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Design and synthesis of a highly selective, orally active and potent anaplastic lymphoma kinase inhibitor (CH5424802)

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ABSTRACT

Anaplastic lymphoma kinase (ALK) receptor tyrosine kinase is considered an attractive therapeutic target for human cancers, especially non-small cell lung cancer (NSCLC). Our previous study revealed that 8,9-side-chains of 6,6-dimethyl-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole scaffold crucially affected kinase selectivity, cellular activity, and metabolic stability. In this work, we optimized the side-chains and identified highly selective, orally active and potent ALK inhibitor CH5424802 (**18a**) as the clinical candidate.

1. Introduction

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase belonging to the insulin receptor superfamily, which has recently emerged as an attractive target for cancer therapy. ALK was originally identified as a part of the fusion gene nucleophosmin (NPM)-ALK resulting from a t(2;5) chromosomal translocation in anaplastic large cell lymphomas (ALCL).[1] NPM-ALK is detected in approximately 75% of all ALK-positive ALCL and is implicated in the pathogenesis of ALCL.[2] Other ALK fusion genes have been identified in not only ALCL but also in inflammatory myofibroblastic tumor (IMT),[3] diffuse large B-cell lymphoma (DLBCL)[4] and non-small cell lung cancer (NSCLC).[5] Among them, a fusion gene of ALK with echinoderm micro-tubule-associated protein-like 4 (EML4) is considered to be the most remarkable discovery. The fusion gene was found to be a key oncogenic factor in EML4-ALK-positive NSCLC, like breakpoint cluster region-Abelson (BCR-ABL) in chronic myeloid leukemias (CML).[6] The fusion gene has been detected in approximately 5% of NSCLC patients.[7] Furthermore, genetic mutations and amplification of the ALK gene have been discovered to cause childhood neuroblastoma.[8,9] These findings have prompted drug discovery research of ALK inhibitors for treatment of various ALK-positive cancers. Recently, the U.S. Food and Drug Administration (FDA) has approved a MET/ALK dual inhibitor crizotinib (Pfizer Inc.) for treatment of patients with ALK-positive NSCLC.[10] Since we consider that a high selectivity is ideal for targeted cancer therapies in terms of safety, our discovery research has focused on finding an inhibitor with high ALK selectivity.

In the previous study, we identified a potent and selective ALK inhibitor **15c**, which has 6,6-dimethyl-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole scaffold, by chemical modification of HTS hit compound **1** (Fig. 1). Conversion of 3-ethoxy group into cyano group and oxygen atom at 5-position into nitrogen atom resulted in drastically improved inhibitory activity against ALK enzyme.[11] *N*-(Oxetan-3-yl)piperazine at 8-position contributed to the improved metabolic stability and its cationic nitrogen is crucial for potent anti-proliferative activity against NPM-ALK-positive cell-line, KARPAS-299.[12] Additionally, the substituent at 9-position of **15c**, which recognizes a particularly wide-open surface in the E_0 region[13] of ALK protein, improved kinase selectivity.[14] So far we have investigated the derivatives bearing side-chains with cationic nitrogen only at 8-position and their structural diversity was limited. Therefore, further exploration of side-chains is needed for the clinical candidate selection.

We report here the results of derivatization focused on side-chains at 8- and 9-positions. As a result, we identified the clinical candidate CH5424802 (**18a**),[15] which has more potent anti-proliferative activity and a more suitable pharmacokinetic profile than those of **15c**. Pharmacokinetics data of **18a** is also described in this paper.

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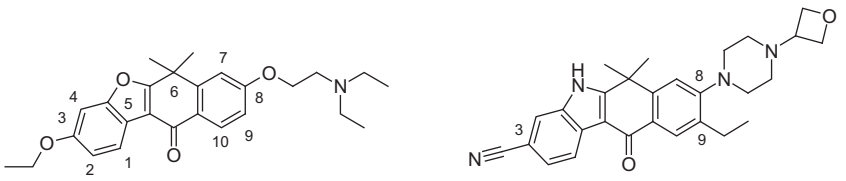


HTS hit compound 1

ALK IC_{50} = 1336 nM

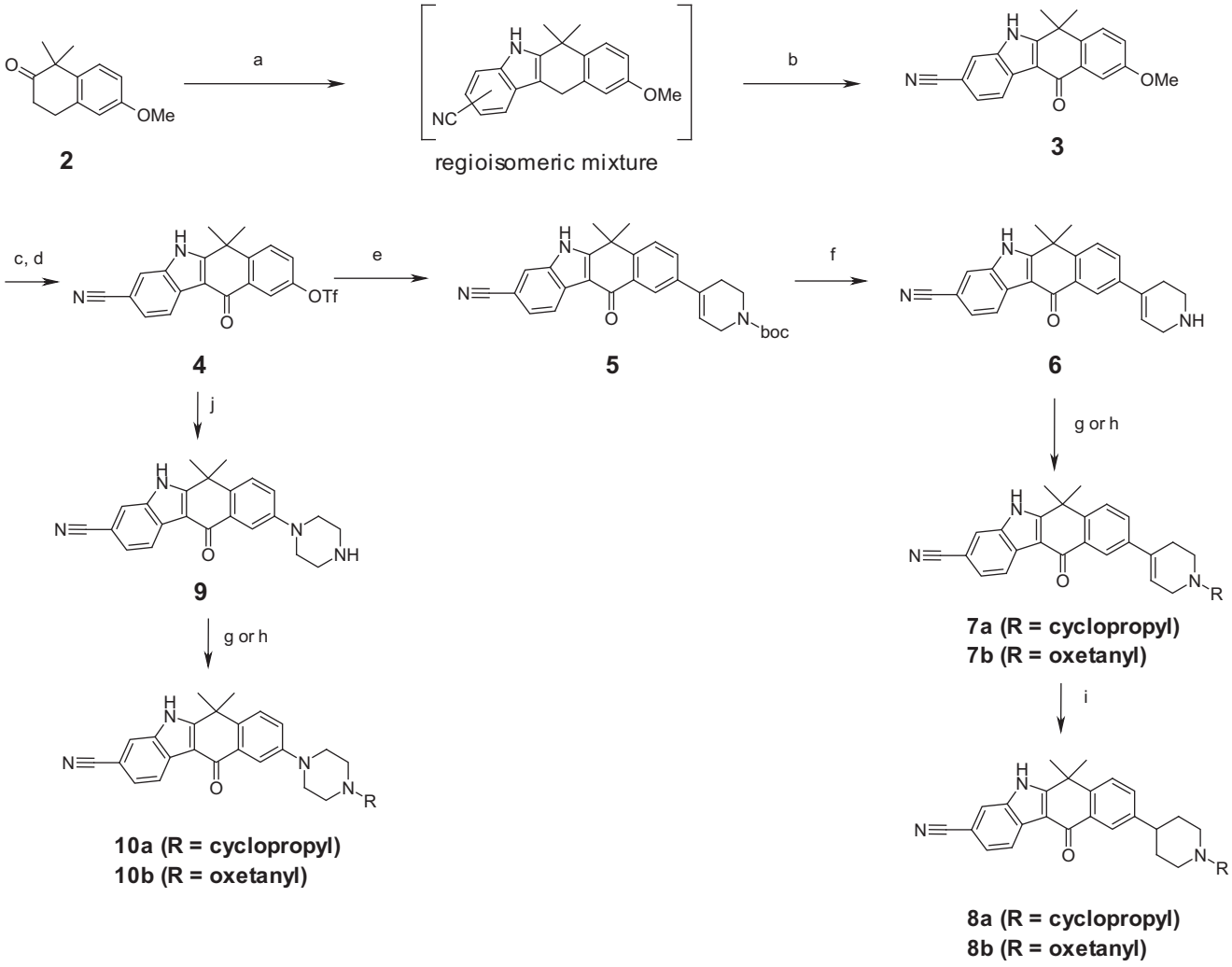
Optimized compound 15c

ALK IC_{50} = 2.9 nM
KARPAS-299 IC_{50} = 12.8 nM
F(monkey) = 28.2%

Figure 1. HTS hit and optimized compound.

