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Article

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Discovery of inhibitors that overcome the G1202R ALK Resistance Mutation

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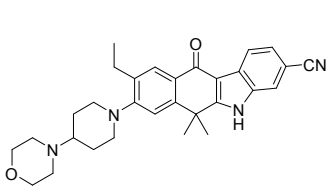
KEYWORDS. alectinib, anaplastic lymphoma kinase (ALK), echinoderm microtubule-associated protein-like 4 (eml4), non-small cell lung cancer (NSCLC)

ABSTRACT: The treatment of patients with advanced non-small cell lung cancer (NSCLC) harboring chromosomal rearrangements of anaplastic lymphoma kinase (ALK) has been revolutionized by the development of crizotinib, a small molecule inhibitor of ALK, ROS1, and MET. However, resistance to crizotinib inevitably develops through a variety of mechanisms leading to relapse both systemically and in the central nervous system (CNS). This has motivated the development of 'second generation' ALK inhibitors, including alectinib and ceritinib that overcome some of the mutations leading to resistance. However, most of the reported ALK inhibitors do not show inhibition of the G1202R mutant, which is one of the most common mutations. Herein, we report the development of a structural analogue of alectinib (JH-VIII-157-02) that is potent against the G1202R mutant as well as a variety of other frequently observed mutants. In addition, JH-VIII-157-02 is capable of penetrating the CNS of mice following oral dosing.

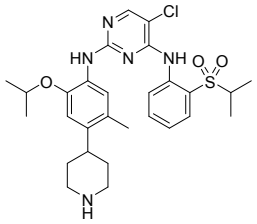
Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase in the insulin receptor superfamily. Expression of ALK in normal human tissue is limited to a subset of neural cell types[1]. No essential role for ALK has been found in mammals. ALK knockout mice live a full life span without any obvious abnormalities, but display a resistance to depressive phenotypes[2]. However, aberrent expression and hyperactivation of ALK due to translocations or point mutations have been shown to be oncogenic in a large variety of cancers[3]. Deregulation of ALK was first identified in anaplastic large cell lymphoma (ALCL), a subtype of non-Hodgkins lymphoma, in which the t(2;5)(p32;q35) chromosomal translocation involving the ALK gene was first described[4]. This translocation results in the fusion of nucleophosmin (NPM) to a truncated form of ALK, which results in a constitutive activation of the kinase domain leading to an "oncogene-addicted" state in several tumor types, including inflammatory myofibroblastic tumors (IMT)[5,6], diffuse large B cell lymphoma (DLBCL)[7], squamous cell carcinoma[8], and non-small-cell lung carcinoma (NSCLC)[9]. Germline mutations in ALK are the cause of the majority of hereditary neuroblastoma cases, and ALK activating mutations and/or gene amplifications are functionally relevant in high-risk sporadic neuroblastoma[10,11]. Lung cancers with ALK rearrangements are highly sensitive to ALK tyrosine kinase inhibition, further underscoring the notion that such cancers are addicted to ALK kinase activity. The multitargeted kinase inhibitor crizotinib was approved by the FDA in 2011 to treat patients with advanced NSCLC harboring ALK rearrangements[3]. However, despite a high response rate of 60% in ALK rearranged NSCLC, most patients develop resistance to crizotinib after 1 year of treatment[12,13]. In particular, the central nervous system (CNS) is one of the most common sites of relapse in patients with ALK-positive NSCLC[14,15]. Studies of lung cancers harboring ALK rearrangements with acquired resistance to crizotinib have identified ALK fusion gene amplification and secondary ALK kinase domain mutations in approximately one third of cases[16,17,18]. The most frequently identified secondary mutations are L1196M (gatekeeper mutation), G1269A, 1151T-ins, L1152R, C1156Y, G1202R, F1174L, and S1206Y[16,19,20,21,22,21,22]. While L1196M is the most frequently identified secondary mutation in crizotinib resistant patients (22%–36%)[16,18,23], the distribution changes dramatically in ceritinib and alectinib treated patients. ceritinib is significantly more potent against the majority of crizotinib resistant

mutants but is ineffective against G1202R and F1174C/V. Hence, those two mutants tend to be the only ones identified in ceritinib resistant patients. In one study, 20% of ceritinib resistant patients harbored the G1202R mutation only, 10% harbored the F1174C/V mutation only, 10% of patients harbored both G1202R and F1174C mutations, and 60% presented with a wild type ALK kinase domain[12]. Hence, in this study G1202R is the leading ALK secondary mutation causing resistance to ceritinib, accounting for 75% of all identified ALK secondary mutations in ceritinib resistant patients. As alectinib resistant patients slowly emerge, a similar profile to that of ceritinib resistance has been reported, identifying the G1202R and F1174V mutations in patients[24]. Several second generation ALK inhibitors have been developed in an attempt to overcome resistance due to these secondary mutants, however, the G1202R mutant confers resistance to all clinical stage ALK inhibitors[25] Table 1. Herein, we report the development of a structural analogue of alectinib (JH-VIII-157-02) that is potent against the G1202R mutant as well as the most common reported mutants, Table 1.

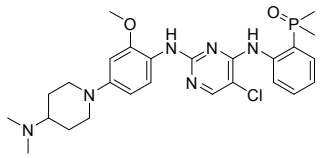
Table 1. Cell-based phospho-ALK IC50 Data of Common Secondary Alk Mutations for Clinical Alk Inhibitors



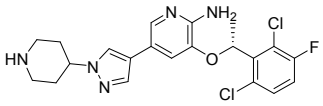
1 AF802 (Alectinib)



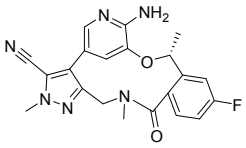
2 LDK378 (Ceritinib)



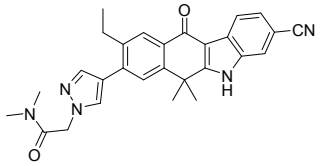
3 AP26113 (Brigatinib)



4 Crizotinib (Xalkori)



5 PF-06463922



6 JH-VIII-157-02

IC50 (nM)

Compd	EA wt	EA C1156Y	EA F1174L	EA L1196M	EA L1152R	EA 1151Tins	EA G1202R	EA G1269A	EA S1206Y	Untransduced Ba/F3
1 AF802 (Alectinib)	2	2	3	90	169	72	207	9	2	$>10^3$
2 LDK378 (Ceritinib)	41	164	101	64	2747	668	444	57	33	5512
3 AP26113 (Brigatinib)	5	3	12	3	2300	142	56	2	12	1921
4 Crizotinib (Xalkori)	56	144	81	549	645	857	328	512	65	927
5 PF-06463922*	1.3	1.6	0.2	21	9.0	38	77	15	4.2	N/A
6 JH-VIII-157-02	2	2	2	58	196	107	2	3	2	591

EA=EML4-ALK,*Published data[26]

EML4-ALK^{WT} or secondary mutant transformed Ba/F3 cells or untransduced Ba/F3 control were treated with ALK inhibitors in a dose escalation MTS assay and assessed for viability after 72 hours. Untransduced Ba/F3 cells served as a cytotoxicity control. Average IC50 values (n=3) are shown.

We chose to target the ALK G1202R mutant using the alectinib scaffold based on the co-crystal structure of alectinib and EML4-ALK (PDB: 3AOX)[27] Figure 1a. We conducted a molecular modeling study incorporating the 1202R mutation and found that the morpholino piperidine ring of alectinib is predicted to be in very close proximity to the G1202 residue, and therefore is likely responsible for the loss of activity in the G1202R mutant due to steric clash with the 1202R residue, Figure 1b. We imagined replacing the bulky morpholino piperidine ring with smaller substituents would avoid this steric clash and result in a potent inhibitor of the G1202R mutant. This coupled with the commercial availability of the tetracyclic core **37** provided a convenient entry point to the development of a potent inhibitor of the ALK G1202R mutant.

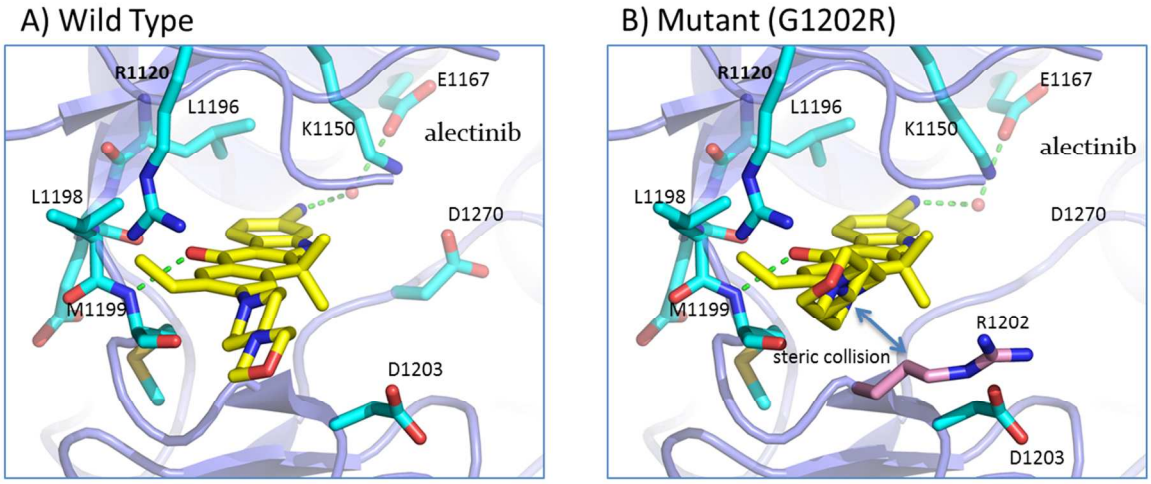


Figure 1. Co-crystal structure of **1** in complex with wild-type ALK and docked pose of **1** with a molecular model of ALK-G1202R. (A) Binding conformation of **1** (yellow stick) in the ATP binding site of ALK-wt (PDB 3AOX). Hydrogen bonds are indicated by dashed lines. (B) Binding conformation of **1** in the ATP binding site of ALK with a model of the G1202R mutation. Hydrogen bonds are indicated by dashed lines.

