LSD1 with the K_D values of 3.83 μM and 0.249 μM, respectively (Fig. 3A). Having confirmed compounds **5d** and **5m** as potent LSD1 inhibitors, the time dependent assay of compounds **5d** and **5m** against LSD1 was further performed. As shown in Fig. 3B, compound **5m** may inhibited LSD1 time-dependently, while compound **5d** can inactivated LSD1 in a time independent manner.

2.6. Enzyme selectivity assay

Compounds **5d** and **5m** were further evaluated for their activity against LSD1's homologies MAO-A and MAO-B, and clorgyline and R-(−)-deprenyl were employed as the positive controls, respectively. These results are summarized in Table 4. Compound **5d** had weak effect on MAO-A and MAO-B, with IC_{50} values of 34.1 and 69.4 μM, respectively, exhibiting selectivity of >36.7-fold against MAOA/B. Compound **5m** showed no significant inhibition against MAO-A and MAO-B at the concentration of 100 μM, showing excellent selectivity of >61-fold. These results indicated that

Table 2
Antiproliferative activity of target compounds against two human cancer cell lines.

Compounds	[a]IC_{50} (μM) or inhibition % at 10 μM	
	HCT-116	MGC-803
5a	3.89 ± 0.31	2.71 ± 0.19
5b	3.36 ± 0.19	2.81 ± 0.22
5c	2.04 ± 0.34	1.92 ± 0.25
5d	1.45 ± 0.16	1.42 ± 0.11
5e	8.44 ± 0.72	6.35 ± 0.43
5f	1.61 ± 0.09	2.10 ± 0.13
5g	1.70 ± 0.12	1.74 ± 0.23
5h	5.14 ± 0.81	4.55 ± 0.40
5i	>10	>10
5j	2.39 ± 0.16	1.91 ± 0.27
5k	>10	>10
5l	3.56 ± 0.38	3.51 ± 0.29
5m	0.80 ± 0.06	0.77 ± 0.09
5n	4.23 ± 0.72	5.99 ± 0.67
5o	>10	8.82 ± 0.73
5p	>10	>10
5q	>10	>10
5r	>10	>10
5s	1.67 ± 0.09	2.51 ± 0.22
6a	>10	>10
6b	3.91 ± 0.38	3.93 ± 0.26
8	1.91 ± 0.11	3.68 ± 0.27
12	>10	>10
16a	>10	>10
16b	>10	>10
16c	2.99 ± 0.22	2.24 ± 0.16
16d	4.68 ± 0.36	5.46 ± 0.33
16e	3.41 ± 0.19	3.36 ± 0.18
GY-046	43.3%@ 10 μM	35.5%@10 μM
SAHA	1.85 ± 0.15	1.54 ± 0.20

[a] The proliferation-inhibitory effects of compounds in HCT116 and MGC-803 cells were measured after 72 h treatment. IC_{50}Values represent a mean ± SD of at least three independent assays.

compounds **5d** and **5m** exhibited remarkable selectivity for LSD1 over MAO-A/B.

2.7. HDAC isoforms inhibition

To explore the inhibitory activity toward other HDAC isoforms, compounds **5d** and **5m** were evaluated against HDAC1, HDAC2, HDAC5, HDAC6, HDAC8 and HDAC11 with **SAHA** and **LBH589** as the reference drugs. As depicted in Table 5, besides HDAC1/6, compound **5d** and **5m** also displayed potent activity for HDAC2 with IC_{50} values of 934 nM and 404 nM, respectively, showing a >11-fold selectivity in favor of HDAC6 versus HDAC2. In addition, compound **5m** also exhibited modest potency toward HDAC8 (IC_{50} = 8.78 μM) and HDAC11 (IC_{50} = 4.93 μM), while compound **5d** showed moderate HDAC8 inhibition (IC_{50} = 4.62 μM) and displayed no significant HDAC11 inhibition at 10 μM. With the exception of HDAC5 and HDAC11, compound **5d** and **5m** were less active than SAHA against all other tested HDAC isoforms.

2.8. Intracellular target modulation

Based on the enzymatic assay and antitumor activity assay results, compound **5d** and **5m** were selected for further evaluation to examine whether they exerted on-target activity in a cellular setting. The ability of compounds **5d** and **5m** to inhibit cellular activity of LSD1 was accessed by analyzing the expression levels of H3K4Me1/2 and H3K9Me2 that regulated by LSD1's demethylase activity, using **ORY-1001** as a positive compound. Human MGC-803 cancer cells were treated with compound **5d**, **5m** or **ORY-1001** at indicated concentrations for 48 h. An equivalent volume of DMSO was added as the blank control. As depicted in Fig. 4A, in agreement with their LSD1 inhibitory potencies in enzymatic assays, compounds **5d a**nd **5m** dose-dependently upregulated H3K4Me1/2 and H3K9Me2 levels in MGC-803 cells, demonstrating that compounds **5d** and **5m** were effective LSD1 inhibitors at the cellular level. Next, compounds **5d** and **5m** were subjected for evaluation of intracellular HDAC inhibitory activity by investigating their effects on the acetylation levels of histone H3 (H3-Ac), a biomarker of HDAC1/2/3 inhibition. As shown in Fig. 4B, both compounds **5d** and **5m** potently inhibited the deacetylation of histone H3 in a dose-dependent manner.

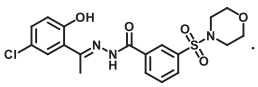
In addition, in order to further validate that compounds **5d** and **5m** are cell-active LSD1 inhibitors, RT-qPCR analysis of CD86, CD14 and CD11b, surrogate cellular biomarkers for LSD1 activity [64,65], were performed. As shown in Table 6, after treatment of MGC-803 cells with **5d a**nd **5m** for 48 h, significant induction of CD86, CD14 and CD11b mRNA amount were observed, indicating the ability to block LSD1 activity in cells.

2.9. Apoptotic assay

To investigate whether the novel LSD1/HDAC dual inhibitors can lead to cell death by apoptosis, the apoptotic effects of selected compounds **5d** and **5m** as well as reference compound SAHA were evaluated by annexin V-FITC/propidium iodide (PI) assay in MGC-803 cells. MGC-803 cells were incubated with different concentrations (0, 2.5 and 5.0 μM) of compounds **5d** and **5m** or SAHA (5.0 μM) for 48 h. As shown in Fig. 5A, compound **5d** induced apoptotic death in MGC-803 cells in a dose-dependent manner, the percentage of apoptotic cells was 4.9% (control), 33.2% (2.5 μM) and

Table 3
Antiproliferative activity of compound **5m** against six gastric cancer cell lines.

Compounds	IC_{50} (μM)[a]					
	AGS	BGC-823	MKN-1	MKN-45	NCI−N87	SNU-1
5m	1.56 ± 0.12	1.50 ± 0.11	0.40 ± 0.17	1.16 ± 0.08	0.23 ± 0.06	1.01 ± 0.02
ORY-1001	>50	>50	>50	>50	>50	>50
SP-2509[b,c]	6.19 ± 0.79	8.08 ± 0.96	8.98 ± 0.97	26.7 ± 1.48	10.54 ± 0.82	8.03 ± 0.73
SAHA	N.D.[d]	1.85 ± 0.10	N.D.	N.D.	1.36 ± 0.11	1.72 ± 0.07
SP-2509 + **SAHA**[e]	N.D.	1.39 ± 0.05	N.D.	N.D.	1.28 ± 0.04	1.52 ± 0.09

[a] IC_{50} Values represent a mean ± SD of at least three independent assays.
[b] LSD1: IC_{50} = 13 nM according to Ref. [63].
[c] The structure of SP-2509:  .
[d] N.D.: Not determined.
[e] The molar ratio of SP-2509 and SAHA was set to 1:1.

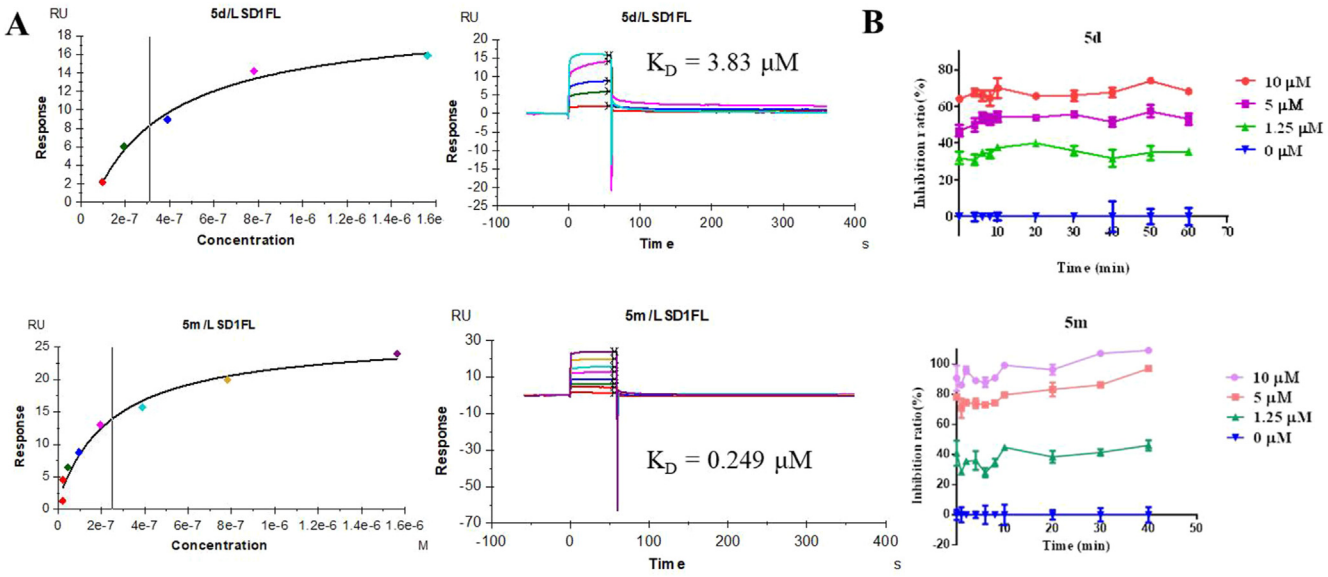


Fig. 3. Inhibitory properties of compounds **5d** and **5m** against LSD1 activity *in vitro*. (A) SPR based sensorgram of compounds **5d** and **5m**. (B) Time dependent assay of compounds **5d** and **5m** against LSD1. The stable progress curves for the LSD1 inhibition were obtained by indicated concentrations of compounds **5d** and **5m** treatment.

Table 4
In vitro inhibition of MAO-A, MAO-B and selectivity index for LSD1.

Compounds	IC_{50} (μM)[a]			Selectivity index
	LSD1	MAO-A	MAO-B	
5d	0.93 ± 0.03	34.1 ± 2.11	69.4 ± 3.45	>36.7
5m	1.64 ± 0.21	>100	>100	>61.0
Clorgyline	[c]N.D.	[b]0.0019	N.D.	–
R-(−)-deprenyl	N.D.	N.D.	[b] 0.069	–

[a] IC_{50} Values represent a mean ± SD of at least three independent assays.
[b] Values are the mean of at least two independent experiments, and the SD value are <20% of the mean.
[c] N.D.: Not determined.

Table 5
Inhibition of HDAC isoforms by compounds **5d** and **5m**

Compounds	IC_{50} (μM)[a]					
	HDAC1	HDAC2	HDAC5	HDAC6	HDAC8	HDAC11
5d	0.22	0.934	>10	0.047	4.62	>10
5m	0.37	0.404	9.40	0.015	8.78	4.93
SAHA	0.014	0.018	N.D.[b]	0.009	0.261	>10
LBH589	N.D.	N.D.	0.29	N.D.	N.D.	N.D.

[a] Values are the mean of at least two independent experiments, and the SD values are <20% of the mean.
[b] N.D.: Not determined.

71.9% (5.0 μM), respectively. The ability of compound **5m** to induce apoptosis was stronger than that of compound **5d**, the percentage of apoptotic cells was 4.3% (control), 77.9% (2.5 μM) and 82.2% (5.0 μM), respectively. The result revealed that compounds **5d** and **5m** could efficiently induce apoptosis of MGC-803 cells. At the concentration of 5.0 μM, the apoptotic induction activity of compounds **5d** and **5m** were comparable to that of SAHA(73.8%).

To further illustrate the mechanism of compounds **5m** and **5d**-induced apoptosis, the expressions of pro-apoptotic protein Bax and anti-apoptotic protein Bcl-2, two key proteins associated with the mitochondrial apoptotic pathway, were investigated by Western blot analysis. Treatment of MGC-803 cells with indicated concentrations of compounds **5d** and **5m** for 48 h significantly increased the expression of pro-apoptotic Bax, together with down-regulation of anti-apoptotic Bcl-2 in a dose-dependent manner (Fig. 5B).

2.10. Colony formation assay

To further investigate the cellular activity of the compounds **5d** and **5m**, their ability to inhibit the colony formation was determined in MGC-803 cancer cells. **ORY-1001** and **SAHA** were used as positive compounds. As reported in Fig. 6, compounds **5d** and **5m** could effectively inhibited the colony formation of MGC-803 cancer cells in a dose dependent manner, especially compound **5m** remarkably reduced colony formation at a dose of 0.6 μM, and more potent than the reference compound **ORY-1001** and **SAHA** at the same concentration.

2.11. Migration ability assay

Considering LSD1 plays important roles in cell migration, the migration inhibitory potency of compounds **5d** and **5m** on MGC-803 cancer cells were investigated by well-established wound healing assay and transwell assay, **ORY-1001** and **SAHA** were used as reference compounds. As depicted in Fig. 7A, treatment with various concentrations of compounds **5d** and **5m** for 12 or 24 h, significantly suppressed MGC-803 cell migration both dose- and time dependently, more potent than **ORY-1001**, which was further conformed using a transwell assay. As shown in Fig. 7B, a remarkable decrease in cell migration was observed after incubation with **5d** or **5m** in a dose-dependent manner. Notably, although the HDAC inhibitory activity of compound **5m** was much lower than that of SAHA, the MGC-803 cells migration was more potently suppressed by **5m** as compared to SAHA. Furthermore, the expression levels of tumor migration- and invasion-associated proteins at different concentrations of **5d** or **5m** were examined by Western blot analysis. We observed that compounds **5d** and **5m** remarkably down-regulated Vimentin, and up-regulated ZO-1 and E-cadherin expressions in MGC-803 cells (Fig. 7C). Taken together, these results indicated the promising potential of compounds **5d** and **5m** to produce an anti-metastasis effect in MGC-803 cells.

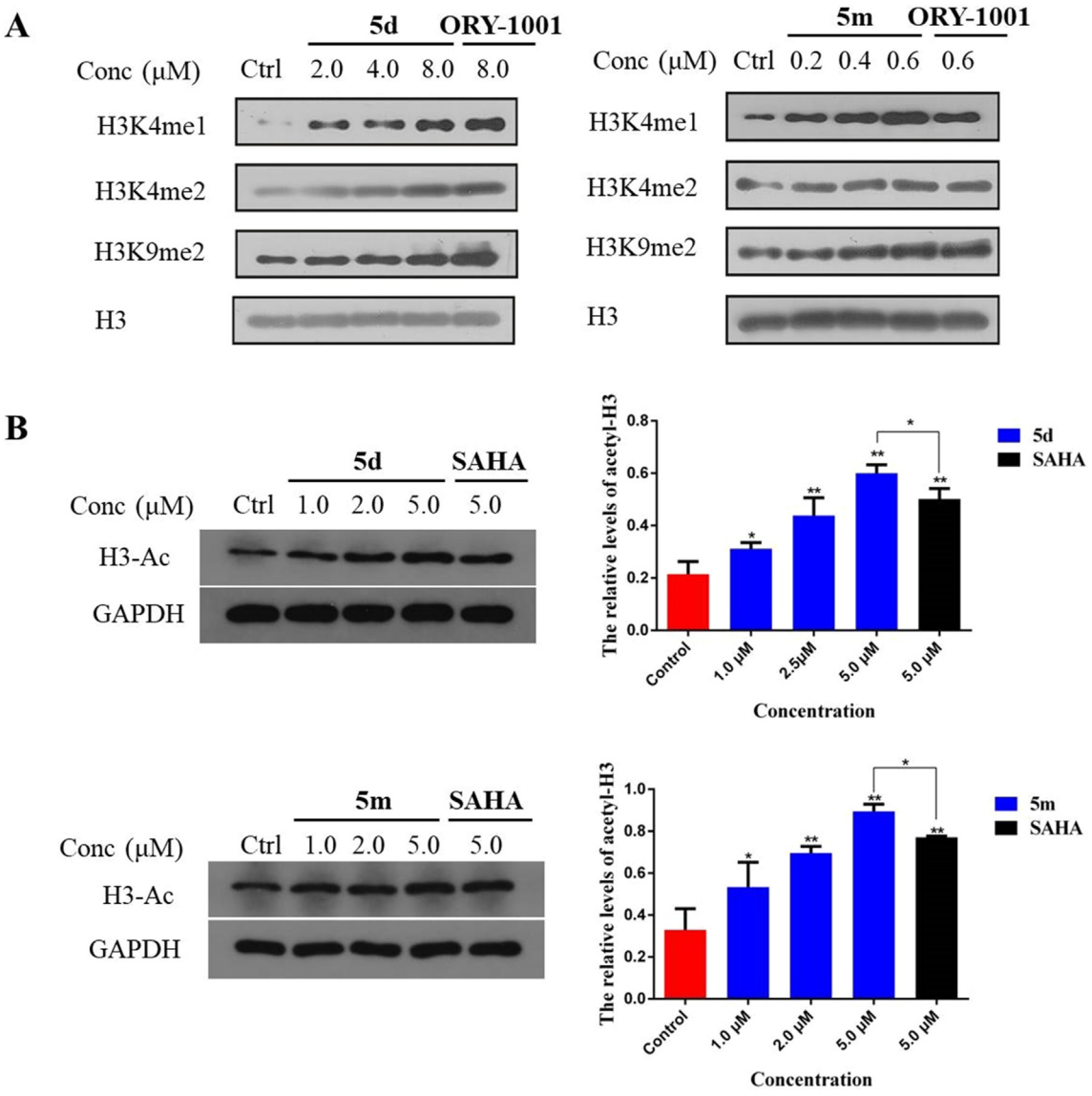


Fig. 4. Inhibitory effects of compounds **5d** and **5m** against LSD1 and HDAC in MGC-803 cells. (A) Western blot analysis of H3K4Me1/2 and H3K9Me2 after treated with **5d**, **5m** and the reference compound **ORY-1001** at indicated concentration for 48 h in MGC-803 cells. H3 was used as an endogenous control to normalize data. (B) Western blot analysis of acetylated histone H3 after treated with **5d**, **5m** and SAHA at indicated concentration for 48 h in MGC-803 cells. *$P < 0.05$, **$P < 0.01$.