2. Chemistry

The preparation of 9-monosubstituted derivatives from the known compound **2**[16] is shown in Scheme 1. The Fischer indole ring formation of **2** gave a 1:1 mixture of regio-isomers (1- and 3-CN derivatives). The crude mixture was treated with DDQ, and the desired isomer **3** was isolated by HPLC. A methoxy group at 9-position was converted into a triflate group by demethylation with pyridinium hydrochloride at 190 °C followed by treatment with triflic anhydride. The triflate **4** was used as a common intermediate. Compound **4** was treated with 3,6-dihydro-2*H*-pyridine-1-Boc-4-boronic acid pinacol ester under Suzuki coupling conditions at 80 °C to give



Scheme 1. Preparation of 9-substituted derivatives. Reagents and conditions: (a) 3-cyanophenylhydrazine hydrochloride, TFA, 90 °C; (b) DDQ, THF/H_2O, 0 °C; (c) pyridinium hydrochloride, 190 °C; (d) trifluoromethanesulfonic anhydride, pyridine, rt; (e) 3,6-dihydro-2*H*-pyridine-1-Boc-4-boronic acid pinacol ester, $Pd(PPh_3)_2Cl_2$, Na_2CO_3, DME/H_2O, 80 °C; (f) TFA, CH_2Cl_2, rt; (g) (1-ethoxycyclopropoxy)trimethylsilane, $NaBH_3CN$, AcOH, THF/2,2,2-trifluoroethanol, 60 °C; (h) 3-oxetanone, $NaBH_3CN$, AcOH, THF/MeOH, rt; (i) 10% Pd/C, H_2 gas, MeOH, rt; (j) piperazine, $Pd_2(dba)_3$, JohnPhos, K_3PO_4, DMA, 80 °C.

compound **5**. After removal of Boc group with trifluoroacetic acid, reductive alkylation with (1-ethoxycyclopropoxy)trimethylsilane or 3-oxetanone afforded corresponding *N*-cyclopropyl derivative **7a** and *N*-(oxetan-3-yl) derivatives **7b**, respectively. Hydrogenation of **7a** and **7b** with catalytic palladium on carbon gave 9-(piperidin-4-yl) derivatives **8a** and **8b**, respectively. In addition, common intermediate **4** was treated with piperazine under Buchwald–Hartwig coupling conditions at 80 °C to give compound **9**. Reductive alkylation of **9** under the same condition as that of **7a** and **7b** gave 9-(piperazin-4-yl) derivatives **10a** and **10b**, respectively.

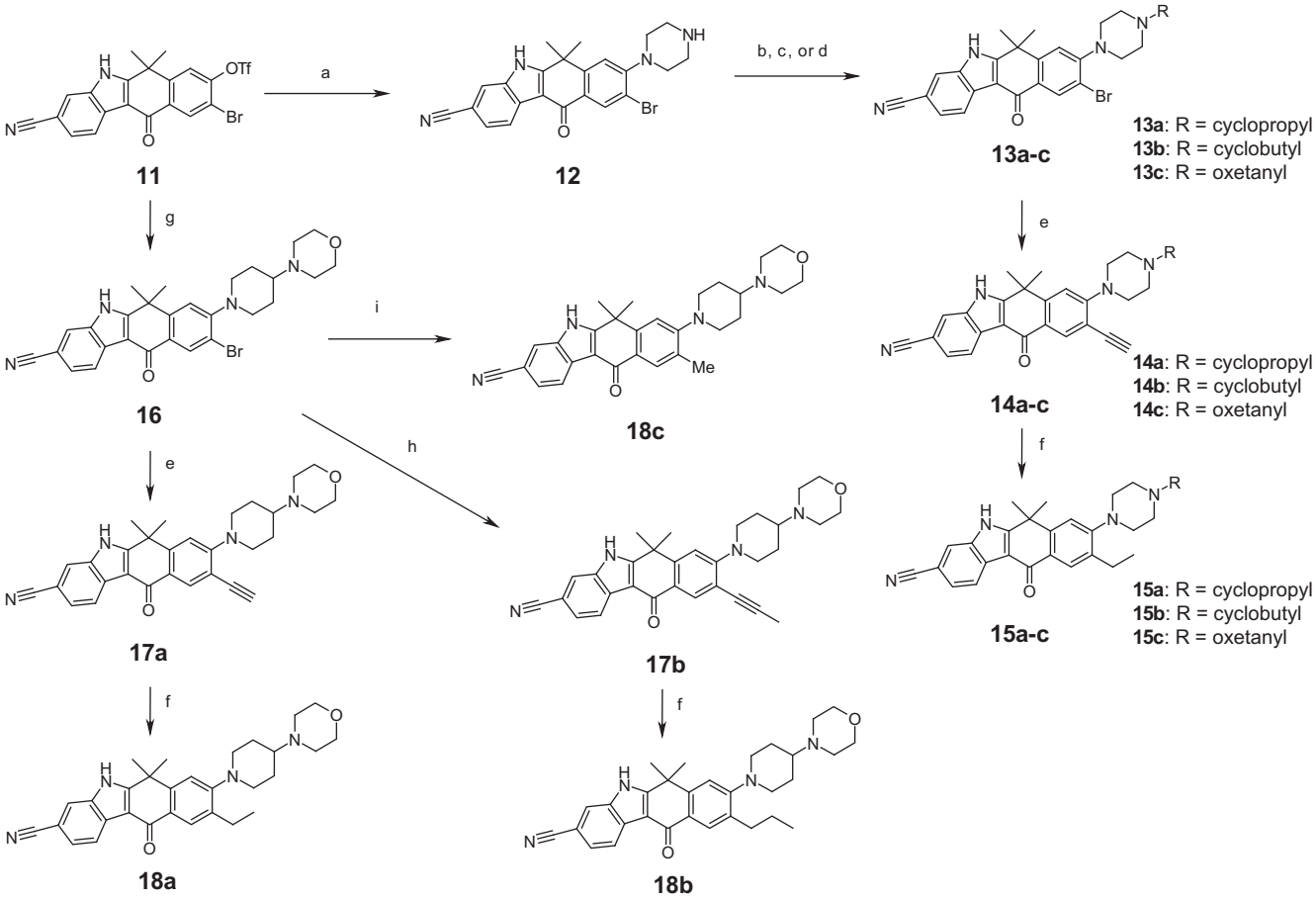
The preparation of 8,9-disubstituted derivatives from a versatile intermediate **11**[14,17] is shown in Scheme 2. The triflate **11** was treated with piperazine to give 8-(piperazin-4-yl) derivative **12**. The reductive alkylation of **12** with (1-ethoxycyclopropoxy)trimethylsilane, cyclobutanone or 3-oxetanone afforded corresponding N-alkylated compounds **13a**, **13b** and **13c**, respectively. N-alkylated derivatives **13a–c** were treated with TIPS-acetylene under conventional Pd-catalyzed cross-coupling conditions to give compounds **14a–c**. Hydrogenation of **14a–c** with catalytic palladium on carbon afforded the corresponding 9-ethyl derivatives **15a–c**. The intermediate **11** was treated with 4-morpholinopiperidine to give compound **16**. The derivatization of **16** at 9-position was conducted under Pd-catalyzed cross-coupling conditions. Compound **18c** was prepared with trimetylboroxine, $Pd(PPh_3)_4$ and base. Compounds **18a** and **18b** were prepared by a synthetic strategy similar to that of **15c**.

