Bioorganic & Medicinal Chemistry 22 (2014) 5513–5529

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis and structure–activity relationships of novel, potent, orally active hypoxia-inducible factor-1 inhibitors

Satoshi Nagao [a,*], Yoshinobu Yamane [a], Setsuo Funasaka [a], Keigo Tanaka [a], Kazuki Miyazaki [a], Yoshihiko Kotake [a], Jun-ichi Kamata [a], Saori Watanabe-Miyano [a], Osamu Toyama [a], Yoichi Ozawa [a], Yoshiharu Mizui [a,b], Kiyoshi Okamoto [a], Daisuke Ito [a]

[a] Eisai Co., Ltd, 5-1-3 Tokodai, Tsukuba-shi, Ibaraki 300-2635, Japan
[b] H3 Biomedicine Inc., 300 Technology Square, Cambridge, MA 02139, USA

ARTICLE INFO

Article history:
Received 2 June 2014
Revised 14 July 2014
Accepted 14 July 2014
Available online 30 July 2014

Keywords:
Hypoxia-inducible factor-1
Hypoxia
Structure–activity relationships
Cancer
Inhibitor
Small molecule

ABSTRACT

Hypoxia-inducible factor-1 (HIF-1) is the chief transcription factor regulating hypoxia-driven gene expression. HIF-1 overexpression is associated with poor prognosis in several cancers and therefore represents an attractive target for novel antitumor agents. We explored small molecule inhibitors of the HIF-1 pathway. Using high-throughput-screening, we identified benzanilide compound **1** (IC_{50} = 560 nM) as a seed. Subsequent extensive derivatization led to the discovery of compounds **43a** and **51d**, with anti-HIF-1 activities in vitro (IC_{50} = 21 and 0.47 nM, respectively), and in vivo. Additionally, **43a** (12.5–100 mg/kg) also displayed in vivo anti-tumor efficacy, without influencing body weight.

1. Introduction

It is generally accepted that cancer cells in hypoxic regions resist existing chemotherapies and radiation therapy,[1–3] and are associated with an increased risk of invasion, metastasis, treatment failure, and patient mortality.[3–5] Hypoxia in itself produces various disadvantages for both normal and cancer cells, such as decreased energy production and induction of apoptosis. However, cancer cells can adapt to the hypoxic environment by activating the hypoxia-inducible factor (HIF) pathway to induce the expression of a wide variety of genes involved in glycolysis, angiogenesis, hematopoiesis, survival pathways, and invasion.[6–10] HIF-1 is a transcription factor, which consists of an α/β heterodimer. HIF-1α is an oxygen-regulated protein and HIF-1β is a constitutively expressed protein, irrespective of oxygen concentration. Under normoxic conditions, the von Hippel–Lindau (VHL) protein recognizes and binds to HIF-1α, which is hydroxylated by prolyl hydroxylase-domain proteins (PHD) 1–3 on proline residues 402 and/or 564. Subsequently, HIF-1α is polyubiquitinated and rapidly degraded by the 26S proteasome. Under hypoxic conditions, however, HIF-1α is highly stabilized, dimerizes with HIF-1β, and translocates to the nucleus. The HIF-1 complex then becomes transcriptionally activated and contributes to cancer malignancy.[6–12]

Overexpression of HIF-1α has been reported in many types of cancers, including colon, breast, lung, gastric, skin, ovarian, pancreatic, prostate and renal carcinomas.[13] It is significantly associated with patient mortality in several different types of cancers affecting the brain, breast, cervix, and lung.[5] These findings indicated that HIF-1α represented an attractive therapeutic target for a wide range of cancers and considerable effort has therefore been devoted to the identification of HIF-1 inhibitors. Various types of small molecules have been reported to inhibit the HIF-1 pathway.[14–16] However, no HIF-1 pathway inhibitors have yet been approved for clinical use and there is still a need for a novel compound with potent HIF-1 inhibitory activity.

The present study describes our identification of novel HIF-1 inhibitors. High-throughput screening (HTS) using a cell-based reporter assay[17] identified *N*-(4-iodophenyl)-4-(morpholinomethyl)-benzamide (compound **1**), which had submicromolar activity (IC_{50} = 560 nM) under hypoxic conditions. This compound reduced

Abbreviations: HIF-1, hypoxia-inducible factor-1; VHL, von Hippel–Lindau; PHD, prolyl hydroxylase; HTS, high-throughput screening; NBS, *N*-bromosuccinimide; MNBA, 2-methyl-6-nitrobenzoic anhydride; DIBAL, diisobutylaluminum hydride; VEGF-PLAP, vascular endothelial growth factor-placental alkaline phosphatase.

* Corresponding author. Tel.: +81 (0)29 847 5882; fax: +81 (0)29 847 5677.
E-mail address: s-nagao@hhc.eisai.co.jp (S. Nagao).

http://dx.doi.org/10.1016/j.bmc.2014.07.020
0968-0896/

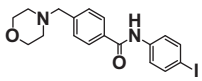
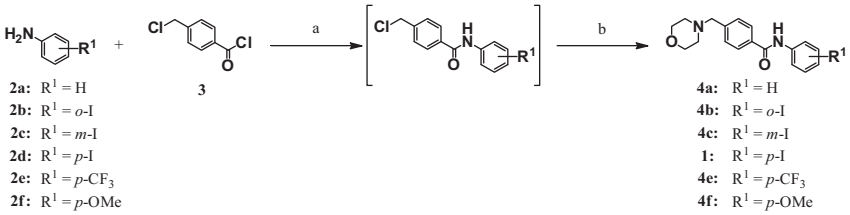


Figure 1. Benzanilide compound **1**.

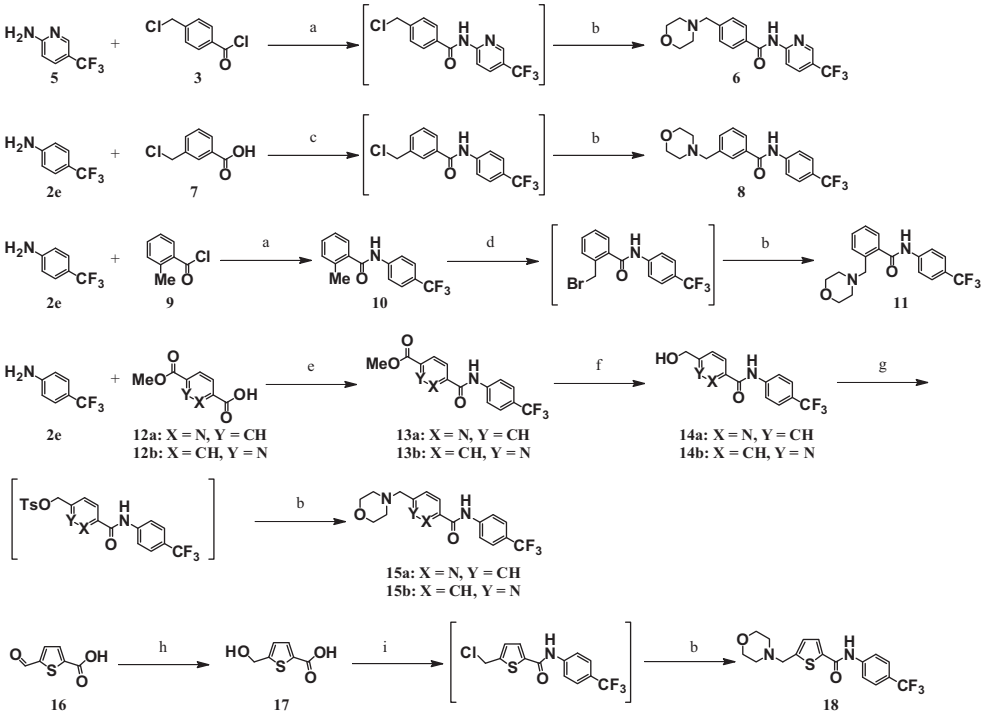
the HIF-1α protein level without affecting its mRNA level.[18] Based on these data, compound **1** was derivatized to produce compounds that showed in vitro activity at subnanomolar concentrations and in vivo efficacy in the U251 mouse xenograft model, without changes in body weight.

2. Chemistry

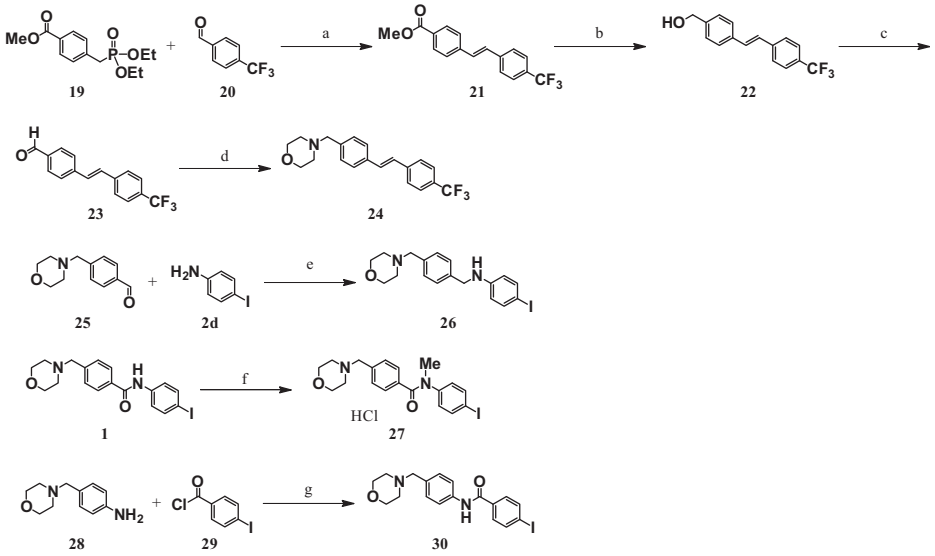
First, we explored the optimal substitution of the *para*-iodo moiety of benzanilide compound **1** (Fig. 1) to create more potent compounds. Derivatization of benzanilide was conducted as shown in Scheme 1. Benzanilide derivatives, **1** and **4a–c**,**e**,**f**, were synthesized by condensation followed amidation. To investigate the optimal position of a substituent, *ortho*-, *meta*-, and *para*-benzylmorpholine were prepared for the amide structure using similar synthetic routes (Scheme 2).[19] To explore an alternative to the benzanilide structure, we synthesized compounds bearing a heteroaromatic core. Condensation was carried out either using 2-methyl-6-nitrobenzoic anhydride (MNBA) or via acid chloride from **12**, followed by reduction using lithium borohydride. Subsequent tosylation and amination with morpholine gave **6**, **15a–b** with pyridine, and **18** with thiophene. These benzanilide compounds had some sub-optimal physicochemical properties, as they were highly crystalline, and/or raised concerns regarding their chemical/metabolic amide stability. With the aim of improving these physicochemical properties, we explored isosteres of the amide moiety or alternatives to morpholine. *E*-Olefine **21** was synthesized from phosphonate **19**[20] and aldehyde **20** using a conventional Horner–Wadsworth–Emmons reaction.[21] The subsequent reduction of methyl ester, oxidation of alcohol and reductive amination afforded the desired *E*-olefine compound **24** (Scheme 3).[22–24] Compounds **26**, **27**, and **30** were synthesized from the already known compounds **25**, **1**, and **28**, respectively. Compounds **32a–d** and **35a–d** were synthesized via nucleophilic substitution to benzylchloride **31** using the amine derivative (Scheme 4).[25] Compound **38** without benzylamine was formed using **2d** and **36**. Sonogashira cross-coupling reactions with aromatic or cyclic aliphatic acetylene using Pd and CuI as catalysts produced the corresponding compounds **41a–e** and **43a–b**, respectively (Scheme 5).[26–28] Compounds **51a**,**c**,**d** were prepared from



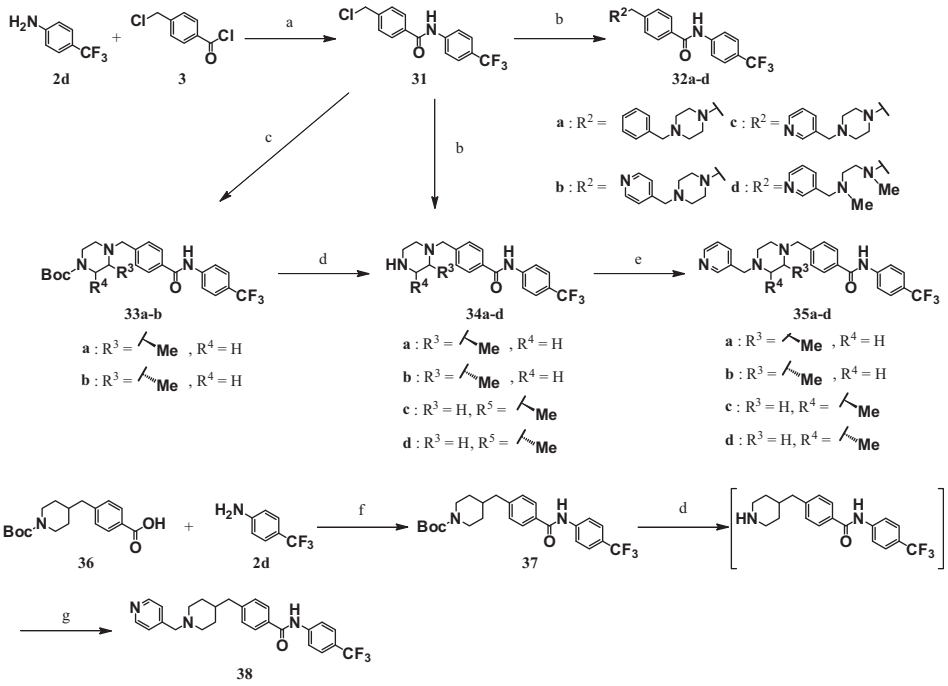
Scheme 1. Reagents and conditions: (a) saturated $NaHCO_3$ aq, THF, room temperature (RT) or Et_3N, THF, RT; (b) morpholine, RT or morpholine, THF, RT.



Scheme 2. Reagents and conditions: (a) saturated $NaHCO_3$ aq, THF, room temperature (RT) or Et_3N, THF, 0 °C; (b) then morpholine, RT, or morpholine, Et_3N, DMF, RT; (c) $SOCl_2$, reflux then saturated $NaHCO_3$ aq, THF, RT; (d) NBS, benzoyl peroxide, CCl_4, reflux; (e) $SOCl_2$, reflux then Et_3N, THF, RT or MNBA, Et_3N, DMAP, DCM, RT; (f) $LiBH_4$, THF, RT; (g) TsCl, DMAP, pyridine, Et_3N, THF–DCM, RT; (h) $NaBH_4$, MeOH, 0 °C to RT; (i) $SOCl_2$, reflux then H_2N–C_6H_4–CF_3, Et_3N, THF, RT.



Scheme 3. Reagents and conditions: (a) NaH, THF, 0 °C to room temperature (RT); (b) DIBAL, toluene, −78 °C to RT; (c) MnO_2, DCM, RT; (d) morpholine, $NaBH(OAc)_3$, AcOH, 1,2-dichloroethane, RT; (e) $NaBH(OAc)_3$, THF, RT; (f) MeI, NaH, DMF, RT then 4 N HCl in EtOAc; (g) pyridine, THF, RT.



