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Synthesis and SAR of 6-chloro-4-fluoroalkylamino-2-heteroaryl-5-(substituted)phenylpyrimidines as anti-cancer agents

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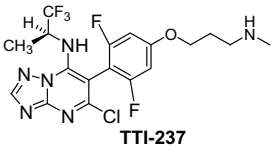
ABSTRACT

The synthesis and SAR of a series of 6-chloro-4-fluoroalkylamino-2-heteroaryl-5-(substituted)phenylpyrimidines as anti-cancer agents are described. This series of 2-heteroarylpyrimidines was developed by modifying a series of anti-tumor [1,2,4]triazolo[1,5-*a*]pyrimidines and 2-cyanoaminopyrimidines we reported earlier. For the 2-heteroaryl group, the best activity is obtained when the heteroaryl group has a nitrogen atom at the *ortho*-position to the pyrimidyl core. The structure–activity relationship for the rest of the molecule in this 2-heteroarylpyrimidine series mimics that of the [1,2,4]triazolo[1,5-*a*]pyrimidine series. Like triazolopyrimidines and 2-cyanoaminopyrimidines, the 2-heteroarylpyrimidines retain the capability to overcome multidrug resistance due to Pgp. Mechanism of action studies showed that the lead compounds behaved in the same manner as triazolopyrimidines and 2-cyanoaminopyrimidines. The lead compounds in this series are more potent than the corresponding triazolopyrimidines in vitro and in vivo. Compound **21** (PTI-868) showed tumor growth inhibition in several nude mouse xenograft models, and was selected to advance to preclinical development.

1. Introduction

Anti-cancer agents that bind tubulin have shown great promise in cancer treatments and have attracted much interest in chemotherapy development.[1,2] These agents are categorized depending on their mechanism of action on the microtubule dynamics and on their binding sites on tubulin. Most of these agents inhibit polymerization of tubulin to microtubules, and they are further categorized as vinca alkaloid site binders and colchicines site binders.[3] Taxoids, on the other hand, promote tubulin polymerization and stabilize microtubules.[4] Later, several other natural products, for example, epothilones,[5] discodermolide,[6] and eleutherobin,[7] were discovered that bind at the taxoid site and show taxoid-like microtubule-stabilizing activity.[8–10] Other natural products, for example, laulimalide[11] and peloruside,[12] however, show taxoid-like microtubule-stabilizing activity, but do not bind at the taxoid site.[13,14] Laulimalide and peloruside are synergistic with paclitaxel, but not with each other.[15–17]

Recently, we reported the synthesis and structure–activity relationship of a series of triazolopyrimidines **1**, which act as anti-cancer agents by interfering with the microtubule dynamics in a unique manner.[18,19] 5-Chloro-6-{2,6-difluoro-4-[3-(methylamino)propoxy]phenyl}-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (TTI-237),[18,19] the lead compound in this series, entered Phase I clinical trials. We then modified the triazolopyrimidine core **1** by N^1-methylation, followed by opening of the five-membered triazolo ring (e.g., **2**). A series of 2-cyanamidopyrimidines[20] (e.g., **3**) was thus obtained and shown to retain in vitro potency observed with the triazolopyrimidines. We then further modified the series by mimicking the 2-cyanamido group with more stable and pharmaceutically acceptable heteroaryls (**4**, Scheme 1). We now report synthesis and structure–activity relationship of this series of 6-chloro-4-fluoroalkylamino-2-heteroaryl-5-(substituted)phenylpyrimidines as anti-cancer agents.[21]



TTI-237

2. Chemistry

Compounds **8**–**26** were prepared,[22] as shown in Scheme 2. Cyclization of diethyl 2,4,6-trifluorophenylmalonate **5**[23] with carboxamidine **6**, followed by chlorination with $POCl_3$ provided

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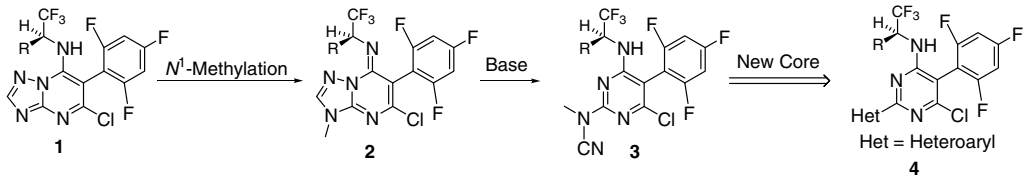
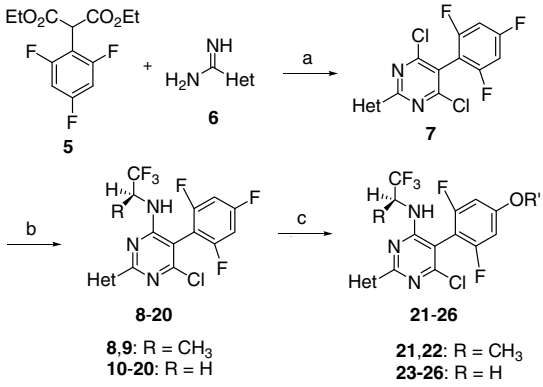


Scheme 1. From [1,2,4]triazolo[1,5-*a*]pyrimidines to 2-heteroarylpyrimidines.

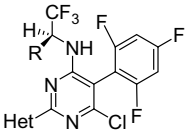


Scheme 2. Synthesis of **8**–**26**. Reagents and conditions: (a) i—tributylamine, 180 °C; ii—$POCl_3$, reflux; (b) $CF_3CH(R)NH_2$, DMF; (c) R′OH, NaH, DMSO.

dichloropyrimidine **7**. Replacement of one of the two equivalent chloro groups with fluoroalkylamines yielded the desired compounds **8**–**20** (Table 1). Compounds **21**–**26** (Table 2) were prepared by further treatment of compounds **8**–**11** with the corresponding alcohols, in the presence of sodium hydride in DMSO. As in the triazolopyrimidine series, the 4-fluoro group on the phenyl ring could be replaced cleanly in the presence of the 2- and 6-fluoro groups.

Compounds **29**–**33** (Tables 1 and 2), in which the five-membered heteroaryl groups are connected to the pyrimidine core through a C–N bond, were prepared as shown in Scheme 3. Cyclization of diethyl 2,4,6-trifluorophenylmalonate **5** with urea, followed by chlorination with $POCl_3$, provided trichloropyrimidine **27**. Replacement of the 2-chloro group with the N–H containing five-membered heteroaryl groups yielded compound **28**. Further

Table 1
Inhibition of COLO 205 cell proliferation by compounds **8**–**20** and **29**–**31**

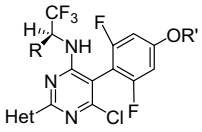


8-20 and **29-31**

Compound	R	Het	IC_{50} (nM)[a]
8	CH_3	2-Pyrazinyl	51 ± 2
9	CH_3	2-Pyridinyl	29 ± 6
10	H	2-Pyrazinyl	84 ± 11
11	H	2-Pyrimidinyl	265 (*n* = 1)
12	H	4-Pyridinyl	>5000 (*n* = 2)
13	H	3-Pyridinyl	3930 ± 373
14	H	2-Pyridinyl	39 ± 8
15	H	2-Quinolinyl	250 ± 23
16	H	1-Isoquinolinyl	4680 ± 251
17	H	3-Isoquinolinyl	119 ± 4
18	H	2-Thienyl	3465 (*n* = 1)
19	H	2-Furyl	232 (*n* = 1)
20	H	2-(1-Methylimidazolyl)	77 ± 22
29	H	1-Imidazolyl	3030 ± 111
30	H	1-Pyrazolyl	53 ± 5
31	CH_3	1-Pyrazolyl	19 ± 6
Paclitaxel			3.3 ± 1
Vincristine			2.6 ± 0.5

[a] Concentration needed to inhibit cell growth by 50% as determined from the dose–response curve. Determinations of each IC_{50} value were conducted at 10 concentrations, and in triplicate. Triplicate values at each concentration agreed, on average, within 10%.