Table 6
Relative mRNA level of CD86, CD14 and CD11b in MGC-803 induced by **5d** and **5m**

Compounds	Cellular data target modulation		
	CD86	CD14	CD11b
5d[b]	2.37 ± 0.16[a]	1.75 ± 0.07	3.11 ± 0.08
5d[c]	3.53 ± 0.38	4.25 ± 0.19	5.19 ± 0.51
5m[d]	1.73 ± 0.37	3.66 ± 0.42	2.30 ± 0.37
5m[b]	4.28 ± 0.39	4.99 ± 0.41	4.25 ± 0.80

[a] Data are expressed as fold increase compared to the vehicle (DMSO) and reported as mean value of at least two biological replicates, measured after 48 h of treatment, with Standard Deviation.
[b] Compound was tested at the dose of 5 μM.
[c] Compound was tested at the dose of 10 μM.
[d] Compound was tested at the dose of 2.5 μM.

2.12. ADMET properties assay

In silico absorption, distribution, metabolism, excretion and toxicity (ADMET) properties prediction of compounds **5d** and **5m** was performed by using molinspiration property calculator. The results presented in Table 7 showed that compounds **5d** and **5m** followed Lipinski's rule of five, a preliminary test for drug-likeness of compounds, indicating that these two compounds would not be expected to cause problems with oral bioavailability. Total polar surface area (TPSA) is a key property closely related to drug bioavailability and molecules with TPSA >140 $Å^2$ are generally considered to have low bioavailability. Compounds **5d** and **5m** showed favorable TPSA of 62.2 and 82.5 $Å^2$ and ABS % of 80.5% and 87.5%, respectively, suggesting that these two compounds theoretically should have good permeability and oral absorption.

The inhibition of human cytochrome P450 isoforms including CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 by compounds **5d** and **5m** were determined to evaluate their effects on liver function, because these enzymes are crucial in the drug metabolism. As shown in Table 8, compound **5m** showed no obvious inhibition against all tested cytochrome P450 isoforms at 10 μM, indicating

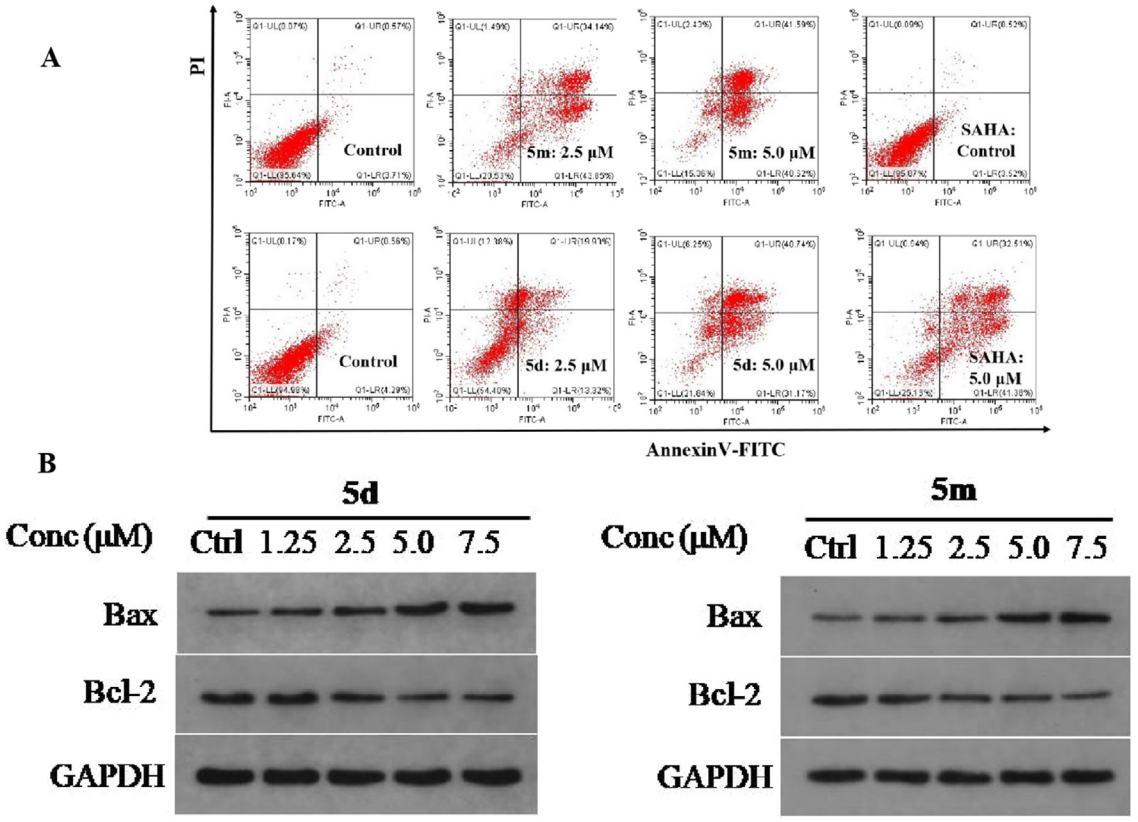


Fig. 5. (A) Propidium iodide/annexin V assay for the detection of early and late apoptotic MGC-803 cancer cells after treatment with the indicated concentrations of compounds **5d** and **5m** or SAHA for 48 h. (B) Western blots showing the expression levels of Bcl-2 and Bax proteins after treatment with indicated concentrations of **5d** and **5m** for 48 h.

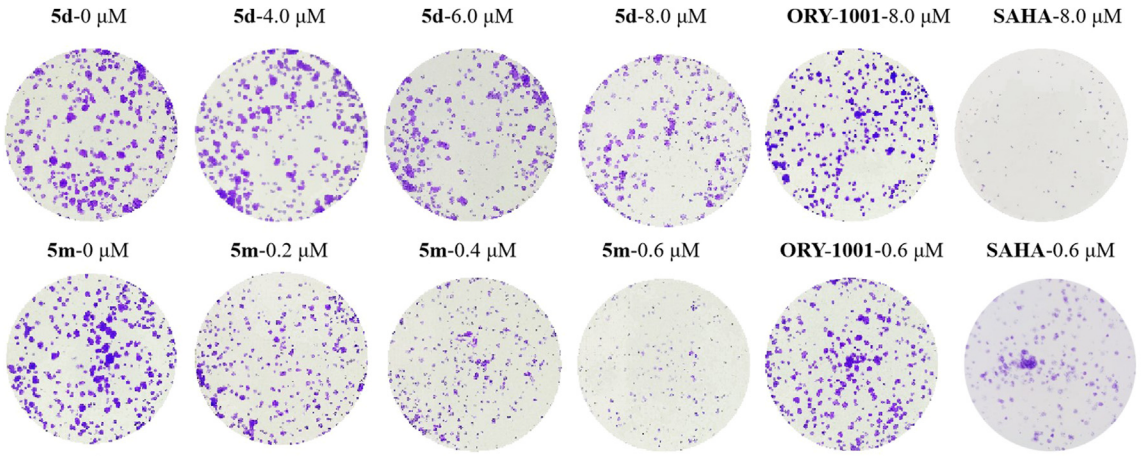


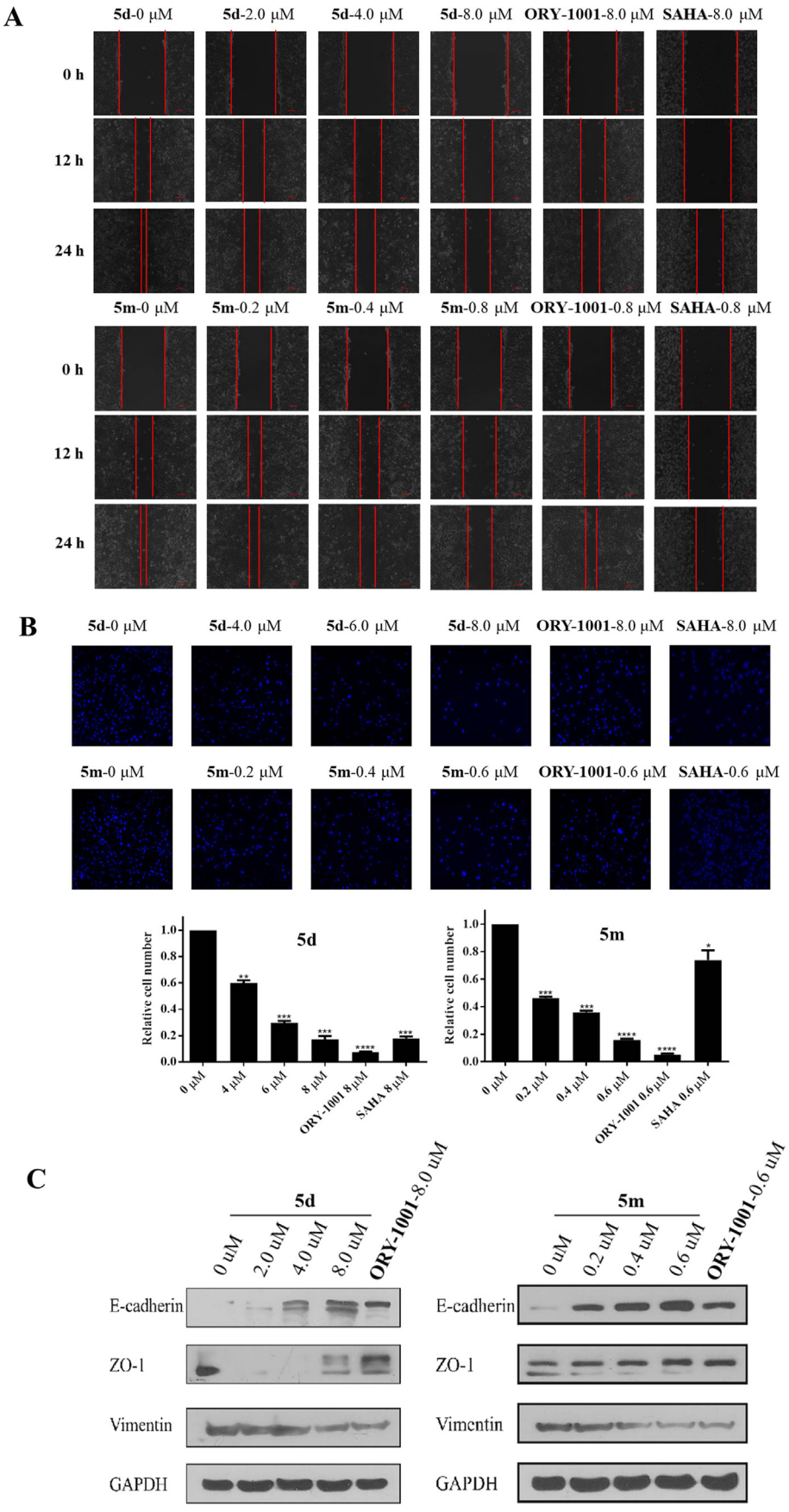
Fig. 6. Colony formation assay for MGC-803 cells dosed with compounds **5d**, **5m**, **ORY-1001** and **SAHA** at indicated concentration for 7 days.

good safety profiles regarding this aspect. Compound **5d** had a very weak effect on CYP1A2, CYP2C9, CYP2C19 and CYP2D6, while exhibited 87.9% inhibition against CYP3A4 at 10 μM.

The *in vitro* metabolic stability of compounds **5d** and **5m** were evaluated with human liver microsomes (HLM) and rat liver microsomes (RLM). Verapami was used as the reference compound. As depicted in Table 9, compounds **5d** and **5m** were not very stable in RLM, as shown by $T_{1/2}$ of 6.9 and 17.2 min, respectively. In HLM, compounds **5d** and **5m** exhibited $T_{1/2}$ of 33.0 and 51.3 min, respectively, indicating acceptable metabolic stability.

2.13. Molecular docking

To gain insights into the mechanism of dual LSD1/HDAC inhibition and to elucidate the binding mode of the most active compounds **5d** and **5m,** molecular docking calculations were performed using Glide of Maestro. The results were shown in Fig. 8. The Fig. 8A and D indicated that **5m** and **5d** were well docked at FAD site. Carbonyl in hydroximic acid of compound **5m** formed hydrogen bond as H-bond acceptor with Ser289 (Ser289NH···O =, bond length 1.8 Å). Hydroxyl in hydroximic acid formed hydrogen bond as H-bond donor with Thr624 (Thr624 = O··· HO, bond length


A
5d-0 μM
5d-2.0 μM
5d-4.0 μM
5d-8.0 μM
ORY-1001-8.0 μM
SAHA-8.0 μM
0 h
12 h
24 h
5m-0 μM
5m-0.2 μM
5m-0.4 μM
5m-0.8 μM
ORY-1001-0.8 μM
SAHA-0.8 μM
0 h
12 h
24 h
B
5d-0 μM
5d-4.0 μM
5d-6.0 μM
5d-8.0 μM
ORY-1001-8.0 μM
SAHA-8.0 μM
5m-0 μM
5m-0.2 μM
5m-0.4 μM
5m-0.6 μM
ORY-1001-0.6 μM
SAHA-0.6 μM
5d
Relative cell number
1.0
0.8
0.6
0.4
0.2
0.0
0 μM
4 μM
6 μM
8 μM
ORY-1001 8 μM
SAHA 8 μM
5m
Relative cell number
0 μM
0.2 μM
0.4 μM
0.6 μM
ORY-1001 0.6 μM
SAHA 0.6 μM
C
5d
0 uM
2.0 uM
4.0 uM
8.0 uM
ORY-1001-8.0 uM
5m
0 uM
0.2 uM
0.4 uM
0.6 uM
ORY-1001-0.6 uM
E-cadherin
ZO-1
Vimentin
GAPDH
E-cadherin
ZO-1
Vimentin
GAPDH

1.9 Å). In **5m**, hydrophobic 2-fluoro-4-methyl benzene and Trp751 formed π-π stacking interaction, pyridine and Tyr761 also formed π-π stacking interaction. Carbonyl in hydroximic acid of compound **5d** formed hydrogen bond as H-bond acceptor with Ser289 (Ser289NH···O = , bond length 1.8 Å). Hydroxyl in hydroximic acid formed hydrogen bond as H-bond donor with Thr624 (Thr624 = O···HO, bond length 1.9 Å). Phenol and pyridine in **5d** formed π-π stacking interaction with Trp751 and Tyr761, respectively.

As shown in Fig. 8B and E, compounds **5m** and **5d** well entered the pocket of HDAC1. The direct interaction between zinc-binding group (ZBG) of HDAC inhibitors and zinc ion in active sites was necessary for the production of inhibitory activity. The hydroximic acid of compound **5m** acted as ZBG coordinated to the catalytic Zn^{2+} of HDAC1. In addition, hydroximic acid formed H-bond with Gly149 (Gly149 = O···HN, bond length 1.9 Å) and His178 (His178NH···O = , bond length 2.1 Å), respectively. Benzene connected with hydroximic acid formed π-π stacking interaction with His141. Hydroimic acid of compound 5d formed coordination bond as ZBG with Zn^{2+}. Hydroimic acid formed H-bond with Gly149 (Gly149 = O···HN, bond length 1.9 Å) and His178 (His178NH···O = , bond length 2.1 Å) respectively. Hydroxy in Phenol formed H-bond with Asp99 (Asp99 = O···HO, bond length 1.6 Å). Benzene connected to hydroximic acid with His141 formed π-π stacking interaction.

