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# Synthesis and biological evaluation of imidazo[1,2-*a*]pyridine derivatives as novel PI3 kinase p110α inhibitors

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**Abstract**—3-{1-[(4-Fluorophenyl)sulfonyl]-1*H*-pyrazol-3-yl}-2-methylimidazo[1,2-*a*]pyridine, **2a**, was discovered in our chemical library as a novel p110α inhibitor with an $IC_{50}$ of 0.67 μM, through screening in a scintillation proximity assay. Optimization of the substituents of **2a** increased the p110α inhibitory activity by more than 300-fold (**2g**: $IC_{50}$ = 0.0018 μM). Further structural modification of **2g** afforded thiazole derivative **12**, which has potent p110α inhibitory activity ($IC_{50}$ of 0.0028 μM) and is highly selective for p110α over other PI3K isoforms. Compound **12** also inhibited serum-induced cell proliferation of A375 and HeLa cells in vitro with $IC_{50}$ values of 0.14 μM and 0.21 μM, respectively, and suppressed tumor growth by 37% in a mouse HeLa xenograft model when dosed intraperitoneally at 25 mg/kg. These results suggest that selective p110α inhibitors may have potential as cancer therapeutic agents.


## 1. Introduction

Phosphoinositide 3-kinase (PI3K) is an enzyme that catalyzes phosphorylation of the 3-hydroxyl position of phosphatidylinositides (PIs), and the resulting 3-phosphorylated phospholipids are known to activate the cell survival kinase PKB/Akt, leading to cell proliferation and survival.[1–4] Negative regulation of PI3K signaling is mediated by PTEN, a lipid-phosphatase that dephosphorylates PI3K products. Loss of PTEN protein or function has been found in a large number of human cancers[5,6] and mutation of PTEN is one of the most common mutations in human cancers.[7]

The family of PI3K enzymes[8–10] is divided into three classes: class I, II, and III; and class I is further subclassified into classes Ia and Ib. Class Ia PI3Ks, which include p110α, p110β, and p110δ, are activated by receptor tyrosine kinases and are thought to play crucial roles in cell proliferation via the growth factor-tyrosine kinase pathway.[11] The PIK3CA gene that encodes p110α is amplified and overexpressed in ovarian and other cancers,[12,13] and is also frequently mutated in many different cancers.[14–17] Thus, class Ia PI3Ks, and particularly p110α, are potential therapeutic targets for cancer, and inhibitors of these enzymes are expected to be useful in cancer treatment.

Reported PI3K inhibitors include the fungal metabolite wortmannin and the flavonoid-related compound LY294002. Although wortmannin is a potent PI3K inhibitor with an $IC_{50}$ in the low nanomolar range, it has low in vitro anti-tumor activity, probably due to instability.[18,19] LY294002 is more stable, but it is a relatively weak PI3K inhibitor with an $IC_{50}$ of 0.63 μM.[20] Furthermore, both wortmannin and LY294002 exhibit no selectivity among PI3K isoforms, and therefore the discovery of isoform-specific and therapeutically useful p110α inhibitors is an important goal.[21,22] We have

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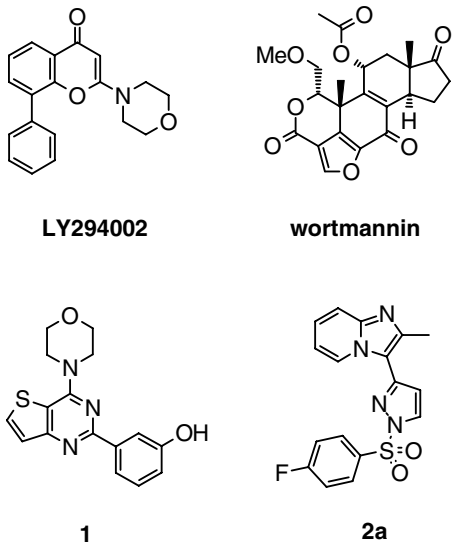
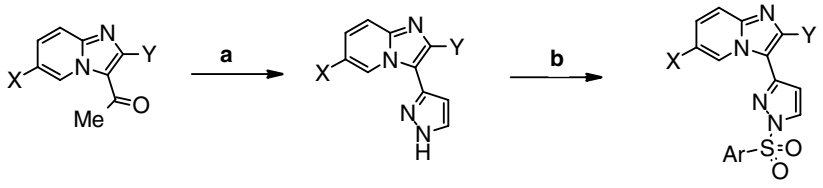



**Figure 1.** Structure of PI3K inhibitors.

found that the thieno[3,2-*d*]pyrimidine derivative **1** is a highly selective p110α inhibitor that potently inhibits tumor cell proliferation in vitro.[23] However, the PK profile of **1** was considered to be insufficient for inhibitory activity of tumor cell growth in vivo, since the half-life of **1** was less than 10 min when dosed intraperitoneally in mice. Therefore, to obtain a potent p110α inhibitor that is effective in vivo, we have focused our efforts on structural modification of another type of p110α inhibitor, **2a**, which was discovered in our chemical library and found to inhibit p110α with an $IC_{50}$ of 0.67 μM. We now report the synthesis and evaluation of novel imidazo[1,2-*a*]pyridine derivatives based on **2a**, and show that these molecules are potent and selective p110α inhibitors.

## 2. Chemistry

As shown in Scheme 1, the sulfonyl pyrazole derivatives **2a**–**g** were prepared from the 1-(imidazo[1,2-*a*]pyridin-3-yl)ethanones **3a**–**d**. Condensation of **3a**–**d**

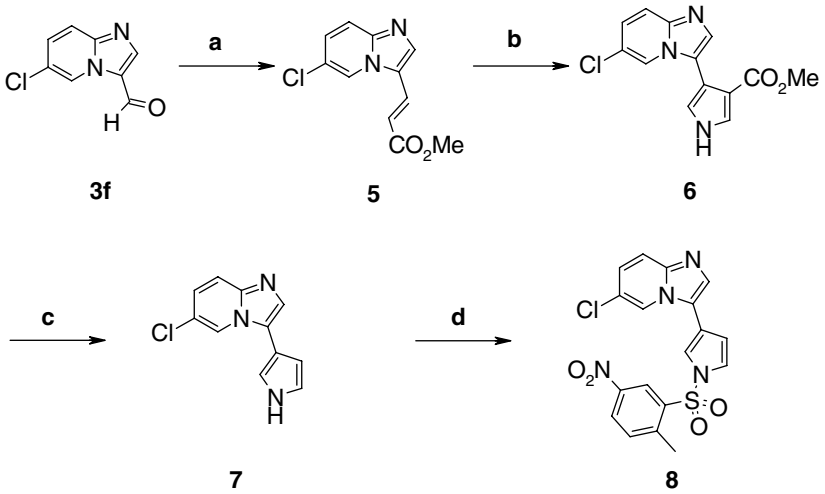


**3a**: X = H, Y = Me
**3b**: X = Cl, Y = Me
**3c**: X = Br, Y = Me
**3d**: X = Br, Y = H

**4a**: X = H, Y = Me
**4b**: X = Cl, Y = Me
**4c**: X = Br, Y = Me
**4d**: X = Br, Y = H

**2a**: X = H, Y = Me, Ar = 4-fluorophenyl
**2b**: X = Cl, Y = Me, Ar = 4-fluorophenyl
**2c**: X = Cl, Y = Me, Ar = 4-nitrophenyl
**2d**: X = Cl, Y = Me, Ar = 3-nitrophenyl
**2e**: X = Cl, Y = Me, Ar = 2-methyl-5-nitrophenyl
**2f**: X = Br, Y = Me, Ar = 2-methyl-5-nitrophenyl
**2g**: X = Br, Y = H, Ar = 2-methyl-5-nitrophenyl

**Scheme 1.** Reagents and conditions: (a) (i) $Me_2NCH(OMe)_2$, reflux; (ii) $H_2NNH_2$ hydrate, EtOH, reflux; (b) substituted benzenesulfonyl chloride, pyridine.