Compound **18d** was prepared from the reported compound **19**[11,17] by S_NAr reaction with 4-morpholinopiperidine. (Scheme 3)

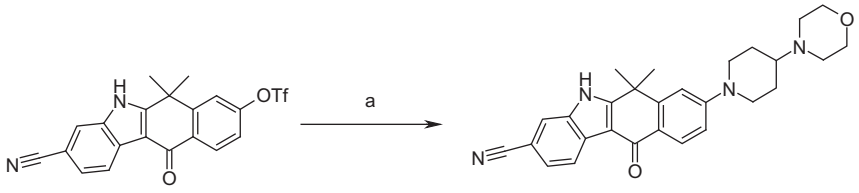
3. Results and discussion

Our previous study revealed that cationic nitrogen in a side-chain was essential for cellular activity, and substitution at 9-position improved kinase selectivity.[11,14] We expected that derivatives bearing a cationic side-chain at 9-position would achieve both high cellular activity and selectivity. Because *N*-(oxetan-3-yl)piperidin-4-yl and *N*-(oxetan-3-yl)piperazin-4-yl side-chains at 8-position showed high metabolic stability,[11] we prepared derivatives bearing the same side-chains at 9-position. In order to clarify the role of oxetane group, the corresponding *N*-cyclopropyl derivatives were also prepared. The inhibitory activity against ALK, other off-target kinases and KARPAS-299, as well as the in vitro clearance (CL) values in the presence of NADPH are summarized in Table 1.

As expected, 9-monostubtituted derivatives (**8a–b** and **10a–b**) showed very weak inhibitory activity against off-target proteins just like the previously identified 9-ethyl derivative **15c**. However, inhibitory activity against ALK was weaker than that of **15c**. The results showed that bulky side-chains like N-substituted piperidine (**8a–b**) and piperazine **(10a–b**) caused steric repulsion to amino acid residues of not only off-target proteins but also ALK in the E_0 region.[13] Thus, monosubstitution at 9-position was not able to realize both high cellular activity and selectivity. On the other hand, the comparable CL values of derivatives bearing *N*-cyclopropyl piperidine (**8a**) and piperazine (**10a**) suggested other side-chains than *N*-oxetanyl side-chains would be applicable. On the basis of this hypothesis, we prepared derivatives bearing various side-chains at 8-position with 9-ethyl group, which was the

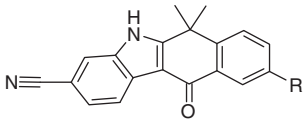


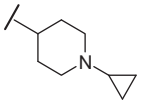
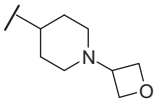
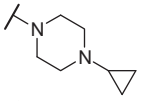
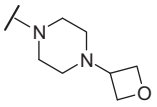
Scheme 2. Preparation of 8,9-disubstituted derivatives. Reagents and conditions: (a) piperazine, NMP, 120 °C; (b) (1-ethoxycyclopropoxy)trimethylsilane, $NaBH_3CN$, AcOH, THF/MeOH, 60 °C; (c) cyclobutanone, $NaBH(OAc)_3$, THF, rt; (d) 3-oxetanone, $NaBH_3CN$, AcOH, THF/MeOH, rt; (e) TIPS-acetylene, $Pd(CH_3CN)_2Cl_2$, X-Phos, Cs_2CO_3, CH_3CN, 80 °C, and then TABF, THF, rt; (f) 10%, Pd/C, H_2 gas, THF/MeOH, rt; (g) 4-morpholinopiperidine, NMP, 120 °C; (h) propyne gas, $Pd(CH_3CN)_2Cl_2$, X-Phos, Cs_2CO_3, CH_3CN, 80 °C; (i) trimethylboroxine, $Pd(PPh_3)_4$, K_2CO_3, DMF, 100 °C.



Scheme 3. Preparation of 8-substituted derivative (**18d**). Reagents and conditions: (a) 4-morpholinopiperidine, NMP, 120 °C.

Table 1
In vitro activity of 9-substituted derivatives



Compd	R	IC_{50} (nM)					CL (μL/min/mg)	
		ALK	KDR	KIT	MET	KARPAS-299	Human	Mouse
8a		44.0	882	>5000	>5000	121	59.1	20.0
8b		67.0	2521	>5000	>5000	86.9	38.6	38.6
10a		233	>5000	>5000	>5000	238	16.4	24.9
10b		69.3	>5000	>5000	>5000	151	14.2	20.6

optimum 9-substituent among 8-(*N*-oxetan-3-ly)piperazin-4-yl derivatives.[14] Their inhibitory activity against the four kinases and against KARPAS-299, and the in vitro metabolic stability are summarized in Table 2.

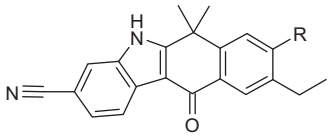
The prepared compounds (**15a–c** and **18a**) showed the same range of kinase selectivity and in vitro metabolic stability. Compared to 9-monosubstituted derivatives (Table 1), CL values of 8,9-disubstituted derivatives are relatively low. The result suggests that 9-ethyl group would block metabolism of 8-side-chain due to its bulkiness. Furthermore, unexpectedly, 4-morpholinopiperidin-1-yl derivative (**18a**) showed the strongest anti-proliferative activity against KARPAS-299. Since the derivatives **15a–c** and **18a** had similar Caco-2 cell permeability and solubility in FaSSIF (data not shown), the reason for strong anti-proliferative activity of **18a** is unclear. This finding of 4-morpholinopiperidin-1-yl side-chain prompted us to investigate a combination of this side-chain and various 9-alkyl groups as a final step in clinical candidate selection. The results are shown in Table 3.

The results indicate that the optimum substituent at 9-position is ethyl group (**18a**). Compound **18a** showed high target selectivity and the strongest anti-proliferative activity against KARPAS-299 out of all the derivatives we have prepared. In vitro metabolic stability of **18a** was very high. On the other hand, 9-unsubstituted derivative **18d** and 9-*n*-propyl compound **18b** had higher CL values than those of **18a**. In addition, 9-unsubstituted and 9-methyl derivatives (**18d** and **18c**) showed lower ALK selectivity than that of 9-ethyl derivative **18a**. These findings are consistent with previously reported SAR of derivatives bearing *N*-(oxetan-3-yl)piperazin-4-yl side-chain at 8-position.[14] Based on in vitro results, we selected **18a** as a final candidate and conducted further evaluation.

We have already reported kinase selectivity profile and in vivo efficacy of **18a** against ALK-positive ALCL and NSCLC xenograft models.[15] The kinase selectivity of compound **18a** was comparable to that of previously reported ALK inhibitor **15c**. Compound **18a** showed weak or no inhibition against 24 kinases except for ALK. Additionally, highly potent antitumor activity by oral administration of **18a** was observed in both the NPM-ALK-positive ALCL xenograft model (KARPAS-299, tumor growth inhibition (TGI) = 119% at 20 mg/kg) and the EML4-ALK-positive NSCLC xenograft model (NCI-H2228, TGI = 168% at 20 mg/kg).