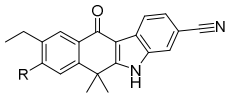
RESULTS AND DISCUSSION

To assess the potency of our newly synthesized compounds, we tested them against Ba/F3 cells in a single point inhibition assay at a concentration of 1 μM (Table 2). Compounds that showed potent inhibition of the G1202R mutant without showing potent inhibition of untransduced Ba/F3 cells in the single point inhibition assay were then taken forward and tested against a panel of the most common secondary ALK mutants to determine cellular phospho-ALK IC50's (Table 3).

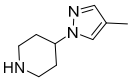
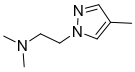
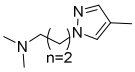
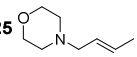
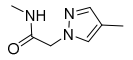
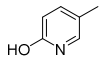
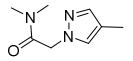
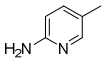
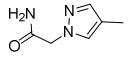
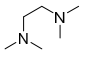
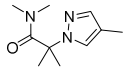
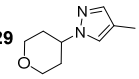
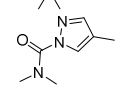
We began by replacing the morpholino piperidine ring with small aromatic heterocycles including pyrazoles (**8** and **9**), methyl pyrazole (**7**), isoxazole (**10**), furan (**11**), thiophene (**12**), triazole (**13**), pyridines (**14** and **15**), and pyrimidine (**16**). We hypothesized that these compounds might be active because they were smaller and possess a basic nitrogen capable of forming a hydrogen bonding with the guanidine moiety of the 1202R. Compounds **7**, **8** and **9** were quite potent against the G1202R mutant in a single point inhibition assay at a concentration of 1 μM. Compounds lacking a basic nitrogen (**11** and **12**) showed much less inhibitory activity. Compounds **7**, **8** and **9** were then tested against a panel of the most common secondary ALK mutants to obtain IC50s, where we found that these compounds were indeed potent against the G1202R mutant with IC50's ranging from 21 to 32 nM. However, they lost activity against all of the other mutants compared to alectinib. We then synthesized smaller Alkyl ring versions of the morpholino piperidine tail of alectinib, including dimethylamino piperidine (**17**), piperazine (**18**), methylpiperazine (**19**), and morpholine (**20**). We thought that by shortening the morpholino piperidine ring that we might be able to avoid the steric clash caused by the 1202R mutation while maintaining potency against the other common ALK mutants. Additionally, we introduced diols (**22** and **23**), the carboxylic acid (**24**), the cyano group (**21**) and the open-ring version of methylpiperazine (**29**) since they are even smaller than the pyrazoles and are potentially capable of forming hydrogen bonds with the guanidine moiety of the 1202R, however, these compounds were much less potent against the G1202R mutant in the single point inhibition assay. The diminished activity is likely due to a loss of structural rigidity compared to the aromatic heterocycles. The allylmorpholine derivative (**25**) was prepared with the rationale that the double bond would avoid steric clash with 1202R, while still possessing a solubilizing group, however, this compound showed no activity in the single point inhibition assay. Compounds **17** and **19** showed potent inhibition at 1 μM, however, when IC50's were calculated for these compounds, we observed a loss of potency against the G1202R mutant and an increase in potency against the other secondary mutants. This suggested that an aromatic ring was necessary to avoid steric clash with the 1202R. We then decided to make substituted pyridines (**26** and **27**) and substituted pyrazoles (**6**, **29-33, 35 and 36**) in order to avoid the steric clash from 1202R and introduce a group capable of forming additional hydrogen bonds to increase potency. The substituted pyrazoles (**6**, **29-33, 35 and 36**) were quite potent against the G1202R mutant at 1μM. Interestingly, compound **33** showed potent activity against untransduced Ba/F3 cells and therefore, IC50's were not calculated for this compound. Compound **6** was extremely potent against the G1202R mutant with an IC50 of 2 nM, which is a 100 fold increase in cellular potency compared to alectinib. The activity of compound **6** was further tested in Ba/F3 cells transformed with CD74-ROS or EGFR exon 19 deletion as specificity controls, untransduced Ba/F3 cells as cytotoxicity control and other common EML4-ALK crizotinib resistant secondary mutants, many of which are unavailable in primary cell lines (see supplementary information Figure 2 and Table 1). In addition to potently inhibiting the G1202R mutant, compound **6** exhibited improved potency against the L1152R mutant in

Ba/F3 cells. The potency and on-target inhibition of phospho-EML4-ALK by compound **6** was further confirmed by Western blotting in NIH-3t3 cells transformed with wild type EML4-ALK or its crizotinib resistant secondary mutants (Figure 2B-C), showing in particular the potent inhibitory activity against the G1202R mutant.

Table 2. Single Point Inhibition Assay of Alectinib Analogues against EML4-ALK^{WT}, EML4-ALK^{G1202R} and Untransduced Ba/F3 Cells



% Viability at 1 μM

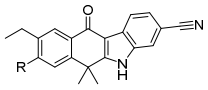
Compd	R	EA wt	EA G1202R	Parental Ba/F3	Compd	R	EA wt	EA G1202R	Parental Ba/F3	Compd	R	EA wt	EA G1202R	Parental Ba/F3	Compd	R	EA wt	EA G1202R	Parental Ba/F3
7		1	1	65	**14**		2	5	132	**22**		4	39	54	**30**		1	1	5
8		0	1	17	**15**		1	3	81	**23**		4	53	59	**31**		1	1	34
9		1	1	17	**16**		3	4	150	**24**	COOH	57	39	68	**32**		0	0	30
10		2	3	121	**17**		2	9	18	**25**		93	103	120	**33**		0	0	1
11		6	8	112	**18**		1	7	74	**26**		4	12	5	**6**		0	0	71
12		12	10	41	**19**		0.1	0.1	37	**27**		12	42	62	**34**		38	12	91
13		6	7	88	**20**		2	4	68	**28**		2	7	83	**35**		0	0	71
					21	CN	19	28	70	**29**		0	0.2	54	**36**		0	0	44

EA=EML4-ALK

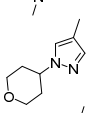
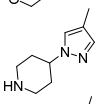
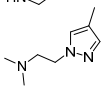
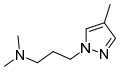
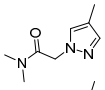
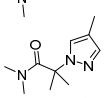
EML4-ALK^{WT} or EML4-ALK^{G1202R} transformed Ba/F3 cells or untransduced Ba/F3 control were treated with a single dose (1 μM) of alectinib analogues. Percent viability of untreated control for each compound was determined by MTS assay after 72 hours.

To better understand the structure activity relationships, we performed a molecular modeling study based upon the co-crystal structure of ALK with alectinib (PDB: 3AOX).[27] This study suggested that compound **6** makes the same backbone hinge contact as alectinib, however, compound **6** forms two additional hydrogen bond interactions between the guanidine moiety of R1120 and the carbonyl group of the dimethyl acetamide group Figure 3a. Furthermore, in the G1202R mutant, compound **6** forms an additional hydrogen bond interaction between the guanidine moiety of R1202 and the nitrogen of the pyrazole ring Figure 3b. The modeling study predicted that the methylene spacer between the pyrazole ring and the dimethylacetamide moiety is required for the carbonyl amide of **6** to interact with the guanidine moiety of R1120.

Table 3. IC50's of Select Alectinib Analogues Against a Panel of the Most Common Secondary EML4-ALK Mutants



IC50 (nM)

Compd	R	EA wt	EA C1156Y	EA F1174L	EA L1196M	EA L1152R	EA 1152Tins	EA G1202R	EA G1269A	EA S1206Y	Untrasnduced Ba/F3
7		61	150	84	370	5750	4409	21	200	104	1651
8		64	59	94	422	284	376	22	94	60	564
9		50	91	81	273	167	289	32	123	55	545
10		59	116	131	634	345	925	82	186	728	7324
13		115	177	188	629	345	821	80	256	1609	3014
17		2	2	2	10	695	53	214	3	2	6255
19		2	9	27	199	617	203	478	50	2	5646
29		78	58	105	535	781	620	20	177	53	924
30		40	24	87	262	297	161	37	146	29	768
31		2	24	54	80	548	77	8	11	6	1338
32		14	8	19	94	286	67	14	23	11	831
6		2	2	2	58	196	107	2	3	2	591
35		6	12	14	91	219	115	2	39	8	577
36		56	58	112	483	711	628	25	153	59	1647

EA = EML4-ALK

EML4-ALK^{WT} or secondary mutant transformed Ba/F3 cells or untransduced Ba/F3 control were treated with selected alectinib analogues in a dose escalation MTS assay and assessed for viability after 72 hours. Untransduced Ba/F3 cells served as a cytotoxicity control.