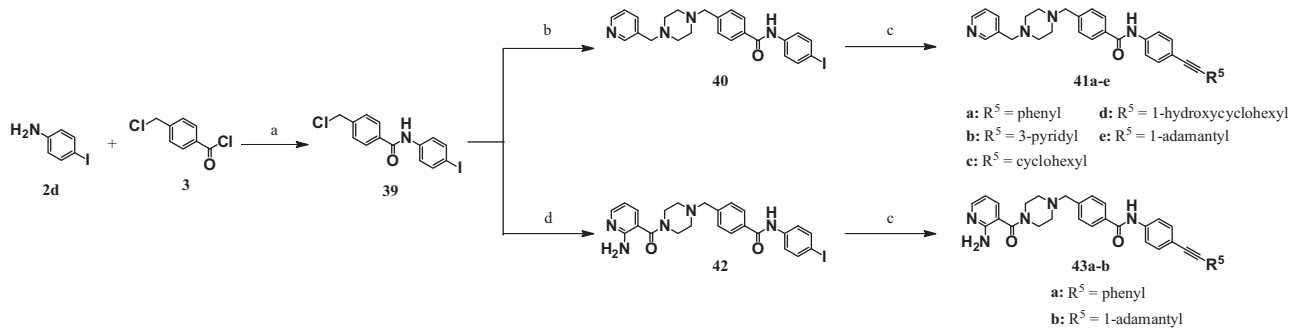
Scheme 4. Reagents and conditions: (a) Et_3N, THF, 0 °C to room temperature (RT); (b) amine, Et_3N, DMF, RT; (c) 2-methylpiperazine Et_3N, DMF, 60 °C; (d) TFA, RT or TFA, DCM, 0 °C to RT; (e) 3-(chloromethyl)pyridine hydrochloride, Et_3N, DMF, 60 °C; (f) MNBA, Et_3N, DMAP, dichloromethane, RT; (g) 4-pyridinecarboxaldehyde, $NaBH(OAc)_3$, AcOH, 1,2-dichloroethane, THF, RT.

benzylchloride **50** and chiral piperazine **47a**,**c**,**d**, which were synthesized as shown in Scheme 6.[29,30]

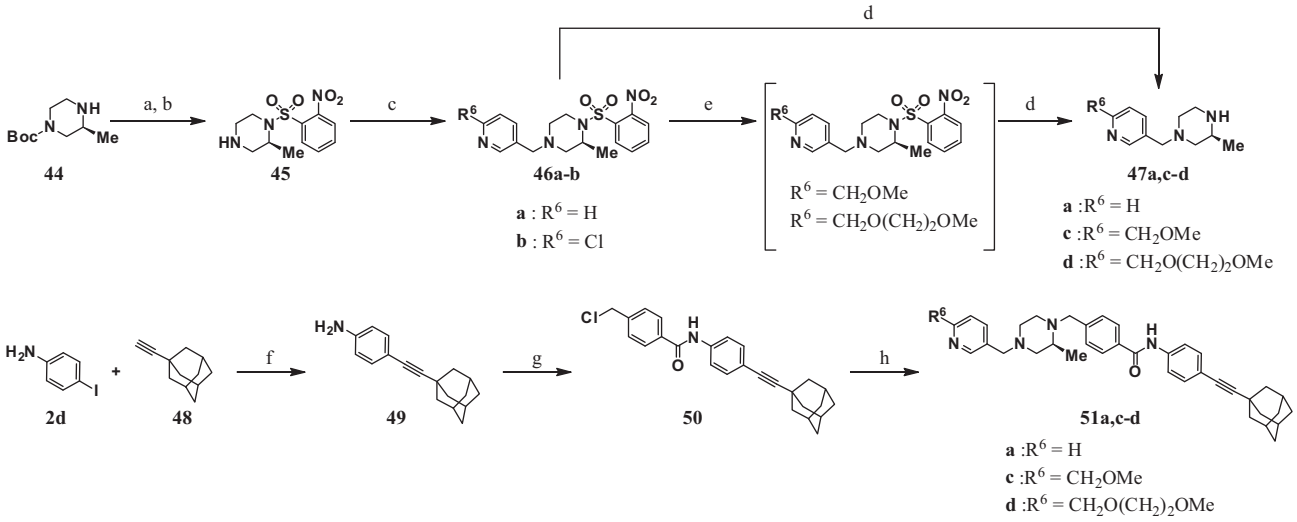
3. Results and discussion

The in vitro activities of the benzanilide derivatives on HIF-1 transcription, quantified using a human glioma cell line (U251) expressing a PLAP reporter gene under the control of a VEGF promoter containing the active HIF-1 binding site (VEGF-PLAP), are summarized in Tables 1–6. Table 1 highlights the effect of modification of the *para*-iodophenyl moiety. The importance of the *para*-substituent was indicated from the lower activity obtained using **4a**, **4b**, and **4c**. Replacement of the *para*-iodophenyl moiety with the hydrophilic *para*-methoxy group in compound **4f** resulted in a 25-fold decrease in potency compared to compound **1**, whereas *para*-trifluoromethyl moiety **4e** was slightly more potent than compound **1**. This result indicated that a hydrophobic moiety on the *para*-position of aniline was required to elicit potent HIF-1 inhibitory activity.

To investigate whether the phenyl moiety of the benzanilide compounds could be replaced by another cyclic moiety, we synthesized pyridine derivatives **6**, **15a**–**b** and a thiophene derivative

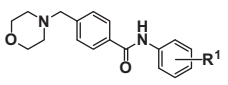


Scheme 5. Reagents and conditions: (a) saturated $NaHCO_3$ aq, THF, room temperature (RT) or Et_3N, THF, RT; (b) 3-pyridylmethylpiperazine, Et_3N, DMF, RT; (c) HC≡C-R^5, $PdCl_2(PPh_3)_2$ or $Pd(PPh_3)_4$, CuI, Et_3N, DMF, RT or 100 °C; (d) (2-aminopyridin-3-yl)(piperazin-1-yl)methanone, Et_3N, DMF, 50 °C.



Scheme 6. Reagents and conditions: (a) 2-nitrobenzene-1-sulfonyl chloride, Et_3N, THF, 0 °C to room temperature (RT); (b) TFA, DCM, 0 °C to RT; (c) 3-(chloromethyl)pyridine, Et_3N, DMF, 60 °C; or 2-chloro-5-(chloromethyl)pyridine, Et_3N, DMF, 60 °C; (d) mercaptoacetic acid, LiOH, DMF, RT; (e) $MeOCH_2SnBu_3$ or $MeO(CH_2)_2OCH_2SnBu_3$, $Pd(PPh_3)_4$, DMF, 120 °C; (f) $PdCl_2(PPh_3)_2$, CuI, Et_3N, DMF, RT; (g) 4-chloromethylbenzoyl chloride, Et_3N, THF, 0 °C; (h) piperazine derivatives, Et_3N, DMF, 70 °C.

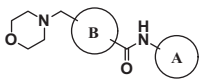
Table 1
Effect of substitution of R^1 on in vitro biological activity



No.	R^1	VEGF/PLAP IC_{50} (μM)
4a	H	5.4
4b	*o*-I	>20
4c	*m*-I	2.2
1	*p*-I	0.56
4e	*p*-CF_3	0.28
4f	*p*-OMe	13.9

18. As shown in Table 2, a heteroaromatic ring, including pyridine and thiophene, resulted in a >5-fold decrease in potency relative to compound **4e**, except for picolinamide **15a**. One possible explanation for the comparable inhibitory activity of **15a** and **4e** is that the basicity/polarity of the pyridine nitrogen was compromised by the adjacent carboxamide functionality. However, the picolinamide **15a** showed unacceptable toxicity compared with the benzanilide **4e** in the mouse model (data not shown) and we therefore selected benzanilide. Replacement of the six membered

Table 2
Effect of ring A and B on in vitro biological activity



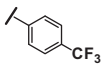
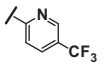
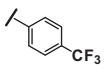
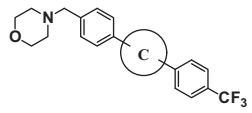
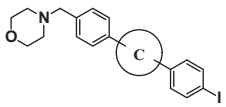
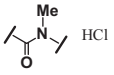
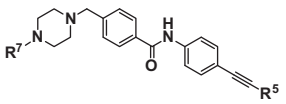
No.	A	B	VEGF/PLAP IC_{50} (μM)
4e			0.28
6			4.0
8			>10
11			>10
15a			0.23
15b			4.5
18			1.6

Table 3
Effect of ring C on in vitro biological activity

No.	C	VEGF/PLAP IC_{50} (μM)
		
4e		0.28
24		0.88
		
1		0.56
26		2.5
27		2.2
30		2.5

benzene/pyridine rings with five membered heterocyclic rings, such as thiophene, led to decreased activity. Additionally, replacement with *meta*- and *ortho*-substitutions **8** and **11** resulted in >35-fold decrease in potency, relative to compound **4e**. These results suggested that *para*-substitution in ring B was required for potent HIF-1 inhibitory activity. The *E*-olefin compound **24** showed moderate inhibitory activity (Table 3). In contrast, the aniline **26** and methylated amide hydrochloride **27** showed less HIF-1 inhibition than did amide **1**. In addition, retro amide **30** resulted in an approximately 5-fold decrease in potency, compared to the seed compound **1**.

Table 5
Effects of substitution of R^5 and R^7 on in vitro biological activity

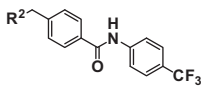


No.	R^5	R^7	VEGF/PLAP IC_{50} (μM)
41a	Ph		0.0079
41b	3-Pyridyl		0.034
41c			0.0031
41d			>0.2
41e			0.00097
43a	Ph		0.021
43b			0.0058

The structure–activity relationships (SAR) of R^2 are highlighted in Table 4. Replacement of the morpholine group with a benzylpiperazine group in compound **32a** resulted in a >10-fold increase in potency, relative to compound **4e**. The pyridylmethylpiperazine groups, such as 4-pyridyl and 3-pyridyl analogues, also had equipotent inhibitory activity to benzylpiperazine **32a**. The importance of an amine moiety at the benzylic position was demonstrated by compound **38** which lacked a nitrogen atom at this position and exhibited >10-fold decrease in potency, relative to compound **32b**. Compound **32d** possessed an open piperazine ring and showed an approximate 9-fold decrease in potency relative to compound **32c**. Compound **35a**, where a chiral methyl moiety was introduced in the piperazine ring, exhibited more potent HIF-1 inhibition compared to **32c**.

An increase in potency was observed with the *para*-phenylacetylene **41a**, compared to compound **32c** (Table 5). The potency of

Table 4
Effect of substitution of R^2 on in vitro biological activity



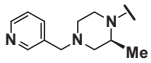
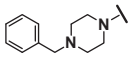
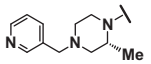
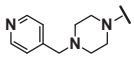
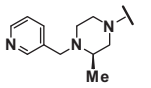
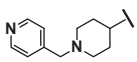
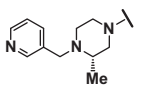
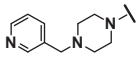
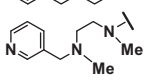
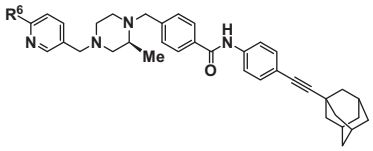
No.	R^2	VEGF/PLAP IC_{50} (μM)	No.	R^2	VEGF/PLAP IC_{50} (μM)
4e		0.28	**35a**		0.0033
32a		0.013	**35b**		0.013
32b		0.0079	**35c**		0.030
38		0.11	**35d**		0.035
32c		0.014			
32d		0.12			

Table 6
Effects of substitution of R^6 on in vitro biological activity



No.	R^6	VEGF/PLAP IC_{50} (μM)
51a	H	0.0026
51c	CH_2OMe	0.0011
51d	$CH_2O(CH_2)_2OMe$	0.00047

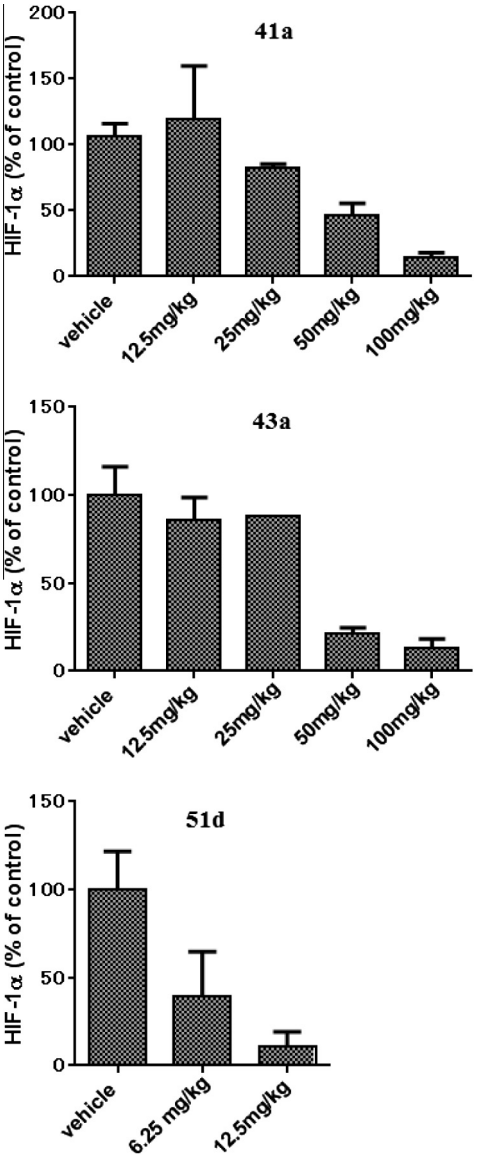


Figure 2. Pharmacodynamic profiles of **41a**, **43a**, and **51d**. Mice bearing U251 tumor xenografts were treated with a single dose of **41a**, **43a**, or **51d** at the indicated dosage. Tumors were harvested 24 h after dosing. The amounts of HIF-1α in the tumors were measured by an ELISA and expressed as a percentage of the vehicle control. Data points represent the mean of three animals.

41a led us to explore hydrophobic substituents further. The cyclohexylacetylene **41c** and adamantylacetylene **41e** showed at least 2-fold improvement in potency, relative to phenylacetylene **41a**. In particular, adamantylacetylene **41e** exhibited the highest potency (IC_{50} = 0.97 nM). In contrast, 3-pyridyl acetylene **41b** and hydroxycyclohexylacetylene **41d** resulted in 4-fold and over 25-fold reductions in potency relative to phenylacetylene **41a**, respectively. These data clearly indicated that hydrophobic substituents on the *para*-position of the benzanilide moiety were essential for potent HIF-1 inhibitory activity. Compounds **43a** and **43b** with 2-aminonicotinic amide also displayed good activity (IC_{50} = 21 nM and 5.8 nM, respectively), however they were slightly less effective than compounds **41a** and **41e**, bearing the 3-pyridylmethyl moiety.

Finally, benzanilide **51a**, synthesized to incorporate the positive SARs described above, exhibited potent HIF-1 inhibition, as expected (Table 6). Furthermore, we explored the effect of substitution on pyridine in **51a** and discovered that substitution of the 6-position on pyridine effectively increased inhibitory activity. In particular, analogue **51d** with a methoxyethoxymethyl moiety showed a sub-nM IC_{50}. This compound was >1000 times more potent than seed compound **1**.

The benzanilide derivatives **41a**, **43a**, and **51d** with effective HIF-1 inhibitory activity in vitro were evaluated for suppression of HIF-1α protein level in vivo. Tumor samples from the mouse xenograft model where **41a** and **43a** had been administered orally at a dosage of 50 and 100 mg/kg displayed significantly less HIF-1α protein, but HIF-1α mRNA was not reduced at all,[18] 24 h after treatment (Fig. 2). Treatment with **51d**, which had shown the most potent in vitro activity, strongly reduced HIF-1α levels at doses of 6.25 and 12.5 mg/kg. However body weight decreased to 92% compared with control at the dose of 12.5 mg/kg of **51d** (data not shown). Next, we evaluated the antitumor efficacy of **43a** in the U251 mouse xenograft model (Fig. 3). Daily oral administration of 12.5–100 mg/kg **43a** for 11 days significantly delayed tumor growth, without significant body weight loss. Compound **43a** also suppressed HIF-1α protein levels 3 h after oral administration of 12.5 mg/kg (Fig. 4), and could be administered daily for approximately 50 days at 50 mg/kg in survival model using a U251 intracranial xenograft (data not shown). The detailed mechanism underlying HIF-1α inhibition by these benzanilide compounds was unclear. To investigate whether HIF-1α synthetic or degradation pathways were affected by these compounds, we experimented with **41a** in a present of an iron chelator and proteasome inhibitor. The degradation of HIF-1α needs to activate prolyl hydroxylase, which is activated in the presence of iron (II) and oxygen. Iron chelators, such as deferoxamine and cobalt, have been reported to interfere with the degradation of HIF-1α by suppressing prolyl hydroxylase. As a result, these induce HIF-1α accumulation under normoxic conditions. Interestingly, compound **41a** inhibited the induction of HIF-1α protein accumulation under hypoxic conditions, but it did not inhibit HIF-1α accumulation induced by deferoxamine (Fig. 5). On the other hand, proteasome inhibitors, such as MG-132, prevented HIF-1 complex degradation by inhibiting the ubiquitin-proteasome system. Compound **41a** did not suppress hypoxia induced HIF-1α protein accumulation in the presence of proteasome inhibitor MG-132 (Fig. 6). Additionally, in vitro and in vivo experiments showed no change of HIF-1α mRNA in the presence of compound **41a**.[18] These results suggested that benzanilide compounds may enhance the degradation of HIF-1α under hypoxic conditions through accelerating the proline hydroxylation dependent ubiquitin–proteasome pathway but not synthetic

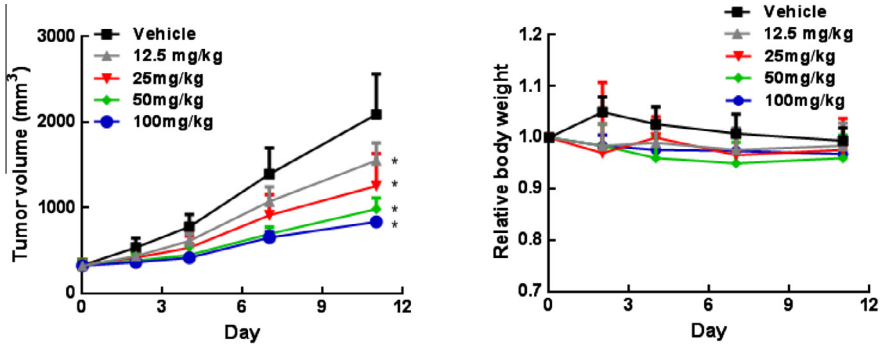


Figure 3. Compound **43a** suppressed U251 tumor xenograft growth without changes in body weight. Mice bearing U251 xenografts received oral administration of **43a** or vehicle daily for 11 days. The *y*-axis indicates tumor volume (mm^3). Data points represent the mean of five animals, $^*P < 0.05$ compared with the vehicle control; bars represent the standard deviation.

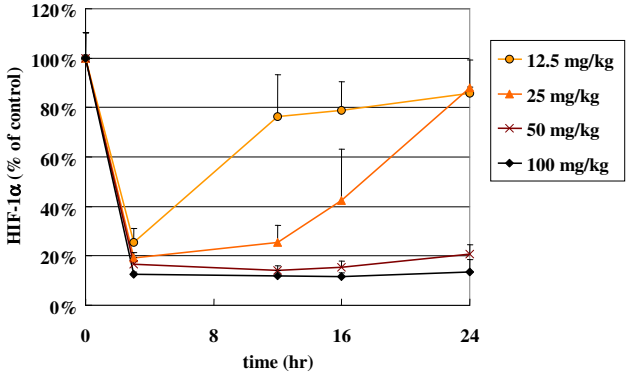


Figure 4. The time-course of changes in the amount of HIF-1α protein in U251 xenografts following **43a** treatment. U251 tumor-bearing mice were orally treated with indicated dosage of **43a**. Tumors were collected at 3, 12, 16 and 24 h after treatment. The amount of HIF-1α protein in total tumor extracts were determined with ELISA. Means ± SD are shown.