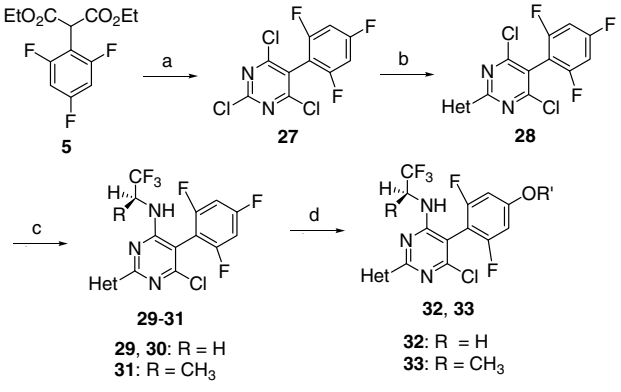
Table 2
Inhibition of COLO 205 cell proliferation by compounds **21**–**26**, **32**, and **33**



21-26,**32**, and **33**

Compound[a]	R	Het	R′	IC_{50} (nM)[b]
21	CH_3	2-Pyrazinyl	$-(CH_2)_3NHCH_3$	6.9 ± 2.2
21[c]	CH_3	2-Pyrazinyl	$-(CH_2)_3NHCH_3$	8.9 ± 0.7
22[c]	CH_3	2-Pyridinyl	$-(CH_2)_3NHCH_3$	5.2 ± 1.3
23	H	2-Pyrimidinyl	$-(CH_2)_3N(CH_3)_2$	49 (n = 1)
24	H	2-Pyrazinyl	$-(CH_2)_3N(CH_3)_2$	22 ± 5
24[c]	H	2-Pyrazinyl	$-(CH_2)_3N(CH_3)_2$	18 ± 3
25	H	2-Pyrazinyl	$-(CH_2)_2N(CH_3)_2$	128 ± 47
26	H	2-Pyrazinyl	$-(CH_2)_4N(CH_3)_2$	32 ± 14
32	H	1-Imidazolyl	$-(CH_2)_3N(CH_3)_2$	682 ± 232
33[c]	CH_3	1-Pyrazolyl	$-CH_2)_3NHCH_3$	2.4 ± 0.3
Paclitaxel				3.3 ± 1
Vincristine				2.6 ± 0.5

[a] Unless indicated otherwise, compounds were tested as the free base.
[b] Concentration needed to inhibit cell growth by 50% as determined from the dose–response curve. Determinations of each IC_{50} value were conducted at 10 concentrations, and in triplicate. Triplicate values at each concentration agreed, on average, within 10%.
[c] Compounds were tested as the HCl salts.



Scheme 3. Synthesis of **29**–**33**. Reagents and conditions: (a) i—urea, NaH, EtOH, 80 °C, ii—$POCl_3$, reflux; (b) Het-H, K_2CO_3, DMF; (c) $CF_3CH(R)NH_2$, *N*,*N*-diisopropylethylamine, DMF; (d) R′OH, NaH, DMSO.

replacement of one of the two remaining equivalent chloro groups with the fluoroalkylamines yielded the desired compounds **29**–**31**. Compounds **32** and **33** (Table 2) were prepared by further treatment of compounds **29** and **31** with the corresponding alcohols, in the presence of sodium hydride in DMSO.

To confirm the regiochemistry from **27** to **28**, an alternative synthesis[22] of **29** was carried out (Scheme 4). Cyclization of diethyl 2,4,6-trifluorophenylmalonate **5** with thiourea provided **34**. Selective methylation of the mercapto group, followed by chlorination with $POCl_3$ yielded **36**. Replacement of one of the two equivalent chloro groups with 2,2,2-trifluoroethylamine gave **37**. Oxidation of the thiomethyl group with mCPBA led to a methylsulfonyl group (in **38**) that was replaced by imidazole to get **29**, which was spectroscopically identical with the material obtained from Scheme 3.

3. Results and discussion

Compounds **8**–**26** and **29**–**33** were evaluated in the COLO 205 cytotoxicity assay[18,19] for their ability to inhibit cancer cell proliferation. As shown in Table 1, for the heteroaryl group attached to the 2-position of the pyrimidine ring, the best activity is obtained when the heteroaryl group has a nitrogen atom at the position *ortho*- to the pyrimidyl core. With such a nitrogen atom, both a five-membered ring and a six-membered ring can render high potency. Thus, the highest potency was observed with a 2-pyrazinyl (**8** and **10**), a 2-pyridinyl (**9** and **14**), a 1-pyrazolyl (**30** and **31**), or a 2-(1-methylimidazolyl) (**20**). Interestingly, compound **11**, with a 2-pyrimidinyl ring and thus two nitrogen atoms *ortho*- to the pyrimidyl core (at the 2- and 6-positions), was less potent than compound **10**. Compounds that lack a nitrogen atom at the position *ortho*- to the pyrimidyl core (**12**, **13**, **18**, **19**, and **29**) showed much lower potency. We further modified the 2-pyridyl group by fusing an additional phenyl ring in all three possible orientations (**15**–**17**). The potency for these isomeric analogs varies greatly. The most potent compound among the three, **17**, is slightly less potent than **10**, suggesting there is space in the binding pocket to accommodate substituents at the 4- and 5-positions on the pyridyl ring. As a head-piece, (*S*)-2,2,2-trifluoro-1-methylethylamine (as in the triazolopyrimidines) seems to provide higher potency than observed with 2,2,2-trifluoroethylamine (**8** vs **10**, **9** vs **14**, and **31** vs **30**).

Selected compounds in Table 1 were further modified by replacing the 4-fluoro group with methylamino alkylalcohols, a strategy proven successful in the triazolopyrimidine series.[18] Comparable IC_{50} values were obtained when a given compound was tested either as a free base or as a hydrogen chloride salt. As shown in Table 2, compounds with proper methylamino alkylalcohol tails are more potent than their corresponding trifluorophenyl analogs (in Table 1). A 4- to 8-fold increase in potency is achieved with a three-methylene unit tether (**21** vs **8**, **22** vs **9**, **23** vs **11**, **24** vs **10**, **32** vs **29**, and **33** vs **31**). A three-methylene unit is optimal for potency. Compound **26**, with a four-methylene unit, is less potent than compound **24**, while compound **25**, with a two-methylene unit, shows further decreased potency. This observation is in agreement with SAR observed in the [1,2,4]triazolo[1,5-*a*]pyrimidine[18] and the 2-cyanoaminopyrimidine series.[20]