When the PK properties of **18a** were determined in rats and monkeys, the compound demonstrated favorable plasma clearance and oral bioavailability in both species (Table 4). Compared to oral bioavailability of **15c** (F = 28.2%, in monkey), that of **18a** is considered to be more suitable for an oral drug. In principle, low oral bioavailability in animal studies leads to high variability of plasma concentrations in human. In addition, compared to **15c**, compound **18a** showed comparable in vivo efficacy at more than 10 times

Table 2
In vitro activity of 8-substituted-9-ethyl derivatives



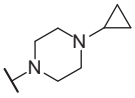
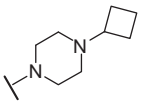
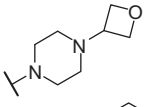
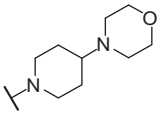
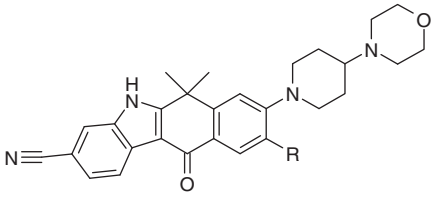
Compd	R	IC_{50} (nM)					CL (μL/min/mg)	
		ALK	KDR	KIT	MET	KARPAS-299	Human	Mouse
15a		5.2	4210	>5000	>5000	45.8	22.1	3.3
15b		2.4	3124	>5000	>5000	22.4	15.6	4.4
15c		2.9	>5000	>5000	>5000	12.8	17.0	4.5
18a (CH5424802)		1.9	1400	>5000	>5000	3.0	17.7	8.7

Table 3
In vitro activity of **18a** and neighboring compounds



Compd	R	IC_{50} (nM)					CL (μL/min/mg)	
		ALK	KDR	KIT	MET	KARPAS-299	Human	Mouse
18d	H	16.0	759	2991	>5000	26.5	106.4	72.0
18c	Me	1.1	186	1057	>5000	4.8	23.0	15.8
18a(CH5424802)	Et	1.9	1400	>5000	>5000	3.0	17.7	8.7
18b	*n*-Pr	1.1	>5000	>5000	>5000	26.1	17.1	58.5

lower AUC in mice (data not shown). The result indicates the strong potency of **18a**.

Finally, highly selective, orally active and potent **18a** was selected as the clinical candidate for the treatment of ALK-positive tumors. Overall, our derivatives showed no overt toxicity. Thus, toxicological parameters were not used as major criteria for candidate selection.

4. Conclusion

The chemical modification of **15c** focusing on side-chains resulted in the identification of CH5424802 (**18a**). The compound showed high kinase selectivity and strong anti-proliferative activity against KARPAS-299 with an IC_{50} value of 3.0 nM. Furthermore, oral administration of **18a** at 20 mg/kg displayed significant tumor regression without body weight loss in an established ALK fusion gene-positive NSCLC xenograft model in mice. The PK profile of **18a** in monkeys was favorable as a clinical candidate. Currently, **18a** is being evaluated in phase I/II clinical trials for the treatment of ALK-positive NSCLC.

5. Experimental

5.1. Chemistry

All solvents and reagents were obtained commercially. ^{1}H and ^{13}C NMR spectra were recorded on a VARIAN 400-MR or a JEOL JNM-EX270 spectrometer, and chemical shifts are expressed as δ units using tetramethylsilane as an internal standard. The spectral splitting patterns are described as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet; and bs, broad singlet peak. High resolution mass spectra (HRMS) were measured with a Thermo Fisher Scientific LTQ Orbitrap XL MS spectrometer using an ESI source coupled to a Waters HPLC system

Table 4
Pharmacokinetic profiles of CH5424802 (**18a**) in rats and monkeys

Species	F^c (%)	po			iv		
		AUC (ng·h/mL)	C_{max} (ng/mL)	$T_{1/2}$ (h)	CL_{tot} (mL/min/kg)	V_{dss} (L/kg)	$T_{1/2}$ (h)
Rat[a]	65.2	1400	60.8	12.6	7.79	9.33	17.8
Monkey[b]	50.4	696	55.8	8.38	6.04	5.28	10.4

[a] Dose: po and iv at 1 mg/kg ($n = 3$).
[b] Dose: po and iv at 0.5 mg/kg ($n = 2$).
[c] Oral bioavailability.

operating in reversed phase with an ACQUITY UPLC BEH C18 (1.7 μm, 2.1 × 50 mm) column. Flash column chromatography was performed with Biotage SNAP Cartridges or SILICYCLE SiliaSep packed columns. Preparative HPLC was conducted with a SunFire Prep C18 OBD (5 μm, 30 × 50 mm) column (Mobile phase, CH_3CN/H_2O with 0.05% TFA).

5.1.1. 9-Methoxy-6,6-dimethyl-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (3)

A mixture of **2** (2.70 g, 13.22 mmol), 3-cyanophenylhydrazine (2.11 g, 15.86 mmol), and trifluoroacetic acid (54 mL) was heated at 90 °C for 3.5 h. The reaction mixture was cooled, and then evaporated under reduced pressure. The residue was neutralized by saturated aqueous $NaHCO_3$, and extracted with AcOEt/THF. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dissolved in AcOEt/*n*-hexane (2:1) and the solution was maintained at room temperature for 14 h. A precipitate was removed by filtration, and a filtrate was evaporated under reduced pressure to give the desired compound roughly purified, which was used in the next reaction without further purification. To a solution of the compound in THF (20 mL) and water (2 mL) was added DDQ (3.0 g, 11.91 mmol). After stirring for 1.5 h at 0 °C, the reaction mixture was dissolved with CPME. The organic layer was washed with aqueous NaOH and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by preparative HPLC to give **3** (488.4 mg, 12% in two steps from **2**). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.79 (1H, s), 8.33 (1H, d, J = 8.2 Hz), 8.02 (1H, s), 7.81 (1H, d, J = 8.6 Hz), 7.69 (1H, d, J = 3.0 Hz), 7.63 (1H, dd, J = 8.3, 1.4 Hz), 7.28 (1H, dd, J = 8.7, 3.0 Hz), 3.87 (3H, s), 1.74 (6H, s); HRMS (ESI), *m/z* Calcd for $C_{20}H_{16}N_2O_2$+H: 317.1285, Found: 317.1287.

5.1.2. Trifluoro-methanesulfonic acid 3-cyano-6,6-dimethyl-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazol-9-yl ester (4)

A mixture of **3** (240 mg, 0.75 mmol) and pyridinium hydrochloride (1.75 g, 15.1 mmol) was stirred under microwave heating at 190 °C for 1 h. The reaction mixture was dissolved in water, and extracted with AcOEt. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was used in the next reaction without further purification. To a solution of the residue in pyridine (6.0 mL) was added trifluoro-methanesulfonic anhydride (316 μL, 2.27 mmol) at room temperature. After stirring for 1 h at room temperature, the reaction mixture was poured into water, and then extracted with AcOEt. The combined organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (*n*-hexane/AcOEt) to yield **4** (200 mg, 61% in two steps from **3**). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.95 (1H, s), 8.31 (1H, d, J = 8.2 Hz), 8.15 (2H, m), 8.05 (1H, s), 7.87 (1H, dd, J = 9.0, 2.7 Hz), 7.65 (1H, d, J = 8.2 Hz), 1.80 (6H, s); HRMS (ESI), *m/z* Calcd for $C_{20}H_{13}F_3N_2O_4S$+H: 435.0621, Found: 435.0620.