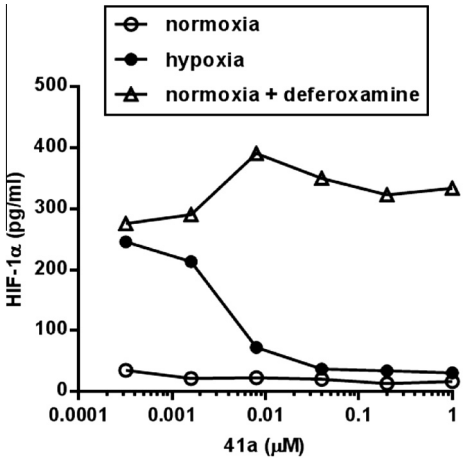


Figure 5. Compound **41a** failed to suppress the deferoxamine induced HIF-1α protein accumulation. Untransfected U251 cells (4×10^4 cells/well in 96-well plates) were incubated for 6 h with different concentrations of **41a** under either normoxia (21% O_2), hypoxia (2% O_2), or normoxia in the presence of 100 μM deferoxamine. The amounts of HIF-1α protein were then measured by ELISA. Data points represent the mean of two wells.

pathway. Further studies are needed to clarify the precise mechanism of benzanilide action on the HIF-1 pathway.

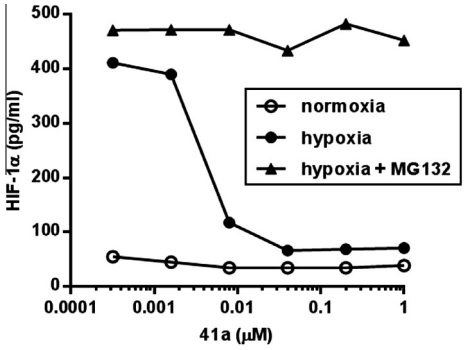


Figure 6. Compound **41a** failed to suppress hypoxia-induced HIF-1α protein accumulation in the presence of proteasome inhibitor MG132. Untransfected U251 cells (4×10^4 cells/well in 96-well plates) were incubated with different concentrations of **41a** for 6 h under either normoxia (21% O_2), hypoxia (2% O_2), or hypoxia in the presence of 25 μM MG132. The amounts of HIF-1α protein were then measured by ELISA. Data points represent the mean of two wells.

4. Conclusions

We explored the development of potent and orally active HIF-1 pathway inhibitors using benzanilide analogues, starting from the HTS hit compound **1**. SAR analyses indicated that: (1) a hydrophobic moiety was preferred at the 4-position of aniline in compound **1**; (2) the piperazinomethyl and carboxyanilide needed to be in a *para*-configuration; and (3) benzyl- or pyridylmethyl-piperazine, instead of morpholine, improved HIF-1 pathway inhibition. In particular, adamantyl-acetylene and (*S*)-4-((6-substituted-4-pyridin-3-yl)methyl)-2-methylpiperazine moieties dramatically improved the compounds' anti-reporter expression activity in vitro and **51d** achieved a sub-nM IC_{50}. Furthermore, we confirmed that representative benzanilide compounds decreased HIF-1α protein levels in vivo.

We discovered a HIF-1 inhibitor with fairly potent in vitro and in vivo activity. As the detailed mechanism underlying these effects is still unclear, additional studies are required for further development of this compound series.

5. Experimental sections

5.1. Chemistry

5.1.1. General methods

^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance spectrometer (operating at

600 MHz for ^{1}H and 150 MHz for ^{13}C). Chemical shifts were expressed in ppm (δ) from the residual $CHCl_3$ signal at δ_H 7.26 ppm and δ_C 77.0 ppm in $CDCl_3$ or the residual CHD_2SOCD_3 signal at δ_H 2.50 ppm and δ_C 39.5 ppm in CD_3SOCD_3 (s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, and br = broad). Coupling constants (J) are given in Hertz (Hz). High-resolution mass spectra (HRMS) were recorded on a Thermo Fisher LTQ Orbitrap XL spectrometer using electrospray ionization (ESI) and a Waters GCT Premier using electron ionization (EI). Column chromatography was carried out using silica gel 60 (spherical) (40–50 μm, Kanto Chemical Co., Inc.), Chromotorex (200–350 mesh, Fuji Silysia Chemical Ltd) and a Hi-Flash™ column (silica gel and NH-silica gel, Yamazen Corporation). Chemicals and solvents used in the study were commercially available.

5.1.2. 4-(Morpholinomethyl)-*N*-phenylbenzamide (4a)

To a stirred solution of **2a** (100 mg, 0.529 mmol) in THF (2 mL) and saturated $NaHCO_3$ aqueous solution (0.4 mL) was added dropwise 4-(chloromethyl)benzoyl chloride (100 mg, 0.529 mmol) in tetrahydrofuran (THF) (0.5 mL) at 0 °C and stirred at 0 °C for 30 min. Morpholine (231 μL, 2.65 mmol) was added and the reaction mixture was stirred at room temperature overnight. The reaction mixture was then poured into saturated $NaHCO_3$ and extracted with ethyl acetate (EtOAc). The organic extract was washed with brine, dried over Na_2SO_4, filtered and then concentrated. The crude solid was washed with *n*-hexane/diethyl ether and filtered to produce compound **4a** as a white solid (141 mg, 0.477 mmol, 90.2%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.46 (br s, 4H), 3.56 (s, 2H), 3.72 (t, J = 4.5 Hz, 4H), 7.13–7.18 (m, 1H), 7.37 (t, J = 7.8 Hz, 2H), 7.46 (d, J = 7.9 Hz, 2H), 7.64 (d, J = 7.8 Hz, 2H), 7.78–7.85 (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 53.6, 62.9, 67.0, 120.1, 124.5, 127.0, 129.1, 129.4, 133.9, 137.9, 142.2, 165.5; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{18}H_{21}N_2O_2^+$: 297.1598, found: 297.1603.

5.1.3. *N*-(2-Iodophenyl)-4-(morpholinomethyl)benzamide (4b)

The title compound was prepared from **2b** using a method analogous to that described for **4a** in 33.9% yield as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.47 (br s, 4H), 3.58 (s, 2H), 3.73 (t, J = 4.5 Hz, 4H), 6.88 (dd, J = 7.8, 7.8 Hz, 1H), 7.41 (t, J = 7.8 Hz, 1H), 7.50 (d, J = 7.9 Hz, 2H), 7.82 (d, J = 7.8 Hz, 1H), 7.93 (d, J = 7.9 Hz, 2H), 8.28 (br s, 1H), 8.46 (d, J = 7.8 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 53.7, 62.9, 67.0, 90.1, 121.7, 126.0, 127.2, 129.4, 129.5, 133.4, 138.3, 138.8, 142.7, 165.1; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{18}H_{20}IN_2O_2^+$: 423.0564, found: 423.0567.

5.1.4. *N*-(3-Iodophenyl)-4-(morpholinomethyl)benzamide (4c)

The title compound was prepared from **2c** using a method analogous to that described for **4a** in 32.3% yield as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.45 (br s, 4H), 3.56 (s, 2H), 3.72 (t, J = 4.5 Hz, 4H), 7.09 (dd, J = 8.2, 8.2 Hz, 1H), 7.44–7.50 (m, 3H), 7.62 (dd, J = 8.2, 1.1 Hz, 1H), 7.74–7.85 (m, 3H), 8.05 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 53.7, 62.9, 67.0, 94.2, 119.3, 127.0, 128.8, 129.4, 130.5, 133.4, 133.5, 139.1, 142.6, 165.4; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{18}H_{20}IN_2O_2^+$: 423.0564, found: 423.0566.

5.1.5. *N*-(4-Iodophenyl)-4-(morpholinomethyl)benzamide (1)

The title compound was prepared from **2d** using a method analogous to that described for **4a** in 94.2% yield as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.41–2.50 (m, 4H), 3.56 (s, 2H), 3.69–3.75 (m, 4H), 7.43 (d, J = 8.0 Hz, 2H), 7.46 (d, J = 8.0 Hz, 2H), 7.67 (d, J = 8.0 Hz, 2H), 7.76–7.84 (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 53.6, 62.9, 67.0, 87.7, 121.9, 127.0, 129.4, 133.5, 137.7, 138.0, 142.6, 165.4; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{18}H_{20}IN_2O_2^+$: 423.0564, found: 423.0565.

5.1.6. 4-(Morpholinomethyl)-*N*-(4-(trifluoromethyl)phenyl) benzamide (4e)

The title compound was prepared from **2e** using a method analogous to that described for **4a** in 86.5% yield as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.47 (br s, 4H), 3.58 (s, 2H), 3.73 (t, J = 4.5 Hz, 4H), 7.49 (d, J = 8.3 Hz, 2H), 7.63 (d, J = 8.7 Hz, 2H), 7.78 (d, J = 8.7 Hz, 2H), 7.84 (d, J = 8.3 Hz, 2H), 7.98 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 53.6, 62.9, 66.9, 119.7, 124.1, 126.3, 126.4, 127.1, 129.5, 133.3, 141.0, 142.7, 165.6; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{19}H_{20}F_3N_2O_2^+$: 365.1471, found: 365.1472.

5.1.7. *N*-(4-Methoxyphenyl)-4-(morpholinomethyl)benzamide (4f)

The title compound was prepared from **2f** using a method analogous to that described for **4a** in 83.4% yield as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.45 (br s, 4H), 3.55 (s, 2H), 3.72 (t, J = 4.7 Hz, 4H), 3.81 (s, 3H), 6.91 (d, J = 8.7 Hz, 2H), 7.45 (d, J = 8.1 Hz, 2H), 7.53 (d, J = 8.7 Hz, 2H), 7.73 (br s, 1H), 7.81 (d, J = 8.1 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 53.6, 55.5, 63.0, 67.0, 114.3, 122.0, 127.0, 129.3, 131.0, 133.9, 142.1, 156.6, 165.4; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{19}H_{23}N_2O_3^+$: 327.1703, found: 327.1707.

5.1.8. 4-(Morpholinomethyl)-*N*-(5-(trifluoromethyl)pyridin-2-yl)benzamide (6)

To a stirred solution of **5** (85.6 mg, 0.528 mmol) in THF (2 mL) were added triethylamine (147 μL, 1.06 mmol) and 4-(chloromethyl)benzoyl chloride **3** (100 mg, 0.528 mmol) at 0 °C. At the same temperature, the mixture was stirred for 200 min. To the stirred reaction mixture was added morpholine (460 μL, 5.28 mmol) at room temperature. The reaction mixture was stirred at room temperature for 38 h. The mixture was diluted with water and EtOAc. The organic extract was washed with brine, dried over Na_2SO_4, filtrated and then concentrated. The residue was purified with column chromatography on NH silica gel (*n*-heptane/EtOAc = 3:1–1:2). The resulting solid was washed with *n*-hexane/Et_2O and filtrated to afford the title compound **6** as a white solid (56.7 mg, 0.155 mmol, 29.4%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.46 (br s, 4H), 3.58 (s, 2H), 3.73 (t, J = 4.7 Hz, 4H), 7.51 (d, J = 8.1 Hz, 2H), 7.89 (d, J = 8.1 Hz, 2H), 7.98 (dd, J = 8.9, 2.1 Hz, 1H), 8.51–8.58 (m, 2H), 8.74 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 53.7, 62.9, 67.0, 113.4, 122.7, 123.5, 127.3, 129.5, 132.4, 135.8, 143.4, 145.4, 154.1, 165.6; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{18}H_{19}F_3N_3O_2^+$: 366.1424, found: 366.1423.

5.1.9. 3-(Morpholinomethyl)-*N*-(4-(trifluoromethyl)phenyl) benzamide (8)

A stirred solution of 3-(chloromethyl)benzoic acid **7** (100 mg, 0.586 mmol) in $SOCl_2$ (5 mL) was refluxed for 85 min. The reaction mixture was evaporated. To a stirred solution of **2e** (94.4 mg, 0.586 mmol) in THF (4 mL) and saturated $NaHCO_3$ aqueous solution (1 mL) was added prepared acid chloride in THF at room temperature. The reaction mixture was stirred at room temperature for 100 min. To the reaction mixture was added morpholine (511 μL, 5.86 mmol). The reaction mixture was stirred at room temperature for 20 h. The mixture was diluted with water and EtOAc. The organic extract was washed with brine, dried over Na_2SO_4, filtrated and then concentrated. The crude solid was washed with *n*-hexane/Et_2O and filtrated to afford the title compound **8** as a white solid (172 mg, 0.472 mmol, 80.6%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.47 (br s, 4H), 3.57 (s, 2H), 3.72 (t, J = 4.7 Hz, 4H), 7.46 (dd, J = 7.7 Hz, 1H), 7.55 (d, J = 7.7 Hz, 1H), 7.63 (d, J = 8.3 Hz, 2H), 7.76–7.82 (m, 3H), 7.86 (s, 1H), 8.02 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 53.7, 63.0, 66.9, 119.7, 124.1, 125.9, 126.3, 126.4, 127.7, 128.9, 133.0, 134.6, 139.0, 141.0,

165.8; HRMS–ESI– m/z $[M+H]^+$ calcd for $C_{19}H_{20}F_3N_2O_2^+$:365.1471, found: 365.1471.

5.1.10. 2-Methyl-*N*-(4-(trifluoromethyl)phenyl)benzamide (10)

To a stirred solution of **2e** (300 mg, 1.86 mmol) in THF (6.0 mL) were added triethylamine (519 μL, 3.72 mmol) and *o*-toluoyl chloride **9** (291 μL, 2.23 mmol) at 0 °C. The mixture was stirred at room temperature for 3 h. The mixture was diluted with water and EtOAc. The organic extract was washed with brine, dried over Na_2SO_4, filtrated through a NH silica gel pad and then concentrated to afford the title compound **10** as a colorless solid (quant.).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.51 (s, 3H), 7.25–7.31 (m, 2H), 7.39 (dd, J = 7.6, 7.6 Hz, 1H), 7.48 (d, J = 7.6 Hz, 1H), 7.58–7.66 (m, 3H), 7.74 (d, J = 7.9 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 19.8, 119.4, 124.1, 126.0, 126.3, 126.4, 126.5, 130.7, 131.5, 135.8, 136.7, 141.0, 168.1; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{15}H_{13}F_3NO^+$: 280.0944, found: 280.0945.

5.1.11. 2-(Morpholinomethyl)-*N*-(4-(trifluoromethyl)phenyl) benzamide (11)

To a stirred solution of **10** (100 mg, 0.358 mmol) in CCl_4 (4 mL) were added *N*-bromosuccinimide (70.1 mg, 0.394 mmol) and benzoyl peroxide (4.34 mg, 0.0179 mmol) at room temperature. The stirred reaction mixture was refluxed for 110 min. The reaction mixture was cooled to room temperature and filtrated. The filtrate was evaporated. To the residue was added THF (1 mL) and morpholine (311 μL, 3.57 mmol) at room temperature. The reaction mixture was stirred at room temperature for overnight. The mixture was diluted with water and EtOAc. The organic extract was washed with brine, dried over Na_2SO_4, filtrated and then concentrated. The residue was purified with column chromatography on NH silica gel (*n*-heptane/EtOAc = 5:1). The resulting solid was washed with *n*-hexane/Et_2O and filtrated to afford the title compound **11** as a white solid (15.4 mg, 0.0423 mmol, 11.8%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.59 (br s, 4H), 3.68 (s, 2H), 3.79 (t, J = 4.3 Hz, 4H), 7.24 (d, J = 7.4 Hz, 1H), 7.43–7.50 (m, 2H), 7.63 (d, J = 8.3 Hz, 2H), 7.82 (d, J = 8.3 Hz, 2H), 8.03 (dd, J = 7.4, 1.5 Hz, 1H), 11.87 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 52.8, 62.8, 66.7, 120.1, 124.1, 126.0, 126.3, 128.9, 130.8, 131.6, 132.1, 132.6, 137.0, 142.0, 166.9; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{19}H_{20}F_3N_2O_2^+$: 365.1471, found: 365.1468.

5.1.12. Methyl 6-((4-(trifluoromethyl)phenyl)carbamoyl) nicotinate (13a)

A stirred mixture of 6-(methoxycarbonyl)nicotinic acid (300 mg, 1.66 mmol) in $SOCl_2$ (3 mL) was refluxed for 40 min. The reaction mixture was evaporated. To a stirred solution of 4-aminobenzotrifluoride (250 μL, 1.99 mmol) in THF (6 mL) were added triethylamine (1.16 mL, 8.3 mmol) and prepared acid chloride in THF at room temperature. The reaction mixture was stirred at room temperature for 50 min. The mixture was diluted with water and EtOAc. The organic extract was washed with brine, dried over Na_2SO_4, filtrated and then concentrated. The crude solid was washed with *n*-heptane/Et_2O and filtrated to afford the title compound **19a** as a white solid (360 mg, 1.11 mmol, 66.9%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 4.01 (s, 3H), 7.66 (d, J = 8.3 Hz, 2H), 7.92 (d, J = 8.3 Hz, 2H), 8.39 (d, J = 7.9 Hz, 1H), 8.53 (dd, J = 7.9, 1.5 Hz, 1H), 9.22 (d, J = 1.5 Hz, 1H), 10.16 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 52.8, 119.5, 122.2, 124.1, 126.4, 126.4, 128.7, 139.1, 140.4, 149.3, 152.2, 161.3, 164.9; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{15}H_{12}F_3N_2O_3^+$: 325.0795, found: 325.0792.