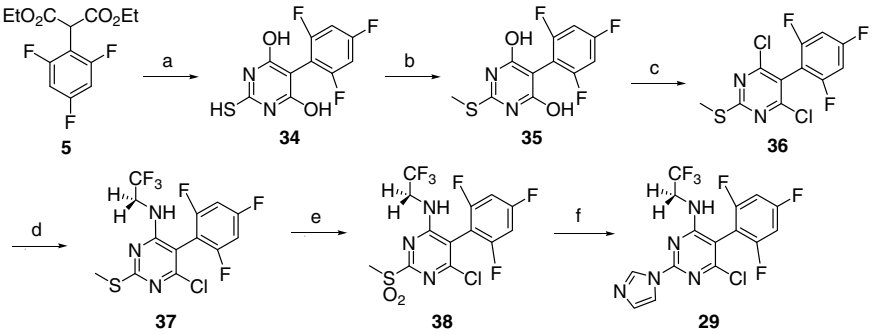
Compounds **21**, **22**, and **33** were further studied in the mechanism of action studies[18–20] and were shown to behave in the same manner as was observed with the [1,2,4]triazolo[1,5-*a*]pyrimidines and 2-cyanoaminopyrimidines.[18–20] They promoted tubulin polymerization using either MAP-rich tubulin or pure tubulin, and did not cause microtubule depolymerization in vitro, a phenomenon similar to the effect of paclitaxel. Competitive binding studies with [H]vinblastine, [^{3}H]colchicine, and [^{3}H]paclitaxel showed that, like their [1,2,4]triazolo[1,5-*a*]pyrimidine counterparts,[18,19] these phenylpyrimidines did not inhibit the binding of [^{3}H]colchicine or [^{3}H]paclitaxel effectively. Instead, they inhibited the binding of [^{3}H]vinblastine.[19] These findings strongly suggest that 2-heteropyrimidines and [1,2,4]triazolo[1,5-*a*]pyrimidines bind at the same site of tubulin with the same specific binding mode. Cell cycle analysis of compounds **21**, **22**, and **33** showed apoptosis induction at low compound concentration and G2/M block at high compound concentration, a pattern that is similar to what was observed with [1,2,4]triazolo[1,5-*a*]pyrimidines[19] and paclitaxel.[24]

Like the [1,2,4]triazolo[1,5-*a*]pyrimidines[18,19] and 2-cyanoaminopyrimidines,[20] compounds **21**, **22**, **24**, and **33** were able to overcome resistance due to P-glycoprotein (Pgp). As shown in Table 3,

Table 3
Inhibition of KB, KB 8.5 and KB V1 cell proliferation by compounds **21**, **22**, **24**, and **33**

Compound[a]	IC_{50}[b] (nM)			Ratio[c]	
	KB	KB 8.5	KB V1	KB 8.5/KB	KB V1/KB
21	7.0 ± 0.5	24 ± 1	405 ± 27	3.4	58
22	6.2 ± 0.4	21 ± 3	423 ± 19	3.4	68
24	20 ± 3	24 ± 2	99 ± 4	1.2	5.0
33	2.4 ± 0.1	32 ± 4	569 ± 36	13	237
Paclitaxel	2.5 ± 0.1	26 ± 0.1	2013 ± 108	10	805
Vincristine	2.2 ± 0.6	58 ± 16	2035 ± 25	26	925

[a] Compounds were tested as the HCl salts.
[b] Concentration needed to inhibit cell growth by 50% as determined from the dose–response curve. Determinations of each IC_{50} value were conducted at 10 concentrations, and in duplicate. Duplicate values at each concentration agreed, on average, within 10%.
[c] Ratio = IC_{50} on KB 8.5 or KB V1 cells/IC_{50} on KB cells. A ratio of about 1 indicates no resistance.



Scheme 4. Alternative synthesis of **29**. Reagents and conditions: (a) thiourea, *n*-Bu_3N, 150 °C; (b) CH_3I, NaOH; (c) $POCl_3$, reflux; (d) $CF_3CH_2NH_2$, *N*,*N*-diisopropylethylamine, DMF; (e) mCPBA; (f) Imidazole, K_2CO_3, DMF.

these compounds showed relatively low IC_{50} ratios between the KB line and KB 8.5 and KB V1 lines. The KB lines[25] express different amounts of the Pgp membrane pump which causes resistance to the action of many cytotoxic compounds, including paclitaxel and vincristine, via enhanced efflux from the cell. The parental KB line has very little or no expression of Pgp, KB 8.5 expresses moderate levels of the protein, and KB V1 expresses artificially high levels. The change in IC_{50} values in these lines reflects the ability of Pgp to recognize and export a potential drug agent. Compounds **21**, **22**, **24**, and **33** showed much lower resistance ratios, and thus much lower levels of recognition by Pgp than paclitaxel and vincristine.

The lead compound in this series, compound **21**, has very desirable pharmaceutical properties. Its water solubility at pH 7.4 is 540 μg/mL (measured by shake flask method[26]), enough for dosing directly in saline with no special formulation needed. The metabolic stability of PTI-868 (1 μM) was determined using liver microsomes obtained from female nude mice, male CD-1 mice, male and female Sprague–Dawley rats, male beagle dogs, male cynomolgus monkeys and male humans (1 mg/mL). Incubations were conducted for 30 min at 37 °C in the presence of NADPH. It is metabolically stable in rat, dog, monkey, human and female nude mouse liver microsomes, with $t_{1/2}$ values greater than 60 min. The pharmacokinetic parameters were determined using a non-compartmental analysis module (Model 201 and 202) of the pharmacokinetic software package WinNonlin, ver. 3.1 (Pharsight, CA). The program applies a model-independent approach and the standard methods described by Gibaldi and Perrier.[27] In PK studies in rat, the compound showed a high volume of distribution (14 L/kg), moderate clearance (21 mL/min/kg), long half-life (8.6 h), and high bioavailability (~100%).

In in vivo xenograft studies, compound **21** showed good efficacy against several human tumor models, including H460 non-small cell lung carcinoma (Fig. 1) and human Lox melanoma (Fig. 2). The compound was selected to advance to preclinical development, based on its pharmaceutical properties and efficacies in in vivo xenograft studies.

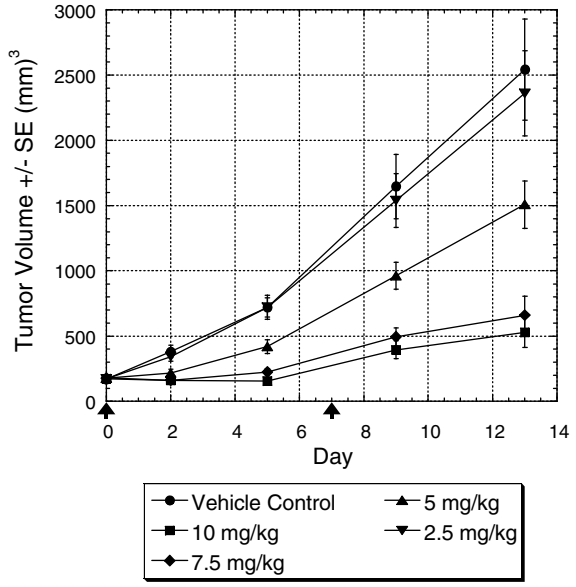


Figure 1. In vivo anti-tumor activity with Compound **21** against H460 human non-small cell lung carcinoma (NSCLC). The compound was administered to tumor-bearing mice on days 0 and 7 after staging (indicated by arrows) by oral gavage. The control group received vehicle only (Klucel) by iv injection. Each group contained nine animals. One animal died in the 10 mg/kg group.