5.1.3. 4-(3-Cyano-6,6-dimethyl-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazol-9-yl)-3,6-dihydro-2*H*-pyridine-1-carboxylic acid tert-butyl ester (5)

A mixture of **4** (1.0 g, 2.30 mmol), 3,6-dihydro-2*H*-pyridine-1-Boc-4-boronic acid pinacol ester (0.78 g, 2.53 mmol), sodium carbonate (0.73 g, 6.91 mmol), $Pd(PPh_3)_2Cl_2$ (80.8 mg, 0.12 mmol), DME (20 mL), and water (4.0 mL) was stirred for 2 h at 80 °C under nitrogen atmosphere. The reaction mixture was then cooled, diluted with AcOEt, washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (*n*-hexane/AcOEt) to give **5** (1.07 g, quant). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.80 (1H, s), 8.34 (1H, d, J = 8.4 Hz), 8.22 (1H, s), 8.03 (1H, s), 7.87 (1H, d, J = 8.4 Hz), 7.79 (1H, d, J = 8.4 Hz), 7.64 (1H, d, J = 7.9 Hz), 6.24–6.33 (1H, m), 4.02–4.07 (2H, m), 3.56–3.61 (2H, m), 2.53–2.58 (2H, m), 1.77 (6H, s), 1.44 (9H, s); HRMS (ESI), *m/z* Calcd for $C_{29}H_{29}N_3O_3$+H: 468.2281, Found: 468.2285.

5.1.4. 6,6-Dimethyl-11-oxo-9-(1,2,3,6-tetrahydro-pyridin-4-yl)-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (6)

To a solution of **5** (1.07 g, 2.30 mmol) in CH_2Cl_2 (15 mL) was added trifluoroacetic acid (7.5 mL, excess amount). After stirring for 1 h at room temperature, the reaction mixture was evaporated under reduced pressure. The residue was dissolved with 4 M HCl in 1,4-dioxane. The resulting solution was evaporated under reduced pressure to give **6** as HCl salt. The salt was dissolved with water/THF, and then neutralized by anion-exchange resin cartridge (PL-HCO3 MP-Resin, VARIAN). The eluent was evaporated under reduced pressure to give **6** as a free form (828 mg, 98%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.90 (1H, s), 8.94 (1H, s), 8.33 (1H, d, J = 7.9 Hz), 8.26 (1H, s), 8.04 (1H, s), 7.92 (1H, d, J = 8.4 Hz), 7.83 (1H, d, J = 8.4 Hz), 7.64 (1H, d, J = 7.9 Hz), 6.30–6.36 (1H, m), 3.77–3.86 (2H, m), 3.33–3.43 (2H, m), 2.73–2.82 (2H, m), 1.78 (6H, s); HRMS (ESI), *m/z* Calcd for $C_{24}H_{21}N_3O$+H: 368.1757, Found: 368.1754.

5.1.5. 9-(1-Cyclopropyl-1,2,3,6-tetrahydro-pyridin-4-yl)-6,6-dimethyl-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (7a)

To a solution of **6** (100 mg, 0.27 mmol) in trifluoroethanol/THF/AcOH (3 mL/1.0 mL/0.1 mL) were added (1-ethoxycyclopropoxy)trimethylsilane (378 μL, 1.89 mmol) and sodium cyanoborohydride (84.8 mg, 1.35 mmol) at room temperature. After stirring for 4 h at 60 °C, the reaction mixture was cooled to room temperature, and neutralized by saturated aqueous $NaHCO_3$. The resulting mixture was extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by preparative HPLC to yield **7a** as a white solid (46 mg, 41%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.79 (1H, s), 8.33 (1H, d, J = 8.8 Hz), 8.20 (1H, s), 8.02 (1H, s), 7.84 (1H, d, J = 8.4 Hz), 7.77 (1H, d, J = 8.4 Hz), 7.63 (1H, d, J = 8.4 Hz), 6.26–6.31 (1H, m), 3.24–3.30 (2H, m), 2.82–2.87 (2H, m), 1.77 (6H, s), 0.44–0.50 (2H, m),

0.35–0.41 (2H, m); HRMS (ESI), *m/z* Calcd for $C_{27}H_{25}N_3O$+H: 408.2070, Found: 408.2067.

5.1.6. 6,6-Dimethyl-9-(1-oxetan-3-yl-1,2,3,6-tetrahydro-pyridin-4-yl)-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (7b)

To a solution of **6** (100 mg, 0.27 mmol) in THF/THF/AcOH (2.0 mL/2.0 mL/0.5 mL) were added 3-oxetanone (55 μL, 1.08 mmol) and sodium cyanoborohydride (33.9 mg, 0.54 mmol) at room temperature. After stirring for 3 h at room temperature, the reaction mixture was dissolved with water, and extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (CH_2Cl_2/AcOEt) to give **7b** as a light-yellow solid (79.6 mg, 69%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.81 (1H, bs), 8.34 (1H, d, J = 8.2 Hz), 8.22 (1H, d, J = 1.8 Hz), 8.03 (1H, s), 7.76–7.90 (2H, m), 7.64 (1H, dd, J = 8.2, 1.8 Hz), 6.25–6.34 (1H, m), 4.60 (2H, dd, J = 6.6, 6.0 Hz), 4.52 (2H, dd, J = 6.6, 6.0 Hz), 3.57 (1H, t, J = 6.0 Hz), 3.03 (2H, m), 2.55 (4H, m), 1.77 (6H, s); HRMS (ESI), *m/z* Calcd for $C_{27}H_{25}N_3O_2$+H: 424.2020, Found: 424.2016.

5.1.7. 9-(1-Cyclopropyl-piperidin-4-yl)-6,6-dimethyl-11-oxo- 6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (8a)

To a solution of **7a** (34.0 mg, 0.08 mmol) in MeOH (2.0 mL), 10% palladium on carbon (20 mg, 60% w/w) was added at room temperature. After being stirred vigorously under hydrogen gas for 4.5 h at room temperature, the reaction mixture was filtered through Celite. The filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (AcOEt/MeOH) to yield **8a** as a white solid (14.2 mg, 41%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.77 (1H, s), 8.33 (1H, d, J = 7.9 Hz), 8.05 (1H, s), 8.01 (1H, s), 7.80 (1H, d, J = 8.4 Hz), 7.62 (1H, d, J = 8.4 Hz), 7.59 (1H, d, J = 8.4 Hz), 3.02–3.10 (2H, m), 2.60–2.70 (1H, m), 2.24–2.36 (3H, m), 1.70–1.84 (7H, m), 1.55–1.69 (3H, m), 0.40–0.47 (2H, m), 0.29–0.36 (2H, m); HRMS (ESI), *m/z* Calcd for $C_{27}H_{27}N_3O$+H: 410.2227, Found: 410.2226.

5.1.8. 6,6-Dimethyl-9-(1-oxetan-3-yl-piperidin-4-yl)-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (8b)

Compound **8b** was prepared from **7b** following the same procedure as described for **8a** (56%, a white solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.78 (1H, s), 8.34 (1H, d, J = 8.4 Hz), 8.08 (1H, s), 8.02 (1H, s), 7.82 (1H, d, J = 8.4 Hz), 7.59–7.65 (2H, m), 4.56 (2H, t, J = 6.2 Hz), 4.47 (2H, t, J = 6.2 Hz), 3.59–3.62 (1H, m), 3.39–3.46 (1H, m), 2.79–2.86 (2H, m), 2.69–2.63 (1H, m), 1.79–1.94 (4H, m), 1.65–1.79 (7H, m); HRMS (ESI), *m/z* Calcd for $C_{27}H_{27}N_3O_2$+H: 426.2176, Found: 426.2178.

