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Synthesis and biological evaluation of sulfonylhydrazone-substituted imidazo[1,2-*a*]pyridines as novel PI3 kinase p110α inhibitors

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Abstract—We have previously reported the imidazo[1,2-*a*]pyridine derivative **4** as a novel p110α inhibitor; however, although **4** is a potent inhibitor of p110α enzymatic activity and tumor cell proliferation in vitro, it is unstable in solution and ineffective in vivo. To increase stability the pyrazole of **4** was replaced with a hydrazone and a moderately potent p110α inhibitor **7a** was obtained. Subsequent optimization of **7a** afforded exceptionally potent p110α inhibitors, including **8c** and **8h**, with IC_{50} values of 0.30 nM and 0.26 nM, respectively; to the best of our knowledge, these compounds are the most potent PI3K p110α inhibitors reported to date. Compound **8c** was also stable in solution and exhibited significant anti-tumor effectiveness in vivo.

1. Introduction

Phosphoinositide 3-kinases (PI3Ks) have emerged as potential targets for anti-cancer therapy.[1–3] PI3K signaling is negatively regulated by PTEN, which is one of the most commonly mutated proteins in human cancers,[4–6] suggesting that inhibitors of PI3Ks have potential as anti-cancer agents. PI3Ks are divided into three major classes based on their primary structure and mechanism of activation: classes I, II, and III.[7–10] Class I PI3Ks are further divided into class Ia enzymes: p110α, p110β, and p110δ, which are activated by tyrosine kinase receptors; and the class Ib enzyme p110γ, which is activated by a G protein-coupled receptor. The class II PI3Ks C2α, C2β, and C2γ are characterized by the presence of a C2 domain at the C terminus. Regarding class III PI3Ks, the mechanism of activation is still not understood, but at least two different complexes of this protein have been reported. Among the PI3K isoforms, PI3K p110α expression correlates most strongly with cancer progression, since amplification[11,12] and frequent mutation[13–16] of the *PIK3CA* gene that encodes PI3K p110α have been observed in several cancers.

Examples of non-isoform-specific PI3K inhibitors include wortmannin and LY294002, and we have reported several series of potent and isoform-specific PI3K p110α inhibitors represented by **1**, **2**, and **4** (Fig. 1).[17–19] Compound **4** contains an imidazo[1,2-*a*]pyridine ring and was derived from lead compound **3**, which was discovered in high-throughput screening. Although **4** has excellent in vitro potency as a p110α inhibitor, it is unstable in solution and ineffective in vivo. The instability is probably due to cleavage of the pyrazole–sulfone linkage, and therefore we focused our efforts on replacement of the pyrazole ring of **4** with other substituents. Introduction of hydrazones gave a novel series of p110α inhibitors, and here we report the synthesis and evaluation of a new series of hydrazone-containing PI3K p110α inhibitors that are exceptionally potent, selective, and effective in vivo.

Keywords: PI3 kinase; p110α; Inhibitor; Anti-cancer agent.
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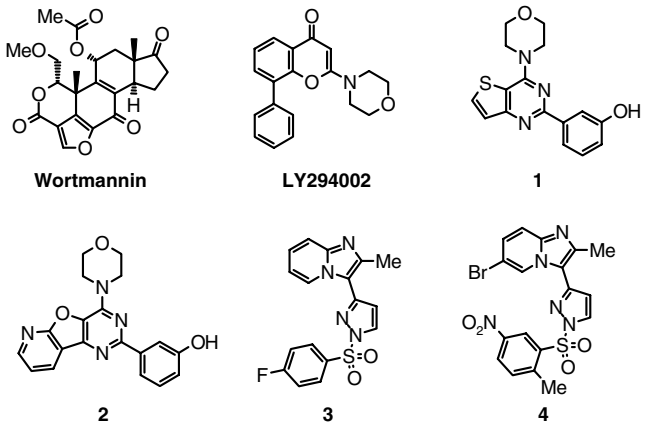


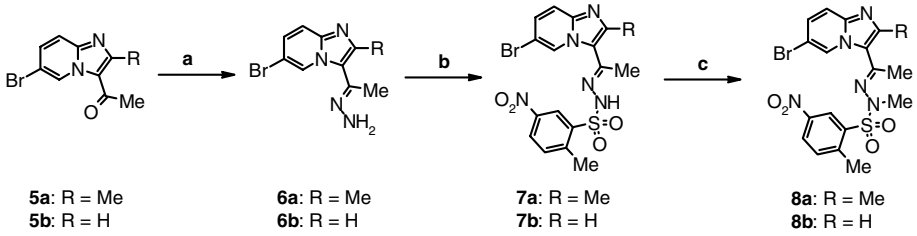
Figure 1. Structures of PI3K inhibitors.