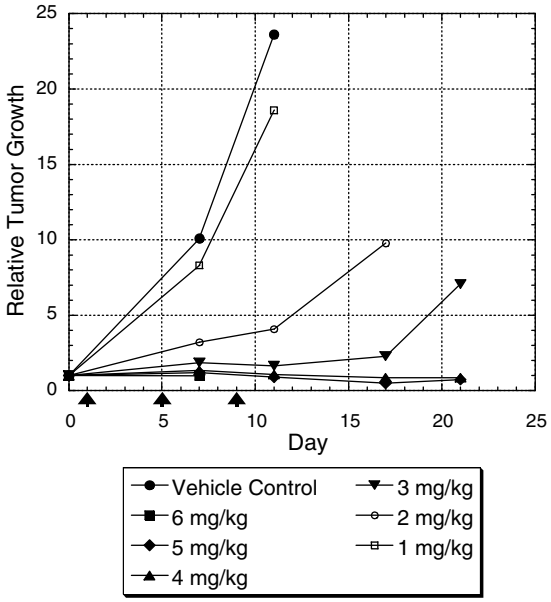


Figure 2. In vivo anti-tumor activity with Compound **21** against Lox melanoma. The compound was administered to tumor-bearing mice on days 1, 5 and 9 after staging (indicated by arrows) by intravenous injection. The control group received vehicle only by iv injection. Vehicle was 0.9% saline. Each group contained five animals. Relative tumor growth = mean tumor mass on day d/mean tumor mass of that group on day 0. Three animals died in the 6 mg/kg group, and two died in each of the 5 mg/kg and 4 mg/kg groups.

4. Conclusion

By modifying a series of [1,2,4]triazolo[1,5-*a*]pyrimidine antitumor agents, we developed a series of 2-heteroarylpyrimidines as anti-cancer agents. For the 2-heteroaryl group, the best activity is obtained when the heteroaryl group has a nitrogen atom at the position *ortho*- to the pyrimidyl core. The structure–activity relationship for the rest of the molecule in this 2-heteroarylpyrimidine series parallels that of the [1,2,4]triazolo [1,5-*a*]pyrimidine and 2-cyanoaminopyrimidine series. Like triazolopyrimidines and 2-cyanoaminopyrimidines, the 2-heteroarylpyrimidines have the capability to overcome multidrug resistance due to Pgp. The lead compounds in this 2-heteroarylpyrimidine series promote tubulin polymerization and inhibit microtubule depolymerization in vitro. They inhibit binding of [^{3}H]vinblastine, but not [^{3}H]colchicine or [^{3}H]paclitaxel, as was observed with the triazolopyrimidines and 2-cyanoaminopyrimidines. These findings strongly suggest that this series of 2-heteroarylpyrimidines binds at the same site and in the same binding mode as the triazolopyrimidines and 2-cyanoaminopyrimidines do. The lead compounds in this series are more potent than the corresponding triazolopyrimidines in vitro and in vivo. Compound **21** showed tumor growth inhibition in several nude mouse xenograft models, and was selected to advance to preclinical development.

5. Experimental

5.1. Chemistry: general

All reactions were conducted under nitrogen atmosphere with magnetic stirring. Chromatographic purifications were performed using Baker 40-μm silical gel. Melting points were determined in open capillary tubes on a Meltemp melting point apparatus and

are uncorrected. ^{1}H NMR spectra were recorded with a Bruker Avance 400 spectrometer at 400 MHz. Chemical shifts are quoted in parts per million from internal standard tetramethylsilane. Mass spectra were obtained using a Micromass LCT mass spectrometer operating at 20 eV. Elemental analyses were performed on a Perkin-Elmer 2400 CHN analyzer instrument.

5.2. 2-Pyrazinecarboxamidine hydrochloride (6)

To methyl alcohol (20 mL) was added sodium (109 mg, 4.74 mmol) with stirring. After disappearance of the solid, 2-pyrazinecarbonitrile (5.0 g, 47.6 mmol) was added. The mixture was stirred at room temperature for 6 h, and ammonium chloride (2.8 g, 52.3 mmol) was added. The mixture is then stirred at room temperature for 18 h. Diethyl ether (50 mL) was added to the reaction mixture, and the precipitates were collected by filtration. The solid was washed with diethyl ether (2×) and dried in vacuum oven to give 6.5 g (86%) of **6** as a hydrochloride salt as a white solid. MS: *m/z* 123.1 (M+H).

5.3. 4,6-Dichloro-2-pyrazin-2-yl-5-(2,4,6-trifluorophenyl)pyrimidine (7)

A mixture of diethyl 2-(2,4,6-trifluorophenyl)malonate[23] (870 mg, 3.0 mmol), 2-pyrazinecarboxamidine (**6**) hydrochloride (500 mg, 3.15 mmol), and 600 mg of tributylamine was stirred under nitrogen atmosphere at 180 °C for 1 h and cooled to room temperature. The mixture was cooled to room temperature and treated with 1.0 N hydrochloric acid. The precipitates were collected by filtration, washed with water and dried to give 2-pyrazin-2-yl-5-(2,4,6-trifluorophenyl)pyrimidine-4,6-diol as a tan solid (401 mg), which was used directly in the next step.

A mixture of 2-pyrazin-2-yl-5-(2,4,6-trifluorophenyl)pyrimidine-4,6-diol (401 mg) in phosphorous oxychloride (5 mL) and 2,6-lutidine (1 mL) was heated at 110 °C for 16 h. The excess phosphorous oxychloride was removed in vacuo, and the resulting residue was dissolved in ethyl acetate. The organic layer was washed with water and saturated sodium chloride, dried over magnesium sulfate, and concentrated. The residue was purified by flash chromatography over silica gel, eluting with a gradient of 20% ethyl acetate in hexanes to 33% ethyl acetate in hexanes. Concentration provided 201 mg (19%) of **7** as a red solid: mp 73–75 °C; ^{1}H NMR ($CDCl_3$) δ 6.89 (m, 2H), 8.78 (d, J = 2 Hz, 1H), 8.85(dd, J = 2, 1 Hz, 1H), 9.76 (d, J = 1 Hz, 1H); MS (ES+): *m/z* 356.9 (M+1). Anal. Calcd for $C_{14}H_5Cl_2F_3N_4$: C, 47.09, H, 1.41, N, 15.69. Found: C, 47.48, H, 1.62, N, 15.29.

5.4. 6-Chloro-2-pyrazin-2-yl-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl]-5-(2,4,6-trifluorophenyl)pyrimidin-4-amine (8)

A mixture of **7** (205 mg, 0.57 mmol), (1 S)-2,2,2-trifluoro-1-methylethylamine hydrogen chloride[28] (298 mg, 2 mmol), and *N*,*N*-diisopropylethylamine (258 mg, 2 mmol) in *N*,*N*-dimethylformamide (5 mL) was stirred at 90 °C in a sealed tube for 18 h. The reaction mixture was partitioned between ethyl acetate and saturated sodium chloride. The organic layer was washed with saturated sodium chloride (3×), dried over magnesium sulfate, and concentrated. The residue was purified by flash chromatography over silica gel, eluting with a gradient of 20% ethyl acetate in hexanes to 50% ethyl acetate in hexanes. Concentration provided 111 mg (45%) of **8** as a light yellow solid: mp 168–172 °C; ^{1}H NMR (DMSO-d_6) δ 1.35 (d, J = 7 Hz, 3H), 5.52 (m, 1H), 7.47 (m, 2H), 7.71 (d, J = 9 Hz, 1H), 8.85 (m, 2H), 9.58 (d, J = 1 Hz, 1H); MS (ES+): *m/z* 434.1 (M+1). Anal. Calcd for $C_{17}H_{10}ClF_6N_5$: C, 47.08, H, 2.32, N, 16.15. Found: C, 47.14, H, 2.32, N, 16.08.