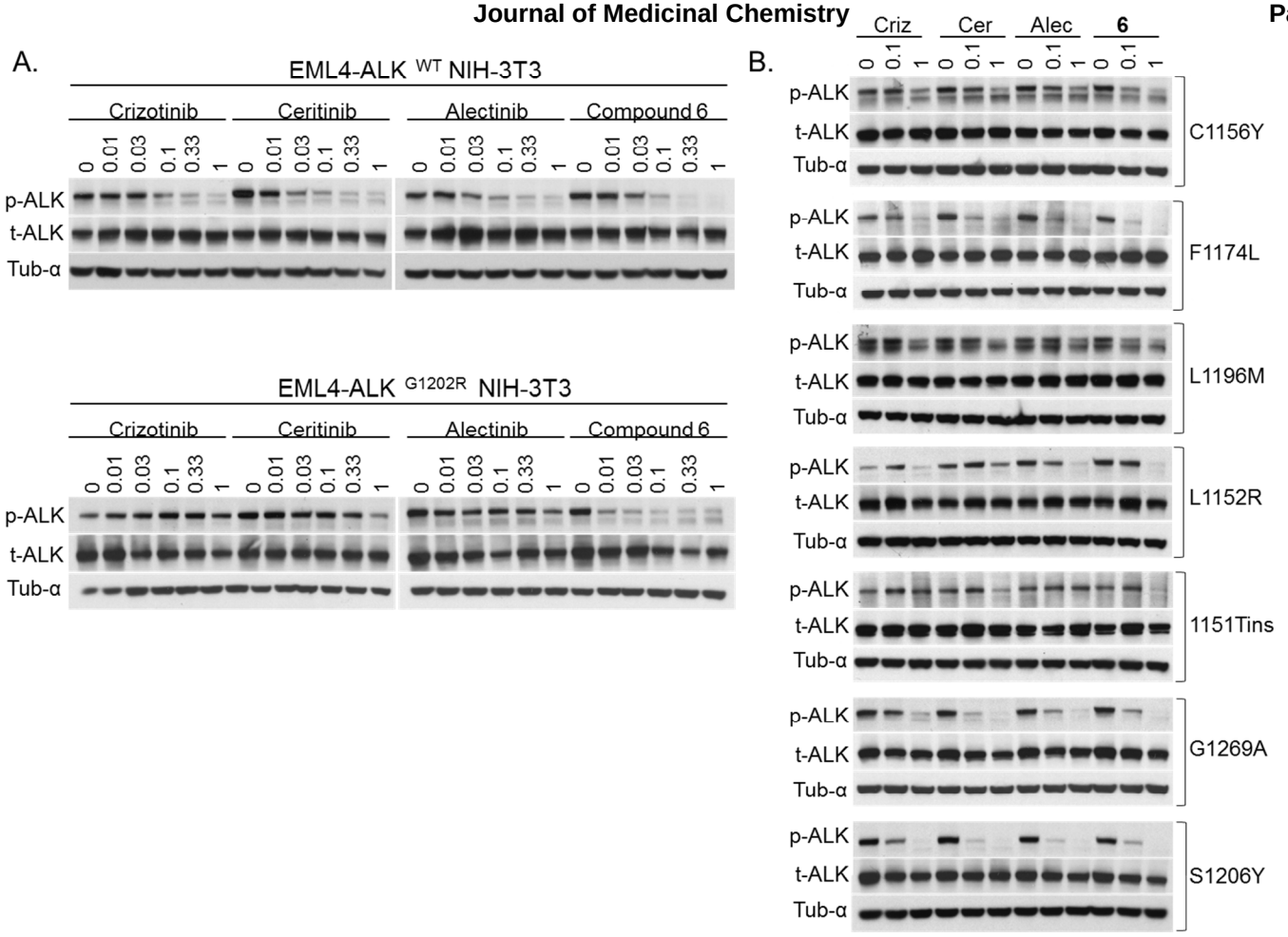


Figure 2. Western blotting in NIH-3t3 cells transformed with wild type EML4-ALK or its crizotinib resistant secondary mutants. (A) Western blotting of crizotinib, ceritinib, alectinib and Compound **6** against EML4-ALK ^{wt} NIH-3T3 and EML4-ALK ^{G1202R} NIH-3T3. (B) Western blotting of crizotinib, ceritinib, alectinib and Compound **6** against crizotinib resistant mutants.

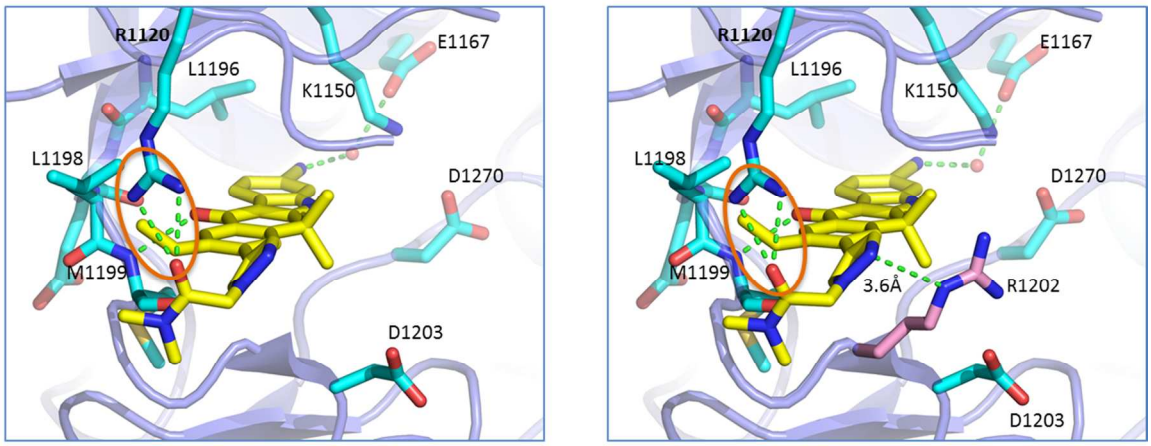


Figure 3. Molecular docking study of **6** with wild-type ALK and a model of G1202R ALK. (A) Binding conformation of **6** (yellow sticks) in the ATP binding site of ALK-wt (blue ribbon). Hydrogen bonds are indicated by dashed lines. (B) Predicted binding conformation of **6** (yellow sticks) in the ATP binding site of ALK-G1202R. Hydrogen bonds are indicated by dashed lines.

To evaluate the inhibitory activity of these new ALK kinase inhibitors against different ALK fusion and mutant ALK kinases, the most potent compounds (**31**, **32** and **6**) as well as the clinical stage ALK inhibitors alectinib, ceritinib (LDK378), AP26113 (Brigatinib) and crizotinib (Xalkori) were tested against a panel of cell lines derived from NSCLC (H3122, DFCI76,

and DFCI114) (Table 4, see also supplementary information Table 1, and figure 2), and neuroblastoma (Kelly, LAN-1, SH-SY5Y (F1174L), SK-N-SH (F1174L), LAN-5 (R1275Q), SMS-KCNR (R1275Q), CHLA-20 (R1275Q), SK-N-BE2 (wt), SK-N-FI (wt), and SK-N-AS (wt) Table 4 (see also supplementary information figure 3). These selected cell lines showed varied patterns of sensitivity to the growth inhibitory activity of **31**, **32** and **6**. This likely reflects a combination of the degree to which the antiproliferative activity is 'on-target' to ALK versus other targets of these compounds and the degree to which each of these cell lines are dependent upon ALK kinase activity. Compounds **6** and **32** possessed submicromolar EC50s across the entire panel of cell lines. Compound **6** showed a marked increase in potency against all of the neuroblastoma cell lines and the ALK TKI sensitive H3122 cells, and was comparable to alectinib against the L1152R EML4-ALK mutant DFCI76 cell line. The L1152R EML4-ALK mutant Ba/F3 cells were more potently inhibited by compound **6** than alectinib (see supplementary information Table 1, Figure 1) possibly due to the fact that in DFCI76 the EML4-ALK activity of compound **6** was masked by the activation of EGFR signaling, an additional known resistance mechanism in DFCI76. The mutant EGFR PC9 cell line was not inhibited by compound **6,** further demonstrating the on-target effect of this compound. Interestingly, compound **32** was more potent in the neuroblastoma cell lines than compound **6.** Further testing is underway to understand the difference in potencies between the two compounds.

Table 4. Antiproliferative EC50's of Compounds Tested Against a Panel of Nsclc and Neuroblastoma Cell Lines.

Cell Line	MYCN	Histology	EC50 (nM)						
			31	**32**	**6**	**1** Alectinib	**2** LDK378	**3** AP26113	**4** Crizotinib
H3122		NSCLC	13	9	5	9	15	5	32
DFCI76 (L1152R)		NSCLC	45	30	19	511	72	30	233
DFCI114 (G1269A)		NSCLC	535	863	419	207	18	9	1615
Kelly (F1174L)	Amplified	Neuroblastoma	164	91	147	434	142	127	211
LAN-1 (F1174L)	Amplified	Neuroblastoma	494	265	571	2004	549	2853	1346
SH-SY5Y (F1174L)	Non-Amplified	Neuroblastoma	451	264	413	1150	186	986	523
SK-N-SH (F1174L)	Non-Amplified	Neuroblastoma	252	161	245	872	303	1988	370
LAN-5 (R1275Q)	Amplified	Neuroblastoma	152	83	192	617	122	790	232
SMS-KCNR (R1275Q)	Amplified	Neuroblastoma	129	74	133	765	92	535	179
CHLA-20 (R1275Q)	Non-Amplified	Neuroblastoma	119	92	218	430	363	8667	439
SK-N-BE2 (wt)	Amplified	Neuroblastoma	1149	752	623	1554	593	2928	710
SK-N-FI (wt)	Non-Amplified	Neuroblastoma	914	567	973	2401	349	2645	1469
SK-N-AS (wt)	Non-Amplified	Neuroblastoma	871	465	775	2139	1045	776	1473

Cells were seeded at 4000 per well in 96 well plates and exposed to each compound in triplicate at 1 nM to 10 μM for 72 hours. Cell viability was evaluated using CellTiter-Glo Luminescent Cell Viability Assay (Promega) following manufacturer's instruction. IC_{50} values were calculated by nonlinear regression (variable slope) using GraphPad Prism 5 software. Each experiment was repeated for at least twice.

