Received: 10 March 2021 | Revised: 18 April 2021 | Accepted: 19 April 2021

DOI: 10.1002/jccs.202100100

ARTICLE

JOURNAL OF THE CHINESE CHEMICAL SOCIETY

Design, synthesis, and in vitro/vivo anticancer activity of 4-substituted 7-(3-fluoro-4-methoxybenzyl)-7*H*-pyrrolo[2,3-*d*] pyrimidines

Pei-Yi Tsai[1] | **Gong-Siang Hu**[2] | **Po-Hsun Huang**[2] | **Huei-Lin Jheng**[1] | **Chi-Hsuan Lan**[2,3] | **You-Sin Chen**[2,3] | **Jia-Ming Chang**[1] | **Shih-Hsien Chuang**[1] | **Jiann-Jyh Huang**[2,3]

[1]Development Center for Biotechnology, National Biotechnology Research Park, Taipei City, Taiwan

[2]Department of Applied Chemistry, National Chiayi University, Chiayi City, Taiwan

[3]The Training and Research Institute of Food and Agriculture, National Chiayi University, Chiayi City, Taiwan

Correspondence
Jiann-Jyh Huang, Department of Applied Chemistry, National Chiayi University, No. 300, Syuefu Road, Chiayi City 60004, Taiwan.
Email: lukehuang@mail.ncyu.edu.tw

Funding information
Ministry of Science and Technology of R.O.C., Grant/Award Number: 105-2113-M-415-001

Abstract

In this paper, we report the design and synthesis of 4-substituted 7-(3-fluoro-4-methoxybenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidines of scaffold **6** as anticancer agents. A total of 19 derivatives of scaffold **6** bearing a C-4 alkoxy, dialkylamino, alkyl, vinyl, or phenyl substituent were synthesized and evaluated. Among them, compound **6q** having a C-4 ethyl group and a benzylic methyl group showed the most potent in vitro anticancer activity, inhibiting the proliferation of Hela, MDA-MB-231, and MDA-MB-426 cancer cells at submicromolar concentrations (GI_{50}: 0.11–0.58 μM). Compound **6q** arrested the cell cycle of MDA-MB-231 at G_2/M phase, and showed in vivo activity on nude mice bearing MDA-MB-231 xenografts. Compound **6q** has served as an anticancer lead for further optimization.

KEYWORDS

anticancer, colchicine, combretastatin, ottelione A, pyrrolopyrimidine, tubulin

1 | INTRODUCTION

Ottelione A (**1**, Figure 1), isolated from *Ottelia alismoides* collected in the Nile Delta,[1] is a *cis*-hydrindanone featuring a carbonyl-conjugated diene and a 3-hydroxyl-4-methoxybenzyl group. Compound **1** shows extremely potent anticancer activity, which can inhibit the proliferation of NCI-60 cancer cells at picomolar to nanomolar concentrations.[1,2] By binding to colchicine-binding site to inhibit tubulin polymerization,[1,2] the mechanism of action of **1** is similar to that of colchicine (**3**)[3] and combretastatin A-4 (**4**).[4] Combretastatin A-4 phosphate (**5**), a prodrug of **4** classified as a vascular disrupting agent,[5] is currently investigated in phase-III clinical trials for the treatment of cancers.

Due to the promising anticancer activity, various synthetic methods have been reported for the preparation of **1**[6–15] and are comprehensively reviewed.[16] Our group has demonstrated a radical cyclization methodology for the enantioselective total synthesis for **1**.[13] In the following study,[17] we established the structure–activity relationship of **1** using the synthetic methodology. Compound **2**, the desvinyl and fluoro analog of **1**, was generated and found 6–38-fold more potent than **1**. Inhibition of colchicine binding and tubulin polymerization was observed for **2** in biochemical assays, which suggested that **2** also bound to colchicine-binding site similar to **1** and **3**–**5**. In addition, our study demonstrated that the exocyclic double bond was essential for the potency of **1** and **2**.[17]

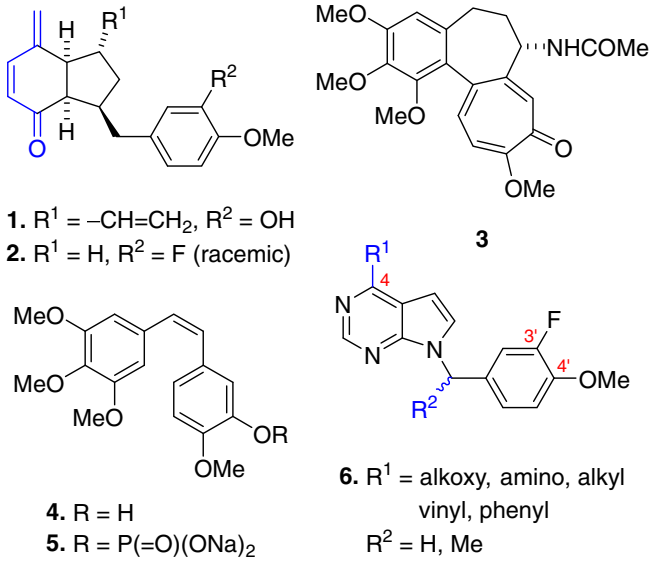
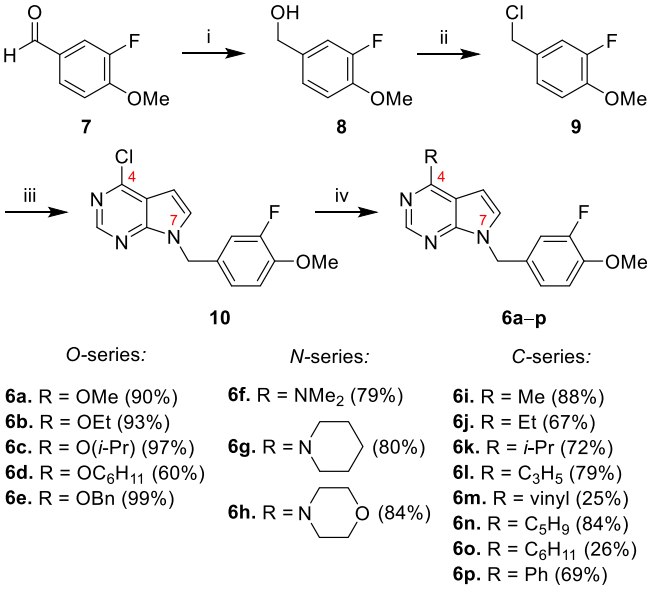


FIGURE 1 Structures of ottelione A (**1**), ottelione A synthetic derivative **2**, colchicine (**3**), combretastatin A-4 (**4**), combretastatin A-4 phosphate (**5**), and 4-substituted 7-(3-fluoro-4-methoxybenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidines **6** reported in this study



SCHEME 1 Synthesis of **6a**–**p** bearing a C-4 alkoxy, dialkylamino, alkyl, vinyl, or phenyl substituent. Reagents and conditions: (i) $NaBH_4$, MeOH, 93%. (ii) $SOCl_2$, Et_2O, 95%. (iii) 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine, NaH, DMF, 93%. (iv) For **6a**–**c**: KOH, alcohol, 70 °C; for **6d**: KOH, cyclohexanol, dioxane, 70 °C; for **6e**: NaH, BnOH, THF, 0 °C; for **6f**–**h**: dialkylamine, EtOH, 70 °C; for **6i**–**l** and **6n**–**p**: RMgBr, $PdCl_2$(dppf), THF; for **6m**: $Bu_3SnCH{=}CH_2$, $Pd(PPh_3)_2Cl_2$, LiCl, DMF, 100 °C

An irreversible nucleophilic 1,6-addition would be responsible for their potency.[18,19] Although both **1** and **2** show potent in vitro activity, they might not be active in vivo due to their inherent instability in serum. However, the structural information of **2** suggested that 7-(3-fluoro-4-methoxybenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine of scaffold **6** (a pyrrolopyrimidine, Figure 1) having a C-4 alkoxy, amino, alkyl, vinyl, and phenyl group could mimic the structure of **2** to inhibit tubulin polymerization. Several pyrrolopyrimidines[20,21] and indoles[22] structurally similar to **2** have been reported to have anticancer activity. For scaffold **6**, a methyl group at the benzylic position could create steric hindrance and direct the benzyl group to the different positions. Enhancement of anticancer activity might be observed.