5.5. 6-Chloro-2-pyridin-2-yl-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl]-5-(2,4,6-trifluorophenyl)pyrimidin-4-amine (9)

According to the procedure used to prepare **8**, starting from 2-cyanopyridine, **9** was obtained as a white solid: mp 230–232 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (d, J = 7 Hz, 3H), 4.53 (d, J = 10 Hz, 1H), 5.34 (m, 1H), 6.89 (m, 2H), 7.43 (m, 1H), 7.87 (m, 1H), 8.42 (d, J = 8 Hz, 1H), 8.85 (m, 1H); MS (ES+): *m/z* 433.0 (M+1). Anal. Calcd for $C_{18}H_{11}ClF_6N_4$: C, 49.96, H, 2.56, N, 12.95. Found: C, 50.01, H, 2.57, N, 12.77.

5.6. 6-Chloro-2-pyrazin-2-yl-*N*-(2,2,2-trifluoroethyl)-5-(2,4,6-trifluorophenyl)pyrimidin-4-amine (10)

According to the procedure used to prepare **8**, reaction of **7** with 2,2,2-trifluoroethylamine provided **10** in 94% yield as a white solid: mp 189–190 °C; ^{1}H NMR (DMSO-d_6) δ 4.35 (m, 2H), 7.48 (t, J = 9 Hz, 2H), 7.97 (t, J = 6 Hz, 1H), 8.84 (m, 2H), 9.57 (s, 1H); MS (ES+): *m/z* 419.9 (M+1). Anal. Calcd for $C_{16}H_8ClF_6N_5$: C, 45.79, H, 1.92, N, 16.69. Found: C, 46.07, H, 2.23, N, 16.29.

5.7. 6-Chloro-*N*-(2,2,2-trifluoroethyl)-5-(2,4,6-trifluorophenyl)-2,2′-bipyrimidin-4-amine (11)

According to the procedure used to prepare **10**, starting from 2-cyanopyrimidine, **11** was obtained as a tan solid: mp 245–250 °C; ^{1}H NMR (DMSO-d_6) δ 4.28 (m, 2H), 7.49 (m, 2H), 7.69 (t, J = 5 Hz, 1H), 7.95 (t, J = 6 Hz, 1H), 9.03 (d, J = 5 Hz, 2H); MS (ES+): *m/z* 420.0 (M+1). Anal. Calcd for $C_{16}H_8ClF_6N_5$: C, 45.79, H, 1.92, N, 16.69. Found: C, 46.00, H, 1.91, N, 16.59.

5.8. 6-Chloro-2-pyridin-4-yl-*N*-(2,2,2-trifluoroethyl)-5-(2,4,6-trifluorophenyl)pyrimidin-4-amine (12)

According to the procedure used to prepare **10**, starting from 4-cyanopyridine, **12** was obtained as a tan solid: mp 180–183 °C; ^{1}H NMR ($CDCl_3$) δ 4.33 (m, 2H), 4.90 (t, J = 6 Hz, 1H), 6.93 (m, 2H), 8.25 (d, J = 4 Hz, 2H), 8.79 (d, J = 4 Hz, 2H); MS (ES+): *m/z* 419.0 (M+1). Anal. Calcd for $C_{17}H_9ClF_6N_4$): C, 48.76, H, 2.17, N, 13.38. Found: C, 49.03, H, 2.21, N, 13.26.

5.9. 6-Chloro-2-pyridin-3-yl-*N*-(2,2,2-trifluoroethyl)-5-(2,4,6-trifluorophenyl)pyrimidin-4-amine (13)

According to the procedure used to prepare **10**, starting from 3-cyanopyridine, **13** was obtained as a dark oil: ^{1}H NMR ($CDCl_3$) δ 4.33 (m, 2H), 4.91 (t, J = 6 Hz, 1H), 6.91 (m, 2H), 7.43 (m, 1H), 8.66 (m, 1H), 8.74 (m, 1H), 9.61 (m, 1H); MS (ES+): *m/z* 419.1 (M+1). Anal. Calcd for $C_{17}H_9ClF_6N_4$·0.25EtOAc: C, 49.05, H, 2.52, N, 12.71. Found: C, 49.18, H, 2.68, N, 12.68.

5.10. 6-Chloro-2-pyridin-2-yl-*N*-(2,2,2-trifluoroethyl)-5-(2,4,6-trifluorophenyl)pyrimidin-4-amine (14)

According to the procedure used to prepare **10**, starting from 2-cyanopyridine, **14** was obtained as a white solid: mp > 200 °C; ^{1}H NMR ($CDCl_3$) δ 4.36 (m, 2H), 4.95 (t, J = 6 Hz, 1 H), 6.88 (m, 2H), 7.44 (m, 1H), 7.88 (m, 1H), 8.43 (d, J = 8 Hz, 1H), 8.85 (m, 1H); MS (ES+): *m/z* 419.0 (M+1). Anal. Calcd for $C_{17}H_9ClF_6N_4$: C, 48.76, H, 2.17, N, 13.38. Found: C, 48.91, H, 2.21, N, 13.23.

5.11. 6-Chloro-2-quinolin-2-yl-*N*-(2,2,2-trifluoroethyl)-5-(2,4,6-trifluorophenyl)pyrimidin-4-amine (15)

According to the procedure used to prepare **10**, starting from 2-quinolinecarbonitrile, **15** was obtained as a white solid: mp 260–

264 °C; ^{1}H NMR ($CDCl_3$) δ 4.41 (m, 2H), 5.07 (t, J = 6 Hz, 1H), 6.86 (m, 2H), 7.64 (m, 1H), 7.78 (m, 1H), 7.89 (d, J = 8 Hz, 1H), 8.35 (t, J = 8 Hz, 2H), 8.51 (m, 1H); MS (ES+): m/z 469.0 (M+1). Anal. Calcd for $C_{21}H_{11}ClF_6N_4{\cdot}0.2C_6H_{14}$: C, 54.86, H, 2.86, N, 11.53. Found: C, 54.81, H, 2.67, N, 11.48.

5.12. 6-Chloro-2-isoquinolin-1-yl-*N*-(2,2,2-trifluoroethyl)-5-(2,4,6-trifluorophenyl)pyrimidin-4-amine (16)

According to the procedure used to prepare **10**, starting from 1-isoquinolinecarbonitrile, **16** was obtained as a light yellow solid: mp 225–226 °C; ^{1}H NMR ($CDCl_3$) δ 4.28 (m, 2H), 4.98 (t, J = 6 Hz, 1H), 6.92 (m, 2H), 7.62 (m, 1H), 7.74 (m, 1H), 7.80 (d, J = 6 Hz, 1H), 7.92 (d, J = 8 Hz, 1H), 8.49 (d, J = 9 Hz, 1H), 8.70 (d, J = 6 Hz, 1H); MS (ES+): m/z 469.0 (M+1). Anal. Calcd for $C_{21}H_{11}ClF_6N_4$: C, 53.80, H, 2.37, N, 11.95. Found: C, 54.17, H, 2.10, N, 11.66.