**3f** **5** **6**

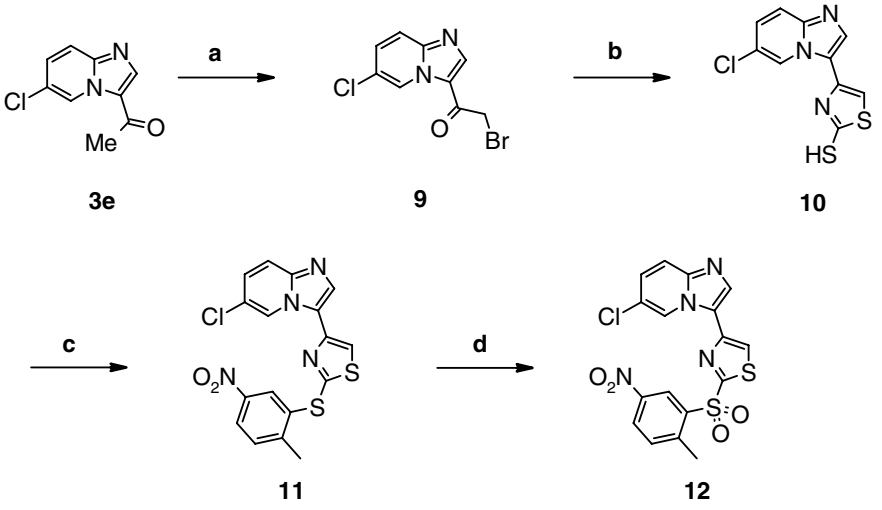
**7** **8**

**Scheme 2.** Reagents and conditions: (a) $K_2CO_3$, $(EtO)_2P(O)CH_2CO_2Me$, DMF; (b) NaH, TosMIC, DMSO/$Et_2O$; (c) (i) KOH, MeOH/$H_2O$; (ii) 2-aminoethanol, Δ; (d) NaH, 2-methyl-5-nitrobenzenesulfonyl chloride, THF.

with 1,1-dimethoxy-*N*,*N*-dimethylmethanamine and cyclization of the resulting enamines with hydrazine hydrate gave pyrazole derivatives **4a**–**d**. Sulfonylation of **4a**–**d** with the appropriate arylsulfonyl chlorides afforded **2a**–**g**.

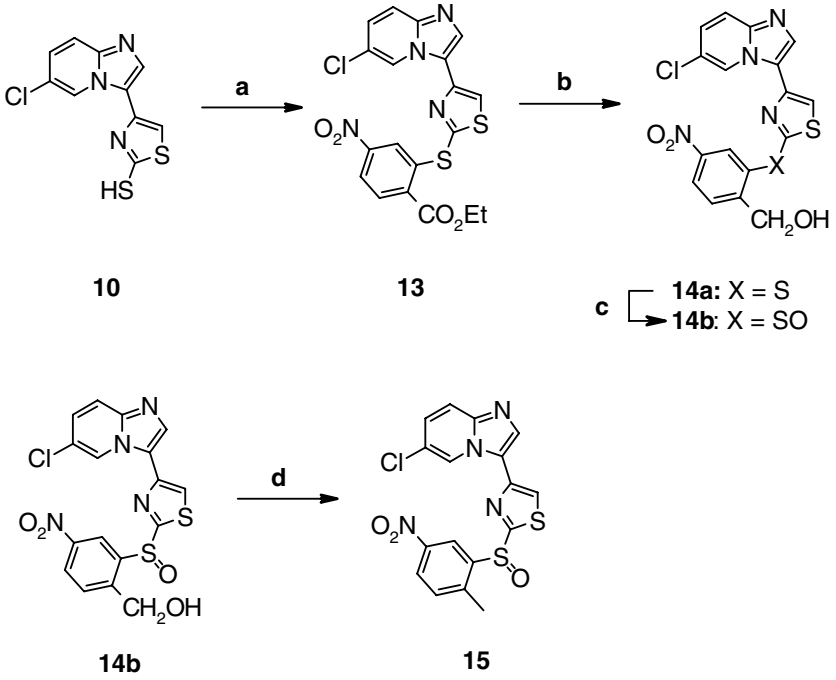
The pyrrole derivative **8** was synthesized from the aldehyde **3f**, as shown in Scheme 2. Horner–Emmons olefination of **3f** gave the ester **5**, which was treated with TosMIC in the presence of NaH to give the cyclized product **6**.[24] Hydrolysis and subsequent decarboxylation by heating in 2-aminoethanol afforded **7**,[25] which was treated with sulfonyl chloride to provide the desired pyrrole derivative **8**.

The thiazole derivatives **11** and **12** were synthesized as shown in Scheme 3. The ketone **3e** was converted to the α-bromoketone **9** via bromination under acidic conditions. Reaction of **9** with ammonium dithiocarbamate in methanol followed by cyclization in acetic acid gave the thiol **10**. A subsequent coupling reaction of **10** with a diazonium salt afforded sulfide derivative **11**,[26] which was oxidized with 30% $H_2O_2$ in acetic acid to give the desired sulfone derivative **12**.




3e 9 10 11 12

**Scheme 3.** Reagents and conditions: (a) $Br_2$, HBr/AcOH; (b) (i) $NH_4H_2NCS_2$, MeOH; (ii) AcOH, reflux; (c) NaH, DMSO, then 2-methyl-5-nitrobenzenediazonium tetrafluoroborate; (d) 30% $H_2O_2$, AcOH, Δ.

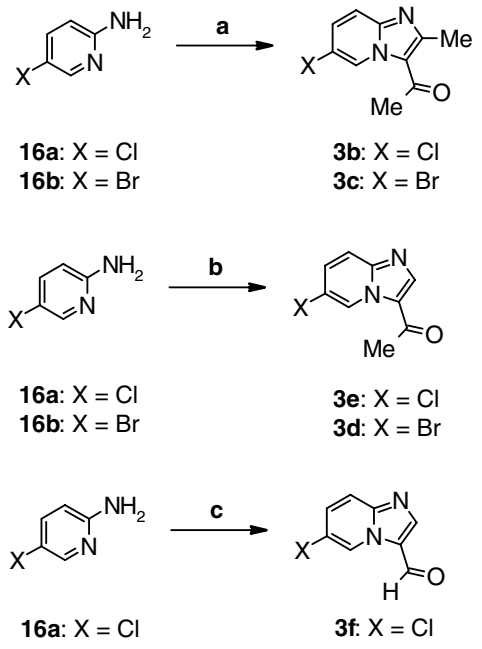



10 13 14a: X = S; 14b: X = SO 14b 15

**Scheme 4.** Reagents and conditions: (a) ethyl 2-fluoro-4-nitrobenzoate, NaH, DMF, Δ; (b) DIBAL-H, $CH_2Cl_2$; (c) *m*-CPBA, $CH_2Cl_2$; (d) (i) MsCl, $Et_3N$, THF; (ii) $NaBH_4$, DMSO.

The sulfoxide derivatives **14b** and **15** were prepared from the thiol **10**, as shown in Scheme 4. Displacement of the fluorine in ethyl 2-fluoro-4-nitrobenzoate with **10** and subsequent reduction of the ester group with DIBAL-H gave the sulfide derivative **14a**. Oxidation of **14a** with *m*-CPBA gave sulfoxide **14b**, and mesylation and subsequent reduction of **14b** with $NaBH_4$ in DMSO gave **15**.[27]

The ketones **3b–e** and the aldehyde **3f**, which were used as starting materials, were synthesized as depicted in Scheme 5. Cyclization of commercially available **16a** and **16b** with 3-chloropentane-2,4-dione in refluxing EtOH gave the 2-methylimidazo[1,2-*a*]pyridines **3b** and **3c**. Compound **3d** and **3e**, which have no substituent at C2, were synthesized by condensation of **16b** and **16a** with dimethylformamide dimethylacetal followed by treatment with bromoacetone.[28] Cyclization of **16a** with bromomalonaldehyde gave the aldehyde **3f**.