5.1.9. 6,6-Dimethyl-11-oxo-9-piperazin-1-yl-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (9)

A mixture of **4** (100 mg, 0.23 mmol), piperazine (59.4 mg, 0.69 mmol), K_3PO_4 (132 mg, 0.46 mmol), $Pd_2(dba)_3$ (10.5 mg, 0.01 mmol), JohnPhos (7.1 mg, 0.02 mmol), and DMA (2.0 mL) was stirred for 2.5 h at 80 °C under nitrogen atmosphere. The reaction mixture was then cooled, diluted with AcOEt and water, washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by preparative HPLC to give **9** as a yellow solid (22.9 mg, 27%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.06 (1H, bs), 8.33 (1H, d, J = 8.4 Hz), 8.01 (1H, s), 7.74 (1H, d, J = 8.8 Hz), 7.69 (1H, s), 7.36 (1H, d, J = 8.8 Hz), 3.28–3.36 (4H, m), 3.03–3.11 (4H, m), 1.74 (6H, s); HRMS (ESI), *m/z* Calcd for $C_{23}H_{22}N_4O$+H: 371.1866, Found: 371.1865.

5.1.10. 9-(4-Cyclopropyl-piperazin-1-yl)-6,6-dimethyl-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (10a)

Compound **10a** was prepared from **9** following the same procedure as described for **7a** (42%, a yellow solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.74 (1H, s), 8.33 (1H, d, J = 8.8 Hz), 8.01 (1H, s), 7.60–7.71 (3H, m), 7.32 (1H, d, J = 8.6 Hz), 3.15–3.22 (4H, m), 2.68–2.75 (4H, m), 1.72 (6H, s), 1.63–1.71 (1H, m), 0.43–0.49 (2H, m), 0.33–0.38 (2H, m); HRMS (ESI), *m/z* Calcd for $C_{26}H_{26}N_4O$+H: 411.2179, Found: 411.2176.

5.1.11. 6,6-Dimethyl-9-(4-oxetan-3-yl-piperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (10b)

Compound **10b** was prepared from **9** following the same procedure as described for **7b** (41%, a white solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.74 (1H, s), 8.33 (1H, d, J = 8.4 Hz), 8.01 (1H, s), 7.59–7.73 (3H, m), 7.34 (1H, d, J = 6.6 Hz), 4.58 (2H, t, J = 6.6 Hz), 4.49 (2H, t, J = 6.0 Hz), 3.43–3.50 (1H, m), 3.22–3.28 (4H, m), 2.41–2.47 (4H, m), 1.72 (6H, s); HRMS (ESI), *m/z* Calcd for $C_{26}H_{26}N_4O_2$+H: 427.2129, Found: 427.2122.

5.1.12. 9-Bromo-6,6-dimethyl-11-oxo-8-piperazin-1-yl-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (12)

To a solution of **11** (6.0 g, 11.7 mmol) in NMP (75 mL) was added piperazine (10.1 g, 117 mmol). After stirring for 30 min at 120 °C, the reaction mixture was cooled and poured into water to afford precipitation. The precipitate was filtered off and dried under reduced pressure to yield **12** as a white solid (3.9 g, 74%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.30 (1H, d, J = 7.9 Hz), 8.28 (1H, s), 8.00 (1H, s), 7.61 (1H, d, J = 7.9 Hz), 7.41 (1H, s), 3.32 (2H, bs), 3.01–3.10 (4H, m), 2.85–2.91 (4H, m), 1.76 (6H, s); HRMS (ESI), *m/z* Calcd for $C_{23}H_{21}BrN_4O$+H: 449.0972, Found 449.0973.

5.1.13. 9-Bromo-8-(4-cyclopropyl-piperazin-1-yl)-6,6-dimethyl-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (13a)

Compound **13a** was prepared from **12** following the same procedure as described for **7a** (25%, a light-yellow solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.22–8.30 (2H, m), 8.00 (1H, s), 7.56 (1H, d, J = 7.9 Hz), 7.43 (1H, s), 3.30(1H, d, J = 5.8 Hz), 3.11 (4H, s), 2.75 (4H, s), 1.75 (6H, s), 0.47 (2H, d, J = 5.8 Hz), 0.34 (2H, d, J = 5.8 Hz); HRMS (ESI), *m/z* Calcd for $C_{26}H_{25}BrN_4O$+H: 489.1285, Found: 489.1285.

5.1.14. 9-Bromo-8-(4-cyclobutyl-piperazin-1-yl)-6,6-dimethyl-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (13b)

To a solution of **12** (25 mg, 0.06 mmol) in THF (1.0 mL) were added cyclobutanone (14.2 μL, 0.19 mmol) and sodium triacetoxyborohydride (53.6 mg, 0.25 mmol) at room temperature. After stirring for 2.5 h at room temperature, the resulting mixture was filtered, and concentrated under reduced pressure. The residue was purified by preparative HPLC to yield **13b** as a white solid (10.9 mg, 34%).^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.23–8.29 (2H, m), 8.00 (1H, s),7.55 (1H, d, J = 7.9 Hz), 7.45 (1H, s), 4.04–4.15 (1H, m), 3.10–3.20 (4H, m), 2.39–2.48 (4H, m), 1.97–2.06 (2H, m), 1.78–1.88 (2H, m), 1.77 (6H, s), 1.61–1.72 (2H, m); HRMS (ESI), *m/z* Calcd for $C_{27}H_{27}BrN_4O$+H: 503.1441, Found: 503.1436.

5.1.15. 9-Bromo-6,6-dimethyl-8-(4-oxetan-3-yl-piperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (13c)

Compound **13c** was prepared from **12** following the same procedure as described for **7b** (73%, an off-white solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.83 (1H, s), 8.30 (1H, d, J = 7.9 Hz), 8.28 (1H, s), 8.02 (1H, s), 7.62 (1H, d, J = 7.9 Hz), 7.48 (1H, s), 4.56–4.61 (2H, m), 4.46–4.51 (2H, m), 3.47–3.56 (1H, m), 3.15–3.24

(4H, m), 2.44–2.54 (4H, m), 1.78 (6H, s); HRMS (ESI), *m/z* Calcd for $C_{26}H_{25}BrN_4O_2$+H: 505.1234, Found 505.1235.

5.1.16. 8-(4-Cyclopropyl-piperazin-1-yl)-9-ethynyl-6,6-dimethyl-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (14a)

Compound **14a** was prepared from **13a** following the same procedure as described for **14c** (19%, a light-yellow solid).

^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.76 (1H, bs), 8.31 (1H, d, J = 8.1 Hz), 8.15 (1H, s), 8.01 (1H, s), 7.61 (1H, dd, J = 8.1, 1.5 Hz), 7.24 (1H, s), 4.52 (1H, s), 3.28–3.36 (4H, m), 3.17 (1H, d, J = 5.3 Hz), 2.70–2.77 (4H, m), 1.76 (6H, s), 0.47 (2H, d, J = 5.3 Hz), 0.36 (2H, d, J = 5.3 Hz); HRMS (ESI), *m/z* Calcd for $C_{28}H_{26}N_4O$+H: 435.2179, Found: 435.2177.

5.1.17. 8-(4-Cyclobutyl-piperazin-1-yl)-9-ethynyl-6,6-dimethyl-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (14b)

Compound **14b** was prepared from **13b** following the same procedure as described for **14c** (57%, a light-yellow solid).^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.85 (1H,s), 8.31 (1H, d, J = 7.9 Hz), 8.20 (1H, s),8.03 (1H, s), 7.62 (1H, d, J = 7.9 Hz), 7.35 (1H, s), 4.62 (1H, s), 3.94–4.03 (2H, m), 3.79–3.89 (1H, m), 3.48–3.54 (2H, m), 3.27–3.38 (2H, m), 2.96–3.16 (2H, m), 2.30–2.41 (2H, m), 2.16–2.26 (2H, m), 1.72–1.85 (8H, m); HRMS (ESI), *m/z* Calcd for $C_{29}H_{28}N_4O$+H: 449.2336, Found: 449.2339.