5.13. 6-Chloro-2-isoquinolin-3-yl-*N*-(2,2,2-trifluoroethyl)-5-(2,4,6-trifluorophenyl)pyrimidin-4-amine (17)

According to the procedure used to prepare **10**, starting from 3-isoquinolinecarbonitrile, **17** was obtained as a white solid: mp 275–277 °C; ^{1}H NMR ($CDCl_3$) δ 4.44 (m, 2H), 4.93 (t, J = 6 Hz, 1H), 6.88 (m, 2H), 7.71 (m, 1H), 7.77 (m, 1H), 8.03 (d, J = 8 Hz, 1H), 8.05 (t, J = 8 Hz, 1H), 8.87 (s, 1H), 9.45 (s, 1H); MS (ES+): m/z 469.0 (M+1). Anal. Calcd for $C_{21}H_{11}ClF_6N_4{\cdot}0.1EtOAc{\cdot}0.1C_6H_{14}$: C, 54.34, H, 2.74, N, 11.52. Found: C, 54.41, H, 2.71, N, 11.22.

5.14. 6-Chloro-2-thien-2-yl-*N*-(2,2,2-trifluoroethyl)-5-(2,4,6-trifluorophenyl)pyrimidin-4-amine (18)

According to the procedure used to prepare **10**, starting from 2-thiophenecarbonitrile, **18** was obtained as a white solid: mp 130–135 °C; ^{1}H NMR ($CDCl_3$) δ 4.28 (m, 2H), 4.73 (t, J = 6 Hz, 1H), 6.88 (m, 2H), 7.14 (dd, J = 5, 3 Hz, 1H), 7.51 (dd, J = 5, 1 Hz, 1H), 8.03 (dd, J = 3, 1 Hz, 1H); MS (ES+): m/z 424.0 (M+1). Anal. Calcd for $C_{16}H_8ClF_6N_3S{\cdot}0.2C_6H_{14}$: C, 46.84, H, 2.47, N, 9.53. Found: C, 46.94, H, 2.18, N, 9.17.

5.15. 6-Chloro-2-(2-furyl)-*N*-(2,2,2-trifluoroethyl)-5-(2,4,6-trifluorophenyl)pyrimidin-4-amine (19)

According to the procedure used to prepare **10**, starting from 2-furonitrile, **19** was obtained as a white solid: mp 175–177 °C; ^{1}H NMR ($CDCl_3$) δ 4.31 (m, 2H), 4.77 (t, J = 6 Hz, 1H), 6.57 (dd, J = 3, 2 Hz, 1H), 6.88 (m, 2H), 7.36 (dd, J = 3, 1 Hz, 1H), 7.64 (dd, J = 2, 1 Hz, 1H); MS (ES+): m/z 408.0 (M+1).

5.16. 6-Chloro-2-(1-methyl-1*H*-imidazol-2-yl)-*N*-(2,2,2-trifluoroethyl)-5-(2,4,6-trifluorophenyl)pyrimidin-4-amine (20)

1-Methyl-2-imidazole carboxaldehyde (1.7 g, 15.4 mmol) was stirred in methyl alcohol (10 mL). *N,N*-Dimethylhydrazine (1.4 g, 23.3 mmol) was then added. The mixture was stirred for 5 h, and the resulting hydrazone solution was added dropwise into a solution of magnesium monoperoxyphtalate hexahydrate (23.9 g, 80%, 38.6 mmol) in methyl alcohol (20 mL) at 0 °C. The resulting reaction mixture was allowed to warm to room temperature overnight and concentrated. The residue was diluted with water, then extracted with methylene chloride (3×). The combined organic was washed with saturated sodium chloride, dried over magnesium sulfate and concentrated. The residue was purified by flash chromatography, eluting with 40% ethyl acetate in hexanes to give 1-methyl-2-imidazole carbonitrile as a yellow oil.

To methyl alcohol (10 mL) in a sealable tube was added sodium hydride (440 mg, 11 mmol) with stirring. 1-Methyl-2-imidazole carbonitrile (1.18 g, 11 mmol) was added. The mixture was stirred at room temperature for 20 h, and ammonium chloride (588 mg, 11 mmol) was added. The tube was then sealed and stirred at 80 °C for 8 h, and cooled to room temperature. The mixture was filtered, and the filtrate was concentrated. The residue was treated with 1% methyl alcohol in diethyl ether, and the precipitates were collected by filtration and dried to give 1.6 g of 1-methyl-2-imidazole carboxamidine hydrochloride as a gray solid. MS: m/z 125.2 (M+H).

According to the procedure used to prepare **10**, starting from 1-methyl-2-imidazole carboxamidine hydrochloride, **20** was obtained as a light yellow solid: mp >230 °C; ^{1}H NMR (DMSO-d_6) δ 4.04 (s, 3H), 4.25 (m, 2H), 7.10 (s, 1H), 7.42 (s, 1H), 7.46 (m, 2H), 7.82 (t, J = 6 Hz, 1H); MS (ES+): m/z 422.0 (M+1). Anal. Calcd for $C_{10}H_{16}ClF_6N_5{\cdot}0.25EtOAc{\cdot}0.15C_6H_{14}$: C, 47.08, H, 3.11, N, 15.34. Found: C, 47.44, H, 2.75, N, 15.04.

5.17. 6-Chloro-5-{2,6-difluoro-4-[3-(methylamino)propoxy]-phenyl}-2-pyrazin-2-yl-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl]-pyrimidin-4-amine (21)

To a solution of **8** (251 mg, 0.58 mmol) and 3-(methylamino)propan-1-ol (267 mg, 3.0 mmol) in dimethylsulfoxide (3 mL) at room temperature was added sodium hydride (60% in mineral oil, 120 mg, 3.0 mmol). The mixture was stirred at 60 °C for 2 h, cooled to room temperature, and partitioned between ethyl acetate and saturated sodium chloride. The organic layer was washed with saturated sodium chloride (3×), dried over magnesium sulfate, and concentrated. The residue was purified by flash chromatography over silica gel, eluting with a gradient of ethyl acetate to 50% methyl alcohol in ethyl acetate. Concentration provided 181 mg (62%) of **21** as a light tan solid: mp 48–50 °C. The product thus obtained was dissolved in methylene chloride and filtered. Into the filtrate was bubbled hydrogen chloride gas. Concentration provided 210 mg of hydrogen chloride salt of **21** as a red solid: mp 68–70 °C; ^{1}H NMR (DMSO-d_6) δ 1.35 (d, J = 8 Hz, 3H), 2.11 (m, 2H), 2.59 (t, J = 5 Hz, 3H), 3.06 (m, 2H), 4.19 (t, J = 6 Hz, 2H), 5.53 (m, 1H), 6.97 (m, 2H), 7.67 (d, J = 8 Hz, 1H), 8.75 (br s, 2H), 8.83 (m, 2H), 9.57 (s, 1H); MS (ES+): m/z 503.0 (M+1). Anal. Calcd for $C_{21}H_{20}ClF_5N_6O{\cdot}2.0HCl{\cdot}0.45EtOAc$: C, 44.50, H, 4.19, N, 13.66. Found: C, 44.83, H, 4.18, N, 14.00.