Herein, we report the synthesis and biological study for the pyrrolopyrimidines of scaffold **6**. We first screened various C-4 substituents, which could provide the anticancer activity of **6**, and then explored the effect of benzylic methyl on the potency of **6**. Our study eventually led to **6q** having a C-4 ethyl group and a benzylic methyl group that showed submicromolar in vitro anticancer activity. The effect of **6q** on the cell cycle of cancer cells was studied to predict its possible mechanism of action. Finally, the in vivo activity of **6q** was investigated to evaluate the potential of **6q** as an anticancer lead for further optimization.

2 | RESULTS AND DISCUSSION

2.1 | Synthesis of 4-substituted 7-(3-fluoro-4-methoxybenzyl)-7*H*-pyrrolo [2,3-d]pyrimidines 6a–s

Scheme 1 presents the synthesis of **6a**–**p** bearing a C-4 alkoxy (*O*-series **6a**–**e**), dialkylamino (*N*-series **6f**–**h**), or alkyl/vinyl/phenyl (*C*-series **6i**–**p**) substituent. 3-Fluoro-4-methoxybenzaldehyde (**7**) served as the starting material and was reduced by $NaBH_4$. The reaction gave benzyl alcohol **8** in 93% yield. The hydroxyl group in **8** was transformed into a chloro group using $SOCl_2$, providing benzyl chloride **9** in 95% yield. Alkylation of 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine by **9** afforded 4-chloropyrrolopyrimidine **10** in 93% yield. To prepare **6a**–**e** (*O*-series), **10** was reacted with the corresponding alcohol to replace its C-4 chloro group. The reaction of **10** with KOH in MeOH, EtOH, or *i*-PrOH at 70 °C gave **6a**–**c** in 90–97% yields. Heating **10** with cyclohexanol and KOH in dioxane provided **6d** in 60% yield. Compound **6e** having a 4-benzyloxy substituent was obtained in 99% yield from the reaction of **10** with BnOH and NaH in THF.

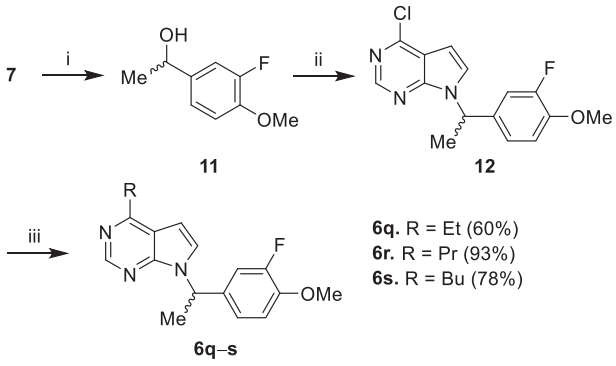
To prepare **6f**–**h** having a C-4 dialklylamino group (*N*-series), **10** was heated with dimethylamine, piperidine, or

morpholine in EtOH. The reaction gave the corresponding **6f**–**h** in 79–84% yields. The reaction of **10** with Grignard reagents in the presence of catalytic $PdCl_2(dppf)$[23] (Kumada coupling) gave the corresponding **6i**–**l** and **6n**–**p** bearing an alkyl or a phenyl group in 26–88% yields. For C-4 vinyl substituted **6m**, **10** was coupled with tributyl(vinyl)tin by Stille coupling[24] using $Pd(PPh_3)_2Cl_2$/LiCl/DMF catalytic system. The reaction gave **6m** in 25% yield.

Scheme 2 illustrates the synthesis of C-4 alkyl substituted pyrrolopyrimidines **6q**–**s** featuring a benzylic methyl substituent. Aldehyde **7** (Scheme 1) also served as the starting material, which was reacted with methylmagnesium bromide to give secondary alcohol **11** in 96% yield. The hydroxyl group in **11** was converted to a chloro group using $SOCl_2$, and the generated benzyl chloride alkylated 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine by NaH in DMF. The two consecutive reactions gave 4-chloropyrrolopyrimidine **12** in 23% total yield. Coupling of **12** with Grignard reagents gave the corresponding racemic **6q**–**s** having a C-4 ethyl, propyl, or butyl group in 60–93% yields.

2.2 | Potency of 6a–p on the cell viability of Hela, MDA-MB-231, and MDA-MB-468

We first studied the potency of **6a**–**p** on the cell viability on Hela (cervical), MDA-MB-231 (breast), and MDA-MB-468 (breast) cancer cells at the concentration of 1.0 μM after a 96-hr incubation. The results are summarized in Figure 2. For 4-alkoxy derivatives **6a**–**d** having a OMe, OEt, O(*i*-Pr), and OC_6H_{11} substituent, the viability of the three cells was not reduced significantly (71–100%). Although not potent, **6a**–**d** were more active for Hela (viability 71–97%) than MDA-MB-231 (94–100%) and MDA-MB-468 (82–99%). Compound **6e** with a C-4 OBn substituent was the most potent analog among **6a**–**e** to inhibit Hela (viability: 63%). However, **6e** was inactive for MDA-MB-231 (105% viability) and MDA-MB-468 (92% viability). Compounds **6a**–**e** having a C-4 alkoxy group were not further derived as they did not reduce the viability of the three cells to below 50%.



SCHEME 2 Synthesis of **6q**–**s** bearing a C-4 alkyl group and a benzylic methyl group. Reagents and conditions: (i) MeMgBr, THF, 96%. (ii) (a) $SOCl_2$, Et_2O; (b) 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine, NaH, DMF, 23% (two steps). (iii) RMgBr, $PdCl_2(dppf)$, THF

We then evaluated the potency of C-4 dialkylamino pyrrolopyrimidines **6f** (dimethylamino), **6g** (piperidinyl), and **6h** (morpholino) (Scheme 1 and Figure 2). Unfortunately, they showed marginal effect on the cell viability (90–100%) of the three cancer cells. Considering the inactivity of C-4 alkoxy and dialkylamino derivatives **6a**–**h**, we synthesized and evaluated **6i**–**p** that have a C-4 alkyl, vinyl, or phenyl substituent. Compound **6i** having a C-4 methyl substituent did not reduce the viability of the three cancer cells to below 50%, though it was more active for Hela (viability: 65%). On the other hand, **6j** with a C-4 ethyl group showed greatly improved potency, reducing the viability of Hela, MDA-MB-231, and MDA-MB-468 to 22, 38, and 13%, respectively. Increasing the size of the C-4 substituent of **6j** to isopropyl (**6k**), cyclopropyl (**6l**), vinyl (**6m**), cyclohexyl (**6o**), and phenyl (**6p**) deactivated **6k**–**m**, **6o**, and **6p** (viability: 67–99%), except that **6n** having a cyclopentyl was active for MDA-MB-468 (viability: 23%). These results indicated that the C-4 position of **6** only tolerates a narrow range of substituents. Only ethyl group provided **6j** with notable anticancer activity for Hela, MDA-MB-231, and MDA-MB-468 (GI_{50}: <1.0 μM).