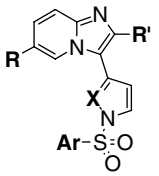

**Scheme 5.** Reagents and conditions: (a) 3-chloropentane-2,4-dione, EtOH, reflux; (b) (i) $Me_2NCH(OMe)_2$, reflux; (ii) bromoacetone, MeOH; (c) $BrCH(CHO)_2$, MeCN, Δ.

## 3. Results and discussion

The lead compound, **2a**, had an $IC_{50}$ of 0.67 μM for inhibition of p110α in an enzymatic scintillation proximity assay (SPA). Under our assay conditions, LY294002 inhibited p110α with an $IC_{50}$ of 0.63 μM, which was comparable with that of **2a**.

As shown in Table 1, inhibitory activity against p110α was retained with introduction of a chloro group into the imidazopyridine ring of **2a** at C6 (**2b**: $IC_{50}$ = 0.76 μM). Replacement of the electron-withdrawing fluorine in **2b** with a nitro group resulted in a loss of p110α activity (**2c**: $IC_{50} > 30$ μM), while the 3-nitro derivative **2d** exhibited 2-fold more potent activity than **2a** and **2b**. A dramatic increase in p110α inhibitory activity was achieved by modification of the benzene ring of **2d**: compound **2e**, which has a methyl group on the phenyl ring of **2d**, was 100-fold more potent against p110α (**2e**: $IC_{50}$ = 0.0028 μM) and also showed potent inhibitory activity against A375 cell proliferation ($IC_{50}$ = 0.83 μM). This suggests that interactions of the methyl group and the oxygen of the sulfone play an extremely important role in p110α inhibition. The inhibitory activities against p110α and cell proliferation were retained with substitution of the chloro group on the imidazopyridine of **2e** with a bromo group (**2f**: $IC_{50}$ against p110α = 0.0031 μM; $IC_{50}$ against A375

**Table 1.** Inhibition of p110α activity of 3-(1*H*-pyrazol-3-yl)imidazo[1,2-*a*]pyridine derivatives




| Compound | R | R′ | X | Ar | $IC_{50}$[c] (μM) | |
|---|---|---|---|---|---|---|
| | | | | | p110α | A375 |
| LY294002[a] | | | | | 0.63 | 8.4 |
| **2a**[a] | H | Me | N | 4-Fluorophenyl | 0.67 | 23 |
| **2b**[a] | Cl | Me | N | 4-Fluorophenyl | 0.76 | NT |
| **2c**[a] | Cl | Me | N | 4-Nitrophenyl | >30 | NT |
| **2d**[a] | Cl | Me | N | 3-Nitrophenyl | 0.28 | NT |
| **2e**[a] | Cl | Me | N | 2-Methyl-5-nitrophenyl | 0.0028 | 0.83 |
| **2f**[a] | Br | Me | N | 2-Methyl-5-nitrophenyl | 0.0031 | 0.73 |
| **2g**[a] | Br | H | N | 2-Methyl-5-nitrophenyl | 0.0018 | 0.17 |
| **8**[b] | Cl | H | CH | 2-Methyl-5-nitrophenyl | 0.10 | 12 |

[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% both for p110α enzyme and A375 cell proliferation assays.

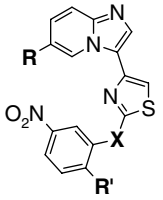
cells = 0.73 μM). Removal of the methyl group at C2 on the imidazopyridine ring further increased inhibitory activities against p110α and cell proliferation (**2g**: $IC_{50}$ against the enzyme = 0.0018 μM; $IC_{50}$ against cell growth = 0.17 μM). Notably, the pyrrole derivative **8** was much less potent than the pyrazole derivatives **2e** or **2g**, indicating that a nitrogen at the 2-position on the pyrazole ring is crucial for p110α inhibitory activity.

The pyrazole derivatives, including **2e**, **2f**, and **2g**, were extremely potent p110α inhibitors, as described above; however, they were found to be unstable in solution, in which they degraded into the desulfonylated pyrazole and benzenesulfonic acid. Furthermore, they were not effective in vivo in xenograft models. Thus, our efforts shifted to obtaining more stable thiazole derivatives such as **11**, **12**, and **15**, which have a carbon-sulfone linkage instead of a nitrogen-sulfone linkage.

The results for the thiazole derivatives are shown in Table 2. The sulfide derivative **11** was approximately 45-fold less potent than the pyrazole derivative **2g**, and the sulfoxide **15** was about 2-fold more potent than the sulfide **11**. A further increase in p110α activity was observed for the sulfone derivative **12**, which exhibited almost the same potencies to **2g** in the enzyme ($IC_{50}$ of 0.0028 μM) and cellular ($IC_{50}$ of 0.14 μM) assays. This result suggests that interactions with the oxygen of the sulfone and the methyl group on the benzene ring are also important for exerting potent p110α inhibitory activity in thiazole derivatives. The sulfoxide derivative **14b**, which has a hydroxymethyl group, also showed more potent p110α inhibitory activity ($IC_{50}$ of 0.020 μM) than the corresponding methyl derivative **15**, however it did not show cellular activity.

Compound **12** was evaluated further, since it was the most potent p110α inhibitor in thiazole derivatives. To check selectivity for p110α, **12** and LY294002 were evaluated against other PI3K isoforms (Table 3). LY294002 showed no selectivity between p110α and p110β (class Ia) and only 3- to 4-fold selectivity for p110α over p110γ (class Ib) and PI3K C2β (class II). In contrast, **12** showed about 60-fold selectivity for p110α ($IC_{50}$ = 0.0028 μM) over p110β ($IC_{50}$ = 0.17 μM) and was approximately 80-fold and 35-fold more selective for p110α versus p110γ ($IC_{50}$ = 0.23 μM) and PI3K C2β ($IC_{50}$ = 0.10 μM), respectively. These data show that **12** is a highly isoform-selective p110α inhibitor (Fig. 1).

**Table 2.** Inhibition of p110α activity of 3-(1,3-thiazol-4-yl)imidazo[1,2-*a*]pyridine derivatives




| Compound[a] | R | –X– | R′ | $IC_{50}$[b] (μM) | |
|---|---|---|---|---|---|
| | | | | p110α | A375 |
| **11** | Cl | –S– | Me | 0.082 | 3.37 |
| **15** | Cl | –SO– | Me | 0.031 | 0.27 |
| **12** | Cl | $–SO_2–$ | Me | 0.0028 | 0.14 |
| **14b** | Cl | –SO– | $CH_2OH$ | 0.020 | 19 |

[a] HCl salt.
[b] $IC_{50}$ values represent means of at least two separate determinations with typical variations of less than ±20% both for p110α enzyme and A375 cell proliferation assays.

**Table 3.** Inhibition of PI3K isoforms by LY294002 and **12**

| Compound | $IC_{50}$[c] (μM) | | | |
|---|---|---|---|---|
| | p110α | p110β | p110γ | PI3K C2β |
| LY294002[a] | 0.63 | 0.34 | 1.6 | 2.1 |
| **12**[b] | 0.0028 | 0.17 | 0.23 | 0.10 |

[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%.