As shown in Fig. 8C and F, compounds **5m** and **5d** entered the active site of HDAC6 well, and their ZBG (hydroximic acid) formed coordination bond with Zn^{2+} in the active site. The hydroximic acid in compound **5m** formed H-bond with His573 (His573 N···HO, bond length 2.0 Å) and Tyr745 (Tyr745OH···O = , bond length 1.9 Å), respectively. Benzene connected with hydroximic acid formed π-π stacking interaction with His574 and Pe583. The hydroximic acid in compound **5d** formed H-bond with His573 (His573 N···HO, bond length 1.9 Å) and Tyr745 (Tyr745OH···O = , bond length 1.9 Å), respectively. Hydroxy in phenol as H-bond donor formed H-bond with Asn645 (Asn645 = O···HO, bond length 1.9 Å). Benzene connected with hydroximic acid formed π-π stacking interaction with His574, Phe583 and Phe643, respectively.

3. Conclusion

In conclusion, a series of styrylpyridine derivatives bearing hydroxamic acid moiety was designed and identified as novel dual LSD1/HDAC inhibitors. The representative compounds **5d** and **5m** potently inhibited HDAC6 with nanomolar potency as well as HDAC1/2 with IC_{50} values in the submicromolar concentration range. Compounds **5d** and **5m** also exhibited strong inhibition of LSD1 with IC_{50} values of 0.93 and 1.64 μM, respectively, confirmed by SPR assay with K_D values of 3.83 and 0.294 μM, respectively, and showed high selectivity against the homologous MAO-A and MAO-B enzymes. In MGC-803 cancer cells, these two compounds dose-dependently upregulated the expression of H3K4Me1/2, H3K9Me2, and H3-Ac, indicating the simultaneous inhibition of intracellular LSD1 and HDAC. *In vitro* cell growth inhibition assay showed that compounds **5d** and **5m** exerted potent anti-proliferative activities against MGC-803 and HCT-116 cancer cells. In particular, **5m** strongly suppressed the proliferation of all tested six gastric cancer cells with IC_{50} values ranging from 0.23 to 1.56 μM, more potent than the reference compounds **ORY-1001** and **SP-2509**. Moreover, compared with an equimolar mixture of **SAHA** and **SP-2509**, compound **5m** showed superior antiproliferative effect against NCI—N87 cancer cells. Western blot analysis confirmed that compounds **5d** and **5m** effectively modulated apoptotic-related and EMT-related proteins including Bcl-2, Bax, Vimentin, ZO-1 and E-cadherin in a dose-dependent manner, and then significantly promoted apoptosis and inhibited the migration in MGC-803 cancer cells. In addition, compounds **5d** and **5m** possessed better inhibitory effect on colony formation of MGC-803 cells than **ORY-1001** and **SAHA**. Furthermore, compounds **5d** and **5m** showed acceptable metabolic stability in human liver microsomes and displayed no significant P450 isoforms inhibitory activity at 10 μM. Docking study of compounds **5d** and **5m** suggested that they bound tightly to the binding pocket of HDAC1/6 and LSD1, consistent with the results of enzymatic assays. This study highlighted the advantages of LSD1/HDAC dual inhibitors as an efficient strategy for the treatment of gastric cancer. Further structural optimization and anticancer activity studies are in progress in our laboratory.

Table 7
Calculated physicochemical parameters of compounds **5d** and **5m**

Comp	Lipinski's Parameters					TPSA[f]	ABS%[g]
	LogP[a]	MW[b]	HBA[c]	HBD[d]	nviolations[e]		
5d	3.7	332.3	5	3	0	82.5	80.5
5m	4.5	348.4	4	2	0	62.2	87.5

[a] Calculated lipophilicity.
[b] Molecular weight.
[c] Number of hydrogen bond acceptor.
[d] Number of hydrogen bond donor.
[e] Number of violation from Lipinski's rule of five.
[f] Total polar surface area.
[g] Percentage of absorption.

4. Experimental section

4.1. Chemistry

4.1.1. General procedures

Reagents and solvents were purchased from commercial sources, when necessary, were purified and dried by standard methods. Melting points were determined on an X-5 micromelting apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance III HD 400 MHz and 100 MHz spectrometer at room temperature, using TMS as an internal standard. Chemical shifts were reported in ppm (d). Spin multiplicities were described as s (singlet), brs (broad singlet), d (doublet), dd (double doublet), dt (double triplet), td (triple doublet), t (triplet), or m (multiplet). Coupling constants were reported in hertz (Hz). High resolution mass spectrometry (HRMS) was recorded on a Bruker MicrOTOF-Q III Micro mass spectrometer by electrospray ionization (ESI). Flash chromatography was performed on 200—300 mesh silica gel with the indicated solvent systems (Qingdao Haiyang Chemical, China). ^{1}H NMR, ^{13}C NMR, and HRMS data for the final target compounds were provided in the supplementary data.

4.1.2. (E)-methyl 4-(2-(2-bromopyridin-4-yl)vinyl)benzoate (*3a*)

To a stirred solution of compound **1a** (376 mg, 2 mmol) and compound **2a** (601.1 mg, 2.1 mmol) in dry DMF (10 mL) was added t-BuOK (673.3 mg, 6 mmol) at 0 °C. Then, the above reaction mixture was stirred for 0.5 h at room temperature. The mixture was

Fig. 7. Changes on the migration of MGC-803 cells under treatment of various doses of **5d**, **5m ORY-1001** and **SAHA**. (A) Effect of compounds **5d** and **5m** on the migration in MGC-803 cells by wound healing assay. (B) Effect of compounds **5d** and **5m** on the migration in MGC-803 cells by transwell assay. *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$, ****$P < 0.001$, compared with the blank control group. (C) The changes on expression levels of EMT related proteins induced by **5d** and **5m** in MGC-803 cells.

Table 8
Inhibitory activity against P450 isoforms of compounds **5d** and **5m**

Compounds	% inhibition at 10 μM[c]				
	CYP1A2	CYP2C9	CYP 2C19	CYP 2D6	CYP 3A4
5d	48.4	13.1	18.7	15.0	87.9
5m	0.95	11.7	22.8	5.8	47.6
α-Naphthoflavone[a]	90.9@ 0.2 μM	N.D.[b]	N.D.	N.D.	N.D.
Sulfaphenazole[a]	N.D	79.9@1.0 μM	ND	N.D.	N.D.
Ticlopidine[a]	N.D.	N.D.	67.0@3.0 μM	N.D.	N.D.
Quinidine[a]	N.D.	N.D.	N.D.	91.3@1.0 μM	N.D.
Ketoconazole[a]	N.D.	N.D.	N.D.	N.D.	65.9@0.1 μM

[a] Positive control.
[b] N.D.: Not determined.
[c] Values are the mean of at least two independent experiments, and the SD value are <20% of the mean.

Table 9
The *in vitro* metabolic stability of compounds **5d** and **5m** in HLM and RLM.

Compound	Species	$T_{1/2}$[a] (min)	CL_{int}[b] (μL/min/mg)
5d	Rat	6.9	505.42
	Human	33.0	105.06
5m	Rat	17.2	201.91
	Human	51.3	67.38
Verapami[c]	Rat	12.7	273.93
	Human	26.9	128.80

[a] Half-life.
[b] Clearance.
[c] Positive control.

poured into cold water (50 mL), the resultant precipitate was filtered, washed with water, dried and purified by silica gel column chromatography (Petroleum ether: EtOAc = 5: 1) to afford the pure product **3a** (396.4 mg) as a white powder, Yield: 62.3%, Mp:106–107 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (d, 1H, J = 5.2 Hz), 8.08 (d, 2H, J = 8.4 Hz), 7.60–7.57 (m, 3H), 7.34–7.29 (m, 2H), 7.04 (d, 1H, J = 16.4 Hz), 3.95 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.57, 150.44, 146.96, 143.04, 139.95, 133.46, 130.39, 130.20, 127.05, 126.87, 125.16, 120.09, 52.27. HRMS (ESI) calcd for $C_{15}H_{13}BrNO_2$ $[M + H]^+$: 318.0124, Found: 318.0126.

*4.1.2.1. (E)-methyl 4-(2-(pyridin-4-yl)vinyl)benzoate (**3b**).* The title compound (**3b**) was synthesized using isonicotinaldehyde (**1b**) and methyl 4-((diethoxyphosphoryl)methyl)benzoate (**2a**) in a manner similar to that described for the synthesis of compound **3a**. White powder, Yield: 76.1%, Mp: 101–102 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (d, 2H, J = 6.0 Hz), 8.05 (d, 2H, J = 8.4 Hz), 7.58 (d, 2H, J = 8.4 Hz), 7.37 (d, 2H, J = 6.4 Hz), 7.30 (d, 1H, J = 16.4 Hz), 7.10 (d, 1H, J = 16.4 Hz), 3.92 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.65, 150.30, 144.00, 140.48, 131.98, 130.12, 129.97, 128.44, 126.87, 121.01, 52.20. HRMS (ESI) calcd for $C_{15}H_{13}NNaO_2$ $[M + Na]^+$: 262.0838, Found: 262.0841.

*4.1.2.2. (E)-methyl 3-(2-(2-bromopyridin-4-yl)vinyl)benzoate (**3c**).* The title compound (**3c**) was synthesized using 2-bromoisonicotinaldehyde (**1a**) and methyl 3-((diethoxyphosphoryl)methyl)benzoate (**2b**) in a manner similar to that described for the synthesis of compound **3a**. White powder, Yield: 45.4%, Mp: 73–74 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (d, 1H, J = 5.2 Hz), 8.21 (t, 1H, J = 2.0 Hz), 8.00 (dt, 1H, J_1 = 1.2 Hz, J_2 = 8.0 Hz), 7.70 (dt, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 7.57 (s, 1H), 7.48 (t, 1H, J = 8.0 Hz), 7.35–7.30 (m, 2H), 7.03 (d, 1H, J = 16.4 Hz), 3.96 (s, 3H). HRMS (ESI) calcd for $C_{15}H_{12}BrNNaO_2$ $[M + Na]^+$: 339.9944, Found: 339.9943.

*4.1.2.3. (E)-methyl 4-(3-bromostyryl)benzoate (**3d**).* The title compound (**3d**) was synthesized using 3-bromobenzaldehyde (**1c**) and methyl 4-((diethoxyphosphoryl)methyl)benzoate (**2a**) in a manner similar to that described for the synthesis of compound **3a**. White powder, Yield: 75.6%, Mp: 117–118 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.97 (d, 2H, J = 8.4 Hz), 7.89 (t, 1H, J = 2.0 Hz), 7.74 (d, 2H, J = 8.4 Hz), 7.64 (dt, 1H, J_1 = 1.2 Hz, J_2 = 8.0 Hz), 7.51–7.48 (m, 1H), 7.43 (d, 2H, J = 4.0 Hz), 7.37 (t, 1H, J = 8.0 Hz), 3.86 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.41, 141.90, 139.66, 131.31, 131.20, 130.09, 129.65, 129.38, 129.04, 127.28, 126.38, 122.76, 52.57. HRMS (ESI) calcd for $C_{16}H_{13}BrNaO_2$ $[M + Na]^+$: 338.9991, Found: 338.9996.

*4.1.2.4. (E)-methyl 4-(2-(2-bromo-5-fluoropyridin-4-yl)vinyl)benzoate (**3e**).* The title compound (**3e**) was synthesized using 2-bromo-5-fluoroisonicotinaldehyde (**1d**) and methyl 4-((diethoxyphosphoryl)methyl)benzoate (**2a**) in a manner similar to that described for the synthesis of compound **3a**. White powder, Yield: 52.9%, Mp: 177–178 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, 1H, J = 2.0 Hz), 8.07 (d, 2H, J = 8.4 Hz), 7.66 (d, 1H, J = 5.2 Hz), 7.62 (d, 2H, J = 8.4 Hz), 7.40 (d, 1H, J = 16.4 Hz), 7.19 (d, 1H, J = 16.4 Hz), 3.94 (s, 3H). HRMS (ESI) calcd for $C_{15}H_{12}BrFNO_2$ $[M + H]^+$: 336.0030, Found: 336.0026.

*4.1.2.5. (E)-methyl 3-(4-((E)-2-(2-(2-hydroxyphenyl)pyridin-4-yl)vinyl)phenyl) acrylate (**11**).* The title compound (**11**) was synthesized using compound **10** and (E)-methyl 3-(4-((diethoxyphosphoryl) methyl)phenyl)acrylate in a manner similar to that described for the synthesis of compound **3a**. White powder, Yield: 58.7%, Mp: 149–150 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.28 (s, 1H), 8.60 (d, 1H, J = 5.2 Hz), 8.41 (s, 1H), 8.14 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 7.83–7.79 (m, 3H), 7.74 (d, 2H, J = 8.0 Hz), 7.69 (d, 1H, J = 16.0 Hz), 7.64 (dd, 1H, J_1 = 1.2 Hz, J_2 = 5.2 Hz), 7.47 (d, 1H, J = 16.0 Hz), 7.36–7.31 (m, 1H), 6.99–6.92 (m, 2H), 6.72 (d, 1H, J = 16.0 Hz), 3.75 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.13, 159.83, 157.82, 146.99, 146.88, 144.32, 138.60, 134.86, 133.98, 131.91, 129.46, 128.10, 127.62, 127.42, 119.87, 119.27, 119.23, 118.58, 118.42, 117.31, 51.99. HRMS (ESI) calcd for $C_{23}H_{19}NNaO_3$ $[M + Na]^+$: 380.1257, Found: 380.1256.

*4.1.2.6. (E)-methyl 4-(2-(5-bromopyridin-3-yl)vinyl)benzoate (**14**).* The title compound (**14**) was synthesized using 5-bromonicotinaldehyde (**13**) and methyl 4-((diethoxyphosphoryl) methyl)benzoate (**2a**) in a manner similar to that described for the synthesis of compound **3a**. White powder, Yield: 80.1%, Mp: 116–117 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, 1H, J = 2.0 Hz), 8.57 (d, 1H, J = 2.0 Hz), 8.06 (d, 2H, J = 8.4 Hz), 8.00 (t, 1H, J = 2.0 Hz), 7.58 (d, 2H, J = 8.4 Hz), 7.19 (d, 1H, J = 16.4 Hz), 7.10 (d, 1H, J = 16.4 Hz), 3.93 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.65, 149.88, 146.75, 140.46, 135.24, 134.17, 131.16, 130.16, 129.88, 126.68,

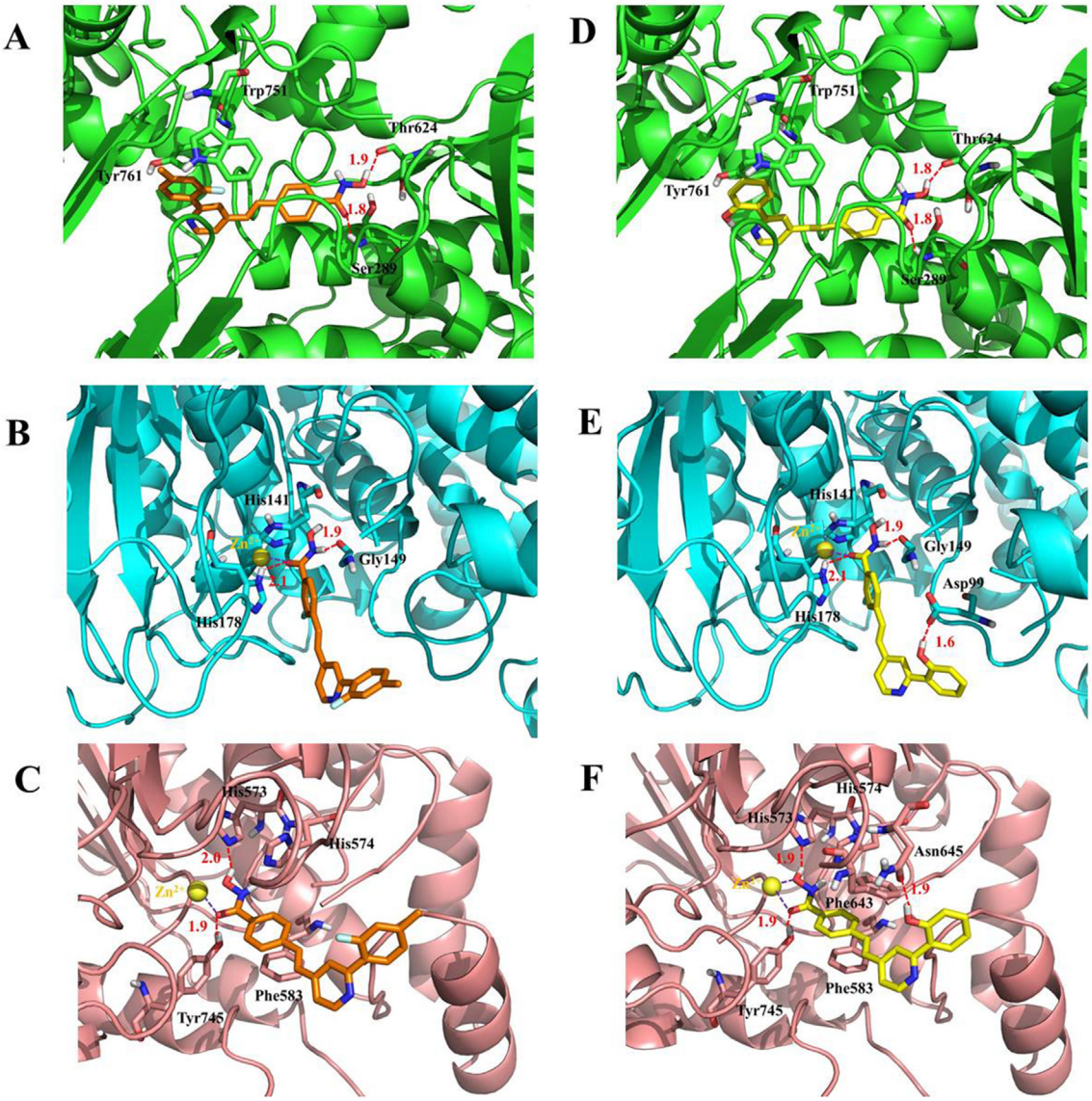


Fig. 8. Small molecules were denoted by stick and proteins were presented by cartoon. The carbon atoms of compound **5m** and **5d** were orange and yellow, the nitrogen atoms and the oxygen atoms were blue and red, respectively. The red dotted line and purple dotted lines represented H-bond and coordination bonds. A, B and C mean the binding modes of **5m** in the active sites of LSD1 (green), HDAC1 (cyan) and HDAC6 (pink), respectively. D, E and F stand for the binding patterns of **5d** in the active sites of LSD1, HDAC1 and HDAC6, respectively.