2. Chemistry

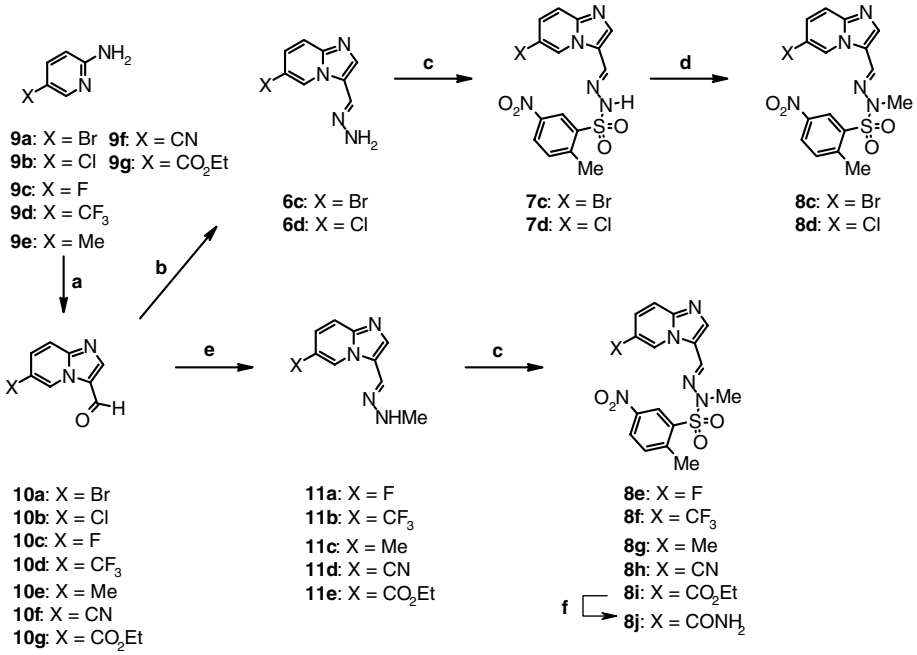
As shown in Scheme 1, the 6-bromoimidazo[1,2-*a*]pyridine derivatives **7a**, **7b**, **8a**, and **8b** were prepared from ketones **5a** and **5b**. Condensation of **5a** and **5b** with hydrazine hydrate in refluxing ethanol, followed by treatment with 2-methyl-5-nitrobenzenesulfonyl chloride, afforded sulfonylhydrazone derivatives **7a** and **7b**, which were subjected to methylation using MeI to give **8a** and **8b**, respectively. Imidazo[1,2-*a*]pyridine derivatives **8c**–**j** were synthesized as shown in Scheme 2. Aldehydes **10a**–**g** were prepared from the 2-aminopyridines **9a**–**g** by cyclization with bromomalonaldehyde.[18] **8c** and **8d** were synthesized from aldehydes **10a** and **10b** by the same method as that for synthesis of **8a** and **8b**. Compounds **8e**–**i** were synthesized from the suitable aldehydes **10c**–**g** by condensation with methylhydrazine followed by sulfonylation to give the desired compounds **8e**–**i**. The carboxamide derivative **8j** was prepared from the corresponding ester **8i** by hydrolysis and subsequent condensation with ammonia. The commercially unavailable 2-aminopyridine **9f** was synthesized by dehydration of **12** with TFAA, as shown in Scheme 3. Compounds **7a**–**b** and **8a**–**j** were all obtained as *E* isomers. The stereochemistry of each of the compounds was determined by the NMR (NOE) and X-ray analysis.

3. Results and discussion

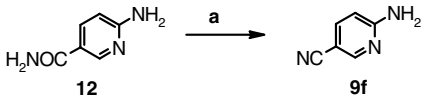
Compound **4** is a potent p110α inhibitor in vitro with an IC_{50} of 3.1 nM, but is unstable in solution and does not inhibit tumor growth in vivo. Since the instability is



Scheme 1. Reagents and conditions: (a) H_2NNH_2 hydrate, EtOH, reflux; (b) 2-methyl-5-nitrobenzenesulfonyl chloride, pyridine; (c) MeI, NaH, DMF.



Scheme 2. Reagents and conditions: (a) $BrCH(CHO)_2$, MeCN, Δ; (b) H_2NNH_2 hydrate, EtOH, reflux; (c) 2-methyl-5-nitrobenzenesulfonyl chloride, pyridine; (d) MeI, NaH, DMF; (e) H_2NNHMe, EtOH, Δ; (f) i—LiOH, EtOH, ii—CDI, NH_4OH.



Scheme 3. Reagents: (a) TFAA, K_2CO_3, then water.

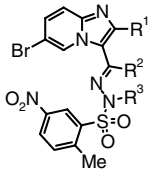
probably due to cleavage of the sulfonyl-pyrazole linkage, we replaced the pyrazole ring of **4** with other groups to connect the imidazo[1,2-*a*]pyridine ring and the 2-methyl-5-nitrophenylsulfone group. Introduction of a hydrazone instead of the pyrazole afforded a moderately potent p110α inhibitor **7a** with an IC_{50} of 0.40 μM, comparable with that of LY294002 (Table 1). Our focus then shifted to increasing the p110α inhibitory activity of **7a** by further structural modification.

First, the methyl groups at R^1 or R^2 of **7a** were removed (Table 1). Demethylation at R^1 on **7a** gave **7b** as a 20-fold more potent p110α inhibitor (IC_{50}: 0.017 μM). The potency of **7b** was beyond our expectation, since the increase in inhibitory activity caused by removal of the methyl group in the corresponding pyrazole derivative was only 1.7-fold.[18] Removal of the methyl group at R^2 of **7b** resulted in retention of p110α inhibitory activity in **7c** (IC_{50}: 0.021 μM).