As a result, the in vitro GI_{50} values of **6j** were measured (Table 1). Compound **6j** inhibited Hela, MDA-MB-231, and MDA-MB-468 with GI_{50} of 0.81, 0.40, and 0.25 μM, respectively. As the benzyl group in **1** and **2** (Figure 1) should be more distant from the plane of fused cyclohexenone–cyclopentane moiety, we synthesized and evaluated **6q**, the benzylic methylated analog of **6j**. Compound **6q** showed around twofold increased potency (GI_{50}: 0.11–0.58 μM) compared to **6j** (GI_{50}: 0.25–0.81 μM). Nevertheless, increasing the size of the C-4 alkyl in **6q** did not improve the potency: **6r** with a propyl group and **6s** with a butyl group have GI_{50} of 1.2–2.9 and 0.52–3.0 μM, respectively. Unfortunately, the more potent analogs **6q**–**s** from this study were less active than **3** (GI_{50}: 0.082–0.21 μM, Table 1).

2.3 | Cell cycle study of 6q on MDA-MB-231 using flow cytometry

As **6q** had the most potent in vitro anticancer activity for the three cancer cells (GI_{50}: 0.11–0.58 μM, Table 1), its effect on the cell cycle of MDA-MB-231 was explored

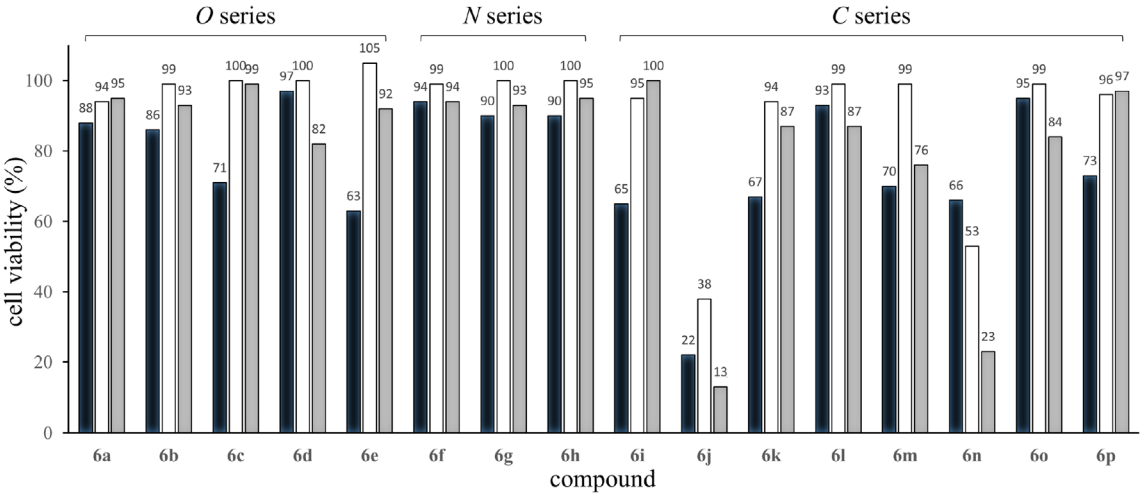
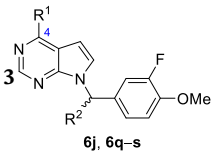


FIGURE 2 Cell viability of Hela (black), MDA-MB-231 (white), and MDA-MB-468 (gray) cancer cells treated with **6a–p** at the concentration of 1.0 μM for 96 hr

TABLE 1 In vitro anticancer activity of **6j**, **6q–s**, and **3**



6j, **6q–s**

Compd	R^1	R^2	In vitro anticancer GI_{50} (μM)		
			Hela	MDA-MB-231	MDA-MB-468
6j	Et	H	0.81	0.40	0.25
6q	Et	Me	0.58	0.20	0.11
6r	Pr	Me	1.2	2.9	1.7
6s	Bu	Me	3.0	0.52	0.69
3	—	—	0.21	0.10	0.082

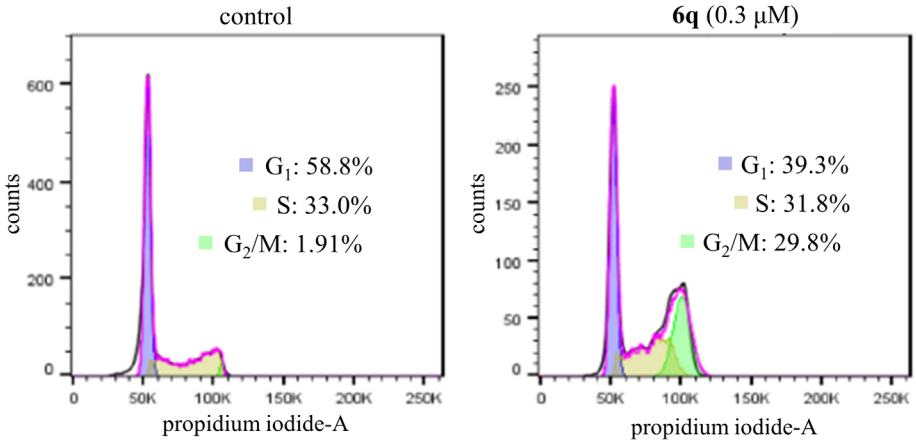


FIGURE 3 Cell cycle profile of MDA-MB-231 cells treated with 0.3 μM of **6q** for 24 hr

using flow cytometry (Figure 3). Compared to control, **6q** arrested the cell cycle at the G_2/M phase at the concentration of 0.3 μM after a 24-hr treatment. The population of cells at the G_2/M phase increased from 1.91 to 29.8%, while the population at the G_1 phase decreased from 58.8 to 39.3%. Similar populations of cells at the S phase were observed in control (33.0%) and treatment (31.8%) groups. The effect of **6q** on cell cycle was similar to that of **3**[25,26] and **4**.[27,28] The results suggested that **6q** worked on the G_2/M phase to show its anticancer activity, possible due

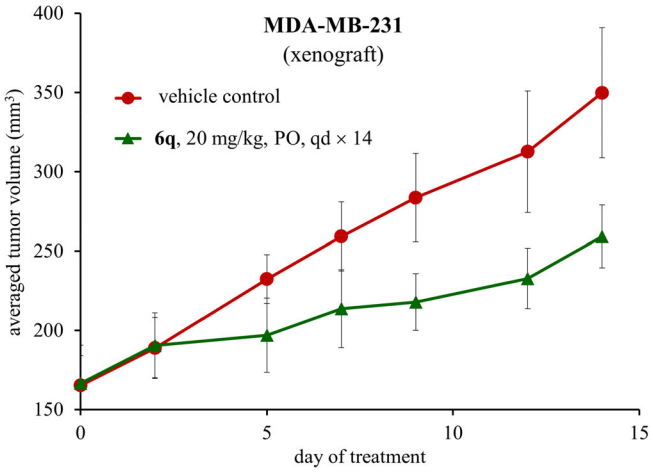


FIGURE 4 In vivo anticancer activity of **6q**. The tumor growth inhibition (1 − T/C) for **6q** at day 14 was 27.6%

to inhibiting tubulin polymerization by binding to the colchicine binding site.

2.4 | In vivo anticancer activity of 6q

To assess the potential of **6q** as an anticancer lead for further optimization, a pilot in vivo study for **6q** was conducted on nude mice bearing human MDA-MB-231 xenografts. The result is shown in Figure 4. Compound **6q** showed moderate tumor growth inhibition when administered PO once per day at a dose of 20 mg/kg for 14 consecutive days. The tumor growth inhibition (1 − T/C) was 27.6% at day 14. No significant weight loss was observed in mice administered with **6q** (compared to vehicle). Although the result from in vivo study excluded **6q** to enter development phase, it suggested that the scaffold of **6q** has the potential to be optimized as an orally administered drug. The ongoing study carried out in our group is to separate the two enantiomers of **6q** and derive the unexplored C-3 position.