125.74, 121.08, 52.23. HRMS (ESI) calcd for $C_{15}H_{13}BrNO_2$ $[M + H]^+$: 318.0124, Found: 318.0122.

*4.1.3. (E)-methyl 3-(2-(2-(5-fluoro-2-hydroxyphenyl)pyridin-4-yl) vinyl)benzoate (**4a**)*

To a solution of compound **3c** (332.2 mg, 1.0 mmol) in toluene (7 mL), ethanol (1.5 mL), and H_2O (7 mL) was added K_2CO_3 (1.04 g, 7.5 mmol) followed by $Pd(PPh_3)_4$ (58 mg, 0.050 mmol) and (5-fluoro-2-hydroxyphenyl)boronic acid (202.7 mg, 1.3 mmol) under N_2 in a 50 mL two-necked flask. The reaction mixture was stirred at 95°Cfor 5 h, and then cooled to room temperature. To the reaction mixture was added aqueous NH_4Cl (10 mL), extracted by EtOAc for three times, dried over Na_2SO_4, and evaporated in vacuum to afford the crude product, which was purified by flash chromatography on silica gel with (Petroleum ether: EtOAc = 4: 1) to give compound **4a** as a yellowish white powder (290.7 mg). Yield: 80.3%, Mp: 119–120 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.06 (s, 1H), 8.49 (d, 1H, J = 5.6 Hz), 8.26 (s, 1H), 8.03 (d, 1H, J = 7.6 Hz), 7.87 (s, 1H), 7.75 (d, 1H, J = 7.6 Hz), 7.55 (dd, 1H, J_1 = 2.8 Hz, J_2 = 10.0 Hz), 7.50 (t, 1H, J = 7.6 Hz), 7.44–7.38 (m, 2H), 7.18 (d, 1H, J = 16.4 Hz), 7.07–7.03 (m, 1H), 7.00–6.96 (m, 1H), 4.43 (q, 2H, J = 7.2 Hz), 1.44 (t, 3H, J = 7.2 Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.23, 157.30 (d, $J_{C\text{-}F}$ = 2.0 Hz), 156.91 (d, $J_{C\text{-}F}$ = 236.0 Hz), 156.18 (d, $J_{C\text{-}F}$ = 2.0 Hz), 146.34, 146.28, 136.12, 133.33, 131.29, 129.97, 129.03, 128.14, 126.79, 119.55 (d, $J_{C\text{-}F}$ = 8.1 Hz), 118.99, 118.91 (d, $J_{C\text{-}F}$ = 7.1 Hz), 118.43 (d, $J_{C\text{-}F}$ = 23.2 Hz), 116.74, 111.76 (d, $J_{C\text{-}F}$ = 24.2 Hz), 61.30, 14.38. HRMS (ESI) calcd for $C_{22}H_{19}FNO$ $[M + H]^+$: 364.1343, Found: 364.1345.

*4.1.3.1. (E)-methyl 3-(2-(2-(2-hydroxyphenyl)pyridin-4-yl)vinyl) benzoate (**4b**).* The title compound (**4b**) was synthesized using compound **3c** and (2-hydroxyphenyl)boronic acid in a manner similar to that described for the synthesis of compound **4a**. Yellowish white powder, Yield: 57.4%, Mp: 142–143 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.31 (s, 1H), 8.60 (d, 1H, J = 5.2 Hz), 8.46 (s,

1H), 8.28 (t, 1H, $J = 1.6$ Hz), 8.16 (dd, 1H, $J_1 = 1.2$ Hz, $J_2 = 8.0$ Hz), 7.99 (dt, 1H, $J_1 = 1.2$ Hz, $J_2 = 8.0$ Hz), 7.95 (dt, 1H, $J_1 = 1.2$ Hz, $J_2 = 7.6$ Hz), 7.91 (d, 1H, $J = 16.4$ Hz), 7.66 (dd, 1H, $J_1 = 1.2$ Hz, $J_2 = 5.2$ Hz), 7.62 (t, 1H, $J = 8.0$ Hz), 7.48 (d, 1H, $J = 16.4$ Hz), 7.36–7.31 (m, 1H), 6.99–6.93 (m, 2H), 3.91 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.48, 159.87, 157.84, 146.97, 146.85, 137.18, 133.69, 131.92, 131.88, 130.80, 129.93, 129.76, 128.36, 127.51, 127.41, 119.94, 119.22, 118.43, 117.31, 52.77. HRMS (ESI) calcd for $C_{21}H_{18}NO_4$ $[M + H]^+$: 332.1281, Found: 332.1288.

*4.1.3.2. (E)-methyl4-(2-(2-(5-fluoro-2-hydroxyphenyl)pyridin-4-yl) vinyl)benzoate(**4c**).* The title compound (**4c**) was synthesized using compound **3a** and (5-fluoro-2-hydroxyphenyl)boronic acid in a manner similar to that described for the synthesis of compound **4a**. Yellowish white powder, Yield: 69.2%, Mp: 164–165 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.05 (s, 1H), 8.62 (d, 1H, $J = 5.2$ Hz), 8.47 (s, 1H), 8.04–8.01 (m, 3H), 7.88 (d, 1H, $J = 16.4$ Hz), 7.81 (d, 2H, $J = 8.4$ Hz), 7.66 (d, 1H, $J = 5.6$ Hz), 7.48 (d, 1H, $J = 16.4$ Hz), 7.19 (td, 1H, $J_1 = 3.2$ Hz, $J_2 = 8.4$ Hz), 6.94 (dd, 1H, $J_1 = 4.8$ Hz, $J_2 = 8.8$ Hz), 3.87 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.32, 156.68 (d, $J_{C\text{-}F} = 3.0$ Hz), 156.02, 155.60 (d, $J_{C\text{-}F} = 234.3$ Hz), 147.23, 146.76, 141.17, 133.75, 130.23, 129.83, 128.81, 127.79, 120.80, 119.68 (d, $J_{C\text{-}F} = 7.1$ Hz), 119.51 (d, $J_{C\text{-}F} = 8.1$ Hz), 118.69 (d, $J_{C\text{-}F} = 23.2$ Hz), 117.67, 113.18 (d, $J_{C\text{-}F} = 25.3$ Hz), 52.66. HRMS (ESI) calcd for $C_{21}H_{15}FNO_3$ $[M - H]^-$: 348.1041, Found: 348.1042.

*4.1.3.3. (E)-methyl 4-(2-(2-(2-hydroxyphenyl)pyridin-4-yl)vinyl) benzoate (**4d**).* The title compound (**4d**) was synthesized using compound **3a** and (2-hydroxyphenyl)boronic acid in a manner similar to that described for the synthesis of compound **4a**. Yellowish white powder, Yield: 71.1%, Mp: 155–156 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.25 (s, 1H), 8.62 (d, 1H, $J = 5.2$ Hz), 8.44 (s, 1H), 8.15 (dd, 1H, $J_1 = 1.6$ Hz, $J_2 = 8.0$ Hz), 8.03 (d, 2H, $J = 8.0$ Hz), 7.88–7.82 (m, 3H), 7.66 (dd, 1H, $J_1 = 1.2$ Hz, $J_2 = 5.2$ Hz), 7.53 (d, 1H, $J = 16.4$ Hz), 7.36–7.31 (m, 1H), 6.99–6.93 (m, 2H), 3.88 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.33, 159.81, 157.87, 147.05, 146.60, 141.22, 133.52, 131.93, 130.22, 129.81, 129.02, 127.81, 127.44, 120.00, 119.25, 118.42, 117.50, 52.66. HRMS (ESI) calcd for $C_{21}H_{16}NO_4$ $[M - H]^-$: 330.1136, Found: 330.1138.

*4.1.3.4. (E)-methyl 4-(2-(2-(2-methoxyphenyl)pyridin-4-yl)vinyl) benzoate (**4e**).* The title compound (**4e**) was synthesized using compound **3a** and (2-methoxyphenyl)boronic acid in a manner similar to that described for the synthesis of compound **4a**. White powder, Yield: 74.2%, Mp: 132–133 °C. ^{1}H NMR (400 MHz,$CDCl_3$) δ 8.68 (d, 1H, $J = 5.2$ Hz), 8.05 (d, 2H, $J = 8.4$ Hz), 7.88 (s, 1H), 7.77 (dd, 1H, $J_1 = 2.0$ Hz, $J_2 = 7.6$ Hz), 7.60 (d, 2H, $J = 8.4$ Hz), 7.41–7.36 (m, 1H), 7.34–7.30 (m, 2H), 7.17 (d, 1H, $J = 16.4$ Hz), 7.09 (dt, 1H, $J_1 = 0.8$ Hz, $J_2 = 7.6$ Hz), 7.03 (d, 1H, $J = 8.0$ Hz), 3.93 (s, 3H), 3.88 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.72, 156.95, 156.90, 149.85, 143.73, 140.78, 131.58, 131.18, 130.12, 130.07, 129.82, 129.19, 129.06, 126.85, 122.83, 121.09, 118.78, 111.44, 55.74, 52.19. HRMS (ESI) calcd for $C_{22}H_{20}NO_3$ $[M + H]^+$: 346.1438, Found: 346.1437.

*4.1.3.5. (E)-methyl 4-(2-(2-(3-hydroxyphenyl)pyridin-4-yl)vinyl) benzoate (**4f**).* The title compound (**4f**) was synthesized using compound **3a** and (3-hydroxyphenyl)boronic acid in a manner similar to that described for the synthesis of compound **4a**. White powder, Yield: 65.6%, Mp: 198–199 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.60 (s, 1H, $J = 5.2$ Hz), 8.64 (d, 1H, $J = 5.2$ Hz), 8.13 (s, 1H), 8.02 (d, 2H, $J = 8.0$ Hz), 7.82 (d, 2H, $J = 8.0$ Hz), 7.78 (d, 1H, $J = 16.4$ Hz), 7.60–7.54 (m, 3H, $J = 8.4$ Hz), 7.50 (d, 1H, $J = 16.4$ Hz), 7.32 (t, 1H, $J = 8.0$ Hz), 6.87 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 8.0$ Hz), 3.87 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.36, 158.23, 157.15, 150.38, 145.27, 141.45, 140.49, 132.30, 130.17, 129.56, 129.45, 127.67, 120.30, 118.01, 117.82, 116.60, 113.94, 52.64. HRMS (ESI) calcd for $C_{21}H_{16}NO_3$ $[M - H]^-$: 330.1139, Found: 330.1136.

*4.1.3.6. (E)-methyl 4-(2-(2-(3-(hydroxymethyl)phenyl)pyridin-4-yl) vinyl)benzoate (**4g**).* The title compound (**4g**) was synthesized using compound **3a** and (3-(hydroxymethyl)phenyl)boronic acid in a manner similar to that described for the synthesis of compound **4a**. White powder, Yield: 79.1%, Mp: 184–185 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.67 (d, 1H, $J = 4.8$ Hz), 8.18 (s, 1H), 8.13 (s, 1H), 8.03–8.00 (m, 3H), 7.83–7.75 (m, 3H), 7.58–7.46 (m, 3H), 7.42 (d, 1H, $J = 7.6$ Hz), 5.30 (t, 1H, $J = 5.6$ Hz), 4.62 (d, 2H, $J = 5.6$ Hz), 3.87 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.38, 157.26, 150.46, 145.37, 143.56, 141.46, 138.90, 132.34, 130.19, 129.60, 129.50, 128.96, 127.73, 127.69, 125.44, 125.21, 120.27, 118.05, 63.42, 52.63. HRMS (ESI) calcd for $C_{22}H_{20}BrN_2O_3$ $[M + H]^+$: 346.1436, Found: 346.1436.

*4.1.3.7. (E)-methyl 4-(2-([2,3′-bipyridin]-4-yl)vinyl)benzoate (**4h**).* The title compound (**4h**) was synthesized using compound **3a** and pyridin-3-ylboronic acid in a manner similar to that described for the synthesis of compound **4a**. White powder, Yield: 78.5%, Mp: 152–153 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.35 (d, 1H, $J = 2.0$ Hz), 8.71 (d, 1H, $J = 5.2$ Hz), 8.67 (dd, 1H, $J_1 = 1.2$ Hz, $J_2 = 4.8$ Hz), 8.51 (dt, 1H, $J_1 = 1.6$ Hz, $J_2 = 8.0$ Hz), 8.32 (s, 1H), 8.02 (d, 2H, $J = 8.0$ Hz), 7.85–7.80 (m, 3H), 7.61 (d, 1H, $J = 4.8$ Hz), 7.56 (dd, 1H, $J_1 = 4.8$ Hz, $J_2 = 8.0$ Hz), 7.49 (d, 1H, $J = 16.4$ Hz), 3.88 (s, 3H). HRMS (ESI) calcd for $C_{20}H_{17}N_2O_2$ $[M + H]^+$: 317.1285, Found: 317.1283.

*4.1.3.8. (E)-methyl 4-(2-(2-(4-fluorophenyl)pyridin-4-yl)vinyl)benzoate (**4i**).* The title compound (**4i**) was synthesized using compound **3a** and(4-fluorophenyl)boronic acid in a manner similar to that described for the synthesis of compound **4a**. White powder, Yield: 51.1%, Mp: 116–117 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (s, 4H), 8.01 (s, 3H), 7.80 (s, 4H), 7.35 (s, 2H), 4.33 (s, 3H). HRMS (ESI) calcd for $C_{21}H_{17}FNO_2$ $[M + H]^+$: 334.1238, Found: 334.1239.

*4.1.3.9. (E)-methyl 4-(2-(2-(p-tolyl)pyridin-4-yl)vinyl)benzoate (**4j**).* The title compound (**4j**) was synthesized using compound **3a** and *p*-tolylboronic acid in a manner similar to that described for the synthesis of compound **4a**. White powder, Yield: 81.2%, Mp: 140–141 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (dd, 1H, $J_1 = 0.4$ Hz, $J_2 = 5.2$ Hz), 8.06 (d, 2H, $J = 8.4$ Hz), 7.93 (d, 2H, $J = 8.4$ Hz), 7.77–7.76 (m, 1H), 7.61 (d, 2H, $J = 8.4$ Hz), 7.36 (d, 2H, $J = 16.4$ Hz), 7.34–7.28 (m, 3H), 7.18 (d, 2H, $J = 16.4$ Hz), 3.93 (s, 3H), 2.42 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.70, 158.18, 150.05, 144.72, 140.62, 139.14, 136.48, 131.75, 130.15, 129.90, 129.53, 128.88, 126.87, 126.83, 118.97, 117.94, 52.23, 21.33. HRMS (ESI) calcd for $C_{22}H_{20}NO_2$ $[M + H]^+$: 330.1489, Found: 330.1487.

*4.1.3.10. (E)-methyl 4-(2-(2-(4-(trifluoromethyl)phenyl)pyridin-4-yl) vinyl)benzoate (**4k**).* The title compound (**4k**) was synthesized using compound **3a** and (4-(trifluoromethyl)phenyl)boronic acid in a manner similar to that described for the synthesis of compound **4a**. White powder, Yield: 73.4%, Mp: 142–143 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (d, 1H, $J = 4.8$ Hz), 8.15 (d, 1H, $J = 8.4$ Hz), 8.15 (d, 2H, $J = 8.4$ Hz), 8.08 (d, 2H, $J = 8.4$ Hz), 7.84 (d, 1H, $J = 2.0$ Hz), 7.76 (d, 2H, $J = 8.4$ Hz), 7.64 (d, 2H, $J = 8.4$ Hz), 7.43–7.38 (m, 2H), 7.21 (d, 2H, $J = 16.0$ Hz), 3.94 (s, 3H). HRMS (ESI) calcd for $C_{22}H_{17}F_3NO_2$ $[M + H]^+$: 384.1206, Found: 384.1206.

*4.1.3.11. (E)-methyl 4-(2-(2-(4-methoxyphenyl)pyridin-4-yl)vinyl) benzoate (**4l**).* The title compound (**4l**) was synthesized using compound **3a** and (4-methoxyphenyl)boronic acid in a manner similar to that described for the synthesis of compound **4a**. White powder, Yield: 74.8%, Mp: 118–119 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (d, 1H, $J = 5.2$ Hz), 8.06 (d, 2H, $J = 8.4$ Hz), 7.99 (d, 2H,

J = 8.8 Hz), 7.73 (t, 1H, J = 0.8 Hz), 7.61 (d, 2H, J = 8.4 Hz), 7.35 (d, 1H, J = 16.4 Hz), 7.28 (dd, 1H, J_1 = 1.6 Hz, J_2 = 5.2 Hz), 7.17 (d, 1H, J = 16.4 Hz), 7.02 (d, 2H, J = 8.8 Hz), 3.93 (s, 3H), 3.87 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.70, 160.56, 157.82, 149.98, 144.69, 140.63, 131.87, 131.70, 130.14, 129.89, 128.92, 128.24, 126.86, 118.57, 117.54, 114.15, 55.39, 52.23. HRMS (ESI) calcd for $C_{22}H_{20}NO_3$ [M + H]$^+$: 346.1438, Found: 346.1436.