A methyl group was then introduced at R^3 in **7a**–**c** to give compounds **8a**–**c**. Methylation at R^3 of **7a** gave **8a**, which showed a 2.4-fold increase in p110α inhibitory activity (IC_{50}: 0.17 μM), but methylation at R^3 of **7b** resulted in a compound (**8b**) with decreased p110α inhibitory activity (IC_{50}: 0.081 μM). However, methylation at R^3 of **7c** gave an unexpectedly large 70-fold increase in inhibitory activity over **7c**, with compound **8c** having an IC_{50} of 0.30 nM. Moreover, **8c** exhibited highly potent inhibitory activity against serum-induced proliferation of A375 human melanoma cells, with an IC_{50} of 0.058 μM.

Next, the bromo group of **8c** at the C6 position of the imidazo[1,2-*a*]pyridine ring was replaced with other substituents (Table 2). Introduction of fluoro and chloro as less bulky halogens gave reduced potency against p110α compared to the bromo derivative **8c**, with a 2.5-fold and an 18-fold drop in potency, respectively. Trifluoromethyl, methyl, ethyl ester, and carboxamide derivatives **8f**, **g**, **i**, and **j** were also less potent than **8c**, but the cyano derivative **8h** was an extremely potent p110α inhibitor (IC_{50}: 0.26 nM) and also a potent inhibitor of proliferation of A375 human melanoma cells (IC_{50}: 0.033 μM). In contrast to the large differences in p110α inhibitory activities among **8c**–**h** in cell-free assays, their inhibitory activities in the cell-based assay were relatively similar, with IC_{50}s ranging from 0.033 to 0.18 μM. The trifluoromethyl derivative **8f** was 54-fold less potent than **8h** in the enzymatic assay, whereas in the cell-based assay **8f** was only 2.7-fold less potent than **8h**, suggesting that **8f** may have improved cell permeability compared to **8h**. It is possible that the cell activity may be due in part to effects on mTOR and DNA-PK, as reported for **8c**.[20] This requires further mechanistic studies.

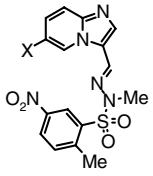
Table 1. Inhibition of p110α activity by imidazopyridine derivatives



Compound[a]	R^1	R^2	R^3	IC_{50}[c] (μM)	
				p110α	A375
LY294002				0.63	8.4
3				0.67	23
4				0.0031	0.73
7a	Me	Me	H	0.40	NT[d]
7b	H	Me	H	0.017	12
7c	H	H	H	0.021	9.0
8a	Me	Me	Me	0.17	NT[d]
8b	H	Me	Me	0.081	20
8c[b]	H	H	Me	0.00030	0.058

[a] Free base.
[b] HCl salt.
[c] IC_{50} values represent means of at least two separate determinations with typical variations of less than ±20%.
[d] NT, not tested.

Table 2. Inhibition of p110α activity by imidazopyridine derivatives



Compound[a]	X	IC_{50}[b] (μM)	
		p110α	A375
8c	Br	0.00030	0.058
8d	Cl	0.00077	0.056
8e	F	0.0053	0.18
8f	$-CF_3$	0.014	0.090
8g	Me	0.0060	0.12
8h	–CN	0.00026	0.033
8i	$-CO_2Et$	0.34	9.35
8j	$-CONH_2$	0.78	8.96

[a] HCl salt.
[b] IC_{50} values represent means of at least two separate determinations with typical variations of less than ±20%.

Table 3. Isoform selectivity of compound **8c** against PI3Ks

Compound	IC_{50}[c] (μM)			
	p110α	p110β	p110γ	PI3K C2β
LY294002[a]	0.63	0.34	1.6	2.1
8c[b]	0.00030	0.85	0.040	0.10
1[b]	0.0025	0.016	0.66	0.22
2[b]	0.0036	0.0030	0.25	0.010

[a] Free base.
[b] HCl salt.
[c] IC_{50} values represent means of at least two separate determinations with typical variations of less than ±20%.

Table 4. Inhibition of tumor cell proliferation in vitro by compound **8c**

Compound	IC_{50}[b] (μM)				
	A375 (Melanoma)	HeLa (Cervix)	A549 (Breast)	MCF7 (Breast)	MCF7 ADR-res
8c[a]	0.058	0.051	0.069	0.019	0.039
Doxorubicin	NT[c]	NT[c]	NT[c]	0.0071	17
Paclitaxel	NT[c]	NT[c]	NT[c]	0.0018	8.2

[a] HCl salt.
[b] IC_{50} values represent means of at least two separate determinations with typical variations of less than ±20%.
[c] NT, not tested.

Compound **8c** was evaluated further as a representative and potent example of this series. First, its selectivity profile over several PI3K isoforms was investigated (Table 3). For comparison, data for the p110α inhibitors **1** and **2**, which we identified previously,[17,19] are also listed in Table 3. Compound **8c** showed excellent selectivity for p110α over other PI3K isoforms, while the derivatives with morpholinopyrimidine as a common motif showed relatively lower isoform selectivity for p110α. Next, the anti-proliferative effect of **8c** was examined in various cancer cell lines (Table 4). These data showed that **8c** is a potent inhibitor of tumor-cell growth and has activity against MCF7 ADR-res cells, in which well-known anti-cancer agents such as doxorubicin and paclitaxel are ineffective due to overexpression of P-glycoprotein.[21,22]

We first tried to investigate the stability of **8c** in aqueous phosphate buffer (pH 6.8); however, this proved to be difficult because of the poor solubility and precipitation of **8c**. Therefore, the stability of **8c** and **4** was tested in methanol, as shown in Figure 2; 37% of **4** decomposed in methanol at 37 °C over 24 h, whereas **8c** was completely stable under the same conditions, probably due to the absence of the pyrazole–sulfone linkage. After confirmation of the improved stability of **8c**, the in vivo activity was evaluated in a HeLa human cervical cancer xenograft model in nude mice. As shown in Figure 3, **8c** dosed intraperitoneally at 50 mg/kg daily for 2 weeks markedly suppressed tumor growth by 62% in this model, without causing weight loss. The in vivo efficacy of **8c** may be explained partly by the improved stability as well as the exceptionally potent p110α inhibitory activity.