3 | CONCLUSIONS

We designed and synthesized a series of 4-substituted 7-(3-fluoro-4-methoxybenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidines **6a–s** as anticancer agents. Among them, **6q** having a C-4 ethyl and a benzylic methyl was the most potent, which inhibited the proliferation of Hela, MDA-MB-231, and MDA-MB-468 cancers cells at submicromolar concentrations. Compound **6q** arrested the cell cycle of MDA-MB-231 at G_2/M phase and was active in vivo. It has served as a lead compound for further optimization.

4 | EXPERIMENTAL

4.1 | General

Starting materials and reagents were used as purchased without further purification. Column chromatography was performed on Merck Reagents Silica Gel 60 (particle size of 0.063–0.200 mm, 70–230 mesh ASTM) using EtOAc and hexanes as the eluent. Proton (400 MHz) and carbon-13 (100 MHz) NMR spectra were obtained on an Agilent 400-MR NMR spectrometer using $CDCl_3$ as the solvent. EI-MS were recorded on an Agilent 5973 mass spectrometer detector coupled with an Agilent 6890 Series GC System. High-resolution mass spectra were obtained on an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific). Compounds **6q–s** were found not optically active by means of Jasco P-1010 polarimeter.

4.2 | (3-Fluoro-4-methoxyphenyl) methanol (8)

A solution of 3-fluoro-4-methoxybenzaldehyde (**7**, 1.183 g, 7.675 mmol, 1.0 equiv) in MeOH (15 ml) was slowly added with $NaBH_4$ (0.4350 g, 11.50 mmol, 1.5 equiv) in an ice bath. The reaction mixture was stirred at room temperature for 60 min. The solution was concentrated under reduced pressure and the residue was redissolved in EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography to give **8** (1.112 g, 7.121 mmol) in 93% yield: ^{1}H NMR δ 7.08 (d, J = 12 Hz, 1 H), 7.03 (d, J = 8.5 Hz, 1 H), 6.92 (t, J = 8.5 Hz, 1 H), 4.57 (s, 2 H), 3.87 (s, 3 H), 2.11 (brs, 1 H); ^{13}C NMR δ 152.3 (d, J = 244.7 Hz), 147.0 (d, J = 10.5 Hz), 133.9 (d, J = 5.0 Hz), 122.7 (d, J = 4.0 Hz), 115.0 (d, J = 18.3 Hz), 113.3, 64.4, 56.3; EI-MS: m/z 156 (M^+, 100), 155 (44), 139 (50), 135 (16), 127 (70), 125 (51), 112 (43), 97 (28), 96 (17), 77 (21).

4.3 | 4-(Chloromethyl)-2-fluoro-1-methoxybenzene (9)

A solution of **8** (3.013 g, 19.30 mmol, 1.0 equiv) in anhydrous Et_2O (64 ml) was slowly added with $SOCl_2$ (2.8 ml, 38 mmol, 2.0 equiv) in an ice bath. The reaction mixture was stirred at room temperature for 3.0 hr. The solution was slowly poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography to

give **9** (3.197 g, 18.31 mmol) in 95% yield: ^{1}H NMR δ 7.13 (d, J = 11.7 Hz, 1 H), 7.09 (d, J = 8.5 Hz, 1 H), 6.92 (t, J = 8.5 Hz, 1 H), 4.53 (s, 2 H), 3.89 (s, 3 H); ^{13}C NMR δ 152.1 (d, J = 245.5 Hz), 147.5 (d, J = 10.5 Hz), 130.3 (d, J = 7.0 Hz), 124.6 (d, J = 3.0 Hz), 116.5 (d, J = 18.8 Hz), 113.2 (d, J = 2.1 Hz), 56.2, 45.5; EI-MS: m/z 176 (34), 174 (M^+, 91), 140 (47), 139 (100), 107 (22), 105 (22), 96 (64), 95 (36), 77 (52), 75 (23).

4.4 | 1-(3-Fluoro-4-methoxyphenyl) ethan-1-ol (11)

A solution of **7** (0.566 g, 3.67 mmol, 1.0 equiv) in THF (12 ml) was added with MeMgBr (11.0 ml, 1.0 M in THF, 11.0 mmol, 3.0 equiv) in a period of 10 min at 0 °C. The reaction mixture was stirred at room temperature for 1.0 hr. The solution was quenched with H_2O and extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography to give **11** (0.603 g, 3.54 mmol) in 96% yield: ^{1}H NMR δ 7.09 (d, J = 12.3 Hz, 1 H), 7.04 (d, J = 8.5 Hz, 1 H), 6.90 (t, J = 8.5 Hz, 1 H), 4.80 (q, J = 6.4 Hz, 1 H), 3.86 (s, 3 H), 2.10 (brs, 1 H), 1.44 (d, J = 6.4 Hz, 3 H); ^{13}C NMR δ 152.3 (d, J = 244.0 Hz), 146.7 (d, J = 10.8 Hz), 138.9 (d, J = 5.3 Hz), 121.0 (d, J = 3.5 Hz), 113.3, 113.2, 69.4, 56.3, 25.0; EI-MS: m/z 170 (M^+, 78), 156 (23), 155 (100), 153 (30), 127 (93), 112 (68), 109 (13), 95 (16), 83 (18), 77 (15).

4.5 | 4-Chloro-7-(3-fluoro-4-methoxybenzyl)-7*H*-pyrrolo[2,3-*d*] pyrimidine (10)

A solution of 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (1.276 g, 8.150 mmol, 1.03 equiv) in DMF (16 ml) was slowly added with NaH (0.351 g, 8.78 mmol, 1.1 equiv) in an ice bath. The solution was then added with **9** (1.377 g, 7.887 mmol, 1.0 equiv), and the resulting reaction mixture was stirred at room temperature for 12 hr. The solution was quenched with H_2O and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography to give **10** (2.147 g, 7.360 mmol) in 93% yield: ^{1}H NMR δ 8.66 (s, 1 H), 7.20 (d, J = 3.6 Hz, 1 H), 7.00–6.93 (m, 2 H), 6.90 (t, J = 8.2 Hz, 1 H), 6.62 (d, J = 3.6 Hz, 1 H), 5.37 (s, 2 H), 3.85 (s, 3 H); ^{13}C NMR δ 152.3 (d, J = 246.1 Hz), 152.2, 151.0, 150.9, 147.6 (d, J = 10.5 Hz), 129.0 (d, J = 6.0 Hz), 128.8, 123.6 (d, J = 4.0 Hz), 117.5, 115.6 (d, J = 18.9 Hz), 113.5 (d, J = 1.0 Hz), 100.1, 56.2, 47.6; HRMS calcd for $[C_{14}H_{11}ClFN_3O + H]^+$: 292.0647, found 292.0636.

4.6 | 4-Chloro-7-[1-(3-fluoro-4-methoxyphenyl)ethyl]-7*H*-pyrrolo[2,3-*d*] pyrimidine (12)

Compound **12** was prepared by the identical method for **9** to **10** using **11** as the starting material. Yield: 23%; ^{1}H NMR δ 8.63 (s, 1 H), 7.24 (d, J = 3.6 Hz, 1 H), 7.01–6.97 (m, 2 H), 6.91–6.87 (m, 1 H), 6.61 (d, J = 3.6 Hz, 1 H), 6.13 (q, J = 7.1 Hz, 1 H), 3.84 (s, 3 H), 1.87 (d, J = 7.2 Hz, 3 H); ^{13}C NMR δ 152.5 (d, J = 142.0 Hz), 150.8, 150.5, 150.4, 147.1 (d, J = 10.5 Hz), 133.4 (d, J = 5.6 Hz), 126.3, 122.2 (d, J = 3.6 Hz), 117.4, 114.2 (d, J = 19.0 Hz), 113.2 (d, J = 2.1 Hz), 99.7, 56.0, 52.2, 20.2; HRMS calcd for $[C_{15}H_{13}ClFN_3O + H]^+$: 306.0804, found 306.0791.