5.1.13. Methyl 5-((4-(trifluoromethyl)phenyl)carbamoyl) picolinate (13b)

A mixture of 6-(methoxycarbonyl)nicotinic acid **12b** (300 mg, 1.66 mmol), 2-methyl-6-nitrobenzoic anhydride (572 mg, 1.66 mmol), triethylamine (694 μL, 4.98 mmol) and 4-dimethylaminopyridine (203 mg, 1.66 mmol) in DCM (15 mL) was stirred at room temperature for 10 min. To the stirred mixture was added 4-aminobenzotrifluoride (309 μL, 2.49 mmol). The mixture was stirred at room temperature for 20.5 h. The mixture was diluted with water and EtOAc. The organic extract was washed with brine, dried over Na_2SO_4, filtrated and then concentrated. The crude solid was washed with *n*-heptane/Et_2O and filtrated to afford the title compound **13b** as a light brown solid (400 mg, 1.23 mmol, 74.3%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 4.06 (s, 3H), 7.67 (d, J = 8.5 Hz, 2H), 7.81 (d, J = 8.5 Hz, 2H), 8.09 (br s, 1H), 8.28 (d, J = 8.2 Hz, 1H), 8.37 (dd, J = 8.2, 1.8 Hz, 1H), 9.20 (d, J = 1.8 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 53.3, 120.1, 123.9, 125.1, 126.5, 127.2, 132.9, 136.6, 140.2, 147.9, 150.5, 163.0, 164.7; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{15}H_{12}F_3N_2O_3^+$: 325.0795, found: 325.0793.

5.1.14. 5-(Hydroxymethyl)-*N*-(4-(trifluoromethyl)phenyl) picolinamide (14a)

To a stirred solution of **13a** (500 mg, 1.54 mmol) in THF (30 mL) was added 2 M $LiBH_4$ in THF solution (3.08 mL, 6.16 mmol) at room temperature. The reaction mixture was stirred at room temperature for 80 min. The mixture was diluted with water and EtOAc. The aqueous layer was extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over Na_2SO_4, filtrated and then concentrated. The residue was purified with column chromatography on NH silica gel (*n*-heptane/EtOAc = 1:1). The target fractions were collected and evaporated to afford the title compound **14a** as a white solid (269 mg, 0.908 mmol, 58.9%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.97–2.10 (m, 1H), 4.86 (br s, 2H), 7.64 (d, J = 8.3 Hz, 2H), 7.88–7.95 (m, 3H), 8.28 (d, J = 7.9 Hz, 1H), 8.62 (d, J = 1.1 Hz, 1H), 10.16 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 62.4, 119.3, 122.4, 124.1, 126.1, 126.4, 136.1, 139.7, 140.7, 146.6, 148.6, 162.2; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{14}H_{12}F_3N_2O_2^+$: 297.0845, found: 297.0845.

5.1.15. 6-(Hydroxymethyl)-*N*-(4-(trifluoromethyl)phenyl) nicotinamide (14b)

The title compound was prepared from **13b** using a method analogous to that described for **14a** in 42.3% yield as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.46 (br s, 1H), 4.87 (s, 2H), 7.45 (d, J = 7.9 Hz, 1H), 7.66 (d, J = 8.3 Hz, 2H), 7.79 (d, J = 8.3 Hz, 2H), 7.96 (br s, 1H), 8.17–8.26 (m, 1H), 9.06 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 64.2, 120.0, 120.5, 124.0, 126.5, 126.9, 129.0, 136.0, 140.5, 146.9, 163.1, 163.7; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{14}H_{12}F_3N_2O_2^+$: 297.0845, found: 297.0844.

5.1.16. 5-(Morpholinomethyl)-*N*-(4-(trifluoromethyl)phenyl) picolinamide (15a)

To a stirred solution of **14a** (269 mg, 0.908 mmol) in DCM (6 mL) and THF (1 mL) were added 4-dimethylaminopyridine (20 mg, 0.164 mmol), *p*-toluenesulfonyl chloride (1.2 g, 6.29 mmol) and pyridine (110 μL, 1.36 mmol) at room temperature. The reaction mixture was stirred at room temperature for 25 min. To the stirred mixture was added triethylamine (253 μL, 1.83 mmol). The mixture was stirred at room temperature for 70 min. The mixture was diluted with water and EtOAc. The organic extract was washed with brine, dried over Na_2SO_4, filtrated and then concentrated. To the residue in DMF (5 mL) were added morpholine (791 μL, 9.07 mmol) and triethylamine (1.26 mL, 9.07 mmol) at room temperature. The mixture was stirred at room temperature for 10 min. The mixture was diluted with water and EtOAc. The organic extract was washed with brine, dried over Na_2SO_4, filtrated and then concentrated. The residue was purified with column chromatography on NH silica gel (*n*-heptane/EtOAc = 12:1–4:1). The target fractions were collected and

evaporated. The resulting solid was washed with *n*-hexane/Et_2O and filtrated to afford the title compound **15a** as a white solid (196 mg, 0.535 mmol, 59.0%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.43–2.53 (m, 4H), 3.61 (s, 2H), 3.70–3.76 (m, 4H), 7.64 (d, J = 8.3 Hz, 2H), 7.88–7.95 (m, 3H), 8.26 (d, J = 7.9 Hz, 1H), 8.58 (d, J = 1.1 Hz, 1H), 10.16 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 53.6, 60.3, 66.9, 119.3, 122.3, 124.1, 126.0, 126.4, 137.4, 138.3, 140.8, 148.4, 148.6, 162.3; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{18}H_{19}F_3N_3O_2^+$: 366.1424, found: 366.1422.

5.1.17. 6-(Morpholinomethyl)-*N*-(4-(trifluoromethyl)phenyl)-nicotinamide (15b)

The title compound was prepared from **14b** using a method analogous to that described for **15a** in 29.7% yield as a light yellow solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.49–2.57 (m, 4H), 3.72–3.77 (m, 6H), 7.61 (d, J = 7.9 Hz, 1H), 7.65 (d, J = 8.7 Hz, 2H), 7.78 (d, J = 8.7 Hz, 2H), 8.02 (s, 1H), 8.19 (dd, J = 7.9, 2.1 Hz, 1H), 9.04 (d, J = 2.1 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 53.8, 64.6, 66.9, 119.9, 123.2, 123.9, 126.4, 126.8, 128.7, 135.8, 140.5, 147.4, 162.5, 163.9; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{18}H_{19}F_3N_3O_2^+$: 366.1424, found: 366.1418.

5.1.18. 5-(Hydroxymethyl)thiophene-2-carboxylic acid (17)

To a stirred solution of 5-formyl-2-thiophenecarboxylic acid **16** (5.0 g, 32.0 mmol) in MeOH (160 mL) was added $NaBH_4$ (1.82 g, 48.0 mmol) at 0 °C under nitrogen. The mixture was stirred at room temperature for 8 h. To the reaction mixture was added acetone and then evaporated. The residue was poured into 2 N HCl diluted with EtOAc. The aqueous layer was extracted with EtOAc. The combined organic extracts were washed with brine, dried over $MgSO_4$, filtrated and then concentrated. The crude solid was washed with *n*-hexane/Et_2O and filtrated to afford the title compound **17** as a light brown solid (quant.).

^{1}H NMR (600 MHz, DMSO-d_6): δ = 4.66 (s, 2H), 5.66 (br s, 1H), 6.99 (d, J = 3.8 Hz, 1H), 7.58 (d, J = 3.8 Hz, 1H), 12.90 (br s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 58.5, 124.3, 132.6, 133.0, 154.4, 163.0; HRMS–ESI m/z $[M+H]^+$ calcd for $C_6H_7O_3S^+$: 159.0110, found: 159.0112.

5.1.19. 5-(Morpholinomethyl)-*N*-(4-(trifluoromethyl)phenyl)-thiophene-2-carboxamide (18)

A stirred solution of **17** (100 mg, 0.631 mmol) in $SOCl_2$ (2 mL) was refluxed for 1 h. The reaction mixture was evaporated. To a stirred solution of **2e** (102 mg, 0.631 mmol) in THF (3 mL) were added triethylamine (440 μL, 3.16 mmol) and prepared acid chloride in THF at room temperature. The reaction mixture was stirred at room temperature for 35 min. To the reaction mixture was added morpholine (550 μL, 6.31 mmol). The reaction mixture was stirred at room temperature for 22 h. The mixture was diluted with water and EtOAc. The organic extract was washed with brine, dried over Na_2SO_4, filtrated and then concentrated. The crude solid was washed with *n*-hexane/Et_2O and filtrated to afford the title compound **18** as a brown solid (96 mg, 0.259 mmol, 41.1%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.53 (br s, 4H), 3.70–3.76 (m, 6H), 6.95 (d, J = 3.8 Hz, 1H), 7.53 (d, J = 3.8 Hz, 1H), 7.61 (d, J = 8.7 Hz, 2H), 7.73 (d, J = 8.7 Hz, 2H), 7.77 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 53.5, 57.7, 66.9, 119.6, 124.0, 126.2, 126.3, 126.4, 129.1, 137.3, 140.8, 149.1, 160.0; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{17}H_{18}F_3N_2O_2S^+$: 371.1036, found: 371.1033.

5.1.20. (*E*)-Methyl 4-(4-(trifluoromethyl)styryl)benzoate (21)

To a stirred solution of methyl 4-((diethoxyphosphoryl)methyl)benzoate **19**[20] (5.42 g, 18.9 mmol) in THF (60 mL) was added NaH (757 mg, 18.9 mmol, 60% w/w) at 0 °C under nitrogen. To the mixture was added 4-(trifluoromethyl)benzaldehyde **20** at 0 °C. At the same temperature, the mixture was stirred for 70 min. The reaction mixture was stirred at room temperature for 35 min. The mixture was diluted with saturated NH_4Cl and EtOAc. The organic extract was washed with water and brine, dried over $MgSO_4$, filtrated and then concentrated. The crude solid was washed with *n*-hexane and filtrated to afford the title compound **21** as a white solid (3.92 g, 12.8 mmol, 74.4%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.93 (s, 3H), 7.21 (d, J = 1.9 Hz, 2H), 7.58 (d, J = 8.3 Hz, 2H), 7.62 (s, 4H), 8.05 (d, J = 8.3 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 52.1, 124.1, 125.7, 126.6, 126.9, 129.6, 129.8, 130.1, 130.1, 140.2, 141.0, 166.7; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{17}H_{14}F_3O_2^+$: 307.0940, found: 307.0937.

5.1.21. (*E*)-(4-(4-(Trifluoromethyl)styryl)phenyl)methanol (22)

To a stirred suspension of **21** (1.0 g, 3.27 mmol) in toluene (60 mL) was added dropwise DIBAL in toluene (3.24 mL, 3.27 mmol, 1.01 M) at −78 °C under nitrogen. At the same temperature, the mixture was stirred for 70 min. The stirred mixture was allowed to warm to room temperature. The mixture was stirred for 30 min. The reaction mixture was recooled to −78 °C. At the same temperature, DIBAL in toluene (4.86 mL, 4.91 mmol, 1.01 M) was added to the reaction mixture. The stirred mixture was warmed to room temperature. The mixture was stirred for 30 min. To the stirred mixture was added 5 N HCl. The mixture was stirred for 90 min. The mixture was diluted with EtOAc. The organic extract was washed with water and brine, dried over $MgSO_4$, filtrated and then concentrated. The crude solid was washed with *n*-hexane and filtrated to afford the title compound **22** as a white solid (884 mg, 3.18 mmol, 97.1%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.68 (t, J = 4.8 Hz, 1H), 4.72 (d, J = 4.8 Hz, 2H), 7.12 (d, J = 16.1 Hz, 1H), 7.19 (d, J = 16.1 Hz, 1H), 7.38 (d, J = 7.9 Hz, 2H), 7.53 (d, J = 7.9 Hz, 2H), 7.57–7.63 (m, 4H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 65.1, 124.2, 125.6, 126.6, 127.0, 127.2, 127.4, 129.3, 130.8, 136.1, 140.8, 141.0; HRMS–ESI m/z $[M-H]^+$ calcd for $C_{16}H_{12}F_3O^-$: 277.0846, found: 277.0838.

5.1.22. (*E*)-4-(4-(Trifluoromethyl)styryl)benzaldehyde (23)

To a stirred mixture of **22** (884 mg, 3.18 mmol) in DCM (100 mL) was added manganese (IV) oxide (8.8 g, 101 mmol) at room temperature. The mixture was stirred at room temperature for 75 min. The reaction mixture was filtrated through a pad of Celite®. The filtrate was evaporated to afford the title compound **23** as a white solid (quant.).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.21–7.29 (m, 2H), 7.64 (s, 4H), 7.68 (d, J = 7.9 Hz, 2H), 7.90 (d, J = 7.9 Hz, 2H), 10.02 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 124.1, 125.8, 127.0, 127.2, 129.8, 130.1, 130.3, 130.5, 135.8, 140.0, 142.6, 191.5; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{16}H_{12}F_3O^+$: 277.0835, found: 277.0832.

5.1.23. (*E*)-4-(4-(4-(Trifluoromethyl)styryl)benzyl)morpholine (24)

To a stirred mixture of **23** (879 mg, 3.18 mmol) in 1,2-dichloroethane (30 mL) were added morpholine (416 μL, 4.77 mmol), AcOH (179 μL, 3.18 mmol) and $NaBH(OAc)_3$ (1.01 g, 4.77 mmol) at room temperature. The mixture was stirred at room temperature for 2 h. The reaction mixture was poured into saturated $NaHCO_3$ aqueous solution and diluted with EtOAc. The organic extract was washed with water and brine, dried over Na_2SO_4, filtrated and then concentrated. The crude solid was washed with *n*-hexane and filtrated to afford the title compound **24** as a white solid (834 mg, 2.40 mmol, 75.5%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.46 (br s, 4H), 3.51 (s, 2H), 3.72 (t, J = 4.5 Hz, 4H), 7.10 (d, J = 16.2 Hz, 1H), 7.18 (d, J = 16.2 Hz, 1H), 7.34 (d, J = 8.1 Hz, 2H), 7.49 (d, J = 8.1 Hz, 2H), 7.60 (s, 4H); ^{13}C NMR

(150 MHz, $CDCl_3$): δ = 53.7, 63.1, 67.0, 124.2, 125.6, 126.5, 126.7, 126.9, 129.2, 129.6, 130.9, 135.6, 138.2, 140.8; HRMS–ESI *m*/*z* [*M*+H]$^+$ calcd for $C_{20}H_{21}F_3NO^+$: 348.1570, found: 348.1570.

5.1.24. 4-Iodo-*N*-(4-(morpholinomethyl)benzyl)aniline (26)

To a stirred mixture of 4-(morpholinomethyl)benzaldehyde **25**[22] (100 mg, 0.487 mmol) in THF (2 mL) were added 4-iodoaniline (160 mg, 0.731 mmol) and $NaBH(OAc)_3$ (206 mg, 0.974 mmol) at room temperature. The mixture was stirred at room temperature for 16 h. The reaction mixture was diluted with saturated $NaHCO_3$ aqueous solution and EtOAc. The organic extract was washed with water and brine, dried over Na_2SO_4, filtrated and then concentrated. The residue was purified with column chromatography on silica gel (*n*-heptane/EtOAc = 3:2–1:1). The resulting solid was washed with *n*-hexane and filtrated to afford the title compound **26** as colorless crystals (67.6 mg, 0.166 mmol, 34.0%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.43 (br s, 4H), 3.48 (s, 2H), 3.70 (t, *J* = 4.5 Hz, 4H), 4.07 (br s, 1H), 4.28 (d, *J* = 4.9 Hz, 2H), 6.41 (d, *J* = 8.7 Hz, 2H), 7.27–7.32 (m, 4H), 7.40 (d, *J* = 8.7 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 47.8, 53.6, 63.1, 67.0, 78.1, 115.1, 127.3, 129.5, 137.0, 137.7, 137.8, 147.6; HRMS–ESI *m*/*z* [*M*+H]$^+$ calcd for $C_{18}H_{22}IN_2O^+$: 409.0771, found: 409.0766.

5.1.25. *N*-(4-Iodophenyl)-*N*-methyl-4-(morpholinomethyl)-benzamide hydrochloride (27)

To a stirred solution of *N*-(4-Iodophenyl)-4-(morpholinomethyl)benzamide **4d** (100 mg, 0.237 mmol) in DMF (2 mL) was added NaH (8.53 mg, 0.356 mmol, 60% w/w) washed with pentane at room temperature under nitrogen. The mixture was stirred for 25 min. To the reaction mixture was added MeI (14.8 μL, 0.237 mmol). The mixture was stirred at room temperature for 50 min. The reaction mixture was poured into water and extracted with EtOAc. The organic extract was washed with water and brine, dried over Na_2SO_4, filtrated and then concentrated. The residue was purified with column chromatography on NH silica gel (*n*-heptane/EtOAc = 2:1). The resulting oil was treated with 4 N HCl in EtOAc to afford the title compound **27** as a white solid (101 mg, 0.214 mmol, 90.1%).

^{1}H NMR (600 MHz, DMSO-d_6): δ = 2.95–3.05 (m, 2H), 3.11 (d, *J* = 12.1 Hz, 2H), 3.35 (s, 3H), 3.77 (t, *J* = 11.7 Hz, 2H), 3.91 (d, *J* = 12.1 Hz, 2H), 4.26 (br s, 2H), 7.00 (d, *J* = 8.7 Hz, 2H), 7.33 (d, *J* = 7.6 Hz, 2H), 7.50 (d, *J* = 7.6 Hz, 2H), 7.60 (d, *J* = 8.7 Hz, 2H), 11.27 (br s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 37.6, 50.6, 58.4, 63.0, 92.0, 128.3, 129.3, 130.4, 130.9, 137.2, 137.8, 144.0, 168.8; HRMS–ESI *m*/*z* [*M*+H]$^+$ calcd for $C_{19}H_{22}IN_2O_2^+$: 437.0720, found: 437.0715.