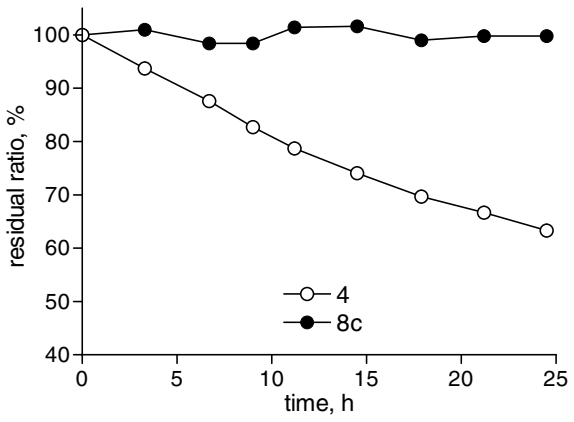


Figure 2. Stability of **4** and **8c** in methanol at 37 °C.

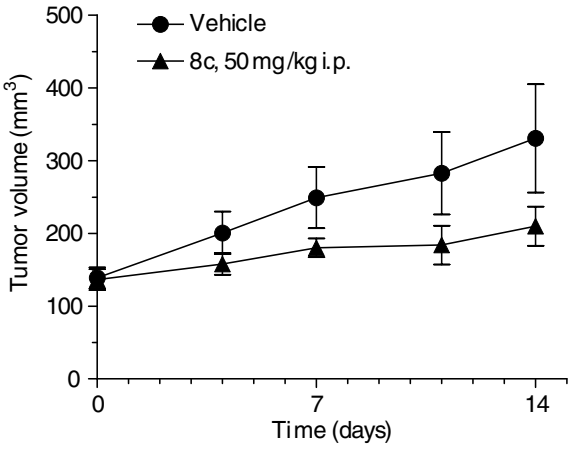


Figure 3. Effect of compound **8c** on the growth of HeLa human cervical tumor xenografts. Compound **8c** suspended in 20% hydroxypropyl-β-cyclodextrin/saline (50 mg/kg) was intraperitoneally administered daily for 2 weeks to nude mice carrying a subcutaneous HeLa xenograft. Error bars show ±SE.

4. Conclusion

We have developed a novel series of PI3K p110α inhibitors that are imidazo[1,2-*a*]pyridines with arylsulfonylhydrazone substituents. Replacement of the pyrazole of **4** with hydrazone and subsequent optimization afforded compounds such as **8c** and **8h**, which are the most potent p110α inhibitors reported to date. Compound **8c** has excellent selectivity for p110α over other PI3K isoforms, and is a potent inhibitor of tumor-cell growth in vitro. Furthermore, **8c** is effective in a HeLa human cervical cancer xenograft model.

5. Experimental

5.1. Chemistry

^{1}H NMR spectra were measured with a JEOL EX400 or GX500 spectrometer; chemical shifts are expressed in δ units using tetramethylsilane as the standard (NMR descriptions: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad peak). Mass spectra were recorded with a Hitachi M-80 or JEOL JMS-DX300 spectrometer. Silica gel column chroma-

tography was performed using Wakogel C-200 or Merck Silica Gel 60.

5.1.1. *N*′-[(1*E*)-1-(6-Bromo-2-methylimidazo[1,2-*a*]pyridin-3-yl)ethylidene]-2-methyl-5-nitrobenzenesulfonohydrazide (7a). A mixture of **5a**[18] (2.0 g, 7.9 mmol) and hydrazine hydrate (2.0 g, 40 mmol) in EtOH (20 mL) was refluxed for 2 d and then concentrated. The residue in pyridine (20 mL) was combined with 2-methyl-5-nitrobenzenesulfonyl chloride (2.4 g, 10 mmol). After stirring for 5 h, the mixture was evaporated and dissolved in a mixture of $CHCl_3$ and water. After separation, the organic layer was dried over $MgSO_4$ and concentrated. The residue was crystallized from $CHCl_3$ to give **7a** as a light brown solid (42% yield): mp 128–130 °C; ^{1}H NMR (DMSO-d_6) δ 2.39 (3H, s), 2.51 (3H, s), 2.79 (3H, s), 7.40–7.46 (1H, m), 7.51 (1H, d, J = 9.8 Hz), 7.74 (1H, d, J = 8.3 Hz), 8.39 (1H, dd, J = 2.4, 8.3 Hz), 8.76 (1H, d, J = 2.4 Hz), 9.15–9.20 (1H, m), 11.41 (1H, br s); FAB MS *m*/*e* $(MH)^+$ 466, 468; Anal. for $C_{17}H_{16}N_5O_4SBr{\cdot}0.05CHCl_3$: Calcd. C, 43.36; H, 3.43; N, 14.83; S, 6.79; Br, 16.92. Found C, 43.65; H, 3.40; N, 14.85; S, 6.58; Br, 16.53.