4.7 | Standard procedure for the synthesis of 6a–e having a C-4 alkoxy substituent

For **6a–c**, **10** (~150 mg, 1.0 equiv) and NaOH (6.0 equiv) were mixed in MeOH, EtOH, or *i*-PrOH (5.0 ml). For **6d**, **10** (~150 mg, 1.0 equiv) was mixed with KOH (2.0 equiv) and cyclohexanol (1.1 equiv) in dioxane. For **6e**, **10** (~150 mg, 1.0 equiv) in THF (2.6 ml) was added with NaH (3.0 equiv) and BnOH (1.2 equiv) at 0 °C. For **6a–d**, the reaction mixture was heated at 70 °C for 12 hr. For **6e**, the reaction mixture was stirred at room temperature for 4.0 hr. The solution was quenched with H_2O and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography to give the target **6a–e** in 60–99% yields.

4.8 | 7-(3-Fluoro-4-methoxybenzyl)-4-methoxy-7*H*-pyrrolo[2,3-*d*] pyrimidine (6a)

Yield: 90%; ^{1}H NMR δ 8.50 (s, 1 H), 6.99 (d, J = 3.6 Hz, 1 H), 6.95–6.86 (m, 3 H), 6.54 (d, J = 3.6 Hz, 1 H), 5.34 (s, 2 H), 4.12 (s, 3 H), 3.84 (s, 3 H); ^{13}C NMR δ 162.9, 152.2 (d, J = 245.7 Hz), 151.6, 150.9, 147.1 (d, J = 10.6 Hz), 129.9 (d, J = 6.0 Hz), 125.4, 123.2 (d, J = 3.0 Hz), 115.2 (d, J = 18.7 Hz), 113.3 (d, J = 2.0 Hz), 105.3, 98.8, 56.1, 53.5, 47.2; HRMS calcd for $[C_{15}H_{14}FN_3O_2 + H]^+$: 288.1143, found 288.1141.

4.9 | 4-Ethoxy-7-(3-fluoro-4-methoxybenzyl)-7*H*-pyrrolo[2,3-*d*] pyrimidine (6b)

Yield: 93%; ^{1}H NMR δ 8.50 (s, 1 H), 7.01 (d, J = 4.0 Hz, 1 H), 6.97–6.87 (m, 3 H), 6.58 (d, J = 4.0 Hz, 1 H), 5.37 (s, 2 H), 4.62 (q, J = 7.1 Hz, 2 H), 3.85 (s, 3 H), 1.50–4.60 (t, J = 7.1 Hz, 3 H); ^{13}C NMR δ 162.7, 153.5, 151.7, 151.0, 147.2 (d, J = 10.6 Hz), 130.0 (d, J = 6.0 Hz), 125.3, 123.2 (d, J = 4.0 Hz), 115.3 (d, J = 18.8 Hz), 113.3 (d, J = 2.0 Hz), 105.4, 99.0, 62.2, 56.1, 47.2, 14.5; HRMS calcd for $[C_{16}H_{16}FN_3O_2 + H]^+$: 302.1299, found 302.1299.

4.10 | 7-(3-Fluoro-4-methoxybenzyl)-4-isopropoxy-7*H*-pyrrolo[2,3-*d*] pyrimidine (6c)

Yield: 97%; ^{1}H NMR δ 8.46 (s, 1 H), 6.97–6.86 (m, 4 H), 6.54 (d, J = 3.6 Hz, 1 H), 5.58 (sept, J = 6.3 Hz, 1 H), 5.33 (s, 2 H), 3.85 (s, 3 H), 1.43 (d, J = 6.3 Hz, 6 H); ^{13}C NMR δ 162.5, 152.3 (d, J = 245.4 Hz), 151.8, 151.1, 147.2 (d, J = 10.6 Hz), 130.1 (d, J = 5.9 Hz), 125.2, 123.3 (d, J = 3.6 Hz), 115.3 (d, J = 18.7 Hz), 113.4 (d, J = 2.0 Hz), 105.7, 99.1, 69.0, 56.1, 47.2, 22.1; HRMS calcd for $[C_{17}H_{18}FN_3O_2 + H]^+$: 316.1456, found 316.1455.

4.11 | 4-(Cyclohexyloxy)-7-(3-fluoro-4-methoxybenzyl)-7*H*-pyrrolo[2,3-*d*] pyrimidine (6d)

Yield: 60%; ^{1}H NMR δ 8.46 (s, 1 H), 6.97–6.86 (m, 4 H), 6.54 (d, J = 3.6 Hz, 1 Hz), 5.35–5.30 (m, 3 H), 3.84 (s, 3 H), 2.12–2.02 (m, 2 H), 1.58–1.79 (m, 2 H), 1.66–1.57 (m, 2 H), 1.52–1.42 (m, 2 H), 1.34–1.28 (m, 2 H); ^{13}C NMR δ 162.5, 152.3 (d, J = 245.5 Hz), 151.8, 151.1, 147.2 (d, J = 10.6 Hz), 130.1 (d, J = 5.9 Hz), 125.1, 123.3 (d, J = 3.6 Hz), 115.3 (d, J = 18.8 Hz), 113.4 (d, J = 2.0 Hz), 105.8, 99.2, 74.0, 56.2, 47.2, 31.8, 25.6, 23.9; HRMS calcd for $[C_{20}H_{22}FN_3O_2 + H]^+$: 356.1769, found 356.1766.

4.12 | 4-(Benzyloxy)-7-(3-fluoro-4-methoxybenzyl)-7*H*-pyrrolo[2,3-*d*] pyrimidine (6e)

Yield: 99%; ^{1}H NMR δ 8.51 (s, 1 H), 7.52–7.51 (m, 2 H), 7.41–7.32 (m, 4 H), 7.00 (d, J = 3.6 Hz, 1 H), 6.96–6.86 (m, 3 H), 6.58 (d, J = 3.6 Hz, 1 H), 5.60 (s, 2 H), 5.35 (s, 2 H), 3.85 (s, 2 H); ^{13}C NMR δ 162.2, 152.0 (d, J = 245.5 Hz), 151.6, 150.7, 146.9 (d, J = 10.5 Hz), 136.5, 129.7 (d, J = 5.8 Hz), 128.2, 127.8, 127.7, 125.3, 123.1 (d, J = 3.4 Hz), 115.1 (d, J = 18.6 Hz), 113.1, 105.2, 98.7, 67.5, 55.7, 47.0; HRMS calcd for $[C_{21}H_{18}FN_3O_2 + H]^+$: 364.1456, found 364.1453.

4.13 | Standard procedure for the synthesis of 6f–h having a C-4 dialkylamino substituent

A solution of **10** (∼150 mg, 1.0 equiv) in EtOH (1.5 ml) was added with dimethylamine, piperidine, or morpholine (1.5 equiv). The solution was heated at 70 °C for 8.0 hr. The solution was quenched with H_2O and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography to give the target **6f–h** in 79–84% yields.

4.14 | 7-(3-Fluoro-4-methoxybenzyl)-*N*,*N*-dimethyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (6f)

Yield: 79%; ^{1}H NMR δ 8.40 (s, 1 H), 6.96–6.86 (m, 4 H), 6.63 (d, J = 3.6 Hz, 1 H), 5.32 (s, 2 H), 3.85 (s, 3 H), 3.44 (s, 6 H); ^{13}C NMR δ 157.4, 152.2 (d, J = 245.2 Hz), 151.4, 150.6, 147.0 (d, J = 10.6 Hz), 130.4 (d, J = 5.8 Hz), 123.2 (d, J = 3.8 Hz), 122.5, 115.2 (d, J = 18.7 Hz), 113.3 (d, J = 1.9 Hz), 103.0, 102.2, 56.1, 46.9, 39.1; HRMS calcd for $[C_{16}H_{17}FN_4O + H]^+$: 301.1459, found 301.1460.