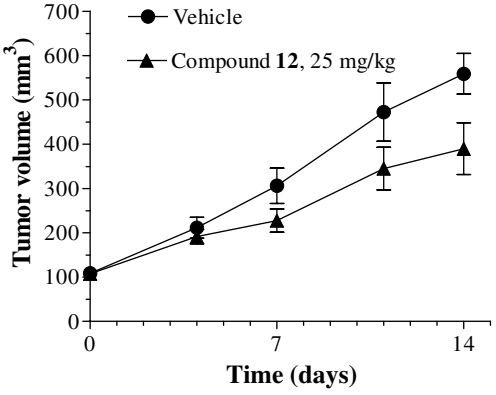



**Figure 2.** HeLa (human cervical cancer cell line) tumor growth during treatment with compound **12** (HCl salt). Compound **12** (25 mg/kg) was intraperitoneally administered daily for 2 weeks to HeLa xenograft nude mice. Compound **12** was suspended in 20% hydroxypropyl-β-cyclodextrin/saline. Tumor growth was suppressed by 37% at 2 weeks after the start of treatment with **12** compared with vehicle alone ($p$ = 0.060)

Finally, the in vivo activity of **12** on tumor growth was evaluated in mice carrying a HeLa tumor xenograft. In HeLa cells, **12** showed antiproliferative activity in vitro ($IC_{50}$ = 0.21 μM) comparable to that observed in A375 cells. Furthermore, the half-life of **12**, as measured by HPLC/MS/MS, was 2.6 h when administered intraperitoneally in mice, and **12** suppressed tumor growth by 37% in HeLa xenograft mice when dosed intraperitoneally at 25 mg/kg once daily for 2 weeks (Fig. 2). No significant weight loss was observed in these mice.

## 4. Conclusion

Structure–activity relationships for PI3K p110α inhibition were examined in a series of imidazo[1,2-*a*]pyridine compounds, among which the thiazole derivative **12**

showed potent p110α inhibitory activity and strong selectivity for p110α over other PI3K isoforms. Compound **12** also inhibited tumor cell growth both in vitro and in vivo, suggesting that PI3K p110α is a potential target in cancer treatment.

## 5. Experimental

### 5.1. Chemistry

$^1$H NMR spectra were recorded on a JEOL EX400 or GX500 spectrometer; chemical shifts are expressed in $\delta$ units using tetramethylsilane as the standard (in the description of the NMR signals, s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad peak). Mass spectra were recorded on a Hitachi M-80 or JEOL JMS-DX300 spectrometer. Silica gel column chromatography was performed with Wakogel C-200 or Merck Silica gel 60.

**5.1.1. 3-{1-[(4-Fluorophenyl)sulfonyl]-1*H*-pyrazol-3-yl}-2-methylimidazo[1,2-*a*] pyridine (2a).** To a suspension of 2-methyl-3-(1*H*-pyrazol-3-yl)imidazo[1,2-*a*]pyridine **4a** (1.8 g, 9.7 mmol) in pyridine (7.1 mL, 88 mmol) was added 4-fluorobenzenesulfonyl chloride (1.9 g, 9.7 mmol). After stirring at reflux for 1 h, the reaction mixture was evaporated, diluted with brine, and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$ and evaporated. The residue was chromatographed on silica gel eluting with $CHCl_3$/MeOH (100:1–50:1), and the obtained solid was washed with a mixture of $CHCl_3$ and hexane to give **2a** (2.4 g, 78%) as a colorless solid. Mp: 159–160 °C; $^1$H NMR (DMSO-$d_6$) $\delta$: 2.55 (3H, s), 7.06 (1H, d, $J$ = 3.0 Hz), 7.13 (1H, dd, $J$ = 1.0, 6.8 Hz), 7.36–7.42 (1H, m), 7.51–7.64 (3H, m), 8.18–8.25 (2H, m), 8.70 (1H, d, $J$ = 3.0 Hz), 9.10–9.16 (1H, m); FAB MS *m/e* $(M+H)^+$ 357; Anal. Calcd for $C_{17}H_{13}N_4O_2SF$: C, 57.29; H, 3.68; N, 15.72; S, 9.00; F, 5.33. Found: C, 56.96; H, 3.68; N, 15.68; S, 9.00; F, 5.37.

**5.1.2. 6-Chloro-3-{1-[(4-fluorophenyl)sulfonyl]-1*H*-pyrazol-3-yl}-2-methylimidazo[1,2-*a*]pyridine (2b).** Compound **2b** was prepared from **4b** and 4-fluorobenzenesulfonyl chloride according to the same procedure as that of **2a**. Compound **2b** was obtained as a colorless solid (84% yield). Mp: 195–196 °C ($CHCl_3$/$Et_2O$); $^1$H NMR (DMSO-$d_6$) $\delta$: 2.58 (3H, s), 7.09 (1H, d, $J$ = 2.9 Hz), 7.44 (1H, dd, $J$ = 2.0, 9.8 Hz), 7.54–7.68 (3H, m), 8.14–8.22 (2H, m), 8.73 (1H, d, $J$ = 2.9 Hz), 9.06–9.09 (1H, m); FAB MS *m/e* $(M+H)^+$ 391; Anal. Calcd for $C_{17}H_{12}N_4O_2SFCl$: C, 52.25; H, 3.09; N, 14.34; S, 8.20; Cl, 9.07, F, 4.86. Found: C, 51.88; H, 2.98; N, 14.25; S, 8.20; Cl, 9.05, F, 4.73.

**5.1.3. 6-Chloro-2-methyl-3-{1-[(4-nitrophenyl)sulfonyl]-1*H*-pyrazol-3-yl}imidazo[1,2-*a*]pyridine (2c).** Compound **2c** was prepared from **4b** and 4-nitrobenzenesulfonyl chloride according to the same procedure as that of **2a**. Compound **2c** was obtained as a yellow solid (56% yield). Mp: 263–264 °C (pyridine); $^1$H NMR (DMSO-$d_6$) $\delta$: 2.54 (3H, s), 7.15 (1H, d, $J$ = 2.9 Hz), 7.47 (1H, dd, $J$ = 1.9, 9.7 Hz), 7.67 (1H, d, $J$ = 9.3 Hz), 8.31–8.37 (2H, m), 8.45–8.51 (2H, m), 8.80 (1H, d, $J$ = 2.9 Hz), 9.07–9.10 (1H, m); FAB MS *m/e* $(M+H)^+$ 418; Anal. Calcd for $C_{17}H_{12}N_5O_4SCl$: C, 48.87; H, 2.89; N, 16.76; S, 7.67; Cl, 8.48. Found: C, 48.90; H, 2.81; N, 16.76; S, 7.65; Cl, 8.45.

**5.1.4. 6-Chloro-2-methyl-3-{1-[(3-nitrophenyl)sulfonyl]-1*H*-pyrazol-3-yl}imidazo[1,2-*a*]pyridine (2d).** Compound **2d** was prepared from **4b** and 3-nitrobenzenesulfonyl chloride according to the same procedure as that of **2a**. Compound **2d** was obtained as a colorless solid (41% yield). Mp: 217–218 °C (pyridine); $^1$H NMR (DMSO-$d_6$) $\delta$: 2.54 (3H, s), 7.13 (1H, d, $J$ = 2.9 Hz), 7.45 (1H, dd, $J$ = 2.0, 9.3 Hz), 7.66 (1H, d, $J$ = 9.3 Hz), 8.02 (1H, t, $J$ = 8.3 Hz), 8.49–8.54 (1H, m), 8.60–8.65 (1H, m), 8.73–8.76 (1H, m), 8.83 (1H, d, $J$ = 3.0 Hz), 9.09–9.12 (1H, m); FAB MS *m/e* $(M+H)^+$ 418; Anal. Calcd for $C_{17}H_{12}N_5O_4SCl$: C, 48.87; H, 2.89; N, 16.76; S, 7.67; Cl, 8.48. Found: C, 48.89; H, 2.70; N, 16.80; S, 7.67; Cl, 8.45.