*4.1.3.12. (E)-methyl4-(2-(2-(2-fluoro-4-methylphenyl)pyridin-4-yl) vinyl)benzoate (**4m**).* The title compound (**4m**) was synthesized using compound **3a** and (2-fluoro-4-methylphenyl)boronic acid in a manner similar to that described for the synthesis of compound **4a**. White powder, Yield: 79.0%, Mp: 139–140 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, 1H, J = 5.2 Hz), 8.06 (d, 2H, J = 8.4 Hz), 7.89 (t, 1H, J = 8.4 Hz), 7.85 (t, 1H, J = 2.0 Hz), 7.61 (d, 2H, J = 8.4 Hz), 7.37–7.32 (m, 2H), 7.17 (d, 1H, J = 16.4 Hz), 7.10–7.07 (m, 1H), 7.02–6.98 (m, 1H), 3.93 (s, 3H), 2.41 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.69, 160.35 (d, $J_{C\text{-}F}$ = 250.2 Hz), 154.18 (d, $J_{C\text{-}F}$ = 2.5 Hz), 150.07, 144.42, 141.37 (d, $J_{C\text{-}F}$ = 8.5 Hz), 140.59, 131.92, 130.67 (d, $J_{C\text{-}F}$ = 3.4 Hz), 130.12, 129.90, 128.75, 126.89, 125.40 (d, $J_{C\text{-}F$ = 3.0 Hz), 124.33 (d, $J_{C\text{-}F$ = 11.5 Hz), 121.90 (d, $J_{C\text{-}F$ = 9.3 Hz), 119.27, 116.70 (d, $J_{C\text{-}F$ = 22.9 Hz), 52.22, 21.20 (d, $J_{C\text{-}F$ = 1.7 Hz). HRMS (ESI) calcd for $C_{22}H_{19}FNO_2$ [M + H]$^+$: 348.1394, Found: 348.1396.

*4.1.3.13. (E)-methyl 4-(2-(2′-hydroxy-[1,1′-biphenyl]-3-yl)vinyl)benzoate (**4n**).* The title compound (**4n**) was synthesized using compound **3d** and (2-hydroxyphenyl)boronic acid in a manner similar to that described for the synthesis of compound **4a**. White powder, Yield: 48.2%, Mp: 158–159 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.57 (s, 1H), 7.96 (d, 2H, J = 8.4 Hz), 7.79–7.75 (m, 3H),7.59 (dt, 1H, J_1 = 1.2 Hz, J_2 = 7.6 Hz), 7.51–7.40 (m, 3H), 7.37 (d, 1H, J = 16.4 Hz), 7.30 (dd, 1H, J_1 = 1.6 Hz, J_2 = 7.6 Hz), 7.19 (td, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 6.96 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.4 Hz), 6.90 (td, 1H, J_1 = 0.8 Hz, J_2 = 7.2 Hz), 3.86 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.49, 154.80, 142.40, 139.62, 136.73, 131.97, 130.86, 130.08, 129.67, 129.14, 128.84, 128.65, 128.16, 127.97, 127.64, 127.10, 125.46, 119.92, 116.50, 52.55. HRMS (ESI) calcd for $C_{22}H_{17}O_3$ [M − H]$^-$: 329.1183, Found: 329.1187.

*4.1.3.14. (E)-methyl 4-(2-(3′-hydroxy-[1,1′-biphenyl]-3-yl)vinyl)benzoate (**4o**).* The title compound (**4o**) was synthesized using compound **3d** and (3-hydroxyphenyl)boronic acid in a manner similar to that described for the synthesis of compound **4a**. White powder, Yield: 71.1%, Mp: 147–148 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.57 (s, 1H), 7.98 (d, 2H, J = 8.4 Hz), 7.89 (t, 1H, J = 1.6 Hz), 7.77 (d, 2H, J = 8.4 Hz), 7.64 (dt, 1H, J_1 = 1.6 Hz, J_2 = 7.6 Hz), 7.55–7.53 (m, 1H), 7.50–7.44 (m, 3H), 7.29 (t, 1H, J = 8.0 Hz), 7.16–7.13 (m, 1H), 7.10 (t, 1H, J = 2.0 Hz), 6.82 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 3.86 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.47, 158.31, 142.35, 141.87, 141.38, 137.63, 131.70, 130.40, 130.10, 129.79, 128.75, 128.11, 127.13, 127.01, 126.32, 125.64, 118.05, 115.07, 114.12, 52.54. HRMS (ESI) calcd for $C_{21}H_{17}O_3$[M − H] $^-$: 329.1183, Found: 329.1181.

*4.1.3.15. (E)-methyl 4-(2-(5′-fluoro-2′-hydroxy-[1,1′-biphenyl]-3-yl) vinyl)benzoate (**4p**).* The title compound (**4p**) was synthesized using compound **3d** and (5-fluoro-2-hydroxyphenyl)boronic acid in a manner similar to that described for the synthesis of compound **4a**. White powder, Yield: 72.6%, Mp: 148–149 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.63 (s,1H), 7.97 (d, 2H, J = 8.4 Hz), 7.83 (t, 1H, J = 2.0 Hz), 7.76 (d, 2H, J = 8.4 Hz), 7.62 (dt, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 7.55–7.42 (m, 3H),7.40 (d, 1H, J = 16.8 Hz), 7.17 (dd, 1H, J_1 = 3.2 Hz, J_2 = 9.6 Hz), 7.04 (td, 1H, J_1 = 3.2 Hz, J_2 = 8.8 Hz), 6.97 (dd, 1H, J_1 = 4.8 Hz, J_2 = 8.8 Hz), 3.86 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.48, 156.13 (d, $J_{C\text{-}F}$ = 235.3 Hz), 151.14 (d, $J_{C\text{-}F}$ = 2.0 Hz), 142.35, 138.48, 136.88, 131.79, 130.09, 129.60, 128.94 (d, $J_{C\text{-}F}$ = 8.1 Hz), 128.94, 128.70, 128.06, 127.84, 127.09, 125.98, 117.37 (d, $J_{C\text{-}F}$ = 8.1 Hz), 116.75 (d, $J_{C\text{-}F}$ = 23.2 Hz), 115.23 (d, $J_{C\text{-}F}$ = 22.2 Hz), 52.53. HRMS (ESI) calcd for $C_{22}H_{16}FNO_3$ [M − H]$^-$: 347.1089, Found: 347.1090.

*4.1.3.16. (E)-methyl 4-(2-(5-fluoro-2-(p-tolyl)pyridin-4-yl)vinyl)benzoate (**4q**).* The title compound (**4q**) was synthesized using compound **3e** and *p*-tolylboronic acid in a manner similar to that described for the synthesis of compound **4a**. White powder, Yield: 61.9%, Mp: 133–134 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, 1H, J = 2.0 Hz), 8.07 (d, 2H, J = 8.4 Hz), 7.87 (d, 2H, J = 8.0 Hz), 7.83 (d, 1H, J = 6.0 Hz), 7.63 (d, 2H, J = 8.4 Hz), 7.45 (dd, 1H, J_1 = 1.6 Hz, J_2 = 16.4 Hz), 7.33–7.28 (m, 3H), 3.94 (s, 3H), 2.41 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.65, 156.16 (d, $J_{C\text{-}F}$ = 259.3 Hz), 154.04 (d, $J_{C\text{-}F}$ = 3.5 Hz), 140.46, 138.98, 138.29 (d, $J_{C\text{-}F}$ = 25.5 Hz), 135.73, 134.20 (d, $J_{C\text{-}F}$ = 5.3 Hz), 132.09 (d, $J_{C\text{-}F}$ = 10.4 Hz), 130.21, 130.16, 129.56, 127.03, 126.66, 121.44 (d, $J_{C\text{-}F}$ = 2.3 Hz), 117.44, 52.26, 21.29. HRMS (ESI) calcd for $C_{22}H_{19}FNO_2$ [M + H]$^+$: 348.1394, Found: 348.1396.

*4.1.3.17. (E)-methyl 4-(2-(5-fluoro-2-(4-(trifluoromethyl)phenyl) pyridin-4-yl)vinyl)benzoate (**4r**).* The title compound (**4r**) was synthesized using compound **3e** and (4-(trifluoromethyl)phenyl) boronic acid in a manner similar to that described for the synthesis of compound **4a**. White powder, Yield: 69.3%, Mp: 164–165 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (d, 1H, J = 2.0 Hz), 8.11–8.07 (m, 4H), 7.91 (d, 1H, J = 6.0 Hz), 7.74 (d, 2H, J = 8.0 Hz), 7.65 (d, 2H, J = 8.0 Hz), 7.49 (d, 1H, J = 16.4 Hz), 7.32 (d, 1H, J = 16.4 Hz), 3.94 (s, 3H). HRMS (ESI) calcd for $C_{22}H_{16}F_4NO_2$ [M + H]$^+$: 402.1112, Found: 402.1112.

*4.1.3.18. (E)-methyl 4-(2-(5-fluoro-2-(2-fluoro-4-methylphenyl)pyridin-4-yl)vinyl)benzoate (**4s**).* The title compound (**4s**) was synthesized using compound **3e** and (2-fluoro-4-methylphenyl)boronic acid in a manner similar to that described for the synthesis of compound **4a**. White powder, Yield: 64.8%, Mp: 141–142 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.71 (d, 1H, J = 2.0 Hz), 8.11 (dd, 1H, J_1 = 1.6 Hz, J_2 = 6.0 Hz), 7.99 (d, 2H, J = 8.4 Hz), 7.86 (d, 2H, J = 8.4 Hz), 7.78 (t, 1H, J = 8.0 Hz), 7.72 (d, 1H, J = 16.8 Hz), 7.49 (d, 1H, J = 16.8 Hz), 7.20 (dd, 1H, J_1 = 1.6 Hz, J_2 = 12.4 Hz), 7.20 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 3.87 (s, 3H), 2.39 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.65, 160.10 (d, $J_{C\text{-}F}$ = 249.7 Hz), 155.95 (d, $J_{C\text{-}F}$ = 260.4 Hz), 149.83 (d, $J_{C\text{-}F}$ = 4.7 Hz), 141.34 (d, $J_{C\text{-}F}$ = 8.4 Hz), 140.46, 138.46 (d, $J_{C\text{-}F}$ = 26.0 Hz), 134.38 (d, $J_{C\text{-}F}$ = 5.5 Hz), 131.81 (d, $J_{C\text{-}F}$ = 10.1 Hz), 130.53 (d, $J_{C\text{-}F}$ = 3.4 Hz), 130.21, 130.14, 129.70, 127.84, 127.06, 125.45 (d, $J_{C\text{-}F}$ = 2.9 Hz), 123.55 (d, $J_{C\text{-}F}$ = 11.5 Hz), 121.44 (d, $J_{C\text{-}F}$ = 10.0 Hz), 121.26 (d, $J_{C\text{-}F}$ = 2.4 Hz), 116.69 (d, $J_{C\text{-}F}$ = 22.9 Hz), 52.25, 21.19 (d, $J_{C\text{-}F}$ = 1.6 Hz). HRMS (ESI) calcd for $C_{22}H_{17}F_2NNaO_2$ [M + Na]$^+$: 388.1120, Found: 388.1119.

*4.1.3.19. (E)-methyl 4-(2-(2-(thiophen-3-yl)pyridin-4-yl)vinyl)benzoate (**7**).* The title compound (**7**) was synthesized using compound **3a** and thiophen-3-ylboronic acid in a manner similar to that described for the synthesis of compound **4a**. Yellow powder, Yield: 79.5%, Mp: 133–134 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, 1H, J = 5.2 Hz), 8.07 (d, 2H, J = 8.0 Hz), 7.95 (d, 1H, J = 2.8 Hz), 7.71–7.68 (m, 2H), 7.62 (d, 2H, J = 8.0 Hz), 7.43–7.41 (m, 1H), 7.35 (d, 1H, J = 16.4 Hz), 7.29 (d, 1H, J = 5.2 Hz), 7.17 (d, 1H, J = 16.4 Hz), 3.94 (s, 3H). HRMS (ESI) calcd for $C_{19}H_{16}NO_2S$ [M + H]$^+$: 322.0896, Found: 322.0893.

*4.1.3.20. 2-(2-hydroxyphenyl)isonicotinaldehyde (**10**).* The title compound (**10**) was synthesized using compound **1a** and (3-hydroxyphenyl)boronic acid in a manner similar to that described for the synthesis of compound **4a**. White powder, Yield: 76.0%, Mp: 136–137 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.72 (s, 1H), 10.15 (s, 1H),

8.75 (dd, 1H, $J_1 = 1.2$ Hz, $J_2 = 5.2$ Hz), 8.31 (t, 1H, $J = 1.2$ Hz), 7.89 (dd, 1H, $J_1 = 1.6$ Hz, $J_2 = 8.0$ Hz), 7.65 (dd, 1H, $J_1 = 1.2$ Hz, $J_2 = 5.2$ Hz), 7.38–7.34 (m, 1H), 7.05 (dd, 1H, $J_1 = 1.6$ Hz, $J_2 = 8.0$ Hz), 6.99–6.94 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.05, 159.91, 159.65, 147.55, 142.78, 132.40, 126.43, 119.47, 119.22, 118.85, 118.55, 118.24. HRMS (ESI) calcd for $C_{12}H_{10}NO_2$ $[M + H]^+$: 200.0706, Found: 200.0709.

*4.1.3.21. (E)-methyl 4-(2-(5-(p-tolyl)pyridin-3-yl)vinyl)benzoate (**15a**).* The title compound (**15a**) was synthesized using compound **14** and *p*-tolylboronic acid in a manner similar to that described for the synthesis of compound **4a**. White powder, Yield: 81.7%, Mp: 181–182 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.80 (d, 1H, $J = 2.0$ Hz), 8.78 (d, 1H, $J = 2.0$ Hz), 8.36 (s, 1H), 8.00 (d, 2H, $J = 8.0$ Hz), 7.78 (d, 2H, $J = 8.0$ Hz), 7.72 (d, 2H, $J = 7.6$ Hz), 7.65 (d, 1H, $J = 16.4$ Hz), 7.52 (d, 1H, $J = 16.4$ Hz), 7.35 (d, 2H, $J = 7.6$ Hz), 3.87 (s, 3H), 2.38 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.40, 147.67, 147.24, 141.92, 138.27, 135.94, 134.33, 132.85, 131.08, 130.18, 130.15, 129.11, 128.14, 127.29, 127.27, 52.59, 21.21. HRMS (ESI) calcd for $C_{22}H_{20}NO_2$ $[M + H]^+$: 330.1489, Found: 330.1487.

*4.1.3.22. (E)-methyl 4-(2-(5-(4-(trifluoromethyl)phenyl)pyridin-3-yl) vinyl)benzoate (**15b**).* The title compound (**15b**) was synthesized using compound **14** and ((4-(trifluoromethyl)phenyl)boronic acid in a manner similar to that described for the synthesis of compound **4a**. White powder, Yield: 69.2%, Mp: 175–176 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (d, 1H, $J = 2.0$ Hz), 8.76 (d, 1H, $J = 2.0$ Hz), 8.07 (d, 2H, $J = 8.4$ Hz), 8.03 (t, 1H, $J = 2.0$ Hz), 7.78 (d, 2H, $J = 8.4$ Hz), 7.74 (d, 2H, $J = 8.4$ Hz), 7.62 (d, 2H, $J = 8.4$ Hz), 7.30 (d, 1H, $J = 16.4$ Hz), 7.25 (d, 1H, $J = 16.4$ Hz), 3.94 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.71, 148.16, 147.56, 141.16, 140.78, 135.40, 132.60, 131.47, 130.43, 130.27 (q, $J_{C\text{-}F} = 32.7$ Hz), 130.17, 129.71, 127.61, 126.91, 126.61, 126.10 (q, $J_{C\text{-}F} = 3.6$ Hz), 124.07 (q, $J_{C\text{-}F} = 273.1$ Hz), 52.22. HRMS (ESI) calcd for $C_{22}H_{17}F_3NO_2$ $[M + H]^+$: 384.1206, Found: 384.1207.