5.1.26. 4-Iodo-*N*-(4-(morpholinomethyl)phenyl)benzamide (30)

To a stirred mixture of 4-(morpholinomethyl)aniline **28**[23,24] (50 mg, 0.260 mmol) in THF (3 mL) were added pyridine (30.8 mg, 0.390 mmol) and 4-iodobenzoyl chloride (76.2 mg, 0.286 mmol) at room temperature. The mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with 1 N NaOH aqueous solution, THF and EtOAc. The organic extract was washed with water and brine, dried over $MgSO_4$, filtrated and then concentrated. The crude solid was recrystallized from EtOAc/Et_2O/*n*-hexane. The suspension was filtrated and washed with Et_2O to afford the title compound **30** as light brown solid (84.0 mg, 0.199 mmol, 76.5%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.44 (br s, 4H), 3.49 (s, 2H), 3.71 (t, *J* = 4.7 Hz, 4H), 7.34 (d, *J* = 8.3 Hz, 2H), 7.53–7.64 (m, 4H), 7.75 (br s, 1H), 7.84 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 53.6, 62.9, 67.0, 98.9, 120.1, 128.6, 129.9, 134.4, 134.4, 136.6, 138.0, 164.9; HRMS–ESI *m*/*z* [*M*+H]$^+$ calcd for $C_{18}H_{20}IN_2O_2^+$: 423.0564, found: 423.0561.

5.1.27. 4-(Chloromethyl)-*N*-(4-(trifluoromethyl)phenyl)-benzamide (31)

The title compound was prepared from **2d** and **3** using a method analogous to that described for **39** in 95.9% yield as a white solid.

^{1}H NMR (600 MHz, DMSO-d_6): δ = 4.85 (s, 2H), 7.61 (d, *J* = 7.9 Hz, 2H), 7.73 (d, *J* = 8.7 Hz, 2H), 7.98 (d, *J* = 7.9 Hz, 2H), 8.01 (d, *J* = 8.7 Hz, 2H), 10.59 (s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 45.3, 120.1, 123.6, 124.3, 125.9, 128.1, 128.8, 134.3, 141.4, 142.7, 165.5; HRMS–ESI *m*/*z* [*M*+H]$^+$ calcd for $C_{15}H_{12}ClF_3NO^+$: 314.0554, found: 314.0550.

5.1.28. 4-((4-Benzylpiperazin-1-yl)methyl)-*N*-(4-(trifluoromethyl)phenyl)benzamide (32a)

The title compound was prepared from **31** using a method analogous to that described for **40** in 84.3% yield as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.49 (br s, 8H), 3.52 (s, 2H), 3.57 (s, 2H), 7.22–7.34 (m, 5H), 7.46 (d, *J* = 8.3 Hz, 2H), 7.62 (d, *J* = 8.3 Hz, 2H), 7.77 (d, *J* = 8.3 Hz, 2H), 7.81 (d, *J* = 8.3 Hz, 2H), 7.94 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 53.0, 53.2, 62.5, 63.0, 119.7, 124.1, 126.3, 126.4, 127.0, 127.0, 128.2, 129.2, 129.5, 133.1, 138.0, 141.0, 143.3, 165.7; HRMS–ESI *m*/*z* [*M*+H]$^+$ calcd for $C_{26}H_{27}F_3N_3O^+$: 454.2101, found: 454.2092.

5.1.29. 4-((4-(Pyridin-4-ylmethyl)piperazin-1-yl)methyl)-*N*-(4-(trifluoromethyl)phenyl)benzamide (32b)

The title compound was prepared from **31** using a method analogous to that described for **40** in 91.9% yield as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.50 (br s, 8H), 3.51 (s, 2H), 3.59 (s, 2H), 7.25–7.28 (m, 2H), 7.46 (d, *J* = 8.3 Hz, 2H), 7.62 (d, *J* = 8.7 Hz, 2H), 7.78 (d, *J* = 8.7 Hz, 2H), 7.83 (d, *J* = 8.3 Hz, 2H), 8.02 (br s, 1H), 8.53 (d, *J* = 5.7 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 53.1, 53.1, 61.7, 62.5, 119.7, 123.9, 124.1, 126.2, 126.4, 127.1, 129.4, 133.2, 141.1, 143.1, 147.6, 149.7, 165.7; HRMS–ESI *m*/*z* [*M*+H]$^+$ calcd for $C_{25}H_{26}F_3N_4O^+$: 455.2053, found: 455.2045.

5.1.30. 4-((4-(Pyridin-3-ylmethyl)piperazin-1-yl)methyl)-*N*-(4-(trifluoromethyl)phenyl)benzamide (32c)

The title compound was prepared from **31** using a method analogous to that described for **40** in 92.3% yield as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.49 (br s, 8H), 3.52 (s, 2H), 3.58 (s, 2H), 7.23–7.28 (m, 1H), 7.46 (d, *J* = 8.3 Hz, 2H), 7.62 (d, *J* = 8.5 Hz, 2H), 7.66 (d, *J* = 7.6 Hz, 1H), 7.78 (d, *J* = 8.5 Hz, 2H), 7.83 (d, *J* = 8.3 Hz, 2H), 8.00 (s, 1H), 8.50 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.54 (d, *J* = 1.4 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 53.0, 53.1, 60.2, 62.4, 119.7, 123.3, 124.1, 126.2, 126.3, 127.1, 129.4, 133.2, 133.6, 136.7, 141.1, 143.2, 148.6, 150.5, 165.7; HRMS–ESI *m*/*z* [*M*+H]$^+$ calcd for $C_{25}H_{26}F_3N_4O^+$: 455.2053, found: 455.2042.

5.1.31. *N*1,*N*2-Dimethyl-*N*1-(pyridin-3-ylmethyl)ethane-1,2-diamine

To a stirred solution of *N*,*N*′-dimethylethylenediamine (1.55 mL, 11.9 mmol) in DMF (8.0 mL) was added triethylamine (3.3 mL, 23.8 mmol) at room temperature under nitrogen. To the mixture was added dropwise 3-bromomethylpyridine hydrobromide (1.0 g, 3.95 mmol) at room temperature. The mixture was stirred at room temperature for 3.5 h. The reaction mixture was diluted with water and $CHCl_3$. The aqueous layer was extracted with $CHCl_3$. The combined organic extracts were dried over $MgSO_4$, filtrated and then concentrated. The residue was purified with column chromatography on NH silica gel (EtOAc/MeOH = 1:0–9:1) to afford the title compound as colorless liquid (562 mg, 3.14 mmol, 79.4%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.19 (s, 3H), 2.42 (s, 3H), 2.55 (t, *J* = 5.9 Hz, 2H), 2.68 (t, *J* = 5.9 Hz, 2H), 3.52 (s, 2H), 7.22–7.30 (m, 1H), 7.66 (d, *J* = 7.9 Hz, 1H), 8.46–8.55 (m, 2H); ^{13}C NMR

(150 MHz, $CDCl_3$): δ = 36.4, 41.9, 49.2, 56.7, 59.9, 123.3, 134.5, 136.5, 148.5, 150.3; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{10}H_{18}N_3^+$: 180.1495, found: 180.1493.

5.1.32. 4-((Methyl(2-(methyl(pyridin-3-ylmethyl)amino) ethyl)amino)methyl)-*N*-(4-(trifluoromethyl)phenyl) benzamide (32d)

The title compound was prepared from **31** using a method analogous to that described for **40** in 72.4% yield as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.26 (s, 3H), 2.31 (s, 3H), 2.39–2.47 (m, 4H), 3.46 (s, 2H), 3.54 (s, 2H), 7.24–7.29 (m, 1H), 7.37 (d, J = 7.9 Hz, 2H), 7.60–7.65 (m, 3H), 7.76 (d, J = 7.9 Hz, 2H), 7.86 (d, J = 8.3 Hz, 2H), 8.11 (s, 1H), 8.50 (dd, J = 4.7, 1.3 Hz, 1H), 9.27 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 43.2, 43.7, 54.4, 54.6, 59.3, 62.6, 119.7, 123.4, 124.2, 125.9, 126.2, 127.4, 129.1, 133.9, 134.5, 136.9, 141.8, 143.8, 148.0, 150.1, 166.9; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{25}H_{28}F_3N_4O^+$: 457.2210, found: 457.2199.

5.1.33. (*S*)-*tert*-Butyl 3-methyl-4-(4-((4-(trifluoromethyl) phenyl)carbamoyl)benzyl)piperazine-1-carboxylate (33a)

The title compound was prepared from **31** using a method analogous to that described for **40** in quant as a colorless solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.12 (d, J = 6.0 Hz, 3H), 1.46 (s, 9H), 2.04–2.17 (m, 1H), 2.43–2.51 (m, 1H), 2.61 (d, J = 11.3 Hz, 1H), 2.81–3.00 (m, 1H), 3.04–3.16 (m, 1H), 3.28 (d, J = 14.0 Hz, 1H), 3.59–3.85 (m, 2H), 4.03 (d, J = 13.2 Hz, 1H), 7.48 (d, J = 7.9 Hz, 2H), 7.63 (d, J = 8.3 Hz, 2H), 7.78 (d, J = 8.3 Hz, 2H), 7.83 (d, J = 7.9 Hz, 2H), 7.97 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 15.3, 28.4, 43.3, 44.2, 50.6, 55.0, 57.7, 79.6, 119.7, 124.1, 126.3, 126.4, 127.1, 129.2, 133.1, 141.0, 144.0, 154.7, 165.6; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{25}H_{31}F_3N_3O_3^+$: 478.2312, found: 478.2301.

5.1.34. (*R*)-*tert*-Butyl 3-methyl-4-(4-((4-(trifluoromethyl) phenyl)carbamoyl)benzyl)piperazine-1-carboxylate (33b)

The title compound was prepared from **31** using a method analogous to that described for **40** in quant as a colorless solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.12 (d, J = 6.4 Hz, 3H), 1.46 (s, 9H), 2.05–2.17 (m, 1H), 2.42–2.51 (m, 1H), 2.61 (d, J = 11.7 Hz, 1H), 2.81–3.00 (m, 1H), 3.04–3.16 (m, 1H), 3.28 (d, J = 13.6 Hz, 1H), 3.59–3.86 (m, 2H), 4.03 (d, J = 13.6 Hz, 1H), 7.47 (d, J = 8.1 Hz, 2H), 7.63 (d, J = 8.3 Hz, 2H), 7.78 (d, J = 8.3 Hz, 2H), 7.83 (d, J = 8.1 Hz, 2H), 7.98 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 15.4, 28.4, 43.3, 44.4, 50.5, 55.1, 57.7, 79.7, 119.7, 124.1, 126.2, 126.4, 127.1, 129.2, 133.1, 141.1, 144.0, 154.7, 165.6; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{25}H_{31}F_3N_3O_3^+$: 478.2312, found: 478.2301.

5.1.35. (*S*)-4-((2-Methylpiperazin-1-yl)methyl)-*N*-(4-(trifluoromethyl)phenyl)benzamide (34a)

A solution of **33a** (530 mg, 1.11 mmol) in trifluoroacetic acid (3 mL) was stirred at room temperature for 55 min. The mixture was evaporated. The residue was diluted with water and 5 N NaOH aqueous solution. The mixture was extracted with EtOAc. The organic extract was washed with brine, dried over Na_2SO_4, filtrated and then concentrated. The crude solid was washed with *n*-heptane/Et_2O to afford the title compound **34a** as a white solid (361 mg, 0.957 mmol, 86.2%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.12 (d, J = 6.0 Hz, 3H), 2.08 (td, J = 10.9, 3.2 Hz, 1H), 2.34–2.43 (m, 1H), 2.56–2.66 (m, 2H), 2.76–2.88 (m, 2H), 2.92 (dd, J = 12.3, 2.1 Hz, 1H), 3.23 (d, J = 14.0 Hz, 1H), 4.09 (d, J = 14.0 Hz, 1H), 7.48 (d, J = 8.1 Hz, 2H), 7.62 (d, J = 8.5 Hz, 2H), 7.78 (d, J = 8.5 Hz, 2H), 7.82 (d, J = 8.1 Hz, 2H), 8.00 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 16.7, 46.4, 52.7, 53.6, 56.7, 58.2, 119.7, 124.1, 126.2, 126.3, 127.0, 129.3, 132.9, 141.1, 144.4, 165.7; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{20}H_{23}F_3N_3O^+$: 378.1788, found: 378.1780.

5.1.36. (*R*)-4-((2-Methylpiperazin-1-yl)methyl)-*N*-(4-(trifluoromethyl)phenyl)benzamide (34b)

The title compound was prepared from **33b** using a method analogous to that described for **34a** in 78.0% as a colorless solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.12 (d, J = 6.4 Hz, 3H), 2.08 (td, J = 10.9, 3.2 Hz, 1H), 2.33–2.44 (m, 1H), 2.56–2.66 (m, 2H), 2.76–2.88 (m, 2H), 2.92 (dd, J = 12.1, 1.9 Hz, 1H), 3.23 (d, J = 13.8 Hz, 1H), 4.09 (d, J = 13.8 Hz, 1H), 7.48 (d, J = 7.9 Hz, 2H), 7.62 (d, J = 8.7 Hz, 2H), 7.78 (d, J = 8.7 Hz, 2H), 7.82 (d, J = 7.9 Hz, 2H), 8.00 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 16.7, 46.4, 52.7, 53.6, 56.7, 58.2, 119.7, 124.1, 126.2, 126.3, 127.0, 129.3, 132.9, 141.1, 144.3, 165.7; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{20}H_{23}F_3N_3O^+$: 378.1788, found: 378.1780.

5.1.37. (*R*)-4-((3-Methylpiperazin-1-yl)methyl)-*N*-(4-(trifluoromethyl)phenyl)benzamide (34c)

The title compound was prepared from **31** using a method analogous to that described for **40** in 64.4% as a colorless solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.02 (d, J = 6.0 Hz, 3H), 1.70 (t, J = 10.6 Hz, 1H), 2.05 (td, J = 11.0, 3.4 Hz, 1H), 2.74 (d, J = 10.6 Hz, 2H), 2.84–2.98 (m, 3H), 3.51–3.60 (m, 2H), 7.48 (d, J = 7.9 Hz, 2H), 7.63 (d, J = 8.5 Hz, 2H), 7.78 (d, J = 8.5 Hz, 2H), 7.83 (d, J = 7.9 Hz, 2H), 7.94 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 20.0, 45.9, 50.5, 53.8, 61.4, 62.9, 119.7, 124.1, 126.3, 126.4, 127.0, 129.5, 133.1, 141.0, 143.3, 165.7; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{20}H_{23}F_3N_3O^+$: 378.1788, found: 378.1778.

5.1.38. (*S*)-4-((3-Methylpiperazin-1-yl)methyl)-*N*-(4-(trifluoromethyl)phenyl)benzamide (34d)

The title compound was prepared from **31** using a method analogous to that described for **40** in 70.8% as a colorless solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.01 (d, J = 6.0 Hz, 3H), 1.70 (t, J = 10.5 Hz, 1H), 2.02–2.08 (m, 1H), 2.74 (d, J = 10.5 Hz, 2H), 2.84–2.98 (m, 3H), 3.51–3.60 (m, 2H), 7.48 (d, J = 7.9 Hz, 2H), 7.63 (d, J = 8.5 Hz, 2H), 7.78 (d, J = 8.5 Hz, 2H), 7.83 (d, J = 7.9 Hz, 2H), 7.95 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 20.0, 45.9, 50.5, 53.8, 61.4, 62.9, 119.7, 124.1, 126.2, 126.4, 127.0, 129.5, 133.1, 141.0, 143.3, 165.7; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{20}H_{23}F_3N_3O^+$: 378.1788, found: 378.1778.

5.1.39. (*S*)-4-((2-Methyl-4-(pyridin-3-ylmethyl)piperazin-1-yl)methyl)-*N*-(4-(trifluoromethyl)phenyl)benzamide (35a)

To a solution of **34a** (200 mg, 0.529 mmol) in DMF (3 mL) were added triethylamine (295 μL, 2.12 mmol) and 3-chloromethylpyridine hydrochloride (95.5 mg, 0.582 mmol) at room temperature. The mixture was stirred at 60 °C for overnight. The residue was diluted with EtOAc and water. The organic extract was dried over Na_2SO_4, filtrated and then concentrated. The residue was purified with column chromatography on NH silica gel (EtOAc/MeOH = 1:0–9:1) to afford the title compound **35a** as a white solid (216 mg, 0.461 mmol, 87.1%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.12 (d, J = 6.4 Hz, 3H), 2.00–2.10 (m, 1H), 2.17–2.28 (m, 2H), 2.53 (br s, 1H), 2.58–2.69 (m, 3H), 3.25 (d, J = 13.6 Hz, 1H), 3.46–3.51 (m, 2H), 4.07 (d, J = 13.6 Hz, 1H), 7.23–7.28 (m, 1H), 7.46 (d, J = 8.1 Hz, 2H), 7.60–7.68 (m, 3H), 7.78 (d, J = 8.3 Hz, 2H), 7.82 (d, J = 8.1 Hz, 2H), 8.00 (br s, 1H), 8.50 (dd, J = 4.7, 1.4 Hz, 1H), 8.53 (d, J = 1.4 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 17.0, 51.3, 53.3, 55.3, 57.7, 60.1, 60.6, 119.7, 123.3, 124.1, 126.2, 126.3, 127.0, 129.3, 133.0, 133.6, 136.7, 141.1, 144.2, 148.6, 150.4, 165.7; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{26}H_{28}F_3N_4O^+$: 469.2210, found: 469.2197.