With the potent anti-proliferative activities of these new ALK inhibitors established, we assessed the selectivity of this scaffold using the KINOME*scan*™ methodology across a panel of 456 kinases (Ambit Biosciences, San Diego, CA)[28]. Compound **6** and **32** were screened at a concentration of 1 μM (see supporting information for complete profiling data). Both compounds were slightly less selective than the parent compound alectinib. Compound **6** was more selective than compound **32** with 34 interactions mapped compared to 39 with an S-score(1) = 0.06, which may explain the increase in cytotoxicity against the neuroblastoma cell lines. Figure 4. Dose – response analysis using compound **6** revealed inhibition of CSNK2A1 <10 μM, IRAK1 with an IC50 = 14 nM, IRAK 4 with an IC50 = 465 nM, CLK4 with an IC50 = 14 nM, RET with an IC50 = 3 nM, RET V804L with an IC50 = 13 nM, and RET V804M with an IC50 = 12 nM. Dose – response analysis using compound **32** revealed inhibition of CSNK2A1 <10 μM, IRAK1 with an IC50 = 15 nM, IRAK 4 with an IC50 = 234 nM, CLK4 with an IC50 = 4 nM, RET with an IC50 = 2 nM, RET V804L with an IC50 = 9 nM, and RET V804M with an IC50 = 23 nM.

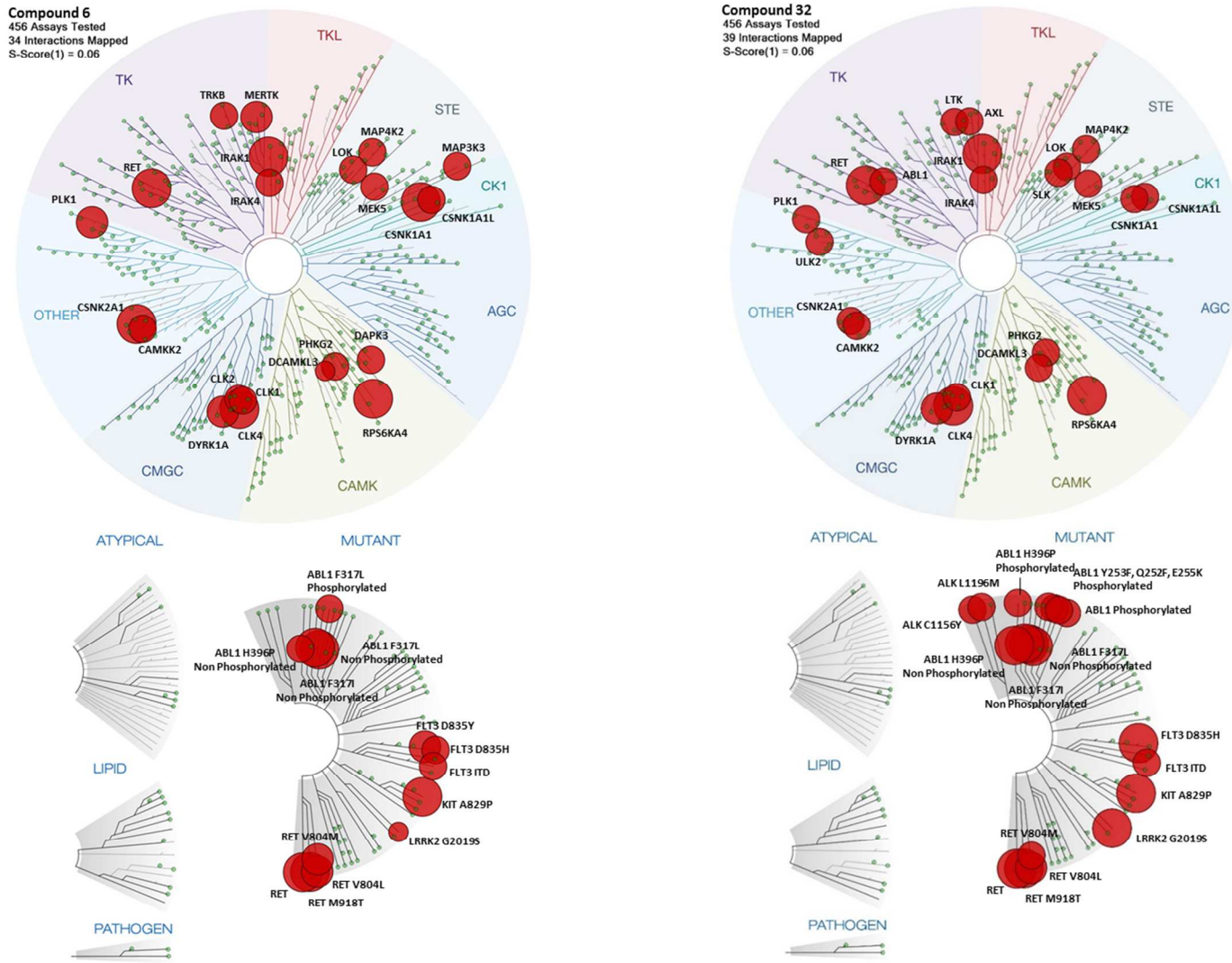


Figure 4. Ambit kinomescan selectivity results for compounds **6** and **32**.

The mouse pharmacokinetic profile of **6** demonstrated good oral bioavailability (87 %F), a half-life of 1.69 hours and a plasma exposure of 64,635 (min*ng/mL, AUClast) following an oral dose of 10 mg/kg (Table 5). Additionally, 2 hours after an oral dose of 10 mg/kg, **6** showed a plasma exposure of 0.34 µM, and a brain exposure of 0.03µM which equates to a low total brain/plasma concentration ratio of 0.1. Because compound **32** was more potent in neuroblastoma cell lines, we decided to evaluate its pharmacokinetic properties. Compound **32** showed lower oral bioavailability (26 %F), a half-life of 4.7 hours and a plasma exposure of 109,909 (min*ng/mL, AUClast) following an oral dose of 10 mg/kg (Table 5). Additionally, 2 hours after an oral dose of 10 mg/kg, 32 showed a plasma exposure of 0.21µM, and a brain exposure of 0.03µM which equates to a low total brain/plasma concentration ratio of 0.14.

Table 5. Pharmacokinetic properties of Compounds 6 and 32.

Compd	Matrix	Route	Dose (mg/kg)	$T_{1/2}$	T_{max} (hr)	C_{max} (ng/mL)	C_{max} (µM)	AUC_{last} (min*ng/mL)	AUC_{last} (µM.hr)	AUC_{INF_obs} (min*ng/mL)	AUC (%Extrap)	Cl_obs (mL/min/kg)	Vss_obs (L/kg)	%F
6	Plasma	i.v.	2	1.64	0.08	35250	75.71	150478	27.56	780621	1.74	3.55	0.38	-
		p.o.	10	1.69	0.83	373	0.8	655320	2.31	67036	3.66	154.83	-	87
32	Plasma	i.v.	2	3.06	0.08	1005	2.16	85807	3.07	96619	12.68	23.27	4.98	-
		p.o.	10	4.79	0.92	640	1.38	109909	3.94	154859	29.71	67.98	-	26

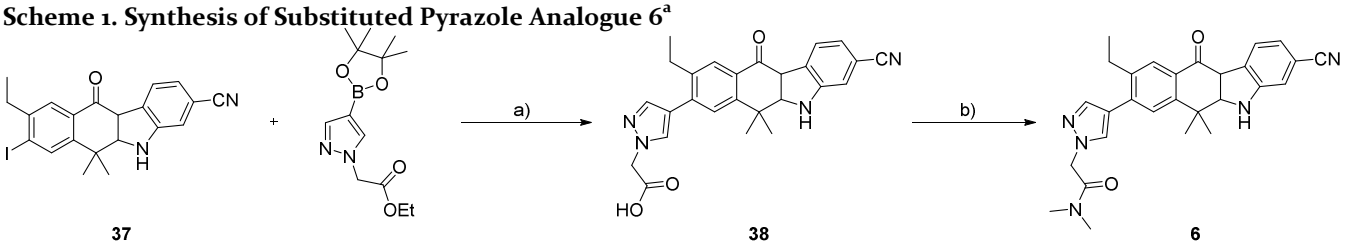
Table 6. In Vivo CNS Availability of Compounds 6 and 32.