4.15 | 7-(3-Fluoro-4-methoxybenzyl)-4-(piperidin-1-yl)-7*H*-pyrrolo[2,3-*d*] pyrimidine (6g)

Yield: 80%; ^{1}H NMR δ 8.38 (s, 1 H), 6.97–6.86 (m, 4 H), 6.53 (d, J = 4.0 Hz, 1 H), 5.31 (s, 2 H), 3.96–3.90 (m, 4 H), 3.85 (s, 3 H), 1.79–1.67 (m, 6 H); ^{13}C NMR δ 162.5, 152.2 (d, J = 245.5 Hz), 151.8, 151.1, 147.1 (d, J = 10.6 Hz), 130.1 (d, J = 5.9 Hz), 125.1, 123.2 (d, J = 3.6 Hz), 115.3 (d, J = 18.8 Hz), 113.3 (d, J = 10.7 Hz), 105.8, 99.1, 56.1, 47.2, 31.8, 25.5, 23.8; HRMS calcd for $[C_{19}H_{21}FN_4O + H]^+$: 341.1772, found 341.1772.

4.16 | 4-[7-(3-Fluoro-4-methoxybenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl] morpholine (6h)

Yield: 84%; ^{1}H NMR δ 8.38 (s, 1 H), 6.95–6.86 (m, 4 H), 6.49 (d, J = 3.6 Hz, 1 H), 5.31 (s, 2 H), 3.97–3.94 (m, 4 H),

3.86–3.84 (m, 7 H); ^{13}C NMR δ 157.0, 152.2 (d, J = 245.5 Hz), 151.1, 151.0, 147.1 (d, J = 10.3 Hz), 130.2 (d, J = 5.9 Hz), 123.5, 122.2 (d, J = 3.6 Hz), 115.2 (d, J = 18.8 Hz), 113.3, 103.0, 101.1, 66.6, 56.1, 47.0, 45.8; HRMS calcd for $[C_{18}H_{19}FN_4O_2 + H]^+$: 343.1565, found 343.1564.

4.17 | Standard procedure for the synthesis of 6i–l and 6n–s having a C-4 alkyl or phenyl substituent

Compound **10** or **12** (∼100 mg, 1.0 equiv) was mixed with Pd(dppf)Cl_2 (0.050 equiv) in THF (1.2 ml). The solution was slowly added with the corresponding Grignard reagent (6.0 equiv) at 0 °C under N_2. The reaction mixture was stirred at room temperature for 12 hr. The solution was quenched with H_2O and extracted with EtOAc. The organic layer was washed with saturated aqueous NH_4Cl, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography to give the target **6i–l** and **6n–s** in 25–93% yields.

4.18 | 7-(3-Fluoro-4-methoxybenzyl)-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (6i)

Yield: 94%; ^{1}H NMR δ 8.82 (s, 1 H), 7.21 (d, J = 3.6 Hz, 1 H), 6.98–6.94 (m, 2 H), 6.91–6.87 (m, 1 H), 6.62 (d, J = 3.6 Hz, 1 H), 5.37 (s, 2 H), 3.84 (s, 3 H), 2.82 (s, 3 H); ^{13}C NMR δ 159.0, 152.3 (d, J = 245.8 Hz), 150.8, 150.1, 147.3 (d, J = 10.6 Hz), 129.6 (d, J = 5.8 Hz), 127.3, 123.5 (d, J = 3.6 Hz), 117.7, 115.4 (d, J = 18.7 Hz), 113.4 (d, J = 1.9 Hz), 100.0, 56.2, 47.1, 21.1; HRMS calcd for $[C_{15}H_{14}FN_3O + H]^+$: 272.1194, found 272.1189.

4.19 | 4-Ethyl-7-(3-fluoro-4-methoxybenzyl)-7*H*-pyrrolo[2,3-*d*] pyrimidine (6j)

Yield: 67%; ^{1}H NMR δ 8.97 (s, 1 H), 7.46 (d, J = 4.0 Hz, 1 H), 7.05–7.01 (m, 2 H), 6.95–6.91 (m, 1 H), 6.87 (d, J = 3.6 Hz, 1 H), 5.45 (s, 2 H), 3.86 (s, 3 H), 3.39 (q, J = 7.6 Hz, 2 H), 1.57 (t, J = 7.6 Hz, 3 H); ^{13}C NMR δ 164.0, 152.2 (d, J = 245.6 Hz), 151.3, 150.2, 147.2 (d, J = 10.5 Hz), 129.6 (d, J = 5.8 Hz), 127.4, 123.4 (d, J = 3.5 Hz), 116.8, 115.4 (d, J = 18.8 Hz), 113.3 (d, J = 1.8 Hz), 99.6, 56.1, 47.0, 28.3, 21.8; HRMS calcd for $[C_{16}H_{16}FN_3O + H]^+$: 286.1350, found 286.1335.

4.20 | 7-(3-Fluoro-4-methoxybenzyl)-4-isopropyl-7*H*-pyrrolo[2,3-*d*] pyrimidine (6k)

Yield: 72%; ^{1}H NMR δ 8.77 (s, 1 H), 7.05 (d, J = 3.6 Hz, 1 H), 6.90–6.88 (m, 2 H), 6.80–6.76 (m, 1 H), 6.52 (d, J = 3.6 Hz, 1 H), 5.27 (s, 2 H), 3.73 (s, 3 H), 3.35 (sept, J = 6.8 Hz, 1 H), 1.33 (d, J = 6.8 Hz, 6 H); ^{13}C NMR δ 167.8, 152.2 (d, J = 245.5 Hz), 151.5, 150.4, 147.2 (d, J = 10.6 Hz), 129.8 (d, J = 5.9 Hz), 127.2, 123.5 (d, J = 3.6 Hz), 116.1, 115.4 (d, J = 18.7 Hz), 113.4 (d, J = 1.9 Hz), 99.6, 56.1, 46.9, 33.7, 21.4; HRMS calcd for $[C_{17}H_{18}FN_3O + H]^+$: 300.1507, found 300.1499.

4.21 | 4-Cyclopropyl-7-(3-fluoro-4-methoxybenzyl)-7*H*-pyrrolo[2,3-*d*] pyrimidine (6l)

Yield: 79%; ^{1}H NMR δ 8.71 (s, 1 H), 7.09 (d, J = 3.6 Hz, 2 H), 6.96–6.86 (m, 3 H), 6.65 (d, J = 3.6 Hz, 2 H), 5.35 (s, 2 H), 3.85 (s, 3 H), 2.38–2.32 (m, 1 H), 1.37–1.34 (m, 2 H), 1.18–1.13 (m, 2 H); ^{13}C NMR δ 164.3, 152.2 (d, J = 245.5 Hz), 151.6, 149.6, 147.2 (d, J = 10.6 Hz), 129.9 (d, J = 5.8 Hz), 127.0, 123.3 (d, J = 3.5 Hz), 117.0, 115.3 (d, J = 18.8 Hz), 113.3 (d, J = 2.1 Hz), 99.4, 56.1, 46.9, 14.4, 10.8; HRMS calcd for $[C_{17}H_{16}FN_3O + H]^+$: 298.1350, found 298.1339.