5.1.18. 9-Ethynyl-6,6-dimethyl-8-(4-oxetan-3-yl-piperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (14c)

A mixture of **13c** (26.7 mg, 0.05 mmol), TIPS-acetylene (17.8 μL, 0.075 mmol), cesium carbonate (81.5 mg, 0.225 mmol), X-Phos (7.56 mg, 0.015 mmol), CH_3CN (1.0 mL), and $Pd(CH_3CN)_2Cl_2$ (1.37 mg, 0.005 mmol) was stirred for 4 h at 85 °C. The reaction mixture was then cooled, diluted with AcOEt, washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was used in the next reaction without further purification. To a solution of the residue in THF (2.0 mL) was added TBAF (74.2 μL, 0.25 mmol). After stirring for 1 h at room temperature, the reaction mixture was diluted with AcOEt, washed with water (×6) and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was triturated with MeOH (1.0 mL). The precipitated solid was filtered off and washed with *n*-hexane to afford **14c** as a brown solid (10.7 mg, 48 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.77 (1H, s), 8.31 (1H, d, J = 7.9 Hz), 8.16 (1H, s), 8.02 (1H, s), 7.61 (1H, d, J = 7.9 Hz), 7.27 (1H, s), 4.55–4.63 (2H, m), 4.46–4.53 (3H, m), 3.47–3.56 (1H, m), 3.35–3.43 (4H, m), 2.43–2.50 (4H, m), 1.78 (6H, s); HRMS (ESI), *m/z* Calcd for $C_{28}H_{26}N_4O_2$+H: 451.2129, Found 451.2127.

5.1.19. 8-(4-Cyclopropyl-piperazin-1-yl)-9-ethyl-6,6-dimethyl-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (15a)

Compound **15a** was prepared from **14a** following the same procedure as described for **15c** (97%, a brown solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.69 (1H, s), 8.32 (1H, d, J = 7.9 Hz), 8.04 (1H, s), 7.99 (1H, s), 7.60 (1H, d, J = 7.9 Hz), 7.36 (1H, s), 2.91–2.99 (4H, m), 2.69–2.78 (4H, m), 1.70–1.78 (7H, m), 0.43–0.52 (2H, m), 0.30–0.37 (2H, m); HRMS (ESI), *m/z* Calcd for $C_{28}H_{30}N_4O$+H: 439.2492, Found: 439.2491.

5.1.20. 8-(4-Cyclobutyl-piperazin-1-yl)-9-ethyl-6,6-dimethyl-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (15b)

Compound **15b** was prepared from **14b** following the same procedure as described for **15c** (76%, a brown solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.80 (1H, s), 8.32 (1H, d, J = 7.9 Hz), 8.10 (1H, s), 8.02 (1H, s), 7.62 (1H, d, J = 7.9 Hz), 7.38 (1H, s), 3.78–3.88 (1H, m), 3.79–3.89 (1H, m), 3.48–3.54 (2H, m), 3.40–3.47 (2H, m), 3.30–3.39 (2H, m), 3.02–3.24 (4H, m), 2.73 (2H, q, J = 7.3 Hz), 2.30–2.41 (2H, m), 2.17–2.26 (2H, m), 1.71–1.86 (8H, m), 1.29 (3H, t, J = 7.3 Hz); HRMS (ESI), *m/z* Calcd for $C_{29}H_{32}N_4O$+H: 453.2649, Found: 453.2648.

5.1.21. 9-Ethyl-6,6-dimethyl-8-(4-oxetan-3-yl-piperazin-1-yl)-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (15c)

To a solution of **14c** (34.0 mg, 0.08 mmol) in MeOH/THF (1.6 mL/2.4 mL), 10% palladium on charcoal (20 mg, 60% w/w) was added at room temperature. After being stirred vigorously under hydrogen gas for 2 h at room temperature, the reaction mixture was filtered through Celite. The filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH) to yield **15c** as a white solid (6.9 mg, 19%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.70 (1H, s), 8.29 (1H, d, J = 8.4 Hz), 8.05 (1H, s), 8.00 (1H, s), 7.61 (1H, d, J = 8.4 Hz), 7.38 (1H, s), 4.55–4.62 (2H, m), 4.45–4.52 (2H, m), 3.48–3.55 (1H, m), 2.98–3.05 (4H, m), 2.71 (2H, q, J = 7.5 Hz), 2.43–2.51 (4H, m), 1.74 (6H, s), 1.26 (3H, t, J = 7.5 Hz); HRMS (ESI), *m/z* Calcd for $C_{28}H_{30}N_4O_2$+H: 455.2442, Found 455.2440.

5.1.22. 9-Bromo-6,6-dimethyl-8-(4-morpholin-4-yl-piperidin-1-yl)-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (16)

To a solution of **11** (737 mg, 1.43 mmol) in NMP (12 mL) was added 4-morpholinopiperidine (1.05 g, 6.17 mmol). After stirring for 3 h at 120 °C, the reaction mixture was cooled and poured into water to afford precipitation. The precipitate was filtered off and dried under reduced pressure to yield **16** as a white solid (748 mg, 98%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.24–8.30 (2H, m), 8.00 (1H, s), 7.59 (1H, d, J = 8.2 Hz), 7.42 (1H, s), 3.45–3.66 (6H, m), 2.80 (2H, t, J = 11.1 Hz), 2.28–2.38 (1H, m), 1.87–1.96 (2H, m), 1.75 (6H, s), 1.56–1.66 (2H, m); HRMS (ESI), *m/z* Calcd for $C_{28}H_{29}BrN_4O_2$+H: 533.1547, Found: 533.1546.

5.1.23. 9-Ethynyl-6,6-dimethyl-8-(4-morpholin-4-yl-piperidin-1-yl)-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (17a)

Compound **17a** was prepared from **16** following the same procedure as described for **14c** (56%, an off-white solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.79 (1H, s), 8.31 (1H, d, J = 7.9 Hz), 8.15 (1H, s), 8.01 (1H, s), 7.61 (1H, d, J = 7.9 Hz), 7.25 (1H, s), 4.51 (1H, s), 3.85–3.95 (2H, m), 3.52–3.69 (4H, m), 2.85 (2H, t, J = 11.7 Hz), 2.28–2.40 (1H, m), 1.86–1.97 (2H, m), 1.77 (6H, s), 1.49–1.69 (2H, m); HRMS (ESI), *m/z* Calcd for $C_{30}H_{30}N_4O_2$+H: 479.2442, Found: 479.2442.

5.1.24. 6,6-Dimethyl-8-(4-morpholin-4-yl-piperidin-1-yl)-11-oxo-9-prop-1-ynyl-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (17b)

A mixture of **16** (100 mg, 0.18 mmol), cesium carbonate (274 mg, 0.84 mmol), X-Phos (13.4 mg, 0.03 mmol), CH_3CN (4.0 mL), and $Pd(CH_3CN)_2Cl_2$ (2.43 mg, 0.01 mmol) was stirred under propyne gas for 5 h at 80 °C. The reaction mixture was cooled, and then triturated with water. The precipitated solid was filtered off and washed with AcOEt/*n*-hexane to yield **17b** as a brown solid (66 mg, 72%).^{1}H NMR (270 MHz, CD_3OD+$CDCl_3$) δ: 8.40 (1H, d, J = 7.8 Hz), 8.24 (1H, s),7.84 (1H, s),7.54 (1H, d, J = 7.8 Hz), 7.14 (1H, s), 3.96–4.01 (2H, m), 3.78 (4H, m), 2.84–2.88 (2H, m), 2.68 (4H, m), 1.73–2.16 (5H, m), 2.16 (3H, s), 1.80 (6H, s); HRMS (ESI), *m/z* Calcd for $C_{31}H_{32}N_4O_2$+H: 493.2598, Found: 493.2595.