Compd	Matrix	Route	Dose (mg/kg)	Time (hr)	Conc. (ng/mL)	Conc. (μM)	Compd	Matrix	Route	Dose (mg/kg)	Time (hr)	Conc. (ng/mL)	Conc. (μM)
6	Plasma	i.v.	2	2	960	2.06	**32**	Plasma	i.v.	2	2	100	0.21
		p.o.	10		159	0.34			p.o.	10		96	0.21
		i.v.	2	8	76	0.16			i.v.	2	8	22	0.05
		p.o.	10		16	0.04			p.o.	10		77	0.17
6	Brain	i.v.	2	2	19	0.04	**32**	Brain	i.v.	2	2	53.8	0.12
		p.o.	10		12	0.03			p.o.	10		12.1	0.03
		i.v.	2	8	2	0.004			i.v.	2	8	35.4	0.08
		p.o.	10		2	0.004			p.o.	10		28.1	0.06

CHEMISTRY

The synthetic route to compound **6** shown in scheme 1. The commercially available starting material **37** was subjected to Suzuki coupling conditions followed by ester hydrolysis to afford the carboxylic acid **38**. Compound **38** was then reacted with dimethylamine HCl and HATU to provide compound **6**. Compounds with an Alkyl heterocyclic ring substituent were prepared by subjecting **37** to Buchwald-Hartwig coupling conditions using the desired amine (scheme 2)

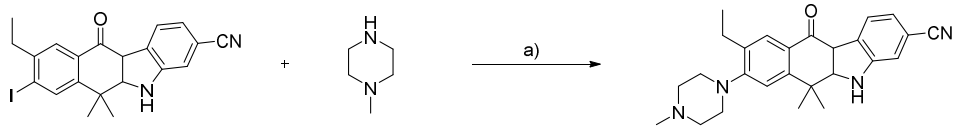
Scheme 1. Synthesis of Substituted Pyrazole Analogue 6[a]



37 **38** **6**

[a]Reagents and conditions: (a) 6 mol% Pd(Dppf)Cl2, 8 mol% *t*-BuXphos, 5 equiv 2M aq Na2CO3, 1,4-Dioxane 100 ° C, 1 h, then 2 equiv LiOH, H2O, rt; b) 1.2 equiv dimethylamine HCl, 2 equiv HATU, 5 equiv DIEA, DMF 54%

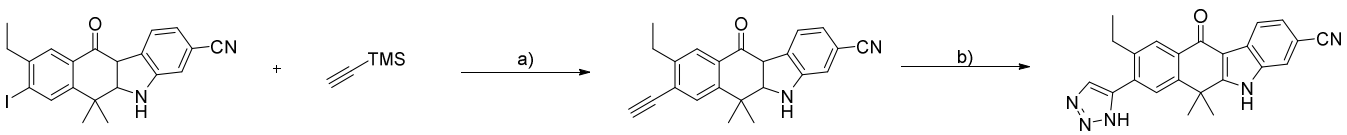
Scheme 2. Synthesis of Alkyl Heterocycle Analogue 19[a]



37 **19**

[a]Reagents and conditions: (a) 6 mol % Pd2(dba)3, 9 mol % Tri (o-tolyl), 8 equiv NaO*t*-bu, 1,4-Dioxane 110 ° C, 2 h, 48 %

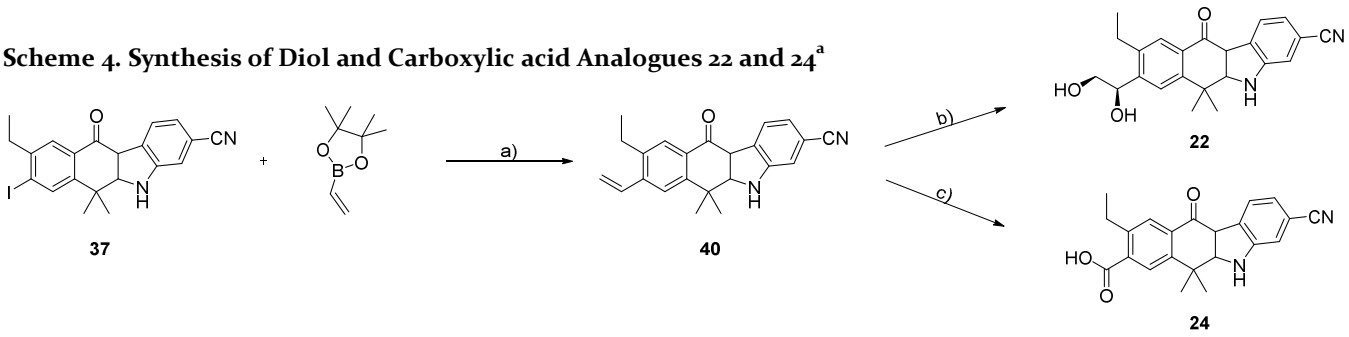
Scheme 3. Synthesis of Triazole Analogue 13[a]



37 **39** **13**

[a]Reagents and conditions: (a) 5 mol% Pd(OAc)2, 12 mol% PPh3, 11 mol % CuI, Et2NH, 90 ° C, 4 h, then 3 equiv TBAF, THF, 4 h; (b) 1.5 equiv TMS-N3, 5 mol % CuI, DMF, MeOH, 100 ° C, 4 h, 36%

Scheme 4. Synthesis of Diol and Carboxylic acid Analogues 22 and 24[a]



[a]Reagents and conditions: (a) 6 mol% Pd(Dppf)Cl2, 8 mol% t-BuXphos, 5 equiv 2M aq Na2CO3, 1,4-Dioxane 100 ° C, 1 h; (b) 5 mol% ad-mix β, *t*-BuOH, H2O, 0 ° C to rt, 12 h, 51 %; (c) 1 mol % OSO4, 4 equiv Oxone, rt, 6 h, 82 %.

CONCLUSION

By modifying the reported ALK inhibitor **1**, we have developed a novel ALK inhibitor that is extremely potent against the ALK G1202R mutant, which confers resistance to all clinical stage ALK inhibitors[25]. In addition, **6** remains potent against the most common secondary ALK mutants and displays strong inhibitory effects across a panel of clinically relevant NSCLC cell lines with ALK mutations. Furthermore, compound **6** displayed good pharmacokinetic properties suggesting the potential for this compound to be used in the treatment of NSCLC. From this study, we also identified compound **32**, which was slightly less potent against ALK G1202R and other secondary mutants, but displayed even stronger inhibitory effects in neuroblastoma cell lines.

EXPERIMENTAL METHODS

Starting materials and other reagents were purchased from commercial suppliers and were used without further purification unless otherwise noted. All reactions were monitored by thin layer chromatography (TLC) with 0.25 mm E. Merck pre-coated silica gel plates (60 F254) and Waters LCMS system (Waters 2489 UV/Visible Detector, Waters 3100 Mass, Waters 515 HPLC pump, Waters 2545 Binary Gradient Module, Waters Reagent Manager, Waters 2767 Sample Manager) using SunFireTM C18 column (4.6 x 50 mm, 5 µm particle size): solvent gradient = 100% A at 0 min, 1% A at 5 min; solvent A = 0.035% TFA in Water; solvent B = 0.035% TFA in CH3CN; flow rate : 2.5 mL/min. Purification of reaction products was carried out by flash chromatography using CombiFlash®Rf with Teledyne Isco RediSep®Rf High Performance Gold or Silicycle SiliaSepTM High Performance columns (4 g, 12 g, 24 g, 40 g, 80 g, or 120 g). The purity of all compounds was over 95% and was analyzed with Waters LCMS system. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Varian Inova-400 (400 MHz for 1H, and 75 MHz for 13C) spectrometer. Chemical shifts are reported relative to chloroform (δ = 7.24) for ^{1}H NMR or dimethyl sulfoxide (δ = 2.50) for ^{1}H NMR and dimethyl sulfoxide (δ = 39.51) for ^{13}C NMR. Data are reported as (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet).

9-ethyl-6,6-dimethyl-8-(1-methyl-1H-pyrazol-4-yl)-11-oxo-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (7)

1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (34 mg, 0.16 mmol) and **37** (60 mg, 0.13 mmol) were dissolved in 1,4-dioxane (5 mL) and 2M Na2CO3 sat. aq. solution (0.17 mL, 0.34 mmol) and thoroughly degassed. Pd(dppf)Cl2 (6 mg, 0.008 mmol) and t-Butyl XPhos (4 mg, 0.005 mmol) were added and mixture was heated to 100 ° C in a sealed vial. After stirring for 1 hour, LC-MS analysis indicated the reaction was finished. The reaction mixture was filtered through celite and purified by reversed-phase HPLC using a gradient of 30-100% CH3CN/H2O with 0.035% TFA to give the desired compound as a beige solid (33 mg, 61% yield). ^{1}H NMR (DMSO-d6, 400 MHz) δ 12.75 (s, 1H), 8.35 (d, J = 8 Hz, 1H), 8.11 (s, 1H) 8.07 (s, 1H), 8.01 (S, 1H), 7.79 (d, J = 8 Hz, 2H), 7.63 (d, J = 8 Hz, 2H), 3.93 (s, 3H), 2.82 (q, J = 7.2 Hz, 2H) 1.79 (s, 6H), 1.17 (t, J = 7.2 Hz, 3H), MS m/z 395.73 [M+1].

9-ethyl-6,6-dimethyl-11-oxo-8-(1H-pyrazol-4-yl)-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (8)

The procedure used to prepare compound **7** was used to prepare compound **8**. Purification by reversed-phase HPLC using a gradient of 40-100% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a beige solid (21 mg, 41% yield). ^{1}H NMR (DMSO-d6, 400 MHz) δ 13.6 (s, 1H), 8.34 (d, J = 8 Hz, 1H), 8.11 (s, 1H) 8.01 (s, 1H), 7.97 (s, 1H), 7.78 (s, 1H), 7.60 (d, J = 7.2 Hz, 1H), 3.46 (br, 1H), 2.85 (q, J = 7.2 Hz, 2H) 1.79 (s, 6H), 1.22 (t, J = 7.2 Hz, 3H), MS m/z 381.65 [M+1].

9-ethyl-6,6-dimethyl-11-oxo-8-(1H-pyrazol-3-yl)-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (9)

The procedure used to prepare compound **7** was used to prepare compound **9**. Purification by reversed-phase HPLC using a gradient of 40-100% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a beige solid (25 mg, 48% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.78 (s, 1H), 8.33 (d, *J* = 8 Hz, 1H), 8.14 (s, 1H), 8.01 (d, *J* = 8 Hz, 2H), 7.83 (s, 1H), 7.76 (s, 1H), 7.64 (d, J = 8 Hz, 1H), 6.89 (m, 1H), 2.81 (q, J = 8 Hz, 2H), 1.85 (s, 6H), 1.21 (t, J = 8 Hz, 3H) MS *m/z* : 381.73 (M + 1).