5.18. 6-Chloro-5-{2,6-difluoro-4-[3-(methylamino)propoxy]-phenyl}-2-pyridin-2-yl-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl]-pyrimidin-4-amine (22)

According to the procedure used to prepare **21**, starting from **9**, hydrogen chloride salt of **17** was obtained as a light yellow solid: mp 55–60 °C; ^{1}H NMR (DMSO-d_6) δ 1.36 (d, J = 7 Hz, 3H), 2.13 (m, 2H), 2.59 (t, J = 5 Hz, 3H), 3.04 (m, 2H), 4.20 (t, J = 6 Hz, 2H), 5.68 (m, 1H), 6.97 (m, 2H), 7.72 (d, J = 9 Hz, 1H), 7.83 (t, J = 6 Hz, 1H), 8.30 (t, J = 8 Hz, 1H), 8.58 (d, J = 8 Hz, 1H), 8.86 (d, J = 4 Hz, 1H), 8.92 (br s, 2H); MS (ES+): m/z 502.0 (M+1). Anal. Calcd for $C_{22}H_{21}ClF_5N_5O{\cdot}2.8HCl{\cdot}0.2EtOAc$: C, 44.06, H, 4.12, N, 11.27. Found: C, 44.32, H, 4.12, N, 11.37.

5.19. 6-Chloro-5-{4-[3-(dimethylamino)propoxy]-2,6-difluorophenyl}-*N*-(2,2,2-trifluoroethyl)-2,2′-bipyrimidin-4-amine (23)

According to the procedure used to prepare **21**, starting from **11**, **23** was obtained as an off-white solid: mp 134–136 °C; ^{1}H NMR (DMSO-d_6) δ 1.91 (m, 2H), 2.20(s, 6H), 2.43 (m, 2H), 4.12 (t, J = 6 Hz, 2H), 4.26 (m, 2H), 6.97 (d, J = 10 Hz, 2H), 7.68 (t, J = 5 Hz, 1H), 7.85 (t, J = 6 Hz, 1H), 9.02 (d, J = 5 Hz, 2H); MS (ES+): m/z 503.1 (M+1). Anal. Calcd for $C_{21}H_{20}ClF_5N_6O{\cdot}0.5EtOAc$: C, 50.51, H, 4.42, N, 15.36. Found: C, 50.24, H, 4.44, N, 15.27.

5.20. 6-Chloro-5-{4-[3-(dimethylamino)propoxy]-2,6-difluorophenyl}-2-pyrazin-2-yl-*N*-(2,2,2-trifluoroethyl)pyrimidin-4-amine (24)

According to the procedure used to prepare **21**, starting from **10**, hydrogen chloride salt of **24** was obtained as a yellow solid: mp 58–62 °C; ^{1}H NMR (DMSO-d_6) δ 2.18 (m, 2H), 2.80 (d, J = 5 Hz, 6H), 3.20 (m, 2H), 4.19 (t, J = 6 Hz, 2H), 4.33 (m, 2H), 7.00 (d, J = 9 Hz, 2H), 7.90 (t, J = 6 Hz, 1H), 8.86 (m, 2H), 9.57 (s, 1H), 10.37 (br s, 1H); MS (ES+): m/z 503.0 (M+1). Anal. Calcd for $C_{21}H_{20}ClF_5N_6O\cdot1.8HCl\cdot0.3EtOAc$: C, 44.82, H, 4.10, N, 14.12. Found: C, 44.80, H, 4.20, N, 14.22.

5.21. 6-Chloro-5-{4-[2-(dimethylamino)ethoxy]-2,6-difluorophenyl}-2-pyrazin-2-yl-*N*-(2,2,2-trifluoroethyl)pyrimidin-4-amine (25)

According to the procedure used to prepare **21**, starting from **10**, **25** was obtained as a white solid: mp 150–155 °C; ^{1}H NMR ($CDCl_3$) δ 2.34 (s, 6H), 2.78 (t, J = 6 Hz, 2H), 4.11 (t, J = 6 Hz, 2H), 4.37 (m, 2H), 5.11 (t, J = 6 Hz, 1H), 6.65 (m, 2H), 8.70 (d, J = 2 Hz, 1H), 8.78 (dd, J = 2, 1 Hz, 1H), 9.64 (d, J = 1 Hz, 1H); MS (ES+): m/z 489.2 (M+1). Anal. Calcd for $C_{20}H_{18}ClF_5N_6O\cdot0.2EtOAc$: C, 49.32, H, 3.90, N, 16.59. Found: C, 49.22, H, 3.56, N, 16.60.

5.22. 6-Chloro-5-{4-[4-(dimethylamino)butoxy]-2,6-difluorophenyl}-2-pyrazin-2-yl-*N*-(2,2,2-trifluoroethyl)pyrimidin-4-amine (26)

According to the procedure used to prepare **21**, starting from **10**, **26** was obtained as a white solid: mp 151–153 °C; ^{1}H NMR ($CDCl_3$) δ 1.70 (m, 2H), 1.87 (m, 2H), 2.29 (s, 6H), 2.38 (t, J = 7 Hz, 2H), 4.03 (t, J = 6 Hz, 2H), 4.35 (m, 2H), 5.18 (t, J = 6 Hz, 1H), 6.62 (m, 2H), 8.70 (d, J = 2 Hz, 1H), 8.78 (dd, J = 2, 1 Hz, 1H), 9.64 (d, J = 1 Hz, 1H); MS (ES+): m/z 517.2 (M+1). Anal. Calcd for $C_{22}H_{22}ClF_5N_6O\cdot0.2EtOAc$: C, 51.23, H, 4.45, N, 15.72. Found: C, 51.38, H, 4.21, N, 15.69.

5.23. 6-Chloro-2-(1*H*-imidazol-1-yl)-*N*-(2,2,2-trifluoroethyl)-5-(2,4,6-trifluorophenyl)pyrimidin-4-amine (29)

To a mixture of diethyl 2-(2,4,6-trifluorophenyl)malonate (580 mg, 2.0 mmol) and urea (360 mg, 6.0 mmol) in ethyl alcohol (10 mL) at room temperature was added sodium hydride (60% in mineral oil, 160 mg, 4.0 mmol). The mixture was then heated at 80 °C for 3 days, cooled to room temperature, and partitioned between ethyl acetate and 1 N hydrochloric acid. The aqueous layer was extracted with ethyl acetate, and the combined organic extracts are dried over magnesium sulfate, and concentrated. The residue was purified by flash chromatography over silica gel, eluting with a gradient of ethyl acetate to 10% methyl alcohol in ethyl acetate. Concentration provided 148 mg (29%) of 5-(2,4,6-trifluorophenyl)pyrimidine-2,4,6-triol as a light tan solid. MS: m/z 257.0 (M−H).