**5.1.5. 6-Chloro-2-methyl-3-{1-[(2-methyl-5-nitrophenyl)-sulfonyl]-1*H*-pyrazol-3-yl}imidazo[1,2-*a*]pyridine (2e).** Compound **2e** was prepared from **4b** and 2-methyl-5-nitrobenzenesulfonyl chloride according to the same procedure as that of **2a**. Compound **2e** was obtained as a yellow solid (34% yield). Mp: 207–208 °C ($Et_2O$); $^1$H NMR (DMSO-$d_6$) $\delta$: 2.55 (3H, s), 2.76 (3H, s), 7.13 (1H, d, $J$ = 2.9 Hz), 7.38–7.43 (1H, m), 7.61–7.66 (1H, m), 7.84 (1H, d, $J$ = 8.8 Hz), 8.54 (1H, dd, $J$ = 2.4, 8.3 Hz), 8.83 (1H, d, $J$ = 2.5 Hz), 8.90 (1H, d, $J$ = 2.9 Hz), 8.94–8.98 (1H, m); FAB MS *m/e* $(M+H)^+$ 432; Anal. Calcd for $C_{18}H_{14}N_5O_4SCl$: C, 50.06; H, 3.27; N, 16.22; S, 7.43; Cl, 8.21. Found: C, 50.07; H, 3.27; N, 16.07; S, 7.54; Cl, 8.19.

**5.1.6. 6-Bromo-2-methyl-3-{1-[(2-methyl-5-nitrophenyl)-sulfonyl]-1*H*-pyrazol-3-yl} imidazo[1,2-*a*]pyridine (2f).** Compound **2f** was prepared from **4c** and 2-methyl-5-nitrobenzenesulfonyl chloride according to the same procedure as that of **2a**. Compound **2f** was obtained as a yellow solid (12% yield). Mp: 202–203 °C ($Et_2O$); $^1$H NMR (DMSO-$d_6$) $\delta$: 2.55 (3H, s), 2.76 (3H, s), 7.13 (1H, d, $J$ = 2.9 Hz), 7.47 (1H, dd, $J$ = 1.9, 9.3 Hz), 7.58 (1H, d, $J$ = 9.3 Hz), 7.85 (1H, d, $J$ = 8.3 Hz), 8.55 (1H, dd, $J$ = 2.4, 8.3 Hz), 8.83 (1H, d, $J$ = 2.5 Hz), 8.89 (1H, d, $J$ = 2.9 Hz), 9.06 (1H, d, $J$ = 2.5 Hz); FAB MS *m/e* $(M+H)^+$ 476, 478; Anal. Calcd for $C_{18}H_{14}N_5O_4SBr$: C, 45.39; H, 2.96; N, 14.70; S, 6.73; Br, 16.78. Found: C, 45.27; H, 2.84; N, 14.67; S, 6.82; Br, 16.65.

**5.1.7. 6-Bromo-3-{1-[(2-methyl-5-nitrophenyl)sulfonyl]-1*H*-pyrazol-3-yl}imidazo[1,2-*a*]pyridine (2g).** Compound **2g** was prepared from **4d** and 2-methyl-5-nitrobenzenesulfonyl chloride according to the same procedure as that of **2a**. Compound **2g** was obtained as a colorless solid (16% yield). Mp: 195–196 °C (AcOEt); $^1$H NMR (DMSO-$d_6$) $\delta$: 2.80 (3H, s), 7.32 (1H, d, $J$ = 2.9 Hz), 7.55 (1H, dd, $J$ = 1.9, 9.3 Hz), 7.73 (1H, d, $J$ = 9.3 Hz), 7.84 (1H, d, $J$ = 8.7 Hz), 8.35 (1H, s), 8.53 (1H, dd, $J$ = 2.5, 8.8 Hz), 8.80–8.85 (2H, m), 9.20–9.24 (1H, m); FAB MS *m/e* $(M+H)^+$ 462, 464; Anal. Calcd for $C_{17}H_{12}N_5O_4SBr$: C, 44.17; H, 2.62; N, 15.15; S, 6.94;

Br, 17.28. Found: C, 44.01; H, 2.58; N, 15.19; S, 6.80; Br, 17.41.

**5.1.8. 1-(6-Chloro-2-methylimidazo[1,2-*a*]pyridin-3-yl)ethanone (3b).** A mixture of 2-amino-5-chloropyridine **16a** (6.4 g, 50 mmol) and 3-chloropentane-2,4-dione (6.7 g, 50 mmol) in ethanol (30 mL) was refluxed for 10 h. After evaporation, the residue was chromatographed on silica gel eluting with $CHCl_3$/MeOH (200:1) to give **3b** (4.4 g, 42%) as a brown solid. $^1$H NMR ($CDCl_3$) $\delta$: 2.63 (3H, s), 2.79 (3H, s), 7.43 (1H, dd, $J$ = 1.9. 9.3 Hz), 7.58 (1H, d, $J$ = 8.8 Hz), 9.82–9.86 (1H, m); FAB MS *m/e* $(M+H)^+$ 209.

**5.1.9. 1-(6-Bromo-2-methylimidazo[1,2-*a*]pyridin-3-yl)ethanone (3c).** Compound **3c** was prepared from 2-amino-5-bromopyridine **16b** according to the same procedure as that of **3b**. Compound **3c** was obtained as a brown solid (44% yield). $^1$H NMR (DMSO-$d_6$) $\delta$: 2.59 (3H, s), 2.72 (3H, s), 7.66–7.76 (2H, m), 9.73–9.76 (1H, m); FAB MS *m/e* $(M+H)^+$ 253, 255.

**5.1.10. 1-(6-Bromolimidazo[1,2-*a*]pyridin-3-yl)ethanone (3d).** Compound **16b** (50 g, 0.29 mol) in 1,1-dimethoxy-*N*,*N*-dimethylmethanamine (55 g, 0.46 mol) was refluxed for 23 h and then evaporated. To a solution of the resulting residue in EtOH (500 mL), 90% 1-bromoacetone (50 g, 0.33 mmol) was added and the reaction mixture was stirred at rt for 18 h. The resulting precipitates were collected and washed with MeOH (30 mL) to give **3d** (22 g, 32%) as a brown solid. $^1$H NMR ($CDCl_3$) $\delta$: 2.59 (3H, s), 7.57 (1H, dd, $J$ = 2.0, 9.8 Hz), 7.66 (1H, $J$ = 8.8 Hz), 8.31 (1H, s), 9.81–9.84 (1H, m); FAB MS *m/e* $(M+H)^+$ 239, 241.

**5.1.11. 1-(6-Chloroimidazo[1,2-*a*]pyridin-3-yl)ethanone (3e).** Compound **3e** was prepared from **16a** according to the same procedure as that of **3d**. Compound **3e** was obtained as a brown solid (25% yield). $^1$H NMR (DMSO-$d_6$) $\delta$: 2.58 (3H, s), 7.66–7.74 (1H, m), 7.78 (1H, d, $J$ = 9.8 Hz), 8.65 (1H, s), 9.52–9.57 (1H, m); FAB MS *m/e* $(M+H)^+$ 195.

**5.1.12. 6-Chloroimidazo[1,2-*a*]pyridine-3-carbaldehyde (3f).** To a mixture of **16a** (14.5 g, 113 mmol) in MeCN (260 mL) was added bromomalonaldehyde (17.4 g, 116 mmol) and the mixture was heated at reflux for 3 h. After evaporation, the residue was dissolved with a mixture of $CHCl_3$ and aqueous $NaHCO_3$. The organic layer was dried over $Na_2SO_4$ and evaporated. The residue was dissolved with EtOH and the insoluble materials were removed by filtration. Then, the filtrate was evaporated and the residue was washed with water to give **3f** (5.18 g, 31%) as a brown solid. $^1$H NMR ($CDCl_3$) $\delta$: 7.54 (1H, d, $J$ = 2.0, 9.7 Hz), 7.75 (1H, d, $J$ = 9.7 Hz), 8.33 (1H, s), 9.59 (1H, d, $J$ = 2.0 Hz), 9.96 (1H, s); FAB MS *m/e* $(M+H)^+$ 181.