5.1.2. *N*′-[(1*E*)-1-(6-Bromoimidazo[1,2-*a*]pyridin-3-yl)ethylidene]-2-methyl-5-nitrobenzenesulfonohydrazide (7b). Compound **7b** was prepared from **5b** using the same procedure as that for **7a**. Compound **7b** was obtained as a colorless solid (28% yield): mp 197–198 °C (MeOH–EtOH); ^{1}H NMR (DMSO-d_6) δ 2.41 (3H, s), 2.79 (3H, s), 7.51 (1H, d, J = 9.8 Hz), 7.67 (1H, d, J = 9.3 Hz), 7.76 (1H, d, J = 9.8 Hz), 8.19 (1H, s), 8.42 (1H, dd, J = 2.4, 8.3 Hz), 8.81 (1H, d, J = 2.0 Hz), 9.24 (1H, s), 11.40 (1H, br s); FAB MS *m*/*e* $(MH)^+$ 452, 454; Anal. for $C_{16}H_{14}N_5O_4SBr{\cdot}0.3C_2H_5OH$: Calcd. C, 42.50; H, 3.50; N, 14.85; S, 6.84; Br, 17.04. Found C, 42.78; H, 3.42; N, 15.03; S, 6.88; Br, 17.14.

5.1.3. *N*′-[(1*E*)-(6-Bromoimidazo[1,2-*a*]pyridin-3-yl)methylene]-2-methyl-5-nitrobenzenesulfonohydrazide (7c). Compound **7c** was prepared from **10a** using the same procedure as that for **7a**. Compound **7c** was obtained as a colorless solid (19% yield): mp 185–186 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ 2.77 (3H, s), 7.56 (1H, dd, J = 2.0, 9.8 Hz), 7.71 (1H, d, J = 9.8 Hz), 7.76 (1H, d, J = 8.3 Hz), 8.06 (1H, s), 8.31 (1H, s), 8.43 (1H, dd, J = 2.4, 8.8 Hz), 8.77 (1H, d, J = 2.4 Hz), 9.00 (1H, d, J = 1.5 Hz), 12.20 (1H, br s); FAB MS *m*/*e* $(MH)^+$ 438, 440; Anal. for $C_{15}H_{12}N_5O_4SBr$: Calcd. C, 41.11; H, 2.76; N, 15.98; S, 7.32; Br, 18.23. Found C, 40.81; H, 2.71; N, 15.96; S, 7.31; Br, 18.28.

5.1.4. *N*′-[(1*E*)-(6-Chloroimidazo[1,2-*a*]pyridin-3-yl)methylene]-2-methyl-5-nitrobenzenesulfonohydrazide (7d). Compound **7d** was prepared from **10b** using the same procedure as that for **7a**. Compound **7d** was obtained as a colorless solid (60% yield): ^{1}H NMR (DMSO-d_6) δ 2.77 (3H, s), 7.49 (1H, dd, J = 2.0, 9.3 Hz), 7.75 (1H, s), 7.77 (1H, s), 8.08 (1H, s), 8.31 (1H, s), 8.43 (1H, dd, J = 2.4, 8.8 Hz), 8.79 (1H, d, J = 2.4 Hz), 8.82 (1H, d, J = 2.0 Hz), 12.20 (1H, br s); FAB MS *m*/*e* $(MH)^+$ 394.

5.1.5. *N*′-[(1*E*)-1-(6-Bromo-2-methylimidazo[1,2-*a*]pyridin-3-yl)ethylidene]-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (8a). Compound **7a** (500 mg, 1.07 mmol) and MeI (198 mg, 1.39 mmol) were added to a suspension of 60% NaH in oil (50 mg) in DMF (5 mL). After stirring for 10 min, the mixture was diluted with EtOAc and washed with water and brine. After evaporation, the residue was subjected to silica gel column chromatography (eluent: $CHCl_3$) and the solid obtained in this procedure was washed with MeOH to give **8a** as a colorless solid (45% yield): mp 216–220 °C; ^{1}H NMR (DMSO-d_6) δ 2.63 (3H, s), 2.66 (3H, s), 2.72 (3H, s), 3.01 (3H, s), 7.56–7.66 (2H, m), 7.83 (1H, s, J = 8.3 Hz), 8.48 (1H, dd, J = 2.5, 8.8 Hz), 8.53 (1H, d, J = 2.5 Hz), 9.49 (1H, d, J = 1.0 Hz); FAB MS *m*/*e* $(MH)^+$ 480, 482; Anal. for $C_{18}H_{18}N_5O_4SBr$: Calcd. C, 45.01; H, 3.78; N, 14.58; S, 6.68; Br, 16.63. Found: C, 44.90; H, 3.72; N, 14.59; S, 6.60; Br, 16.34.

5.1.6. *N*′-[(1*E*)-1-(6-Bromoimidazo[1,2-*a*]pyridin-3-yl)ethylidene]-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (8b). Compound **8b** was prepared from **7b** using the same procedure as that for **8a**. Compound **8b** was obtained as a colorless solid (56% yield): mp 216–220 °C (MeOH–EtOH); ^{1}H NMR (DMSO-d_6) δ 2.63 (3H, s), 2.72 (3H, s), 3.02 (3H, s), 7.66 (1H, dd, J = 2.0, 9.8 Hz), 7.79 (1H, d, J = 9.3 Hz), 7.87 (1H, d, J = 7.8 Hz), 8.48–8.54 (3H, m), 9.34 (1H, d, J = 1.0 Hz); FAB MS *m*/*e* $(MH)^+$ 466, 468; Anal. for $C_{17}H_{16}N_5O_4SBr$: Calcd. C, 43.79; H, 3.46; N, 15.02; S, 6.88; Br, 17.14. Found C, 43.93; H, 3.57; N, 15.13; S, 6.74; Br, 17.13.