5.1.25. 9-Ethyl-6,6-dimethyl-8-(4-morpholin-4-yl-piperidin-1-yl)-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (CH5424802, 18a)

Compound **18a** was prepared from **17a** following the same procedure as described for **15c** (25%, a white solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.70 (1H, s), 8.32 (1H, d, J = 7.9 Hz), 8.04 (1H, s), 8.00 (1H, s), 7.61 (1H, d, J = 8.5 Hz), 7.34 (1H, s), 3.57–3.64 (4H, m), 3.18–3.27 (2H, m), 2.66–2.82 (4H, m), 2.28–2.39 (1H, m), 1.87–1.96 (2H, m), 1.76 (6H, s), 1.53–1.69 (2H, m), 1.29 (3H, t, J = 7.3 Hz); ^{13}C NMR (100 MHz, DMSO-d6) δ: 179.1, 160.0, 155.4, 146.8, 136.2, 135.7, 127.5, 125.8, 124.6, 121.5, 120.4, 119.9, 116.2, 114.4, 109.3, 104.3, 66.4, 60.9, 51.4, 49.3, 36.7, 30.0, 28.3, 22.6, 14.3; HRMS (ESI), m/z Calcd for $C_{30}H_{34}N_4O_2$+H: 483.2755, Found: 483.2754.

5.1.26. 6,6-Dimethyl-8-(4-morpholin-4-yl-piperidin-1-yl)-11-oxo-9-propyl-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (18b)

Compound **18b** was prepared from **17b** following the same procedure as described for **15c** (67%, a white solid). ^{1}H NMR (270 MHz, CD_3OD+$CDCl_3$) δ: 8.41 (1H, d, J = 7.8 Hz), 8.14 (1H, s), 7.84 (1H, s), 7.53 (1H, d, J = 7.8 Hz), 7.31 (1H, s), 3.77 (4H, m), 3.32 (2H, m), 2.66–2.86 (8H, m), 2.05–2.43 (3H, m), 1.79 (6H, s), 1.66–1.79 (4H, m), 1.02 (3H, t, J = 7.3 Hz); HRMS (ESI), m/z Calcd for $C_{31}H_{36}N_4O_2$+H: 497.2911, Found: 497.2904.

5.1.27. 6,6,9-Trimethyl-8-(4-morpholin-4-yl-piperidin-1-yl)-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (18c)

A mixture of **16** (40 mg, 0.07 mmol), trimethylboroxine (20.9 μL, 0.15 mmol), potassium carbonate (31.1 mg, 0.22 mmol), DMF (1.2 mL), and $Pd(PPh_3)_4$ (8.66 mg, 0.01 mmol) was stirred for 14 h at 100 °C. The reaction mixture was then cooled, diluted with AcOEt/THF, washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by preparative HPLC to give **18c** (9.2 mg, 28%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.70 (1H, bs), 8.30–8.33 (1H, d, J = 8.08 Hz), 8.00 (1H, s), 7.95 (1H, s), 7.58–7.61 (1H, m), 7.28 (1H, s), 3.60 (4H, m), 3.26–3.32 (2H, m), 2.69–2.79 (2H, m), 2.32 (3H, s), 1.90–1.95 (2H, m), 1.74 (6 H, s), 1.52–1.65 (2H, m); HRMS (ESI), m/z Calcd for $C_{29}H_{32}N_4O_2$+H: 469.2598, Found: 469.2602.

5.1.28. 6,6-Dimethyl-8-(4-morpholin-4-yl-piperidin-1-yl)-11-oxo-6,11-dihydro-5*H*-benzo[*b*]carbazole-3-carbonitrile (18d)

Compound **18d** was prepared from **19** following the same procedure as described for **16** (12%, a light-yellow solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.73 (1H, s), 8.27–8.31 (1H, m), 7.98–8.02 (1H, m), 7.95–7.97 (1H, m), 7.53–7.58 (1H, m), 7.17–7.21 (1H, m), 6.99–7.05 (1H, m), 3.97–4.05 (2H, m), 3.53–3.59 (4H, m), 2.80–2.90 (2H, m), 2.43–2.51 (4H, m), 2.31–2.40 (1H, m), 1.83–1.92 (2H, m), 1.74 (6H, s), 1.39–1.52 (2H, m); HRMS (ESI), m/z Calcd for $C_{28}H_{30}N_4O_2$+H: 455.2442, Found: 455.2443

5.2. In vitro kinase enzyme assay

ALK protein was purchased from Carna Biosciences. The inhibitory ability was evaluated by examining their ability to phosphorylate substrate peptide (Biotin-EGPWLEEEEEAYGWMDF) in the presence of 30 μM ATP and 10 mM $MgCl_2$ using time-resolved fluorescence resonance energy transfer (TR-FRET) assay. The quantity of enzyme and ATP and the kind of substrate and cation species added in each assay for evaluation of other kinases are given in Supplementary Table S1.

5.3. In vitro cell growth assay

KARPAS-299 cells were treated with various concentrations of assay compounds for 96 h. Cell growth inhibition was determined by Cell Counting Kit-8 assay.

5.4. Microsomal stability assay

One micromolar of each compound was incubated with human (or mouse) liver microsome (0.5 mg protein/mL) in 50 mM phosphate buffer (pH 7.4) containing 1 mM NADPH (the reduced form of nicotinamide adenine dinucleotide phosphate) at 37 °C for 30 min. After the enzyme reaction was terminated with the addition of a threefold volume of acetonitrile, the reaction mixture was centrifuged at 1500 rpm for 10 min. The resultant supernatant was used as a test sample to measure the stability in human (or mouse) liver microsome by quantitating the compound in the sample using LC/MS.

5.5. Determination of pharmacokinetic parameters in rats and monkeys

Sprague–Dawley rats (n = 3 per treatment group) were given **20a** which was dissolved in a vehicle of 10% *N*,*N*-Dimethylacetamide, 20% HP-β-CD (2-hydroxypropyl-β-cyclodextrin), and 0.02 M HCl in water by oral (po) or intravenous (iv) route at a dose of 1 mg/kg. Fasted cynomolgus monkeys (n = 2 per treatment group) were given **20a** which was dissolved in a vehicle of 10% DMSO, 20% HP-β-CD, and 0.02 M HCl in water by po or iv route at a dose of 0.5 mg/kg. Blood samples of each rat were collected with heparin as an anticoagulant at 0.08, 0.25, 1, 2, 4, 7, 24, 48, and 72 h following iv dosing and at 0.50, 1, 2, 4, 7, 24, 48, and 72 h following po dosing. Blood samples of each monkey were collected with heparin as an anticoagulant at 0.08, 0.25, 1, 2, 4, 7, 24, and 48 h following iv dosing and at 0.50, 1, 2, 4, 7, 24, and 48 h following po dosing. Samples were centrifuged and the plasma collected and stored at −80 °C until analysis. Samples were analyzed by LC–MS/MS technique. The pharmacokinetic parameters were calculated by noncompartmental analysis.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.12.021.

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