*4.1.3.23. (E)-methyl 4-(2-(5-(2-fluoro-4-methylphenyl)pyridin-3-yl) vinyl)benzoate (**15c**).* The title compound (**15c**) was synthesized using compound **14** and (2-fluoro-4-methylphenyl)boronic acid in a manner similar to that described for the synthesis of compound **4a**. White powder, Yield: 71.4%, Mp: 140–141 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (d, 1H, $J = 2.0$ Hz), 8.67 (t, 1H, $J = 2.0$ Hz), 8.05 (d, 2H, $J = 8.4$ Hz), 8.01–7.99 (m, 1H), 7.59 (d, 2H, $J = 8.4$ Hz), 7.36 (t, 1H, $J = 8.0$ Hz), 7.23 (s, 2H), 7.08 (dd, 1H, $J_1 = 1.6$ Hz, $J_2 = 8.0$ Hz), 7.03 (dd, 1H, $J_1 = 1.6$ Hz, $J_2 = 11.6$ Hz), 3.93 (s, 3H), 2.42 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.75, 159.72 (d, $J_{C\text{-}F} = 249.3$ Hz), 148.87 (d, $J_{C\text{-}F} = 3.2$ Hz), 147.35, 141.00, 140.95 (d, $J_{C\text{-}F} = 8.0$ Hz), 133.13 (d, $J_{C\text{-}F} = 3.3$ Hz), 132.10, 131.76 (d, $J_{C\text{-}F} = 1.7$ Hz), 130.12, 130.08 (d, $J_{C\text{-}F} = 3.6$ Hz), 129.95, 129.51, 127.26, 126.55, 125.51 (d, $J_{C\text{-}F} = 3.2$ Hz), 122.24 (d, $J_{C\text{-}F} = 13.9$ Hz), 116.87 (d, $J_{C\text{-}F} = 22.2$ Hz), 52.18, 21.19 (d, $J_{C\text{-}F} = 1.6$ Hz). HRMS (ESI) calcd for $C_{22}H_{19}FNO_2$ $[M + H]^+$: 348.1394, Found: 348.1394.

*4.1.3.24. (E)-methyl 4-(2-(5-(2-hydroxyphenyl)pyridin-3-yl)vinyl) benzoate (**15d**).* The title compound (**15d**) was synthesized using compound **14** and (2-hydroxyphenyl)boronic acid in a manner similar to that described for the synthesis of compound **4a**. White powder, Yield: 72.3%, Mp: 201–202 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.81 (s, 1H), 8.75 (d, 1H, $J = 2.4$ Hz), 8.67 (d, 1H, $J = 2.4$ Hz), 8.21 (t, 1H, $J = 2.4$ Hz), 7.99 (d, 2H, $J = 8.4$ Hz), 7.78 (d, 2H, $J = 8.4$ Hz), 7.56 (d, 1H, $J = 16.4$ Hz), 7.51 (d, 1H, $J = 16.4$ Hz), 7.38 (dd, 1H, $J_1 = 2.0$ Hz, $J_2 = 7.6$ Hz), 7.28–7.23 (m, 1H), 7.01 (dd, 1H, $J_1 = 1.2$ Hz, $J_2 = 8.0$ Hz), 6.97–6.92 (m, 1H), 3.86 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.41, 155.05, 149.57, 146.96, 141.97, 134.69, 133.72, 132.19, 130.90, 130.11, 129.96, 129.68, 129.04, 128.40, 127.26, 124.64, 120.10, 116.53, 52.58. HRMS (ESI) calcd for $C_{21}H_{18}NO_3$ $[M + H]^+$: 332.1281, Found: 332.1280.

*4.1.3.25. (E)-methyl 4-(2-(5-(5-fluoro-2-methoxyphenyl)pyridin-3-yl)vinyl)benzoate (**15e**).* The title compound (**15e**) was synthesized using compound **14** and (5-fluoro-2-methoxyphenyl)boronic acid in a manner similar to that described for the synthesis of compound **4a**. White powder, Yield: 76.9%, Mp: 146–147 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (d, 1H, $J = 2.0$ Hz), 8.66 (d, 1H, $J = 2.0$ Hz), 8.05 (d, 1H, $J = 8.4$ Hz), 7.98 (t, 1H, $J = 2.0$ Hz), 7.60 (d, 2H, $J = 8.4$ Hz), 7.23 (s, 2H), 7.11–7.05 (m, 2H), 6.98–6.93 (m, 1H), 3.93 (s, 3H), 3.82 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.76, 157.14 (d, $J_{C\text{-}F} = 240.4$ Hz), 152.80 (d, $J_{C\text{-}F} = 2.2$ Hz), 149.50, 147.27, 141.05, 133.67, 133.26 (d, $J_{C\text{-}F} = 1.6$ Hz), 131.89, 130.12, 129.80, 129.48, 127.95 (d, $J_{C\text{-}F} = 7.5$ Hz), 127.38, 126.54, 117.26 (d, $J_{C\text{-}F} = 23.8$ Hz), 115.53 (d, $J_{C\text{-}F} = 22.7$ Hz), 112.40 (d, $J_{C\text{-}F} = 8.4$ Hz), 56.23, 52.19. HRMS (ESI) calcd for $C_{22}H_{19}FNO_3$ $[M + H]^+$: 364.1343, Found: 364.1347.

*4.1.4. (E)-3-(2-(2-(5-fluoro-2-hydroxyphenyl)pyridin-4-yl)vinyl)-N-hydroxybenzamide (**5a**)*

A solution of hydroxylamine hydrochloride (5.84 g, 85.75 mmol) in anhydrous methanol (45 ml) was added to a solution of KOH (7.0 g, 127.25 mmol) in anhydrous methanol (20 ml) at 0 °C, and stirred for 5 min and the white precipitate formed was filtered. The resultant filtrate (16 ml) was added to a solution of compound **4a** (174.7 mg, 1.0 mmol) in anhydrous CH_2Cl_2 (5 ml) and the mixture was stirred at room temperature for 1 h. The reaction mixture was evaporated under vacuum. The residue was acidified with 1 N HCl to a pH 5–6 and then the resultant precipitate was filtered, washed with water, dried to afford the crude product, which was purified by recrystallization from methanol to afford pure compound **5a** (137 mg, Yield: 39.3%) as a yellow powder, Mp: 207–208 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.11 (s, 1H), 11.33 (s, 1H), 9.15 (s, 1H), 8.62 (d, 1H, $J = 3.6$ Hz), 8.49 (s, 1H), 8.13 (s, 1H), 8.06 (dd, 1H, $J_1 = 2.8$ Hz, $J_2 = 10.8$ Hz), 7.91 (d, 1H, $J = 16.4$ Hz), 7.83 (d, 1H, $J = 7.2$ Hz), 7.74 (d, 1H, $J = 7.2$ Hz), 7.66 (d, 1H, $J = 2.4$ Hz), 7.55 (t, 1H, $J = 7.2$ Hz), 7.43 (d, 1H, $J = 16.4$ Hz), 7.20 (t, 1H, $J = 7.2$ Hz), 6.95 (dd, 1H, $J_1 = 4.8$ Hz, $J_2 = 8.4$ Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.43, 156.71 (d, $J_{C\text{-}F} = 3.0$ Hz), 156.08, 155.69 (d, $J_{C\text{-}F} = 233.3$ Hz), 147.19, 147.11, 136.80, 134.41, 133.97, 130.21, 129.51, 127.49, 126.98, 126.25, 120.68, 119.71 (d, $J_{C\text{-}F} = 8.1$ Hz), 119.52 (d, $J_{C\text{-}F} = 8.1$ Hz), 118.67 (d, $J_{C\text{-}F} = 23.2$ Hz), 117.48, 113.19 (d, $J_{C\text{-}F} = 24.2$ Hz). HRMS (ESI) calcd for $C_{20}H_{16}FN_2O_3$ $[M + H]^+$: 351.1139, Found: 351.1138.

*4.1.4.2. (E)-N-hydroxy-3-(2-(2-(2-hydroxyphenyl)pyridin-4-yl)vinyl) benzamide (**5b**).* The title compound (**5b**) was synthesized using compound **4b** in a manner similar to that described for the synthesis of compound **5a**. White powder, Yield: 47.1%, Mp: 187–188 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.31 (s, 1H), 11.30 (s, 1H), 9.20 (s,1H), 8.60 (d, 1H, $J = 4.0$ Hz), 8.45 (s, 1H), 8.17–8.14 (m, 2H), 7.88–7.81 (m, 2H), 7.74 (d, 1H, $J = 7.2$ Hz), 7.65 (d, 1H, $J = 3.2$ Hz), 7.53 (t, 1H, $J = 7.2$ Hz), 7.44 (d, 1H, $J = 16.0$ Hz), 7.34 (t, 1H, $J = 7.2$ Hz), 6.98–6.93 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.41, 164.27, 159.87, 157.85, 146.99, 146.93, 136.78, 134.20, 131.92, 130.12, 129.46, 127.44, 127.12, 126.18, 119.87, 119.24, 118.43, 117.29. HRMS (ESI) calcd for $C_{21}H_{18}NO_3$ $[M + H]^+$: 333.1234, Found: 333.1234.

*4.1.4.3. (E)-4-(2-(2-(5-fluoro-2-hydroxyphenyl)pyridin-4-yl)vinyl)-N-hydroxybenzamide (**5c**).* The title compound (**5c**) was synthesized using compound **4c** in a manner similar to that described for the synthesis of compound **5a**. Yellowish white powder, Yield: 38.6%, Mp: 210–211 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.06 (s, 1H), 11.29 (s, 1H), 9.10 (s, 1H), 8.63 (d, 1H, $J = 4.8$ Hz), 8.47 (s, 1H), 8.04 (dd, 1H, $J_1 = 3.2$ Hz, $J_2 = 10.4$ Hz), 7.89–7.75 (m, 5H), 7.65 (d, 1H, $J = 5.2$ Hz), 7.46 (d, 1H, $J = 16.4$ Hz), 7.20 (td, 1H, $J_1 = 2.8$ Hz,

J_2 = 8.8 Hz), 6.95 (dd, 1H, J_1 = 4.8 Hz, J_2 = 8.8 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.19, 156.69 (d, J_{C-F} = 2.0 Hz), 155.69 (d, J_{C-F} = 233.3 Hz), 154.53, 147.24, 147.01, 139.22, 134.11, 133.20, 127.99, 127.79, 127.54, 120.74, 119.74 (d, J_{C-F} = 8.1 Hz), 119.52 (d, J_{C-F} = 7.1 Hz), 118.68 (d, J_{C-F} = 23.2 Hz), 117.57, 113.21 (d, J_{C-F} = 25.3 Hz). HRMS (ESI) calcd for $C_{20}H_{14}FN_2O_3$ [M − H]$^-$: 349.0994, Found: 349.0997.

*4.1.4.4. (E)-N-hydroxy-4-(2-(2-(2-hydroxyphenyl)pyridin-4-yl)vinyl) benzamide (**5d**).* The title compound (**5d**) was synthesized using compound **4d** in a manner similar to that described for the synthesis of compound **5a**. White powder, Yield: 69.2%, Mp: 206−207 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.28 (s, 1H), 11.30 (s, 1H), 9.11 (s, 1H), 8.61 (d, 1H, J = 5.2 Hz), 8.43 (s, 1H), 8.15 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 7.86−7.81 (m, 3H), 7.77 (d, 2H, J = 8.0 Hz), 7.65 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 7.48 (d, 1H, J = 16.4 Hz), 7.36−7.31 (m, 1H), 6.98−6.93 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.17, 159.82, 157.83, 147.02, 146.81, 139.23, 133.84, 133.13, 131.93, 127.95, 127.54, 127.42, 119.92, 119.24, 118.42, 117.34. HRMS (ESI) calcd for $C_{20}H_{15}N_2O_3$ [M − H]$^-$: 331.1088, Found: 331.1088.

*4.1.4.5. (E)-N-hydroxy-4-(2-(2-(2-methoxyphenyl)pyridin-4-yl)vinyl)benzamide (**5e**).* The title compound (**5e**) was synthesized using compound **4e** in a manner similar to that described for the synthesis of compound **5a**. Yellowish white powder, Yield: 87.3%, Mp: 176−177 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.30 (s, 1H), 9.10 (s, 1H), 8.64 (d, 1H, J = 5.2 Hz), 7.97 (s, 1H), 7.83−7.55 (m, 7H), 7.45−7.41 (m, 2H), 7.18 (d, 1H, J = 8.8 Hz), 7.08 (t, 1H, J = 7.6 Hz), 3.87 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.22, 157.22, 156.48, 150.08, 144.32, 139.41, 132.84, 132.37, 131.17, 130.61, 128.88, 128.57, 127.85, 127.47, 122.93, 121.04, 119.24, 112.36, 56.12. HRMS (ESI) calcd for $C_{21}H_{19}N_2O_3$ [M + H]$^+$: 347.1390, Found: 347.1394.

*4.1.4.6. (E)-N-hydroxy-4-(2-(2-(3-hydroxyphenyl)pyridin-4-yl)vinyl) benzamide (**5f**).* The title compound (**5f**) was synthesized using compound **4f** in a manner similar to that described for the synthesis of compound **5a**. White powder, Yield: 49.4%, Mp: 203−204 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.29 (s, 1H), 9.58 (s, 1H), 9.10 (s, 1H), 8.64 (d, 1H, J = 5.2 Hz), 8.10 (s, 1H), 7.83 (d, 2H, J = 8.0 Hz), 7.77−7.71 (m, 3H), 7.59−7.53 (m, 3H), 7.45 (d, 1H, J = 16.8 Hz), 7.32 (t, 1H, J = 8.0 Hz), 6.87 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.0 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.24, 158.24, 157.15, 150.36, 145.48, 140.55, 139.47, 132.89, 132.60, 130.16, 128.38, 127.91, 127.41, 120.20, 117.93, 117.83, 116.60, 113.96. HRMS (ESI) calcd for $C_{20}H_{17}N_2O_3$ [M + H]$^+$: 333.1234, Found: 333.1237.

*4.1.4.7. (E)-N-hydroxy-4-(2-(2-(3-(hydroxymethyl)phenyl)pyridin-4-yl)vinyl)benzamide (**5g**).* The title compound (**5g**) was synthesized using compound **4g** in a manner similar to that described for the synthesis of compound **5a**. White powder, Yield: 75.3%, Mp: 181−182 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.28 (s, 1H), 9.09 (s,1H), 8.66 (d, 1H, J = 4.8 Hz), 8.16 (s, 1H), 8.13 (s, 1H), 8.02 (d, 1H, J = 7.2 Hz), 7.82 (d, 2H, J = 8.4 Hz), 7.77−7.71 (m, 3H), 7.56 (d, 1H, J = 4.8 Hz), 7.50−7.40 (m, 3H), 5.30 (t, 1H, J = 5.6 Hz), 4.61 (d, 2H, J = 5.2 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.17, 157.23, 150.44, 145.57, 143.55, 139.43, 138.94, 132.91, 132.63, 128.96, 128.39, 127.90, 127.71, 127.42, 125.44, 125.20, 120.17, 117.95, 63.42. HRMS (ESI) calcd for $C_{21}H_{19}N_2O_3$ [M + H]$^+$: 347.1390, Found: 347.1389.

*4.1.4.8. (E)-4-(2-([2,3′-bipyridin]-4-yl)vinyl)-N-hydroxybenzamide (**5h**).* The title compound (**5h**) was synthesized using compound **4h** in a manner similar to that described for the synthesis of compound **5a**. White powder, Yield: 79.5%, Mp: 152−153 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 1H), 9.35 (d, 1H, J = 2.0 Hz), 9.12 (s, 1H), 8.71 (d, 1H, J = 5.2 Hz), 8.67 (dd, 1H, J_1 = 1.2 Hz, J_2 = 4.4 Hz), 8.51 (dt, 1H, J_1 = 2.0 Hz, J_2 = 8.0 Hz), 8.31 (s, 1H), 7.85 (d, 2H, J = 8.4 Hz), 7.82−7.75 (m, 3H), 7.60 (dd, 1H, J_1 = 1.6 Hz, J_2 = 5.2 Hz), 7.56 (dd, 1H, J_1 = 4.8 Hz, J_2 = 8.0 Hz), 7.45 (d, 1H, J = 16.4 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.18, 154.84, 150.83, 150.43, 148.34, 145.84, 139.34, 134.54, 134.48, 133.12, 133.01, 128.04, 127.94, 127.44, 124.25, 121.14, 118.10. HRMS (ESI) calcd for $C_{19}H_{16}N_3O_2$ [M + H]$^+$: 318.1237, Found: 318.1236.

*4.1.4.9. (E)-4-(2-(2-(4-fluorophenyl)pyridin-4-yl)vinyl)-N-hydroxybenzamide (**5i**).* The title compound (**5i**) was synthesized using compound **4i** in a manner similar to that described for the synthesis of compound **5a**. Yellow powder, Yield: 40.3%, Mp: 186−187 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.28 (s, 1H), 9.11 (s, 1H), 8.65 (d, 1H, J = 4.8 Hz), 8.23 (d,1H, J = 7.6 Hz), 8.21 (d, 1H, J = 7.6 Hz), 8.19 (s, 1H), 7.84 (d, 2H, J = 8.4 Hz), 7.78−7.73 (m, 3H), 7.55 (dd, 1H, J_1 = 1.2 Hz, J_2 = 5.2 Hz), 7.44 (d, 1H, J = 16.4 Hz), 7.38−7.33 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.23, 163.39 (d, J_{C-F} = 247.4 Hz), 156.06, 150.49, 145.67, 139.41, 135.65 (d, J_{C-F} = 3.0 Hz), 132.93, 132.80, 129.25 (d, J_{C-F} = 8.1 Hz), 128.26, 127.92, 127.41, 120.34, 117.66, 116.04 (d, J_{C-F} = 21.2 Hz). HRMS (ESI) calcd for $C_{20}H_{16}FN_2O_2$ [M + H]$^+$: 335.1190, Found: 335.1191.