5.1.40. (*R*)-4-((2-Methyl-4-(pyridin-3-ylmethyl)piperazin-1-yl)methyl)-*N*-(4-(trifluoromethyl)phenyl)benzamide (35b)

The title compound was prepared from **34b** using a method analogous to that described for **35a** in 54.8% as a light brown solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.12 (d, J = 6.0 Hz, 3H), 2.00–2.11 (m, 1H), 2.17–2.28 (m, 2H), 2.53 (br s, 1H), 2.57–2.70 (m, 3H), 3.25 (d, J = 14.0 Hz, 1H), 3.45–3.52 (m, 2H), 4.07 (d, J = 14.0 Hz, 1H), 7.23–7.28 (m, 1H), 7.46 (d, J = 8.1 Hz, 2H), 7.59–7.68 (m, 3H), 7.78 (d, J = 8.3 Hz, 2H), 7.82 (d, J = 8.1 Hz, 2H), 8.01 (br s, 1H), 8.50 (dd, J = 4.9, 1.5 Hz, 1H), 8.53 (d, J = 1.5 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 17.0, 51.3, 53.3, 55.3, 57.7, 60.1, 60.6, 119.7, 123.3, 124.1, 126.2, 126.3, 127.0, 129.3, 133.0, 133.6, 136.7, 141.1, 144.2, 148.6, 150.4, 165.7; HRMS–ESI m/z [M+H]$^+$ calcd for $C_{26}H_{28}F_3N_4O^+$: 469.2210, found: 469.2197.

5.1.41. (*R*)-4-((3-Methyl-4-(pyridin-3-ylmethyl)piperazin-1-yl)methyl)-*N*-(4-(trifluoromethyl)phenyl)benzamide (35c)

The title compound was prepared from **34c** using a method analogous to that described for **35a** in 60.7% as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.13 (d, J = 6.0 Hz, 3H), 1.99–2.10 (m, 1H), 2.17–2.29 (m, 2H), 2.54 (br s, 1H), 2.58–2.70 (m, 3H), 3.22 (d, J = 13.6 Hz, 1H), 3.50–3.57 (m, 2H), 4.02 (d, J = 13.6 Hz, 1H), 7.22–7.28 (m, 1H), 7.46 (d, J = 7.9 Hz, 2H), 7.60–7.67 (m, 3H), 7.78 (d, J = 8.3 Hz, 2H), 7.82 (d, J = 7.9 Hz, 2H), 8.02 (br s, 1H), 8.46–8.51 (m, 1H), 8.54 (d, J = 1.1 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 17.0, 51.1, 53.4, 55.2, 55.3, 60.6, 62.4, 119.7, 123.3, 124.1, 126.2, 126.3, 127.1, 129.4, 133.1, 134.4, 136.6, 141.1, 143.2, 148.4, 150.4, 165.7; HRMS–ESI m/z [M+H]$^+$ calcd for $C_{26}H_{28}F_3N_4O^+$: 469.2210, found: 469.2196.

5.1.42. (*S*)-4-((3-Methyl-4-(pyridin-3-ylmethyl)piperazin-1-yl)methyl)-*N*-(4-(trifluoromethyl)phenyl)benzamide (35d)

The title compound was prepared from **34d** using a method analogous to that described for **35a** in 60.9% as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.13 (d, J = 6.4 Hz, 3H), 1.98–2.10 (m, 1H), 2.17–2.29 (m, 2H), 2.53 (br s, 1H), 2.59–2.69 (m, 3H), 3.22 (d, J = 13.8 Hz, 1H), 3.51–3.55 (m, 2H), 4.01 (d, J = 13.8 Hz, 1H), 7.22–7.28 (m, 1H), 7.45 (d, J = 7.9 Hz, 2H), 7.60–7.67 (m, 3H), 7.78 (d, J = 8.3 Hz, 2H), 7.82 (d, J = 7.9 Hz, 2H), 8.01 (br s, 1H), 8.48 (dd, J = 4.7, 1.2 Hz, 1H), 8.54 (d, J = 1.2 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 16.9, 51.1, 53.4, 55.2, 55.3, 60.6, 62.4, 119.7, 123.3, 124.1, 126.2, 126.3, 127.1, 129.4, 133.1, 134.4, 136.6, 141.1, 143.2, 148.4, 150.4, 165.7; HRMS–ESI m/z [M+H]$^+$ calcd for $C_{26}H_{28}F_3N_4O^+$: 469.2210, found: 469.2196.

5.1.43. *tert*-Butyl 4-(4-((4-(trifluoromethyl)phenyl)carbamoyl)benzyl)piperidine-1-carboxylate (37)

To a stirred solution of 4-((1-(*tert*-butoxycarbonyl)piperidin-4-yl)methyl)benzoic acid **36**[25] (722 mg, 2.26 mmol) in dichloromethane (36.1 mL) were added triethylamine (848 μL, 6.10 mmol), 4-dimethylaminopyridine (276 mg, 2.26 mmol) and 2-methyl-6-nitrobenzoic anhydride (780 mg, 2.26 mmol) at room temperature. The mixture was stirred at room temperature for 25 min. To the mixture was added 4-aminobenzotrifluoride (284 μL, 2.26 mmol). The mixture was stirred at room temperature for overnight. The reaction mixture was diluted with water and EtOAc. The organic extract was dried over $MgSO_4$, filtrated and then concentrated. The residue was purified with column chromatography on silica gel (*n*-heptane/EtOAc = 4:1–2:1) to afford the title compound **37** as a light yellow solid (760 mg, 1.64 mmol, 72.7%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.10–1.21 (m, 2H), 1.45 (s, 9H), 1.55–1.65 (m, 2H), 1.65–1.76 (m, 1H), 2.57–2.69 (m, 4H), 4.08 (br s, 2H), 7.25–7.29 (m, 2H), 7.62 (d, J = 8.7 Hz, 2H), 7.75–7.84 (m, 4H), 7.98 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 28.5, 31.9, 38.0, 43.0, 44.0, 79.4, 119.7, 124.1, 126.2, 126.4, 127.1, 129.6, 132.2, 141.1, 145.1, 154.8, 165.7; HRMS–ESI m/z [M+H]$^+$ calcd for $C_{25}H_{30}F_3N_2O_3^+$: 463.2203, found: 463.2192.

5.1.44. 4-((1-(Pyridin-4-ylmethyl)piperidin-4-yl)methyl)-*N*-(4-(trifluoromethyl)phenyl)benzamide (38)

A solution of **37** (191 mg, 0.413 mmol) in trifluoroacetic acid (3 mL) was stirred at 0 °C for 15 min. The mixture was evaporated. The residue was diluted with EtOAc and saturated $NaHCO_3$ aqueous solution. The organic extract was washed with water and brine, dried over Na_2SO_4, filtrated and then concentrated. The residue was suspended in 1,2-dichloroethane (3 mL) and THF (6 mL). To the suspension were added 4-pyridinecarboxaldehyde (78.9 μL, 0.826 mmol), $NaBH(OAc)_3$ (131 mg, 0.62 mmol) and acetic acid (23.6 μL, 0.413 mmol) at room temperature. The mixture was stirred at room temperature for 110 min. The residue was diluted with EtOAc and water. The organic extract was washed with brine, dried over Na_2SO_4, filtrated and then concentrated. The crude solid was washed with *n*-hexane/Et_2O to afford the title compound **38** as a white solid (138 mg, 0.304 mmol, 73.7%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.29–1.41 (m, 2H), 1.53–1.65 (m, 3H), 1.96 (t, J = 11.2 Hz, 2H), 2.62 (d, J = 6.8 Hz, 2H), 2.83 (d, J = 11.2 Hz, 2H), 3.48 (s, 2H), 7.23–7.30 (m, 4H), 7.62 (d, J = 8.3 Hz, 2H), 7.74–7.83 (m, 4H), 8.02 (br s, 1H), 8.52 (d, J = 5.3 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 32.1, 37.6, 43.0, 53.8, 62.0, 119.7, 123.9, 124.1, 126.2, 126.3, 127.1, 129.6, 132.1, 141.1, 145.5, 147.9, 149.6, 165.7; HRMS–ESI m/z [M+H]$^+$ calcd for $C_{26}H_{27}F_3N_3O^+$: 454.2101, found: 454.2090.

5.1.45. 4-(Chloromethyl)-*N*-(4-iodophenyl)benzamide (39)

To a stirred solution of **2d** (20.0 g, 91.3 mmol) in THF (300 mL) were added triethylamine (25.0 mL, 183 mmol) and 4-(chloromethyl)benzoyl chloride (17.3 g, 91.3 mmol) at 0 °C. The mixture was stirred at room temperature for 3.5 h. The reaction mixture was diluted with water, EtOAc and THF. The organic extract was washed with brine, dried over $MgSO_4$, filtrated and then concentrated. The crude solid was washed with Et_2O and filtrated to afford the title compound **39** as a white solid (32.2 g, 86.7 mmol, 94.9%).

^{1}H NMR (600 MHz, DMSO-d_6): δ = 4.84 (s, 2H), 7.59 (d, J = 8.3 Hz, 2H), 7.61–7.64 (m, 2H), 7.68–7.71 (m, 2H), 7.95 (d, J = 8.3 Hz, 2H), 10.34 (s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 45.4, 87.4, 122.4, 128.0, 128.8, 134.5, 137.3, 138.9, 141.2, 165.2; HRMS–ESI m/z [M+H]$^+$ calcd for $C_{14}H_{12}ClNO^+$: 371.9647, found: 371.9644.

5.1.46. *N*-(4-Iodophenyl)-4-((4-(pyridin-3-ylmethyl)piperazin-1-yl)methyl)benzamide (40)

To a stirred solution of **39** (5.0 g, 13.5 mmol) in DMF (42 mL) were added triethylamine (3.8 mL) and 1-(pyridin-3-ylmethyl)-piperazine (3.2 g, 18.1 mmol) at room temperature under nitrogen. The stirred mixture was heated at 50 °C for 3 h. The reaction mixture was cooled to room temperature and diluted with water, THF and EtOAc. The organic extract was washed with water, dried over Na_2SO_4, filtrated and then concentrated. The crude solid was washed with Et_2O/EtOAc and filtrated to afford the title compound **40** as a white solid (6.33 g, 12.4 mmol, 91.5%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.48 (br s, 8H), 3.52 (s, 2H), 3.56 (s, 2H), 7.23–7.27 (m, 1H), 7.40–7.47 (m, 4H), 7.62–7.70 (m, 3H), 7.80 (d, J = 7.9 Hz, 2H), 7.85 (s, 1H), 8.50 (dd, J = 4.9, 1.3 Hz, 1H), 8.53 (d, J = 1.3 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 53.0, 53.1, 60.2, 62.5, 87.7, 121.9, 123.3, 127.0, 129.4, 133.4, 133.6, 136.7, 137.8, 138.0, 142.9, 148.6, 150.5, 165.5; HRMS–ESI m/z [M+H]$^+$ calcd for $C_{24}H_{26}IN_4O^+$: 513.1146, found: 513.1148.

5.1.47. *N*-(4-(Phenylethynyl)phenyl)-4-((4-(pyridin-3-ylmethyl)piperazin-1-yl)methyl)benzamide (41a)

To a stirred solution of **40** (3.0 g, 5.85 mmol) in DMF (50 mL) were added triethylamine (2.4 mL, 17.3 mmol), CuI (113 mg, 0.593 mmol) and $PdCl_2(PPh_3)_2$ (207 mg, 0.295 mmol) at room

temperature under nitrogen. To the mixture was dropwise added phenylacetylene (1.9 mL, 17.3 mmol) at room temperature. The mixture was stirred at room temperature for 20 h. The reaction mixture was diluted with EtOAc, THF and water. The organic extract was washed with water, dried over $MgSO_4$, filtrated and then concentrated. The residue was purified with column chromatography on NH silica gel (EtOAc). The resulting solid was washed with EtOAc/Et_2O and filtrated to afford the title compound **41a** as a light brown solid (2.61 g, 5.36 mmol, 91.7%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.49 (br s, 8H), 3.52 (s, 2H), 3.57 (s, 2H), 7.23–7.27 (m, 1H), 7.31–7.37 (m, 3H), 7.45 (d, J = 8.1 Hz, 2H), 7.50–7.56 (m, 4H), 7.65 (d, J = 8.3 Hz, 3H), 7.82 (d, J = 8.1 Hz, 2H), 7.89 (s, 1H), 8.50 (dd, J = 4.5, 1.3 Hz, 1H), 8.54 (d, J = 1.3 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 53.0, 53.1, 60.2, 62.5, 89.1, 89.2, 119.1, 119.7, 123.3, 123.3, 127.0, 128.2, 128.3, 129.4, 131.5, 132.5, 133.5, 133.6, 136.7, 138.0, 142.9, 148.6, 150.5, 165.4; HRMS–ESI m/z [M+H]$^+$ calcd for $C_{32}H_{31}N_4O^+$: 487.2492, found: 487.2495.

5.1.48. *N*-(4-(Pyridin-3-ylethynyl)phenyl)-4-((4-(pyridin-3-ylmethyl)piperazin-1-yl)methyl)benzamide (41b)

The title compound was prepared from **40** using a method analogous to that described for **41a** in 85.5% yield as a light yellow solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.49 (br s, 8H), 3.52 (s, 2H), 3.57 (s, 2H), 7.23–7.30 (m, 2H), 7.45 (d, J = 8.3 Hz, 2H), 7.55 (d, J = 7.9 Hz, 2H), 7.64–7.71 (m, 3H), 7.78–7.85 (m, 3H), 8.00 (s, 1H), 8.48–8.57 (m, 3H), 8.76 (d, J = 1.5 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 53.0, 53.1, 60.2, 62.5, 85.7, 92.4, 118.3, 119.8, 120.5, 123.0, 123.3, 127.0, 129.4, 132.6, 133.4, 133.6, 136.7, 138.3, 138.5, 143.0, 148.4, 148.6, 150.5, 152.2, 165.5; HRMS–ESI m/z [M+H]$^+$ calcd for $C_{31}H_{30}N_5O^+$: 488.2445, found: 488.2445.

5.1.49. *N*-(4-(Cyclohexylethynyl)phenyl)-4-((4-(pyridin-3-ylmethyl)piperazin-1-yl)methyl)benzamide (41c)

The title compound was prepared from **40** using a method analogous to that described for **41a** in 76.3% yield as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.29–1.42 (m, 3H), 1.49–1.59 (m, 3H), 1.72–1.80 (m, 2H), 1.84–1.93 (m, 2H), 2.27–2.70 (m, 9H), 3.52 (s, 2H), 3.56 (s, 2H), 7.22–7.28 (m, 1H), 7.39 (d, J = 8.3 Hz, 2H), 7.43 (d, J = 8.1 Hz, 2H), 7.57 (d, J = 8.1 Hz, 2H), 7.65 (d, J = 7.9 Hz, 1H), 7.80 (d, J = 8.3 Hz, 2H), 7.83 (s, 1H), 8.49 (dd, J = 4.7, 1.4 Hz, 1H), 8.53 (d, J = 1.4 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 24.9, 25.9, 29.7, 32.7, 53.0, 53.1, 60.2, 62.5, 80.1, 94.2, 119.6, 120.1, 123.3, 127.0, 129.4, 132.4, 133.6, 136.7, 137.2, 142.7, 148.6, 150.5, 165.4; HRMS–ESI m/z [M+H]$^+$ calcd for $C_{32}H_{37}N_4O^+$: 493.2962, found: 493.2962.

5.1.50. *N*-(4-((1-Hydroxycyclohexyl)ethynyl)phenyl)-4-((4-(pyridin-3-ylmethyl)piperazin-1-yl)methyl)benzamide (41d)

The title compound was prepared from **40** using a method analogous to that described for **41a** in 89.8% yield as a light yellow solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.22–1.36 (m, 1H), 1.52–1.81 (m, 7H), 1.93–2.08 (m, 2H), 2.20–2.84 (m, 9H), 3.52 (s, 2H), 3.56 (s, 2H), 7.23–7.28 (m, 1H), 7.40–7.46 (m, 4H), 7.61 (d, J = 7.9 Hz, 2H), 7.66 (d, J = 7.9 Hz, 2H), 7.80 (d, J = 8.3 Hz, 2H), 7.89 (s, 1H), 8.50 (dd, J = 4.9, 1.5 Hz, 1H), 8.54 (d, J = 1.5 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 23.4, 25.2, 40.1, 53.0, 53.1, 60.2, 62.5, 69.1, 84.0, 92.6, 118.8, 119.6, 123.3, 127.0, 129.4, 132.5, 133.5, 133.6, 136.7, 137.9, 142.8, 148.6, 150.4, 165.4; HRMS–ESI m/z [M+H]$^+$ calcd for $C_{32}H_{37}N_4O_2^+$: 509.2911, found: 509.2913.

5.1.51. *N*-(4-(Adamantan-1-ylethynyl)phenyl)-4-((4-(pyridin-3-ylmethyl)piperazin-1-yl)methyl)benzamide (41e)

The title compound was prepared from **40** using a method analogous to that described for **41a** in 46.3% yield as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.72 (br s, 6H), 1.94–2.02 (m, 9H), 2.48 (br s, 8H), 3.52 (s, 2H), 3.56 (s, 2H), 7.23–7.28 (m, 1H), 7.38 (d, J = 8.7 Hz, 2H), 7.43 (d, J = 7.9 Hz, 2H), 7.56 (d, J = 8.7 Hz, 2H), 7.65 (d, J = 7.9 Hz, 1H), 7.77–7.83 (m, 3H), 8.50 (dd, J = 4.7, 1.4 Hz, 1H), 8.54 (d, J = 1.4 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 28.0, 30.1, 36.4, 42.9, 53.0, 53.1, 60.2, 62.5, 79.0, 98.2, 119.5, 120.1, 123.3, 127.0, 129.4, 132.4, 133.6, 133.6, 136.7, 137.2, 142.7, 148.6, 150.5, 165.3; HRMS–ESI m/z [M+H]$^+$ calcd for $C_{36}H_{41}N_4O^+$: 545.3275, found: 545.3273.