A mixture of 5-(2,4,6-trifluorophenyl)pyrimidine-2,4,6-triol (258 mg, 1.0 mmol) in phosphorous oxychloride (2.5 mL) and 2,6-lutidine (0.5 mL) was heated at 110 °C for 16 h. The excess phosphorous oxychloride was removed in vacuo, and the resulting residue was dissolved in a 1:1 mixture of methylene chloride and hexanes. The organic layer was filtered through hydrous magnesium silicate, and the filtrate was concentrated to provide 104 mg of crude 2,4,6-trichloro-5-(2,4,6-trifluorophenyl)pyrimidine (**27**) as a dark solid. A mixture of the above 2,4,6-trichloro-5-(2,4,6-trifluorophenyl)pyrimidine (**27**, 104 mg), imidazole (23 mg, 0.33 mmol), and potassium carbonate (92 mg, 0.66 mmol) in *N*,*N*-dimethylformamide (2 mL) is stirred at room temperature for 3 h. The mixture was partitioned between ethyl acetate and saturated sodium chloride. The organic layer was washed with saturated sodium chloride (4×), dried over magnesium sulfate, and concentrated. The residue was purified by flash chromatography over silica gel, eluting with a gradient of hexanes to 50% ethyl acetate in hexanes. Concentration provided 71 mg (21%, two steps) of **28** as a tan solid: mp 72–74 °C; ^{1}H NMR ($CDCl_3$) δ 6.88 (m, 2H), 7.20 (s, 1H), 7.87 (s, 1H), 8.61 (s, 1H); MS (ES+): m/z 345.2 (M+1). Anal. Calcd for $C_{13}H_5Cl_2F_3N_4$: C, 45.24, H, 1.46, N, 16.23. Found: C, 45.59, H, 1.71, N, 15.92.

A solution of **28** (35 mg, 0.10 mmol) and 2,2,2-trifluoroethylamine (50 mg, 0.5 mmol) in 2 mL of *N*,*N*-dimethylformamide was stirred at room temperature for 1 h. A saturated sodium chloride solution is added, and the product was extracted with ethyl acetate. The organic solution was washed with saturated sodium chloride (4×), dried over magnesium sulfate, and concentrated. The residue was purified by flash chromatography over silica gel, eluting with a gradient of 20% ethyl acetate in hexanes to 50% ethyl acetate in hexanes. Concentration provided 36 mg (88%) of **29** as a light tan solid: mp 168–170 °C; ^{1}H NMR ($CDCl_3$) δ 4.23 (m, 2H), 5.11 (t, J = 6 Hz, 1H), 6.91 (m, 2H), 7.14 (s, 1H), 7.83 (s, 1H), 8.54 (s, 1H); MS (ES+): m/z 408.2 (M+1). Anal. Calcd for $C_{15}H_8ClF_6N_5$: C, 44.19, H, 1.98, N, 17.18. Found: C, 44.15, H, 1.78, N, 16.83.

5.24. 6-Chloro-2-(1*H*-pyrazol-1-yl)-*N*-(2,2,2-trifluoroethyl)-5-(2,4,6-trifluorophenyl)pyrimidin-4-amine (30)

According to the procedure used to prepare **29**, starting from **27**, **30** was obtained as a light tan solid: mp 150 °C (decomposed); ^{1}H NMR ($CDCl_3$) δ 4.29 (m, 2H), 4.93 (t, J = 6 Hz, 1H), 6.50 (dd, J = 3, 1 Hz, 1H), 6.91 (m, 2H), 7.85 (d, J = 1 Hz, 1H), 8.54 (d, J = 3 Hz, 1H); MS (ES+): m/z 407.9 (M+1). Anal. Calcd for $C_{15}H_8ClF_6N_5$: C, 44.19, H, 1.98, N, 17.18. Found: C, 44.20, H, 1.80, N, 16.81.

5.25. 6-Chloro-2-(1*H*-pyrazol-1-yl)-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl)-5-(2,4,6-trifluorophenyl)pyrimidin-4-amine (31)

According to the procedure used to prepare **29**, starting from **27**, **31** was obtained as a light yellow solid: mp 200–205 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (d, J = 7 Hz, 3H), 4.679 (d, J = 9 Hz, 1H), 5.23 (m, 1H), 6.50 (dd, J = 3, 1 Hz, 1H), 6.90 (m, 2H), 7.85 (d, J = 1 Hz, 1H), 8.53 (d, J = 3 Hz, 1H); MS (ES+): m/z 422.0 (M+1). Anal. Calcd for $C_{16}H_{10}ClF_6N_5$: C, 45.57, H, 2.39, N, 16.61. Found: C, 45.97, H, 2.27, N, 16.40.

5.26. 6-Chloro-5-{4-[3-(dimethylamino)propoxy]-2,6-difluorophenyl}-2-(1*H*-imidazol-1-yl)-*N*-(2,2,2-trifluoroethyl)pyrimidin-4-amine (32)

To a solution of **29** (20 mg, 0.05 mmol) and 3-dimethylamino-1-propanol (103 m g, 1.0 mmol) in dimethylsulfoxide (3 mL) at room temperature was added sodium hydride (60% in mineral oil, 40 mg, 1.0 mmol). The mixture was stirred at 60 °C for 2 h, cooled to room temperature, and partitioned between ethyl acetate and saturated sodium chloride. The organic layer was washed with saturated sodium chloride (3×), dried over magnesium sulfate, and concentrated. The residue was purified by flash chromatography over silica gel, eluting with a gradient of ethyl acetate to 50% methyl alcohol in ethyl acetate. Concentration provided 16 mg (65%) of **32** as a light tan solid: mp 47–49 °C; ^{1}H NMR (DMSO-d_6) δ 1.87 (m, 2H), 2.16 (s, 6H), 2.37 (t, J = 7 Hz, 2H), 4.10 (t, J = 6 Hz, 2H), 4.30 (m, 2H), 6.94 (d, J = 10 Hz, 2H), 7.13 (s, 1H), 7.92 (s, 1H), 8.02 (t, J = 6 Hz, 1H), 8.61 (s, 1H); MS (ES+): m/z 491.1 (M+1). Anal. Calcd for $C_{20}H_{20}ClF_5N_6O\cdot0.25EtOAc$: C, 49.48, H, 4.60, N, 15.46. Found: C, 49.14, H, 4.25, N, 15.26.

5.27. 6-Chloro-5-{2,6-difluoro-4-[3-(methylamino)propoxy]phenyl}-2-(1*H*-pyrazol-1-yl)-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl)-pyrimidin-4-amine (33)

According to the procedure used to prepare **32** and **21**, starting from **31**, hydrogen chloride salt of **33** was obtained as a white solid: mp 110–115 °C; ^{1}H NMR ($CDCl_3$) δ 1.40 (d, J = 6 Hz, 3H), 2.45 (br s, 2H), 2.77 (br s, 3H), 3.24 (br s, 2H), 4.21 (br s, 2H), 4.99 (br s, 1H), 5.19 (br s, 1H), 6.49 (br s, 1H), 6.65 (d, J = 8 Hz, 2H), 7.86 (s, 1H), 8.51 (d, J = 2 Hz, 1H), 9.75 (br s, 2H); MS (ES+): m/z 491.0 (M+1). Anal. Calcd for $C_{20}H_{20}ClF_5N_6O{\cdot}1.2HCl{\cdot}0.35EtOAc$: C, 45.46, H, 4.2 8, N, 14.86. Found: C, 45.71, H, 4.32, N, 14.93.

5.28. Biological evaluation

The protocols for COLO 205 cytotoxicity assay,[18,19] KB, KB 8.5 and KB V1 cellular assays,[25] tubulin polymerization studies,[18,19] competitive tubulin binding studies,[18,19] and cell cycle analysis[18,19] have been reported.

5.29. Xenograft assay

The procedure has been reported.[18] To initiate tumors, cells were injected subcutaneously into the flanks of female NU/NU-Foxn1^{nu} mice from Charles River Laboratories (1×10^6 cells/animal for human H460 non-small cell lung carcinoma, 2×10^6 cells/animal for human LOX melanoma). When tumors attained a mass of about 100 mg, mice were staged, that is, randomized into treatment groups containing 5–10 animals each. Compound vehicles are given in Figure legends.

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