4.22 | 4-Cyclopentyl-7-(3-fluoro-4-methoxybenzyl)-7*H*-pyrrolo[2,3-*d*] pyrimidine (6n)

Yield: 84%; ^{1}H NMR δ 8.83 (s, 1 H), 7.09 (d, J = 3.6 Hz, 2 H), 6.96–6.93 (m, 2 H), 6.89–6.85 (m, 1 H), 6.58 (d, J = 3.6 Hz, 2 H), 5.34 (s, 2 H), 3.83 (s, 3 H), 3.57–3.48 (m, 1 H), 2.15–1.97 (m, 3 H), 1.94–1.85 (m, 2 H), 1.78–1.68 (m, 2 H); ^{13}C NMR δ 166.7, 152.2 (d, J = 245.7 Hz), 151.6, 150.3, 147.2 (d, J = 10.6 Hz), 129.8 (d, J = 5.9 Hz), 127.0, 123.4 (d, J = 3.6 Hz), 116.9, 115.3 (d, J = 18.7 Hz), 113.4 (d, J = 1.7 Hz), 99.8, 56.2, 47.0, 44.8, 32.6, 26.2; HRMS calcd for $[C_{19}H_{20}FN_3O + H]^+$: 326.1663, found 326.1652.

4.23 | 4-Cyclohexyl-7-(3-fluoro-4-methoxybenzyl)-7*H*-pyrrolo[2,3-*d*] pyrimidine (6o)

Yield: 26%; ^{1}H NMR δ 8.82 (s, 1 H), 7.08 (d, J = 3.6 Hz, 2 H), 6.96–6.93 (m, 2 H), 6.85 (t, J = 8.5 Hz, 1 H), 6.58 (d, J = 3.6 Hz, 2 H), 5.32 (s, 2 H), 3.81 (s, 3 H), 3.08–3.02 (m,

1 H), 1.94–1.73 (m, 8 H), 1.47–1.31 (m, 2 H); ^{13}C NMR δ 167.0, 151.5, 152.2 (d, J = 245.6 Hz), 150.5, 147.2 (d, J = 10.6 Hz), 129.8 (d, J = 5.9 Hz), 127.0, 123.4 (d, J = 3.6 Hz), 116.3, 115.4 (d, J = 18.7 Hz), 113.4 (d, J = 2.0 Hz), 99.6, 56.1, 46.9, 44.0, 31.5, 26.4, 25.9; HRMS calcd for $[C_{20}H_{22}FN_3O + H]^+$: 340.1820, found 340.1814.

4.24 | 7-(3-Fluoro-4-methoxybenzyl)-4-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (6p)

Yield: 69%; ^{1}H NMR δ 8.99 (s, 1 H), 8.11 (d, J = 6.8 Hz, 2 H), 7.57–7.49 (m, 3 H), 7.22 (d, J = 3.6 Hz, 1 H), 7.02–6.99 (m, 2 H), 6.93–6.88 (m, 1 H), 6.83 (d, J = 3.6 Hz, 1 H), 5.42 (s, 2 H), 3.86 (s, 3 H); ^{13}C NMR δ 157.5, 152.2 (d, J = 245.7 Hz), 151.5, 151.5, 150.7, 147.2 (d, J = 10.6 Hz), 137.9, 129.9, 129.6 (d, J = 5.9 Hz), 128.7 (d, J = 7.9 Hz), 128.5, 123.4 (d, J = 3.6 Hz), 115.6, 115.4 (d, J = 18.8 Hz), 113.4 (d, J = 2.1 Hz), 100.9, 56.1, 47.0; HRMS calcd for $[C_{20}H_{16}FN_3O + H]^+$: 334.1350, found 334.1339.

4.25 | 4-Ethyl-7-[1-(3-fluoro-4-methoxyphenyl)ethyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (6q)

Yield: 60%; ^{1}H NMR δ 8.88 (s, 1 H), 7.31 (d, J = 3.2 Hz, 1 H), 7.03–7.01 (m, 2 H), 6.93–6.89 (m, 2 H), 6.71 (d, J = 3.2 Hz, 1 H), 6.19 (q, J = 7.1 Hz, 1 H), 3.86 (s, 3 H), 3.21 (q, J = 7.8 Hz, 2 H), 1.89 (d, J = 7.1 Hz, 3 H), 1.49 (t, J = 7.8 Hz, 3 H); ^{13}C NMR δ 164.0, 152.0 (d, J = 245.2 Hz), 151.1, 149.9, 146.9 (d, J = 10.5 Hz), 134.2 (d, J = 5.4 Hz), 124.6, 122.1 (d, J = 3.4 Hz), 116.9, 114.2 (d, J = 18.8 Hz), 113.1, 99.3, 56.0, 51.4, 28.3, 20.3, 12.7; HRMS calcd for $[C_{17}H_{18}FN_3O + H]^+$: 300.1507, found 300.1499.

4.26 | 7-[1-(3-Fluoro-4-methoxyphenyl)ethyl]-4-propyl-7*H*-pyrrlo[2,3-*d*]pyrimidine (6r)

Yield: 93%; ^{1}H NMR δ 8.80 (s, 1 H), 7.16 (d, J = 3.6 Hz, 1 H), 7.02–6.98 (m, 2 H), 6.91–6.87 (m, 1 H), 6.57 (d, J = 3.6 Hz, 1 H), 6.16 (q, J = 7.1 Hz, 1 H), 3.85 (s, 3 H), 2.99 (t, J = 7.6 Hz, 2 H), 1.93–1.84 (m, 5 H), 1.01 (t, J = 7.1 Hz, 3 H); ^{13}C NMR δ 163.0, 152.1 (d, J = 245.2 Hz), 151.2, 149.9, 147.0 (d, J = 10.6 Hz), 134.2 (d, J = 5.5 Hz), 124.6, 122.2 (d, J = 3.5 Hz), 117.6, 114.3 (d, J = 18.9 Hz), 113.2 (d, J = 2.1 Hz), 99.5, 56.1, 51.4, 37.3, 22.1, 20.4, 14.1; HRMS calcd for $[C_{18}H_{20}FN_3O + H]^+$: 314.1663, found 314.1656.

4.27 | 4-Butyl-7-[1-(3-fluoro-4-methoxyphenyl)ethyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (6s)

Yield: 78%; ^{1}H NMR δ 8.80 (s, 1 H), 7.16 (d, J = 3.6 Hz, 1 H), 7.02–6.09 (m, 2 H), 6.91–6.87 (m, 1 H), 6.57 (d, J = 3.6 Hz, 1 H), 6.16 (q, J = 7.1 Hz, 1 H), 3.85 (s, 3 H), 3.01 (t, J = 8.0 Hz, 2 H), 1.87–1.79 (m, 5 H), 1.48–1.38 (m, 2 H), 0.96 (t, J = 7.4 Hz, 3 H); ^{13}C NMR δ 163.3, 152.2 (d, J = 245.4 Hz), 151.2, 150.0, 147.0 (d, J = 10.7 Hz), 134.3 (d, J = 5.4 Hz), 124.6, 122.3 (d, J = 3.5 Hz), 117.5, 114.3 (d, J = 18.9 Hz), 113.3, 99.5, 56.1, 51.5, 35.1, 30.9, 22.7, 20.5, 13.9; HRMS calcd for $[C_{19}H_{22}FN_3O + H]^+$: 328.1820, found 328.1805.