9-ethyl-8-(isoxazol-4-yl)-6,6-dimethyl-11-oxo-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (10)

The procedure used to prepare compound **7** was used to prepare compound **10**. Purification by reversed-phase HPLC using a gradient of 30-100% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a beige solid (31 mg, 60% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.87 (s, 1H), 9.25 (s, 1H), 9.02 (s, 1H), 8.31 (d, *J* = 8 Hz, 1H), 8.13 (s, 1H) 7.98 (s, 1H), 7.84 (S, 1H), 7.6 (d, J = 8 Hz, 1H), 2.75 (q, J = 7.2 Hz, 2H) 1.76 (s, 6H), 1.17 (t, J = 8 Hz, 3H), MS *m/z* : 382.43 [M+1].

9-ethyl-8-(furan-3-yl)-6,6-dimethyl-11-oxo-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (11)

The procedure used to prepare compound **7** was used to prepare compound **11**. Purification by reversed-phase HPLC using a gradient of 20-90% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a beige solid (37 mg, 71% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.81 (s, 1H), 8.34 (d, *J* = 8 Hz, 1H), 8.15 (s, 1H), 7.96 (s, 1H), 7.90, (s, 1H), 7.70 (s, 1H), 7.61 (d, *J* = 8 Hz, 1H), 7.43 (d, *J* = 8 Hz, 1H), 7.32 (d, *J* = 6 Hz, 1H), 2.81 (q, J = 8 Hz, 2H), 1.81 (s, 6H), 1.26 (t, J = 8 Hz, 3H) MS *m/z* : 381.48 (M + 1).

9-ethyl-6,6-dimethyl-11-oxo-8-(thiophen-2-yl)-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (12)

The procedure used to prepare compound **7** was used to prepare compound **12**. Purification by reversed-phase HPLC using a gradient of 20-90% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a beige solid (31 mg, 58% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.78 (s, 1H), 8.36 (d, *J* = 8 Hz, 1H), 8.15 (s, 1H), 8.02 (s, 1H), 7.71 (m, 2H), 7.65 (m, 2H), 7.32 (d, *J* = 6 Hz, 1H), 2.81 (q, J = 8 Hz, 2H), 1.78 (s, 6H), 1.21 (t, J = 8 Hz, 3H) MS *m/z* : 397.26 (M + 1).

9-ethyl-6,6-dimethyl-11-oxo-8-(1H-1,2,3-triazol-5-yl)-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (13)

Ethylnyltrimethylsilane (70 µL, 0.5 mmol) and **37** (200 mg, 0.45 mmol) were dissolved in Diethylamine (2 mL). Triphenylphosphine (15 mg, 0.054 mmol) and CuI (10 mg, 0.05 mmol) were added and the solution degassed. $Pd(OAc)_2$ (5 mg, 0.022 mmol) was added and the mixture heated to 90 °C for 4 h. The mixture was filtered through celite and purified by reversed-phase HPLC using a gradient of 40-100% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a white solid (131 mg, 70% yield). This material was dissolved in THF (5 mL) and TBAF 1M in THF (0.95 mL, 0.95 mmol) was added. The mixture was stirred for 5 h at rt. The reaction was quenched with water and extracted with EtOAc (3 X 50 mL) washed with brine, dried over $MgSO_4$, and condensed to give the Alkyne as a white solid in quantitative yield. TMS-Azide (30µL, 0.22 mmol) was added to a solution of the Alkyne **39** (50 mg, 0.15 mmol) and CuI (3 mg, 0.007 mmol) in a 9:1 mixture of DMF/MeOH (1 mL) and stirred at 100 °C for 4 h. The mixture was filtered through celite and purified by reversed-phase HPLC using a gradient of 40-100% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a white solid (20 mg, 36 % yield). (^{1}H NMR (DMSO-*d6*, 400 MHz) δ 13.15 (s, 1H), 12.06 (br, 1H), 8.31 (d, *J* = 8 Hz, 1H), 8.11 (s, 1H) 8.04 (s, 1H), 7.95 (s, 1H), 7.71 (s, 1H), 7.60 (d, *J* = 7.2 Hz, 1H), 2.91 (q, *J* = 7.2 Hz, 2H) 1.81 (s, 6H), 1.17 (t, *J* = 7.2 Hz, 3H), MS *m/z* 382.19 [M+1].

9-ethyl-6,6-dimethyl-11-oxo-8-(pyridin-4-yl)-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (14)

The procedure used to prepare compound **7** was used to prepare compound **14**. Purification by reversed-phase HPLC using a gradient of 20-90% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a beige solid (27 mg, 50% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.88 (s, 1H), 8.85 (m, 2H), 8.36 (d, *J* = 8 Hz, 1H), 8.23 (s, 1H) 8.04 (s, 1H), 7.78 (s, 1H), 7.65 (d, J = 7.2 Hz, 1H), 2.70 (q, J = 8 Hz, 2H) 1.79 (s, 6H), 1.13 (t, J = 8 Hz, 3H), MS *m/z* 392.31 [M+1].

9-ethyl-6,6-dimethyl-11-oxo-8-(pyridin-3-yl)-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (15)

The procedure used to prepare compound **7** was used to prepare compound **15**. Purification by reversed-phase HPLC using a gradient of 20-90% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a beige solid (30 mg, 57% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.85 (s, 1H), 8.78 (m, 2H), 8.36 (d, *J* = 8 Hz, 1H), 8.22 (s, 1H), 8.13 (d, *J* = 8 Hz, 1H), 8.03 (s, 1H), 7.77 (s, 1H), 7.73 (m, 1H), 7.65 (d, J = 7.2 Hz, 1H), 2.66 (q, *J* = 8 Hz, 2H) 1.79 (s, 6H), 1.13 (t, J = 8 Hz, 3H), MS *m/z* 392.48 [M+1].

9-ethyl-6,6-dimethyl-11-oxo-8-(pyrimidin-5-yl)-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (16)

The procedure used to prepare compound **7** was used to prepare compound **16**. Purification by reversed-phase HPLC using a gradient of 20-90% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a beige solid (26 mg, 49%

yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.83 (s, 1H), 9.28 (s, 1H), 8.95 (s, 2H), 8.36 (d, *J* = 8 Hz, 1H), 8.22 (s, 1H), 8.03 (s, 1H), 7.84 (s, 1H), 7.64 (d, *J* = 7.2 Hz, 1H), 2.67 (q, *J* = 8 Hz, 2H) 1.8 (s, 6H), 1.13 (t, J = 7.2 Hz, 3H), MS *m/z* 393.71 [M+1].

8-(4-(dimethylamino)piperidin-1-yl)-9-ethyl-6,6-dimethyl-11-oxo-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (17)

N,N-dimethylpiperidin-4-amine (30 mg, 0.15 mmol), NaO*t*-Bu (70 mg, 0.73 mmol) and **37** (40 mg, 0.09 mmol) were dissolved in 1,4-Dioxane (3 mL), and the mixture thoroughly degassed. Pd2(dba)3 (5 mg, 0.05 mmol) and tri(o-tolyl) (3 mg, 0.09 mmol) were added. The mixture was heated to 110 °C for 4 hours. LC-MS analysis showed conversion to the desired product. The mixture was filtered and purified by reversed-phase HPLC using a gradient of 10-60% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a brown solid (18 mg, 45% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.76 (s, 1H), 8.31 (d, *J* = 8 Hz, 2H), 8.04 (s, 1H) 7.98 (s, 1H), 7.58 (d, *J* = 8 Hz, 1H), 7.34 (s, 1H), 6.51 (s, 1H), 4.07 (m, 4H), 2.80 (s, 6H), 3.14 (m, 4H), 2.71 (q, J = 7.2 Hz, 2H) 1.74 (s, 6H), 1.23 (t, J = 8 Hz, 3H), MS *m/z* 382.43 [M+1].

9-ethyl-6,6-dimethyl-11-oxo-8-(piperazin-1-yl)-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (18)

The procedure used to prepare compound **17** was used to prepare compound **18**. Purification by reversed-phase HPLC using a gradient of 10-80% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a beige solid (13 mg, 36% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.81 (s, 1H), 8.29 (d, *J* = 8 Hz, 1H), 8.06 (s, 1H) 7.98 (s, 1H), 7.59 (d, *J* = 7 Hz, 1H), 7.35 (s, 1H), 3.14 (m, 4H), 2.71 (q, J = 7.2 Hz, 2H), 2.49 (m, 4H), 1.74 (s, 6H), 1.25 (t, J = 8 Hz, 3H), MS *m/z* 399.69 [M+1].

9-ethyl-6,6-dimethyl-8-(4-methylpiperazin-1-yl)-11-oxo-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (19)

The procedure used to prepare compound **17** was used to prepare compound **19**. Purification by reversed-phase HPLC using a gradient of 10-80% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a beige solid (11 mg, 28% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.75 (s, 1H), 8.27 (d, *J* = 8 Hz, 1H), 8.06 (s, 1H) 7.96 (s, 1H), 7.58 (d, *J* = 7 Hz, 1H), 7.35 (s, 1H), 3.21 (m, 4H), 2.71 (q, J = 7.2 Hz, 2H), 2.10 (m, 4H), 1.76 (s, 6H), 1.17 (t, J = 8 Hz, 3H), MS *m/z* 413.27 [M+1].

9-ethyl-6,6-dimethyl-8-morpholino-11-oxo-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (20)

The procedure used to prepare compound **17** was used to prepare compound **20**. Purification by reversed-phase HPLC using a gradient of 10-80% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a beige solid (14 mg, 38% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.76 (s, 1H), 8.31 (d, *J* = 8 Hz, 1H), 8.02 (s, 1H) 7.97 (s, 1H), 7.76 (d, J = 8 Hz, 1H), 7.58 (s, 1H), 7.34 (S, 1H), 3.78 (m, 2H), 2.98 (m, 2H) 2.71 (q, J = 7.2 Hz, 2H) 1.76 (s, 6H), 1.23 (t, J = 8 Hz, 3H), MS *m/z* 400.49 [M+1].