*4.1.4.10. (E)-N-hydroxy-4-(2-(2-(p-tolyl)pyridin-4-yl)vinyl)benzamide (**5j**).* The title compound (**5j**) was synthesized using compound **4j** in a manner similar to that described for the synthesis of compound **5a**.White powder, Yield: 64.7%, Mp: 230−231 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.29 (s, 1H), 9.11 (s, 1H), 8.63 (d, 1H, J = 5.2 Hz), 8.15 (s, 1H), 8.06 (d, 2H, J = 7.6 Hz), 7.82 (d, 2H, J = 8.0 Hz), 7.76−7.72 (m, 3H), 7.52 (d, 1H, J = 5.2 Hz), 7.44 (d, 1H, J = 16.8 Hz), 7.33 (d, 2H, J = 8.0 Hz), 2.38 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.12, 157.05, 150.39, 145.48, 139.42, 139.14, 136.36, 132.90, 132.58, 129.81, 128.37, 127.89, 127.39, 126.95, 120.02, 117.49, 40.37, 40.16, 39.95, 39.74, 39.53, 21.32. HRMS (ESI) calcd for $C_{21}H_{17}N_2O_2$ [M − H]$^-$: 329.1296, Found: 329.1298.

*4.1.4.11. (E)-N-hydroxy-4-(2-(2-(4-(trifluoromethyl)phenyl)pyridin-4-yl)vinyl)benzamide (**5k**).* The title compound (**5k**) was synthesized using compound **4k** in a manner similar to that described for the synthesis of compound **5a**. White powder, Yield: 57.2%, Mp: 254−255 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.32 (brs, 1H), 9.11 (brs, 1H), 8.80 (d, 1H, J = 5.6 Hz), 8.47 (s, 1H), 8.38 (d, 2H, J = 8.0 Hz), 7.99−7.94 (m, 3H), 7.88−7.84 (m, 3H), 7.79 (d, 2H, J = 8.0 Hz), 7.57 (d, 1H, J = 16.4 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.04, 155.42, 150.75, 145.92, 142.94, 139.25, 133.11, 129.69 (q, J_{C-F} = 31.6 Hz), 128.01, 127.89, 127.80, 127.44, 126.10 (q, J_{C-F} = 3.8 Hz), 124.77 (q, J_{C-F} = 272.8 Hz), 121.30, 118.51. HRMS (ESI) calcd for $C_{21}H_{14}F_3N_2O_2$ [M − H]$^-$: 383.1013, Found: 383.1013.

*4.1.4.12. (E)-N-hydroxy-4-(2-(2-(4-methoxyphenyl)pyridin-4-yl)vinyl)benzamide (**5l**).* The title compound (**5l**) was synthesized using compound **4l** in a manner similar to that described for the synthesis of compound **5a**.White powder, Yield: 61.8%, Mp: 241−242 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.32 (brs, 1H), 9.13 (brs, 1H), 8.61 (d, 1H, J = 5.2 Hz), 8.14−8.11 (m, 3H), 7.84 (d, 1H, J = 8.0 Hz), 7.77−7.71 (m, 3H), 7.49 (dd, 1H, J_1 = 1.6 Hz, J_2 = 5.2 Hz), 7.46 (d, 1H, J = 16.4 Hz), 7.08 (d, 1H, J = 8.0 Hz), 3.84 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.21, 160.67, 156.85, 150.30, 145.40, 139.47, 132.85, 132.48, 131.59, 128.47, 128.42, 127.90, 127.39, 119.49, 117.10, 114.55, 55.70. HRMS (ESI) calcd for $C_{21}H_{17}N_2O_3$ [M − H]$^-$: 345.1245, Found: 345.1244.

*4.1.4.13. (E)-4-(2-(2-(2-fluoro-4-methylphenyl)pyridin-4-yl)vinyl)-N-hydroxybenzamide (**5m**).* The title compound (**5m**) was synthesized using compound **4m** in a manner similar to that described for

the synthesis of compound **5a**. White powder, Yield: 66.2%, Mp: 177–178 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (brs, 1H), 9.13 (brs, 1H), 8.68 (d, 1H, J = 5.2 Hz), 7.92 (d, 1H, J = 2.0 Hz), 7.87–7.81 (m, 3H), 7.78 (d, 2H, J = 8.4 Hz), 7.65 (d, 1H, J = 16.4 Hz), 7.61 (dd, 1H, J_1 = 1.6 Hz, J_2 = 5.2 Hz), 7.46 (d, 1H, J = 16.4 Hz), 7.22–7.15 (m, 2H), 2.39 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.18, 160.15 (d, $J_{C\text{-}F}$ = 248.5 Hz), 153.81 (d, $J_{C\text{-}F}$ = 2.0 Hz), 150.54, 145.16, 141.79 (d, $J_{C\text{-}F}$ = 4.0 Hz), 139.30, 132.91, 132.72, 131.08 (d, $J_{C,\ F}$ = 4.0 Hz), 128.19, 127.84, 127.51, 125.92 (d, $J_{C\text{-}F}$ = 3.0 Hz), 124.60 (d, $J_{C\text{-}F}$ = 11.1 Hz), 121.90 (d, $J_{C\text{-}F}$ = 8.1 Hz), 119.89, 117.03 (d, $J_{C\text{-}F}$ = 22.2 Hz), 21.09. HRMS (ESI) calcd for $C_{21}H_{16}FN_2O_2$ [M − H]$^-$: 347.1201, Found: 347.1200.

*4.1.4.14. (E)-N-hydroxy-4-(2-(2′-hydroxy-[1,1′-biphenyl]-3-yl)vinyl) benzamide (**5n**).* The title compound (**5n**) was synthesized using compound **4n** in a manner similar to that described for the synthesis of compound **5a**. White powder, Yield: 49.3%, Mp: 212–213 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.24 (s, 1H), 9.56 (s, 1H), 9.05 (s, 1H), 7.79–7.76 (m, 3H), 7.70 (d, 2H, J = 8.4 Hz), 7.57 (dt, 1H, J_1 = 1.6 Hz, J_2 = 7.6 Hz), 7.47 (dt, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 7.45–7.40 (m, 2H), 7.35–7.29 (m, 2H), 7.19 (td, 1H, J_1 = 1.6 Hz, J_2 = 8.4 Hz), 6.97 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.4 Hz), 6.90 (td, 1H, J_1 = 1.2 Hz, J_2 = 7.2 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.38, 154.80, 140.35, 139.60, 136.90, 131.92, 130.86, 130.82, 129.42, 129.12, 128.82, 128.02, 127.89, 127.79, 126.81, 125.31, 119.91, 116.50. HRMS (ESI) calcd for $C_{21}H_{16}NO_3$ [M − H]$^-$: 330.1136, Found: 330.1136.

*4.1.4.15. (E)-N-hydroxy-4-(2-(3′-hydroxy-[1,1′-biphenyl]-3-yl)vinyl) benzamide (**5o**).* The title compound (**5o**) was synthesized using compound **4k** in a manner similar to that described for the synthesis of compound **5a**. White powder, Yield: 40.7%, Mp: 187–188 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.22 (s, 1H), 9.57 (s, 1H), 9.06 (s, 1H), 7.86 (t, 1H, J = 1.6 Hz), 7.79 (d, 2H, J = 8.4 Hz), 7.71 (d, 2H, J = 8.4 Hz), 7.62 (td, 1H, J_1 = 1.6 Hz, J_2 = 7.2 Hz), 7.52 (td, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 7.49–7.43 (m, 3H), 7.29 (t, 1H, J = 8.0 Hz), 7.14 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.0 Hz), 7.09 (t, 1H, J = 2.4 Hz), 6.81 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.35, 158.30, 141.91, 141.35, 140.30, 137.80, 132.01, 130.56, 130.40, 129.78, 128.37, 127.80, 126.85, 126.78, 126.17, 125.48, 118.05, 115.06, 114.10. HRMS (ESI) calcd for $C_{21}H_{16}NO_3$ [M − H]$^-$: 330.1136, Found: 330.1134.

*4.1.4.16. (E)-4-(2-(5′-fluoro-2′-hydroxy-[1,1′-biphenyl]-3-yl)vinyl)-N-hydroxybenzamide (**5p**).* The title compound (**5p**) was synthesized using compound **4p** in a manner similar to that described for the synthesis of compound **5a**. White powder, Yield: 36.6%, Mp: 209–210 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.25 (s, 1H), 9.60 (s, 1H), 9.05 (s, 1H), 7.81–7.77 (m, 3H), 7.70 (d, 2H, J = 8.4 Hz), 7.60(dt, 1H, J_1 = 1.6 Hz, J_2 = 7.6 Hz), 7.51(dt, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 7.46–7.41 (m, 2H),7.36 (d, 1H, J = 16.8 Hz), 7.17 (dd, 1H, J_1 = 3.2 Hz, J_2 = 9.6 Hz), 7.03 (td, 1H, J_1 = 3.2 Hz, J_2 = 8.4 Hz), 6.96 (dd, 1H, J_1 = 5.2 Hz, J_2 = 8.8 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.38, 156.12 (d, $J_{C\text{-}F}$ = 235.3 Hz), 151.14, 140.32, 138.44, 137.06, 131.96, 130.65, 129.36, 128.99 (d, $J_{C\text{-}F}$ = 7.1 Hz), 128.91, 128.09, 127.91, 127.80, 126.82, 125.84, 117.37 (d, $J_{C\text{-}F}$ = 8.1 Hz), 116.76 (d, $J_{C\text{-}F}$ = 23.2 Hz), 115.22 (d, $J_{C\text{-}F}$ = 22.2 Hz). HRMS (ESI) calcd for $C_{21}H_{15}FNO_3$ [M − H]$^-$: 348.1041, Found: 348.1041.

*4.1.4.17. (E)-4-(2-(5-fluoro-2-(p-tolyl)pyridin-4-yl)vinyl)-N-hydroxybenzamide (**5q**).* The title compound (**5q**) was synthesized using compound **4q** in a manner similar to that described for the synthesis of compound **5a**. White powder, Yield: 69.3%, Mp: 204–205 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.32 (s, 1H), 9.12 (s, 1H), 8.64 (d, 1H, J = 2.0 Hz), 8.34 (d, 1H, J = 6.0 Hz), 8.05 (d, 2H, J = 8.0 Hz), 7.88–7.79 (m, 5H), 7.44 (d, 1H, J = 16.4 Hz), 7.33 (d, 2H, J = 8.0 Hz), 2.38 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.13, 156.12 (d, $J_{C\text{-}F}$ = 258.6 Hz), 153.26 (d, $J_{C\text{-}F}$ = 5.1 Hz), 139.12, 138.99, 138.46 (d, $J_{C\text{-}F}$ = 25.3 Hz), 135.53, 135.38 (d, $J_{C\text{-}F}$ = 4.0 Hz), 133.26, 132.65 (d, $J_{C\text{-}F}$ = 10.1 Hz), 129.80, 127.93, 127.67, 126.91, 120.11(d, $J_{C\text{-}F}$ = 2.0 Hz), 117.57, 21.29. HRMS (ESI) calcd for $C_{21}H_{16}FN_2O_2$ [M − H]$^-$: 347.1201, Found: 347.1200.

*4.1.4.18. (E)-4-(2-(5-fluoro-2-(4-(trifluoromethyl)phenyl)pyridin-4-yl)vinyl)-N-hydroxybenzamide (**5r**).* The title compound (**5r**) was synthesized using compound **4r** in a manner similar to that described for the synthesis of compound **5a**. White powder, Yield: 61.0%, Mp: 199–200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.33 (s, 1H), 9.13 (s, 1H), 8.73 (d, 1H, J = 1.6 Hz), 8.52 (d, 1H, J = 5.6 Hz), 8.37 (d, 2H, J = 8.4 Hz), 7.91–7.80 (m, 7H), 7.46 (d, 1H, J = 16.4 Hz). HRMS (ESI) calcd for $C_{21}H_{13}F_4N_2O_2$ [M − H]$^-$: 401.0919, Found: 401.0918.

*4.1.4.19. (E)-4-(2-(5-fluoro-2-(2-fluoro-4-methylphenyl)pyridin-4-yl)vinyl)-N-hydroxybenzamide (**5r**).* The title compound (**5s**) was synthesized using compound **4s** in a manner similar to that described for the synthesis of compound **5a**. White powder, Yield: 57.9%, Mp: 178–179 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 1H), 9.11 (s, 1H), 8.71 (d, 1H, J = 1.6 Hz), 8.11 (dd, 1H, J_1 = 1.6 Hz, J_2 = 6.0 Hz), 7.86–7.76 (m, 5H), 7.70 (d, 1H, J = 16.4 Hz), 7.46 (d, 1H, J = 16.4 Hz), 7.21 (d, 1H, J = 12.4 Hz), 7.17 (d, 1H, J = 8.4 Hz), 2.39 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.10, 159.88 (d, $J_{C\text{-}F}$ = 249.0 Hz), 155.98 (d, $J_{C\text{-}F}$ = 259.1 Hz), 149.96, 141.81 (d, $J_{C\text{-}F}$ = 8.5 Hz), 138.99 (d, $J_{C\text{-}F}$ = 2.1 Hz), 138.76, 135.59 (d, $J_{C\text{-}F}$ = 4.9 Hz), 133.31, 132.38 (d, $J_{C\text{-}F}$ = 10.1 Hz), 131.02 (d, $J_{C\text{-}F}$ = 3.0 Hz), 127.88, 127.76, 125.93 (d, $J_{C\text{-}F}$ = 3.0 Hz), 123.88 (d, $J_{C\text{-}F}$ = 11.8 Hz), 121.86 (d, $J_{C\text{-}F}$ = 7.9 Hz), 120.32, 117.01 (d, $J_{C\text{-}F}$ = 22.5 Hz), 21.10. HRMS (ESI) calcd for $C_{21}H_{15}F_2N_2O_2$ [M − H]$^-$: 365.1107, Found: 365.1107.

*4.1.4.20. (E)-4-(2-(2-bromopyridin-4-yl)vinyl)-N-hydroxybenzamide (**6a**).* The title compound (**6a**) was synthesized using compound **3a** in a manner similar to that described for the synthesis of compound **5a**. White powder, Yield: 39.7%, Mp: 138–139 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, 1H, J = 5.2 Hz), 8.07 (d, 2H, J = 8.4 Hz), 7.60–7.58 (m, 3H), 7.35–7.29 (m, 2H), 7.06 (d, 1H, J = 16.4 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.15, 151.18, 148.10, 142.74, 138.98, 134.30, 133.24, 127.89, 127.62, 126.61, 125.23, 121.17. HRMS (ESI) calcd for $C_{14}H_{10}BrN_2O_2$ [M − H]$^-$: 316.9931, Found: 316.9933.

*4.1.4.21. (E)-N-hydroxy-4-(2-(pyridin-4-yl)vinyl)benzamide (**6b**).* The title compound (**6b**) was synthesized using compound **3b** in a manner similar to that described for the synthesis of compound **5a**. White powder, Yield: 86.5%, Mp: 186–187 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.28 (s, 1H), 9.09 (s, 1H), 8.58 (d, 2H, J = 5.6 Hz),7.80 (d, 2H, J = 8.4 Hz), 7.74 (d, 2H, J = 8.4 Hz), 7.62–7.57 (m, 3H), 7.37 (d, 1H, J = 16.4 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.20, 150.56, 144.44, 139.31, 132.90, 132.53, 128.15, 127.87, 127.43, 121.48. HRMS (ESI) calcd for $C_{14}H_{13}N_2O_2$ [M + H]$^+$: 241.0972, Found: 241.0977.

*4.1.4.22. (E)-N-hydroxy-4-(2-(2-(thiophen-3-yl)pyridin-4-yl)vinyl) benzamide (**8**).* The title compound (**8**) was synthesized using compound **7** in a manner similar to that described for the synthesis of compound **5a**. White powder, Yield: 49.1%, Mp: 205–206 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.29 (s, 1H), 9.10 (s, 1H), 8.58 (d, 1H, J = 5.2 Hz), 8.24 (d, 1H, J = 1.2 Hz), 8.11 (s, 1H), 7.84–7.81 (m, 3H), 7.84–7.81 (m, 2H), 7.76–7.67 (m, 4H), 7.47 (d, 1H, J = 4.8 Hz), 7.40 (d, 1H, J = 16.4 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.23, 153.76, 150.42, 145.45, 142.41, 139.40, 132.92, 132.64, 128.29, 127.93, 127.52, 127.39, 126.83, 124.62, 119.97, 117.79. HRMS (ESI) calcd for $C_{18}H_{15}N_2O_2S$ [M + H]$^+$: 323.0849, Found: 323.0845.

*4.1.4.23. (E)-N-hydroxy-3-(4-((E)-2-(2-(2-hydroxyphenyl)pyridin-4-yl)vinyl)phenyl) acrylamide (**12**).* The title compound (**12**) was synthesized using compound **11** in a manner similar to that described for the synthesis of compound **5a**. White powder, Yield: 72.9%, Mp: 189–190 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.30 (s, 1H), 10.81 (s, 1H), 9.10 (s, 1H), 8.59 (d, 1H, J = 5.2 Hz), 8.41 (s, 1H), 8.15 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 7.83–7.62 (m, 6H), 7.50 (d, 1H, J = 16.0 Hz), 7.43 (d, 1H, J = 16.4 Hz), 7.35–7.31 (m, 1H), 6.98–6.92 (m, 2H), 6.53 (d, 1H, J = 15.6 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.14, 159.84, 157.81, 146.96, 138.18, 137.62, 135.73, 134.12, 131.90, 128.55, 128.17, 127.42, 127.06, 119.97, 119.83, 119.26, 119.23, 118.41, 117.24. HRMS (ESI) calcd for $C_{22}H_{19}N_2O_2$ [M + H]$^+$: 359.1390, Found: 359.1396.