5.1.7. *N*′-[(1*E*)-(6-Bromoimidazo[1,2-*a*]pyridin-3-yl)methylene]-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide hydrochloride (8c). The free base form of **8c** was prepared from **7c** using the same procedure as that for **8a** (43% yield). 4 N HCl/EtOAc (0.15 mL) was added to a solution of the free base of **8c** (260 mg, 0.57 mmol) in MeOH (20 mL) and $CHCl_3$ (20 mL). After evaporation, the solid was recrystallized from MeOH to give **8c** (HCl salt) as a colorless solid (42% yield): mp 205–208; ^{1}H NMR (DMSO-d_6) δ 2.69 (3H, s), 3.48 (3H, s), 7.76–7.89 (3H, m), 8.23 (1H, s), 8.33 (1H, s), 8.46 (1H, dd, J = 2.4, 8.3 Hz), 8.74 (1H, d, J = 2.4 Hz), 9.22 (1H, s); FAB MS *m*/*e* $(MH)^+$ 452, 454; Anal. for $C_{16}H_{14}N_5O_4SBr{\cdot}HCl{\cdot}2H_2O$: Calcd. C, 36.62; H, 3.65; N, 13.35; S, 6.11; Br, 15.23; Cl, 6.76. Found C, 36.73; H, 3.27; N, 13.31; S, 6.11; Br, 15.39; Cl, 6.61.

5.1.8. *N*′-[(1*E*)-(6-Chloroimidazo[1,2-*a*]pyridin-3-yl)methylene]-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide hydrochloride (8d). The free base form of **8d** was prepared from **7d** using the same procedure as that for **8a**. 4 N HCl/EtOAc was added to a solution of the free base of **8d** in EtOH and the mixture was evaporated and recrystallized from MeOH to give **8d** (HCl salt) as a colorless solid (48% overall yield): mp 216–217 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ 2.68 (3H, s), 3.50 (3H, s), 7.73–7.78 (1H, dd, J = 2.0, 9.3 Hz), 7.80 (1H, d, J = 8.3 Hz), 7.88–7.94 (1H, m), 8.26 (1H, s), 8.33 (1H, s), 8.48 (1H, dd, J = 2.4, 8.3 Hz), 8.77 (1H, d, J = 2.5 Hz), 8.99–9.02 (1H, m); FAB MS *m*/*e* $(MH)^+$

408; Anal. for $C_{16}H_{14}N_5O_4SCl{\cdot}HCl{\cdot}0.8H_2O$: Calcd. C, 41.69; H, 3.25; N, 15.33; S, 6.94; Cl, 15.38. Found C, 41.89; H, 3.65; N, 15.27; S, 6.99; Cl, 15.46.

5.1.9. *N*′-[(1*E*)-(6-Fluoroimidazo[1,2-*a*]pyridin-3-yl)methylene]-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide hydrochloride (8e). A mixture of **9c** (1.0 g, 8.9 mmol) and bromomalonaldehyde (2.7 g, 18 mmol) in EtOH (10 mL) was refluxed for 18 h. After evaporation, the residue was washed with EtOAc and Et_2O. A mixture of the resulting crude aldehyde **10c** and methylhydrazine (0.38 mL, 11 mmol) in EtOH (30 mL) was stirred at room temperature for 2 h and then heated at 60 °C for 0.5 h. After evaporation, pyridine (13 mL) and 2-methyl-5-nitrobenzenesulfonyl chloride (2.1 g, 10 mmol) were added to the resulting crude hydrazone and the reaction mixture was stirred for 12 h. After evaporation, the residue was washed with water and EtOH. 4 N HCl/EtOAc (3 mL) was added to a suspension of the resulting solid in EtOH (10 mL). The mixture was concentrated and washed with hot EtOH to give **8e** (382 mg, 12% yield) as a colorless solid: mp 214–216 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ 2.67 (3H, s), 3.49 (3H, s), 7.75–7.85 (2H, m), 7.90–7.99 (1H, m), 8.24–8.29 (1H, m), 8.32 (1H, s), 8.49 (1H, dd, J = 2.5, 8.3 Hz), 8.76–8.83 (1H, m); FAB MS *m*/*e* $(MH)^+$ 392; Anal. for $C_{16}H_{14}N_5O_4SFHCl$: Calcd. C, 44.92; H, 3.53; N, 16.37; S, 7.49; Cl, 8.29; F, 4.44. Found C, 44.77; H, 3.47; N, 16.45; S, 7.49; Cl, 8.28; F, 4.44.