9-ethyl-6,6-dimethyl-11-oxo-6,11-dihydro-5H-benzo[b]carbazole-3,8-dicarbonitrile (21)

The procedure used to prepare compound **17** was used to prepare compound **21**. Purification by reversed-phase HPLC using a gradient of 20-80% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a beige solid (14 mg, 45% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.92 (s, 1H), 8.44 (s, 1H), 8.33 (d, *J* = 8 Hz, 1H), 8.24 (s, 1H) 8.05 (s, 1H), 7.66 (d, *J* = 8 Hz, 1H), 2.92 (q, *J* = 7.2 Hz, 2H), 1.79 (s, 6H), 1.31 (t, *J* = 8 Hz, 3H), MS *m/z* 340.53 [M+1].

9-ethyl-6,6-dimethyl-11-oxo-8-vinyl-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (40)

The procedure used to prepare compound **7** was used to prepare compound **40** (32 mg, 68% yield)

(R)-8-(1,2-dihydroxyethyl)-9-ethyl-6,6-dimethyl-11-oxo-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (22)

Ad-mixβ (206 mg) and **40** (50 mg, 0.15 mmol) were dissolved in a 0 °C solution of H_2O (3 mL) and *t*-BuOH. The mixture was slowly warmed to rt and stirred for 12 h at rt. LC-MS analysis showed complete conversion of the starting material to the desired product. The reaction mixture was filtered and purified by reversed-phase HPLC using a gradient of 20-70% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a white solid (28 mg, 51% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.75 (s, 1H), 8.32 (d, *J* = 8 Hz, 1H), 7.99 (m, 2H), 7.85 (s, 1H), 7.63 (d, *J* = 8 Hz, 1H), 5.38 (d, *J* = 4 Hz, 1H), 4.87 (m, 2H), 3.48 (m, 2H), 2.75 (m, 2H), 1.75 (s, 6H), 1.25 (t, *J* = 7 Hz, 3H), MS *m/z* 375.74 [M+1].

(S)-8-(1,2-dihydroxyethyl)-9-ethyl-6,6-dimethyl-11-oxo-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (23)

The procedure used to prepare compound **22** was used to prepare compound **23** using Ad-mix α instead of Ad-mix β**.** Purification by reversed-phase HPLC using a gradient of 20-70% CH_3CN/H_2O with 0.035% TFA gave the desired compound as a white solid (24 mg, 44% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.82 (s, 1H), 8.34 (d, *J* = 8 Hz, 1H), 8.03 (m, 2H), 7.85 (s, 1H), 7.63 (d, *J* = 8 Hz, 1H), 5.40 (d, *J* = 4 Hz, 1H), 4.78 (m, 2H), 3.69 (m, 2H), 2.68 (m, 2H), 1.81 (s, 6H), 1.13 (t, *J* = 7 Hz, 3H), MS *m/z* 375.68 [M+1].

3-cyano-9-ethyl-6,6-dimethyl-11-oxo-6,11-dihydro-5H-benzo[b]carbazole-8-carboxylic acid (24)

Compound **40** (333 mg, 0.98 mmol) was dissolved in DMF (5 mL). OsO_4 2.5% in *t*-BuOH (100 µL, 0.01 mmol) was added and stirred for 5 minutes. Oxone (2.4 g, 3.9 mmol) added and stirred for 6 hours. LC-MS analysis showed the reaction was complete. Na2SO3 sat. aq. Solution (50 mL) added and stirred for 1 h. Partitioned between EtOAc and 1M HCl. Extracted with EtOAc, washed with brine and condensed. Purification by reversed-phase HPLC using a gradient of 20-80% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a beige solid (285 mg, 82% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.89 (s, 1H), 8.34 (d, *J* = 8 Hz, 1H), 8.13 (d, J = 8 Hz, 2H), 8.03 (s, 1H), 7.64 (d, J = 8 Hz, 1H), 2.98 (q, J = 7.2 Hz, 2H) 1.76 (s, 6H), 1.23 (t, J = 8 Hz, 3H), MS *m/z* 359.43 [M+1].

(E)-9-ethyl-6,6-dimethyl-8-(3-morpholinoprop-1-en-1-yl)-11-oxo-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (25)

(E)-(3-chloroprop-1-en-1-yl)boronic acid (42 mg, 0.34 mmol) and **37** (100 mg, 0.23 mmol) were dissolved in DMF (5 mL). Cs_2CO_3 (370 mg, 1.14 mmol) was added and the mixture thoroughly degassed. $Pd(dppf)Cl_2$ (17 mg, 0.02 mmol) was added and the mixture heated to 100 ° C for 1 hour. LC-MS analysis showed conversion of the startming material to the desired product. The mixture was cooled to rt, and morpholine (60 µL, 0.68 mmol) was added. The mixture was stirred at rt for 2 hours. LC-MS analysis showed conversion to the desired product. The mixture was filtered and purified by reversed-phase HPLC using a gradient of 10-80% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a brown solid (56 mg, 56% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.82 (s, 1H), 8.30 (d, *J* = 8 Hz, 1H), 8.03 (m, 3H), 8.03 (s, 1H), 7.64 (d, *J* = 8 Hz, 1H), 7.19 (d, *J* = 16 Hz, 1H), 6.48 (m, 1H), 3.99 (m, 4H), 2.80 (q, *J* = 8 Hz, 2H) 1.79 (s, 6H), 1.20 (t, J = 7.2 Hz, 3H), MS *m/z* 440.84 [M+1].

9-ethyl-8-(6-hydroxypyridin-3-yl)-6,6-dimethyl-11-oxo-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (26)

The procedure used to prepare compound **7** was used to prepare compound **26**. Purification by reversed-phase HPLC using a gradient of 10-90% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a beige solid (33 mg, 60% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.88 (s, 1H), 8.34 (d, *J* = 8 Hz, 1H), 8.18 (s, 1H), 8.06 (d, *J* = 7 Hz, 2H), 7.98 (d, *J* = 8 Hz, 1H), 7.89 (br, 1H), 7.73 (s, 1H), 7.64 (d, *J* = 8 Hz, 1H), 7.03 (d, *J* = 8 Hz, 1H), 2.68 (q, *J* = 7.2 Hz, 2H), 1.78 (s, 6H), 1.18 (t, *J* = 7 Hz, 3H), MS *m/z* 408.29 [M+1].

8-(6-aminopyridin-3-yl)-9-ethyl-6,6-dimethyl-11-oxo-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (27)

The procedure used to prepare compound **7** was used to prepare compound **27**. Purification by reversed-phase HPLC using a gradient of 10-90% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a beige solid (28 mg, 50% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.78 (s, 1H), 11.84 (br, 1H), 8.36 (d, *J* = 8 Hz, 1H), 8.14 (s, 1H), 7.67 (s, 1H), 7.63 (d, *J* = 8 Hz, 1H), 7.55 (d, *J* = 7.2 Hz, 1H), 7.46 (s, 1H), 6.46 (d, *J* = 8.2 Hz, 1H), 2.69 (q, *J* = 8 Hz, 2H), 1.78 (s, 6H), 1.17 (t, *J* = 7 Hz, 3H), MS *m/z* 407.72 [M+1].

8-((2-(dimethylamino)ethyl)(methyl)amino)-9-ethyl-6,6-dimethyl-11-oxo-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (28)

The procedure used to prepare compound **17** was used to prepare compound **28**. Purification by reversed-phase HPLC using a gradient of 20-90% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a beige solid (12 mg, 32% yield). ^{1}HNMR (400 MHz, DMSO-d_6) *δ* 12.78 (br, 1H), 8.29 (d, *J* = 4 Hz, 1H), 8.05 (s, 1H), 7.99 (s, 1H), 7.58 (d, J = 7.2 Hz, 1H), 7.47 (s, 1H), 2.8 (s, 3H), 2.73 (m, 5H), 2.48 (s, 6H), 2.73 (m, 5H), 1.79 (s, 6H), 0.88 (t, *J* = 8 Hz, 3H) MS *m/z* : 415.33 (M + 1).

9-ethyl-6,6-dimethyl-11-oxo-8-(1-(tetrahydro-2H-pyran-4-yl)-1H-pyrazol-4-yl)-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (29)

The procedure used to prepare compound **7** was used to prepare compound **29**. Purification by reversed-phase HPLC using a gradient of 10-80% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a beige solid (48 mg, 76% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.76 (s, 1H), 8.34 (d, *J* = 8 Hz, 1H), 8.18 (s, 1H), 8.11 (S, 1H), 8.01 (S, 1H), 7.78 (d, J = 8 Hz, 2H), 7.62 (d, J = 8 Hz, 1H), 4.49 (q, J = 7.2 Hz, 1H), 3.98 (m, 4H), 3.50 (m, 4H), 2.85 (q, J = 7.2 Hz, 2H), 1.76 (s, 6H), 1.23 (t, J = 8 Hz, 3H), MS *m/z* 465.84 [M+1]

9-ethyl-6,6-dimethyl-11-oxo-8-(1-(piperidin-4-yl)-1H-pyrazol-4-yl)-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (30)

The procedure used to prepare compound **7** was used to prepare compound **30**. Purification by reversed-phase HPLC using a gradient of 30-100% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a beige solid (38 mg, 60% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.82 (s, 1H), 8.33 (d, *J* = 8 Hz, 1H), 8.13 (d, *J* = 8 Hz, 2H), 8.03 (S, 1H), 7.87 (s, 1H), 7.79 (s, 1H), 7.63 (d, J = 8 Hz, 2H), 4.59 (m, 1H), 3.47 (m, 2H), 3.13 (m, 2H), 2.85 (q, J = 7.2 Hz, 2H), 2.26 (m, 4H), 1.79 (s, 6H), 1.23 (t, J = 8 Hz, 3H), MS *m/z* 464.47 [M+1]

8-(1-(2-(dimethylamino)ethyl)-1H-pyrazol-4-yl)-9-ethyl-6,6-dimethyl-11-oxo-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (31)

The procedure used to prepare compound **7** was used to prepare compound **31**. Purification by reversed-phase HPLC using a gradient of 30-100% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a beige solid (29 mg, 48% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.83 (s, 1H), 8.34 (d, *J* = 8 Hz, 1H), 8.22 (s, 1H), 8.14 (s, 1H), 8.03 (s, 1H), 7.95 (s, 1H), 7.78 (s, 1H), 7.63 (d, J = 8 Hz, 1H), 4.21 (m, 2H), 3.65 (m, 2H), 2.86 (s, 6H), 1.79 (s, 6H), 1.24 (t, J = 8 Hz, 3H), MS *m/z* : 452.57 (M + 1).