*4.1.4.24. (E)-N-hydroxy-4-(2-(5-(p-tolyl)pyridin-3-yl)vinyl)benzamide (**16a**).* The title compound (**16a**) was synthesized using compound **15a** in a manner similar to that described for the synthesis of compound **5a**. White powder, Yield: 67.8%, Mp: 221–222 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.28 (br, 1H), 9.10 (br, 1H), 8.78 (d, 1H, J = 2.0 Hz), 8.76 (d, 1H, J = 2.0 Hz), 8.34 (s, 1H), 7.82 (d, 2H, J = 8.0 Hz), 7.74–7.71 (m, 4H), 7.61 (d, 1H, J = 16.4 Hz), 7.47 (d, 1H, J = 16.4 Hz), 7.35 (d, 2H, J = 7.6 Hz), 2.38 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.24, 147.59, 147.10, 139.89, 138.23, 135.91, 134.40, 133.00, 132.39, 130.88, 130.42, 130.18, 127.84, 127.29, 127.01, 126.98, 21.21. HRMS (ESI) calcd for $C_{21}H_{19}N_2O_2$ [M + H]$^+$: 331.1441, Found: 331.1441.

*4.1.4.25. (E)-N-hydroxy-4-(2-(5-(4-(trifluoromethyl)phenyl)pyridin-3-yl)vinyl)benzamide (**16b**).* The title compound (**16b**) was synthesized using compound **15b** in a manner similar to that described for the synthesis of compound **5a**. White powder, Yield: 70.2%, Mp: 219–220 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.29 (br, 1H), 9.10 (br, 1H), 8.88 (d, 1H, J = 2.0 Hz), 8.86 (d, 1H, J = 2.0 Hz), 8.46 (t, 1H, J = 2.0 Hz), 8.06 (d, 2H, J = 8.0 Hz), 7.90 (d, 2H, J = 8.0 Hz), 7.82 (d, 2H, J = 8.4 Hz), 7.73 (d, 2H, J = 8.4 Hz), 7.65 (d, 1H, J = 16.4 Hz), 7.50 (d, 1H, J = 16.4 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.25, 148.75, 147.48, 141.49, 139.81, 134.64, 133.18, 132.48, 131.65, 130.83, 129.07 (q, $J_{C\text{-}F}$ = 32.0 Hz), 128.40, 127.89, 127.02, 126.73, 126.40 (q, $J_{C\text{-}F}$ = 3.6 Hz), 124.74 (q, $J_{C\text{-}F}$ = 273.1 Hz). HRMS (ESI) calcd for $C_{21}H_{14}F_3N_2O_2$ [M − H]$^-$: 383.1013, Found: 383.1012.

*4.1.4.26. (E)-4-(2-(5-(2-fluoro-4-methylphenyl)pyridin-3-yl)vinyl)-N-hydroxybenzamide (**16c**).* The title compound (**16c**) was synthesized using compound **15c** in a manner similar to that described for the synthesis of compound **5a**. White powder, Yield: 62.0%, Mp: 210–211 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.29 (br, 1H), 9.11 (br, 1H), 8.82 (d, 1H, J = 2.0 Hz), 8.64 (t, 1H, J = 2.0 Hz), 8.22 (d, 1H, J = 1.6 Hz), 7.81 (d, 2H, J = 8.0 Hz), 7.73 (d, 2H, J = 8.4 Hz), 7.59–7.54 (m, 2H), 7.48 (d, 1H, J = 16.4 Hz), 7.50 (d, 1H, J = 16.4 Hz), 7.22 (dd, 1H, J_1 = 1.6 Hz, J_2 = 12.8 Hz), 7.19 (dd, 1H, J_1 = 1.6 Hz, J_2 = 8.0 Hz), 2.40 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.27, 159.53 (d, $J_{C\text{-}F}$ = 247.1 Hz), 148.66 (d, $J_{C\text{-}F}$ = 4.0 Hz), 147.83, 141.37 (d, $J_{C\text{-}F}$ = 8.3 Hz), 139.81, 133.23 (d, $J_{C\text{-}F}$ = 2.5 Hz), 132.88, 132.43, 131.51 (d, $J_{C\text{-}F}$ = 1.6 Hz), 131.03 (d, $J_{C\text{-}F}$ = 3.5 Hz), 130.54, 127.85, 127.04, 126.80, 126.27 (d, $J_{C\text{-}F}$ = 2.9 Hz), 122.29 (d, $J_{C\text{-}F}$ = 13.6 Hz), 117.00 (d, $J_{C\text{-}F}$ = 22.1 Hz), 21.08. HRMS (ESI) calcd for $C_{21}H_{18}FN_2O_2$ [M + H]$^+$: 349.1347, Found: 349.1349.

*4.1.4.27. (E)-N-hydroxy-4-(2-(5-(2-hydroxyphenyl)pyridin-3-yl)vinyl)benzamide (**16d**).* The title compound (**16d**) was synthesized using compound **15d** in a manner similar to that described for the synthesis of compound **5a**. White powder, Yield: 61.2%, Mp: 167–168 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.25 (br, 1H), 9.80 (s, 1H), 9.07 (br, 1H), 8.73 (d, 1H, J = 2.4 Hz), 8.65 (d, 1H, J = 2.4 Hz), 8.19 (t, 1H, J = 2.4 Hz), 7.79 (d, 2H, J = 8.4 Hz), 7.72 (d, 2H, J = 8.4 Hz), 7.51 (d, 1H, J = 16.4 Hz), 7.46 (d, 1H, J = 16.4 Hz), 7.38 (dd, 1H, J_1 = 1.6 Hz, J_2 = 7.6 Hz), 7.27–7.23 (m, 1H), 7.01 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.0 Hz), 6.97–6.92 (m, 1H), 3.86 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.03, 159.79, 154.06, 151.59, 144.68, 139.42, 138.38, 138.27, 137.10, 137.04, 135.65, 134.70, 132.56, 131.98, 131.73, 129.41, 124.86, 121.27. HRMS (ESI) calcd for $C_{20}H_{15}N_2O_3$ [M − H]$^-$: 331.1088, Found: 331.1089.

*4.1.4.28. (E)-4-(2-(5-(5-fluoro-2-methoxyphenyl)pyridin-3-yl)vinyl)-N-hydroxybenzamide (**16e**).* The title compound (**16e**) was synthesized using compound **15e** in a manner similar to that described for the synthesis of compound **5a**. White powder, Yield: 64.4%, Mp: 148–149 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.29 (s, 1H), 9.10 (br, 1H), 8.78 (d, 1H, J = 2.0 Hz), 8.62 (d, 1H, J = 2.0 Hz), 8.20 (t, 1H, J = 2.0 Hz), 7.81 (d, 2H, J = 8.4 Hz), 7.72 (d, 2H, J = 8.4 Hz), 7.55 (d, 1H, J = 16.4 Hz), 7.46 (d, 1H, J = 16.4 Hz), 7.36 (dd, 1H, J_1 = 2.8 Hz, J_2 = 8.8 Hz), 7.30–7.25 (m, 1H), 7.19 (dd, 1H, J_1 = 4.8 Hz, J_2 = 8.8 Hz), 3.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.26, 156.90 (d, $J_{C\text{-}F}$ = 237.4 Hz), 153.15 (d, $J_{C\text{-}F}$ = 2.0 Hz), 149.19, 147.41, 139.85, 133.94, 133.22, 132.62, 132.38, 130.39, 127.93, 127.84, 127.00, 126.88, 117.55 (d, $J_{C\text{-}F}$ = 23.2 Hz), 116.10 (d, $J_{C\text{-}F}$ = 22.2 Hz), 113.64 (d, $J_{C\text{-}F}$ = 8.1 Hz), 56.72. HRMS (ESI) calcd for $C_{21}H_{16}FN_2O_3$ [M − H]$^-$: 363.1150, Found: 363.1151.

4.2. In vitro LSD1、MAO-A and MAO-B inhibition assay

In vitro LSD1 inhibition assays were conducted as previously described [61]. Briefly, the candidate compounds were incubated with the recombinant LSD1 and H3K4me2. After that, the fluorescence was measured at excitation wavelength 530 nm and emission wavelength 590 nm with the addition of Amplex Red and horseradish peroxidase (HRP) in order to evaluate the inhibition rate of the candidate compound. MAO inhibitory activities were determined using a commercialized MAO-Glo assay kit from Promega, according to the manufacturer's protocol.

4.3. In vitro HDAC inhibition assay

In vitro HDAC assays were carried out by Shanghai Sundia MediTech Company, Ltd. in Shanghai, China. The general procedures were as the following: 1x assay buffer (modified Tris Buffer) was prepared. HDAC enzyme was diluted to 1.67x final concentration with 1x assay buffer. Substrate solution was prepared by mixing trypsin and Ac-peptide substrate and diluted to 2.5x final concentration with 1x assay. 250 nL candidate compounds or SAHA were transferred to 384-well plate using Echo550. For control wells, transfer 250 nL DMSO. Then, 15 μL of enzyme solution was added to 384-well plate and pre-incubate with candidate compounds at room temperature for 15 min. For negative control, 15 μL 1x assay buffer was added instead of enzyme solution. 10 μL substrate solution was then added to 384-well to initial the reaction. The fluorescence generated was recorded at 355 nm (excitation) and 460 nm (emission) using Envision (PerkinElmer).

4.4. Surface plasmon resonance (SPR) experiment

Studies of binding kinetics are performed on a Biacore S200 (GE Healthcare, USA). LSD1 is aimed for an immobilization level of approximately 8000 RU with a CM5 sensor chip, and the running buffer is PBS-P (0.2 M phosphate buffer 0.027 M KCl, 1.37 M NaCl, 0.5%Surfactant P20, PH 7.4). The direct binding assay is tested with buffer and sample in 2% DMSO. Then compounds with twofold dilution series are injected for 60s and dissociated for 300s at a flow rate of 30 μL/min. The data is analyzed by Biacore S200 Evaluation Software 1.0.

4.5. In vitro antitumor activity assay

Cancer cells were seeded into 96-well plates at a density of 1.5×10^4 cells per well, treated with the synthesized compounds at different concentrations. After 72 h treatment, the assay plate was equilibrated to room temperature, then 40 mL of CellTiter-Glo®Reagent (Promega) was added to each well, mixed for 2 min on an orbital shaker to induce cell lysis and incubated at room temperature for 10 min to stabilize luminescent signal. The luminescence was recorded using Envision (PerkinElmer).

4.6. Apoptosis detection assay

Cell apoptosis was determined using Annexin-V-FITC/PI apoptosis detection kit (Biovision). MGC-803 cancer cells were seeded in six-well plates and then treated with DMSO, compound **5d** (2.5, 5.0 μM), compound **5m** (2.5, 5.0 μM) or SAHA (5.0 μM) for 48 h. The procedures were conducted according to the manufacturer's recommended procedures. The samples were detected with a CytoFLEX flow cytometer (Beckman).

4.7. Colony formation assay

MGC-803 cells (500 cells/well) were seeded in 24-well plate, 1:1 mixture of RPMI 1640 containing 10% FBS (BI) and Methylcellulose-based medium as the culture medium. Cells were treated with compound **5d**, **5m**, **ORY-1001** or SAHA at indicated concentrations for 7 days. Then cells were stained with DAPI (Sigma) according to the manufacturer's instructions and washed with PBS. The colony formation was imaged using microscope (TS-100, Nikon, Japan).

4.8. Western blot assay

10^6 MGC-803 cells/well were incubated with compounds **5d**, **5m**, SAHA, ORY-1001, or DMSO for 48 h. Then the cells were washed twice with prechilled PBS and lysed in ice-cold RIPA buffer. Lysates were cleared by centrifugation. Protein concentrations were determined using the BCA assay. Equal amounts of cell extracts were then resolved by SDS-PAGE, transferred to nitrocellulose membranes, and probed with H3K4me1 antibody, H3K4me2 antibody, H3K9me2 antibody, Ac-histone H3 antibody, α-tubulin antibody, Ac-α-tubulin antibody, GAPDH antibody, Bax antibody, Bcl-2 antibody, Vimentin antibody, ZO-1 antibody and E-cadherin antibody, respectively. The immunoblots were detected using an enhanced chemical luminescence system.

4.9. Migration ability assay

For the migration assay, Costar® Transwell cell culture chamber (8.0 μm pore size for the porous membrane) was used. Cells and compounds were seeding into the upper chambers containing 1% FBS. Meanwhile, 600 μL medium with 20% FBS was used as chemoattractant in the lower chamber. After incubation for 12 or 24 h, cells passing through the membrane were quantified with high content screening using Hoechst 33258 for cell counting.

4.10. Cytochrome p450 inhibition assay

The CYP inhibition assays were performed by Shanghai Sundia MediTech Company, Ltd. in Shanghai, China. The inhibition rate of compound **5d** and **5m** on CYP activity was determined at a concentration of 10 μM. α-Naphthoflavone (for CYP1A2), Sulfaphenazole (for CYP2C9), Ticlopidineand (for CYP2C19), Quinidine (for CYP2D6) and Ketoconazole (for CYP3A4) served as the positive controls. Phenacetin, Diclofenac, S-Mephenytoin, Dextromethorphan and Testosterone served as the substrates for CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4, respectively.

4.11. Microsome metabolic stability assay

In vitro metabolic stability assay was carried out by Shanghai Sundia MediTech Company, Ltd. in Shanghai, China. The general procedures were as the following: Incubation mixtures containing 0.2 mg/mL Rat or human liver microsomal protein and 1 μM tested compounds or positive control in 100 mM potassium phosphate buffer was prepared. The 0-min samples were prepared by addition of an 80 μL aliquot of each incubation mixture to 400 μL quench reagent consisted of acetonitrile containing tolbutamide and propanolol to precipitate proteins. The samples were vortexed, and then a 20 μL aliquot of the NADPH regenerating system was added in. The reaction was initiated by addition of 130 μL of the NADPH regenerating system to 520 μL of each incubation mixture. The final incubation conditions achieved in 650 μL are: 0.2 mg/mL microsomal protein, 1 μM test article/positive control, 1.3 mM NADP, 3.3 mM glucose 6 phosphate, 0.6 U/mL glucose 6 phosphate dehydrogenase. The mixtures were incubated in a 37°Cwater bath with gentle shaking. A 100 μL aliquot of each mixture was removed at 5, 10, 30, 60 min to a clean 96-well plate which contains 400 μL quench reagent to precipitate proteins, and centrifuged ($5000 \times g$, 10 min). 100 μL of supernatant are taken into 96-well assay plates pre-added with 200 μL ultrapure water, and then analyzed by LC-MS/MS. Intrinsic clearance (CLint) and half-life (T1/2) values were then calculated. Clint $= 0.693/T_{1/2}/0.5$ mg/mL.

4.12. Transcriptional assay

Human gastric cancer MGC-803 cells were cultured in RPMI1640 medium with 10% FBS and treated with different concentration of compounds **5d** or **5m** for 48 h. The total RNA was extracted using Ultrapure RNA Kit (Cowin Biotech Co., Ltd) according to the manufacturer's instructions. After that the purity of the isolated RNA was tested by NanoDrop™ One^c Spectrophotometer (Thermo Fisher Scientific Inc) and then the RNA was reverse transcribed using First-strand cDNA Synthesis Kit (Cowin Biotech Co., Ltd). Finally the mRNA expression was performed by quantitative real-time PCR with ChamQ Universal SYBR® qPCR Master Mix kit (Vazyme Biotech Co., Ltd). The human CD14, CD11B, CD86, and TBP specific primers which previously designed were synthesized by SunYa Biotech Co., Ltd.

4.13. Molecular docking

The 3D structures of compounds **5d** and **5m** were constructed in Sketch module of SYBYL-X2.0. Gastieger-Huckel charges were added to compounds **5d** and **5m** under Tripos force field, and then the energy was minimized. The maximum number of iterations was set to 1000, and the convergence criterion was limited to 0.001 kcal $mol^{-1}Å^{-1}$. Molecular docking was performed using Glide of Maestro. First, we downloaded LSD1 (PDB code: 2V1D), HDAC1 (PDB code: 4BKX), HDAC6 (PDB code: 5EEI) crystal structures from PDB bank, then deleted crystal water and added hydrogen atoms to entire complex. Secondly, LSD1, HDAC1, HDAC6 receptor-grid files were generated, the edge length of box was set to 20 Å × 20 Å x 20 Å. Finally, **5m** and **5d** were used to do docking with three receptors, respectively. The docking accuracy was set to standard precision mode (SP). Each small molecule was set to produce 20 poses, and the highest score pose was selected as the docking result under the condition of the orientation with the largest number of occurrences.

Declaration of competing interest

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

Acknowledgments

This work were supported by National Natural Science Foundation of China (Project No. U1704184 for Y.-C. D.; Science and Technology Tackling Key Project of Henan Province (Project No.182102310214, for Y.-C. D). The authors thank Dr. Meiting Wang from East China Normal University for offering Schordinger software support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.113453.

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