5.1.10. *N*′-[(1*E*)-(6-Trifluoromethylimidazo[1,2-*a*]pyridin-3-yl)methylene]-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide hydrochloride (8f). Compound **8f** was prepared from **9d** using the same procedure as that for **8e**. Compound **8f** was obtained as a colorless solid (11% yield): mp 207–209 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ 2.67 (3H, s), 3.48 (3H, s), 7.76 (1H, d, J = 8.3 Hz), 7.79–7.86 (1H, m), 8.00 (1H, d, J = 9.3 Hz), 8.21–8.27 (1H, m), 8.36 (1H, s), 8.42 (1H, dd, J = 2.4, 8.3 Hz), 8.71 (1H, d, J = 2.5 Hz), 9.62 (1H, s); FAB MS *m*/*e* $(MH)^+$ 442; Anal. for $C_{17}H_{14}N_5O_4SF_3{\cdot}HCl$: Calcd. C, 42.73; H, 3.16; N, 14.66; S, 6.71; Cl, 7.42; F, 11.93. Found C, 42.81; H, 3.03; N, 14.91; S, 6.66; Cl, 7.20; F, 12.04.

5.1.11. *N*,2-Dimethyl-*N*′-[(1*E*)-(6-methylimidazo[1,2-*a*]pyridin-3-yl)methylene]-5-nitrobenzenesulfonohydrazide hydrochloride (8g). Compound **8g** was prepared from **9e** using the same procedure as that for **8e**. Compound **8g** was obtained as a colorless solid (14% yield): mp 235–238 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ 2.37 (3H, s), 2.69 (3H, s), 3.50 (3H, s), 7.79 (1H, d, J = 8.8 Hz), 7.84 (1H, dd, J = 1.5, 9.3 Hz), 7.95 (1H, d, J = 9.3 Hz), 8.33 (1H, s), 8.40 (1H, s), 8.45 (1H, dd, J = 2.5, 8.3 Hz), 8.76 (1H, d, J = 2.9 Hz), 9.04 (1H, s); FAB MS *m*/*e* $(MH)^+$ 388; Anal. for $C_{17}H_{17}N_5O_4SHCl$: Calcd. C, 48.17; H, 4.28; N, 16.52; S, 7.56; Cl, 8.36. Found C, 47.90; H, 4.18; N, 16.69; S, 7.54; Cl, 8.13.

5.1.12. *N*′-[(1*E*)-(6-Cyanoimidazo[1,2-*a*]pyridin-3-yl)methylene]-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide hydrochloride (8h). Compound **8h** was prepared from **9f** using the same procedure as that for **8e**. Compound **8h** was obtained as a colorless solid (9% yield): mp 236–238 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ 2.68 (3H, s), 3.47 (3H, s), 7.75–7.83 (3H, m), 7.94 (1H, d, J = 9.3 Hz), 8.22 (1H, s), 8.36 (1H, s), 8.43 (1H, dd, J = 2.5, 8.3 Hz), 8.71 (1H, d, J = 2.5 Hz), 9.43 (1H, s); FAB MS *m*/*e* $(MH)^+$ 399; Anal. for $C_{17}H_{14}N_6O_4S{\cdot}HCl$: Calcd. C, 46.95; H, 3.48; N, 19.33; S, 7.37; Cl, 8.15. Found C, 46.95; H, 3.34; N, 19.55; S, 7.02; Cl, 8.09.

5.1.13. Ethyl 3-((*E*)-{methyl[(2-methyl-5- nitrophenyl)sulfonyl]hydrazono}methyl)imidazo[1,2-*a*]pyridine-6-carboxylate hydrochloride (8i). Compound **8i** was prepared from **9g** using the same procedure as that for **8e**. Compound **8i** was obtained as a colorless solid (28% yield): mp 174–176 °C (MeOH); ^{1}H NMR (DMSO-d_6) δ 1.40 (3H, t, J = 6.8 Hz), 2.71 (3H, s), 3.48 (3H, s), 4.42 (3H, q, J = 6.8 Hz), 7.73 (1H, d, J = 8.3 Hz), 7.94 (1H, d, J = 9.3 Hz), 8.07 (1H, dd, J = 2.0, 9.6 Hz), 8.30 (1H, s), 8.34 (1H, s), 8.36 (1H, dd, J = 2.4, 8.8 Hz), 8.79 (1H, d, J = 2.4 Hz), 10.01–10.04 (1H, m); FAB MS *m*/*e* $(MH)^+$ 446; Anal. for $C_{19}H_{19}N_5O_6S{\cdot}HCl$: Calcd. C, 47.35; H, 4.18; N, 14.53; S, 6.65; Cl, 7.36. Found C, 47.04; H, 4.18; N, 14.39; S, 6.65; Cl, 7.26.

5.1.14. 3-((*E*)-{Methyl[(2-methyl-5-nitrophenyl)sulfonyl]-hydrazono}methyl)imidazo[1,2-*a*]pyridine-6-carboxamide hydrochloride (8j). A solution of LiOH hydrate (510 mg, 12 mmol) in water (5 mL) was added to a solution of free base of **8i** (2.8 g, 6.3 mmol) in a mixture of EtOH (30 mL), and water (5 mL). After stirring for 23 h, the reaction mixture was acidified with 4 N HCl/EtOAc and then evaporated. The resulting carboxylic acid was prepared as a suspension in THF (50 mL) and CDI (2.4 g, 15 mmol) was added. After stirring at room temperature for 2.5 h, 28% aqueous NH_4OH (50 mL) was added and the reaction mixture was stirred for 2 d. The mixture was concentrated and washed with hot EtOH suspended in MeOH, acidified with 4 N HCl/EtOAc (2 mL), evaporated, and washed with MeOH–EtOH to give **8j** as a colorless solid (1.74 g, 53% yield): mp 220–223 °C (MeOH–EtOH); ^{1}H NMR (DMSO-d_6) δ 2.68 (3H, s), 3.50 (3H, s), 7.68 (1H, br s), 7.73 (1H, d, J = 8.3 Hz), 7.98 (1H, d, J = 9.3 Hz), 8.21 (1H, dd, J = 1.0, 9.3 Hz), 8.30 (1H, br s), 8.36 (1H, s), 8.38 (1H, dd, J = 2.5, 8.3 Hz), 8.42 (1H, s), 8.74 (1H, d, J = 2.4 Hz), 9.73 (1H, s); FAB MS *m*/*e* $(MH)^+$ 417; Anal. for $C_{17}H_{16}N_6O_5S{\cdot}HCl{\cdot}0.2H_2O$: Calcd. C, 44.73; H, 3.84; N, 18.41; S, 7.02; Cl, 7.77. Found C, 44.69; H, 3.72; N, 18.37; S, 6.99; Cl, 7.55.