5.1.52. *tert*-Butyl 4-(2-aminonicotinoyl)piperazine-1-carboxylate

To a solution of *tert*-butyl 1-piperazinecarboxylate (5.18 g, 27.8 mmol) in DMF (40 mL) were added triethylamine (7.4 mL, 53.4 mmol), 2-aminonicotinic acid (3.7 g, 26.8 mmol) and benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (14.2 g, 32.1 mmol) at room temperature under nitrogen. The stirred mixture was heated at 60 °C for 2 h. The reaction mixture was cooled to room temperature and diluted with water and EtOAc. The aqueous layer was extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over Na_2SO_4, filtrated and then concentrated. The crude solid was purified with column chromatography on NH silica gel (*n*-heptane/EtOAc 1:1–1:2) to afford the title compound as a light orange solid (7.67 g, 25.0 mmol, 93.4%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.47 (s, 9H), 3.44–3.51 (m, 4H), 3.58 (br s, 4H), 5.18 (s, 2H), 6.67 (dd, J = 7.5, 4.9 Hz, 1H), 7.35 (dd, J = 7.5, 1.6 Hz, 1H), 8.13 (dd, J = 4.9, 1.6 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 28.4, 44.2, 80.4, 113.0, 113.1, 136.3, 150.1, 154.5, 157.1, 169.0; HRMS–ESI m/z [M+H]$^+$ calcd for $C_{15}H_{23}N_4O_3^+$: 307.1765, found: 307.1745.

5.1.53. 4-((4-(2-Aminonicotinoyl)piperazin-1-yl)methyl)-*N*-(4-iodophenyl)benzamide (42)

To a *tert*-butyl 4-(2-aminonicotinoyl)piperazine-1-carboxylate (1.94 g, 6.33 mmol) was cooled to 0 °C. At the same temperature, a cooled trifluoroacetic acid (36 mL, 485 mmol) was added. The mixture was stirred at 0 °C for 50 min. The mixture was diluted with toluene and then evaporated. To the mixture were added DMF (24 mL), triethylamine (6.8 mL, 49.1 mmol) and 4-(chloromethyl)-*N*-(4-iodophenyl)benzamide (1.8 g, 4.84 mmol) at room temperature under nitrogen. The stirred mixture was heated at 50 °C for 5 h, cooled to room temperature. The reaction mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with water, THF and EtOAc. The organic extract was washed with water, dried over Na_2SO_4, filtrated and then concentrated. The crude solid was washed with Et_2O/EtOAc and filtrated to afford the title compound **42** as a light brown solid (2.22 g, 4.10 mmol, 84.7%).

^{1}H NMR (600 MHz, DMSO-d_6): δ = 2.41 (br s, 4H), 3.28–3.64 (m, 6H), 5.91 (s, 2H), 6.58 (dd, J = 7.5, 4.9 Hz, 1H), 7.35 (dd, J = 7.5, 1.8 Hz, 1H), 7.46 (d, J = 8.3 Hz, 2H), 7.62 (d, J = 9.0 Hz, 2H), 7.68 (d, J = 9.0 Hz, 2H), 7.90 (d, J = 8.3 Hz, 2H), 7.99 (dd, J = 4.9, 1.8 Hz, 1H), 10.29 (s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 52.5, 61.3, 67.0, 87.2, 111.7, 114.0, 122.4, 127.7, 128.7, 133.5, 136.0, 137.2, 139.0, 141.9, 149.1, 156.0, 165.4, 167.2; HRMS–ESI m/z [M+H]$^+$ calcd for $C_{24}H_{25}IN_5O_2^+$: 542.1047, found: 542.1046.

5.1.54. 4-((4-(2-Aminonicotinoyl)piperazin-1-yl)methyl)-*N*-(4-(phenylethynyl)phenyl)benzamide (43a)

To a stirred solution of **42** (100 mg, 0.185 mmol) in DMF (1.8 mL) were added triethylamine (39 μL, 0.277 mmol), phenylacetylene (41 μL, 0.369 mmol), CuI (3.8 mg, 0.02 mmol) and $PdCl_2(PPh_3)_2$ (7.3 mg, 0.01 mmol) at room temperature under nitrogen. The mixture was stirred at room temperature for 6 h. The reaction mixture was diluted with EtOAc and water. The

organic extract was washed with brine, dried over Na_2SO_4, filtrated and then concentrated. The residue was purified with column chromatography on NH silica gel (*n*-heptane/EtOAc = 1:3–EtOAc/MeOH = 19:1) to afford the title compound **10a** as a light yellow solid (91.2 mg, 0.177 mmol, 96.0%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.48 (br s, 4H), 3.55–3.77 (m, 6H), 5.15 (s, 2H), 6.65 (dd, *J* = 7.6, 5.0 Hz, 1H), 7.31–7.38 (m, 4H), 7.46 (d, *J* = 8.3 Hz, 2H), 7.50–7.57 (m, 4H), 7.66 (d, *J* = 8.6 Hz, 2H), 7.84 (d, *J* = 8.3 Hz, 2H), 7.91 (s, 1H), 8.07–8.16 (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 53.2, 62.3, 89.1, 89.2, 113.0, 113.4, 119.2, 119.7, 123.3, 127.2, 128.2, 128.3, 129.3, 131.6, 132.5, 133.8, 136.3, 137.9, 142.1, 149.9, 157.0, 165.3, 168.6; HRMS–ESI *m*/*z* [*M*+H]$^+$ calcd for $C_{32}H_{30}N_5O_2^+$: 516.2394, found: 516.2393.

5.1.55. *N*-(4-(Adamantan-1-ylethynyl)phenyl)-4-((4-(2-aminonicotinoyl)piperazin-1-yl)methyl)benzamide (43b)

The title compound was prepared from **42** using a method analogous to that described for **43a** in 59.8% yield as a light yellow solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.72 (br s, 6H), 1.93–2.01 (m, 9H), 2.48 (br s, 4H), 3.52–3.75 (m, 6H), 5.15 (s, 2H), 6.65 (dd, *J* = 7.6, 4.9 Hz, 1H), 7.35 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.39 (d, *J* = 8.7 Hz, 2H), 7.45 (d, *J* = 8.1 Hz, 2H), 7.56 (d, *J* = 8.1 Hz, 2H), 7.78–7.85 (m, 3H), 8.11 (dd, *J* = 4.9, 1.5 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 28.0, 30.1, 36.4, 42.9, 53.2, 62.3, 79.0, 98.2, 113.0, 113.4, 119.5, 120.2, 127.1, 129.3, 132.4, 133.9, 136.3, 137.1, 142.0, 149.9, 157.0, 165.2, 168.6; HRMS–ESI *m*/*z* [*M*+H]$^+$ calcd for $C_{36}H_{40}N_5O_2^+$: 574.3177, found: 574.3175.

5.1.56. (*S*)-2-Methyl-1-((2-nitrophenyl)sulfonyl)piperazine (45)

To a stirred solution of (*S*)-*tert*-butyl 3-methylpiperazine-1-carboxylate (2.01 g, 10 mmol) in THF (20 mL) were added triethylamine (2.8 mL, 20.2 mmol) and 2-nitrobenzenesulfonyl chloride (2.72 g, 12.3 mmol) at 0 °C under nitrogen. The mixture was stirred at room temperature for 70 min. The mixture was diluted with EtOAc and water. The organic extract was washed with brine, dried over $MgSO_4$, filtrated and then concentrated. The residue was purified with column chromatography on NH silica gel (EtOAc only) to afford the product A.

To a stirred solution of the product A in dichloromethane (20 mL) was added trifluoroacetic acid (7.5 mL, 101 mmol) at room temperature. The mixture was stirred at room temperature for 85 min. The mixture was evaporated. The residue was diluted with EtOAc and saturated $NaHCO_3$ aqueous solution. The aqueous layer was extracted with EtOAc. The combined organic extracts were dried over $MgSO_4$, filtrated and then concentrated. The residue was purified with column chromatography on NH silica gel (EtOAc/MeOH = 1:0–9:1) to afford the title compound **45** as a yellow solid (quant.).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.29 (d, *J* = 6.8 Hz, 3H), 2.70–2.80 (m, 2H), 2.91–3.00 (m, 2H), 3.28 (td, *J* = 12.7, 3.4 Hz, 1H), 3.49 (d, *J* = 13.2 Hz, 1H), 3.99–4.08 (m, 1H), 7.62–7.73 (m, 3H), 8.04–8.10 (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 15.2, 41.5, 45.9, 49.6, 50.6, 124.3, 130.9, 131.7, 133.4, 133.9, 147.8; HRMS–ESI *m*/*z* [*M*+H]$^+$ calcd for $C_{11}H_{16}N_3O_4S^+$: 286.0856, found: 286.0850.

5.1.57. (*S*)-2-Methyl-1-((2-nitrophenyl)sulfonyl)-4-(pyridin-3-ylmethyl)piperazine (46a)

To a stirred solution of **45** (485 mg, 1.70 mmol) in DMF (5.0 mL) were added triethylamine (711 μL, 5.10 mmol) and 3-chloromethylpyridine hydrochloride (423 mg, 2.58 mmol) at room temperature under nitrogen. The stirred mixture was heated at 80 °C for 5 h. The mixture was diluted with EtOAc and water. The organic extract was washed with water and brine, dried over Na_2SO_4, filtrated and then concentrated. The residue was purified with column chromatography on NH silica gel (*n*-heptane/EtOAc = 3:1–1:3) to afford the title compound **46a** as light yellow oil (545 mg, 1.45 mmol, 85.0%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.30 (d, *J* = 6.8 Hz, 3H), 2.13 (td, *J* = 11.5, 3.6 Hz, 1H), 2.28 (dd, *J* = 11.3, 3.6 Hz, 1H), 2.57 (d, *J* = 11.3 Hz, 1H), 2.76 (d, *J* = 11.3 Hz, 1H), 3.34–3.46 (m, 2H), 3.47–3.61 (m, 2H), 4.06–4.14 (m, 1H), 7.22–7.29 (m, 1H), 7.62–7.73 (m, 4H), 8.06 (dd, *J* = 7.4, 1.7 Hz, 1H), 8.47–8.56 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 16.2, 41.1, 50.0, 53.0, 57.5, 59.8, 123.4, 124.3, 130.9, 131.7, 133.4, 133.8, 136.3, 147.8, 148.8, 150.2; HRMS–ESI *m*/*z* [*M*+H]$^+$ calcd for $C_{17}H_{21}N_4O_4S^+$: 377.1278, found: 377.1265.

5.1.58. (*S*)-4-((6-Chloropyridin-3-yl)methyl)-2-methyl-1-((2-nitrophenyl)sulfonyl)piperazine (46b)

To a stirred solution of **45** (1.98 g, 6.59 mmol) in DMF (15 mL) were added triethylamine (2.0 mL, 14.3 mmol) and 2-chloro-5-chloromethylpyridine (1.28 g, 7.91 mmol) at room temperature under nitrogen. The stirred mixture was heated at 60 °C for 5.5 h. The mixture was diluted with EtOAc and water. The organic extract was washed with water and brine, dried over Na_2SO_4, filtrated and then concentrated. The residue was purified with column chromatography on NH silica gel (*n*-heptane/EtOAc = 4:1–1:1) to afford the title compound **46b** as yellow oil (quant.).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.29 (d, *J* = 6.4 Hz, 3H), 2.15 (td, *J* = 11.6, 3.5 Hz, 1H), 2.28 (dd, *J* = 11.3, 3.5 Hz, 1H), 2.54 (d, *J* = 11.0 Hz, 1H), 2.74 (d, *J* = 11.3 Hz, 1H), 3.35–3.43 (m, 2H), 3.47–3.53 (m, 1H), 3.57 (d, *J* = 13.2 Hz, 1H), 4.06–4.14 (m, 1H), 7.26–7.31 (m, 1H), 7.61–7.72 (m, 4H), 8.07 (dd, *J* = 7.4, 1.8 Hz, 1H), 8.29 (d, *J* = 1.8 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 16.1, 41.0, 49.9, 53.0, 57.4, 58.9, 124.1, 124.4, 130.9, 131.8, 132.5, 133.5, 133,7, 139.1, 147.8, 149.8, 150.5; HRMS–ESI *m*/*z* [*M*+H]$^+$ calcd for $C_{17}H_{20}ClN_4O_4S^+$: 411.0888, found: 411.0878.

5.1.59. (*S*)-3-Methyl-1-(pyridin-3-ylmethyl)piperazine (47a)

To a stirred solution of **46a** (314 mg, 0.834 mmol) in DMF (8.0 mL) were added lithium hydroxide (200 mg, 8.34 mmol) and mercaptoacetic acid (290 μL, 4.17 mmol) at room temperature under nitrogen. The mixture was stirred at room temperature for 24 h. The mixture was diluted with THF and EtOAc. The resulting suspension was filtrated through a pad of Celite®. The filtrate was evaporated. The residue was purified with column chromatography on NH silica gel (EtOAc/MeOH = 1:0–4:1) to afford the title compound **47a** as yellow oil (80.6 mg, 0.421 mmol, 50.5%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.01 (d, *J* = 6.4 Hz, 3H), 1.70 (t, *J* = 10.4 Hz, 1H), 2.04 (td, *J* = 11.2, 3.0 Hz, 1H), 2.73 (d, *J* = 11.2 Hz, 2H), 2.83–2.98 (m, 3H), 3.45–3.54 (m, 2H), 7.23–7.29 (m, 1H), 7.67 (d, *J* = 7.6 Hz, 1H), 8.50 (d, *J* = 4.9 Hz, 1H), 8.54 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 19.9, 45.8, 50.5, 53.6, 60.5, 61.2, 123.3, 133.6, 136.7, 148.5, 150.5; HRMS–ESI *m*/*z* [*M*+H]$^+$ calcd for $C_{11}H_{18}N_3^+$: 192.1495, found: 192.1493.

5.1.60. (*S*)-1-((6-(Methoxymethyl)pyridin-3-yl)methyl)-3-methylpiperazine (47c)

To a stirred solution of **46b** (465 mg, 1.13 mmol) in DMF (5.0 mL) were added tributyl(methoxymethyl)stannane[30] (550 mg, 1.64 mmol) and tetrakis(triphenylphosphine)palladium(0) (131 mg, 0.113 mmol) at room temperature under nitrogen. The stirred mixture was heated at 120 °C for 6 h. The mixture was diluted with water and EtOAc. The organic extract was washed with brine, dried over Na_2SO_4, filtrated and then concentrated. The residue was purified with column chromatography on NH silica gel (*n*-heptane/EtOAc = 3:1–2:3) to afford the product A.

To a stirred solution of the product A in DMF (3.0 mL) were added lithium hydroxide (134 mg, 5.61 mmol) and mercaptoacetic acid (195 μL, 2.81 mmol) at room temperature under nitrogen. The mixture was stirred at room temperature for 24 h. The mixture was

diluted with THF and EtOAc. The resulting suspension was filtrated through a pad of Celite®. The filtrate was evaporated. The residue was purified with column chromatography on NH silica gel (EtOAc/MeOH = 1:0–4:1) to afford the title compound **47c** as yellow oil (57.0 mg, 0.242 mmol, 21.4%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.01 (d, J = 6.4 Hz, 3H), 1.68 (t, J = 10.4 Hz, 1H), 2.03 (td, J = 11.2, 3.2 Hz, 1H), 2.73 (d, J = 11.0 Hz, 2H), 2.82–2.97 (m, 3H), 3.45–3.52 (m, 5H), 4.58 (s, 2H), 7.38 (d, J = 7.9 Hz, 1H), 7.68 (dd, J = 7.9, 1.7 Hz, 1H), 8.48 (d, J = 1.7 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 19.9, 45.8, 50.5, 53.6, 58.8, 60.3, 61.1, 75.4, 121.0, 132.3, 137.5, 149.8, 157.1; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{13}H_{22}N_3O^+$: 236.1757, found: 236.1750.

5.1.61. Tributyl((2-methoxyethoxy)methyl)stannane

To a stirred solution of diisopropylamine (5.0 mL, 35.7 mmol) in THF (80 mL) was added *n*-BuLi (11.0 mg, 28.6 mmol, 2.59 M) at −78 °C under nitrogen. At the same temperature, the mixture was stirred for 1 h. To the mixture was added tri-*N*-butyltin hydride (7.0 mL, 26.0 mmol) at −78 °C. The mixture was stirred at 0 °C for 30 min. The mixture was recooled to −78 °C. To the mixture was 2-methoxyethoxymethyl chloride (3.5 mL, 30.9 mmol), followed by stirred at the same temperature for 30 min. The stirred mixture was warmed to room temperature over 4 h. The mixture was diluted with saturated NH_4Cl aqueous solution and Et_2O. The aqueous layer was extracted with Et_2O. The combined organic extracts were washed with brine, dried over Na_2SO_4, filtrated and then concentrated. The residue was purified with column chromatography on silica gel (*n*-heptane/EtOAc = 1:0–4:1) to afford the title compound as colorless oil (5.72 g, 15.1 mmol, 58.0%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.85–0.92 (m, 15H), 1.23–1.34 (m, 6H), 1.45–1.55 (m, 6H), 3.37 (s, 3H), 3.46–3.53 (m, 4H), 3.77–3.81 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 9.1, 13.7, 27.3, 29.1, 59.1, 62.6, 71.9, 74.5; HRMS–EI m/z $[M+H]^+$ calcd for $C_{12}H_{27}O_2Sn^{\cdot+}$: 323.1028, found: 323.0999.