**5.1.13. 2-Methyl-3-(1*H*-pyrazol-3-yl)imidazo[1,2-*a*]pyridine (4a).** A mixture of 1-(2-methylimidazo[1,2-*a*]pyridin-3-yl)ethanone **3a**[29] (5.4 g, 31 mmol) and 1,1-dimethoxy-*N*,*N*-dimethylmethanamine (9.1 mL, 53 mmol) was heated at 120 °C for 14 h. After cooling to room temperature, EtOH (30 mL) and hydrazine hydrate (4.5 mL, 93 mmol) were added and the reaction mixture was refluxed for 3 h. The mixture was diluted with water and extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$ and evaporated. The resulting solid was washed with $Et_2O$ to give **4a** (1.75 g, 28%) as a brown solid. $^1$H NMR (DMSO-$d_6$) $\delta$: 2.51 (3H, s), 6.65 (1H, d, $J$ = 2.4 Hz), 6.80–7.10 (1H, m), 7.13–7.40 (1H, m), 7.45–7.65 (1H, m), 7.95 (1H, d, $J$ = 2.4 Hz), 9.00–9.35 (1H, m), 13.18 (1H, br s); FAB MS *m/e* $(M+H)^+$ 199.

**5.1.14. 6-Chloro-2-methyl-3-(1*H*-pyrazol-3-yl)imidazo-[1,2-*a*]pyridine (4b).** 1-(6-Chloro-2-methylimidazo[1,2-*a*]pyridin-3-yl)ethanone**3b** (4.5 g, 22 mmol) in 1,1-dimethoxy-*N*,*N*-dimethylmethanamine (9 mL, 68 mmol) was refluxed for 4.5 h. After cooling, MeOH (50 mL) was added to the mixture and the insoluble matearials were removed by filtration. The filtrate was concentrated, and hydrazine hydrate (2.4 mL, 49 mmol) and EtOH (25 mL) were added to the resulting residue. After refluxing for 1 h, the reaction mixture was filtered and the filtrate was allowed to cool. Then, water (50 mL) was added to it and the resulting precipitates were collected to give **4b** (3.5 g, 68%) as a brown solid. $^1$H NMR (DMSO-$d_6$) $\delta$: 2.56 (3H, s), 6.68–6.72 (1H, m), 7.33 (1H, dd, $J$ = 1.9, 9.3 Hz), 7.61 (1H, d, $J$ = 9.8 Hz), 7.99 (1H, s), 9.42–9.47 (1H, m), 13.26 (1H, br s); FAB MS *m/e* $(M+H)^+$ 233.

**5.1.15. 6-Bromo-2-methyl-3-(1*H*-pyrazol-3-yl)imidazo-[ 1,2-*a*]pyridine (4c).** Compound **4c** was prepared from **3c** according to the same procedure as that of **4b**. Compound **4c** was obtained as a brown solid (54%). $^1$H NMR (DMSO-$d_6$) $\delta$: 2.56 (3H, s), 6.70 (1H, d, $J$ = 2.5 Hz), 7.39 (1H, dd, $J$ = 2.0, 9.3 Hz), 7.56 (1H, d, $J$ = 9.3 Hz), 8.00 (1H, s), 9.53 (1H, s), 13.26 (1H, s); FAB MS *m/e* $(M+H)^+$ 276, 278.

**5.1.16. 6-Bromo-3-(1*H*-pyrazol-3-yl)imidazo[1,2-*a*]pyridine (4d).** Compound **4d** was prepared from **3d** according to the same procedure as that of **4b**. Compound **4d** was obtained as a brown solid (34% yield). $^1$H NMR (DMSO-$d_6$) $\delta$: 6.81–6.86 (1H, m), 7.42–7.48 (1H, m), 7.68 (1H, d, $J$ = 9.3 Hz), 7.90–7.95 (1H, m), 8.10 (1H, s), 9.65 (1H, s), 13.16 (1H, br s); FAB MS *m/e* $(M+H)^+$ 263, 265.

**5.1.17. Methyl (2*E*)-3-(6-chloroimidazo[1,2-*a*]pyridin-3-yl)acrylate (5).** To a mixture of **3f** (5.2 g, 29 mmol) in DMF (55 mL) were added methyl (diethoxyphosphoryl)acetate (7.2 g, 34 mmol) and $K_2CO_3$ (5.0 g, 36 mmol) and the reaction mixture was stirred at 60 °C for 27 h. After evaporation, the residue was washed with water to give **5** (3.5 g, 70%) as a brown solid. $^1$H NMR (DMSO-$d_6$) $\delta$: 3.75 (3H, s), 6.66 (1H, d, $J$ = 15.6 Hz), 7.40–7.48 (1H, m), 7.72 (1H, d, $J$ = 9.2 Hz), 8.09 (1H, d, $J$ = 15.6 Hz), 8.37 (1H, s), 9.19 (1H, s); FAB MS *m/e* $(M+H)^+$ 237.

**5.1.18. Methyl 4-(6-chloroimidazo[1,2-*a*]pyridin-3-yl)-1*H*-pyrrole-3-carboxylate (6).** To a suspension of 60% NaH (540 mg, 14 mmol) were added dropwose a solution

of **5** (2.7 g, 29 mmol) and TosMIC (2.2 g, 11 mmol) in a mixture of DMSO (32 mL) and $Et_2O$ (16 mL). After stirring at room temperature for 1 h, the reaction mixture was evaporated and the residue was washed with water and then $Et_2O$ to give **6** (2.5 g, 80%) as a brown solid. $^1H$ NMR (DMSO-$d_6$) $\delta$: 3.57 (3H, s), 7.10–7.18 (1H, m), 7.28 (1H, dd, $J$ = 1.2, 10.0 Hz), 7.50–7.72 (3H, m), 7.99 (1H, s), 11.91 (1H, br s); FAB MS *m/e* $(M+H)^+$ 276.

**5.1.19. 6-Chloro-3-(1*H*-pyrrol-3-yl)imidazo[1,2-*a*]pyridine (7).** To a mixture of **6** (800 mg, 2.9 mmol) in a mixture of MeOH (5 mL) and water (5 mL) was added KOH (1.6 g, 29 mmol). After stirring for 3 h, the mixture was neutralized with 1 N HCl and the insoluble materials were removed. After evaporation, the residue was dissolved in 2-aminoethanol (5 mL) and heated at 200 °C for 1 h. After the reaction mixture was allowed to cool, water was added. The resulting precipitates were collected to give **7** (203 mg, 32%) as a brown solid. $^1H$ NMR (DMSO-$d_6$) $\delta$: 6.44–6.50 (1H, m), 6.93–6.99 (1H, m), 7.24 (1H, dd, $J$ = 2.0, 9.6 Hz), 7.31–7.37 (1H, m), 7.59–7.70 (2H, m), 8.48–8.52 (1H, m), 11.26 (1H, br s); FAB MS *m/e* $(M-H)^-$ 216.