5.1.15. 2-Amino-5-cyanopyridine (9f). TFAA (18 g, 86 mmol) was slowly added to a mixture of **12** (5.0 g, 37 mmol) and Et_3N (15 g, 148 mmol) at 0 °C. After stirring at room temperature for 4 h, the reaction mixture was evaporated and diluted with brine and EtOAc. The organic layer was separated, dried over $MgSO_4$, and evaporated. K_2CO_3 (5.5 g, 40 mmol), MeOH (90 mL) and water (30 mL) were added to the resulting residue and the reaction mixture was stirred overnight and then evaporated. The residue was dissolved in brine and EtOAc, and the organic layer was separated, dried over $MgSO_4$, and concentrated to give **9f** (3.5 g, 80% yield) as a brown solid: ^{1}H NMR ($CDCl_3$) δ 5.01 (2H, br s), 6.50 (1H, d, J = 8.8 Hz), 7.62 (1H, d, J = 2.5,

8.8 Hz), 8.36 (1H, d, $J = 2.5$ Hz); FAB MS *m/e* $(MH)^+$ 120.

5.1.16. 6-Bromoimidazo[1,2-*a*]pyridine-3-carbaldehyde (10a). Bromomalonaldehyde (6.0 g, 40 mmol) was added to a mixture of **9a** (6.0 g, 35 mmol) in MeCN (60 mL) and the reaction was allowed to proceed at 75 °C for 15 min. After addition of EtOH (10 mL) the mixture was refluxed for 3 h. Following concentration of the mixture, the residue was suspended in a mixture of $CHCl_3$ and water. Insoluble materials were removed by filtration and then the organic layer was dried over $MgSO_4$ and evaporated to give **5c** (3.3 g, 42% yield) as a brown solid: ^1H NMR (DMSO) δ: 7.84–7.89 (2H, m), 8.56 (1H, s), 9.49 (1H, s), 9.96 (1H, s); FAB MS *m/e* $(M+H)^+$ 225, 227.

5.2. Scintillation proximity assay (SPA) for p110α, p110β, p110γ, and PI3K C2β

GST-tagged bovine p110α, GST-tagged human p110β, His-tagged p110γ, and Glu-tagged PI3K C2β were expressed in an Sf9/Baculovirus system and purified as fusion proteins. The test compounds dissolved in DMSO (0.5 μL) and each enzyme were mixed in 25 μL of buffer solution (p110α, β, γ assay: 20 mM Tris–HCl (pH 7.4), 160 mM NaCl, 2 mM dithiothreitol, 30 mM $MgCl_2$, 0.4 mM EDTA, 0.4 mM EGTA; PI3K C2β assay: 20 mM Tris–HCl (pH 7.4), 160 mM NaCl, 2 mM dithiothreitol, 5 mM $MgCl_2$, 15 mM $CaCl_2$, 0.4 mM EDTA). Then, 25 μL of 5 mM Tris–HCl supplemented with 1 μg PI (Sigma), 0.125 μCi [γ-^{33}P]ATP (Amersham Pharmacia), and 2 μM non-radiolabeled ATP (Sigma) was added to the mixture to initiate the reaction. After allowing the reaction to proceed at room temperature for 120 min, 0.2 mg of wheat germ agglutinin-coated SPA beads (Amersham) in 150 μL PBS was added. The mixture was left to stand for 5 min and then centrifuged at 300*g* for 2 min. Radioactivity was measured using TopCount (Packard). IC_{50} values represent means of at least two separate determinations with typical variations of less than ±20%.

5.3. Proliferation assays

Cells (A375, HeLa, A549, MCF7, and MCF7 ADR-res) were cultured in DMEM with 10% fetal bovine serum and streptomycin/penicillin. Solutions of the test compounds (1 μL) were spotted onto a 96-well culture plate, followed by addition of cells (1×10^4) in 100 μL. After a 46-h incubation, 10 μL of Alamar blue reagent was added to each well. After 2 h, the excitation/emission wavelengths at 544/590 nm were measured using Fluostar. IC_{50} values represent means of at least two separate determinations with typical variations of less than ±20%.

5.4. Xenografts

Female Balb/c-nu/nu mice were used. HeLa human cervical cancer cells (5×10^6) were injected subcutaneously into the hind quarters of the mice. Each group consisted of five animals. When the tumor reached about 100 mm^3 in volume, test compound was administered intraperitoneally. The tumor volume was calculated by the following formula: $1/2 \times (\text{shorter diameter})^2 \times (\text{longer diameter})$. Test compound was suspended in 20% hydroxypropyl-β-cyclodextrin/saline, and doses are given as the free base.

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