4.28 | 7-(3-Fluoro-4-methoxybenzyl)-4-vinyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (6m)

A solution of **10** (0.108 g, 0.370 mmol, 1.0 equiv), $Pd(PPh_3)_2Cl_2$ (14 mg, 0.020 mmol, 0.054 equiv), and LiCl (0.019 g, 0.45 mmol, 1.2 equiv) in DMF (1.2 ml) was slowly added with tributyl(vinyl)tin (175 mg, 0.552 mmol, 1.5 equiv). The reaction mixture was heated at 100 °C for 19 hr under N_2. The solution were quenched with H_2O and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography to give **6m** (0.026 g, 0.092 mmol) in 25% yield: ^{1}H NMR δ 8.87 (s, 1 H), 7.17 (d, J = 3.6 Hz, 1 H), 7.11 (dd, J = 17.6 Hz, 10.8 Hz, 1 H), 6.97–6.95 (m, 2 H), 6.91–6.87 (m, 1 H), 6.68 (d, J = 3.6 Hz, 1 H), 6.64 (dd, J = 17.6 Hz, 1.2 Hz, 1 H), 5.79 (dd, J = 10.8 Hz, 1.2 Hz, 1 H), 5.38 (s, 2 H), 3.85 (s, 3 H); ^{13}C NMR δ 154.3, 152.3 (d, J = 245.9 Hz), 151.7, 151.5, 147.3 (d, J = 10.6 Hz), 133.5, 129.6 (d, J = 4.0 Hz), 128.4, 123.4 (d, J = 3.6 Hz), 123.2, 116.0, 115.4 (d, J = 18.7 Hz), 113.4, 99.5, 56.2, 47.0; HRMS calcd for $[C_{16}H_{14}FN_3O + H]^+$: 284.1194, found 284.1185.

4.29 | Biological study

The in vitro anticancer activity of compounds **6a–s** and **3** (Figure 1 and Table 1) for Hela, MDA-MB-231, and MDA-MB-468 cancer cells was measured on CellTiter96 assay kit (Promega) after a 96-hr incubation as we previously described.[29] The cell cycle analysis of **6q** by flow cytometry (FACS) on MDA-MB-231 cells was performed by our published method.[29] The in vivo anticancer activity of **6q** was conducted on nude mice bearing human MDA-MB-231 xenografts as previously reported.[30]

ACKNOWLEDGMENTS

We are grateful to the Ministry of Science and Technology of R.O.C. (Taiwan, MOST 105-2113-M-415-001) for financial support.

ORCID

Jiann-Jyh Huang https://orcid.org/0000-0003-4336-7913

REFERENCES

[1] S.-E. N. Ayyad, A. S. Judd, W. T. Shier, T. R. Hoye, *J. Org. Chem.* **1998**, *63*, 8102.
[2] C. Combeau, J. Provost, F. Lancelin, Y. Tournoux, F. Prod'homme, F. Herman, F. Lavelle, J. Leboul, M. Vuilhorgne, *Mol. Pharmacol.* **2000**, *57*, 553.
[3] G. Nuki, *Medicine* **2006**, *34*, 417.
[4] Y. Shan, J. Zhang, Z. Liu, M. Wang, Y. Dong, *Curr. Med. Chem.* **2011**, *18*, 523.
[5] R. Smolarczyk, J. Czapla, M. Jarosz-Biej, K. Czerwinski, T. Cicho, *Eur. J. Pharmacol.* **2021**, *891*, 173692.
[6] G. Mehta, K. Islam, *Angew. Chem. Int. Ed.* **2002**, *41*, 2396.
[7] G. Mehta, K. Islam, *Tetrahedron Lett.* **2003**, *44*, 6733.
[8] H. Araki, M. Inoue, T. Katoh, *Org. Lett.* **2003**, *5*, 3903.
[9] H. Araki, M. Inoue, T. Suzuki, T. Yamori, M. Kohno, K. Watanabe, H. Abe, T. Katoh, *Chem. A Eur. J.* **2007**, *13*, 9866.
[10] D. L. J. Clive, D. Liu, *Angew. Chem. Int. Ed.* **2007**, *46*, 3738.
[11] D. L. J. Clive, D. Liu, *J. Org. Chem.* **2008**, *73*, 3078.
[12] M. Y. Lee, K. H. Kim, S. Jiang, Y. H. Jung, J. H. Sim, G.-S. Hwang, D. H. Ryu, *Tetrahedron Lett.* **2008**, *49*, 1965.
[13] C.-H. Chen, Y.-K. Chen, C.-K. Sha, *Org. Lett.* **2010**, *12*, 1377.
[14] D. R. Hookins, R. J. K. Taylor, *Tetrahedron Lett.* **2010**, *51*, 6619.
[15] T. Suzuki, K. Ghozati, D.-Y. Zhou, T. Katoh, H. Sasai, *Tetrahedron* **2010**, *66*, 7562.
[16] T. Katoh, *Nat. Prod. Commun.* **2013**, *8*, 973.
[17] T.-Y. Chang, Y.-P. Tu, W.-Y. Wei, H. Y. Chen, C.-S. Chen, Y.-S. E. Lee, J.-J. Huang, C.-K. Sha, *ACS Med. Lett.* **2012**, *3*, 1075.
[18] A. G. Csákÿ, G. de la Herrán, M. C. Murcia, *Chem. Soc. Rev.* **2010**, *39*, 4080.
[19] J. C. Powers, J. L. Asgian, Ö. D. Ekici, K. E. James, *Chem. Rev.* **2002**, *102*, 4639.
[20] A. Gangjee, O. A. Namjoshi, S. N. Keller, C. D. Smith, *Bioorg. Med. Chem.* **2011**, *19*, 4355.
[21] A. Gangjee, S. Kurup, C. D. Smith, *Bioorg. Med. Chem.* **2013**, *21*, 1180.
[22] J.-P. Liou, Y.-L. Chang, F.-M. Kuo, C.-W. Chang, H.-Y. Tseng, C.-C. Wang, Y.-N. Yang, J.-Y. Chang, S.-J. Lee, H.-P. Hsieh, *J. Med. Chem.* **2004**, *47*, 4247.
[23] T. Hayashi, M. Konish, Y. Kobori, M. Kumada, T. Higuchi, K. Hirotsu, *J. Am. Chem. Soc.* **1984**, *106*, 158.
[24] J. K. Stille, *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 508.
[25] S.-K. Kim, S.-M. Cho, H. Kim, H. Seok, S.-O. Kim, T. K. Kwon, J.-S. Chang, *Exp. Mol. Med.* **2013**, *45*, e19.
[26] A. Ciupa, P. A. de Bank, M. F. Mahon, P. J. Wood, L. Caggiano, *Med. Chem. Commun.* **2013**, *4*, 956.
[27] M. Ma, L. Sun, H. Lou, M. Ji, *Chem. Cent. J.* **2013**, *7*, 179.
[28] M.-Y. Song, Q.-R. He, Y.-L. Wang, H.-R. Wang, T.-C. Jiang, J.-J. Tang, J.-M. Gao, *Int. J. Mol. Sci.* **2020**, *21*, 1817.
[29] C.-C. Chiang, Y.-H. Lin, S. F. Lin, C.-L. Lai, C. Liu, W.-Y. Wei, S.-C. Yang, R.-W. Wang, L.-W. Teng, S.-H. Chuang, J.-M. Chang, T.-T. Yuan, Y.-S. Lee, P. Chen, W.-K. Chi, J.-Y. Yang, H.-J. Huang, C.-B. Liao, J.-J. Huang, *J. Med. Chem.* **2010**, *53*, 5929.
[30] Y.-S. E. Lee, S.-H. Chuang, L. Y. L. Huang, C.-L. Lai, Y.-H. Lin, J.-Y. Yang, C.-W. Liu, S.-c. Yang, H.-S. Lin, C.-c. Chang, J.-Y. Lai, P.-S. Jian, K. Lam, J.-M. Chang, J. Y. N. Lau, J.-J. Huang, *J. Med. Chem.* **2014**, *57*, 4098.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Tsai P-Y, Hu G-S, Huang P-H, et al. Design, synthesis, and in vitro/vivo anticancer activity of 4-substituted 7-(3-fluoro-4-methoxybenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidines. *J Chin Chem Soc*. 2021;1–10. https://doi.org/10.1002/jccs.202100100