**5.1.20. 6-Chloro-3-{1-[(2-methyl-5-nitrophenyl)sulfonyl]-1*H*-pyrrol-3-yl}imidazo[1,2-*a*]pyridine hydrochloride (8).** To a solution of **7** (203 mg, 0.93 mmol) in THF (5 mL) was added 60% NaH (90 mg, 2.3 mmol) and the mixture was stirred at room temperature for 0.5 h. Then, after addition of 2-methyl-5-nitrobenzensulfonyl chloride (400 mg, 1.7 mmol), the reaction mixture was stirred for 2 h. The mixture was evaporated and chromatographed on silica gel with eluting $CHCl_3$/MeOH (20/1). The collected fractions were concentrated and the residue was dissolved with MeOH. 4 N HCl/AcOEt (1 mL) was added to the solution and then evaporated. The resulting solid was washed with hot EtOH to give HCl salt of **8** (169 mg, 44%) as a colorless solid. Mp: 199–203 °C; $^1H$ NMR (DMSO-$d_6$) $\delta$: 2.70 (3H, s), 6.99–7.05 (1H, m), 7.75–7.80 (1H, m), 7.82 (1H, d, $J$ = 8.7 Hz), 7.94–8.00 (1H, m), 8.05 (1H, d, $J$ = 9.3 Hz), 8.31 (1H, s), 8.42–8.56 (2H, m), 8.66 (1H, d, $J$ = 2.4 Hz), 8.93 (1H, s); FAB MS *m/e* $(M+H)^+$ 417; Anal. Calcd for $C_{18}H_{13}N_4O_4SClHCl$: C, 47.69; H, 3.11; N, 12.36; S, 7.07; Cl, 15.64. Found: C, 47.46; H, 3.09; N, 12.16; S, 7.03; Cl, 15.69.

**5.1.21. 2-Bromo-1-(6-chloroimidazo[1,2-*a*]pyridin-3-yl)ethanone hydrobromide (9).** To a suspension of **3e** (10 g, 51 mmol) in 30% HBr/AcOH (100 mL) was added $Br_2$ (9.8 g, 50 mmol) at room temperature. After stirring for 32 h, the precipitates were collected by filtration and washed with EtOH and $Et_2O$ to give **9** (16 g, 90%) as a colorless solid. $^1H$ NMR (DMSO-$d_6$) $\delta$: 4.65–4.90 (2H, s), 7.90–8.15 (2H, m), 8.90–9.23 (1H, m), 9.55–9.70 (1H, m).

**5.1.22. 4-(6-Chloroimidazo[1,2-*a*]pyridin-3-yl)-1,3-thiazole-2-thiol (10).** To a suspension of **9** (15 g, 42 mmol) in MeOH (150 mL), ammonium dithiocarbamate (7.0 g, 64 mmol) was added at room temperature. After stirring for 15 min, the solid was collected by filtration. After the obtained solid in AcOH (40 mL) was refluxed for 1 h, water (40 mL) was added to the reaction mixture and the resulting colorless solid was collected and washed with hot MeOH (70 mL) to give **10** as a colorless solid (8.0 g, 71%). $^1H$ NMR (DMSO-$d_6$) $\delta$: 7.42–7.50 (2H, m), 7.75 (1H, d, $J$ = 9.7 Hz), 8.02 (1H, s), 8.72 (1H, d, $J$ = 1.9 Hz), 13.71 (1H, br s); FAB MS *m/e* $(M+H)^+$ 268.

**5.1.23. 6-Chloro-3-{2-[(2-methyl-5-nitrophenyl)thio]-1,3-thiazol-4-yl}imidazo[1,2-*a*]pyridine hydrochloride (11).** To a suspension of 2-methyl-5-nitroaniline (1.0 g, 6.6 mmol) in 6 N HCl (5 mL), a solution of $NaNO_2$ (480 mg, 7.0 mmol) in water (5 mL) was added dropwise below 0 °C. After stirring for 15 min, a solution of $NaBF_4$ (1.0 g, 9.1 mmol) in water (5 mL) was added and the reaction mixture was stirred at room temperature for 0.5 h. The resulting diazonium salt was collected and dried under vacuum. To a suspension of 60% NaH (140 mg, 3.5 mmol) in DMSO (20 mL), **10** (950 mg, 3.5 mmol) was added and stirred at 70 °C for 15 min. To the resulting red solution, diazonium salt was added portionwise at room temperature. After stirring for 10 min, $CHCl_3$ and saturated aqueous $NaHCO_3$ were added to the reaction mixture and the insoluble materials were removed by filtration through a bed of Celite. After the filtrate was separated, the organic layer was dried over $MgSO_4$ and evaporated. The obtained residue was chromatographed on silica gel eluting with hexane/AcOEt (2:3) to give free base of **11** as a brown oil (270 mg, 19%). To a solution of free base of **11** (270 mg, 0.67 mmol) was added 4 N HCl/AcOEt (0.3 mL) and the mixture was evaporated. The residue was crystallized from EtOH to give hydrochloride salt of **11** (120 mg, 8%) as a pink solid. Mp: 180–182 °C; $^1H$ NMR (DMSO-$d_6$) $\delta$: 2.59 (3H, s), 7.78–7.86 (2H, m), 7.98 (1H, d, $J$ = 9.8 Hz), 8.32–8.38 (2H, m), 8.52 (1H, d, $J$ = 2.4 Hz), 8.64 (1H, s), 9.14–9.17 (1H, m); FAB MS *m/e* $(M)^+$ 402; Anal. Calcd for $C_{17}H_{11}N_4O_2S_2Cl0.95HCl$: C, 46.67; H, 2.75; N, 12.81; S, 14.66; Cl, 15.80. Found: C, 46.34; H, 2.75; N, 12.48; S, 14.45; Cl, 15.55.

**5.1.24. 6-Chloro-3-{2-[(2-methyl-5-nitrophenyl)sulfonyl]-1,3- thiazol-4-yl}imidazo[1,2-*a*]pyridine hydrochloride (12).** To free base of **11** (260 mg, 0.645 mmol), AcOH (10 mL) and 30% $H_2O_2$ (10 mL) were added and the reaction mixture was stirred at 70 °C for 9.5 h. After the mixture was diluted with water (50 mL) and $CHCl_3$ (50 mL), the organic layer was washed with 5% $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$, and dried over $MgSO_4$. After evaporation, the residue was chromatographed on silica gel with eluting $CHCl_3$/MeOH (100:1) and the collected fractions were concentrated. To the solution of the residue in $CHCl_3$ (5 mL) and MeOH (5 mL), 4 N HCl/AcOEt (0.2 mL) was added and the mixture was evaporated to give a colorless solid. The solid was washed with hot MeOH and $Et_2O$ to give HCl salt of **12** (110 mg, 36%) as a colorless solid. Mp: 221–223 °C; $^1H$ NMR (DMSO-$d_6$) $\delta$: 2.84 (3H, s), 7.76 (1H, d, $J$ = 1.9, 9.8 Hz), 7.88 (1H, d, $J$ = 8.8 Hz), 7.94 (1H, d, $J$ = 9.8 Hz), 8.55–8.60 (1H, m), 8.63 (1H, s), 8.84–8.88 (2H, m), 9.02–9.06 (1H, m); FAB MS *m/e* $(M)^+$ 434; Anal. Calcd for $C_{17}H_{11}N_4O_4S_2ClHCl$: C, 43.32; H, 2.57; N, 11.89; S, 13.61; Cl, 15.04. Found: C, 43.04; H, 2.57; N, 11.78; S, 13.66; Cl, 15.30.