8-(1-(3-(dimethylamino)propyl)-1H-pyrazol-4-yl)-9-ethyl-6,6-dimethyl-11-oxo-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile (32)

The procedure used to prepare compound **7** was used to prepare compound **32**. Purification by reversed-phase HPLC using a gradient of 30-100% CH_3CN/H_2O with 0.035% TFA to give the desired compound as a beige solid (31 mg, 49% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 13.05 (s, 1H), 8.36 (d, *J* = 8 Hz, 1H), 8.18 (s, 1H), 8.11 (S, 1H), 8.15 (S, 1H), 8.06 (S,

1H), 7.90 (S, 1H), 7.82 (S, 1H), 7.62 (d, J = 8 Hz, 1H), 4.32 (m, 2H), 3.15 (m, 2H), 2.86 (m, 5H), 2.27 (m, 2H), 1.83 (s, 6H), 1.26 (t, J = 8 Hz, 3H); ^{13}C NMR 100 MHz (DMSO-d_6) δ 179.61, 160.69, 150.93, 146.15, 139.55, 139.25, 136.12, 136.26, 130.09, 128.13, 127.41, 126.17, 125.25, 122.09, 120.52, 116.95, 109.94, 105.11, 54.78, 48.95, 42.70, 36.75, 30.35, 26.31, 25.38, 15.20; MS *m/z* 466.37 [M+1]

2-(4-(3-cyano-9-ethyl-6,6-dimethyl-11-oxo-6,11-dihydro-5H-benzo[b]carbazol-8-yl)-1H-pyrazol-1-yl)-N-methylacetamide (33)

The procedure used to prepare compound **7** was used to prepare compound **33**. Purification by reversed-phase HPLC using a gradient of 20-100% CH3CN/H2O with 0.035% TFA to give the desired compound as a beige solid (27 mg, 44% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.87 (s, 1H), 8.51 (S, 1H), 8.31 (d, *J* = 8 Hz, 1H), 8.13 (d, *J* = 8 Hz, 2H), 8.02 (s, 1H), 7.86 (S, 1H), 7.64 (d, J = 8 Hz, 1H), 6.43 (s, 1H), 5.32 (s, 2H), 3.27 (s, 3H), 2.83 (q, *J* = 8 Hz, 2H), 1.80 (s, 6H), 1.22 (t, *J* = 8 Hz, 3H), MS *m/z* 452.59 [M+1].

2-(4-(3-cyano-9-ethyl-6,6-dimethyl-11-oxo-6,11-dihydro-5H-benzo[b]carbazol-8-yl)-1H-pyrazol-1-yl)-N,N-dimethylacetamide (6)

The procedure used to prepare compound **7** was used to prepare compound **6**. Purification by reversed-phase HPLC using a gradient of 20-100% CH3CN/H2O with 0.035% TFA to give the desired compound as a beige solid (34 mg, 54% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.77 (s, 1H), 8.57 (S, 1H), 8.34 (d, *J* = 8 Hz, 1H), 8.13 (d, *J* = 8 Hz, 2H), 8.02 (s, 1H), 7.86 (S, 1H), 7.64 (d, J = 8 Hz, 1H), 5.21 (s, 2H), 3.01 (s, 3H), 2.89 (s, 3H), 2.83 (q, J = 8 Hz, 2H), 1.80 (s, 6H), 1.22 (t, J = 8 Hz, 3H); ^{13}C NMR 100 MHz (DMSO-d_6) δ 179.61, 167.01, 160.63, 147.78, 146.10, 139.58, 138.93, 136.61, 136.16, 131.37, 129.83, 128.13, 127.3, 126.22, 125.33, 122.09, 120.42, 116.85, 109.97, 105.15, 53.34, 36.74, 36.36, 35.68, 30.39, 26.32, 15.27; MS *m/z* 466.19 [M+1]

2-(4-(3-cyano-9-ethyl-6,6-dimethyl-11-oxo-6,11-dihydro-5H-benzo[b]carbazol-8-yl)-1H-pyrazol-1-yl)acetamide (34)

The procedure used to prepare compound **7** was used to prepare compound **34**. Purification by reversed-phase HPLC using a gradient of 20-100% CH3CN/H2O with 0.035% TFA to give the desired compound as a beige solid (24 mg, 40% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.86 (s, 1H), 8.63 (S, 1H), 8.25 (d, *J* = 8 Hz, 1H), 8.08 (d, *J* = 8 Hz, 2H), 7.96 (s, 1H), 7.87 (S, 1H), 7.64 (d, J = 8 Hz, 1H), 5.31 (s, 2H), 2.87 (q, J = 8 Hz, 2H), 1.77 (s, 6H), 1.18 (t, J = 8 Hz, 3H); MS *m/z* 438.59 [M+1]

2-(4-(3-cyano-9-ethyl-6,6-dimethyl-11-oxo-6,11-dihydro-5H-benzo[b]carbazol-8-yl)-1H-pyrazol-1-yl)-N,N,2-trimethylpropanamide (35)

The procedure used to prepare compound **7** was used to prepare compound **35**. Purification by reversed-phase HPLC using a gradient of 10-100% CH3CN/H2O with 0.035% TFA to give the desired compound as a beige solid (38 mg, 57% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.76 (s, 1H), 8.33 (d, *J* = 8 Hz, 1H), 8.22 (s, 1H), 8.11 (s, 1H), 8.02 (s, 1H), 7.87 (s, 1H), 7.82 (s, 1H), 7.64 (d, J = 8 Hz, 1H), 2.85 (q, J = 8 Hz, 2H), 1.81 (s, 6H), 1.77 (s, 6H), 1.19 (t, J = 8 Hz, 3H)MS *m/z* : 494.61 (M + 1).

4-(3-cyano-9-ethyl-6,6-dimethyl-11-oxo-6,11-dihydro-5H-benzo[b]carbazol-8-yl)-N,N-dimethyl-1H-pyrazole-1-carboxamide (36)

The procedure used to prepare compound **7** was used to prepare compound **36**. Purification by reversed-phase HPLC using a gradient of 20-100% CH3CN/H2O with 0.035% TFA to give the desired compound as a beige solid (31 mg, 51% yield). ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 12.77 (s, 1H), 8.52 (S, 1H), 8.34 (d, *J* = 8 Hz, 1H), 8.13 (d, *J* = 8 Hz, 2H), 8.02 (s, 1H), 7.86 (S, 1H), 7.63 (d, J = 8 Hz, 1H), 2.83 (q, J = 8 Hz, 2H), 2.51 (S, 6H), 1.85 (s, 6H), 1.22 (t, J = 8 Hz, 3H) MS *m/z* 452.68 [M+1]

ASSOCIATED CONTENT

Ba/F3 cellular IC50 curves for crizotinib, ceritinib, alectinib and compound **6**. Cellular IC50 curves for patient derived NSCLC cells and neuroblastoma cells for compound **6**. Cellular IC50's with standard deviations for crizotinib, ceritinib, alectinib and compound **6**. Full Ambit profiling for Compound **6** and **32**. This material is available free of charge via the internet at http://pubs.acs.org

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ABBREVIATIONS

ALK, anaplastic lymphoma kinase; ALKi, anaplastic lymphoma kinase inhibitor; NPM, nucleoplasmin; EML4, echinoderm microtubule-associated protein-like 4; ALCL, anaplastic large cell lymphoma; NSCLC, non-small-cell lung cancer; DLBCL, diffuse large b-cell lymphoma; IMT, microfibroblastic tumor.

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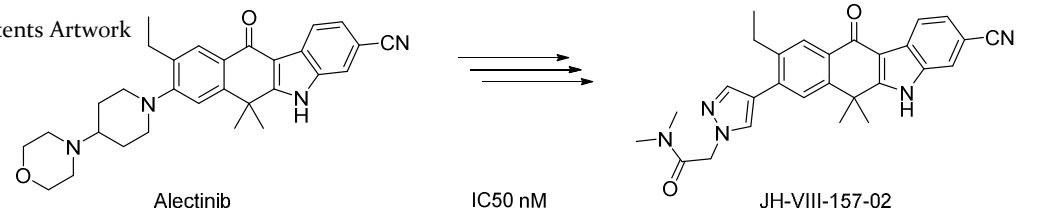
Table of Contents Artwork



Alectinib		IC50 nM	JH-VIII-157-02	
EML4-ALK^{WT}	EML4-ALK^{G1202R}		EML4-ALK^{WT}	EML4-ALK^{G1202R}
2	207		2	2