5.1.62. (*S*)-1-((6-((2-methoxyethoxy)methyl)pyridin-3-yl)methyl)-3-methylpiperazine (47d)

The title compound was prepared from **46b** using a method analogous to that described for **47c** in 35.1% as yellow oil.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.01 (d, J = 6.4 Hz, 3H), 1.68 (t, J = 10.4 Hz, 1H), 2.03 (td, J = 11.0, 3.0 Hz, 1H), 2.72 (d, J = 11.0 Hz, 2H), 2.82–2.97 (m, 3H), 3.39–3.42 (m, 3H), 3.44–3.52 (m, 2H), 3.62 (t, J = 4.7 Hz, 2H), 3.73 (t, J = 4.7 Hz, 2H), 4.68 (s, 2H), 7.44 (d, J = 7.9 Hz, 1H), 7.67 (dd, J = 7.9, 1.5 Hz, 1H), 8.48 (d, J = 1.5 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 19.9, 45.8, 50.5, 53.6, 59.1, 60.3, 61.1, 70.1, 71.9, 74.0, 121.2, 132.3, 137.5, 149.7, 157.2; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{15}H_{26}N_3O_2^+$: 280.2020, found: 280.2012.

5.1.63. 4-(Adamantan-1-ylethynyl)aniline (49)

The title compound was prepared from **2d** and **48** using a method analogous to that described for **41a** in 76.1% yield as a brown solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.70 (br s, 6H), 1.92–2.00 (m, 9H), 3.69 (br s, 2H), 6.56 (d, J = 8.7 Hz, 2H), 7.18 (d, J = 8.7 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 28.1, 30.0, 36.5, 43.1, 79.5, 96.0, 113.7, 114.7, 132.8, 145.7; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{18}H_{22}N^+$: 252.1747, found: 252.1737.

5.1.64. *N*-(4-(Adamantan-1-ylethynyl)phenyl)-4-(chloromethyl)benzamide (50)

The title compound was prepared from **49** using a method analogous to that described for **31** in 85.3% yield as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.72 (br s, 6H), 1.93–2.01 (m, 9H), 4.63 (s, 2H), 7.39 (d, J = 8.7 Hz, 2H), 7.50 (d, J = 8.1 Hz, 2H), 7.56 (d, J = 8.7 Hz, 2H), 7.80 (s, 1H), 7.85 (d, J = 8.1 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 28.0, 30.1, 36.4, 42.9, 45.3, 78.9, 98.3, 119.6, 120.3, 127.4, 128.9, 132.4, 134.8, 136.9, 141.3, 164.9; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{26}H_{27}ClNO^+$: 404.1776, found: 404.1761.

5.1.65. *N*-(4-((Adamantan-1-ylethynyl)phenyl)-4-(((*S*)-2-methyl-4-(pyridin-3-ylmethyl)piperazin-1-yl)methyl)benzamide (51a)

The title compound was prepared from **50** and **47a** using a method analogous to that described for **40** in 21.3% yield as a yellow solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.11 (d, J = 6.4 Hz, 3H), 1.72 (br s, 6H), 1.94–2.08 (m, 10H), 2.16–2.27 (m, 2H), 2.52 (br s, 1H), 2.56–2.70 (m, 3H), 3.24 (d, J = 13.6 Hz, 1H), 3.48 (s, 2H), 4.07 (d, J = 13.6 Hz, 1H), 7.21–7.28 (m, 1H), 7.38 (d, J = 8.5 Hz, 2H), 7.44 (d, J = 7.9 Hz, 2H), 7.57 (d, J = 8.5 Hz, 2H), 7.65 (d, J = 7.6 Hz, 1H), 7.77–7.84 (m, 3H), 8.49 (dd, J = 4.7, 1.4 Hz, 1H), 8.53 (d, J = 1.4 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 16.9, 28.0, 30.1, 36.4, 42.9, 51.3, 53.3, 55.3, 57.7, 60.1, 60.6, 79.0, 98.1, 119.5, 120.1, 123.3, 126.9, 129.3, 132.4, 133.4, 133.6, 136.7, 137.2, 143.7, 148.6, 150.4, 165.4; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{37}H_{43}N_4O^+$: 559.3431, found: 559.3411.

5.1.66. *N*-(4-(Adamantan-1-ylethynyl)phenyl)-4-(((*S*)-4-((6-(methoxymethyl)pyridin-3-yl)methyl)-2-methylpiperazin-1-yl)methyl)benzamide (51c)

The title compound was prepared from **50** and **47c** using a method analogous to that described for **40** in 79.0% yield as light yellow amorphous.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.11 (d, J = 6.0 Hz, 3H), 1.72 (br s, 6H), 1.93–2.08 (m, 10H), 2.19–2.26 (m, 2H), 2.51 (br s, 1H), 2.57–2.69 (m, 3H), 3.19–3.27 (m, 1H), 3.48 (s, 5H), 4.06 (d, J = 13.6 Hz, 1H), 4.57 (s, 2H), 7.35–7.40 (m, 3H), 7.44 (d, J = 8.3 Hz, 2H), 7.56 (d, J = 8.0 Hz, 2H), 7.66 (dd, J = 7.9, 1.7 Hz, 1H), 7.76–7.83 (m, 3H), 8.47 (d, J = 1.7 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 17.0, 28.0, 30.1, 36.4, 42.9, 51.3, 53.3, 55.3, 57.7, 58.8, 59.8, 60.5, 75.4, 79.0, 98.1, 119.5, 120.1, 121.0, 126.9, 129.3, 132.3, 132.4, 133.4, 137.2, 137.4, 143.8, 149.8, 157.1, 165.4; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{39}H_{47}N_4O_2^+$: 603.3694, found: 603.3669.

5.1.67. *N*-(4-(Adamantan-1-ylethynyl)phenyl)-4-(((*S*)-4-((6-((2-methoxyethoxy)methyl)pyridin-3-yl)methyl)-2-methylpiperazin-1-yl)methyl)benzamide (51d)

The title compound was prepared from **50** and **47d** using a method analogous to that described for **40** in 52.3% yield as light yellow amorphous.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.11 (d, J = 6.0 Hz, 3H), 1.72 (br s, 6H), 1.94–2.06 (m, 10H), 2.18–2.25 (m, 2H), 2.51 (br s, 1H), 2.57–2.69 (m, 3H), 3.23 (d, J = 14.0 Hz, 1H), 3.40 (s, 3H), 3.47 (s, 2H), 3.59–3.64 (m, 2H), 3.70–3.74 (m, 2H), 4.06 (d, J = 14.0 Hz, 1H), 4.68 (s, 2H), 7.38 (d, J = 8.7 Hz, 2H), 7.41–7.46 (m, 3H), 7.56 (d, J = 8.0 Hz, 2H), 7.66 (dd, J = 7.9, 1.7 Hz, 1H), 7.76–7.83 (m, 3H), 8.45 (d, J = 1.7 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 17.0, 28.1, 30.1, 36.4, 42.9, 51.3, 53.3, 55.3, 57.7, 59.1, 59.8, 60.5, 70.2, 71.9, 74.0, 79.0, 98.1, 119.5, 120.1, 121.2, 126.9, 129.2, 132.3, 132.4, 133.4, 137.2, 137.5, 143.8, 149.7, 157.2, 165.4; HRMS–ESI m/z $[M+H]^+$ calcd for $C_{41}H_{51}N_4O_3^+$: 647.3956, found: 647.3929.

5.2. Biology

5.2.1. Cells and compounds

Human U251 glioma cells were purchased from the Riken Cell Bank (Ibaraki, Japan). U251/vascular endothelial growth factor (VEGF)-placental alkaline phosphatase (PLAP) cells harbored a plasmid in which the PLAP reporter gene was under the control of a VEGF promoter, as described previously.[17] The cells were

cultured in Dulbecco's modified Eagle's medium, supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 and either 21% O_2 (normoxic conditions) or 2% O_2 (hypoxic conditions).

For the cellular assays, benzanilide compounds were prepared in dimethyl sulfoxide (DMSO) and then diluted in the culture medium. The final concentration of DMSO in any incubation mixture did not exceed 0.2% (v/v). For the animal studies, **41a**, **43a**, and **51d** were formulated in DMSO/Tween-80 (35:65), which was diluted 5-fold with saline immediately before administration.

Following exposure to the compounds indicated, untransfected U251 cells in 96-well plates were solubilized in 100 μL lysis buffer (50 mM Tris [pH 7.5], 300 mM NaCl, 10% glycerol, 3 mM EDTA, 1 mM $MgCl_2$, 20 mM beta-glycerophosphate, 25 mM NaF, 1% Triton X-100, and 1 mM Na_3VO_4). The lysates were diluted fivefold with PBS containing 1% bovine serum albumin, and 100 μL was assayed for HIF1α using the human/mouse total HIF-1α DuoSet IC enzyme-linked immunosorbent assay (ELISA, R&D systems, Minneapolis, MN) according to the manufacturer's protocol.

5.2.2. Cell-based HIF-1 reporter assay

HIF-1 reporter activity was determined as previously described.[17] Briefly, U251/VEGF-PLAP cells were seeded onto 96-well plates at 4.0×10^4 cells/well. After overnight incubation, the test compounds were added. The plates were incubated under hypoxic conditions for an additional 18 h. PLAP activity in the culture supernatant was then determined.

5.2.3. Human tumor xenograft model

Untransfected U251 cells were injected subcutaneously into the flank or hind leg of female BALB/cA (nu/nu) athymic mice, and allowed to grow to ~300 mm^3. Tumor volumes were determined as length × $(\text{diameter})^2/2$, where length was the longest dimension and diameter was the shortest dimension. All experiments were approved by the Animal Care and Use Committee at the Eisai Tsukuba Research Laboratories (Ibaraki, Japan).

5.2.4. Pharmacodynamic studies

Mice bearing untransfected U251 tumor xenografts received a 10 mL/kg single oral administration of **41a**, **43a**, and **51d**. Animals were sacrificed 24 h after dosing, and the tumors were removed, frozen in liquid nitrogen, and fragmented using a Multi-beads Shocker (Yasui Kikai, Osaka, Japan). To determine the amount of HIF-1α present, the fragmented tumors were lysed in lysis buffer [50 mM Tris (pH 7.5), 300 mM NaCl, 10% glycerol, 3 mM EDTA, 1 mM $MgCl_2$, 20 mM β-glycerophosphate, 25 mM NaF, 1% Triton X-100, and 1 mM Na_3VO_4) and centrifuged at 1500×*g* for 5 min. The total protein concentration of the sample supernatant was determined using a BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA). The HIF-1α concentration in the supernatant was determined by ELISA, as described above (Section 5.2.1),[18] and normalized to the total protein concentration.

5.2.5. Antitumor studies

Mice bearing untransfected U251 xenografts were stratified into groups of 5 animals with approximately equal mean tumor volumes. The animals received a 10 mL/kg oral administration of **43a** or vehicle once daily for 11 days. The tumor volumes were measured twice weekly, and the relative tumor volumes (the ratio of tumor volume to initial size before treatment) were calculated.

Acknowledgments

The authors would like to thank the colleagues who helped to generate all of the data reported in this manuscript. In particular, special thanks are owed to Makoto Asano for a commitment to the Project, Eri Ena, Satoko Sasaki, and Yumi Asai for NMR and HRMS analyses.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.07.020.

References and notes

1. Brown, J. M.; Wilson, W. R. *Nat. Rev. Cancer* **2004**, *4*, 437.
2. Vaupel, P.; Thews, O.; Hoeckel, M. *Med. Oncol.* **2001**, *18*, 243.
3. Wouters, A.; Pauwels, B.; Lardon, F.; Vermorken, J. B. *Oncologist* **2007**, *12*, 690.
4. Semenza, G. L. *Trends Mol. Med.* **2002**, *8*, S62.
5. Semenza, G. L. *Drug Discovery Today* **2007**, *12*, 853.
6. Semenza, G. L. *Nat. Rev. Cancer* **2003**, *3*, 721.
7. Patiar, S.; Harris, A. L. *Endocr. Relat. Cancer* **2006**, *13*, S61.
8. Vaupel, P.; Mayer, A. *Cancer Metastasis Rev.* **2007**, *26*, 225.
9. Semenza, G. L. *Oncogene* **2010**, *29*, 625.
10. Tsai, Y.-P.; Wu, K.-J. *J. Biomed. Sci.* **2012**, *19*, 102.
11. Harris, A. L. *Nat. Rev. Cancer* **2002**, *2*, 38.
12. Giaccia, A.; Siim, B. G.; Johnson, R. S. *Nat. Rev. Drug Dis.* **2003**, *2*, 1.
13. Zhong, H.; De Marzo, A. M.; Laughner, E.; Lim, M.; Hilton, D. A.; Zagzag, D.; Buechler, P.; Isaacs, W. B.; Semenza, G. L.; Simons, J. W. *Cancer Res.* **1999**, *59*, 5830.
14. Lin, X.; David, C. A.; Donnelly, J. B.; Michaelides, M.; Chandel, N. S.; Huang, X.; Warrior, U.; Weinberg, F.; Tormos, K. V.; Fesik, S. W.; Shen, Y. *PNAS* **2008**, *105*, 174.
15. Ellinghaus, P.; Heisler, I.; Unterschemmann, K.; Haerter, M.; Beck, H.; Greschat, S.; Ehrmann, A.; Summer, H.; Flamme, I.; Oehme, F.; Thierauch, K.; Michels, M.; Hess-Stumpp, H.; Ziegelbauer, K. *Cancer Med.* **2013**, *2*, 611.
16. Xia, Y.; Choi, H. K.; Lee, K. *Eur. J. Med. Chem.* **2012**, *49*, 24.
17. Sakai, T.; Sameshima, T.; Matsufuji, M.; Kawamura, N.; Dobashi, K.; Mizui, Y. *J. Antibiot. (Tokyo)* **2004**, *57*, 173.
18. Okamoto, K.; Ito, D.; Miyazaki, K.; Watanabe, S.; Tohyama, O.; Yokoi, A.; Ozawa, Y.; Asano, M.; Kawamura, T.; Yamane, Y.; Nagao, S.; Funasaka, S.; Kamata, J.; Kotake, Y.; Aoki, M.; Tsukahara, N.; Mizui, Y.; Tanaka, I.; Sawada, K. *Int. J. Mol. Med.* **2012**, *29*, 541.
19. Shinkai, H.; Ito, T.; Iida, T.; Kitao, Y.; Yamada, H.; Uchida, I. *J. Med. Chem.* **2000**, *43*, 4667.
20. Kitazume, T.; Lin, J. T.; Takeda, M.; Yamazaki, T. *J. Am. Chem. Soc.* **1991**, *113*, 2123.
21. Nagarathnam, D. *J. Heterocycl. Chem.* **1992**, *29*, 953.
22. Hall, M. J.; McDonnell, S. O.; Killoran, J.; O'Shea, D. F. *J. Org. Chem.* **2005**, *70*, 5571.
23. Shiraishi, M.; Aramaki, Y.; Seto, M.; Imoto, H.; Hishikawa, Y.; Kanzaki, N.; Okamoto, M.; Sawada, H.; Nishimura, O.; Baba, M.; Fujino, M. *J. Med. Chem.* **2000**, *43*, 2049.
24. Tsou, H.-R.; Otteng, M.; Tran, T.; Floyd, M. B., Jr.; Reich, M.; Birnberg, G.; Kutterer, K.; Ayral-Kaloustian, S.; Ravi, M.; Nilakantan, R.; Grillo, M.; McGinnis, J. P.; Rabindran, S. K. *J. Med. Chem.* **2008**, *51*, 3507.
25. Imamura, S.; Ichikawa, T.; Nishikawa, Y.; Kanzaki, N.; Takashima, K.; Niwa, S.; Iizawa, Y.; Baba, M.; Sugihara, Y. *J. Med. Chem.* **2006**, *49*, 2784.
26. Lee, J.; Kang, S. U.; Lim, J. O.; Choi, H. K.; Jin, M. K.; Toth, A.; Pearce, L. V.; Tran, R.; Wang, Y.; Szabo, T.; Blumberg, P. M. *Bioorg. Med. Chem.* **2004**, *12*, 371.
27. Kashiwagi, Y.; Chiba, S.; Ikezoe, H.; Anzai, J. *Synlett* **2004**, 2513.
28. Christiansen, E.; Urban, C.; Merten, N.; Liebscher, K.; Karlsen, K. K.; Hamacher, A.; Spinrath, A.; Bond, A. D.; Drewke, C.; Ullrich, S.; Kassack, M. U.; Kostenis, E.; Ulven, T. *J. Med. Chem.* **2008**, *51*, 7061.
29. Aicher, T. D.; Anderson, R. C.; Gao, J.; Shetty, S. S.; Coppola, G. M.; Stanton, J. L.; Knorr, D. C.; Sperbeck, D. M.; Brand, L. J.; Vinluan, C. C.; Kaplan, E. L.; Dragland, C. J.; Tomaselli, H. C.; Islam, A.; Lozito, R. J.; Liu, X.; Maniara, W. M.; Fillers, W. S.; DelGrande, D.; Walter, R. E.; Mann, W. R. *J. Med. Chem.* **2000**, *43*, 236.
30. Buchwald, S. L.; Nielsen, R. B.; Dewan, J. C. *Organometallics* **1989**, *8*, 1593.