**5.1.25. Ethyl 2-{[4-(6-chloroimidazo[1,2-*a*]pyridin-3-yl)-1,3-thiazol-2-yl]thio}-4-nitrobenzoate (13).** To a suspension of 60% NaH (1.8 g, 45 mmol) in DMF (200 mL), **10** (11 g, 41 mmol) was added and the mixture was stirred at room temperature for 0.5 h. To the reaction mixture, ethyl 2-fluoro-4-nitrobenzoate (10 g, 47 mmol) was added. After stirring at 90 °C for 7 h, the reaction mixture was concentrated and dissolved with a mixture of THF (100 mL) and AcOEt (400 mL). The solution was washed with brine, dried over $MgSO_4$, and evaporated. The residue was subjected to silica gel column chromatography ($CHCl_3$) and the obtained solid was washed with MeOH (100 mL) to give **13** (4.8 g, 25%) as a pale yellow solid. $^1$H NMR (DMSO-$d_6$) $\delta$: 1.29–1.36 (3H, m), 4.35–4.43 (2H, m), 7.37–7.43 (1H, m), 7.75 (1H, d, $J$ = 9.2 Hz), 8.16 (1H, d, $J$ = 2.0 Hz), 8.21–8.31 (3H, m), 8.47 (1H, s), 9.12 (1H, d, $J$ = 1.9 Hz); FAB MS $m/e$ $(M+H)^+$ 461.

**5.1.26. (2-{[4-(6-Chloroimidazo[1,2-*a*]pyridin-3-yl)-1,3-thiazol-2-yl]thio}-4-nitrophenyl)methanol hydrochloride (14a).** To a suspension of **13** (4.7 g, 10 mmol) in $CH_2Cl_2$ (50 mL) was added dropwise 1 M DIBAL-H/toluene (50 mL) below 5 °C and the mixture was stirred at room temperature for 1 h. The reaction was quenched with MeOH and diluted with saturated aqueous $NaHCO_3$ and $CHCl_3$. After filtration through a bed of Celite, the organics of the filtrate was dried over $MgSO_4$ and concentrated. The residue was subjected to column chromatography on silica gel ($CHCl_3$/MeOH = 100:1) to give free base of **14a** (2.0 g, 47%) as a pale yellow solid. The free base of **14a** (250 mg, 0.60 mmol) was dissolved with a mixture of MeOH (40 mL) and $CHCl_3$ (40 mL). To the solution was added 4 N HCl/AcOEt (0.3 mL) and the mixture was evaporated. The resulting solid was washed with EtOH and recrystallized from MeOH to give HCl salt of **14a** (122 mg, 45%) as a colorless solid. Mp: 193–194 °C; $^1$H NMR (DMSO-$d_6$) $\delta$: 4.78 (2H, s), 7.83 (1H, dd, $J$ = 2.0. 8.8 Hz), 7.94–8.01 (2H, m), 8.36 (1H, s), 8.46 (1H, dd, $J$ = 2.5, 8.3 Hz), 8.52 (1H, d, $J$ = 2.4 Hz), 8.63 (1H, s), 9.12–9.16 (1H, m); FAB MS $m/e$ $(M)^+$ 418; Anal. Calcd for $C_{17}H_{11}N_4O_3S_2ClHCl$: C, 44.84; H, 2.66; N, 12.30; S, 14.08; Cl, 15.57. Found: C, 44.90; H, 2.67; N, 12.02; S, 14.12; Cl, 15.48.

**5.1.27. (2-{[4-(6-Chloroimidazo[1,2-*a*]pyridin-3-yl)-1,3-thiazol-2-yl]sulfinyl}-4-nitrophenyl)methanol hydrochloride (14b).** To a suspension of free base of **14a** (115 mg, 0.275 mmol) in $CH_2Cl_2$ (15 mL) was added 70–75% *m*-CPBA (180 mg). After stirring for 1 h, $CHCl_3$ (20 mL) was added and the resulting solid was collected. The obtained solid was dissolved with THF (50 mL), MeOH (20 mL), and $CHCl_3$ (20 mL). To the solution was added 4 N HCl/AcOEt (40 μL) and the mixture was evaporated. The resulting solid was recrystallized from MeOH/$Et_2O$ to give HCl salt of **14b** (45 mg, 35%) as a colorless solid. mp: 198–200 °C; $^1$H NMR (DMSO-$d_6$) $\delta$: 4.86–5.00 (2H, m), 7.80–8.00 (3H, m), 8.42–8.50 (1H, m), 8.61 (1H, s), 8.66 (1H, s), 8.78 (1H, d, $J$ = 2.4 Hz), 9.16–9.20 (1H, m); FAB MS $m/e$ $(M)^+$ 434; Anal. Calcd for $C_{17}H_{11}N_4O_4S_2ClHCl$: C, 43.32; H, 2.57; N, 11.89; S, 13.61; Cl, 15.04. Found: C, 43.01; H, 2.48; N, 11.67; S, 13.57; Cl, 14.86.

**5.1.28. 6-Chloro-3-{2-[(2-methyl-5-nitrophenyl)sulfinyl]-1,3-thiazol-4-yl}imidazo[1,2-*a*]pyridine hydrochloride (15).** To a suspension of free base of **14b** (360 mg, 0.83 mmol) in THF (15 mL) were added $Et_3N$ (300 mg, 3.0 mmol) and methanesulfonyl chloride (170 mg, 1.5 mmol). After stirring for 0.5 h, DMSO (15 mL) and $NaBH_4$ (200 mg, 5.3 mmol) were added to the reaction mixture. After stirring for 0.5 h, the mixture was evaporated and diluted with AcOEt and brine. After separation, the organic layer was dried over $MgSO_4$ and evaporated. The residue was purified with column chromatography on silica gel ($CHCl_3$/MeOH = 200:1). The obtained solid was dissolved with a mixture of $CHCl_3$ (100 mL), THF (50 mL), and MeOH (50 mL). To the solution was added 4 N HCl/AcOEt (0.1 mL) and the mixture was evaporated. The resulting solid was recrystallized from MeOH/$Et_2O$ to give HCl salt of **15** (49 mg, 13%) as a colorless solid. Mp: 204–206 °C; $^1$H NMR (DMSO-$d_6$) $\delta$: 2.75 (3H, s), 7.73–7.82 (2H, m), 7.95 (1H, d, $J$ = 9.8 Hz), 8.36 (1H, dd, $J$ = 2.4, 8.3 Hz), 8.57 (1H, s), 8.61 (1H, d, $J$ = 2.4 Hz), 8.67 (1H, s), 9.12–9.16 (1H, m); FAB MS $m/e$ $(M+H)^+$ 419; Anal. Calcd for $C_{17}H_{11}N_4O_3S_2ClHCl$: C, 44.84; H, 2.66; N, 12.30; S, 14.08; Cl, 15.57. Found: C, 44.86; H, 2.76; N, 12.38; S, 14.09; Cl, 15.28.

### 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, and 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 $CaCl_2$, and 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, and the mixture was left to stand for 5 min and then centrifuged at 300*g* for 2 min. The radioactivity was measured using TopCount (Packard). $IC_{50}$ values are given as means of at least two separate determinations with typical variations of less than ±20%.

### 5.3. In vitro proliferation assays (A375, HeLa cells)

Cells were cultured in DMEM with 10% fetal bovine serum and 1% streptomycin/penicillin. The test compound in a volume of 1 μL was spotted onto a 96-well culture plate followed by addition of cells ($1 \times 10^4$) up to a volume of 100 μL. After incubation for 46 h, 10 μL of Alamar Blue reagent was added to each well, and after a

further 2 h absorption was measured using Fluostar at excitation/emission wavelengths of 544/590 nm. The reported $IC_{50}$ values are means of at least two separate determinations with typical variations of less than ±20%.

### 5.4. Xenografts

HeLa cells ($5 \times 10^6$) were subcutaneously injected into the hind quarters of female Balb/c-nu/nu mice. The group receiving the compound and the vehicle group each included five animals. Test compound or vehicle was intraperitoneally administered after the volume of the tumor reached about 100 $mm^3$. The tumor volume was calculated by the following formula: $1/2 \times (\text{short diameter})^2 \times (\text{long diameter})$. The test compound was suspended in 20% hydroxypropyl-β-cyclodextrin/saline, with doses calculated as the free base.

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